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SEED GERMINATION STUDIES IN *RHODODENDRON MADDENII* HOOK.F. AND RHODODENDRON NIVEUM HOOK.F.

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SUMMARY

The genus *Rhododendron* constitutes a very important dominant combination in temperate, sub-alpine and alpine region of the Sikkim Himalaya. The present investigation was undertaken to examine the effect of various physical and chemical agents and plant growth regulators for enhancing uniform seed germination. Among the various plant growth regulators and chemicals tried, only a few could significantly influence seed germination over control. Seeds of *Rhododendron maddenii, R. niveum* in MS medium treated with GA_3 (250 µM) recorded maximum germination. The BAP did not enhance the seed germination in *R. maddenii;* BAP (250 µM) was infact inhibitory. On the other hand, in *R. niveum,* BAP enhanced seed germination. The combined treatments of gibberellins and BAP resulted in reduced germination in *R. maddenii* and enhanced germination in *R. niveum.* Among nitrogenous compounds, KNO_3 decreased germination in *R. maddenii,* and increased in *R. niveum*. However KOH solution was found to be beneficial in both concentrations. Seed germination percentage and seed vigour were decreased with the increase of storage time.

Key words: **Plant growth regulators, *Rhododendron,* seed germination**

INTRODUCTION

The genus *Rhododendron* (family Ericaceae) comprises about 1000 species mainly inhabiting a vast section of south-eastern Asia stretching from north-western Himalaya through Nepal, north-eastern India, eastern Tibet, northern Burma and western and central China (Leach 1961). The genus constitutes a very important dominant combination of forest types in cool temperate and sub-alpine region, and also on the alpine meadows of the Sikkim Himalaya (Pradhan and Lachungpa 1990). *Rhododendron* has a major use in landscaping, accent and woodland planting. It supports a wide range of biodiversity. Therefore, this genus could be regarded as a keystone element in these high altitude areas. *R. maddenii* and *R. niveum* are important for community and commercial (tourism) in trekking corridors of the Sikkim Himalaya. Both species are endemic to Sikkim and Bhutan (*R. maddenii* is also found in China) between 2900 to 3650 m amsl (above mean sea level) and needs state protection. *R. niveum* is the state tree of Sikkim (Tiwari and Chauhan 2005). There is a great potential of these plant species in horticulture.

It is now widely accepted that the physiological quality of seed, defined in terms of percentage, rate and uniformity of germination, has a major impact on the efficiency and production. It has been recognized that

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a quick method to predict seed viability can improve the seed processing and storage. Seeds of some plants (Agrawal *et al*. 1973) showed positive correlation between germination percentage and viability percentage as determined by tetrazolium test (one of the rapid test for seed quality). Small seeds (like Rhododendrons) require suitable medium supplemented with nutrient and carbohydrates source for vigorous *in vitro* seed germination and growth (Bhattacharjee *et al.* 1999). The use of plant growth substances and chemicals to break seed dormancy and to synchronize seed germination is well known (Vanstaden *et al*. 1987, Whitehead & Sutcliffe 1995). Physical parameters such as light and temperature also influence the germinability of the seeds. Regeneration of rhododendrons under natural condition through seeds are erratic due to prevailing harsh climatic condition; largely limited number of viable seeds germinates, and germination is also not uniform. Seed size widely influences seedling fitness, and small seeds with very low reserves of nutrients could be potentially disadvantageous to rhododendrons. Taking note of the above, present investigation was undertaken to examine the effect of various physical and chemical agents and plant growth regulators (PGR's) for uniform and enhance seed germination and storage of seeds.

MATERIAL AND METHODS

Mature fruits (capsule) of both species were collected from different localities situated between 2000 to 4500 m amsl in the Sikkim Himalaya; the capsules were collected between September to December 2000 and 2001 as the period of maturity varied for both species. These were air dried for one week and the seeds were collected from ruptured capsules and stored in small bottles / polythene bags in the laboratory under normal room conditions. The temperature during storage varied from 15 to 30^{0}C (room temperature) with light intensity ranging between 30-50 μmol m^{-2} s^{-1} during daytime. Some of the seeds were kept at 4 ^{0}C in dark for storage experiment.

Viability test: Seeds were subjected to tetrazolium test (Agrawal *et al.* 1973), immediately after collection and at six month intervals after storage for determination of seed viability. The topographical staining pattern of different parts of seeds was recorded under a dissection microscope.

Imbibition: One hundred air dried seeds (in triplicate) were weighed collectively, allowed to imbibe water in beakers (50 ml water, 20^{0}C in dark) and at regular intervals seeds were removed, wiped dry with the help of blotting paper and their fresh weight determined. Once the weight was recorded the seeds were transferred back into beakers containing water.

Experiments with PGR's, physical and chemical agents: Following surface disinfection with $HgCl_2$ solution (0.1%) for 10 minutes, seeds were washed thoroughly with double distilled water (x2) and placed in beaker containing 25 ml of various test solutions (24h at 20 ^{0}C in dark). Stock solution of all chemicals were made in water or in 50 per cent ethanol (*v/v*) and diluted with distilled water before treatments. The seeds were then washed with autoclaved double distilled water (x2) and incubated in MS hormone free medium (Murashige and Skoog 1962). All cultures were incubated at 20^{0}C± 2^{0}C and 75± 5% RH under a 12h photoperiod, with a light intensity 60 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes. Some culture tubes / vials were wrapped with dark carbon paper to maintain dark condition. Seeds kept for germination in dark were taken out in dim light (yellowish light with the light intensity about 15-20 μmol m^{-2} s^{-1}) for 1-2 minutes after every week to record germination. Experiments were also done in 10 ^{0}C and 30 ^{0}C under light and dark condition. To study the effect of media, seeds were incubated in petridishes on the layer of filter paper, and distilled water was used as a germination media. Three replicates per treatment with 100 seeds in each replicate were studied for each species. Seeds showing radicle emergence were recorded as germinated. Germination was monitored at weekly intervals up to a period of 16 weeks (i.e. the last recorded germination). The experiment was repeated once.

Effect of seed storage on viability: Viability of the stored seeds was monitored by germination test following the method described earlier at intervals of six months for a period of one year.

The chemicals used were of AR grade from Qualigens Chemicals Pvt. Ltd., Mumbai while PGR's was from Sigma Chemical Company, St. Louis, USA.

RESULTS

Seed viability testing : All parts of freshly collected seeds took stain in the majority of seeds but this declined with storage. In both species all parts took stain in over 70 % seeds reflecting a high degree of viability in freshly collected seeds. The values were found to decrease with storage over a period of one year. In general, staining pattern and seed viability appeared to be similar in both the species (Table 1).

Imbibition: The imbibition data reveals that the seeds absorb water quite rapidly within the first 24 h with 230 per cent increase for *R. maddenii* and 500 per cent increase for *R. niveum* over initial weight (initial weight for *R. maddenii* was 17mg/100 seeds and 3mg/100 seeds for *R. niveum*); uptake reached a plateau 251percent for *R. maddenii* and 525 percent for *R. niveum* in next 2 days (48 hours) (Table 2). Therefore, a pre-soaking treatment of 24h was used to ensure sufficient uptake of PGR's and chemicals for influencing germination.

Effect of temperature and light : The germination behaviour of *R. maddenii* and *R. niveum* is shown in Table 3. In general, the observations reported here reveal the fact that both species germinate at higher temperatures and under light condition with considerable variations in the percentage germination and the time taken for germination. Time taken for germination of seeds of both species were 3-12 weeks depending upon species under light condition at 20 and 30 ^{0}C temperature. At 10 ^{0}C under light and 10, 20 & 30 ^{0}C under dark condition no germination took place. Germinated seedling vigour was observed by health quality, percentage survival of seedling and their growth. Seedlings germinated at 30 ^{0}C under light condition were not seems to be healthy and showed poor growth.

Effect of PGR's and chemicals: The rate of seed germination in *R. maddenii* and *R. niveum* following various treatments is summarized in Table 4 and 5. Among the various plant growth regulators and chemicals tried, only a few could significantly influence seed germination over control. Seeds of *R. maddenii* and *R. niveum* in MS hormone free medium and MS with GA_3 (Gibberellic Acid) (250 µM) treated were recorded with maximum germination, although higher concentration of GA_3 (250µM) could significantly enhance (65% compared to 40% in control) germination in *R. maddenii* and *R. niveum* (43% in compared 25% in control). The cytokinin BAP did not enhance the seed germination in *R. maddeni*; BAP (250 µM) was infact inhibitory. On the other hand, in *R. niveum* BAP enhanced the seed

Table 1. The staining pattern of seeds of *R. maddenii* and *R. niveum* after collection and in six months intervals.

Staining status	% Seed stained (Storage at 4 ^{0}C)						% Seed stained (Storage between 15 to 30 ^{0}C)					
	R. maddenii			*R. niveum*			*R. maddenii*			*R. niveum*		
	0 MAS*	6 MAS	12 MAS	0 MAS	6 MAS	12 MAS	0 MAS	6 MAS	12 MAS	0 MAS	6 MAS	12 MAS
All parts of seeds well stained	78	36	30	82	25	15	74	36	30	80	30	25
All parts stained partially	12	30	18	08	25	10	20	40	35	08	30	30
Cotyledons not stained properly	09	06	18	06	13	21	06	20	24	09	28	25
All parts unstained	01	28	34	04	37	54	00	04	11	03	12	20

*MAS = Months After Storage

Table 2. Imbibition status in *R. maddenii* and *R. niveum*

Time (h)	Weight (mg)	
	R. maddenii	***R. niveum***
01	17	3
02	18	3
04	22	5
06	22	5
12	23	7
18	36	14
24	40 (230%)	16 (500%)
36	41	17
48	42 (251 %)	17 (525%)

germination (33% in BAP (25 µM) and 38% in BAP 250 µm compared to 25% in control). The combined treatments of gibberellins and BAP resulted in reduced germination in *R. maddenii* and enhanced germination in *R. niveum*. KNO_3 (Potassium Nitrate) decreased germination in *R. maddenii*, and increased in *R. niveum*. However, KOH (Potassium hydroxide) solution was found to be beneficial in both concentrations. The first sign of germination (3%) was observed in *R. maddenii* in 2nd week following treatment with 250 µM KOH; this value increased to 16% in 4th week (compared to 20% in control) reaching as high as 45% in 16th week (compared to 40% in control). The first sign of germination in *R. niveum* was observed in 4th week following treatments with KOH and KNO_3 (250 µm) (compared to 6th week in control)

Effect of seed storage on germination and viability : The germination pattern of stored seeds of *R. maddenii* and *R. niveum* at 4^0C is summarized in Table 6. Seed germination percentage and seed vigour (percentage survival of germinated seedling) were decreased with increase of storage time. Pattern of the effect of PGR's and chemicals on seed germination were seems to be similar, as observed in case of freshly collected seeds. Immediately after harvest, the seeds of both species were viable and gradually lost viability over time.

DISCUSSION

The seeds of *R. maddenii* and *R. niveum* were viable and gradually lost viability over time (Table 6). Since these plants grow at higher altitudes and seeds are exposed to sub-zero temperature and snowfall during the winter, like other alpine seeds, chilling requirement appears to be essential for germination in the following spring; this is suggested by the fact that seeds stored at 4°C remained viable for some time. Seeds of other alpine plants also exhibit a period of after ripening as in rhododendrons (Nautiyal *et al*. 1987). The dormancy breaking and germination stimulating effect of low temperature could possibly be under control of increased synthesis and sensitivity of seeds to plant growth regulators (PGR's) such as gibberellins, cytokinins and ethylene (Whitehead and Sutclife 1995). Amongst the various PGR's and chemicals tested (Table 4 and 5) only GA_3 (250 µM) and KOH (25µM and 250 µM) enhanced germination in both species, while BAP enhanced seed germination in *R. niveum* and reduced in *R. maddenii*.

Table 3. Germination percentage of seeds of *R. maddenii* and *R. niveum* at various temperatures under light and dark conditions.

Species	At 20 ºC		At 30 ºC	
	Time taken for germination (First to last seed)	**% Germination**	**Time taken for Germination (First to last seed)**	**% Germination**
R. maddenii	6-8 weeks	65±3	3-5 weeks	70#±5
R. niveum	10-12 weeks	43±2	5.5-8 weeks	52#±3

Per cent germination shown are in average ± standard errors.

At 10 ^{0}C temperature under light condition and 10 ^{0}C, 20 ^{0}C & 30 ^{0}C under dark condition no germination took place.

Seedlings germinated at 30 ^{0}C under light condition were not seems to be healthy and showed poor growth.

Table 4. Percentage seed germination in *R. maddenii* following various treatments

Treatments	Germination (%)			
	4 WAI*	8 WAI	12 WAI	16 WAI
Control	20 ±2	40 ±1	40 ±1	40 ±1
GA_3 (25 µM)	21 ±3	42 ±6	42 ±6	42 ±6
GA_3 (250 µM)	15 ±3	65 ±3	65 ±3	65 ±3
BAP (25 µM)	08 ±4	34 ±2	36 ±3	38 ±2
BAP (250 µM)	08 ±5	31 ±2	32 ±1	32 ±1
GA_3 + BAP (25 µM each)	20 ±3	36 ±3	36 ±3	36 ±3
GA_3 + BAP (250 µM each	20 ±2	29 ±1	29 ±1	29 ±1
KNO_3 (25 µM)	14 ±2	35 ±2	35 ±2	35 ±2
KNO_3 (250 µM)	11 ±1	25 ±3	25 ±3	25 ±3
KOH (25 µM)	17 ±5	42 ±2	42 ±2	42 ±2
KOH (250 µM)	16 ±2	45 ±1	45 ±1	45 ±1

Values shown are in average ± standard errors
* WAI = weeks after inoculation

Table 5. Percentage seed germination in *R. niveum* following various treatments

Treatments	Germination (%)			
	4 WAI*	8 WAI	12 WAI	16 WAI
Control	0 ±0	25 ±0	25 ±0	25 ±0
GA_3 (25 µM)	0 ±0	26 ±4	26 ±4	26 ±4
GA_3 (250 µM)	0 ±0	40 ±3	43 ±2	43 ±2
BAP (25 µM)	0 ±0	25 ±2	30 ±0	33 ±2
BAP (250 µM)	0 ±0	33 ±2	36 ±1	38 ±3
GA_3 + BAP (25 µM each)	0 ±0	32 ±3	33 ±2	33 ±2
GA_3 + BAP (250 µM each	0 ±0	28 ±5	28 ±5	28 ±5
KNO_3 (25 µM)	0 ±0	36 ±1	36 ±1	36 ±1
KNO_3 (250 µM)	2 ±0.4	32 ±2	32 ±2	32 ±2
KOH (25 µM)	0 ±0	36 ±1	36 ±1	36 ±1
KOH (250 µM)	5 ± 0	40 ±0	40 ±0	40 ±0

Values shown are in average ± standard errors
* WAI = weeks after inoculation

Table 6. Percent germination in stored seeds of *R. maddenii* and *R. niveum* at 4^0C following various treatments

Treatments	*R. maddenii* % Germination		*R. niveum* % Germination	
	6 MAS*	12 MAS	6 MAS	12 MAS
Control	35 ±2	26 ±1	20 ±3	08 ±2
GA_3 (25 µM)	37 ±3	30 ±0	20 ±2	12 ±1
GA_3 (250 µM)	47 ±5	30 ±3	31 ±2	25 ±0
BAP (25 µM)	23 ±2	16 ± 3	30 ±0	12 ±2
BAP (250 µM)	27 ±2	09 ±2	27 ±3	07 ±0
GA_3 + BAP (25 µM each)	24 ±1	13 ±3	28 ±4	11 ±2
GA_3 + BAP (250 µM each)	25 ±0	18 ±2	26 ±2	10 ±0
KNO_3 (25 µM)	33 ±3	29 ±3	33 ±3	31 ±3
KNO_3 (250 µM)	23 ±3	17 ±2	28 ±1	24 ±2
KOH (25 µM)	31 ±2	25 ±0	28 ±2	11 ±1
KOH (250 µM)	34 ±3	25 ±0	32 ±3	25 ±0

Values shown are in average ± standard errors
* MAS= months after storage

The endogenous PGR's level in the seeds do vary (Voesenek and Blom 1996) and a variety of developmental processes can be regulated by changes in their concentration and/or modifications in the sensitivity of tissues towards it (Trewaves and Cleland 1983). In this regard it must be noted that lower and physiological concentrations of PGR's should be applied, as in this study. High concentrations may not be effective, e.g. treatments with 5-10 mM GA_3 did not stimulate seed germination in *Podophylum hexandrum* (Chaudhary *et al.* 1996) whereas 250 µM GA_3 could significantly improve it (54% compared to 31% in control) (Nadeem *et al.* 2000). Cytokinins are also involved in breaking of seed dormancy and stimulation of germination (Walker *et al.*1989, Vilalobas and Martin 1992); they may not be directly involved in the breaking of dormancy but play a permissive role by decreasing the level of germination inhibitors and making the seed more sensitive to gibberellins (Walker *et al*. 1989). Such a role of cytokinins can be ascribed in this study as BAP could enhance germination in *R. niveum,* whereas an inhibitory influence in *R. maddenii*. Since an interplay of endogenous PGR's plays a significant role in the

regulation of seed germination, it may be relevant to determine the endogenous PGR levels during the course of seed storage and examine if a relationship exists between PGR content and seed viability. The inhibitory influence of cotyledons is likely to be regulated by endogenous PGR's in *R. maddenii* (Pandey *et al.* 2000).

The nitrogenous compound KNO_3 used in this study markedly enhanced germination (32-36%) in *R. niveum*; however, in *R. maddenii* KNO_3 was inhibitory. The use of chemicals like KNO_3, KOH and Thiourea to stimulate germination also has been reported by Chaudhary *et al.* (1996) and McIntyre *et al.* (1996). The physiological role is, however, still not clear. It had been proposed that oxidized forms of these chemicals promote germination by causing a shift in respiratory metabolism to the pentose phosphate pathway (Roberts and Smith 1977). More recently, based on investigation with *Avena fatua* seeds, these chemicals (50 and 100 μM) were found to promote germination of dormant caryopses by accumulating in the embryo where it acts osmotically to increase water uptake. This led them to suggest that these chemical combine an osmotic role on water uptake with a positive effect on protein synthesis (McIntyre *et al*. 1996). Further studies would be needed to understand the mode of action of these compounds. The results of this study indicate that use of KOH and GA_3 (BAP and KNO_3 to a certain extent) is useful for advancing the time, synchronizing and stimulating the seed germination in these two species.

In general, the germination in both the species was favoured by high temperature and continuous light with some variation in the percentage of germination (Table 3) and the time taken for onset of germination (Table 4 and 5). This supports the earlier reports for Himalayan alpine and temperate species and north American alpine and southern hemisphere plant species (Amen 1996, Semwal and Purohit 1980). The germination under high temperature and light is of adaptive advantage and ensures the establishment of seedlings in harsh climatic conditions. From the results of storage experiments it is likeky that the seeds of rhododendrons are sensitive to desiccation. The results of tetrazolium tests (Table 1) generally agreed with the germination tests. The observed slow staining and lesser degree of intensity could be reflection of the loss of viability due to desiccation, exposure to temperatures as well as aging. New viable seeds also reduced the clarity of tetrazolium chloride solution with dull red leachates.

Since both species have been considered as rare and endangered and large-scale removal still continues at rates well over natural regeneration (Tiwari and Chauhan 2006), special attention needs to be given for propagation and conservation. Systematic propagation via well defined protocol for seed germination would go a long way in achieving mass scale propagation/ conservation.

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REFERENCES

Agrawal, P.K., Karihaloo, J.L., Ahmed, S.M.M. and Gupta, P.C. (1973). Predicting germinability in maize, wheat and paddy seeds on the basis of tetrazolium test. *Seed Res.* **1:** 83-85.

Amen, R.D. (1996). The extent and role of seed dormancy in alpine plants. *Q. Rev. Biol.* **41:** 271-281.

Bhattacharjee, S., Khan, H.A. and Reddy, P.V. (1999). Effect of various sucrose levels on i*n vitro* seed germination of *Phalaenopsis* Hybrids. *J. Hill Res.* **12**: 58-60.

Chaudhary, D.K., Kaul, B.L. and Khan, S., (1996) Breaking seed dormancy of *Podophyllum hexandrum* Royal ex *Camb* (Syn.) *P. emodi* wall. Ex *Honigberger*). *J. Non Timber Forest Products* **3** : 10-12.

Leach, D.G. (1961). Rhododendrons of the World. Charles Scribners's Sons, New York.

McIntyre, G. I., Cessna, A.J. and Hsiao, A. I. (1996) Seed dormancy in *Avena fatua:* Interacting effects of nitrate, water and seed coat injury. *Physiol. Plant.* **97** : 291-302.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15** : 473-497.

Nadeem, M., Palni, L.M.S., Purohit, A.N., Pandey, H. and Nandi, S.K. (2000). Propagation and conservation of *Podophyllum hexandrum* Royal: An important medicinal herb. *Biol. Cons.* **92** : 121-129.

Nautiyal, M.C., Rawat, A. S., Bhadula, S.K. and Purohit, A.N. (1987). Seed germination in *Podophyllum hexandrum*. *Seed Res.* **15** : 206-209.

Pradhan, U.C. and Lachungpa M.L. (1990). Sikkim Himalayan Rhododendrons. Primulaceae Books, Kalimpong, West Bengal.

Pandey, H., Nandi, S.K., Nadeem, M. and Palni, L.M.S. (2000). Chemical stimulation of seed germination in *Aconitum heterophyllum* Wall. and *A. balfourii* Stapf. Important himalayan species of medicinal value. *Seed Sci. Tech.* **28:** 39-48.

Roberts, E., H. and Smith, R. D. (1977). Dormancy and pentose phosphate pathway. In : A. Khan (ed.), The Physiology and Biochemistry of Seed Dormancy and Germination, pp. 384 -411. North Holland Publication Company, Amsterdam.

Semwal, J.K. and Purohit, A.N. (1980) Germination of Himalayan alpine and temperate potentilla. *Proc. Indian Acad. Sci., Plant Sci.* **89**: 61-65.

Tiwari, O.N. and Chauhan,U.K. (2005). Genus *Rhododendron* status in Sikkim Himalaya: An assessment. *J. American Rhododendron Soc.* **59**: 147-158.

Tiwari, O.N. and Chauhan, U.K. (2006). Rhododendron conservation in Sikkim Himalaya. *Curr. Sci.* **90** : 532-541.

Trewavas, A.J. and Cleland, R.E.(1983). Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? *Trends in Biochem. Sci.* **8**: 354-357.

Vanstaden, J., Manning, J.C. and Dickens, C.W.S.(1987). A phylogenetic analysis of the role of plants hormones in the development and germination of legume seeds. In: C.H. Stirton (ed.), Advances in Legume Systematics, pp. 387-432. Royal Botanical Garden, Kew.

Vilalobas, N. and Martin, L. (1992). Involvement of cytokinins in the germination of chickpea seeds. *Plant Growth Regul.* **11**: 277-291.

Voesenek, L.A.C.J. and Blom, W.P.M. (1996). Plant and hormones: An ecophysiological view on timing and plasticity. *J. Eco.* **84**: 111-119.

Walker, M.A., Roberts, D.R., Waite, J.L. and Dumbroff, E.B. (1989). Relationship among cytokinin, ethylene, and polyamines during the stratification – Germination process in seeds of *Acer saccharum*. *Physiol. Plant.* **76**: 326-332.

Whitehead, C.S. and Sutcliffe, M.A.(1995). Effect of low temperatures and different growth regulators on seed germination in *Cyclopia species*. *J. Plant Physiol.* **147**: 107-112.