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Effect of Seed Pre-Treatments on Germination of *Swertia chirayita* — A Rare Medicinal Plant

ARVIND BHATT, R.S. RAWAL AND UPPEANDRA DHAR

G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora 263 643
arvin_bhatt@rediffmail.com

ABSTRACT Effect of seed pre-treatments on per cent germination and mean germination time (MGT) of different seed lots of *Swertia chirayita* revealed that GA_3 (100 ppm) improved germination and reduced mean germination time (MGT) significantly ($p<0.05$). Variability was recorded in germination response ($p<0.05$) among populations, and possible reasons for such variation are discussed.

Key words: *Swertia chirayita*, critically rare, mean germination time, GA_3

Swertia chirayita (Roxb. ex Fleming) Karsten (Family - Gentianaceae), a rare medicinal plant [1], is an erect annual herb. It is distributed between 1200-3000m asl in the Himalaya from Kashmir to Bhutan, and in the Khasia hills in Meghalaya. The whole plant is of medicinal value and used as a blood purifier, in skin diseases, as bitter tonic for fever and indigestion, laxative, anthelmentic, antidiarrhoetic, antiperiodic and tonic for gout and in bronchial asthma [2]. The presence of xanthones, used as antituberculous, is also reported [3, 4].

Demand for *S. chirayita* is high in the pharmaceutical industry. For instance, besides local supplies, each year about 60-100 metric ton of Chirayita is imported into India [5]. The demand of *S. chirayita* raw material is largely met from wild through destructive harvesting. As a result, the species has reached critically rare status. Also, poor availability of species in wild has resulted in higher adulteration rate to meet the increasing demand. In view of this, the studies on germination and establishing a practicable germination package assumes a great significance. Realizing this need, the present study attempts to address issues related to: (i) improvement of germination percentage and reduction in Mean Germination Time (MGT), (ii) analyze the extent of variation in germination responses among seeds of different populations.

MATERIALS AND METHODS

Seeds of *S. chirayita* were collected in October 2002 (reddish brown seeds regarded to be the mature) [6] from four locations [viz. Kanchula and Duggalbitha - Uttaranchal; Pullag and Dora - Himachal Pradesh (Table 1)]. Immediately after collection, seeds were dried at room temperature (20°C ± 2°C) for 7 days and stored in brown paper bags until the start of germination experiment (December 2002). The mean seed weight showed only marginal difference between the studied populations. Ten seeds at random in three replicates from each population were studied for measuring seed length and width [7].

Seeds from each population were surface sterilized in 0.5 per cent aqueous solution of mercuric chloride for two minutes and then rinsed thoroughly by distilled water before soaking in different concentrations (100, 200, 400 ppm) of chemicals (i. e., GA_3, IAA and KNO_3 for 18h). Germination tests were conducted using 75 seeds in each set of experiment (3 replicates of 25 seeds each). Seeds were sown in plastic Petri plates (95mm x 17mm) containing moistened filter paper

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(Whattman No. 1) and kept in growth chamber at (25°C ± 2°C). Suitable control of untreated seeds were also maintained. All the experiments were conducted in complete randomized block design. Analysis of variance (ANOVA) was applied using SYSTAT [8] for all the experiments. Least significant difference was estimated separately for comparison of treatment and population means [9].

RESULTS AND DISCUSSION

The maximum mean seed size (length and width) was recorded for P_3 and minimum for P_1 population (Table 1). However, there was no influence of mean seed size on germination percentage for different populations. The control set (untreated seeds) from different populations showed poor germination (Table 2) response (maximum P_2-33.3% and minimum P_4-25.3%). However, the variation among populations was significant ($p < 0.05$).

The germination was significantly ($P < 0.001$) more in 100 ppm GA_3 treated seeds in all populations (Table 2). Highest germination (78.7%) in P_2 and P_4 population was significantly ($p < 0.05$) more than the control (untreated seeds) and P_1 and P_3 populations (Table 2). As compared to control (untreated seed) GA_3 (100 ppm) also reduced the mean germination time (MGT) significantly in all the populations. However, maximum reduction was obtained for P_2 population (MGT 19 days), which was lower than the other populations. The best response under application of GA_3 (100 ppm) treatment is in agreement with previous study wherein seeds of *S. chirayita*, treated with 50-400 ppm of GA_3 showed higher (72-98%) germination [10]. Previous studies on *S. chirayita* have reported improvement of germination up to 91.0 per cent through prechilling and up to 100 per cent through GA_3 (400 ppm) treatments. However, variations among populations was not studied. Therefore, the success in the replicability of such results remains doubtful as compared to multiple population investigations. For example, in the present study, best germination (78.7%) is achieved through 100 ppm GA_3 treatment in population P_2 and P_4. This was significantly better response than the responses of seeds from other locations. This suggests that the probability of getting better results in a particular treatment may remain confined to a specific seed source.

IAA treatments improved germination significantly only in 400 ppm for seeds from P_2 (61.0%) and P_4 (50.6%) population as compared to control (untreated seeds). While comparing different populations, the maximum improvement obtained for P_2 (61.0%) at 400 ppm, was significantly higher as compared to P_1 and P_3 populations under same treatment (Table 2). IAA treatments also reduced

Table 1. Location and general features of the identified populations of *Swertia chirayita*

Population	Mean seed weight (mg)	Mean seed length (mm)	Mean seed width (mm)	Moisture content (%)	Habitat characteristics
P_1-Dugalbitha (2440 m asl)	10.1	808.13	598.66	12.2	Open area with *Rhododendron arboreum* and *Quercus leucotrichophora*
P_2-Pullag (2650m)	9.5	825.84	618.93	15.0	Moist gentle slope with *Abies pindrow* and *Acer* spp
P_3-Kanchula (2650m)	9.9	842.17	619.04	13.9	Moist slope with *A. pindrow*, *Acer* spp and *Quercus* spp
P_4-Dora (2500m)	10.4	827.23	612.24	10.5	Gentle slope with *A. pindrow* and *Acer* spp

Table 2. **Effect of different pre-treatments on germination percentage of *Swertia chirayita***

Treatment (ppm)	Populations				LSD (p<0.05)	F
	P_1	P_2	P_3	P_4		
Control	29.33	33.33	26.66	25.33	8.00	2.33*
GA_3 100	53.33	78.66	64.00	78.66	13.60	9.80**
200	33.33	38.66	36.00	57.33	19.66	2.66*
400	32.00	50.66	36.00	42.67	8.11	12.18**
IAA 100	32.00	45.00	38.66	30.66	29.55	NS
200	36.00	46.00	37.33	40.00	29.99	NS
400	38.00	61.00	36.00	50.66	15.08	7.18**
KNO_3 100	40.00	64.00	42.66	60.00	15.48	7.00**
200	48.00	76.00	41.33	66.66	21.79	5.85**
400	46.66	66.66	40.00	65.33	14.54	10.15**
LSD (p<0.05)	16.22	23.21	11.75	11.75		
F	2.15*	3.93**	5.81**	5.81**		

*p<0.05, **p<0.01.

mean germination time but the responses varied across the concentration in different populations. Only P_2 and P_4 population showed significant reduction in MGT as compared to control (untreated seeds). Different concentration of IAA showed variations in response in different populations. As such, IAA treatments have been used to stimulate germination in many species [11-13]. However, the treatment can not be generalised for the species as the present study does not show uniform results across different populations.

KNO_3 treatment also improved germination significantly ($p < 0.05$) as compared to control (untreated seeds) in almost all the populations. The maximum improvement (76.0%) was observed for P_2 population under 200 ppm, which was significantly higher as compared to P_1 and P_3 populations (Table 2). Further increase in concentration decreased germination. However, this decrease was not significant ($p > 0.05$). The application of nitrogenous compounds, particularly KNO_3 to stimulate germination has also been reported previously [14, 15]. KNO_3 plays a critical role in increasing physiological efficiency of the seed [16]. Inconsistent germination due to KNO_3 application in the present study is in agreement with Qaderi and Cavers [17], who found different degree of germination pattern in different populations of *Onopordum acanthium*.

Considering variation in responses under different treatments for specific populations, P_2 and P_4 responded the best. Almost all the germination enhancing treatments (i.e. GA_3 100 ppm, IAA 400 ppm and KNO_3 200 ppm) improved the germination per cent of P_2 and P_4 significantly as compared to other populations. Compared to untreated seeds, most of the treatments reduced Mean Germination Time (MGT) (Table 3).

Table 3. **Effect of best responding treatments on MGT (days) of *Swertia chirayita***

Treatment (ppm)	Populations				LSD (p<0.05)	F
	P_1	P_2	P_3	P_4		
T_1	24.78	25.73	26.18	28.30	3.69	NS
T_2	20.38	19.00	20.43	19.93	2.75	NS
T_3	25.69	23.17	23.72	22.53	3.92	NS
T_4	20.46	20.60	19.30	21.65	3.95	NS
LSD(P<0.05)	4.68	1.99	4.62	2.68		
F	4.40*	26.63**	5.62**	22.32**		

p<0.05, **p<0.01, NS: non significant; T_1-control (untreated seeds), T_2-GA_3, T_3-IAA, T_4-KNO_3.

As GA_3 and KNO_3 behave similarly in the present study with respect to germination enhancement of the species, it may be attributed to the physiological dormancy [18]. Such dormancy is most often reported to be overcome by chemicals including potassium nitrate [19] and gibberellins [20, 21]. Using the germination protocols developed in the present study, production of large number of seedlings can be attempted to meet raw material demand through cultivation. The variation in germination in different lots of the species seems helpful in identifying the best seed source for mass multiplication. Such population can be marked and conserved *in situ* as seed production areas.

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