

Research Note

Effects of moist-chilling and GA₃ applications on seed germination of three *Pedicularis* species from Yunnan, China

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Summary

Most species of the genus *Pedicularis* L. have rather high ornamental value and some species are medicinal plants. Little is known about the germination requirements of these species and it is necessary to obtain fundamental information about seeds germination for the purpose of introduction and cultivation. Six stratification (4°C and darkness) durations (15, 30, 45, 60, 75, 90 days) plus control, as well as 3 GA₃ solution concentrations (100, 500, 1000 mg/L) were tested on seeds of *Pedicularis rex*, *P. rhinanthoides*, and *P. longiflora* var. *tubiformis*. The optimal germination conditions varied between species, while as a whole, the highest germination percentage was obtained from treatments of 500-1000 mg/L GA₃ followed by 15-30days stratification, and the lowest value of mean germination time was obtained from treatments of 100-1000ppm GA₃ followed by 15-day stratification.

Experimental and discussion

The genus *Pedicularis* L. (Orobanchaceae) is considered to be one of the largest angiosperm genera in the northern hemisphere. It is mainly distributed in cold, high-latitude or montane habitats (Yang *et al.*, 1998; Wang *et al.*, 2003). Most species of *Pedicularis* are attractive, colorful plants with beaked and long-blooming flowers. Plants of this genus have great potential as ornamental plants (Wang and Tang, 2005). Furthermore, chemical constituents from many species of the genus have special pharmacological effects (Wang and Jia, 1995, 1996; Liao *et al.*, 1999; Yang *et al.*, 2006). Some *Pedicularis* species have rather high medical value in traditional Chinese medicine (Zhu, 1997; Guan *et al.*, 2006). However, cultivation of *Pedicularis* has never been achieved. The present study was undertaken to determine the effects of GA₃ and moist-chilling on seed germination of *Pedicularis rex* C.B. Clarke ex Maxim., *P. rhinanthoides* Schrenk, and *P. longiflora* Rudolph var. *tubiformis*, and to find optimal germination conditions for these species.

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Seeds of the three species were collected in mid-September, 2006 from Zhongdian, Yunnan (27°54'N, 99°38'E). Tested seeds were cleaned by blowing and were stored in envelopes under room conditions until the initiation of experiments one month later. Before experiments, seeds were surface sterilized in 1% sodium hypochlorite solution for 5 minutes, and then rinsed 5-7 times with distilled water. The effects of moist-chilling and GA₃ were tested in a two-factor experiment. The first factor consisted of four GA₃ solution concentrations: seeds were imbibed for 24h with 0, 100, 500, 1000 mg/L GA₃ solution at 25°C in darkness. The second factor consisted of seven stratification durations: seeds were treated with 0, 15, 30, 45, 60, 75 and 90 days of moist-chilling at 4°C in darkness.

Stratification media was sand moistened with distilled water. After treatments, seeds were cultured in 9 cm Petri dishes with two layers of filter paper moistened with distilled water. Whenever it was needed to keep filter papers moist, additional water was added. Petri dishes were placed in a growth chamber at 10/20°C alternating temperature (12 hours: 12 hours), with cool fluorescent light (66% illuminate degree of 12000 LX) during the period at higher temperature. Each treatment was replicated three times and 25 seeds were used in each replication. Germination was recorded daily and a seed was considered germinated when the radicle was approximately 2mm long. Germinated seeds were discarded from the Petri dishes daily.

When no further germination took place for 5 continuous days, germination percentage (GP) and mean germination time (MGT) were calculated as:

$$GP = (\sum n_i / N)$$

$$MGT(\text{days}) = \sum (t_i * n_i) / \sum n_i$$

Where t_i is the number of days from experiment starting, n_i is the number of seeds germinated at each day and N is the total number of seeds tested. All percentage data sets were arcsine transformed before analysis. The data were analyzed using SPSS 11.5 software. After conducting an analysis of variance (General Linear Model), the S-N-K criterion was used to detect significant differences among the treatments at 5% level of significance.

Untreated seeds had very low germination percentage (12% or less). Both moist-chilling and GA₃ applications significantly improved germination percentage of all three species (table 1). For *P. rex*, the highest germination percentages were obtained from the treatments of 500 mg/L GA₃ followed by 15 or 30-day stratification (62.7 and 65.3%, respectively). For *P. rhinanthoides*, the highest germination percentage (100%) was obtained from the treatments of 500 mg/L GA₃ followed by 0-30 days stratification, or the treatment of 1000 mg/L GA₃ without stratification. For *P. longiflora* var. *tubiformis*, the highest germination percentages were obtained from the treatments of 1000 mg/L GA₃ followed by 0 or 15-day stratification (94.7 and 96.0%, respectively).

Stratification durations had significant effect on mean germination time of all three species, while GA₃ treatments had no significant effect on mean germination time of all three species (table 2). For *P. rex*, the lowest value of mean germination time (7.61d) was obtained from the treatment of 100 mg/L GA₃ followed by 15-day stratification. For *P. rhinanthoides* and *P. longiflora* var. *tubiformis*, the lowest value of mean germination time

Table 1. Effects of different treatments on germination percentage(%) of seeds of 3 *Pedicularis* species.

Species	Stratification duration(day)	GA ₃ solution concentration (mg/L)			Total by duration
		0	100	500	
<i>P. rex</i>	0	6.67±2.31	20.00±5.66	26.00±8.49	22.67±2.31
	15	41.33±15.14	37.33±6.11	62.67±2.31	56.00±8.00
	30	52.00±6.93	52.00±10.58	65.33±11.55	62.67±11.55
	45	36.00±6.93	48.00±13.86	50.67±4.62	54.67±2.31
	60	45.33±4.62	37.33±12.86	48.00±13.86	50.67±6.11
	75	25.33±6.11	14.67±2.31	37.33±6.11	30.67±6.11
	90	26.67±4.62	16.00±0.00	24.00±4.00	16.00±4.00
	Total by concentration	33.33±15.77a	32.80±16.45a	45.80±16.85b	41.90±18.3b
	0	4.00±0.00	69.00±25.38	100.00±0.00	100.00±0.00
<i>P. rhinanthoides</i>	15	46.67±16.17	92.00±6.93	100.00±0.00	94.67±4.62
	30	78.67±2.31	97.33±2.31	100.00±0.00	96.00±4.00
	45	90.67±2.31	93.33±4.62	82.67±8.33	81.33±8.33
	60	85.33±11.55	81.33±8.33	46.67±6.11	33.33±8.33
	75	76.00±10.58	54.67±9.24	28.00±0.00	21.33±2.31
	90	73.33±14.05	38.67±12.86	9.33±6.11	8.00±0.00
	Total by concentration	64.95±29.96a	74.91±23.46b	65.00±36.60ab	64.80±36.70ab
	0	12.00±5.66	65.00±17.40	80.00±5.66	94.67±4.62
	15	14.67±2.31	40.00±13.86	82.67±12.22	96.00±4.00
<i>P. longiflora</i> var. <i>tubiformis</i>	30	44.00±6.93	61.33±8.33	81.33±4.62	92.00±8.00
	45	72.00±6.93	92.00±0.00	77.33±11.55	76.00±10.58
	60	72.00±8.00	60.00±10.58	68.00±10.58	41.33±4.62
	75	57.33±2.31	49.33±4.62	61.33±2.31	36.00±10.58
	90	48.00±4.00	41.33±20.53	32.00±10.58	13.33±2.31
	Total by concentration	47.40±23.04a	58.73±19.84b	68.40±18.98c	64.19±32.39c

Data are means and standard deviations. Different letters represent significant different treatment means by S-N-K test at 5% level of significance.

Table 2. Effects of different treatments on mean germination time (day) of seeds of 3 *Pedicularis* species.

Species	Stratification duration(day)	GA ₃ solution concentration (mg/L)			Total by duration
		0	100	500	
<i>P. rex</i>	0	9.61±0.56	9.39±1.02	10.39±1.43	9.94±0.95
	15	8.42±0.94	7.61±0.66	8.49±0.99	8.90±0.62
	30	9.67±0.87	9.41±0.17	9.25±0.59	9.38±0.75
	45	9.91±1.38	9.25±0.61	9.83±1.23	10.18±0.61
	60	12.10±1.99	11.96±1.97	11.69±0.46	13.41±0.82
	75	11.38±1.38	13.89±1.21	12.00±0.38	17.11±0.27
	90	15.36±1.59	13.67±2.10	13.84±2.19	13.06±3.14
	Total by concentration	10.99±2.49a	10.74±2.55a	10.81±2.04a	11.73±3.01a
	0	11.50±4.95	6.28±0.75	5.80±0.23	5.11±0.31
<i>P. rhinanthoides</i>	15	4.82±0.52	3.83±0.27	3.48±0.26	3.20±0.33
	30	5.48±0.24	4.67±0.15	4.67±0.15	4.34±0.64
	45	5.19±0.05	5.23±0.08	5.40±0.25	6.00±0.47
	60	5.45±0.31	5.95±0.17	6.46±0.76	8.28±1.23
	75	5.92±0.86	7.23±0.31	8.05±1.62	7.32±1.65
	90	5.76±0.16	8.18±1.43	9.17±1.89	6.50±2.12
	Total by concentration	6.04±2.24a	5.93±1.49a	6.16±2.08a	5.80±1.90a
	0	8.57±0.94	7.57±0.51	7.18±0.69	7.08±0.54
	15	6.11±1.01	6.83±0.38	5.92±0.52	4.92±0.39
<i>P. longiflora</i> var. <i>tubiformis</i>	30	7.23±0.65	7.17±0.28	6.99±0.25	5.48±0.04
	45	7.22±0.34	7.72±0.28	6.45±0.74	7.12±1.40
	60	8.19±0.30	7.91±0.43	8.12±0.33	8.10±0.51
	75	7.46±0.48	7.46±0.50	7.77±0.69	8.14±0.17
	90	7.90±1.69	8.10±0.85	8.59±1.05	9.50±1.88
	Total by concentration	7.53±1.07a	7.54±0.58a	7.29±1.06a	7.19±1.70a

Data are means and standard deviations. Different letters represent significant different treatment means by S-N-K test at 5% level of significance.

(3.26d and 4.92d, respectively) were all obtained from the treatment of 1000 mg/L GA₃ followed by 15-day stratification. It was noticeable that treatments of 15-day stratification resulted in the lowest mean germination time for all three species.

In conclusion, both moist-chilling and GA₃ applications are effective methods to improve germination in all species tested, indicating the dormancy in these seeds might be physiological. Germination does not seem to be the obstacle for cultivation. Li *et al.* (2007) presented that GA₃ slightly increased germination of some *Pedicularis* species. However, GA₃ soaking applications in the present experiment were much longer (24h instead of 2h) and more significant results were achieved. This research showed different result from other holoparasitic members of Orobanchaceae, which needed specific signals from host plants for germination (Batchvarova *et al.*, 1999). The species studied in our research seemed to be independent of these signals.

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