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Research Article
Influence of Light Intensity and Photoperiod on the Seed Germination of Four *Rhododendron* Species in Taiwan

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Abstract

Background and Objective: There are 15 native *Rhododendron* species in Taiwan, among which 11 species are endemic and compose 73% of these native species. Although researchers predominantly use cuttings to propagate *Rhododendron* shrubs, there are no studies on the seed germination of *Rhododendron* species. The objective of this study was to evaluate the seed germination of four *Rhododendron* species in Taiwan under different light intensities and photoperiods. **Materials and Methods:** Two experiments on the seed germination percentage of *R. breviperulatum*, *R. kanehirai*, *R. ovatum* and *R. simsii* were conducted in this study. The first experiment was to identify the seed germination percentage of these four *Rhododendron* species using different light intensities (0, 700, 1400 and 3200 lux). The second experiment was to clarify the seed germination percentage of these four *Rhododendron* species using different photoperiods (0, 1, 4 and 16 h). All statistical analyses were performed using Statistical Package for the Social Science (SPSS12.0) for Windows software program. The data were analyzed using Tukey's multiple range test at the $p \leq 0.05$ significance level. **Results:** After 30 days, no seed germination occurred in darkness. The highest average seed germination percentages were all observed at 700 lux: *R. breviperulatum* (83.3%), *R. kanehirai* (68.9%), *R. ovatum* (85.6%) and *R. simsii* (92.2%). The highest average germination percentages of seeds were observed in *R. breviperulatum* at 16 h (83.3%), *R. kanehirai* at 1 h (60.0%), *R. ovatum* at 16 h (84.4%) and *R. simsii* at 16 h (85.6%). According to the results, these four *Rhododendron* species required light for germination. There were significant differences ($p<0.05$) in the seed germination of these four *Rhododendron* species for light intensity greater than 700 lux. Similar results were observed with photoperiods. **Conclusion:** The seed germination percentage of *R. breviperulatum*, *R. ovatum* and *R. simsii* increased with increasing photoperiod.

Key words: Germination, intensity, photoperiod, *Rhododendron breviperulatum*, *Rhododendron kanehirai*, *Rhododendron ovatum*, *Rhododendron simsii*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rhododendron is a large genus of the family Ericaceae[1,2]. Approximately 1,200 native *Rhododendron* species have been reported worldwide[3]. In Taiwan, much attention has been paid to the conservation of endemic *Rhododendron* species. Current propagation of *Rhododendron* species is carried out via cuttings[4,5], which can lead to a narrower genetic makeup of *Rhododendron* in Taiwan and decrease the genetic diversity of *Rhododendron* species. The best genetic conservation strategy is through sexual propagation[6].

The number of seedlings that emerge from the soil can help determine plant distribution and abundance[7-9]. Different factors of light such as light intensity, light quality and photoperiod can influence seed germination[10]. Olson[11] and Cho *et al.*[12] demonstrated that light is essential to seed germination of *Rhododendron* species. In terms of light quality, red light (660 nm) can promote seed germination[13]. Experiments have also been conducted on the daily number of hours required and the interaction of light and temperature[14-21] and previous studies have reported that the best photoperiod for *Rhododendron* seed germination is 24 h[14-16, 21-23].

There have been no studies on the seed germination of *Rhododendron* in Taiwan. The purpose of this study was to evaluate the rate and trend of seed germination of four *Rhododendron* species in Taiwan under four different light intensities and four different photoperiods.

MATERIALS AND METHODS

Seed preparation and treatment: Four native *Rhododendron* species of Taiwan, *Rhododendron breviperulatum* Hayata, *R. kanehirai* Wilson, *R. ovatum* Planch and *R. simsii* Planch., were examined. Mature seed capsules were collected in the fall of 2012.

Rhododendron breviperulatum is endemic to Taiwan. Seeds of *R. breviperulatum* were collected from the Guandao mountain located in central Taiwan (longitude, 121°00'48.7" E; latitude, 24°01'18.3" N; altitude, 1,590 m). *Rhododendron kanehirai* is also endemic to Taiwan. Seeds of *R. kanehirai* were collected from the Wu-Shi-Keng area located in central Taiwan (longitude, 120°56'53.5" E; latitude, 24°16'24.5" N; altitude, 1,008 m). *Rhododendron ovatum* is native to Taiwan. Seeds of *R. ovatum* were collected from the Da-Ken area located in central Taiwan (longitude, 120°44'0.7" E; latitude, 24°10'39.1"N; altitude, 1,008 m). *Rhododendron simsii* is also native to Taiwan. Seeds of *R. simsii* were collected from the Wu-Shi-Keng area located in central Taiwan (longitude, 120°56'53.5"E; latitude, 24°16'24.5" N; altitude, 1,008 m). All of the collected seed capsules were air-dried at room temperature until seed release. The released seeds were then collected, placed into paper bags and refrigerated at 4°C.

The chaff and other debris were removed using sieves. After their surface was cleaned, the seeds of these four *Rhododendron* species were sterilized with 30% solution of hydrogen peroxide for 90 sec, rinsed 3 times with sterilized distilled water and soaked overnight in sterilized distilled water. Afterward, 30 seeds of each species were sown in a 9 cm petri dishes and each treatment combination was performed with three replicates[24].

Experimental light intensity: All seeds were incubated daily under the following light intensities: 0, 700, 1400 and 3200 lux. For 0 lux (L0), the petri dishes were completely wrapped with aluminum foil. For 700 lux (L1), the dishes were placed underneath two black net layers and for 1400 lux (L2), the dishes were placed underneath one black net layer. For 3200 lux (L3), the dishes were directly exposed to light. All light intensities were measured using a photometer YF-170 (Tenmars) and all treatments were conducted under the same photoperiod and temperature in the growth chamber (16/8 h, 25/20°C light/dark)[25,26].

Experimental photoperiod: All seeds were incubated daily under the following photoperiods: 0 (continuous darkness, P0), 1 h (P1), 4 h (P4) and 16 h (P16). For 0 h (P0), the petri dishes were completely wrapped with aluminum foil. With the exception of P0, all photoperiod treatments began at 8 am under the same light intensity and temperature (3200 lux, 25/20°C) in the growth chamber.

Statistical analysis: The methods used in this study to evaluate the rate of seed germination of the *Rhododendron* species were modified from those of Hebert *et al.*[23]. The number of germinated seeds from total darkness was counted after 30 days of incubation. Seeds were considered germinated when their radicles were $\geq$1 mm in length. All statistical analyses were performed using Statistical Package for the Social Science for Windows (SPSS 12.0) (Illinois, USA) software program. All of the data represent the means of three separate experiments $\pm$ standard error (n = 3). The data were analyzed using Tukey's multiple range test at the $p \leq 0.05$ significance level.

RESULTS

The seeds of the four *Rhododendron* species were treated under different light intensities (0, 700, 1400 and 3200 lux) and photoperiods (0, 1, 4 and 16 h) during germination. These results are described as follows (Fig. 1, Table 1, 2).

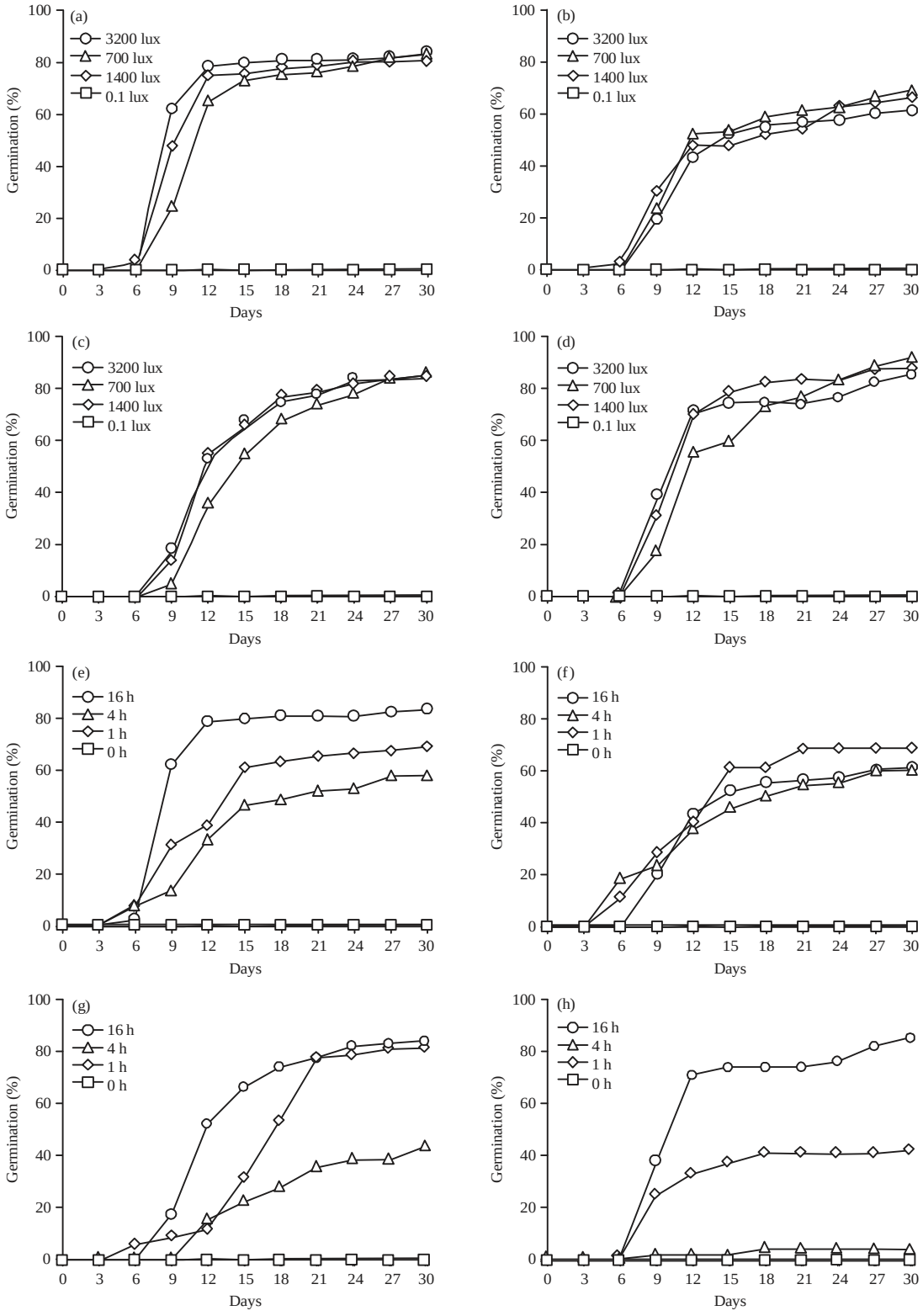

Fig. 1(a-h): Influence of light intensity and photoperiod on seed germination of four native *Rhododendron* species in Taiwan for 30 days (a): *R. breviperulatum*, (b): *R. kanehirai*, (c): *R. ovatum* and (d): *R. simsii*, germinated with daily light intensities of total darkness (L0), 700 lux (L1), 1400 lux (L2) or 3200 lux (L3), (e): *R. breviperulatum*, (f): *R. kanehirai*, (g): *R. ovatum*, (h): *R. simsii* germinated with daily photoperiods of total darkness (P0), 1 h (P1), 4 h (P4) or 16 h (P16)

Table 1: Influence of light intensity on seed germination of four *Rhododendron* species in Taiwan for 30 days

Specie	Treatment	Light intensity (lux)	Average germination (%)	Time for first germination (days)
R. breviperulatum	L0	0	0[b]	-
	L1	700	83.3±5.8[a]	9
	L2	1,400	81.1±10.7[a]	6
	L3	3,200	83.3±3.3[a]	6
R. kanehirai	L0	0	0[b]	-
	L1	700	68.9±6.9[a]	9
	L2	1,400	66.7±3.3[a]	6
	L3	3,200	61.1±3.9[a]	9
R. ovatum	L0	0	0[b]	-
	L1	700	85.6±3.9[a]	9
	L2	1,400	84.4±8.4[a]	9
	L3	3,200	84.4±7.7[a]	9
R. simsii	L0	0	0[b]	-
	L1	700	92.2±7.7[a]	9
	L2	1,400	87.8±1.9[a]	6
	L3	3,200	85.6±1.9[a]	9

L0: Light intensity is 0 lux, L1: Light intensity is 700 lux, L2: Light intensity is 1,400 lux, L3: Light intensity is 3,200 lux, Values in the same column with different letters were different at 5% significant level±standard error

Light intensity: Light intensity had high significant effect ($p<0.05$) on the average germination percentage of *R. breviperulatum* after 30 days of incubation (Table 1, Fig. 1a). Seeds of the L0 treatment did not germinate after 30 days of incubation. In the L1 treatment, the initial germination was observed on the ninth day. The average germination percentage of L1 after 30 days of incubation was 83.3±5.8%. In the L2 treatment, germination occurred on the sixth day and the average germination pe rcentage of L2 after 30 days of incubation was 81.1±10.7%. In L3, germination occurred on the sixth day and the average germination percentage of L3 after 30 days of incubation was 83.3±3.3%.

Light intensity had a strong significant effect ($p<0.05$) on the average germination percentage of *R. kanehirai* after 30 days of incubation (Table 1, Fig. 1b). No germination was observed in the L0 treatment after 30 days of incubation. The initial germination of the L1 treatment was on the ninth day and the average germination percentage of L1 after 30 days of incubation was 68.9±6.9%. The germination of L2 was first observed on the sixth day. The average germination percentage of L2 after 30 days of incubation was 66.7±3.3%. The germination of L3 treatment was first observed on the 9th day and the average germination percentage after 30 days of incubation of L3 was found to be 61.1±3.9%.

Light intensity had high significant effect ($p<0.05$) on the average germination percentage of *R. ovatum* after 30 days of incubation (Table 1, Fig. 1c). No germination was observed in the L0 treatment after 30 days of incubation. The initial germination in the L1 treatment occurred on the 9th day. The average germination percentage of L1 after 30 days of incubation was 85.6±3.9%. Germination in the L2 treatment was first observed on the ninth day and the average germination percentage of L2 after 30 days of incubation was 84.4±8.4%. The beginning of germination in the L3 treatment was first observed on the ninth day and the average germination percentage of L3 after 30 days of incubation was 84.4±7.7%.

Light intensity had high significant effect ($p<0.05$) on the average germination percentage of *R. simsii* after 30 days of incubation (Table 1, Fig. 1d). After 30 days of incubation, germination did not occur in the L0 treatment. The initial germination in the L1 treatment was observed on the ninth day of incubation and the average germination percentage of L1 after 30 days of incubation was 92.2±7.7%. The beginning of germination in L2 was observed on the sixth day of incubation. The average germination percentage of L2 after 30 days of incubation was 87.8±1.9%. Germination was first observed in the L3 treatment on the ninth day of incubation and the average germination percentage of L3 after 30 days of incubation was 85.6±1.9%.

Photoperiod: Photoperiod also had a strong significant effect ($p<0.05$) on the average germination percentage of *R. breviperulatum* after 30 days of incubation (Table 2, Fig. 1e). In the P0 treatment, no germination occurred after

Table 2: Influence of photoperiod on seed germination of four native *Rhododendron* species in Taiwan for 30 days

	Treatment	Photoperiods (h)	Average germination (%)	Time for first Specie germination (days)
R. breviperulatum	P0	0	0[d]	-
	P1	1	57.8±5.1[c]	6
	P4	4	68.9±1.9[b]	6
	P16	16	83.3±3.3[a]	6
R. kanehirai	P0	0	0[b]	-
	P1	1	60.0±5.8[a]	6
	P4	4	68.9±5.1[a]	6
	P16	16	61.1±3.9[a]	9
R. ovatum	P0	0	0[c]	-
	P1	1	43.3±12.0[b]	12
	P4	4	81.1±8.4[a]	6
	P16	16	84.4±7.7[a]	9
R. simsii	P0	0	0[c]	-
	P1	1	4.4±3.9[c]	9
	P4	4	42.2±10.2[b]	6
	P16	16	85.6±1.9[a]	9

L0: Photoperiod is 0 h, P1: Photoperiod is 1 h, P4: Photoperiod is 4 h, P16: Photoperiod is 16 h. Values in the same column with different letters are different at 5% significant level±standard error

30 days of incubation. In the P1 treatment, the initial germination occurred on the sixth day. The average germination percentage of P1 after 30 days of incubation was 57.8±5.1%. In the P4 treatment, germination was first observed on the sixth day and the average germination percentage of P4 after 30 days of incubation was 68.9±1.9%. In the P16 treatment, the initial germination occurred on the sixth day. The average germination percentage of P16 after 30 days of incubation was 83.3±3.3%.

Photoperiod also had a strong significant effect ($p<0.05$) on the average germination percentage of *R. kanehirai* after 30 days of incubation (Table 2, Fig. 1f). No germination was found in the L0 treatment after 30 days of incubation. The initial germination of the P1 treatment was observed on the sixth day. The average germination percentage of P1 after 30 days of incubation was 60.0±5.8%. Germination of the P4 treatment was first observed on the sixth day and the average germination percentage of P4 after 30 days of incubation was 68.9±5.1%. The initial germination of the P16 treatment occurred on the ninth day. The average germination percentage of P16 after 30 days of incubation was 61.1±3.9%.

Photoperiod also strongly significantly affected ($p<0.05$) the average germination percentage of *R. ovatum* after 30 days of incubation (Table 2, Fig. 1g). No germination was observed in the P0 treatment after 30 days of incubation. The first germination in the P1 treatment occurred on the twelfth day. The average germination percentage of P1 after 30 days of incubation was 43.3±12.0%. Germination in the P4 treatment was first observed on the sixth day and the average germination percentage of P4 was 81.1±8.4%. The initial germination in the P16 treatment was observed on the ninth day and the average germination percentage of P16 after 30 days of incubation was 84.4±7.7%.

Photoperiod also had a strong significant effect ($p<0.05$) on the average germination percentage of *R. simsii* after 30 days of incubation (Table 2, Fig. 1h). Germination did not occur in the P0 treatment after 30 days of incubation. Germination in the P1 treatment was first observed on the ninth day of incubation. The average germination percentage of P1 after 30 days of incubation was 4.4±3.9%. The initial germination in the P4 treatment was observed on the sixth day of incubation and the average germination percentage of P4 after 30 days of incubation was 42.2±10.2%. Germination in the P16 treatment was first observed on the ninth day of incubation and the average germination percentage of P16 after 30 days of incubation was 85.6±1.9%.

DISCUSSION

Regarding *R. breviperulatum*, L3 and L1 had the highest average germination percentages (83.3±3.3 and 83.3±5.8%, respectively) compared to those of L2 (81.1±10.7%) and L0 (0%). The Tukey analysis indicated that the values of L1, L2 and L3 were significantly higher ($p<0.05$) than those of L0. However, L1, L2 and L3 did not significantly differ from each other ($p<0.05$) (Table 1).

For *R. kanehirai*, L1 had higher average germination percentages (68.9±6.9%) than did L2 (66.7±3.3%), L3 (61.1±3.9%) and L0 (0%). Although the average germination percentage of L1, L2 and L3 was significantly higher ($p<0.05$) than that of L0 according to the Tukey analysis, L1, L2 and L3 were not significantly different from one another ($p<0.05$) (Table 1).

For *R. ovatum*, L1, L2 and L3 had higher average germination percentages (85.6±3.9, 84.4±8.4 and 84.4±7.4%, respectively) than did L0 (0%). According to the

Tukey analysis, it was found that L1, L2 and L3 were significantly higher ($p<0.05$) than L0. However, L1, L2 and L3 did not significantly differ from one another ($p<0.05$) (Table 1).

Regarding *R. simsii*, L1 had the highest average germination percentage (92.2±7.7%) compared to that of L2 (87.8±1.9%), L3 (85.6±1.9%) and L0 (0%). L1, L2 and L3 were significantly higher ($p<0.05$) than L0 according to the Tukey analysis. Nevertheless, L1, L2 and L3 were not significantly different from each other ($p<0.05$) (Table 1).

The light intensity experiments indicated that light increases seed germination percentage and is required to promote germination, as no seed germination occurred in the dark treatment of the four *Rhododendron* species. As such, the *Rhododendron* species studied required light for germination, especially at amounts up to 700 lux and all of the highest average germination percentages were observed at 700 lux.

The results of this study (Table 2, Fig. 1) indicate the benefits of light and agree with previous reports of seeds (*R. agastum*, *R. decorum*, *R. delavayi* and *R. irroratum*) exposed to full light and half light having higher seed germination rates than those under deep shade[27]. In addition, other studies on the seed germination of *Rhododendron* species (*R. chapmanii*[19], *R. vaseyi*[20] and *R. protistum* var. *giganteum*[19]) also support these results.

Regarding *R. breviperulatum* (Table 2), P16 had higher average germination percentages (83.3±5.8%) than did P4 (68.9±1.9%), P1 (57.8±5.1%) and P0 (0%). The Tukey analysis indicated that P16 was significantly higher ($p<0.05$) than P4, P1 and P0. All of these four treatments were significantly different among each other ($p<0.05$).

For *R. kanehirai* (Table 2), P4 had a higher average germination percentage (68.9±5.1%) than did P16 (61.1±3.9%), P1 (60.0±5.8%) and P0 (0%). The average germination percentages of P1, P4 and P16 were significantly higher ($p<0.05$) than that of P0 according to the Tukey test and P1, P2 and P3 were not significantly different from one another ($p<0.05$).

With respect to *R. ovatum* (Table 2), P16 had a higher average germination percentage (84.4±7.7%) than did P4 (81.1±8.4%), P1 (43.3±12.0%) and P0 (0%). According to the Tukey analysis, the values of germination percentage of P16, P4 and P1 were significantly higher ($p<0.05$) than those of P0. Although P1, P4 and P16 did not significantly differ from each other, P1 was significantly different from both P4 and P16 ($p<0.05$).

Regarding *R. simsii* (Table 2), P16 had the highest average germination percentage (85.6±1.9%) compared to that of P4 (42.2±10.2%), P1 (4.4±3.9%) and P0 (0%). The germination percentage of P16 was significantly higher ($p<0.05$) than that of P4, P1 and P0 according to the Tukey test.

The results of this study suggested that seed germination of *R. breviperulatum*, *R. ovatum* and *R. simsii* would increase with increasing photoperiod (Table 2, Fig. 1). Similar results were also discovered in *R. calendulaceum* (Michx.) Torr., *R. catawbiense* Michx., *R. chapmanii* A. Gray, *R. maximum* L., *R. protistum* var. *giganteum* and *R. vaseyi* A. Gray[14,15,20-23,27].

CONCLUSION

The results of this study confirmed that different light intensities did not play a significant role in seed germination; however, the effects of photoperiod varied with different *Rhododendron* species.

SIGNIFICANCE STATEMENT

This study discovers that light is a limiting factor for seed germination of *Rhododendron breviperulatum*, *R. kanehirai*, *R. ovatum* and *R. simsii*. This study will also help researchers in understanding that germination percentage of *Rhododendron breviperulatum*, *R. ovatum* and *R. simsii* is affected by photoperiod.

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