Seed Germination of *Rhododendron calophytum* Planch. in Response to Temperature, Light, and GA_3©

Bing Zhao
Northwest A&F University, Yangling, 712100, China

Jin-Ying Dong and Donglin Zhang
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: donglin@uga.edu

INTRODUCTION

Rhododendron calophytum Planch., commonly named large leaf *Rhododendron* or meili *Rhododendron* is in the *Ericaceae* family, *Rhododendron* genera. It is an endemic evergreen plant with beautiful flowers, found in high mountains at altitudes of 1,300 to 4,000 m in south-west China (Ran et al., 2010). This includes the Qinling Mountains, where the species is beneficial for helping to maintain the stability of the ecosystem. *Rhododendron calophytum* germplasm is endangered because of excessive excavation activities. Additionally, few cultivars are cultivated and utilized in modern city landscape.

In order to protect *R. calophytum* from extinction, help maintain its diversity, and utilization of its multiple-color landscape cultivars, it is necessary to develop propagation systems for *R. calophytum*. Seed propagation can be used to protect germplasm and enrich genetic diversity of the species (Zhang et al., 2010). More importantly, for wild resources, seedlings of diverse populations are more easily adapted to new environments than seedlings collected and transplanted from the mountains.

External factors such as light and temperature are known to affect seed germination. Studies of how environmental factors affect seed germination in *Rhododendron* genus have provided various results. Some species need higher temperatures to germinate (Antonidaki-Giatromanolaki et al., 2008; Sajad et al., 2012; Fan et al., 2011). Conversely, lower temperatures are favorable for germination of other species (Vologdina, 2006; Zhang et al., 2007).

Phytohormones can be used for breaking seed dormancy. Germination requirements of many *Rhododendron* species indicate that the seeds have a non-deep, simple, morphophysiological dormancy, but gibberellic acid (GA_3) application can increase seed germination of selected *Rhododendron* species (Tiwari and Chauhan, 2007; Gao et al., 2010; Su et al., 2011).

There are studies on *R. calophytum* using tissue culture (Luo et al., 2007), chemical analysis (Tian et al., 2010) and cultivation management (Si et al., 2012), but few on seed propagation.

The objective of the present study was to develop seed propagation systems for germinating *R. calophytum* by manipulating photoperiod, temperature, and GA_3 treatments. Establishing standard germination procedures is important for conservation strategies, including providing future breeding material of *R. calophytum*.

MATERIALS AND METHODS

Seed Source

Mature capsules of *R. calophytum* were harvested from Niu Beiliang district of Qinling Mountain at the end of November in 2012. The capsules were air-dried at room temperature until the seed released. Seed was collected, put into paper bags and refrigerated at 4°C (39°F) until experimentation.

Experimental Design

The main treatment effects were: light, photoperiod, and GA_3, each of which was treated as a single factorial. There were three photoperiod treatments were 0, 16 light/8 dark, and 24 h light. The three temperatures treatments were 20, 30, and 20°C (16 h)/30°C (8 h).

The six GA_3 treatments were 0, 200, 400, 600, 800, and 1,000 mg·L. Each treatment was replicated three times with 100 seeds per replicate, n=3.

Seed Germination Tests

Germination tests were performed in incubators (Conviron A1000, Canada) with automatic temperature and light control. Seeds were immersed in GA_3 for 24 h, then rinsed three times with deionized water and dried on filter paper. Finally, 100 seeds were counted out and put inside 12-cm petri dish containing absorbent gauze and filter paper. To control fungal contamination, seeds were periodically transferred to clean petri dishes, and the absorbent gauze and filter paper were changed and moistened with new deionized water every day. The germination dishes were randomly rearranged daily to avoid effects of potential temperature and light differences and other factors. The germinated seeds were recorded everyday for 6 d. Seeds were considered to have germinated as soon as radicle emerged from seed coats. Germination performance was evaluated according to germination percentage and germination vigor.

Statistical Analysis

Germination percentage and germinating vigor were calculated with the Excel software. Germination percentage GP = (n/N) × 100% ,where n is the number of germinated seeds, N is the number of seeds tested; germinating vigor (GV) is the germination percentage on the 11^{th} day, final day of test run.

RESULTS AND DISCUSSION

Seeds Morphology Observation

Seeds were small and flat, with an oval or long oval shape. There were obvious vertical stripes on the surface of seed and developed wings around the seed. The average length and width was around 0.2 and 0.1 cm, respectively. The seed coat was brown and weighed on average around 0.2 g.

Effect of Different Temperature on Germination of *Rhododendron calophytum*

Temperature was an important environmental factor affecting seed germination. Temperature of 30°C for 24 or 16 h depressed germination, while the optimal temperature (for control GA_3 treatment) was 20°C for 24 h (Fig. 1). Initial germination occurred after Day 8, and peaked 5 or 6 days later (Fig. 1). The results showed that there was little difference among the effects of the three temperature on the starting germination time and germination speed. While seed germination of *Cynanchum bungei* (Zhang, 2012) and *Rhododendron delavayi* (Duan et al., 2007) showed that the higher the temperature, the earlier seeds began to germinate (Zhang, 2012). There were significant difference among the effects of the three temperature regimes on germination percentage and vigor (Table 1). Both germination percentage and vigor were significantly highest at 20°C, and lowest at 30°C is the lowest (Table 1). The optimal lower temperature response may be due to this species being adapted to low temperatures, since wild populations of *R. calophytum* are found in mountainous areas with heights of 1,300 to 4,000 m and cooler temperatures. Our results are in agreement with seed germination of *R. molle* (Shi et al., 2010), but contrast to *R. irroratum* (Fan et al., 2011); the later findings reported that germination speed and germination vigor were best at 30°C. In our study, the germination vigor at 20°C was significantly greater than the higher temperature regimes, emphasizing the importance of seed propagation and cultivation of this species under cooler conditions.

Effect of Different Light Time on Germination of *Rhododendron calophytum*

A 24-h light exposure delayed seed germination of all GA_3 treatments until after Day 8, whereas seed exposed to 0 and 16-h light germinated by Day 8 (Fig. 2). At the end of the 13-day experimental runs, the final percent germination and germination vigor was greatest at 0- and 16-h light exposure and lowest at 24-h light exposure (Table 1). While

our results agree with Roberts (1973), they conflict with the benefits of light on germination percentage and germination vigor of four other *Rhododendron* species that require light for germination (Zhang, 2012).

Effect of Different Gibberellic Acid Concentration on Germination of *Rhododendron calophytum*

Compared with the control, seeds treated with GA_3 had greater germination percentage and germination vigor (Table 1, Fig. 1). Seed treated with GA_3 germinated after Day 8. While 200 $mg \cdot L^{-1}$ GA_3 had greater germination percentage and vigor than the control, the greatest benefit occurred with 400-1,000 $mg \cdot L$ GA_3. There were no significant differences among 400-1000 $mg \cdot L$ GA_3. Under the adverse conditions of 30°C for light exposure of 24-h to 16-h, there was a trend in 800 $mg \cdot L^{-1}$ GA_3 having the greatest benefit (Fig. 1). This suggests that a range of 400 to 800 $mg \cdot L^{-1}$ GA_3 is beneficial for seed germination.

The control did not germinate under darkness (0-h light) and also 24 h light; the germination percentage of the control under 16-h light was also lower than that of the seeds treated with GA_3 treatment (Fig. 2). Seeds of *R. jiulongshanense* and *R. annae* treated with 200 $mg \cdot L^{-1}$ GA_3 for 15 min had increased germination percentage and vigor (Gao et al., 2010), whereas in our study 200 $mg \cdot L^{-1}$ GA_3 was only marginally effective, i.e., GA_3 treatments of 400-800 $mg \cdot L^{-1}$ were more effective.

This research demonstrated seed propagation is a valuable method for the reproduction and conservation of *R. calophytum*. The high germination percentage obtained with this protocol will facilitate the conservation and development of new cultivars of *R. calophytum* germplasm.

ACKNOWLEDGEMENTS

This research was conducted in woody ornamental plant lab at the University of Georgia in USA. Funding for this research was provided by National Natural Science Foundation of China (k305021110), Shaanxi Natural Science Foundation (2012JQ3008), and construction project of Forestry Department of Shaanxi Province Shan (2011(70)).

Literature Cited

Antonidaki-Giatromanolaki, A., Dragassaki, M., Papadimitriou, M. and Vlahos, I. 2008. Effects of stratification, temperature and light on seed germination of *Colchicum macrophyllum* B.L. Burtt. Prop. Ornam. Plants 8:105-107.

Duan, Xu, Chen, X. and Zhao, Y.Y. 2007. Study of the seed germination of *Rhododendron delavayi*. J. Anhui. Agri. Sci. 35:9199-9200.

Fan, C.L., Chen, X. and Xing, J.N. 2011. Effects of different treatments on seed germination of *Rhododendron.* Seed 30:106-108.

Gao, G.L., Long, X.Q., Hu, X.J., Lu, X.L. and Chen, X. 2010. Effect of GA on two species of Alpine *Rhododendron* seed germination. Seed 5:22-25.

Luo, P., Zhuang, P. and Bai, J. 2007. Tissue culture of *Rhododendron decorum* Franch， *Rhododendron calophytum* Franch. and *Rhododendron discolor* Franch. Plant Physiol. Commun. 2:326-328.

Roberts, E.H. 1973. Predicting the storage life of seed. Seed Sci. Technol. 1:449-514.

Ran, F., Wu, C.C., Peng, G.Q., Korpelainen, H. and Li, C.Y. 2010. Physiological differences in *Rhododendron calophytum* seedlings regenerated in mineral soil or on fallen dead wood of different decaying stages. Plant Soil 337:205-215.

Sajad, R., Shailesh, P. and Nautiyal, S. 2012. Studies of seed germination in four *Rhododendron* species of Garhwal Himalayas. Indian Forest. 138:284-288.

Shi, D.H. and Chen, X. 2010. Effect of different treatments on *Rhododendron molle* G.D seed germination. Seed 9:91-94.

Su, J.L., Li, C., Chen, L., Liu, X.Q., Chen, S.P. and He, L.S. 2011. Effects of different pretreatment methods on seed germination of *Rhododendron aureum* and *R. parvifolium*. J. Plant Resour. Environ. 20:64-69.

Si, G.C., Zhang, Y.L., Gu, X., Wang, Y.Q. and Zhao, B. 2012. Study on cutting propagation technology of QinLing Wild *Rhododendron calophytum*. North. Hort. 3:77-79.
Tian, P., Fu, X.L., Zhuang, P., Bai, J. and Chen, F. 2010. Analysis on the volatile oils from *Rhododendron calophytum* Franch. by GC-MS. Chinese J. Appl. Environ. Biol. 5:734-737.
Tiwari, O.N. and Chauhan, U.K. 2007. Seed germination studies in *Rhododendron maddenii* Hook.f. and *Rhododendron niveum* Hook.f. Indian J. Plant Physiol. 12:50-56.
Vologdina, O.S. 2006. Biology of *Rhododendron dauricum*, *R. mucronulatum* and *R. sichotense* (*Ericaceae*) seeds germination. Rastitel'nye Resursy. 42:55-60.
Zhang, H.Y. 2012. Seed germination and early seedling growth of *Cynanchum bungei* Decne (*Asclepiadaceae*) in response to photoperiod, temperature, and seed size. HortScience 47:1338-1341.
Zhang, J.L., Wu, Y.W., Wu, H.Z., Zhao, H.Z. and Wang, Y.Y. 2012. Studies on seed germination of four species of Subgen. Hymeanthes (Blume) K. Koch *Ericaceae* in Yunnan. J. Agricul. Univ. 27:875-881.
Zhang, F., Yu, S.L. and Wang, J.H. 2010. Studies on the photosynthetic characteristics and its relationship to yield in Radix *Cynanchum bungei* Decne. J. Nuclear Agr. Sci. 24:176-180.

Table 1. Effect of temperature, light time, and gibberellic acid (GA_3) concentration on germination of *Rhododendron calophytum*.

Factors	Germination percentage (%)	Germination vigor (%)	Factors	Germination (%)	Germination vigor (%)
Light (h)	**	**	GA_3 ($mg \cdot L^{-1}$)	**	**
24	62.3 a	52.0 a	0	14.5 a	9.1a
16	83.4 b	76.5 b	200	64.7 b	58.6 b
0	80.4 b	76.8 b	400	72.9 c	64.2 c
Temperature (°C)	**	**	600	74.5 c	67.7 c
20	87.8 a	77.4 a	800	79.4 cd	74.7 cd
30	5.0 b	4.3 b	1000	76.7 cd	70.0 cde
20/30	62.3 c	52.0 c			

** Different letter has significant difference at 0.01 level.

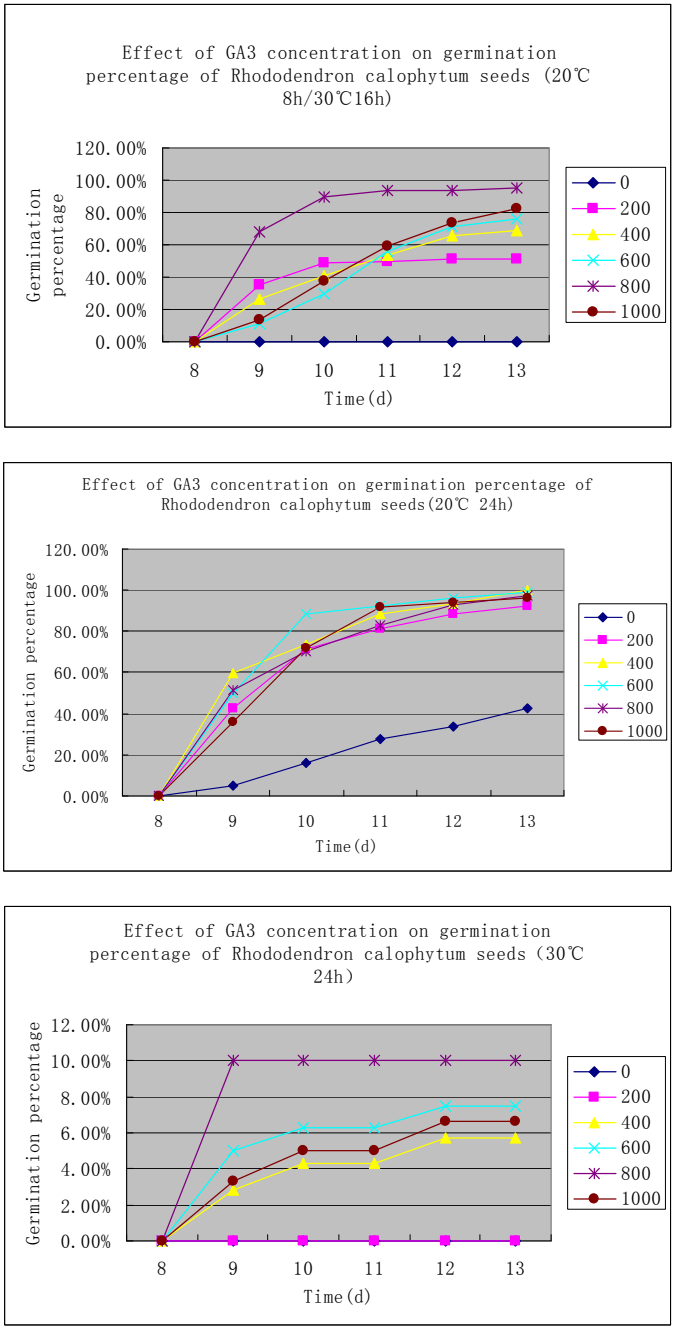

Fig. 1. Effect of six gibberellic acid (GA_3) concentrations under three temperature regimes on germination percentage of *Rhododendron calophytum*.

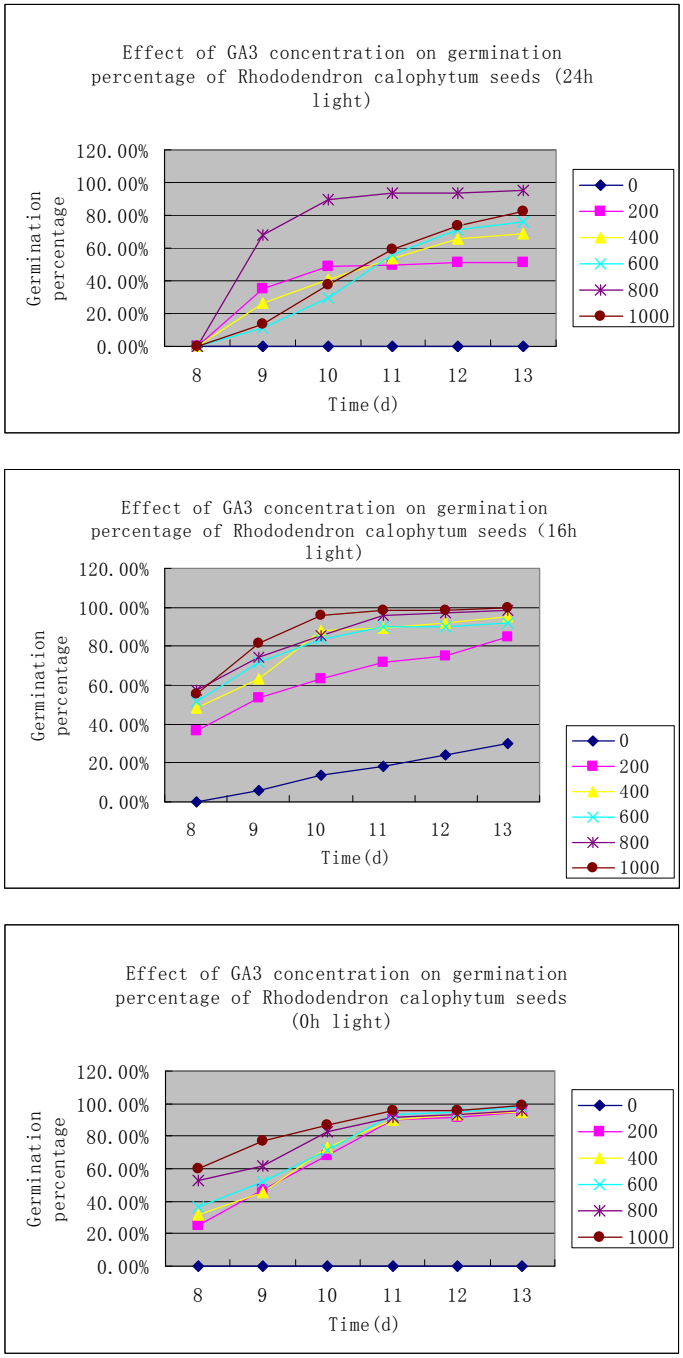

Fig. 2. Effect of six gibberellic acid (GA_3) concentrations and three photoperiod regimes on germination percentage of *Rhododendron calophytum*.