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Temperature Effects and Anatomical Characteristics on Supercooling Ability and Water Content of *Rhododendron yedoense* Maxim. var. *poukhanense* Nakai and *R. simsii* Planch.

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산철쭉과 영산홍의 過冷却 能力과 水分含量에 관한 온도 영향 및 해부학적 특성

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ABSTRACT

Twigs bearing floral buds of *Rhododendron yedoense* var. *poukhanense(Ryp)* and *R. simsii(Rs)*, hardy species in Korea, were used to investigate the cold hardiness in relation to the developmental and anatomical characteristics, and the changes of water content. In floral buds of both species, the reproductive organs, pistils and stamens, matured within the bud scales in early October to prepare for a cold acclimation. The ray parenchymatous areas occupied in xylem were 41% in *Ryp* and 38% *Rs*. As the storage temperature is raised from 2 to 17°C, water content increased more highly in *Rs* than in *Ryp*. Exotherm temperature of floral bud in *Ryp* was generally lower than that in *Rs*. The result supports that the higher cold-hardiness is achieved owing to the earlier maturation of floral buds, to the larger area of ray parenchyma in xylem, and to the lesser increase of water content as the storage temperature is raised.

INTRODUCTION

Dormancy period and cold hardiness are different in each plant. Water content, cooling rate and season are important factors affecting on the supercooling ability(George *et al.*, 1974; Graham and Mullin, 1976; Kaku *et al.*, 1981 a,b). A high supercooling ability in the pine needles depends on the small area of ice nucleation sites at xylem(Kaku, 1975). Since ray parenchyma strongly affects on water metabolism, the destruction of them causes the death of trees (Dixon, 1910; Overton, 1911). Supercoolig ability is thus supposed to be correlated with the abundance of ray parenchyma in xylem. Supercooling abilily and water content in *Rhododendron* species were compared at different storage temperatures (Kaku *et al.*, 1982). In spite of the possibility of correlation between the anatomical characteristics and the supercooling ability, however, there has been no intensive research on this problem, especially in *Rhododendron*.

Ryp is deciduous and mainly found in Korean peninsula and the Thushima island, whereas *Rs* is evergreen and distributed from the southern part of China to Kagoshima prefecture of Japan. *Rs* seems to be a native in Korea but the natural habitat has never been found and the possibility of introduction from China can not be excluded. *Rs* has recently pulled attraction owing to its beautiful color for an ornament.

The present study aims to relate the anatomical characteristics to cold hardiness, and to characterize the effect of storage temperature on the supercooling ability and water content of flower buds in the two species.

MATERIALS AND METHODS

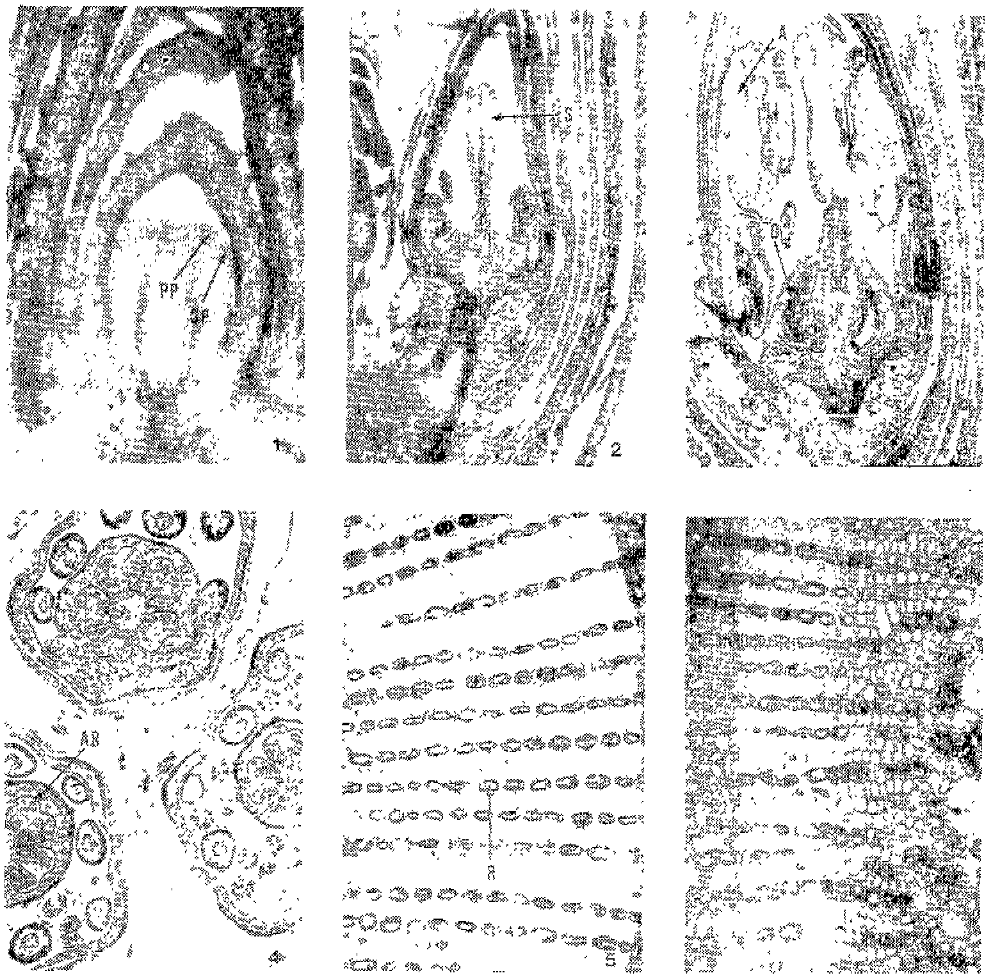
Rhododendron yedoense Maxim. var. *poukhanense* Nakai was collected from natural habitats in Jeonju and *R. simsii* Planch. from an ornamental garden near Jeonju. The samples, two- or three-year-old twigs having flower buds, were collected biweekly from May to October, 1981. For the anatomical research, the materials were fixed in the formalin-acetic acid-alcohol(FAA), dehydrated in the ethyl-butyl alcohol series and embedded in paraffin. Embedded materials were sectioned into 10 μm in thickness with a rotary microtome, stained in Heidenheim's hematoxylin, safranin and light green, and the permanent preparations were made.

For the measurement of cooling ability and water content, twigs were collected during the winter season, enclosed in polyethylene bags saturated with water vapor, and stored at 2 to 3°C until used for the exotherm analysis. A differential thermal analysis(DTA) was used to determine the exotherm temperature distribution(ETD) of florets in the excised whole flower buds. The apparatus of DTA was described by Kaku *et al.* (1980) in detail. The cooling rate was 1°C per hour by using a programatic regulator(Chino BF-121) attached to the freezer throughout the experiment.

Water content was determined by drying the samples at 90°C for 24 hours and was expressed as the percentage of the fresh weight.

RESULTS AND DISCUSSION

Anatomy of flower buds. Anatomical investigations of the floral buds in the two species revealed that the developmental patterns of all floral parts were almost identical, as represented in the micro-photographs of *Ryp* at four different stages (Figs. 1~4). Sepal and petal primordia appeared in early July (Fig. 1) and rapidly elongated in late July. Stamens appeared in early August and carpels in the middle of August(Fig. 2), and fastly



Figs. 1~6. Microphotographs of sectional floral buds and xylem of *Rhododendron*.
SP, sepal primordia ; PP, petal primordia ; S, style ; O, ovule ;
R, ray parenchyma ; A, anther ; AR, archesporial cell.
Figs. 1~4. Floral buds of *R. yedoense* var. *poukhanense* at different times.

Fig. 1. Sepal and petal primordia initiated, covered with bud scales, in early July, (×75).

Fig. 2. Stamen and carpel elongated in mid-August. (×30).

Fig. 3. Anthers and an ovary maturated including pollen grains and ovules, in early September, (×30).

Fig. 4. A cross section of the floral buds in early October, archesporial cells developed in ovules, (×30).

Fig. 5. Ray parenchyma of xylem in the twig axis of *R. yedoense*, (×290).

Fig. 6. Ray parenchyma of xylem in the twig axis of *R. simsii*, (×290).

grew until the late August. In the early September(Fig. 3), when pollen grains started to be developed, and tetrad grains were abundant. Sepals and petals almost finished maturing to ready for an anthesis. In the ovary at this time, ovules also distinctly appeared. Archesporial cells in the ovules appeared in early October(Fig. 4) and remained without further differentiation until the next March.

On the basis of the present anatomical studies, all the development of floral parts in both species ceased in early or at least in the middle of October, although it did somewhat earlier in *Ryp* than in *Rs*. This may suggest they are prepared for a cold acclimation in October. The result accords well with the fact that hardy species characteristically stop growing early in the fall and begin acclimating to cold, while non-hardy species continue growing even in the late fall(McKenzie *et al.*, 1974). All the floral parts of section azalea(kurume), accomplish development in October(Goi, 1980), as the same as in the two species. However, the initiation of sepals and petals starts in the middle of August, one and a half month later than the above species. This temporal discrepancy might due to the specific aspect as well as geographic difference.

Anatomical research of xylem. Ray parenchyma of xylem in the axis was mainly observed in the two species. In *Ryp* the ray parenchyma occupied about 41% area of the xylem(Fig. 5) and in *Rs* about 38%(Fig. 6). *R. scabrum*, one of the southern species in Japan, occupied smaller area(31%, unpblished data) than the above two species. Ray parenchyma takes part in water metabolism as an important role(Dixon, 1910) and therefore, will be closely connected with cold hardiness. Exotherm temperature of *R. scabrum* was about -16°C(Kaku *et al.*, 1982), *Ryp* -25°C and *Rs* -23°C in floral bud(Fig. 7), and this tendency shows very similar to xylem respectively(Kaku *et al.*, 1982). These data suggest that *Ryp* and *Rs* possess a greater resistance to supercool than *R. scabrum* as well as *Ryp* than *Rs*.

The fact that larger the area of ray parenchyma in *Rhododendron* spp., the higher the supercooling ability, would be due to the strong effect of water metabolism. In addition, since the larger the area of ray parenchyma the smaller the area of xylem may be allowed, the larger the area of ray parenchyma, the smaller the area of ice nucleation sites would be occupied at the xylem(Kaku, 1975), and accordingly, the higher the rate of supercooling would be achieved.

Effects of 5°C and 17°C storage-temperatures on winter buds for *Ryp* and *Rs*.

① Water content: Floret water content of *Ryp* stored at 5°C for four weeks was slightly higher than that of the fresh control. Those of the samples stored at 17°C for the same period, however, markedly increased as compared with the fresh control(Table 1). In the water content of axes, there was no remarkable difference among the fresh control and the samples stored at 5°C and 17°C. On the other hand, the water contents of scales stored at 17°C were much higher than those of fresh control and 5°C.

The floret water contents of *Rs* stored at 5°C slightly increased as compared with

Table 1. Changes of water contents(mg) within bud tissues in reponse to storage temperatures

Taxa storage temperature	Floret	Scale	Axis
R. yedoense var. *poukhanense*			
Control(December)	210.4±20.5	112.8± 8.0	96.8± 9.0
5°C for 4 weeks	217.9±25.8	120.5± 7.8	101.0±10.2
17°C for 4 weeks	262.1±22.1	168.1±10.2	119.3± 8.6
R. simsii			
Control(December)	190.7± 9.3	82.6± 5.3	81.3± 7.5
5°C for 4 weeks	212.3±16.0	98.7± 6.0	108.9±11.7
17°C for 4 weeks	307.1±23.0	160.6± 3.7	127.6±13.2

those of the fresh materials(Table 1). Those of florets and scales stored at 17°C markedly surpassed those of the control and 5°C sample. Those of axis at 17°C were slightly higher than the control and 5°C ones.

In the less hardy species of *Rhododendron*, the increase of floret water content at the different storage temperatures was generally higher than the hardy species. Therefore, the hardy species was suggested to be well adapted to the changes of storage temteratures. Such a tendency shows very similar to other *Rhododendron* species(Kaku *et al.*, 1982).

② Freezing pattern and ETD: In *Ryp* ETDs of the samples stored at 5°C for four weeks were slightly higher than those of the fresh control. Those of the samples stored at 17°C showed an almost continuous freezing(Fig. 7). ETDs of florets stored at 5°C for eight weeks exhibited the same tendency as at 5°C for four weeks, and those of 17°C for eight weeks showed only a continuous freezing.

In *Rs* ETDs of florets in the fresh control were clustered into two groups ranging from -8 to -10°C and from -22 to -24°C(Fig. 7). Those of florets at 5°C for 4 weeks were a slightly higher than those of the control but the high group of ETDs did not appear. The samples stored at 17°C also showed only a continuous freezing.

In *Ryp* some of the samples stored at 17°C for four weeks showed a cluster of ETDs with some separated ones, whereas in *Rs* the samples showed only a continuous freezing. Therefore, *Ryp* could be said to be more adapted to a low temperature than *Rs*. In *Rs* having two groups of floret ETDs would be interpreted as an adaptation, i.e., in the case that all flower buds cannot survive during the winter season, one group of florets would be protected while the other freezed.

The measurements of ETDs support all the above mentioned suggestions on the relationship between the ETDs and the other factors dealt in the present study. The high cold-hardiness in the two *Rhododendron* species, was likely to be resulted from the early development of floral buds in the fall, from the possession of a large ray parenchymatous area and from the little increase of water content as the storage temperature

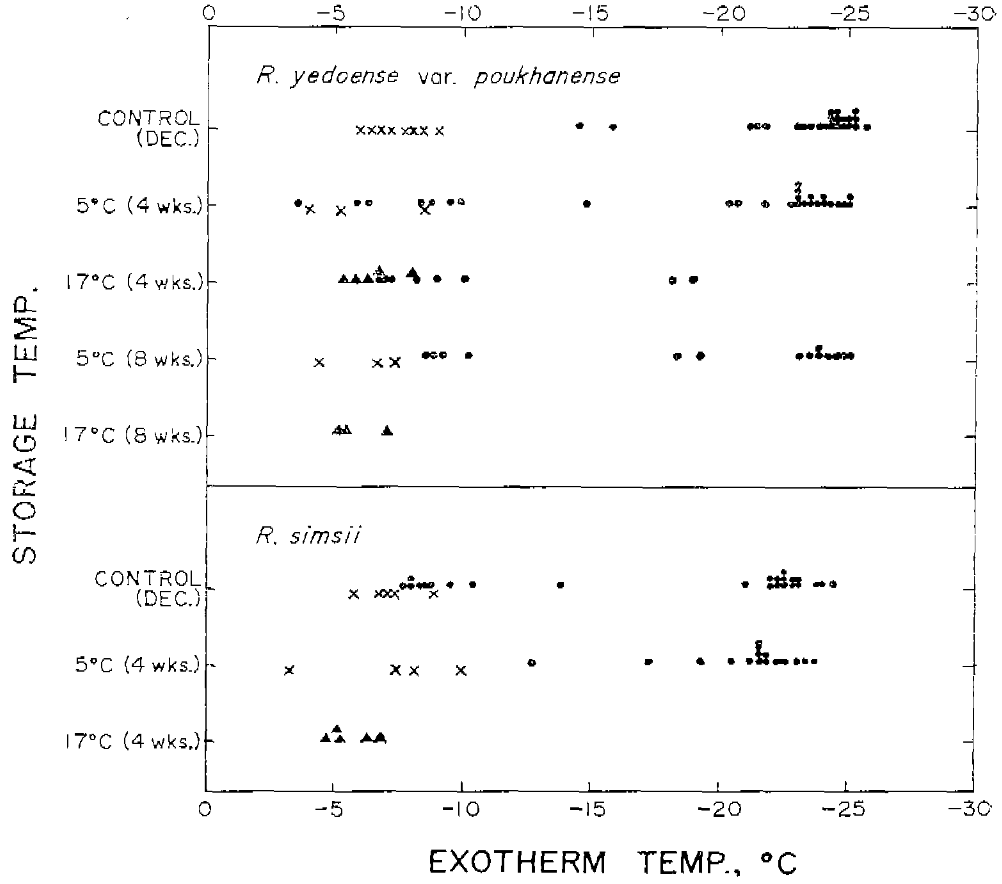


Fig. 7. Changes of freezing pattern in flower buds for *R. yedoense* var. *poukhanense* and *R. simsii* during changes of storage temperature.
▲, continuous freezing; x, exotherm temperature of bud scale;
●, exotherm temperature of florets.

became lower. In other words, the present study strongly supports the fact the cold-hardiness of *Rhododendron* spp. is a function of at least all the above factors. Further investigations with a wide range of samples, specifically as well as geographically, would elucidate the relationship among the factors.

摘 要

산철쭉(*Rhododendron yedoense* var. *poukhanense*)과 영산홍(*R. simsii*)의 耐寒性 研究를 分化 및 해부학적 측면에서 실시하였다. 두 종의 花芽에 있어서 雄蕊와 雌蕊의 成長은 10月初에 멈추게 되며, 이 때 목부의 放射柔組織은 산철쭉에 있어서 41%, 영산홍에 있어서 38%의 면적을 차지한 것으로 나타났다. 저장온도를 2°C에서 17°C로 높임에 따라 수분함량은 산철쭉에 비해서 영산홍에서 높았으며, 산철쭉의 발열온도는 영산홍의 것보다 일반적으로 낮았다.

결론적으로 耐寒性이 강한 것은 花芽의 성숙이 빠르고 放射柔組織의 면적이 크며 온도의 증가에 따

은 수분함량의 증가가 낮은 것이 그 요인으로 생각된다.

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