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Dormancy and germination in *Rosa multibracteata* Hemsl. & E. H. Wilson

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ABSTRACT

Most of the achenes produced by *Rosa multibracteata* Hemsl. & E. H Wilson are dormant on maturity and require pretreatment to stimulate germination. To investigate the mechanism of dormancy and to develop effective methods of improving germination, roles of the pericarp, testa, and embryo of *R. multibracteata* in regulating dormancy were studied by investigating the effect of different pretreatments on germination. The effects of temperature and water stress were also tested with achenes treated by warm plus cold stratification. In freshly harvested achenes, pericarps are permeable and the embryo fully developed, which eliminates the possibility of physical, morphological, or morphophysiological dormancy. Germination percentage remained low (<5%) despite softening the pericarp or even removing it fully. However, fully removing the testa improved germination significantly (39%), indicating the possible presence of germination inhibitors in the testa. Dry storage, scarification with sulphuric acid (H_2SO_4), and warm stratification proved ineffective by themselves but when combined with cold stratification, improved germination and shortened the cold stratification period needed to break dormancy. Dry storage for 68 weeks followed by cold stratification for 16 or 24 weeks resulted in maximum germination (72–79%) among all the treatments. In achenes scarified with H_2SO_4, germination increased with an increase in the duration of cold stratification. Neither gibberellic acid (GA_3) nor 'smoke water' (water through which smoke had been bubbled for 2 h) had any positive effect on germination even on seeds that had been mechanically scarified or stratified. Both high temperature and water stress lowered germination in achenes treated with warm plus cold stratification. Our results suggest that *R. multibracteata* achenes have an intermediate physiological dormancy, and that dry storage for 68 weeks followed by cold stratification for 16 or 24 weeks is the best method for propagating *R. multibracteata* from seed.

1. Introduction

Rose is one of the most important commercial crops worldwide because of its value in landscape gardening, as an ornamental plant, as a medicinal plant, and as food and also because the plant is adapted to a wide range of habitats (Hornero-Méndez and Minguez-Mosquera, 2000; Uggla, 2004; Winther et al., 2005). Conventionally, rose is propagated mainly by such vegetative methods as stem cutting, layering, budding, grafting, and tissue culture (Pati et al., 2006). All these methods are associated with various problems such as shortage of rootstocks and longer production time. Seed propagation of roses is used for breeding new cultivars, restoring native plants, selecting rootstocks, and, in some varieties, for producing the hips; however, seed propagation is difficult because of the low germination percentage, a result of prolonged seed dormancy (Tincker and Wisley, 1935; Xu et al., 1993; Bo et al., 1995; Hoşafçı et al., 2005).

The dormancy in rose achenes and delayed germination may be due to the hard pericarp, inhibitors in pericarp and testa, and physiological barriers in the embryo (Jackson and Blundell, 1963; Densmore and Zasada, 1977; Bo et al., 1995). The pericarp is permeable, although it sometimes prevents full imbibition (Svejda, 1972; Xu et al., 1993; Bo et al., 1995). The barrier in the form of a hard pericarp contributes to dormancy in some rose achenes (Bhanuprakash et al., 2004) but is not its sole cause, since cracking the pericarp fails to break the dormancy in some other achenes (Tincker and Wisley, 1935; Svejda, 1968). Moreover, Bo et al. (1995) report that high concentrations of abscisic acid (ABA) in the pericarp and testa of rose achenes may inhibit germination. It has been confirmed that embryos in the achenes are fully developed and have no morphological dormancy (Bo et al., 1995; Jackson and Blundell, 1963). Further, physiological barriers to germination in embryos have been overcome by cold stratification in a number of rose species (Tincker and Wisley, 1935; Svejda, 1968; Densmore

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and Zasada, 1977). The mechanism of dormancy in rose achenes is thus a complex phenomenon and the few studies that have been conducted have focused on only a few species. Therefore, a better understanding of dormancy in rose achenes would contribute to successful propagation of roses from seeds.

A higher percentage of germination in rose seeds is possible only when the dormancy is overcome. Current attempts to release the dormancy have centred on two approaches, namely (a) eliminating the mechanical barrier in the form of the pericarp, which restricts the growth of embryo and its access to water and air and (b) reducing the period of after-ripening required by the embryo (Stewart and Semeniuk, 1965). Treatments involving soaking achenes in concentrated H_2SO_4, exposing them to an oxygen-rich environment (100% oxygen) (Tincker and Wisley, 1935; Svejda, 1968; Zlesak, 2005) or to various chemicals (such as GA_3), and dry storage or cold stratification alone (Stewart and Semeniuk, 1965; Semeniuk and Stewart, 1966) have had little success. However, a combination of different treatments – for example, H_2SO_4 scarification combined with cold stratification (Svejda, 1968; Densmore and Zasada, 1977; Bhanuprakash et al., 2004) or a combination of warm and cold stratification (Semeniuk and Stewart, 1966; Svejda, 1968; Densmore and Zasada, 1977) – may greatly improve germination. However, the efficacy of different pretreatments in stimulating germination varies with the species (Semeniuk and Stewart, 1966; Bhanuprakash et al., 2004).

It is important to know the kind of seed dormancy for successful propagation of horticultural plants, but presently most publications on seed dormancy do not indicate the kind of dormancy that was investigated (Baskin and Baskin, 1998). Lack of an internationally acceptable system of specifying dormancy may have discouraged researchers from investigating the different kinds of seed dormancy. Baskin and Baskin (2004) propose a new classification system for seed dormancy that consists of five types of dormancy: physiological, morphological, morphophysiological, physical, and combinatorial. Definition of these various classes of dormancy is based on a number of attributes such as permeability of the seed coat (or the fruit) to water (impermeable or permeable), morphology of the embryo (underdeveloped or fully developed), and physiological responses of whole seeds to temperature or to a sequence of temperatures. The new system of classifying seed dormancy makes it possible to determine the type of dormancy by investigating the effect of various pretreatments on germination.

Rosa multibracteata Hemsl. & E. H. Wilson is a perennial shrub found in Sichuan and Yunnan provinces of China (Yu, 1985). The plant produces its characteristically pink flowers from May to July and the red fruits in August and September. The leaves, pollen, and fruits are of great economic value as a source of vitamins and as constituents of medicines (He et al., 1994; Chen et al., 2000). *R. multibracteata* is abundant in arid and semi-arid habitats where it checks soil erosion and provides food and shelter to animals. The plant may be a good candidate for restoring vegetation to these dry areas. However, paucity of information on seed dormancy and germination greatly limits the utility of the species.

The objectives of this study were to investigate the mechanism of dormancy and to develop effective methods of improving germination in *R. multibracteata*. In particular, we wanted to (1) determine kind of dormancy in this species according to the system developed by Baskin and Baskin (2004); (2) understand the role of the pericarp, testa, and embryo in the dormancy; and (3) determine the effects of temperature and water stress during incubation on the germination of *R. multibracteata* achenes subjected to warm plus cold stratification. The results will be helpful in large-scale propagation of the species and facilitate its economic exploitation and ecological restoration.

2. Material and methods

2.1. Collection of achenes and measuring their physical attributes

Hips (the fleshy hypanthium) of *R. multibracteata* were collected from at least 30 plants on 1 October 2005 from the dry Minjiang River valley (32°02′N, 103°40′E, 2370 m a.s.l.) in Maoxian county, Sichuan, China. The area is semi-arid with a typical dry climate (Zhang, 1992) characterized by low and unpredictable rainfall, rapid and intense evaporation, and infertile soil (Bao et al., 1999). The mean annual temperature is 11 °C, mean annual rainfall is 494 mm, and mean annual evapo-transpiration is 1019 mm (Liu et al., 1996). Immediately after collection, the achenes were extracted manually from the hips and mixed thoroughly. Only those achenes that sunk in water, and therefore assumed to be mature and viable, were used in the experiments. After drying the achenes for 3 days in the open, the achenes were stored at room temperature (10–25 °C) until needed (a period that amounted to less than 2 weeks). Table 1 presents data on such physical attributes as length, mass, and moisture content of the achenes.

2.2. Experiment 1: imbibition by achenes

To study imbibition and physical dormancy, imbibition was monitored in mechanically scarified and non-treated (control) achenes. A small portion of the pericarp on the side opposite to the radicle was carefully removed with a scalpel to obtain mechanically scarified achenes. As replications, three lots, each comprising 100 scarified achenes and 100 non-scarified achenes, were placed on a double layer of filter paper moistened with 10 ml distilled water in individual Petri dishes 9 cm in diameter, which served as growth chambers, and incubated in the dark at 25 °C for different durations: 0, 1, 3, 9, 24, 48, 72, 96, and 120 h. After incubation, the achenes were blotted dry, weighed to the nearest milligram, and returned to the growth chambers. The percentage increase in achene mass was determined as described by Baskin et al. (2004).

2.3. Experiment 2: removal of the pericarp and testa

To examine the role of the hard pericarp and testa in seed dormancy, the achenes were scarified by any of the four methods: mechanically with a scalpel as described above, using H_2SO_4, by fully removing the pericarp, and by fully removing the testa. Each replication consisted of 25 achenes. For scarification with H_2SO_4, achenes were soaked in concentrated H_2SO_4 for 2, 4, or 6 h and then washed thoroughly with tap water. For removing the pericarp, a scalpel was used to carefully excise the seeds, whereas the testa was removed with tweezers to obtain excised embryos. The treated achenes were sterilized in 5% (v/v) sodium hypochlorite solution for 10 min and washed three times with sterile

Table 1
Achene traits of *R. mulbraceata* (mean ± S.E.)

Achene length (mm)	5.15 ± 0.11
Achene width (mm)	2.89 ± 0.06
Pericarp thickness (mm)	0.64 ± 0.04
Achene mass (mg)	20.01 ± 0.37
Seed:achene ratio (%)	22.95 ± 0.44
Pericarp:achene ratio (%)	77.05 ± 0.44
Achene water content (%)	8.22 ± 0.08
Percentage of sunken achenes (%)	72.50 ± 1.20
Viability of sunken achenes (%)	54.19 ± 0.90

20 achenes were measured for achene length, achene width and pericarp thickness, 6 replications of 100 achenes each for achene mass, achene water content and percentage of sunken achenes and 4 replications of 20 achenes each for seed:achene ratio, pericarp:achene ratio and viability of sunken achenes.

distilled water. The achenes were placed in Petri dishes as described earlier. All Petri dishes were then incubated in a growth chamber under a cycle of 14 h of light (about 30 μmol m^{-2} s^{-1} provided by fluorescent lamps) at 20 °C and 10 h of darkness at 10 °C to approximate the field conditions in springtime. To avoid any effect due to the position of a dish in the chamber, the Petri dishes were rearranged at random every 2 days. Visible growth of the radicle was taken as germination. Germination was recorded every day, and the experiment continued until no new achenes germinated during 5 consecutive days. The viability of ungerminated achenes was determined by using standard tetrazolium tests (Moore, 1962).

2.4. Experiment 3: dry storage and such storage combined with cold stratification

Achenes were stored dry at ambient temperature (10–25 °C) for 1, 13, 28, 50, and 68 weeks to test the effect of storage under dry conditions on dormancy release. To test whether such storage shortens the duration of cold stratification, fresh achenes and those stored dry for 68 weeks were stratified for 16 and 24 weeks. Three replications of 50 achenes each were used for each treatment, and the achenes tested for germination under conditions identical to those described for Experiment 2.

2.5. Experiment 4: chemical pre-soaking of scarified achenes

Two separate experiments were conducted to determine the effects of GA_3 (gibberellic acid) and 'smoke water' (water that had been brought in close contact with smoke by bubbling it through the water) on dormancy. Mechanically scarified achenes were used in this experiment to eliminate the physical barrier to the emerging radicle posed by the hard pericarp. The GA_3 treatments comprised three concentrations of GA_3 (100, 250, and 500 ppm) and the 'smoke water' treatments comprised two dilutions of smoke water (1:50 and 1:500). For each treatment, four replications of 25 achenes each were soaked in each of the solutions mentioned above or in distilled water (control) for 24 h. The smoke water was prepared as follows. Smoke was generated in a metal drum by slowly burning, under controlled conditions, a mixture of dry and fresh native plant materials typically found growing along with communities of *R. multibracteata*. The smoke, after cooling, was bubbled through 10 l water for 120 min. The achenes for each treatment were tested for germination as described for Experiment 2.

2.6. Experiment 5: cold stratification treatments

A two-factor factorial design with four treatments (control, darkness, smoke water, and GA_3) and five durations of cold stratification (4, 8, 12, 16, and 24 weeks) was used to determine the effect of various treatments on dormancy. The achenes were first soaked for 24 h in distilled water (for the control and darkness treatments), in smoke water diluted to 1:50, or in 250 ppm GA_3 solution; mixed thoroughly with sphagnum moss (1 part of seed and 4 parts of sphagnum moss, v/v) moistened with the corresponding solutions; and then packed in polythene bags. All the bags were stored at 5 °C under light except those with achenes for the dark treatment, which were wrapped in aluminium foil. The bags were opened every 4 weeks for aeration during stratification period, at which time water was added as needed to keep sphagnum moss moist. Three replications, each comprising 50 achenes for each treatment, were used for the germination test (as described for Experiment 2) after stratification for 4, 8, 12, 16, and 24 weeks.

2.7. Experiment 6: H_2SO_4 scarification combined with cold stratification

Experiment 6 was a combination of two treatments, namely scarification with H_2SO_4 and cold stratification that was conducted to determine if the combination would be better than each of the two treatments carried out separately for promoting achene germination. A pretreatment experiment indicated that scarification with H_2SO_4 for 6 h would destroy some achene embryos. Therefore, achenes scarified for 4 h, thoroughly mixed with moistened sphagnum moss (1 part of seed and 4 parts of sphagnum moss, v/v), packed in polythene bags, and stored at 5 °C. Three replications of 50 achenes each were conducted for the germination test (as described for Experiment 2) after 4, 8, 12, and 16 weeks.

2.8. Experiment 7: warm plus cold stratification

Three treatments, namely warm stratification at 25 °C, cold stratification at 5 °C and warm plus cold stratification, were included in the experiment to determine the effect of warm stratification on achene dormancy release. The achenes were mixed with moistened sphagnum moss (1 part of seed and 4 parts of sphagnum moss, v/v) and stored at 25 or 5 °C for 12 weeks for the warm stratification and cold stratification treatment. In the third treatment, storage at 25 °C for 4 weeks was followed by that at 5 °C for 8 weeks. Germination tests consisted of three replications with 50 achenes in each treatment.

Another set of the achenes treated with warm plus cold stratification as described above was used to determine the effects of temperature and water stress on achene germination. Achenes were tested at six constant temperatures (5, 10, 15, 20, 25, and 30 °C) and four combinations of temperatures that fluctuated between low and high (5/15, 10/20, 15/25 and 20/30 °C) at 10 h nights and during 14 h days. Light (about 30 μmol m^{-2} s^{-1}) was provided by fluorescent lamps. Three replications, with 50 achenes each, were placed at each temperature regime.

The levels of water stress were set up as negative water potentials: 0, −0.2, −0.4, −0.66, −0.86, and −1.20 MPa (Michel and Kaufmann, 1973). Each treatment comprised three replications of 50 achenes each. The achenes were placed in glass Petri dishes (9 cm in diameter) lined with a double layer of filter paper moistened with 5 ml of the PEG-6000 solution of appropriate strength, depending on the corresponding water potential, and incubated under a cycle of 14 h of light (about 30 μmol m^{-2} s^{-1}) at 20 °C and 10 h of darkness at 10 °C. The filter papers and water or the osmotic solutions in each Petri dish were replaced every other day, which alternated with days on which 0.2 ml water was added to each Petri dish to maintain the water potentials at the set levels.

2.9. Data analysis

Two germination parameters were determined, namely germination percentage (GP) and the coefficient of rate of germination (CRG). The germination percentage is the proportion, expressed as percentage, of germinated seeds to the total number of viable seeds that were tested. The CRG, also expressed as percentage, was computed as

$$\mathrm{CRG} = \left[\frac{\sum n}{\sum (n \times d)}\right] \times 100$$

where n is number of seeds completing germination on day d and d is the time in days starting from day 0, the day of starting the germination test (Mamo et al., 2006).

The GP and CRG were arcsine-square-root-transformed prior to the analysis. The GP and CRG in Experiment 5 were analysed using

the univariate process of general linear model (GLM) with the treatments, the durations of the cold stratification, and their interaction as factors. A one-way ANOVA was employed to compare the effect of other treatments, temperature, and water potential regimes on the GP and CRG. Treatments that produced zero germination in any of the replications were excluded to satisfy the ANOVA assumption of equal variances. When significant differences were noted, multiple comparisons of the means were made with Tukey's test at 0.05 probability level. Relationships among variables were determined using Pearson's correlations coefficient test at 0.05 probability level.

3. Results

The pericarp of *R. multibracteata* was light yellow and the testa was dark brown. The embryo was morphologically mature with the embryonic bud, hypocotyls, the radicle, and cotyledons clearly visible. Other traits of the achenes are shown in Table 1.

3.1. Experiment 1: achene imbibition

Both scarified and intact achenes imbibed water, and increased in mass with an increase in imbibition period. The quantity of water imbibed and the rate of imbibition were slightly higher in scarified achenes (Fig. 1).

3.2. Experiment 2: effect of removal of pericarp or testa

Freshly harvested achenes of *R. multibracteata* were dormant, and all intact achenes failed to germinate. Mechanical scarification, scarification with H_2SO_4, or full removal of the pericarp did not increase the germination percentage significantly whereas removing the testa increased it significantly (37.78 ± 2.22%) when compared to that in achenes in which the pericarp had been removed (Table 2); however, the seedlings were abnormal.

3.3. Experiment 3: effect of dry storage and such storage combined with cold stratification

Dry storage did not improve germination, and no achenes germinated in any of the treatments that consisted of varying the length of storage. However, dry storage preceding cold stratification stimulated germination. After 16 or 24 weeks stratification at 5 °C, achenes that had been stored dry for 68 weeks germinated to 72.01 ± 3.86 and 79.26 ± 4.33%, respectively, compared to 8.62 ± 1.21 and 38.17 ± 4.41%, respectively of freshly harvested achenes.

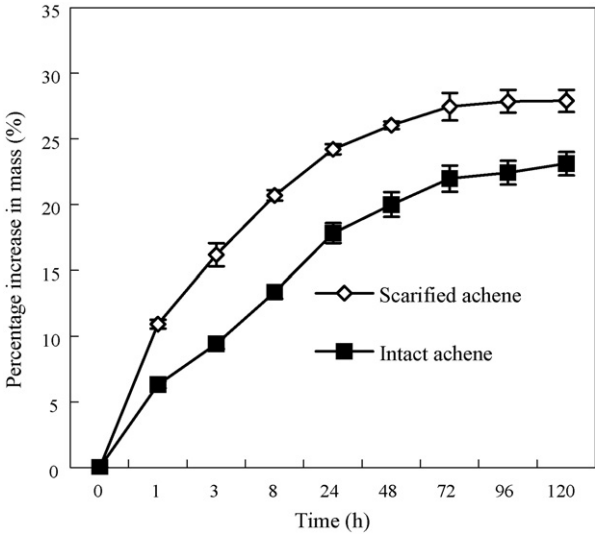

Fig. 1. Percentage increase in mass of *R. multibracteata* of intact and mechanically scarified achenes incubated in distilled water at 25 °C with 14 h under light and 10 h in dark. Bars represent the standard errors.

Table 2
Effects of mechanical scarification, H_2SO_4 scarification, fully pericarp removal and testa removal on germination percentage of *R. mulbraceata* achenes (mean ± S.E.)

Treatments		Germination percentage (%)
Mechanical scarification	Distilled water	1.00 ± 1.00
	100 ppm GA_3	0
	250 ppm GA_3	3.00 ± 1.00
	500 ppm GA_3	0
	1/500 smoke water	0
	1/50 smoke water	4.00 ± 2.83
H_2SO_4 scarification	2 h	0
	4 h	0
	6 h	0
Full removal of pericarp		5.00 ± 1.91
Full removal of testa		37.78 ± 2.22

3.4. Experiment 4: effect of mechanical scarification combined with GA_3 or smoke water

Neither GA_3 nor smoke water, regardless of the concentration or dilution, improved germination in scarified achenes (Table 2).

3.5. Experiment 5: effect of cold stratification treatments

Although treatments involving cold stratification combined with other factors (photoperiods, smoke water, GA_3, and control) or different durations of cold stratification and their interaction significantly affected the germination rate, the duration of cold stratification alone significantly affected germination percentage ($P < 0.05$) (Table 3). Overall, prolonged cold stratification significantly increased both the rate and percentage of germination regardless of the stratification treatments: achenes that had been subjected to cold stratification for less than 12 weeks did not germinate at all and at 16 weeks, less than 10% did, regardless of the stratification treatment except in the case of GA_3 treatment, in which the value was 13.5 ± 3.3%. On the other hand, in achenes that had been stratified for 24 weeks the germination percentage was nearly five times – and the rate of germination nearly twice – that in achenes stratified for 16 weeks (Fig. 2).

3.6. Experiment 6: effect of H_2SO_4 scarification combined with cold stratification

Significant differences in the percentage ($F_{3,\ 8} = 8.798$, $P = 0.006$) and rate ($F_{3,\ 8} = 18.079$, $P = 0.001$) of germination were

Table 3
ANOVA table for germination percentage and coefficient of rate of germination (CRG) of *R. multibraceata* achenes exposed to four treatments and two stratification times

Source	d.f.	Mean square	*F*	Sig.
Germination percentage				
A: Stratification time	1	5121.3	102.56	0.000
B: Treatment	3	145.8	2.92	0.066
A × B	3	68.7	1.385	0.286
CRG				
A: Stratification time	1	217.2	87.03	0.000
B: Treatment	3	52.5	21.04	0.000
A × B	3	14.9	5.97	0.006

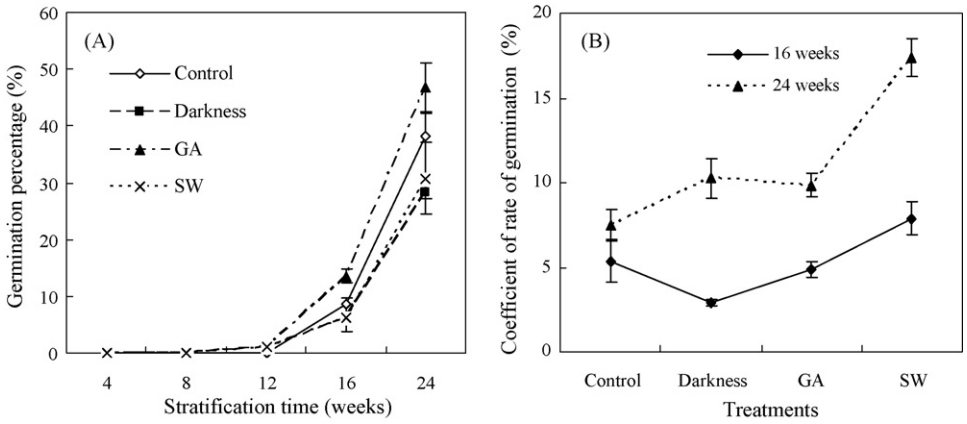

Fig. 2. Germination percentage (A) and rate (B) of *R. multibracteata* achenes stratified at 5 °C for different periods and incubated for 14 h under light (20 °C) and 10 h in dark (10 °C). *Note*: Control, achenes moistened with distilled water and stratified at 5 °C under light; Darkness, achenes moistened with distilled water and stratified at 5 °C in dark; GA, achenes moistened with 250 ppm GA_3 solution and stratified at 5 °C under light; SW, achenes moistened with 1/50 smoke water and stratified at 5 °C under light.

observed in H_2SO_4 scarified achenes stratified for different durations (Fig. 3). The germination percentage in scarified achenes increased initially with the duration of stratification, peaking at 70.1 ± 9.2% in achenes stratified for 12 weeks, and then decreased to 43.0 ± 9.8% in those stratified for 16 weeks. The germination rate also increased with the duration, peaked at 12 weeks of stratification, and decreased thereafter (Fig. 3).

3.7. Experiment 7: effects of warm plus cold stratification

Warm plus cold stratification was more effective in breaking achene dormancy than either of warm or cold stratification alone ($P < 0.001$): The germination percentage was zero in achenes subjected to warm or cold stratification for 12 weeks but increased to 50.1 ± 5.9% (and the rate of germination to 9.6 ± 0.7%) in those that had been first stratified at 25 °C for 4 weeks and then at 5 °C for 8 weeks.

There were significant differences in germination percentage ($F_{5,\ 12} = 13.201$, $P < 0.001$) and rate ($F_{5,\ 12} = 94.543$, $P < 0.001$) among different constant-temperature regimes. At higher constant temperatures, the germination percentage decreased but its rate increased (Fig. 4A and C). Constant temperature had a linear relationship with germination percentage (Fig. 5A) and an exponential relationship with the rate of germination (Fig. 5B).

When temperatures fluctuated between days and nights, germination percentage was not affected significantly ($F_{3,\ 9} = 1.163$ $P = 0.382$) and the rate of germination was affected only slightly ($F_{3,\ 9} = 7.281$, $P = 0.011$). At higher temperatures, the percentage decreased but the rate increased (Fig. 4B and D).

Water stress significantly decreased both the percentage ($F_{3,\ 8} = 27.325$, $P < 0.0001$) and the rate ($F_{3,\ 8} = 16.294$, $P = 0.001$) of germination. There was no significant difference in germination percentages between −0.20 and 0 MPa; at −0.40 MPa, the percentage was significantly lower than that in control but higher than that in achenes at −0.66 MPa; and at −0.86 MPa, no achene germinated at all. The rate of germination decreased continuously with decreasing water potential (Fig. 6).

4. Discussion

4.1. Dormancy kind in the achenes

The embryo of *R. multibracteata* is fully developed, suggesting that the achenes have neither morphological dormancy nor morphophysiological dormancy. And since intact achenes are permeable, neither physical dormancy nor combinatorial dormancy appears likely, according to the classification proposed by Baskin and Baskin (2004). Therefore, the achenes most likely have

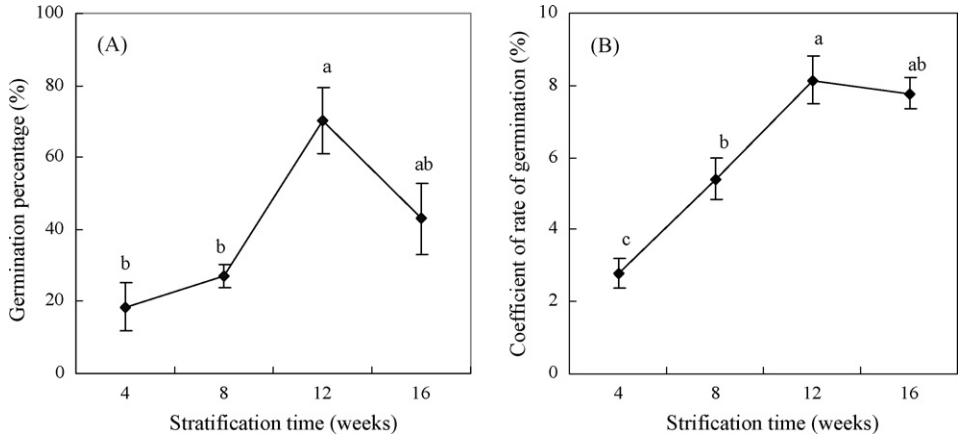

Fig. 3. Germination percentage (A) and rate (B) of *R. multibracteata* achenes scarified with H_2SO_4 for 4 h followed by cold stratification at 5 °C for different periods and then incubated for 14 h under light at 20 °C and 10 h in dark at 10 °C. Bars represent the standard errors. Different letters indicate significant differences among stratification times (Tukey's test, $P \leqq 0.05$).

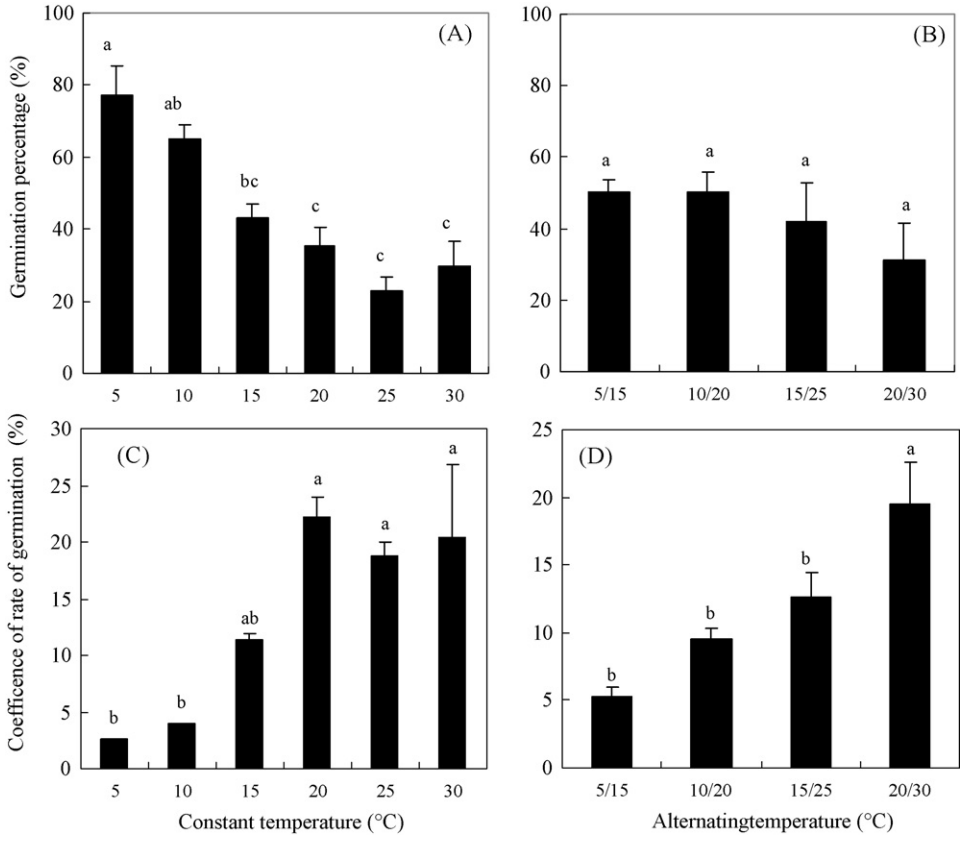

Fig. 4. Germination percentage and rate of *R. multibracteata* achenes stratified at 25 °C for 4 weeks followed by 5 °C for 8 weeks and incubated in continuous (A and C) and fluctuation temperatures (B and D) for 14 h under light at 20 °C and 10 h in dark at 10 °C. Bars represent the standard errors. Different letters indicate significant differences among temperatures (Tukey's test, $P \leqq 0.05$).

some type of physiological dormancy, an inference substantiated by the stimulatory effect of cold stratification on germination. Further, only $37.78 \pm 2.22\%$ of the excised embryos of *R. multibracteata* produced seedlings (which were abnormal), and GA_3 did not promote germination—neither alone nor when combined with scarification or stratification. Dry storage and warm stratification shortened the duration of cold stratification required for high germination percentages. Therefore, achenes of *R. multibracteata* possess an intermediate physiological dormancy as defined by Baskin and Baskin (2004).

It is difficult to compare the relative levels of dormancy across different species of rose because different studies have used different methods to break the dormancy (Jackson and Blundell, 1963, 1965; Svejda and Poapst, 1972; Xu et al., 1993; Bhanuprakash et al., 2004). Cold stratification at about 5 °C, the most common treatment for breaking dormancy in rose achenes, can probably be a benchmark. The physiological dormancy in *R. multibracteata* is likely to be deeper than that in many other roses: for example, at 4.4 °C, such roses as *R. mutiflora* and *R. setigera* 'Beltsville' need 30 days of cold stratification; *R. wichuraiana* needs

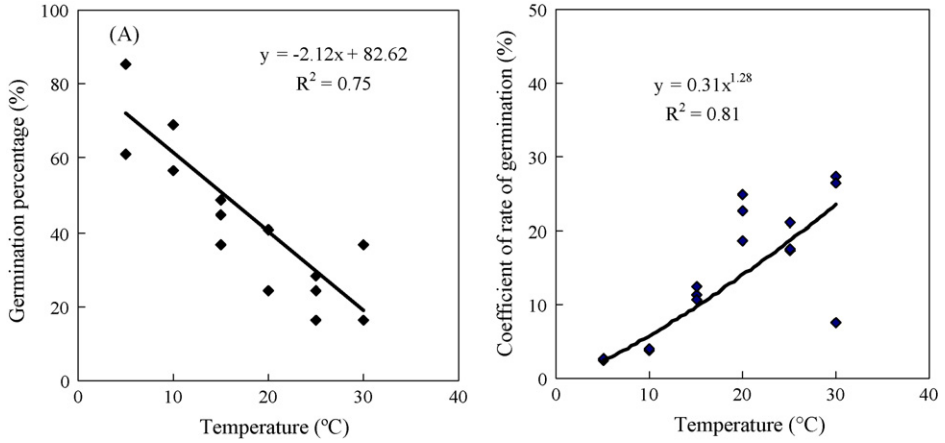

Fig. 5. Relationships of germination percentage (A) and rate (B) with various constant temperatures for *R. multibracteata* achenes stratified at 25 °C for 4 weeks followed by 5 °C for 8 weeks and then incubated in different constant temperatures for 14 h under light at 20 °C and 10 h in dark at 10 °C.

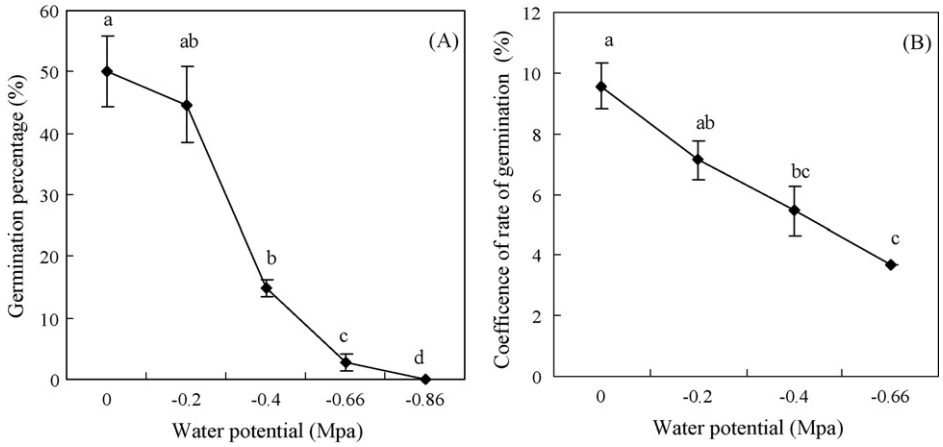

Fig. 6. Germination percentage (A) and rate (B) of *R. multibracteata* achenes stratified at 25 °C for 4 weeks followed by 5 °C for 8 weeks and incubated at different water potentials with 14 h under light at 20 °C and 10 h in dark at 10 °C. Bars represent the standard errors. Different letters indicate significant differences among water potentials (Tukey's test, $P \leqq 0.05$).

45 days; and *R. setigera* 'Serena' and *R.* × *reversa* need 90 days to obtain maximum germination percentages (Stewart and Semeniuk, 1965). On the other hand, the dormancy level in *R. multibracteata* does not appear to be as deep as that in *R. nutkana*, which recorded 65% germination after cold stratification for 1 year (Semeniuk and Stewart, 196 6).

4.2. The role of pericarp, testa, and embryo in dormancy release

Physiological dormancy is mostly caused by the double, or the so-called physiological germination inhibiting mechanism (PIM): decreased embryo activity and the inhibitory effect of covers (Nikolaeva, 2001; Baskin and Baskin, 2004). Results obtained in this study present strong evidence that the pericarp, the testa, and the embryo play important roles in regulating achene dormancy. Pericarps of *R. multibracteata* achenes are permeable, as is the case with other rose achenes such as *R. roxburghii*, *R. omeiensis*, and *R. rogusa* (Tincker and Wisley, 1935; Svejda, 1972; Xu et al., 1993). However, although permeable, the pericarp in *R. multibracteata* does affect water absorption by the embryo, as shown by the slower and decreased imbibition in intact achenes than that in scarified achenes. A similar result was reported in *R. rogusa* (Svejda, 1972). Moreover, germination was better after scarification with H_2SO_4 or warm stratification followed by cold stratification at 5 °C than that after cold stratification alone in the present study (Figs. 3 and 4), suggesting that the pericarp probably offered a mechanical barrier for embryo growth or limited the exposure of inhibitors in the pericarp, testa or embryo (Svejda, 1968; Bo et al., 1995). Also, neither breaking down the pericarp with H_2SO_4 or mechanical scarification nor fully removing the pericarp improved germination, which indicates that other parts of achenes are also involved in regulating the dormancy. The testa may also inhibit germination, since removing it increased germination percentage significantly (Table 2). The negative effect of the testa on germination can be attributed to some inhibitory substances in the testa and not to its role as a mechanical barrier or in restricting access to water (Bo et al., 1995). Lastly, the appearance of light brown patches on the filter paper after the achenes with testa had been incubated for several days also points to the presence of some inhibitory substances in the testa, as has been observed with *R. rugosa* as well (Jackson and Blundell, 1963).

The obligatory requirement for prolonged cold stratification to break the dormancy supports the hypothesis that strong dormancy probably resides in the embryo of *R. multibracteata*. In some rose achenes with mild dormancy, relatively high germination percentages have been obtained by scarification (Bhanuprakash et al., 2004), dry storage (Zlesak, 2005), or GA_3 treatment. However, in *R. multibracteata*, germination did not exceed 5% in any of the treatments without cold stratification except the one in which the testa had been fully removed. The effects of GA_3 on dormancy vary among different rose species (Xu et al., 1993; Liu et al., 2001; Tillberg, 1983; Hoşafçı et al., 2005). In the present study, neither GA_3 nor smoke water improved germination even when combined with mechanical scarification or cold stratification.

4.3. Comparison of the effects of various treatments on germination

Every treatment in our study was less effective singly than in combination. Scarification with H_2SO_4 followed by cold stratification resulted in high germination percentage. This treatment has been found to be effective in other rose species as well (Densmore and Zasada, 1977; Bhanuprakash et al., 2004). However, seedlings produced by achenes that had been scarified with H_2SO_4 were less vigorous (Baskin and Baskin, 1998), and the treatment was less effective than warm plus cold stratification or dry storage combined with cold stratification, considering the economic and environmental implications. Since the highest germination percentage was obtained when dry storage for 68 weeks was followed by cold stratification at 5 °C for over 16 weeks, the method can be preferred to other methods so long as the required time is available. Warm plus cold stratification appears to be another effective method to break dormancy in rose achenes (Semeniuk and Stewart, 1966; Svejda, 1968; Densmore and Zasada, 1977); in the present study, the method proved significantly better than either of the stratifications alone for stimulating both the percentage and the rate of germination. Contrary to several other reports that germination percentage increases with incubation temperature (Baskin et al., 2002; Lohengrin and Arroyo, 2000; Giménez-Benavides et al., 2005), our results show that high temperatures inhibit germination. In our study, warm plus cold stratification did not break the dormancy fully; about 50% of the achenes remained dormant. Stewart and Semeniuk (1965) reported that if the duration of cold stratification is near the required minimum, high temperatures suppress germination. Warm plus cold stratification might contribute to the significant decrease in germination percentage and rate caused by water stress (Fig. 6).

4.4. Recommendation on propagation

In the present work, dry storage for 68 weeks followed by cold stratification at 5 °C for 16 weeks (72.01 ± 3.86%) or 24 weeks (79.26 ± 4.33%), scarification with H_2SO_4 combined with cold stratification for 12 weeks (70.1 ± 9.2%), warm plus cold stratifications (50.1 ± 5.9%) greatly stimulated germination in *R. multibracteata*. When adequate time is available, we recommend dry storage followed by cold stratification. Achenes can be collected in October, stored dry for 14 months indoors, and sown directly in the field in December of the following year. Alternatively, achenes may be collected in October and sown immediately in the field, the period from October to November simulating warm stratification and the period from December to February simulating cold stratification.

In conclusion, *R. multibracteata* has an intermediate physiological dormancy in which the pericarp, the testa, and the embryo all play important roles. The most effective method to break achene dormancy is dry storage for 68 weeks followed by cold stratification at 5 °C for 24 weeks. Dry storage, scarification with H_2SO_4, and warm stratification preceding cold stratification significantly stimulate germination. The achenes germinate better at low temperatures regardless of whether they remain constant or fluctuate. Water stress decreases germination. Further work should address optimal dormancy-breaking conditions to ensure achenes can germinate over a wide range.

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