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Levels of physiological dormancy and methods for improving seed germination of four rose species

Zhiqiong Zhou, Weikai Bao*

Key Laboratory of Ecological Restoration, Chengdu Institute of Biology, Chinese Academy of Sciences, 610041 Chengdu, PR China

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ABSTRACT

Low seed germination is a major problem in commercial rose propagation and breeding and is species-dependent. The present work selected four rose species previously un-examined to explore effective methods for improving seed germination and the relevant dormancy mechanism and its levels in seven experiments. The results showed that both pulp and achenes from the four rose shrubs had chemical substances that significantly inhibited seed germination with the inhibitory effect was more pronounced in pulp extract than of achenes. Single treatments of H_2SO_4 scarification, short-term cold stratification (<16 weeks) or warm stratification were less effective in breaking dormancy as indicated by lower germination index than their combinations. Comprehensive comparisons showed that among the six treatments the most effective for breaking dormancy was H_2SO_4 scarification followed by warm plus cold stratification, then H_2SO_4 scarification followed by cold stratification and finally warm plus cold stratification. Scarification with H_2SO_4 for 2–4 h ordinal followed by warm stratification at 20 °C for 4 weeks and cold stratification at 5 °C for 8 weeks was the best pretreatment for increasing seed germination percentage for *Rosa multibracteata* (81.4 ± 2.9%), *Rosa hugonis* (13.1 ± 6.0%), and *Rosa filipes* (62.7 ± 5.7%); and H_2SO_4 scarification for 4 h followed by cold stratification at 5 °C for 12 weeks was the best pretreatment for *Rosa sericea* (46.7 ± 8.7%). Our results suggest that these four species have only physiological dormancy caused by integrative roles of pulp, pericarp and embryo. The level of physiological dormancy was ranked as *R. hugonis* > *R. sericea* > *R. filipes* > *R. multibracteata*.

1. Introduction

Roses are a group of important garden species of the genus *Rosa*, within the family Rosaceae. It contains about 200 species of perennial plants and 95 rose species are distributed in China of which 65 species are endemic (Wu et al., 2005). The low germination percentage of its achenes is a major problem in commercial rose propagation and breeding (Buckley, 1985; Zlesak, 2005).

Previous studies have found that many treatments can improve the germination of rose achenes, including scarification with H_2SO_4, use of enzymes and microflora, dry storage, cold stratification, warm stratification and their combinations (Yambe and Takeno, 1992; Morpeth and Hall, 2000; Zhou et al., 2008). For some species or varieties with relatively non-deep seed dormancy, e.g. *Rosa soulieana*, *Rosa hybrida* 'Happiness', *Rosa multiflora* and *Rosa corymbifera* 'Laxa' (Bhanuprakash et al., 2004; Zhou et al., 2008), single treatments can often effectively promote germination. However, for most rose species with deep dormancy, e.g. *Rosa nutkana*, *Rosa gallica* 'Ekta' and *Rosa acicularis*, only a combination of different treatments, e.g. H_2SO_4 scarification followed by cold stratification (Svejda, 1968; Densmore and Zasada, 1977) or warm plus cold stratification (Semeniuk and Stewart, 1966; Svejda, 1968; Densmore and Zasada, 1977) can greatly improve germination. Thus, effective methods for improving seed germination vary among roses (Semeniuk and Stewart, 1966; Bhanuprakash et al., 2004) depending on their dormancy levels. Understanding the dormancy level of different rose species will contribute to choosing the appropriate method for improving seed germination and counteracting the dormancy mechanism.

Dormancy and delayed germination in rose achenes may be caused by inhibitors in the pulp and in the achene (Buckley, 1985; Bo et al., 1995), hardness of the pericarp (Jackson and Blundell, 1963; Bhanuprakash et al., 2004) and physiological barriers in the embryo (Densmore and Zasada, 1977; Buckley, 1985; Zhou et al., 2009). Attempts to break the dormancy have centered on two approaches: (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 (Zhou et al., 2009). The first approach involves scarification with H_2SO_4 (Xu et al., 1993; Zhou et al., 2008), use of enzymes (Yambe and Takeno, 1992) and microflora (Morpeth and Hall, 2000). The other approach reduces the period of after-ripening

* Corresponding author. Tel.: +86 28 85231656/85229223; fax: +86 28 85222753.
E-mail addresses: zhouzq@cib.ac.cn (Z. Zhou), baowk@cib.ac.cn (W. Bao).

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by use of GA_3 and cold stratification, which has been proved to be the simplest and most effective method (Zhou et al., 2009; Jackson and Blundell, 1963). Since seed dormancy is incompletely understood, significant experimentation is necessary to determine treatments that give near maximum germination.

To our knowledge, the four rose shrubs, *Rosa multibracteata*, *Rosa hugonis*, *Rosa sericea* and *Rosa filipes*, common in southwestern mountains of China have not been investigated in relation to their levels of physiological dormancy and methods for improving seed germination, except for one study (Zhou et al., 2009). Thus, the objectives of this study were to develop effective methods for improving seed germination and to investigate the mechanism of dormancy. In particular, we aimed to (1) compare the effects of different pretreatments on breaking dormancy in achenes; (2) analyze the roles of pulp, pericarp and embryo in regulating germination and dormancy; and (3) determine the class and level(s) of dormancy in the achenes.

2. Materials and methods

2.1. Hip collection and achene treatment

Hips of *R. multibracteata* and *R. hugonis* were collected from the arid Minjiang valley in Maoxian County, Sichuan, China. This arid area is characterized by low and unpredictable rainfall, rapid and intense evaporation, and infertile soil (Bao et al., 1999). Hips of *R. sericea* and *R. filipes* were collected from the middle mountain area, about 5 km from the collection sites of *R. multibracteata* and *R. hugonis*. The location is about 31°42′N and 103°51′E, with altitude of 1820–1850 m. The mean annual values of rainfall, evapotranspiration and the temperature were within 494–700 mm, 1019–1048 mm and 10.1–11.8 °C, respectively.

Hips (pulpy hypanthia) of the four species were collected from at least 30 plants at the sites when seed matured in 2006 (early August to mid-October). Immediately after collection, achenes were manually extracted from the hips and mixed thoroughly. Only achenes that sank in water and cut to determine maturity and viability were used. After drying for 3 d outdoors, the achenes were stored at room temperature (10–25 °C) until experimental pretreatments were initiated (within 2 weeks).

2.2. Physical trait measurement

Morphological characteristics of 10 newly harvested achenes of each species were observed to determine whether the embryo was fully developed or not. Moreover, to characterize the achenes we determined achene mass, percentage of achenes that sank in water, and viability of naturally harvested achenes. Achene mass was obtained for six of 100-achene replicates using an analytical balance (precision 0.01 mg), percentage of sunken achenes by placing achenes in tap water for 3–5 min, and viability by using the standard tetrazolium test (Moore, 1962).

2.3. Extracts of the pulp and achenes and seed germination

The effects of the extracts of pulp and of achenes on germination of *Brassica campestris* L. were investigated to determine whether inhibitors were present in the pulp or achene for each of the four species. Extracts were prepared as follows. Freshly ripe hips were separated into pulp and achenes. Ten grams of pulp and of achenes were extracted by soaking in 100 mL of deionized water at 25 °C for 24 h in a shaker to give a concentration of 0.1 g fresh tissue mL^{-1}. The extracts were filtered through four layers of cheesecloth to remove the fiber debris and centrifuged at 3000 rpm for 4 h (Chon et al., 2002). The supernatant was filtered again using a 0.2-mm filterware unit. Fresh extracts were kept in a refrigerator at 2 °C until used.

Seed germination tests were conducted for each of the two extracts. Treatments and control were arranged in a completely randomized design with three replications, each of 50 achenes (in one Petri dish). *B. campestris* seeds were surface-sterilized with 5% (v/v) sodium hypochlorite solution for 10 min, rinsed three times with distilled water and then evenly placed on two layers of filter paper in sterilized 9-cm-diameter Petri dishes. Of extract, 5 mL was added to each Petri dish containing *B. campestris* seeds, with distilled water as the control. Seeds were incubated at 25 °C in a growth chamber under a 14/10 h cycle of light/dark (light about 30 $\mu mol\ m^{-2}\ s^{-1}$ from fluorescent lamps). To avoid any effect due to the position of a dish in the chamber, the Petri dishes were randomly rearranged every 2 d. The number of germinated seeds was counted at 24-h intervals for 3 d. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1 mm. The experiment continued for 7 d.

2.4. Experiments for improving germination

To overcome the dormancy imposed by the hard pericarp and embryo, achenes were subjected to H_2SO_4 scarification, warm stratification (for pericarp) and cold stratification (for embryo) and their combinations (Table 1s). Six experiments and 19 treatments were designed for each species in total.

2.4.1. Experiment 1: H_2SO_4 scarification

Achenes were soaked in 98% H_2SO_4 for 2, 4 or 6 h and then washed thoroughly with tap water.

2.4.2. Experiment 2: warm stratification

Warm stratification was implemented by storing achenes with moistened sphagnum moss at 20 °C for 8 weeks. Achenes were pre-soaked in distilled water for 24 h, mixed thoroughly with moistened sphagnum moss (1 achene:4 sphagnum moss, v/v), and then placed into polythene bags which were closed and stored at 5 °C. They were opened every 4 weeks for aeration during stratification, at which time water was added as needed to keep sphagnum moss moist.

2.4.3. Experiment 3: cold stratification

Six durations (4, 8, 12, 16, 24 and 28 weeks) were designed for cold stratification treatments. Achenes were pre-soaked in distilled water for 24 h, mixed thoroughly with moistened sphagnum moss, and then placed into polythene bags which were closed and stored at 5 °C. They were opened every 4 weeks for aeration during stratification, at which time water was added as needed.

2.4.4. Experiment 4: warm plus cold stratification

Warm plus cold stratification were arranged to compare its effect with that of warm stratification and cold stratification on breaking dormancy. Achenes were kept at 20 °C for 8 weeks and then at 5 °C for 8, 12 and 16 weeks.

2.4.5. Experiment 5: H_2SO_4 scarification combined with cold stratification

In this experiment, H_2SO_4 scarification was integrated with cold stratification to determine whether the combination of the two treatments was better than each separately. Experiment 2 indicated that 6 h of H_2SO_4 scarification destroyed some embryos of *R. multibracteata*, *R. hugonis* and *R. sericea*, and that 4 h did so for *R. filipes*. Therefore, achenes of *R. multibracteata*, *R. hugonis* and *R. sericea* were scarified with 98% H_2SO_4 for 4 h, and those of *R. filipes*

Table 1
Treatments applied in the six experiments for improving the germination of rose achenes from the arid Minjiang valley of southwestern China.

Experiments	H_2SO_4 scarification	Warm stratification (20 °C)	Cold stratification (5 °C)
Experiment 1: H_2SO_4 scarification	2 h, 4 h and 6 h		
Experiment 2: warm stratification (20 °C)		8 weeks	
Experiment 3: cold stratification (5 °C)			4, 8, 12, 16, 24, and 28 weeks
Experiment 4: H_2SO_4 scarification combined with cold stratification	2 h for *R. filipes* and 4 h for *R. multibracteata*, *R. hugonis* and *R. sericea*		4, 8, and 12 weeks
Experiment 5: warm plus cold stratification		8 weeks	8, 12 and 16 weeks
Experiment 6: H_2SO_4 scarification combined with warm plus cold stratification	2 h for *R. filipes* and 4 h for *R. multibracteata*, *R. hugonis* and *R. sericea*	4 weeks	4, 8, and 12 weeks

Table 2
Achene traits of four rose species from the arid Minjiang valley of southwestern China.

Species	Achene mass (mg)	Percentage of achenes that sank (%)	Viability of naturally harvested achenes (%)
R. multibracteata	20.0 ± 0.4a	72.5 ± 1.2b	54.2 ± 0.9a
R. hugonis	16.1 ± 0.2ab	84.2 ± 2.0a	53.0 ± 2.1a
R. sericea	12.5 ± 0.2b	81.0 ± 1.1a	34.1 ± 1.8b
R. filipes	6.5 ± 0.2c	73.3 ± 2.4b	40.8 ± 3.4b

Note: Mean (±S.E.) of six replicates of 100 achenes each for achene mass and percentage of achenes that sank and four replicates of 20 achenes each for viability of naturally harvested achenes. Different lowercase letters within the same column indicate significant differences.

Table 3
Germination percentage (GP) (mean ± S.E.) and the coefficient of rate of germination (CRG) (mean ± S.E.) of *Brassica campestris* seeds treated with extracts of pulp and achenes of four rose species, respectively.

Species	GPs (%)		CRGs (%)	
	Pulp	Achene	Pulp	Achene
Control	100.0 ± 0.0aA	100.0 ± 0.0aA	89.9 ± 1.4aA	89.9 ± 1.4aA
R. multibracteata	77.3 ± 1.3bB	99.3 ± 0.7aA	45.6 ± 1.0bB	89.2 ± 2.4aA
R. hugonis	3.3 ± 1.3cB	86.0 ± 4.2bcA	50.0 ± 0.0bA	43.5 ± 0.7cB
R. sericea	0.0 ± 0.0dB	79.3 ± 3.3cA	N.A.	46.7 ± 1.0c
R. filipes	0.0 ± 0.0dB	90.7 ± 1.8bA	N.A.	52.5 ± 0.4b

Note: Different lowercase letters within the same column indicate significant differences between different rose species and control, and different uppercase letters within the same row indicate significant differences between pulp and achene.

for 2 h. Then the scarified achenes were stratified at 5 °C for 4, 8 and 12 weeks, respectively.

2.4.6. Experiment 6: H_2SO_4 scarification combined with warm plus cold stratification

Achenes of *R. multibracteata*, *R. hugonis* and *R. sericea* were treated with 4 h H_2SO_4 scarification, and those of *R. filipes* with 2 h to test the integrated effect of H_2SO_4 scarification and warm plus cold stratification. Scarified achenes were stratified at 20 °C for 4 weeks followed by 4, 8 and 12 weeks at 5 °C.

2.4.7. Seed germination

As mentioned above, for each species there were 2850 achenes in 19 treatments for germination tests, with three replications (each a Petri dish) each of 50 achenes per treatment. The germination process of treated achenes was similar to that used for *B. campestris* seeds (described in Section 2.3), with some exceptions. Achenes were kept in a cycle of 14/10 h of light/dark at 20 °C but not at 25 °C. The germination test continued for 3 months. The viability of ungerminated achenes was determined by the standard tetrazolium test (Moore, 1962).

2.5. Data analysis

Two germination parameters were calculated: germination percentage (GP) and the coefficient of rate of germination (CRG). GP is the proportion (%) of total number of germinated achenes to that of viable tested achenes. CRG (also %) was computed as follows:

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

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. As GP of some treatments in Experiments 3 and 5 was zero, and data did not fulfill the basic assumptions of ANOVA, a Kruskal–Wallis H-test was carried out (Zar, 1996). A one-way ANOVA was employed to compare the effect of species on achene traits. The GP and CRG in *B. campestris* seeds (described in Section 2.3) in Experiments 4 and 6 were analyzed using the univariate process of a general linear model with the issue/treatments, species and their interaction as factors. When significant differences were noted, multiple comparisons of means were made with an LSD test at $P < 0.05$.

3. Results

The embryos in seeds of four rose shrubs examined were morphologically mature (fully developed) with the embryonic plumule bud, hypocotyl, radicle and cotyledons clearly visible. Achene mass was in the following order: *R. multibracteata* > *R. hugonis* > *R.*

Table 4
ANOVA for germination percentage (GP) and the coefficient of the rate of germination (CRG) of *Brassica campestris* seeds treated with extractions of pulp and achenes of four rose species from the arid Minjiang valley of southwestern China.

Source	df	Mean square	*F*	*P*
GP				
A: Tissue	1	22,632.5	2121.8	<0.001
B: Species	4	4802.5	450.2	<0.001
A × B	4	2528.9	237.1	<0.001
CRG				
A: Tissue	1	688.0	148.2	<0.001
B: Species	4	1951.5	420.5	<0.001
A × B	2	1111.7	239.5	<0.001

sericea > *R. filipes*. Percentages of achenes that sunk for *R. hugonis* and *R. sericea* were significantly higher than those of *R. multibracteata* and *R. filipes*. Viabilities of naturally harvested achenes in all species were low; they were significantly greater in *R. multibracteata* and *R. hugonis* than in *R. sericea* and *R. filipes* (Table 2).

3.1. Effect of the extracts of pulp and achenes on germination

Pulp extract of each species significantly decreased GP and CRG of *B. campestris* seeds (Table 3). The inhibitory effect was the strongest for *R. sericea* and *R. filipes* (with 0% GP), followed by *R. hugonis* (3.3%) and *R. multibracteata* (77.3%). Except for *R. multibracteata*, the achene extracts also decreased GP and CRG of *B. campestris*. The degree of inhibition of achene extracts was the lowest for *R. multibracteata*, followed by *R. filipes*, *R. hugonis* and *R. sericea*.

There were highly significant effects ($P < 0.001$) on GP and CRG of *B. campestris* seeds due to tissue extracts (pulp and achenes), species and their interactions (Table 4). The extract of pulp had a stronger inhibitory action than that of achenes, shown by lower GP and CRG in *B. campestris* seeds treated by pulp, regardless of species (Table 3). The degree of inhibition of pulp and achene extracts varied across species, as indicated by the significant interactions of 'Tissue' and 'Species' (Table 4).

3.2. Effect of H_2SO_4 scarification, warm stratification and cold stratification

Freshly harvested achenes were dormant. H_2SO_4 scarification and warm stratification alone produced no germination and so were ineffective in breaking dormancy. GP was low in cold-stratified achenes for all species, with no achenes of *R. hugonis* germinating. Achenes of *R. filipes* (GP = 18.5 ± 5.1%) and *R. sericea* (GP = 12.1 ± 1.1%) only germinated after 28 weeks of stratification. For *R. multibracteata*, no germination occurred for cold stratification <16 weeks, and GP was 11.7 ± 2.0%, 15.2 ± 1.2% and 23.9 ± 6.2% for 16, 24 and 28 weeks, respectively. Further prolonged cold stratification significantly increased GP of *R. multibracteata* (Kruskal–Wallis or KW = 16.12; df = 5; $P = 0.007$) and *R. filipes* (KW = 16.83; df = 5; $P = 0.005$).

3.3. Effect of warm plus cold stratification

Warm plus cold stratification significantly increased GP of *R. multibracteata* (KW = 9.59; df = 3; $P = 0.02$), *R. sericea* (KW = 10.65; df = 3; $P = 0.01$) and *R. filipes* (KW = 9.80; df = 3; $P = 0.02$), but it did not lead to germination in *R. hugonis* regardless of regimes of warm plus cold stratification (Table 5). Overall, the effect of warm plus cold stratification was most pronounced in increasing GP and CRG for *R. multibracteata*, followed by *R. filipes* and *R. sericea*. GP and CRG for *R. multibracteata* and *R. sericea* increased continuously with increased duration of the cold stratification of warm plus cold stratification (Table 5). For *R. filipes*, GP and CRG were significantly

Table 5
Germination percentage (GP) (mean ± S.E.) and the coefficient of rate of germination (CRG) (mean ± S.E.) of four rose species achenes by warm plus cold stratification and then incubated at 20 °C with a cycle of 14/10 h of light/dark.

Parameter	Species	8–0	8–8	8–12	8–16
GPs (%)	*R. multibracteata*	0.0 ± 0.0	47.1 ± 3.4	62.0 ± 3.5	70.0 ± 7.1
	R. hugonis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	R. sericea	0.0 ± 0.0	0.0 ± 0.0	4.5 ± 1.7	13.8 ± 4.3
	R. filipes	0.0 ± 0.0	6.7 ± 0.3	31.2 ± 8.0	21.9 ± 3.8
CRGs (%)	*R. multibracteata*	N.A.	11.8 ± 1.0	15.1 ± 1.7	16.1 ± 0.5
	R. hugonis	N.A.	N.A.	N.A.	N.A.
	R. sericea	N.A.	N.A.	8.5 ± 2.1	25.0 ± 4.5
	R. filipes	N.A.	5.6 ± 0.7	12.1 ± 0.6	12.8 ± 0.8

Note: 8–0 Warm stratification at 20 °C for 8 weeks; 8–8 warm stratification at 20 °C for 8 weeks followed by cold stratification at 5 °C for 8 weeks; 8–12 warm stratification at 20 °C for 8 weeks followed by cold stratification at 5 °C for 12 weeks; 8–16 warm stratification at 20 °C for 8 weeks followed by cold stratification at 5 °C for 16 weeks.

Table 6
ANOVA table for germination percentage (GP) and the coefficient of the rate of germination (CRG) of four rose achenes exposed to H_2SO_4 scarification combined with cold stratification and H_2SO_4 scarification combined with warm plus cold stratification.

Treatment	Parameter	Source	df	Mean square	*F*	*P*
H_2SO_4 scarification combined with cold stratification	GP	A: Species	3	2304.7	63.6	<0.001
		B: Duration[a]	2	2511.1	69.3	<0.001
		A × B	6	243.8	6.7	<0.001
	CRG	A: Species	3	47.6	10.8	<0.001
		B: Duration[a]	2	7.1	1.6	0.227
		A × B	4	6.4	1.5	0.255
H_2SO_4 scarification combined with warm plus cold stratification	GP	A: Species	3	6882.2	122.5	<0.001
		B: Regime[b]	2	1161.5	20.7	<0.001
		A × B	6	160.2	2.9	0.031
	CRG	A: Species	3	212.4	20.0	<0.001
		B: Regime[b]	2	63.3	6.0	0.009
		A × B	5	53.7	5.1	0.003

[a] "Duration" means duration of cold stratification in Experiment 5: 4 weeks, 8 weeks and 12 weeks.
[b] "Regime" means patterns of warm plus cold stratification in Experiment 6.

Table 7
Germination percentage (GP) (mean ± S.E.) and the coefficient of rate of germination (CRG) (mean ± S.E.) of four rose species achenes by H_2SO_4 scarification combined with cold stratification (4–12 weeks duration) and then incubated at 20 °C with a cycle of 14/10 h of light/dark.

Parameters	Species	4	8	12
GPs (%)	*R. multibracteata*	29.5 ± 7.2aB	37.1 ± 4.7aB	66.2 ± 10.1aA
	R. hugonis	0.0 ± 0.0bB	2.8 ± 1.7cB	12.5 ± 5.2dA
	R. sericea	0.0 ± 0.0bC	26.1 ± 1.6bB	46.7 ± 8.7bA
	R. filipes	10.6 ± 4.2bB	27.7 ± 3.4abB	30.3 ± 9.3cA
CRGs (%)	*R. multibracteata*	11.5 ± 1.4aA	12.4 ± 1.2aA	10.1 ± 0.9aA
	R. hugonis	N.A.	6.0 ± 0.7bA	5.2 ± 0.9bA
	R. sericea	N.A.	8.6 ± 0.7abA	8.7 ± 1.8abA
	R. filipes	5.0 ± 1.2bA	8.3 ± 1.0abA	9.3 ± 1.6aA

Note: Different lowercase letters within the same column indicate significant differences between different rose species, and different uppercase letters within the same row indicate significant differences between different cold stratification periods.

Table 8
Germination percentage (GP) (mean ± S.E.) and the coefficient of rate of germination (CRG) (mean ± S.E.) of four rose species achenes by H_2SO_4 scarification combined with warm plus cold stratification and then incubated at 20 °C with a cycle of 14/10 h of light/dark.

Parameter	Regime of warm plus cold stratification	4–4	4–8	4–12
GPs (%)	*R. multibracteata*	65.1 ± 2.3aB	81.4 ± 2.9a A	68.9 ± 1.8aB
	R. hugonis	6.8 ± 2.7cA	13.1 ± 6.0dA	1.9 ± 1.0cA
	R. sericea	14.1 ± 1.7cB	38.9 ± 4.1cA	35.1 ± 7.3bA
	R. filipes	31.6 ± 6.1bB	62.7 ± 5.7bA	55.5 ± 4.5aA
CRGs (%)	*R. multibracteata*	15.9 ± 1.0aB	19.5 ± 0.8Aab	21.0 ± 1.9aA
	R. hugonis	8.6 ± 2.2bcB	23.5 ± 5.0aA	N.A.
	R. sericea	7.0 ± 0.6cA	8.5 ± 0.7cA	6.7 ± 0.4cA
	R. filipes	14.4 ± 1.1bA	12.0 ± 1.0bB	15.2 ± 1.1bA

Note: 4–4 Warm stratification at 20 °C for 4 weeks followed by cold stratification at 5 °C for 4 weeks; 4–8 warm stratification at 20 °C for 4 weeks followed by cold stratification at 5 °C for 8 weeks; 4–12 warm stratification at 20 °C for 4 weeks followed by cold stratification at 5 °C for 12 weeks. Different lowercase letters within the same column indicate significant differences between different rose species, and different uppercase letters within the same row indicate significant differences between different regimes of warm plus cold stratification.

improved when cold stratification increased from 8 to 12 weeks, but GP decreased when cold stratification was prolonged further to 16 weeks.

3.4. Effect of H_2SO_4 scarification combined with cold stratification

Species, duration of cold stratification and their interaction had highly significant effects on GP ($P < 0.001$; Tables 6 and 7). In general, H_2SO_4 scarification followed by cold stratification significantly improved GP of all four species. The GP also increased with cold stratification duration. When H_2SO_4 scarified achenes of *R. multibracteata* and *R. filipes* were stratified for 12 weeks, their respective GPs were about two and three times that of achenes stratified for 4 weeks. For *R. sericea* and *R. hugonis*, there was no germination in scarified achenes stratified for only 4 weeks; however, when cold stratification was extended to 8 and 12 weeks the GP was improved significantly. GP of achenes was greatest for *R. multibracteata*, followed by *R. sericea* and *R. filipes* (they were similar), and finally *R. hugonis* regardless of cold stratification duration (Table 7).

Species alone had a highly significant effect on CRG ($P < 0.001$; Table 6). Comparatively, *R. multibracteata* germinated fastest, with CRG > 10% for all durations of cold stratification. CRGs of *R. sericea* and *R. filipes* were similar and that of *R. hugonis* was least, regardless of cold stratification duration (Table 7).

3.5. Effect of H_2SO_4 scarification combined with warm plus cold stratification

GP and CRG differed significantly for two factors (species and regime) and their interaction when achenes were treated by H_2SO_4 scarification combined with warm plus cold stratification ($P < 0.05$; Table 6). Warm stratification at 20 °C for 4 weeks followed by cold stratification at 5 °C for 8 weeks resulted in the highest GP and CRG in scarified achenes for all species; however, overall GP and CRG decreased when the duration of cold stratification was shorter or longer (i.e. 4 or 12 weeks). The scarified achenes of *R. multibracteata* germinated to the highest GP, followed by *R. filipes*, *R. sericea* and *R. hugonis* regardless of the regime of warm plus cold stratification. The highest GP of scarified achenes of *R. multibracteata* was 81.4 ± 2.9%; however, that of *R. hugonis* was only 13.1 ± 6.0% (Table 8).

4. Discussion

4.1. Comparison of various pretreatments

The single treatments were less effective in breaking dormancy (given by GP and CRG) than their combinations for the four rose species (Tables 5, 7 and 8), consistent with two earlier studies (Semeniuk and Stewart, 1966; Svejda, 1968) for other rose species, e.g. *R. nutkana*, *R. gallica* 'Ekta' and *Rosa rugosa* 'Frau Dagmar Hartopp' and 'Tetonkaha'. Among the combination experiments in breaking dormancy in achenes and increasing GP for four rose species we examined, the most effective was H_2SO_4 scarification followed by warm plus cold stratification, followed by H_2SO_4 scarification combined with cold stratification, and warm plus cold stratification.

4.2. Roles of pulp, pericarp and embryos in regulating dormancy

Lower GP and CRG of *B. campestris* seeds treated by the extracts of rose pulp and achenes (Table 3) indicated that both pulp and achenes of the examined species had chemical substances significantly inhibiting seed germination, confirming that pulp is important in achene dormancy of rose species. It is consistent with the report by Buckley (1985) that inhibitory substances were found in the pulp of some rose species.

It is known that the major effect of H_2SO_4 scarification on germination is to destroy the seed coat, thereby eliminating the mechanical barriers, and the prevention of imbibition of water or air. The greater efficiency in breaking dormancy of cold stratification and warm plus cold stratification, when they were combined with H_2SO_4 scarification, indicated the importance of the pericarp in regulating achene dormancy. Since the pericarp of rose achenes is permeable (Tincker and Wisley, 1935; Svejda, 1972; Xu et al., 1993), and may not limit uptake of water or oxygen by the embryo, it probably provides a mechanical barrier for embryo growth (Svejda, 1968; Bo et al., 1995; Zhou et al., 2008). Furthermore, the hard pericarp was not the sole factor inhibiting germination of the examined rose species since no achenes germinated when treated only by H_2SO_4 scarification. The H_2SO_4 scarification has improved germination of rose species with relatively non-deep seed dormancy, e.g. *R. soulieana*, *R. hybrida* 'Happiness', *R. multiflora* and *R. corymbifera* 'Laxa' (Bhanuprakash et al., 2004; Zhou et al., 2008) but not for those with deep dormancy (Tincker and Wisley, 1935; Xu et al., 1993; Liu et al., 2001). Moreover, an obligatory requirement for cold stratification to break dormancy in the present study confirmed that strong dormancy existed in embryos of the four species. Therefore, the present results provided strong evidence that the pulp (Table 3), pericarp (Tables 7 and 8) and embryo (Tables 5, 7 and 8) played important roles in regulating achene dormancy for the examined species. This further confirms the so-called physiological germination-inhibiting mechanism hypothesized by Baskin and Baskin (2004), in which physiological dormancy is mostly caused by decreased embryo activity and the inhibitory effect of covers.

4.3. Dormancy classification and comparison of taxa

Although a proportion of the embryos in seeds were viable (Table 2), no fresh achenes of the four species germinated, indicating that the achenes were dormant at maturity. All embryos of the species were fully developed, implying that the achenes had neither morphological dormancy nor morphophysiological dormancy. Although we did not explicitly measure imbibition capability by achenes, our observations from the present study and of other researchers demonstrate that rose achenes are water permeable (Svejda, 1972; Xu et al., 1993; Ueda et al., 2000; Zhou et al., 2009), in which case the seeds do not have either physical dormancy or combinational dormancy (Baskin and Baskin, 2004). Thus, the elimination of four of the five classes of dormancy (Baskin and Baskin, 2004) indicates that seeds of all species fit into the fifth class of dormancy, i.e. physiological dormancy. Now the question is what level of physiological dormancy do the seeds have? In the present study, there was no germination of achenes treated by H_2SO_4 or short-term cold stratification (<16 weeks), indicating that none of these achenes had non-deep physiological dormancy. Warm stratification shortened the cold stratification period required for high GP in *R. multibracteata*, *R. filipes* and *R. sericea*, but not for *R. hugonis*, indicating intermediate physiological dormancy in the first three species. However, following cold stratification for 28 weeks or any regime of warm plus cold stratification (Table 5), no achenes of *R. hugonis* germinated, indicating deep physiological dormancy as defined by Baskin and Baskin (2004).

To describe dormancy characteristics of the species, we used (i) 'dormancy pattern' – described by the kinds of environmental events that break dormancy, and (ii) 'dormancy strength' – described by the kind and amount of factor(s) required to break dormancy (Karlsson et al., 2005; Karlsson and Milberg, 2007). 'Dormancy strength', referring to the general pattern, is described as strong to weak, and the extent of dormancy present at any specific moment is referred to as 'degree of dormancy' (Karlsson and Milberg, 2008). We found that the dormancy pattern was similar for *R. multibracteata* and *R. filipes*, since their achenes responded similarly to different pre-treatments. Germination was improved by combinations of H_2SO_4 scarification, warm stratification and short-term cold stratification, but not by them alone. This dormancy pattern has been observed in achenes of other rose species (Semeniuk and Stewart, 1966; Svejda, 1968; Densmore and Zasada, 1977). However, *R. sericea* and *R. hugonis* had different dormancy patterns. Cold stratification did not effectively improve GP regardless of duration. Warm plus cold stratification improved germination of *R. sericea* but not for *R. hugonis*. This requirement for H_2SO_4 scarification or/and warm stratification before cold stratification would effectively postpone seedling emergence in the field until the second spring following achene production.

'Dormancy strength' in achenes varied across the species. The ranking of GP in achenes following different treatments was *R. multibracteata* > *R. filipes* > *R. sericea* > *R. hugonis* (Tables 5, 7 and 8). Therefore, comparatively, *R. hugonis* achenes had the strongest dormancy, followed by *R. sericea*, *R. filipes* and *R. multibracteata*. Cold stratification at about 5 °C, the most common treatment for breaking dormancy for rose, can be a benchmark to compare the level of dormancy in rose achenes (Zhou et al., 2009). To obtain maximum GP, achenes of *R. multiflora* and *R. setigera* 'Beltsville' required 30 d of cold stratification at 5 °C, *R. wichuraiana* 45 d, and *R. setigera* 'Serena' and *R.* × *reversa* 90 d (Stewart and Semeniuk, 1965). However, for *R. gallica* 'Ekta' and *R. rugosa* 'Tetonkaha' only 17.4 and 0.2% achenes germinated after cold stratification at 5 °C for 150 d, respectively (Svejda, 1968); and for *R. acicularis*, only about 2% of achenes germinated following cold stratification at 5 °C for 200 d (Densmore and Zasada, 1977). In the present study, even 28 weeks of stratification at 5 °C did not induce high GP for all species, especially for *R. sericea* and *R. hugonis*. The great variation in germination responses to cold stratification may be caused by differences in achene structure between rose species, providing evidence for the presence of the great variation in dormancy strength and the complexity of the dormancy mechanism in rose.

4.4. Recommendation for propagation

In the present work, the highest GPs of *R. multibracteata* (81.4 ± 2.9%), *R. hugonis* (13.1 ± 6.0%) and *R. filipes* (62.7 ± 5.7%) were obtained by H_2SO_4 scarification followed by warm stratification at 20 °C for 4 weeks and cold stratification at 5 °C for 8 weeks; but for *R. sericea* H_2SO_4 scarification followed by cold stratification at 5 °C for 12 weeks induced the highest GP (46.7 ± 8.7%). Therefore, for *R. multibracteata*, *R. hugonis* and *R. filipes* propagation outdoors, we recommend that achenes can be scarified with 98% H_2SO_4 for 4 h (*R. multibracteata* and *R. hugonis*) or 2 h (*R. filipes*) after collection, warm stratified at 20 °C for 4 weeks in December, and then sown immediately in the field. For *R. sericea*, achenes may be scarified with 98% H_2SO_4 for 4 h after collection, stored dryly indoors until December and sown directly. The period of December–February simulates cold stratification for achenes sown in soil.

5. Conclusion

Some inhibitory substances were present in the pulp and achenes, but the degree of inhibition was higher in extracts of pulp than of achenes. The inhibitory effect of pulp and achene extracts was species-dependent, being lowest in *R. multibracteata*, followed by *R. filipes*, *R. hugonis* and *R. sericea*. Scarification with H_2SO_4, cold stratification, or warm stratification alone did not effectively increase germination. However, combinations of these treatments were more effective in breaking seed dormancy. The most effective treatment to break dormancy for *R. multibracteata*, *R. hugonis* and *R. filipes* was H_2SO_4 scarification followed by warm plus cold stratification (at 20 °C for 4 weeks and at 5 °C for 8 weeks), and for *R. sericea* it was H_2SO_4 scarification followed by cold stratification at 5 °C for

12 weeks. Our results suggested that the achenes of all four species had physiological dormancy and the pattern of dormancy strength was ranked: *R. hugonis* > *R. sericea* > *R. filipes* > *R. multibracteata*.

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