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Effects of Scarification, Stratification and Chemical Treatments on the Germination of *Rosa soulieana* Crépin Achenes

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ABSTRACT

Most rose achenes are dormant at maturity and require pretreatment to stimulate germination. To develop effective methods to improve germination of *Rosa soulieana* Crépin, its achenes were subjected to mechanical and acid (H_2SO_4) scarification, cold stratification (5°C), and GA_3 and smoke water combined with mechanical scarification or stratification. Achene morphology and imbibition also were investigated to help determine the class of dormancy. Freshly harvested *R. soulieana* achenes displayed some dormancy by having a low germination percentage (41.0 ± 1.9%, mean ± SE) and slow germination rate (mean germination time, MGT; 52.0 ± 2.7 days). The embryo was fully developed, and the pericarp was permeable. Thus, the achenes did not have physical, morphological, or morphophysiological dormancy. Mechanical and acid scarification significantly increased germination percentage and rate. Germination percentage increased with an increase in duration of soaking in H_2SO_4, reaching the highest value after 2 h and then decreasing, while germination rate increased continuously with increased time of acid scarification. A short duration (4 weeks) of cold stratification significantly improved germination percentage, while prolonged stratification durations (12-16 weeks) only increased germination rate and had no influence on percentage. Light during stratification had no effect on achene germination. Neither GA_3 nor smoke water improved germination percentage and rate, regardless of combination with scarification or stratification, but highly concentrated GA_3 (500 ppm) and smoke water (1:50) inhibited germination percentage. The highest germination percentage (75.33 ± 5.21%) was obtained when achenes were soaked in H_2SO_4 for 3 h, while the highest germination rate (MGT 9.8 ± 0.4 days) was obtained when achenes were stratified with water in darkness for 16 weeks. The results suggest that *R. soulieana* achenes have non-deep physiological dormancy which can be overcome by scarification or a short period of cold stratification.

Keywords: GA_3, germination requirement, physiological dormancy, Rosaceae, smoke water

INTRODUCTION

Most commercialized roses are traditionally propagated by asexual methods (suckers, hardwood cuttings, semi-hardwood cuttings, layering, budding and grafting) (Horn 1992; Lebris *et al.* 1998), and these methods are usually associated with problems such as limitation of stock plants, virus transmission, and prolonged production time. Seed propagation of rose is used for breeding new cultivars, selected rootstocks and native vegetation restoration (Gudin 2001; Zlesak 2005). Raising seedlings from rose achenes is difficult, because most rose achenes are dormant upon harvest which may be caused by the hard pericarps (Tincker and Wisley 1935; Jackson and Blundell 1963) and physiological barriers within the embryo (Tillberg 1983; Xu *et al.* 1993; Liu *et al.* 2001).

The hard pericarp is water-permeable (Tincker and Wisley 1935; Svejda 1972; Xu *et al.* 1993), and it provides a mechanical barrier for radicle protrusion. Removal of the pericarp of rose achenes by mechanical or chemical scarification can improve germination or shorten the period of cold stratification required to release dormancy (Tincker and Wisley 1935; Svejda 1968; Densmore and Zasada 1977). Moreover, enzymes (Yambe and Takeno 1992) or microflora (Morpeth and Hall 2000) also can weaken the pericarp and improve germination. Cold stratification at approximately 5°C is the treatment most often applied to release rose achene dormancy, however, the period varies among species and cultivars (Stewart and Semeniuk 1965; Semeniuk and Stewart 1966; Svejda 1968; Densmore and Zasada 1977; Anderson and Byrne 2007). Scarification or warm stratification combined with cold stratification has proven to be more effective than cold stratification alone for some species (Svejda 1968; Densmore and Zasada 1977; Bhanuprakash *et al*. 2004; Zlesak 2006). Dormancy is thought to be related to the germination inhibitor abscisic acid (ABA) (Svejda and Poapst 1972; Yambe *et al.* 1992; Bo *et al.* 1995). The effect of GA_3 on germination is variable and depends on the species studied (Xu *et al*. 1993; Liu *et al.* 2001; Bhanuprakash *et al.* 2004). Although smoke water is beneficial in the germination of some species (e.g. Brown and van Staden 1997), there have been no reports on the effect of smoke water on the germination of rose achenes.

Rosa soulieana Crépin is a perennial shrub, occurring in Sichuan, Yunlan, and Anhui provinces and the Tibet autonomous region in China (Yu 1985). Plants may be up to 2-4 m tall. Compact corymbs of white flowers occur between May to July, ripening hips turn orange in August and September, and hips shed from December to the next April. *R. soulieana* is abundant in arid and semi-arid habitats where it plays a crucial role in controlling erosion and providing food and habitat for animals. Thus it may be a good candidate for vegetation restoration in these areas. *R. soulieana* also has important economic potential. Some compounds which combat disease are present in the leaves (Chen *et al.* 2000), and hips contain 0.051% vitamin C, 0.015% carotenes and 0.0015% vitamin E (He *et al.* 1994). However, little information is available on seed dormancy and germination for this important species which limits its propagation and exploitation.

The present work aims to 1) identify methods to improve cumulative germination percentage and rate of *R. soulieana* achenes, 2) determine the kind of dormancy in

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achenes of this species according to the new revised system by Baskin and Baskin (2004), and to 3) understand the role of the pericarp and embryo in dormancy release.

MATERIALS AND METHODS

Achene collection

Ripe hips (fleshy hypanthia) were collected in October 2005 from about 30 plants from the dry valley of the upper Minjiang River (32°02′N, 103°40′E, 2370 m a.s.l.), Maoxian County, Sichuan Province, China. Immediately after collection, achenes were manually extracted from the hips and then mixed thoroughly. Achenes that sunk in water, assumed to be mature and viable, were used in the following experiments. After drying for 3 days in open sunlight (20–25°C), achenes were stored at room temperature (10–25°C) until experimental treatments were initiated (within 2 weeks).

Physical characterization

Anatomical characteristics of achenes were observed with 10 newly harvested achenes to determine the existence of morphological dormancy. Moreover, in order to characterize the achenes of this speices, achene length, width, mass and water content; pericarp thickness, pericarp: achene ratio; seed: achene ratio; percentage of sunken achenes; and viability of sunken achenes were measured. Length and width of 20 randomly selected achenes were measured with vernier calipers. Pericarp thickness was measured from median transverse sections of 20 achenes under a light microscope. Mass and water content of achenes were obtained for six replicates of 100 achenes each using an analytical balance (0.01 mg); water content was based on a fresh weight basis after drying the achene samples in an oven at 80°C for 48 h. Pericarp: achene ratio and seed: achene ratio were determined from four replicates of 20 achenes separated with a scalpel. Percent of sunken achenes was determined by placing achenes in tap water for 3-5 min. Viability of sunken achenes was tested before germination trials using the standard tetrazolium test (Moore 1962).

Experiment 1: imbibition of achenes

To determine if water penetrates into achenes and thus if physical dormancy is present, imbibition was monitored for mechanically scarified and non-treated (control) achenes. Mechanical scarification was performed by carefully removing a small portion of the pericarp on the side opposite to the radicle using a scalpel. Three replicates of 100 scarified and of 100 non-scarified achenes each were put on two layers of filter paper (9 cm in diameter Hangzhou Fuyang Special Paper Industry Co., Ltd) moistened with 10 ml distilled water in a Petri dish (1.5 × 9.0 cm) and placed in a growth chamber (25°C, in the dark). After 0, 1, 3, 9, 24, 48, 72, 96 and 120 h, surfaces of the achenes were blotted dry, weighed to the nearest 0.001 g and returned to the moistened paper in Petri dishes. Percentage increase in achene mass was determined as described by Baskin *et al.* (2004).

Experiment 2: mechanical and H_2SO_4 scarification treatment

To examine the influence of the hard pericarp on germination, four replications of 25 dry achenes each were exposed to mechanical and H_2SO_4 scarification. For mechanical scarification, achenes were treated as described in experiment 1. For H_2SO_4 scarification, achenes were soaked in concentrated H_2SO_4 for 1, 2 or 3 h and then washed thoroughly with tap water. Treated achenes were sterilized in a 5% (v/v) sodium hypochlorite solution for 10 min and then washed three times with sterile distilled water. Achenes were placed in glass Petri dishes (1.2 × 7.0 cm) with two layers of moistened filter paper. All Petri dishes were incubated in a growth chamber, and maintained at a daily cycle of 14 h light (about 30 μmol m^{-2} s^{-1}, provided by cool white fluorescent lamps) at 20°C and 10 h darkness at 10°C, to approximate springtime field conditions. To avoid any effects due to position within the chamber, Petri dishes were rearranged at random every 2 days. Radicle emergence was used as the criterion for germination. Germination was recorded every day, and seedlings were removed when germinated. The experiment continued until no new achenes germinated for 5 consecutive days.

Experiment 3: chemical pre-soaking treatment on scarified achenes

Mechanically scarified achenes were used in this experiment to determine the effects of GA_3 and smoke water on dormancy release to avoid the physical resistance of hard pericarps for radicle emergence. GA_3 treatments consisted of three concentrations (100, 250 and 500 ppm), and smoke water treatment of two concentrations (1:50 and 1:500). The initial smoke water was prepared as follows. Smoke was generated in a metal drum by slow controlled combustion of a mixture of dry and fresh native plant materials in *R. soulieana* communities. Smoke water was prepared by bubbling cooled smoke through 10 L of water for 120 min. For each treatment, four replicates of 25 achenes each were soaked in the above-mentioned solutions and distilled water (the control) for 24 h. Achenes for each treatment were tested for germination as described in experiment 2.

Experiment 4: cold stratification treatments

A two-factor experiment was used to determine the effect of various stratification treatments on dormancy release with factors being achene handling (five treatments, namely control; darkness; smoke water; GA_3; SGA_3, stratification followed soaking in 1000 ppm GA_3 for 48 h) and cold stratification duration (0, 4, 8, 12 and 16 weeks). For the control, darkness, and SGA_3 treatments, achenes were first soaked in distilled water, and in smoke water (1:50 smoke water) and GA_3 (250 ppm GA_3 solution) for 24 h for the smoke water and GA_3 treatments, respectively. Then all soaked achenes were mixed thoroughly with sphagnum (1 seed: 4 sphagnum, v/v) moistened with the corresponding solution that achenes were exposed to for 24 h, and then sealed in polyethylene bags (20 cm in length and 16 cm in width). All bags were stored at 5°C in continual light (about 20 μmol m^{-2} s^{-1}, provided by fluorescent lamps), except for the achenes given the darkness treatment had their bag enclosed within aluminium foil. Every 4 weeks during stratification bags were opened to supply air and water was added to keep the sphagnum moist. Achenes were removed from cold stratification at their prescribed durations (0, 4, 8, 12 and 16 weeks). Three replicates of 50 achenes of each treatment were taken out at each cold stratification duration and the germination environment and data collection continued using the same method as described for experiment 2. For the SGA_3 treatment, achenes were soaked in 1000 ppm GA_3 for 48 h before each germination test.

Data analysis

Two germination parameters were determined: cumulative germination percentage (GP) and mean germination time (MGT).

$GP = N/N_0$

$MGT = \sum nd/N$

where N is the total number of germinated achenes; N_0 is the number of achenes tested; n is the number of achenes germinated between scoring intervals; d is the time in days starting from day 0, the day of starting germination test.

GP and MGT were arcsine square root transformed prior to ANOVA. The effect of mechanical scarification on GP and MGT was analyzed by a paired-sample *t*-test. GP and MGT in experiment 4 were analyzed using the univariate process of General Linear Model (GLM) with achene handling treatments, cold stratification durations and their interaction as factors. A one-way analysis of variance (ANOVA) was employed to compare the effect of smoke water and GA_3 in experiment 3 and the effect of H_2SO_4 scarification in experiment 2 on GP and MGT. When significant differences were noted, multiple comparisons of means were made with Tukey's HSD at the 0.05 probability level.

Table 1 Traits of achenes of *Rosa soulieana*.

Trait	Mean ± SE
Achene length (mm)	4.78 ± 0.10
Achene width (mm)	2.41 ± 0.07
Pericarp thickness (mm)	0.43 ± 0.01
Achene mass (mg)	8.50 ± 0.10
Seed: achene ratio (%)	21.81 ± 5.88
Pericarp: achene ratio (%)	78.19 ± 6.67
Achene water content (%)	10.00 ± 1.35
Percentage of sunken achenes (%)	73.83 ± 3.42
Viability of sunken achenes (%)	75.00 ± 2.89

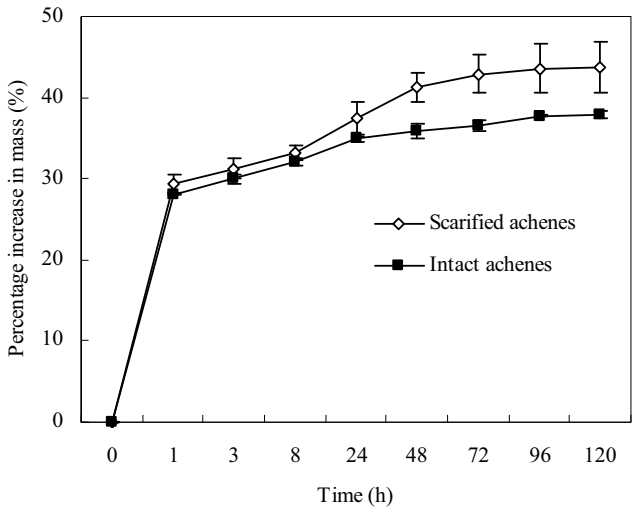

Fig. 1 Percentage increase in mass for intact and mechanically scarified *Rosa soulieana* achenes incubated in distilled water at 25°C with a photoperiod of 14 h light: 10 h dark.

RESULTS

The pericarp of newly harvested *R. soulieana* achenes was brown and the testa was dark brown. The achene has no endosperm and the embryo is clearly morphologically mature with an apparent embryonic bud, hypocotyl, radicle and cotyledons. Other physical traits of achenes are summarized in **Table 1**.

Experiment 1: achene imbibition

Both scarified and intact achenes took up water, and mass increased with an increase in imbibition period. The mass of scarified and intact achenes increased by 29.41 ± 1.17% and 28.10 ± 0.09% in 1 h and 43.72 ± 3.09% and 37.89 ± 0.37% (fully imbibed) in 120 h, respectively (**Fig. 1**). Scarification slightly improved the imbibition quantity and rate of achenes.

Experiment 2: effect of mechanical and H_2SO_4 scarification

For freshly harvested achenes (non-scarified) of *R. soulieana* only 41.0 ± 1.9% of them germinated and did so at a very slow germination rate (MGT 52.0 ± 2.7 days). Mechanical scarification and H_2SO_4 scarification significantly improved the germination percentages ($F_{3, 9} = 9.32$, $P = 0.004$ for H_2SO_4 scarification; t = -7.21, $P < 0.0001$ for mechanical scarification) and rates ($F_{3, 9} = 45.22$, $P < 0.0001$ for H_2SO_4 scarification; t = 9.10, $P < 0.0001$ for mechanical scarification). The effect of H_2SO_4 scarification varied with the duration of the treatment. Soaking in H_2SO_4 for 2 h is best for improving germination percentage and inducing the highest germination percentage (75.3 ± 5.2%) with a quick germination rate (MGT 16.6 ± 4.2 days) (**Fig. 2**).

Experiment 3: effects of GA_3 and smoke water on germination of mechanically scarified achenes

Pre-soaking treatments significantly affected the germination percentages ($F_{4,12} = 7.10$, $P = 0.001$) and rates ($F_{4,12} = 4.84$, $P = 0.006$) of mechanically scarified achenes (**Table 2**). Neither GA_3 solution nor smoke water improved the germination percentage or rate of mechanically scarified achenes. Conversely, a high concentration of GA_3 (500 ppm) and smoke water (1:50) solutions significantly decreased the germination percentage. GA_3 (250 and 500

Table 2 Effect of gibberellic acid (GA_3) and smoke water on cumulative germination percentage and mean germination time of mechanically scarified achenes of *Rosa soulieana* (mean ± SE).

Pre-soaking treatment	Germination percentage (%)	Mean germination time (days)
Distilled water	70.0 ± 3.5 a	19.8 ± 2.3 ab
100 ppm GA_3	70.0 ± 2.1 a	21.1 ± 1.4 ab
250 ppm GA_3	64.0 ± 4.1 ab	18.6 ± 2.4 b
500 ppm GA_3	51.0 ± 1.5 b	16.3 ± 1.8 b
1:500 smoke water	69.0 ± 3.4 a	24.0 ± 0.7 ab
1:50 smoke water	54.0 ± 3.2 b	27.4 ± 1.7 a
F	7.1	4.84
P	0.001	0.006
df	5	5

Means followed by the same lower-case letter within columns are not significantly different from each other (Tukey's HSD, $P < 0.05$).

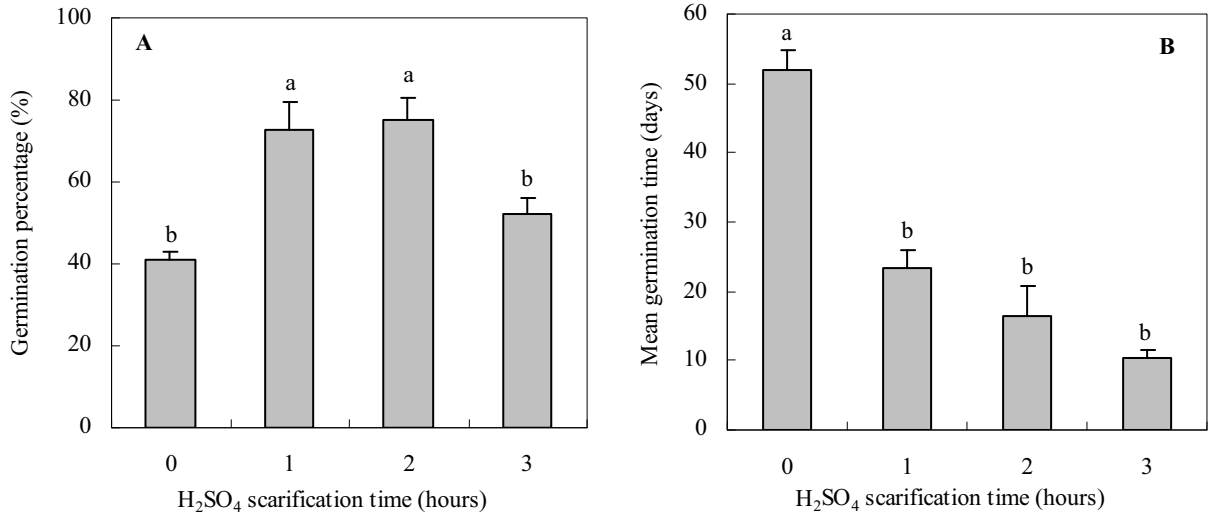

Fig. 2 Effects of H_2SO_4 scarification on cumulative germination percentage (A) and mean germination time (B) of *Rosa soulieana* achenes. Different lower-case letters indicate significant differences among H_2SO_4 scarification durations (Tukey's HSD, $P < 0.05$).

Table 3 ANOVA results on cumulative germination percentage and mean germination time (MGT) of *Rosa soulieana* achenes for different treatments and stratification times.

Source	*df*	*MS*	*F*	*P*
Germination percentage				
A: Treatment	4	209.3	10.884	< 0.0001
B: Stratification time	4	120.7	6.277	0.0003
A*B	16	69.5	3.613	0.0002
Germination rate				
A: Treatment	4	139.5	9.903	< 0.0001
B: Stratification time	4	4499.8	319.527	< 0.0001
A*B	16	47.7	3.385	0.0004

ppm) significantly increased the germination rate, i.e. MGT decreased. However, no significant difference was observed between the germination rates of the scarified achenes soaked in smoke water or in distilled water.

Experiment 4: effect of different stratifications on achene germination

A two-way ANOVA of germination percentage indicated a significant effect for treatment and stratification duration and their interaction (**Table 3**). Overall, all stratification treatments, except for those of GA_3, increased germination percentage compared to achenes without stratification at the shorter stratification durations. However, prolonged stratification (more than 8 weeks) in the water, darkness or SGA_3 treatments tended to decrease germination percentage until it was comparable to the control (**Table 3**). Water and SGA_3 treatments with 4 or 8 weeks stratification, respectively, significantly increased germination percentages relative to 0 weeks stratification, while GA_3 treatments with 4 weeks of stratification significantly decreased it. Light and smoke water during stratification had no effect on germination percentage as no significant difference in germination percentage was observed between water, darkness and smoke water regardless of stratification duration. Soaking achenes in GA_3 (1000 ppm) after stratification did not increase germination percentage. Germination of achenes treated with SGA_3 was comparable to that of the water treatment in each stratification duration (**Table 4**).

A two-way ANOVA for germination rate indicated a significant effect for treatment and stratification duration and their interaction (**Table 3**). All stratification treatments improved germination rate (decreased MGT) relative to no stratification. In each stratification treatment germination rate improved with increasing stratification duration. No significant differences were observed in MGT of each treatment in different stratification durations except for MGT of GA_3 with 4 weeks stratification, which was significantly longer than MGT of other treatments with the same stratification duration (**Table 5**).

DISCUSSION

Embryos of *R. soulieana* are fully developed and no endosperm is present upon harvest, suggesting that the achenes have no morphological or morphophysiological dormancy. On the other hand, intact achenes are water-permeable, indicating that they have no physical or combinational dormancy according to the definitions provided by Baskin and Baskin (2004). Thus, achenes have some level of physiological dormancy, which was substantiated by the stimulatory effect of cold stratification on germination. Furthermore, mechanical and H_2SO_4 scarification promoted germination, cold stratification broke seed dormancy and excised embryos produced normal seedlings (Zhou *et al.* unpublished data) and achenes after-ripened in dry storage (Zhou *et al.* unpublished data). Thus, achenes of *R. soulieana* appear to have non-deep physiological dormancy as defined by Baskin and Baskin (2004).

The *Synstylae* section of *Rosa*, of which *R. soulieana* belongs, appears to have reduced achene dormancy, compared with rose achenes of other sections. First, the germination percentage of freshly matured achenes of this species was as high as 41% without any pretreatment although the germination rate was very slow (**Table 2**), while for many other rose species, no germination occurred without pretreatment (Jackson and Blundell 1963; Xu *et al.* 1993; Liu *et al.* 2001). Moreover, germination of these achenes can be increased significantly by either scarification or short-time (less than 2 months) stratification alone. However, neither scarification nor short-time stratification alone induced ger-

Table 4 Effect of darkness, smoked water, stratification with gibberellic acid (GA_3) and soaking in GA_3 (SGA_3) after stratification along with varying cold stratification durations on cumulative germination percentage of *Rosa soulieana* achenes (mean ± SE).

Stratification time (weeks)	Water[a]	Darkness	Smoke water	GA_3	SGA_3
0	41.0 ± 1.9 bA[b]	45.2 ± 2.3 bA	40.1 ± 1.6 bA	45.2 ± 2.1 bA	41.5 ± 1.9 bA
4	62.7 ± 1.8 aA	58.7 ± 6.6 bA	48.7 ± 4.1 bA	19.3 ± 4.4 cB	61.3 ± 4.7 aA
8	58.0 ± 1.2 aA	47.3 ± 8.5 bA	51.3 ± 3.7 bA	44.0 ± 6.4 bA	58.0 ± 1.2 aA
12	50.0 ± 5.3 bA	50.0 ± 6.1 bA	44.7 ± 2.9 bA	35.3 ± 1.8 bA	43.3 ± 4.7 bA
16	53.3 ± 8.2 bA	56.0 ± 6.4 bA	51.3 ± 4.1 bA	47.3 ± 0.7 bA	45.3 ± 4.4 bA
F	4.05	1.55	2.07	9.80	6.99
P	0.029	0.257	0.153	0.001	0.005
df	4	4	4	4	4

[a] Water: Stratification in distilled water in light; Darkness: Stratification in distilled water in darkness; Smoke water: Stratification in smoke water (1:50) in light; GA_3: Stratification in GA_3 (250 ppm) in light; SGA_3: achene soaking in GA_3 (1000 ppm) for 48 h after stratification in distilled water in light.
[b] Means followed by the same lower-case letter within columns are not significantly different from each other (Tukey's HSD, $P < 0.05$). Means followed by the same upper-case letter within a row are not significantly different from each other (Tukey's test, $P< 0.05$).

Table 5 Effects of darkness, smoked water, stratification with gibberellic acid (GA_3) and soaking in GA_3 after stratification (SGA_3) along with varying cold stratification durations on mean germination time (MGT) (days) of *Rosa soulieana* achenes (mean ± SE).

Stratification time (weeks)	Water[a]	Darkness	Smoke water	GA_3	SGA_3
0	52.0 ± 2.7 aA[b]	50.1 ± 2.5 aA	48.5 ± 1.7 aA	49.2 ± 1.8 aA	46.4 ± 2.2 aA
4	39.0 ± 1.5 bB	37.4 ± 3.3 bB	31.3 ± 3.9 bB	55.6 ± 3.0 aA	42.6 ± 2.1 bB
8	26.9 ± 2.3 cA	20.7 ± 1.0 cA	24.0 ± 0.5 bcA	29.2 ± 1.5 bA	24.5 ± 1.5 cdA
12	15.4 ± 0.2 dA	14.1 ± 0.2 cdA	18.9 ± 0.3 cdA	19.2 ± 1.0 cA	18.0 ± 0.3 cA
16	13.2 ± 0.1 dA	9.8 ± 0.4 dA	15.4 ± 1.5 cdA	17.2 ± 0.6 cA	15.8 ± 0.2 cA
F	57.62	57.62	38.17	55.48	59.63
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
df	4	4	4	4	4

[a] Water: Stratification in distilled water in light; Darkness: Stratification in distilled water in darkness; Smoke water: Stratification in smoke water (1:50) in light; GA_3: Stratification in GA_3 (250 ppm) in light; SGA_3: achene soaking in GA_3 (1000 ppm) for 48h after stratification in distilled water in light.
[b] Means followed by the same lower-case letter within columns are not significantly different from each other (Tukey's test, P <0.05). Means followed by the same upper-case letter within a row are not significantly different from each other (Tukey's HSD, P<0.05).

mination of achenes of some roses, such as *R. davurica*, *R. helenae*, *R. omeiensis*, among others (Xu *et al.* 1993; Liu *et al.* 2001). For other species germination occurred only when rose achenes were exposed to scarification followed by stratification, or long-time stratification (longer than 3 months) (Xu *et al.* 1993; Liu *et al.* 2001). Other rose species belonging to section *Synstylae* such as *R. multiflora* Thunberg ex Murray and *R. setigera* Michaux also exhibit reduced dormancy (Semeniuk and Stewart 1962).

Physiological dormancy is mostly caused by the double, or so-called physiological germination inhibiting mechanism (PIM): decreased embryo activity and inhibiting effect of covers (Nikolaeva 2001; Baskin and Baskin 2004). Results in this study present strong evidence that the pericarp and embryo play important roles in regulating achene dormancy. The pericarps of *R. soulieana* achenes were permeable, which is in agreement with the reports of rose achenes of other species, such as *R. roxburghii* Trattinnick, *R. omeiensis* Rolfe and *R. rugosa* Thunberg (Tincker and Wisley 1935; Svejda 1972; Xu *et al.* 1993). Nevertheless, pericarps of *R. soulieana* exert some limitation on water absorption into the embryo, as shown by slow and decreased imbibition in intact achenes compared to scarified achenes. A similar result has been reported in *R. rugosa* (Svejda 1972). Moreover, mechanical and H_2SO_4 scarification promoted germination in the present study, suggesting that the pericarp probably offered a mechanical barrier for embryo growth or limited the dissipation of inhibitors in the pericarp, testa or embryo (Svejda 1968; Bo *et al.* 1995). Scarification could not improve achene germination of many roses (Svejda 1968; Tincker and Wisley 1935; Xu *et al.* 1993). However, 75% H_2SO_4 scarification for 15 min increased germination percentage of *R. hybrida* 'Happiness' achenes from 0 to 30% (Bhanuprakash *et al*. 2004). In the present study, H_2SO_4 scarification was effective in overcoming dormancy in *R. soulieana* achenes with 1 to 2 h being optimal. The decline in germination percentage with 3 h H_2SO_4 scarification can be attributed to the sensitivity of seeds to the acid which resulted in damage to the embryos. The positive responses of *R. soulieana* achenes to scarification suggest that the hard pericarp is responsible for the low and slow germination of intact achenes. A hard pericarp is an adaptation for achenes to withstand unfavorable conditions such as heat caused by sunlight, the chewing of dispersing animals, severe drought and mechanical damage (Baskin and Baskin 1998; Silvertown 1999).

Stratification is the most common method to overcome dormancy of rose achenes (Buckley 1986). Some rose achenes would ultimately germinate if given sufficiently long periods of cold stratification, but adequate duration and temperatures of stratification for high germination percentage varies among species (Stewart and Semeniuk 1965; Semeniuk and Stewart 1966; Svejda 1968; Densmore and Zasada 1977). Stratification at 5°C significantly increased the germination percentage and rate of *R. soulieana* achenes. A 4-week stratification period was enough to induce a high germination percentage; however, the germination rate was slow (**Tables 4, 5**). Therefore, long-term stratification (12 to 16 weeks) is necessary for rapid and uniform germination of *R. soulieana* achenes. No significant differences were observed in germination percentages and rates of *R. soulieana* achenes stratified in darkness and in light, suggesting that light did not play a crucial role on dormancy release during stratification for this rose species. However, Yambe *et al.* (1995) found that achenes of *R. multiflora* treated with macerating enzymes germinated better under light than in complete darkness. Treatment of achenes with macerating enzymes resulted in splitting of the pericarp along the suture (Yambe and Takeno 1992), aiding exposure of seeds to light when germinated under light. In the present study, *R. soulieana* achenes not treated with enzymes germinated equally after being stratified in darkness or light and suggests that rose seeds rather than the achenes may be positively photoblastic.

GA_3 and smoke water have been reported to improve seed germination of many species (Brown and Staden 1997; Nadjafi *et al.* 2006), but in *R. soulieana* neither GA_3 nor smoke water significantly improved germination, even of scarified achenes. In fact, high concentration of GA_3 and smoke water tended to be toxic. Similar results were found when the achenes were subjected to stratification with GA_3 and smoke water or soaking in GA_3 solution after stratification. Xu *et al*. (1993) found that GA_3 alone or GA_3 combined with KNO_3 could not significantly improve germination of rose achenes such as *R. omeiensis* Rolfe, *R. roxburghii* Tuatt., and *R. rubrifolia* Vill. after cold stratification, which is consistent with the report for *R. davurica* Pall. (Liu *et al.* 2001). But for *R. canina* L. and *R. rugosa*, GA_3 could obviously stimulate germination (Tillberg 1983; Hoşafçı 2005). Therefore, the effect of GA_3 on germination of rose achenes potentially differs across species.

CONCLUSION

In conclusion, *R. soulieana* achenes are permeable, and have no physical dormancy. The dormancy of achenes was characterized as a non-deep physiological dormancy. Both scarification and stratification significantly increased germination percentage and rate. Both hard pericarp and embryo play critical roles in regulating dormancy in *R. soulieana* achenes. Neither GA_3 nor smoke water improved germination percentages and rates even when combined with scarification or stratification; in contrast, they are detrimental to achenes of *R. soulieana* and hampered germination at the highest concentrations. We recommend 3-4 months cold stratification (5°C) for seed propagation of this plant, because mechanical and H_2SO_4 scarification have higher economic and environmental costs than cold stratification. The achenes can be collected in October, stratified at 5°C beginning in December for 3-4 months, and then sown in pots in the greenhouse to raise transplants in early spring of the following year, planted directly in short term nursery beds and transplanted at a later date to their final field locations, or sown directly in field locations.

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