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

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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*.

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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

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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*

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