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Enhancing Seed Germination in Rose (*Rosa rubiginosa* L.)

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Abstract

In the genus *Rosa*, the seed's (achenes) dormancy, due to various factors such the toughness of the pericarp, poses a considerable problem for the production of seedlings. Seeds of *Rosa rubiginosa* L., a wild species in Tunisia, exhibited the same germination difficulties. In this study, different treatments were assessed to overcome the mechanical pericarp resistance and to enhance germination: warm (25°C) or cold (5°C) stratification for 10,11 or 12 weeks, scarification with concentrated sulphuric acid (during 30,45 or 60 mn) or hot water (during 24 h). Evaluation of germination rate was carried out by placing the seeds on Petri dishes at 23°C. Cold stratification for 12 weeks resulted in maximum germination (30.6%) with a germination time about 85 days. Germination percentage remained low (3.6-11%) with warm stratification and the germination time varied from 88 to 101 days according to the duration of stratification. Neither sulphuric acid nor hot water had any positive effect on germination.

Keywords: Germination; *Rosa rubiginosa*; Scarification; Seed; Stratification

Introduction

The genus *Rosa* comprises hundreds of species and thousands of cultivars. Roses are, undoubtedly, one of the most economically important and favorite ornamental plants. Millions of rose bushes are planted in garden or pots and billions of rose cut flowers are sold annually over the world [1]. Rose plants are propagated by seed, stem cutting, grafting, budding and tissue culture [2,3]. 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. Some rose species, for example *Rosa villosa*, has unique genetic characteristics among plant kingdom therefore they can be propagated by seeds to obtain homogeny plant materials. Therefore seed propagation can be the easiest method for these species [4]. However, seed propagation is difficult because of the low germination percentage, a result of prolonged seed dormancy [5-8].

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 [5,9]. The barrier in the form of a hard pericarp contributes to dormancy in some rose achenes [10] but is not its sole cause, since cracking the pericarp fails to break the dormancy in some other achenes [11]. Physiological dormancy is positively correlated to the abscisic acid (ABA) concentration in the pericarp and testa of rose achenes [12,5]. It has been confirmed that embryos in the achenes are fully developed and have no morphological dormancy. Physiological barriers to germination in embryos have been overcome by cold stratification in a number of rose species [13]. Treatments to reduce the mechanical resistance of the pericarp and the physiological dormancy have been attempted in different ways. Scarification by immersion in sulphuric acid (H_2SO_4) promoted seed germination in *Rosa rugosa* [14] and *Rosa dumetorum* [15]. To reduce the ABA content in the achene, the most common treatment is a warm stratification at about 20°C followed by a cold stratification at 2-4°C [16,17].

Germination of rose achenes varies with genotype, prevalent temperatures during seed development, level of maturation at harvest and type of treatment [18,19]. In fact, the temperature influences the rate of embryo development and the thickness of the endocarp [20]. A high temperature and much light during the preceding harvest in hybrid tea rose, resulted in considerably higher germination next year compared to years when harvest was conducted in low temperature and less light [21]. Zimmermann et al. [22] showed that germination rates of *Rosa rubiginosa* achenes varied from one country to another and from one region to another in relation to environmental conditions. Achenes from Central Argentina had a germination rate of 49%, Patagonia (Southern Argentina) and Germany had 25% and Spain had 14%. The highest germination of *Rosa rubiginosa* achenes collected in Sweden was 18.8% [6].

Rosa rubiginosa is wild in northern Tunisia where it grows near streams. It's used for the distillation of flowers for rose water which used in cooking to flavor cookies, tea etc. and in traditional medicine. This species is in endangered due to climate change and its excessive exploitation by local people for harvesting flowers. It is important to restore the species to facilitate ecosystem restoration and to promote its use in particular for its ornamental properties and for its hips which are rich in vitamin C [8] and achenes could be exploited for their essential oils.

The objective of this study is to develop effective method of improving germination in *Rosa rubiginosa*. The results will be helpful in propagation of the species considering that for a commercial rose hip plantation, a large number of plants are necessary. Therefore seed propagation could be a suitable propagation method than the labour intense softwood cuttings. We focused on pregermination treatment to answer the following: (1) is warm or cold stratification better to enhance seed germination and what is the optimal duration? (2) Do scarification with sulphuric acid or hot water has an effect on germination?

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Materials and methods

Plant material

Seeds of *Rosa rubiginosa* L. were harvested in November 2012 from one selected plant located in the region of Susa on the central east coast of Tunisia and its height is more than 2 m.

Experimental protocols

Stratification: Seeds were placed in warm (25°C) or cold (5°C) stratification in moist sand for 10, 11 or 12 weeks. All stratifications were performed in sterile plastic Petri dishes, one for each replication, filled with river sand. During warm or cold stratification, the Petri dishes were kept in dark conditions and maintained humid by spraying distilled water. At the end of the stratification, seeds were washed and wiped carefully to separate from the sand.

Scarification: The scarification effect of concentrated sulphuric acid (H_2SO_4) and hot water on the pericarp was also evaluated. Seeds were immersed in 95-97% H_2SO_4 for 30, 45 or 60 mn. At the end of scarification, seeds were rinsed three times with distilled water. Immersion in hot water (98°C) was for 24 h.

After stratification and scarification, seeds were sown on a double layer of filter paper moistened with distilled water in sterile Petri dishes 8 cm in diameter, and placed in the dark at a temperature of 23°C. As control treatment, seeds were sown directly in the same conditions.

The observation of seeds under a binocular microscope was done to see their evolution.

For all our experiments, three replications of 100 seeds were used for each treatment. A total of 3300 seeds collected from 220 fruits were used. Germinating seeds were recorded every three days from the beginning of the sowing. The seed was considered germinated when the radicle appeared and reached at least 2 mm length (Figure 1).

Data analysis: Percentage of germination was calculated as a ratio between the number of germinated seeds and the total number of seeds sown after stratification or scarification. The mean germination time was calculated according to Ellis and Roberts [23]: $\Sigma (t_i.n_i)/\Sigma n_i$, n_i is the number of seeds germinated on the day t_i, t_i is the number of days counted from the sowing day.

The percentage of germination was subjected to analysis of variance (ANOVA). Differences among treatment means were analysed using Duncan test (SPSS 16.0) for Windows. Differences between means was considered significant when $P<0.05$.

Results

Cold stratification

At the end of cold stratification (5°C) for 10, 11 or 12 weeks and before sowing, the germination percentage of seeds was respectively 0.3, 1.0 and 27.3% (Table 1). After their sowing, the germination percentage of seeds became respectively 8.0, 3.6 and 30.6% (Table 2). The highest germination percentage was obtained at the 12 week-long cold stratification. This parameter was the lowest when the stratification time was 11 weeks. The differences were statistically significant between the three stratification times. The mean germination time was about 85 days for all stratification times (Table 3).

Warm stratification

At the end of warm stratification (25°C), there was no seed's germination for all stratification times (Table 1). After sowing, the

Figure 1: Germinating *Rosa rubiginosa* seeds (achenes).

	10	**11**	**12**
Cold (5°C)	0.3 ± 0.2a	1.0 ± 0.6a	27.3 ± 5.6b
Warm (25°C)	0	0	0

Values in the same line followed by different letters are significantly different ($P<0.05$) according to Duncan test

Table 1: Germination percentage of *Rosa rubiginosa* achenes at the end of stratification.

Treatments	Germination percentage (%)
Cold stratification 10 weeks	8.0 ± 2.8a
11 weeks	3.6 ± 1.5b
12 weeks	30.6 ± 8.6c
Warm stratification 10 weeks	3.6 ± 1.7b
11 weeks	11.0 ± 3.1a
12 weeks	7.3 ± 2.3ab
H_2SO_4 scarification 30 mn	0
45 mn	0
60 mn	0
Hot water scarification	0
Control (non-treated achenes)	0

Values followed by different letters (a, b, c) are significantly different ($P<0.05$) according to Duncan test

Table 2: Effects of cold and warm stratification, H_2SO_4 and hot water scarification on germination percentage of *Rosa rubiginosa* achenes.

	10	**11**	**12**
Cold (5°C)	85.2 ± 3.2a	85.5 ± 3.0a	84.5 ± 2.9a
Warm (25°C)	87.7 ± 4.0a	94.2 ± 4.4ab	100.6 ± 4.6b

Means in the same line followed by different letters are significantly different ($P<0.05$) according to Duncan test

Table 3: Mean germination time (days) of *Rosa rubiginosa* achenes subjected to cold or warm stratification.

germination percentage was the highest (11%) when the stratification time was 11 weeks (Table 2). The lowest germination percentage (3.6%) was obtained at the 10 week-long stratification. The mean germination time increased with the duration of stratification (Table 3). It was 87.7, 94.2 and 100.6 days in achenes stratified for 10, 11 and 12 weeks, respectively.

Scarification

The germination percentage was zero in achenes subjected to H_2SO_4 scarification for 30, 45 or 60 mn (Table 2). Hot water had also no effect on germination. The germination of non-treated seeds (control) was completely inhibited.

Discussion

In order to overcome the seed dormancy in *Rosa rubiginosa*, it has been determined that appropriate stratification temperature is more important compared to other species. The most common treatment to break dormancy of rose seeds is cold stratification at about 5°C [9, 24], and the degree of dormancy varies by species and duration of stratification [25]. The species *Rosa multiflora* and *Rosa setigera* need 30 days of cold stratification; the species *Rosa wichuraiana* needs 45 days of cold stratification; and *Rosa setigera* 'Serena' and *Rosa x reverse* need 90 days of cold stratification to obtain maximum germination percentages [24]. *Rosa rubiginosa* needs 84 days (12 weeks) of cold stratification at 5°C [16].

In our experiments, the highest germination percentage (27.3%) at the end of cold stratification was obtained when the stratification time was 12 weeks. This percentage became 30.6% after seeds sowing and seems satisfactory compared to other species. It was reported that the germination percentages in some rose species are still low. Belletti et al. [25] ranged the germination percentage from 0.5 to 50.3%. This percentage was from 1.8 to 41.5% in *Rosa bracteata* [19] and from 12.9 to 18.8% in *Rosa x hybrida* [26].

Warm stratification permitted a germination percentage relatively low (3.6-11%) but could be considered acceptable compared to other species. In fact, this percentage was 18.8% in *Rosa canina* L., 13.8% in *Rosa pulverulenta* Bieb and 13.5% in *Rosa dumalis Bechst* at 25°C of warm stratification followed by 5°C cold stratification [17]. In *Rosa rubiginosa*, the highest germination percentage (18.8%) was obtained at 20/5°C of warm/cold stratification [6]. Then, our results could be improved by following warm stratification by a cold stratification period of variable duration depending on the species: 1-3 weeks for *Rosa heckeliana* [17], 9 weeks for *R. x hybrida* [26], 12 weeks for *R. rubiginosa* [16] and up to 20 weeks for *R. canina, R. pulverelanta* and *R. dumalis* [17]. To enhance germination in R. rubiginosa seeds, a warm stratification for 12 weeks at 20°C [16] or 20/30°C (darkness/light) [22] followed by a cold stratification for 12 weeks at 5°C or 4/8°C permitted higher germination percentage of 18.8 and 49%, respectively. However, seeds were collected in Sweden and Argentina, respectively. Seed weight can be an indicator of fitness, as heavy seeds may have higher germination rates [27]. Central Argentinean populations of *Rosa rubiginosa* had the highest germination rates and relatively heavy seeds, Patagonian seeds were heavier than seeds from Europe (Germany, Spain) but had low germination rates [22].

Germination has been lacking in seeds subjected to concentrated H_2SO_4 scarification for 30, 45 or 60 mn. Similar results were found even for much longer immersion times. Thus, heavy scarification with 95-97% H_2SO_4 by immersion for 2,4 or 6 h completely inhibited seed germination of *Rosa multibracteata* Hemsl. & E. H. Wilson [9]. Immersion in 95-97% H_2SO_4 for 1, 5 or 10 mn did not inhibit seed germination of *Rosa rugosa* Thunb.; however, no positive effect was found [28].

Hot water treatment had no effect on seed germination of *Rosa rubiginosa*. Younis et al. [29] reported also that no significant effect of hot water treatment was observed on germination of rose seeds.

Conclusion

Rose seeds exhibit difficulties in germination due to strong dormancy. This study suggested that the best treatment to enhance seed germination in *Rosa rubiginosa* is cold stratification at 5°C for 12 weeks. Warm stratification during 11 weeks permitted seed germination (11%) but this percentage could be improved using a combination of warm and cold stratification. Sulphuric acid and hot water treatments inhibited germination. A much longer immersion times or a combination with other methods could be experimented.

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