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A Study on the Aseptic Germination Method for *Rosa rugosa* Seeds

Changli Fu[1,2*], Shutang Xing[3*], Dandan Zhao[1,2*], Dekui Zang[1,2], Yuyuan Wei[1,2], Lanyong Zhao[1,2], Xiaoyan Yu[1,2#]

[1]Forestry College of Shandong Agricultural University, Tai'an, China
[2]Shandong Provincial Research Center of Landscape Engineering Technology for Urban and Rural, Tai'an, China
[3]College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an, China
Email: #yxyxst20040214@163.com

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Abstract

In order to find a method to break dormancy of *Rosa rugosa* seeds and interspecific hybrid seeds between *Rosa rugosa* and *Rosa hybrid* quickly, and accelerate the breeding process of interspecific hybridization between *Rosa rugosa* and *Rosa hybrid*, the influence of concentrated acid, seed maturity, GA_3 (gibberellin) and low temperature (4˚C) on seed germination of *Rosa rugosa* from Muping was researched under aseptic condition. The results showed that aseptic germination can significantly shorten the germination time of *Rosa rugosa* seeds and raise its germination ratio. Before inoculation, concentrated acid treatment greatly increased the germination rate and reduced the contamination rate of the seeds. The higher the degree of maturity of seeds is, the lower the germination rate would be, and the best time for seed to aseptic germination is 60 d after pollination. The addition of GA_3 in 1/2MS medium could promote seeds germination better, and when the concentration of GA_3 was 0.15 mg/L, the seed germination ratio was the highest; the germination time decreased and the seed germination ratio increased gradually as the treatment time at 4˚C lasted longer.

Keywords

Rosa rugosa, Aseptic Germination, GA_3 Treatment, Low Temperature Treatment

1. Introduction

The *Rosa rugosa* is a famous traditional Chinese flower. It is fragrant as well as resistant to cold, drought, pest, disease, salt, and alkali [1] [2] [3]. However, due to its short bloom phase and uniform color and pattern, it has not been widely

*These authors contribute equally.

used in landscaping. In contrast, the *Rosa hybrid* belongs to the same family and genus but comes in many varieties, and has a year-round bloom, rich color, and ornate patterns. The hybridization of the traditional *R. rugosa* with the *R. hybrid* could produce new varieties of *R. rugosa* that were aromatic, highly resistant, and highly ornamental. Nevertheless, the incompatibility between the two plants and the dormancy characteristic of the interspecific hybrid seeds hindered the breeding process seriously [4] [5] [6]

Seeds from plants of the Rosaceae family generally undergo a period of dormancy. Seed dormancy can be interrupted by sand storage under low temperature [7] [8] [9] [10]. Interspecific hybridization between *Rosa rugosa* and *Rosa hybrid* usually results in very low seeding rate due to seriously interspecific incompatibility [11]. As very few hybrid seeds are obtained, it is not convenient to interrupt seed dormancy by sand storage under low temperature. Moreover, a small amount of seeds mixed with sand will make the differentiation difficult, and this will result in a loss of seeds. It takes at least 150 days to interrupt the dormancy of seeds of *Rosa rugosa* or seeds of interspecific hybrid between *Rosa rugosa* and *Rosa hybrid.* All these bring great difficulty to interspecific hybridization between *Rosa rugosa* and *Rosa hybrid.* Therefore, if a quick method for interrupting seed dormancy is found, the interspecific breeding process between *Rosa rugosa* and *Rosa hybrid* will be facilitate greatly.

Previous studies have shown that hormones, concentrated acids and low temperature contribute to the interruption of seed dormancy. 1/2 MS medium is an aseptic seeding substrate and suitable for quantitative and qualitative detections of the effects of hormones, concentrated acids and low temperature on interrupting seed dormancy. Although the seeds of *Rosa rugosa* share similarly dormancy features with the seeds of interspecific hybrid between *Rosa rugosa* and *Rosa hybrid*, the former is much easier to obtain in large quantity than the latter. We performed experiments with the seeds of wild *Rosa rugosa* from Muping, Shandong Province. The effects of treatment with concentrated acids, seed maturity, GA_3 and low temperature (4˚C) on seed germination of *Rosa rugosa* were studied under aseptic conditions. We attempted to develop a quick method for interrupting the dormancy of seeds of interspecific hybrid between *Rosa rugosa* and *Rosa hybrid* and to lay a foundation for facilitating the interspecific breeding process.

2. Materials and Methods

2.1. Materials

The experimental materials were seeds of wild *Rosa rugosa* from Muping, which were planted in *Rosa rugosa* germplasm nursery of Shandong Agricultural University.

2.2. Methods

2.2.1. Seed Disinfection

The *Rosa rugosa* seeds were washed with running water for 2 h and soaked in

75% alcohol on the sterile bench. Then the seeds were soaked in 0.1% $HgCl_2$ solution for 15 min, followed by washing with sterile water for three times. The water was removed from the surface of the seeds with filter papers.

2.2.2. Treatment with Concentrated Acids

In September 2016, *Rosa rugosa* seeds were collected at 90 d after pollination and soaked in different concentrations of concentrated hydrochloric acid and concentrated sulfuric acid, respectively. Table 1 shows different treatments. The seeds treated with concentrated acid were first disinfected following the method described in section 2.2.1 and then inoculated to the 1/2MS medium containing 0.15 mg/L GA_3. The inoculated seeds were preserved at 4˚C for 15d and cultured under light conditions at 22˚C. The contamination rate and germination rate of seeds were determined 20d later. Each treatment consisted of 30 seeds and was repeated for 3 times.

2.2.3. Treatment of Seeds at Different Maturity

The seeds were collected at 60 d (orange red, hard-textured pericarp), 90d (red, softened pericarp) and 120 d (black, shriveled pericarp) after pollination, respectively. After treatment with concentrated sulfuric acid for 4min, the seeds were inoculated to the 1/2 MS medium containing 0.15 mg/L GA_3. The inoculated seeds were preserved at 4˚C for 15 d and cultured under light conditions at 22˚C. The germination time and germination rate of seeds were determined 20 d later. Each treatment consisted of 30 seeds and was repeated for 3 times.

2.2.4. Treatment of Seeds with GA_3

The seeds were collected at 60d after pollination. Some seeds were inoculated to the 1/2MS medium containing different concentrations of GA_3 (0, 0.05, 0.1, 0.15, 0.2 mg/L). Some other seeds were soaked in different concentrations of GA_3 (100, 300, 500, 800, 1000 mg/L) for 24 h and then inoculated to the blank 1/2MS medium. The procedures described in section 2.2.5 were performed to the inoculated seeds.

2.2.5. Treatment of Seeds under Low Temperature (4˚C)

The seeds treated using the method in section 2.2.4 were preserved at 4˚C for 0,

Table 1. List of concentrated acid treatment.

Number	Concentrated acid	processing time (min)
CK	-	-
A1	concentrated hydrochloric acid	30
A2	concentrated hydrochloric acid	35
A3	concentrated hydrochloric acid	40
B1	concentrated sulfuric acid	2
B2	concentrated sulfuric acid	4
B3	concentrated sulfuric acid	6

5, 10 and 15 d, respectively, and cultured under light conditions at 22˚C. The germination time and germination rate of seeds were determined. Each treatment consisted of 30 seeds and was repeated for 3 times.

2.2.6. Data Statistical Analysis

Data statistical analyses were performed using SPSS software.

3. Results and Analysis

3.1. Effect of Concentrated Acid on Aseptic Germination of *Rosa rugosa* Seeds

As shown in Table 2, corrosion seed coat with concentrated hydrochloric acid and concentrated sulfuric acid caused not only a significant reduction in the seed contamination rate during aseptic germination, but also an increase in the seed germination rate. As the treatment proceeding, the seed contamination rate decreased, though the specific effect varied for different acids used. The seed contamination rates at 2, 4 and 6min after treatment with concentrated sulfuric acid (6.15% - 13.16%) were significantly lower than those at 30, 35 and 40 min after the treatment with concentrated hydrochloric acid (60.53% - 69.16%). Therefore, compared with concentrated hydrochloric acid, treatment with concentrated sulfuric acid caused a considerable reduction in the seed contamination rates. Moreover, the seed germination rates first increased and then decreased over time. The seed germination rate was the highest (34.15%) after treatment with concentrated sulfuric acid for 4 min, and it was the lowest (12.45%) after treatment with concentrated sulfuric acid for 6min. In a word, treatment with concentrated sulfuric acid for 4 min is the best scheme of acid corrosion for aseptic germination of *Rosa rugosa* seeds.

3.2. Effect of Seed Maturity on Aseptic Germination of *Rosa rugosa* Seeds

According to Table 3, seed maturity had a significant impact on the germination time and germination rate of *Rosa rugosa* seeds. The germination time was the shortest (15.67%) if the seeds were collected at 60 d after pollination and the germination rate was also the highest (61.57%). As the seed maturity increased,

Table 2. Effect of concentrated acid on aseptic germination of *Rosa rugosa* seeds.

Number	Contamination rate (%)	Germination rate (%)
CK	75.74 ± 1.27 a	1.09 ± 0.16 d
A1	69.16 ± 0.91 b	26.61 ± 1.37 b
A2	63.16 ± 2.26 c	32.49 ± 1.66 a
A3	60.53 ± 3.40 c	25.53 ± 1.09 b
B1	13.16 ± 1.23 d	32.82 ± 1.06 a
B2	10.73 ± 0.82 de	34.15 ± 2.55 a
B3	6.15 ± 0.73 e	12.45 ± 1.01 c

Table 3. Effect of seed maturity on aseptic germination of *Rosa rugosa* seeds.

Maturity	Germination time(d)	Germination rate (%)
60d after pollination	15.67 ± 3.33c	61.57 ± 0.48a
90d after pollination	20.33 ± 3.67b	30.03 ± 0.64b
120d after pollination	31.33 ± 4.33a	7.29 ± 0.32c

the germination time increased as well, while the seed germination rate decreased. The seeds that were collected at 120 d after pollination did not germinate until 31.33 d later, and the germination rate was only 7.29%. Therefore, the best time to collect the seeds for aseptic germination is 60 d after pollination.

3.3. Effect of the Adding Method of GA_3 on Aseptic Germination of *Rosa rugosa* Seeds

Rosa rugosa seeds germinated after inoculation to 1/2MS medium containing different concentrations of GA_3 and culture under light conditions for 25 d. As shown in **Figure 1**, the addition of different concentrations of GA_3 into the culture medium greatly increased the seed germination rates (35.6% - 60.25%). As the concentration of GA_3 increased, the seed germination rates first increased and then decreased. The highest seed germination rate (60.25%) was obtained at a GA_3 concentration of 0.15 mg/L.

The seeds were first soaked in different concentrations of GA_3 for 24 h and then inoculated to the GA_3-free 1/2MS medium under light conditions for 43 d, at which time the seeds germinated. As shown in **Figure 2**, immersing the seeds in different concentrations of GA_3 significantly increased the seed germination rates. As the GA_3 concentration increased, the seed germination rates first increased and then decreased. As compared with the treatment of directly adding GA_3 into the culture medium, the seed germination rates were lower after the soaking treatments in GA_3. The seed germination rate was the highest at a GA_3 concentration of 500 mg/L of all soaking treatments, though it was only 14.87%. This was much lower even than the lowest seed germination rate of all treatments where GA_3 was directly added into the culture medium, which was 35.60%, obtained at 0.05 mg/L GA_3.

In conclusion, compared with the GA_3 soaking treatments, directly adding GA_3 into the 1/2 MS medium better promoted seed germination. The latter not only reduced the germination time, but also increased the seed germination rate.

3.4. Effect of Treatment at 4˚C on Aseptic Germination of *Rosa rugosa* Seeds

According to **Table 4**, treatment at 4˚C had a significant impact on the germination time and also on the seed germination rate. As the treatment proceeded at 4˚C, the germination time decreased, while the seed germination rate increased. The germination time was the shortest (17.33 d) after treatment at 4˚C for 15 d, while the seed germination rate (60.59%) was the highest. Therefore, appropriate

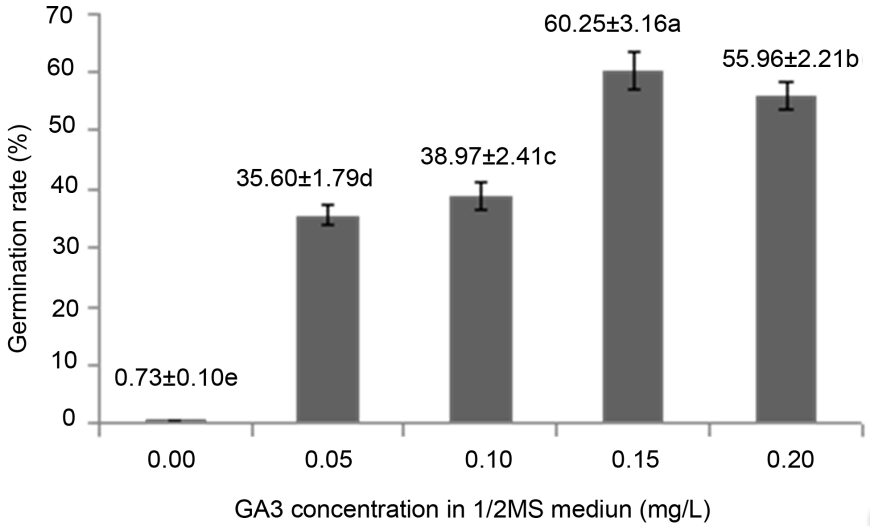

Figure 1. Effect of GA_3 addition in culture medium on seed germination rate.

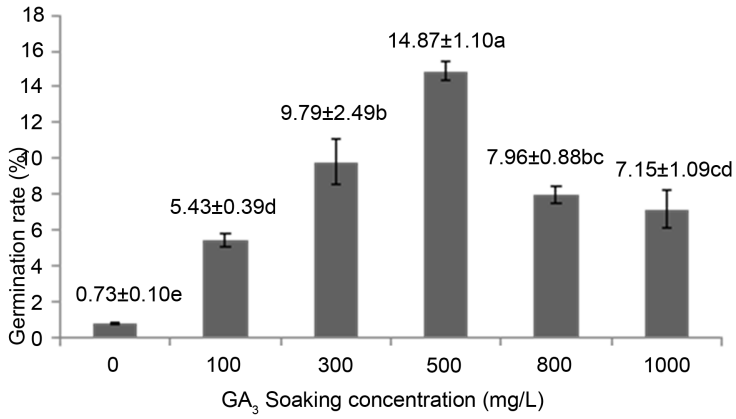

Figure 2. Effect of GA_3 soaking on seed germination rate.

Table 4. Effect of treatment at 4˚C on aseptic germination of *Rosa rugosa* seeds.

Maturity	Germination time(d)	Germination rate (%)
0 d	35.33 ± 5.33a	9.27 ± 0.78d
5 d	25.33 ± 5.67b	36.76 ± 2.12c
10 d	21.67 ± 4.67bc	54.50 ± 3.21b
15 d	17.33 ± 4.33c	60.59 ± 3.22a

low temperature treatment can reduce the germination time and increase the germination rate of *Rosa rugosa* seeds.

4. Discussion

4.1. Effect of Seed Coat on Seed Germination

Many researches indicate that seed dormancy is closely related to seed coat impermeability [12]. The seed coat of *Rosa rugosa* is lignified and hard, which im-

pedes the germination of seeds to some extent [13]. The seed coat can be partially corroded with concentrated hydrochloric acid and sulfuric acid so that the seed husk is thinned, the blockage is removed and the seed coat permeability is increased [14] [15] [16]. Our experiment indicated that concentrated acid treatments not only promoted germination of *Rosa rugosa* seeds, but also disinfected the surface, which is favorable for aseptic germination. This is in accordance with the use of concentrated hydrochloric acid by Han *et al.* to remove the seed coat of *Rosa multiflora* for aseptic germination [17]. However, the contamination rates of seeds treated by concentrated hydrochloric acid and sulfuric acid varied in our study, probably because of different mechanism of corrosion. Concentrated hydrochloric acid can soften the seed coat without changing the thickness of the seed coat. Concentrated sulfuric acid, however, can cause carbonization and thinning of seed coat. The duration of treatment with either acid is the key factor. If the duration is too short, the seed coat permeability will be improved only to a small degree; if the duration is too long, the seeds will be harmed by the acids and lose the ability to germinate.

4.2. Effect of Degree of Maturity of Seeds on Germination

Seed dormancy of *Rosa rugosa* is mainly related to seed coat limit and high level of growth inhibitory substance ABA (abscisic acid) in seeds [18] [19]. Although the content of ABA will decline in the later period of seed maturation, the seed coat impermeability will increase [20]. Therefore, seed dormancy is associated with seed maturity as well. Our experiment, aseptic germination of *Rosa rugosa* seeds with different degree of maturity, indicated that germination time and germination rates of those were different. In the early period of seed maturation, seed embryos, with low level growth inhibitory substance, were developed completely, therefore it was easy to germination and higher germination rate. As the seed matures, the content of growth inhibitory substance increased gradually, the permeability of seed coat became weaker and weaker, and even some seeds might be in dormancy, accordingly, the germination time increased and germination rate decreased gradually.

4.3. Combination Effect of GA3 and Low Temperature (4˚C) Treatment on Seed Dormancy

GA_3, commonly used as germination promoter, can help interrupt seed dormancy [21]. We compared the effects of different addition modes of exogenous GA_3 on the seed germination rate of *Rosa rugosa*. It was found that the germination time was significantly shortened and the germination rate was increased by inoculating the seeds to the 1/2 MS medium containing exogenous GA_3. However, after inoculating the seeds soaked with GA_3 into blank 1/2 MS medium, the germination time was prolonged and the germination rate decreased compared with the former. This is probably because the longer exposure of seeds to exogenous GA_3 added to the medium more effectively interrupts seed dormancy. We also found that exogenous GA_3 alone failed to interrupt seed dormancy of

Rosa rugosa, and the combined treatment under 4˚C is required to reduce germination time and to increase the germination rate.

5. Conclusion

This is the first study on the aseptic germination of *Rosa rugosa* seeds, and the research shows that the aseptic germination can greatly reduce the germination time of *Rosa rugosa* and increase the germination rate. Four factors are related to seed germination during aseptic germination: treatment with concentrated acids, seed maturity, treatment with exogenous GA_3 and low temperature (4˚C). Before inoculation, concentrated acid treatment greatly increased the germination rate and reduced the seed contamination rate. The higher the degree of maturity of seeds, the lower the germination rate would be. The germination rate first increased and then decreased with the increasing concentration of GA_3 added to the medium; it increased gradually as the treatment time at 4˚C germination rate.

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