ACHENE DORMANCY CHARACTERISTICS OF THREE ROSE SPECIES AND THE METHODS FOR IMPROVING GERMINATION

ZHIQIONG ZHOU[1,2], DANDAN WEI[2], XIAOMING LI[2], YULAN PENG[2] AND FUZHONG WU[1*]

[1]*Key Laboratory for Humid Subtropical Eco-Geographical Processes of the Ministry of Education, School of Geographical Sciences, Fujian Normal University, Fuzhou 350007, China*
[2]*Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan, 610041, China*
[*]*Corresponding author's email: wufzchina@fjnu.edu.cn*

Abstract

Roses are a group of world famous garden plants, and strong dormancy exists in the achenes. We characterized the water absorption of achenes, the inhibition of extracts of pulp and achenes, effects of different pretreatments on breaking dormancy to determine the dormancy type and mechanism. Achenes of three rose species had physiological dormancy (PD) based on the water-permeable seed coat and fully developed embryo. *Rosa rubus* had intermeditate PD, and *Rosa longicuspis* and *Rosa helenae* had deep PD. The best treatment for improving germination was warm plus cold stratification, as indicated by higher germination percentage (GP) and lower moldy achene percentage for *R. rubus* (74.9±2.1%) and *R. longicuspis* (25.9±2.1%). For *R. helenae*, GP (36.3±1.5%) was highest treated by H_2SO_4 scarification followed by warm stratification at 25°C for eight weeks and cold stratification at 5°C for sixteen weeks. Prolonged period of cold stratification significantly increased germination speed and moldy achene percentage especially when combined with H_2SO_4 scarification. The physiological dormancy of rose achenes was caused jointly by inhibitory substances in pulp and achenes, mechanical barrier of thick pericarp and physiological dormancy in embryo.

Key words: Dormancy type, Rose, Scarification, Achene dormancy, Cold stratification.

Introduction

Roses (Rosaceae) are one of group of the most popular garden species in the world characterized by beautiful flower and sweet fragrance (Wu *et al*., 2005). Meanwhile, many wild rose species are frequently recommended as ideal species for ecological restoration in degraded ecosystem for their adaptation of withstanding drought stress and impoverished soil (Zhou & He, 2020). Roses are typically propagated by vegetative methods as stem cutting, grafting, and tissue culture for high cost of seed collection in the field, low seed germination, and lack of achene dormancy-release techniques (Pati, 2006). However, vegetative propagation has problems e.g. shortage of rootstocks, long production time and high risk of extermination by disease (Kim, 2019). Propagation by seeds is a preferable choice to increase the genetic diversity and the stability of restored ecosystem. The achenes of most roses exhibit deep dormancy causing difficulties in germination and natural regeneration, which restricts large-scale commercial propagation and breeding (Zlesak, 2005; Yang *et al*., 2017).

A feature of all members of *Rosa* is the hardness of pericarp, which may offer some resistance to germination (Zhou & Bao, 2009; Conev & Sandhu, 2015). The presence of this hard pericarp acts to physically restrain enlargement of embryo and prohibit radicle emergence (Zhou & Bao, 2011; Kim, 2019). Degradation of the pericarp by H_2SO_4 or mechanical scarification, use of enzymes was effective for improving the germination of achenes for species with relative weak dormancy (Zhou *et al*., 2008; Zhou & He, 2020). However, for species with strong dormancy, e.g. *Rosa omeiensis* and *Rosa*

hugonis, H_2SO_4 or mechanical scarification (Zhou & Bao, 2011; Conev & Sandhu, 2015), and even totally remove the pericarp was ineffective for promoting germination (Zhou *et al*., 2009). For these species, physiological barriers in the embryo play a key role in regulating dormancy persistence and release (Zhou *et al*., 2009). A combined treatment that aimed weakening the hard pericarp (by H_2SO_4 scarification or warm stratification) and released physiological barriers in the embryo (by cold stratification) at the same time can effectively promote germination of deep dormancy species (Zhou & Bao, 2011; Sandhu & Conev, 2016). Additionally, extract of pulp and achenes had germination inhibitors, which complex the mechanism of achene dormancy (Bo *et al*., 1995). The correlation of the inhibition of extracts of pulp or achenes and the dormancy level was uncertain (Zhou & Bao, 2011).

Microorganism may play a crucial role in facilitating germination by macerating the hard-coated seed pericarp. Addition of the compost activator during warm plus cold stratification enhanced microbial growth, and consequently resulted in higher germination percentage of *Rosa corymbifera* 'Laxa' (Morpeth & Hall, 2000). Applied microbial nutrition to the germination medium increased the germination percentage of *Rosa omeienisis* remarkably (Han *et al*., 2012). The promoting effect of microbial inoculation on germination has also been observed for *Rosa damascena* (Kazaz *et al*., 2010) and *Crataegous pseudoheterophylla* (Fatemeh *et al*., 2014). The benefit of bacteria on seed germination is attributed to production of metabolites, enzymes and phytohormones and other associated activities such as greater phosphate solubilization and competition in soil and root colonization (Kazaz *et al*., 2010).

The studied species *Rosa helenae*, *Rosa longicuspis*, and *Rosa rubus* are belonging to Section *Synstylae* (Wu et al., 2005). The aim of this study was to determine the achene dormancy type, explore effective treatments that are able to improve germination percentage and speed, and to investigate the mechanism of dormancy. Our research questions were as follows: what is the dormancy type of each rose species? What is the best treatment that is able to break dormancy and improve germination percentage and speed? How do pulp, pericarp and embryo affect seed germination?

Materials and Methods

Plant materials: Mature fruits of *R. helenae, R. longicuspis* and *R. rubus* were collected in mid-October 2019 in the rose garden in Maoxian Mountain Ecosystem Research Station，Sichuan, China (31°41′ N, 103°53′ E, 1,816 masl). The mean annual values of precipitation and temperature were 760.8 mm and 9.8°C, respectively (2005-2015) (Yu, 2019). Fruits were crushed with a heavy wooden bar and then placed in a plastic bucket filled with water for three days to discompose and soften pulp. Achenes cleaning were performed with water to float off the discomposed pulp. Achenes were air-dried in ambient conditions (10-25°C), and stored at 5°C until the start of the experiments (within two weeks).

Achene trait measurement: Ten achenes of each species were halved using a razor blade, and then observed the developmental status of the embryo. Achene length and achene width of 10 randomly selected achenes per species were measured using a digital micro caliper (Mahr 16GN, GRAINGER, Shanghai, China). Pericarp thickness was measured from median transverse sections of the 10 selected achenes under a light microscope (CX21, OLYMPUS, Tokyo, Japan). Achene mass and water content were obtained by weighing five replications of 100 achenes per species to the nearest 0.01 mg using an analytical balance (NJ 07932, OHAUS, New Jersey, USA). Viability of sunken achenes was measured by four replications of 20 achenes using the tetrazolium test (Anon., 2010).

Imbibition of achenes: Water uptake was compared between scarified and intact achenes to detect the water permeability of achenes. Achenes were scratched using a scalpel opposite to the radicle to obtain mechanically scarified achenes. Five replications of 100 scarified and of 100 non-scarified achenes each were used. Each duplicate was placed on two-layer filter papers moisten with 10 ml distilled water, in a 9-cm-diameter Petri dish. All Petri Dishes were put in a growth chamber (25°C, dark). After 0, 1, 3, 9, 24, 48, 72, 96 and 120 h, the achenes was blotted dry, weighed, and returned onto the wet filter papers in Petri dishes as described by Zhou et al., (2009). The percentage increase in air-dried achene mass was determined described by Tang et al., (2019).

Inhibition of extracts of pulp and achene: Achenes were manually removed from freshly collected hips, which were divided to be pulp and achenes. Fresh tissue of concentration of 0.2 g mL^{-1} for pulp and achene was prepared using the method described by Zhou & Bao (2011).

Germination was performed in a germination Chamber at 25°C in the light/dark (12 h/12 h a day). 50 seeds of L*actuca sativa* were placed on two layers of filter paper in 70 mm Petri dish (each treatment with three Petri dishes). Newly germinated seeds were counted and removed every day for 7 d. A seed was considered to have germinated when the radicle protruded at least 1mm.

Seed germination test: The germination process of 2550 treated rose achenes in 17 treatments (Table 1) resembled that for *L. sativa* seeds (in *2.4*), with one exception. Achenes were kept in a cycle of 20°C 12 h light and 10°C 12 h dark. The germination test continued for two months. After the test, non-germinated achenes were cut and evaluated as healthy and dormant or decayed, or empty.

Effect of scarification: Achenes were scratched using a scalpel opposite to the radicle to obtain mechanically scarified achenes. Achenes of each species were soaked in 98% H_2SO_4 for 1.75, 1.75 and 2.5 h respectively according to the pericarp thickness and then washed thoroughly with tap water.

Effect of cold stratification: There were five periods (12, 16, 20, 24 and 28 weeks) for cold stratification treatment. Presoaked achenes with water were mixed with moistened sphagnum moss at a ratio of 1:3 in loosely sealed plastic bags, which were kept in a 5°C refrigerator.

H_2SO_4 scarification combined with cold stratification: H_2SO_4 scarified achenes obtained by the method described as 2.5.1 of each species were stratified at 5°C for 8, 12 and16 weeks respectively.

Table 1. Treatments applied for breaking the achene dormancy of three rose species.

Treatments	H$_2$SO$_4$ Scarification (98%)	Warm stratification (20 ºC)	Cold stratification (5 ºC)
Scarification	H$_2$SO$_4$ scarification: 1.75 h for *R. longicuspis* and *R. rubus* and 2.5 h for *R. helenae*, mechanical scarification		
Cold stratification (5 ºC)			12, 16, 20, 24, and 28 weeks
H$_2$SO$_4$ scarification combined with cold stratification	1.75 h for *R. longicuspis* and *R. rubus* and 2.5 h for *R. helenae*		8, 12 and 16 weeks
Warm plus cold stratification		8 weeks	8, 12 and 16 weeks
H$_2$SO$_4$ scarification combined with warm plus cold stratification	1.75 h for *R. longicuspis* and *R. rubus* and 2.5 h for *R. helenae*	8 weeks	8, 12 and 16 weeks

Warm plus cold stratification: The experiment included two types of medium: water moistened sphagnum moss and the mixture of moistened sphagnum moss and microbial nutrition. Microbial nutrition was prepared as a mixture liquid of KH_2PO_4 ($1.5g.L^{-1}$) $+FeSO_4$ (0.1 $g.L^{-1}$) $+NH_3NO_3$ ($0.7g.L^{-1}$) $+yeast$ extract paste ($0.5g.L^{-1}$) $+soluble$ starch ($5g.L^{-1}$). Achenes of three species were pre-soaked in distilled water for 24 h, mixed thoroughly with two types of stratification medium respectively, and then placed into plastic bags. These bags were sealed and kept in a growth chamber at 25^oC for 8 weeks and then transferred into a 5^oC refrigerator for 8, 12 and 16 weeks, respectively.

H_2SO_4 scarification combined with warm plus cold stratification: H_2SO_4 scarified achenes were warmly stratified at 25^oC for 8 weeks followed by cold stratification at 5^oC for 8, 12 and 16 weeks.

Data analysis

Four parameters were calculated: germination percentage (GP), mean germination time (MGT), dormant achene percentage (DAP) and moldy achene percentage (MAP).

$$GP\ (\%) = (\textstyle\sum n/N) \times 100 \qquad (1)$$

where N is total number of sown achenes, n is number of achenes that were germinated on day d.

$$MGT = \textstyle\sum (n \times d)] / \textstyle\sum n \qquad (2)$$

where d is the number of days counted from the beginning of germination (Fatemeh *et al*., 2014).

$$DAP\ (\%) = (D/N) \times 100 \qquad (3)$$

where D is total number of dormant achenes.

$$MAP\ (\%) = (M/N) \times 100 \qquad (4)$$

where M is total number of moldy achenes.

Effect of species on achene traits were analyzed with one-way ANOVA. T-tests were used for comparing the imbibition quantity of intact and scarified achenes. Effects of extracts, different treatments and species on GP, MGT, DAP and MAP were analyzed using two-way ANOVA. Multiple comparisons of means were made with a LSD test at $p<0.05$ using SPSS 19.0.

Results

Achene traits and imbibition: The seeds of all three species had no endosperm and the embryos were morphologically mature with apparent embryonic bud, hypocotyls, radicle and cotyledons. Three species had significant differences for achene mass, viability of sunken achenes, achene width and pericarp thickness (Table 2).

Both intact and the scarified achenes did take up water. The percentage increase in seed mass increased with prolonging imbibition period for each rose species (Fig. 1). Intact achenes had significant greater imbibition quantity for *R. rubus* ($t=8.548$, $p<0.001$), *R. longicuspis* ($t=2.100$, $p=0.039$) relative to the scarified achenes; and no difference of imbibition quantity was observed

between intact and the scarified achenes ($t=1.682$, $P=0.096$) for *R. helenae*. After imbibing water for 1 h, achene mass of intact achenes increased to 25.06 ± 1.05 %, 37.58 ± 1.13 % and 42.62 ± 0.45 %, while those of scarified achenes increased to 23.86 ± 3.11 %, 34.41 ± 2.03 % and 27.03 ± 0.75 % for *R. helenae*, *R. longicuspis* and *R. rubus* respectively. This result indicates that the pericarp was water permeable for each species.

Extract inhibition o on lettuce germination: Significant effects of tissue extracts, species and their interactions ($p<0.001$) were observed on GP and MGT of *L. sativa* seeds (Table 4). Lower GP was achieved in *L. sativa* seeds treated by pulp than that of achenes regardless of species, indicating a stronger inhibitory action of the extract of pulp (Table 3).

L. sativa seeds treated with pulp extract germinate with lower GP and higher MGT compared with the control (Table 3, Table 4). The inhibition of germination was the strongest for *R. longicuspis* (GP=0%), followed by *R. rubus* (GP=1%) and *R. helenae* (GP=18%). Achene extract also decreased GP and MGT of *L. sativa* seeds, with exception of *R. helenae*. The inhibition degree of pulp and achene extract was similar: *R. longicuspis* > *R. rubus* > *R. helenae* (Table 3).

Effect of scarification, cold stratification and their combination: Untreated intact achenes were dormant. Both mechanical and H_2SO_4 scarification improved GP of *R. rubus*, and only mechanical scarification increased GP of *R. longicuspis*, and no effect of mechanical and H_2SO_4 scarification was observed for *R. R. helenae* (Fig. 2). Cold stratification continuously increased GP of *R. rubus* and *R. longicuspis* but not for *R. helenae* (Table 5, Fig. 3). H_2SO_4 scarification combined with cold stratification was more effective to improve GP than H_2SO_4 scarification or cold stratification treatment solely (Figs. 2, 3, 4). Generally, GP was ranked as *R. rubus*> *R. longicuspis*> *R. helenae* for scarification, cold stratification and their combination treatment (Figs. 2, 3, 4). The MGT of intact and scarified achenes was more than one month. Long period cold stratification (>20 weeks) decreased the MGT of *R. rubus* and *R. longicuspis* but not for *R. helenae*. However, H_2SO_4 scarification combined with cold stratification was significantly decreased MGT of all species (Fig. 4).
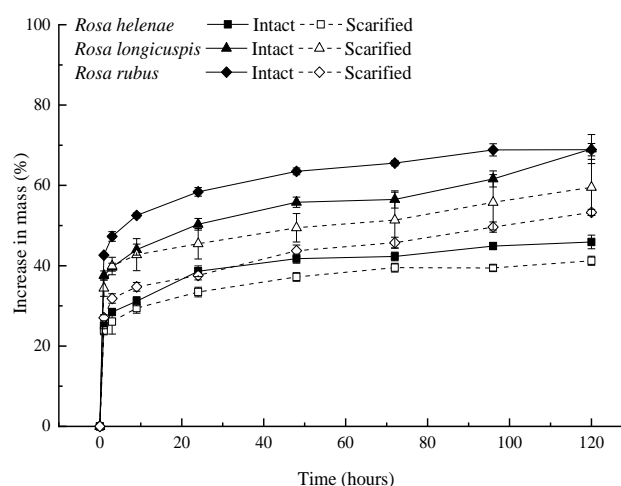

Fig. 1. Imbibition curves for intact and scarified achenes of three rose species. Error bars are mean ± se.

Tables 2. Achene traits (mean ± se) of three rose species.

Species	Achenes mass (mg)	Water content (%)	Viability of sunken achenes (%)	Achene length (mm)	Achene width (mm)	Pericarp thickness (mm)
Rosa helenae	12.11 ± 0.09 a	14.66 ± 0.24	93.33 ± 1.67 a	5.00 ± 0.16	2.98 ± 0.10 a	0.517 ± 0.035 a
Rosa.longicuspis	5.92 ± 0.19 c	15.01 ± 1.40	80.00 ± 5.00 b	4.83 ± 0.16	2.77 ± 0.10 ab	0.386 ± 0.023 b
Rosa rubus	8.04 ± 0.13 b	16.93 ± 0.90	93.33 ± 1.67 a	4.84 ± 0.10	2.61 ± 0.08 b	0.272 ± 0.014 c
F	474.745	1.572	5.400	0.437	3.858	22.834
P	<0.001	0.248	0.046	0.650	0.034	<0.001

Note: different letters within the same column indicate significant differences (LSD test, *p*<0.05)

Table 3. Germination percentage (GP) (mean ± se) and mean germination time (MGT) (mean ± se) of *Lactuca sativa* seeds treated with extracts of pulp and achenes of three rose species.

Species	GPs (%)		MGT (d)	
	Pulp	Achene	Pulp	Achene
Control	100.0 ± 0.0 a	100.0 ± 0.0 a	2.7 ± 0.1 b	2.7 ± 0.1 c
Rosa helenae	18.0 ± 4.7 b	96.9 ± 2.1 a	5.5 ± 0.3 a	3.4 ± 0.3 c
Rosa.longicuspis	0.0 ± 0.0 c	14.6 ± 3.4 d	N.A.	5.9 ± 0.6 b
Rosa rubus	1.0 ± 0.8 c	77.52 ± 6.2 c	N.A.	7.3 ± 0.3 a

Note: different letters within the same column indicate significant differences (LSD test, *p*<0.05)

Table 4. ANOVA for germination percentage (GP) and mean germination time (MGT) of *Lactuca sativa* seeds treated with extracts of pulp and achenes of *Rosa helenae*, *Rosa longicuspis* and *Rosa rubus*.

Source	df	Mean square	F	P
GP				
A: Tissue	1	14390.6	377.9	<0.001
B: Species	2	3904.4	102.5	<0.001
A * B	2	1979.1	52.0	<0.001
MGT				
A: Tissue	1	6.2	14.3	0.005
B: Species	2	11.4	26.3	<0.001
A * B	0	N.A.	N.A.	N.A.

No intact achenes were moldy, while a few (<10%) achenes with mechanical scarification, H_2SO_4 scarification and cold stratification (<28 weeks) were moldy. Most of ungerminated achenes were dormant at the end of the germination test (60 days) (Figs. 2, 3). However, MAP was significantly increased when period of cold stratification was 28 weeks. Combination of H_2SO_4 scarification with cold stratification was significantly increased MAP, which increased with the period of cold stratification. DAP continuous decreased with extending cold stratification for all species regardless of cold stratification or H_2SO_4 scarification combined with cold stratification (Figs. 3, 4).

Effect of warm plus cold stratification and its combination with H_2SO_4 scarification: Warm plus cold stratification resulted in a significant increase in GP for each species. For *R. rubus*, extended period of cold stratification continuously increased the GP, but it did not for *R. helenae* and *R. longicuspis* (Fig. 5). H_2SO_4 scarification combined with warm plus cold stratification significantly decreased GP of *R. rubus* (F =16.9; df = 2; P = 0.001) and *R. longicuspis* (F =26.5; df = 2; *p*<0.001), while increased that of *R. helenae* (F =71.9; df = 2; *p*<0.001) compared with warm plus cold stratification (Figs. 5, 7). MGT decreased continuously with prolonged period of cold stratification for both treatments. Addition microbial nutrition during warm plus cold did not increased GP of each species. It decreased GP of *R. rubus* (F =53.14; df = 2; *p*<0.001) and of *R. longicuspis* (F =7.45; df = 2; *p*=0.015), and had no effect on that of *R. helenae* (F =0.09; df = 2; P = 0.771) (Fig. 6).

For warm plus cold stratification, prolonged period of cold stratification produced no effect on MAP of all species, while slightly decreased DAP of *R. rubus*. Addition microbial nutrition during warm plus cold stratification increased MAP when cold stratification exceeded 12 weeks. H_2SO_4 scarification combined with warm plus cold stratification significantly increased MAP resulting in few dormant achenes at the end of the test (Fig. 6).

Discussion

Rose achenes of each species had physiological dormancy because of the water-permeable seed coat and fully developed embryo. The *R. rubus* likely has intermediate physiological dormancy based on the fact that scarification partly promoting germination (Fig. 2), and H_2SO_4 scarification and warm stratification shortened the period of cold stratification required for high germination (Figs. 4, 5, 7). However, less than 30% GP was attained by any treatment for *R. helenae* and *R. longicuspis* (Figs. 2, 3, 4, 5, 7), indicating that this two species have deep physiological dormancy as defined by Baskin & Baskin (2014). In general, the GP sequence of achenes treated by different methods was *R. rubus* > *R. longicuspis* > *R. helenae* (Figs. 2, 3, 4, 5, 7). Consequently, *R. helenae* achenes relatively had the strongest dormancy, followed by *R. longicuspis* and *R. rubus*.

H_2SO_4 scarification, cold stratification and warm plus cold stratification and their combinations were useful for releasing intermediate and deep physiological dormancy and promoting germination (Zhou & Bao, 2011; Rizwan & Aftab, 2018; Tang *et al.*, 2019; Wawrzyniak *et al.*, 2020). A most interesting thing about seeds with intermediate PD is that warm stratification can shorten the cold stratification period required for breaking dormancy (Baskin & Baskin, 2014). This phenomenon was confirmed in our study for *R. rubus*, which has intermediate physiological dormancy. The achenes with long-term cold stratification (28 weeks) produced GP less than 40%, while 8-week warm stratification followed by 8-week cold stratification produced GP of 64.1%±1.9%.

For *Rosa multibracteata*, warm stratification was also effective in decreasing the length of the cold stratification period (Zhou & Bao, 2011). The most effective treatment for promoting germination for *R. rubus* (74.9±2.1%) was warm stratification at 25°C for eight weeks and cold stratification at 5°C for sixteen weeks.

Treatments required for high GP were different between *R. longicuspis* and *R. helenae*, although both of them had deep PD. For *R. longicuspis*, long-term (24 weeks) cold stratification was effective to produce relative high GP (28.6±2.4%). However, even *R. helenae* achenes with 28-week cold stratification germinated with GP less than 3%. H_2SO_4 scarification combined with warm plus cold stratification produced the highest GP (>23%), indicating that *R. helenae* preferred a more complex treatment.

Duration of cold stratification is a key factor influencing germination of seeds with physiological dormancy (Baskin & Baskin, 2014; Tang *et al*., 2019). In this study, GP and germination speed increased initially with duration of cold stratification, while GP decreased if period was longer than the optimal period. This confirmed previous results about the effect of cold stratification period on germination of rose species (Zhou & Bao, 2011; Chen *et al*., 2013). However, few studies had investigated the causes why GP decreased when cold stratification was too long. In the present study, increased MAP partly contributes to the decreased GP when cold stratification duration exceeded a given period especially for H_2SO_4 scarification combined with cold stratification or warm plus cold stratification. This indicates that prolonged cold stratification duration and using H_2SO_4 scarification may increase the risk of microbial contamination. Therefore, determining the optimal regime of treatments is indispensable for seedling propagation.

Rose achene dormancy is complex, and tissues associated with achene such as pulp, testa and embryo regulated the dormancy break and persistence (Zhou & Bao, 2009; Zhou *et al*., 2009). In this study, the extracts of rose pulp and achenes decreased GP and increased MTG of *L. sativa* seeds (Table 3), suggesting the existence of water-soluble germination inhibitors. Germination inhibitory substance was also observed in pulp and seeds for other species (Zhou & Bao, 2011; Li & Min, 2020).
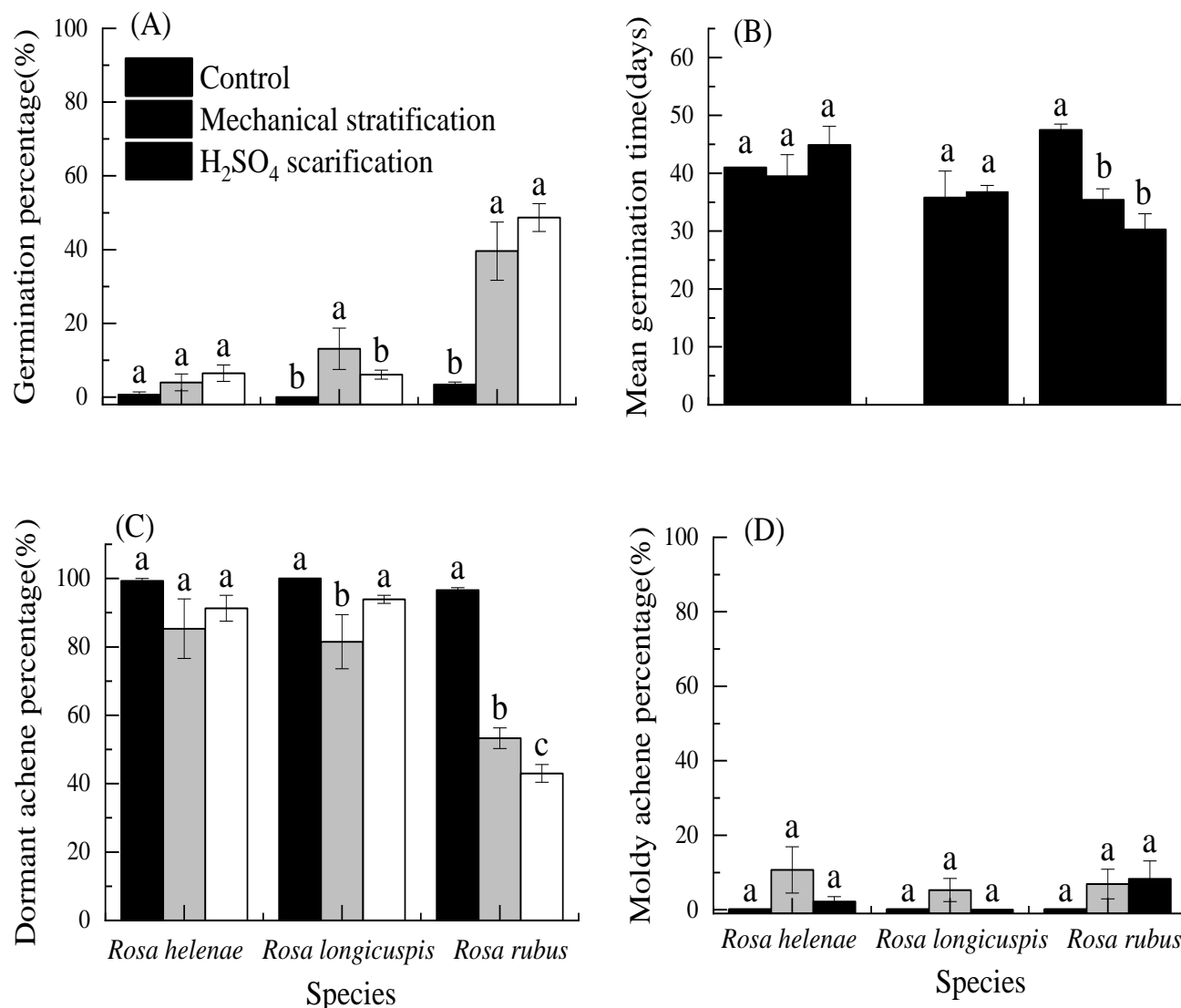

Fig. 2. Germination parameters (mean ± se) of rose achenes treated by mechanical scarification or H_2SO_4 scarification. Different letters within the same species indicate significant differences (LSD test, *p*<0.05).
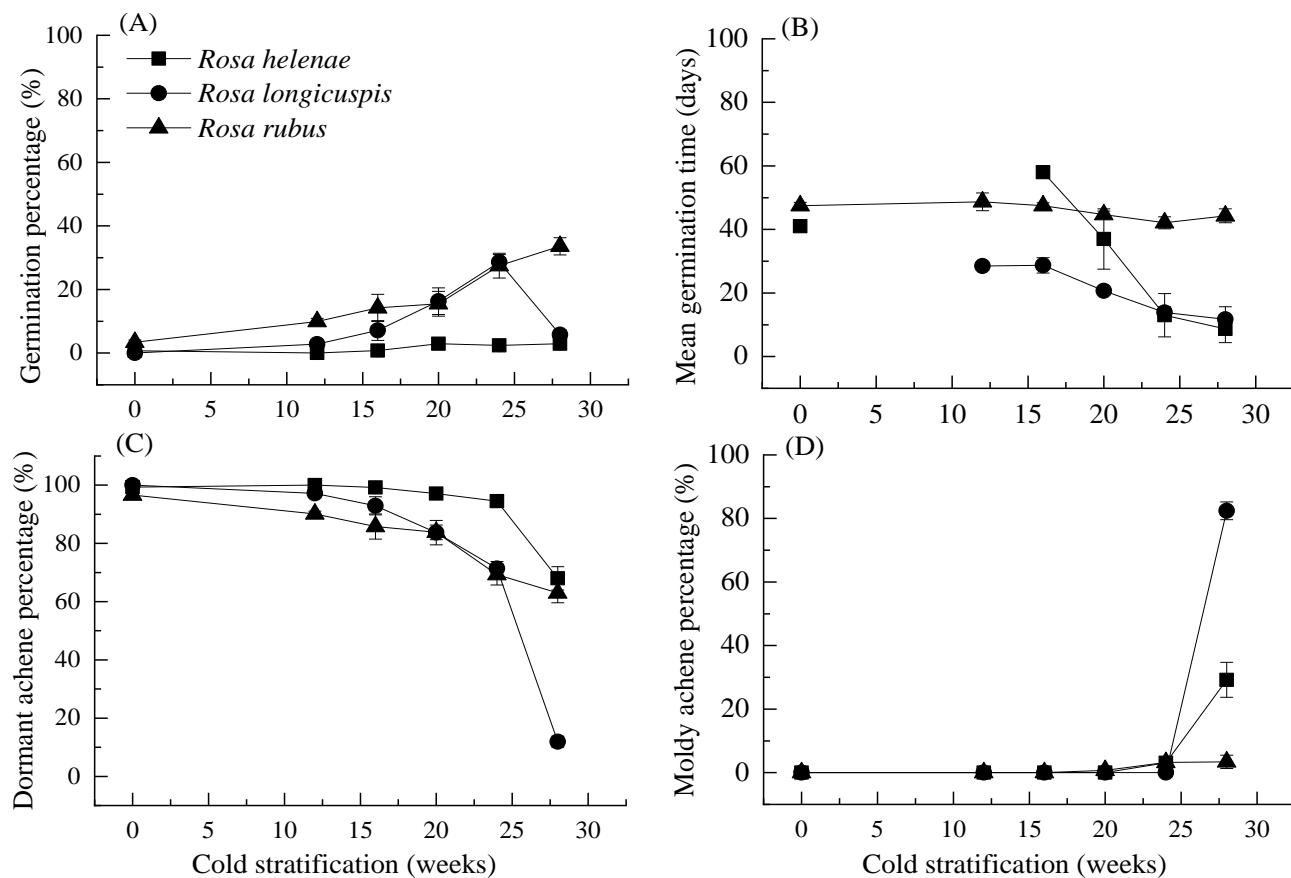
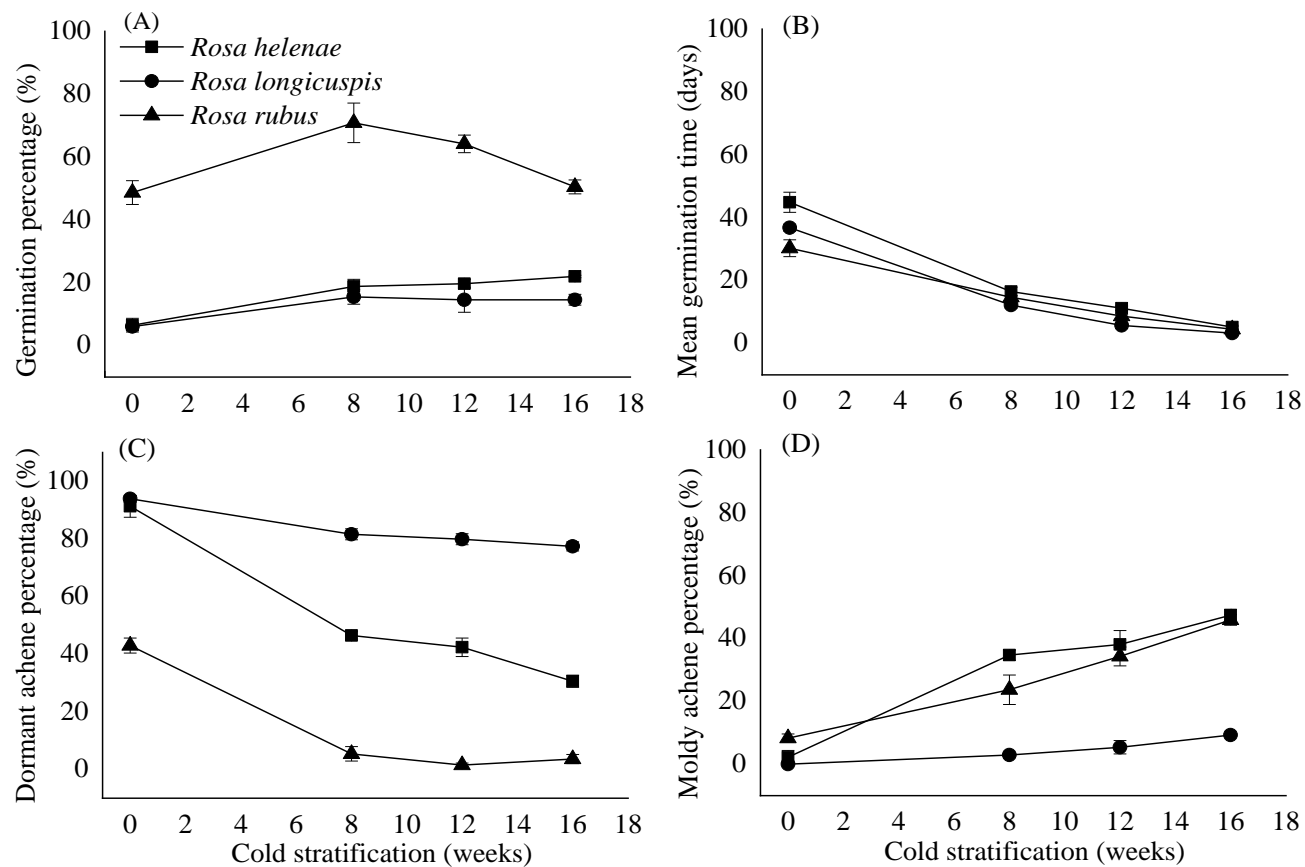Fig. 3. Germination parameters (mean ± se) of rose achenes pretreated cold stratification (0-28 weeks).

Fig. 4. Germination parameters (mean ± se) of rose achenes pretreated with H_2SO_4 scarification combined with cold stratification (0-16 weeks).
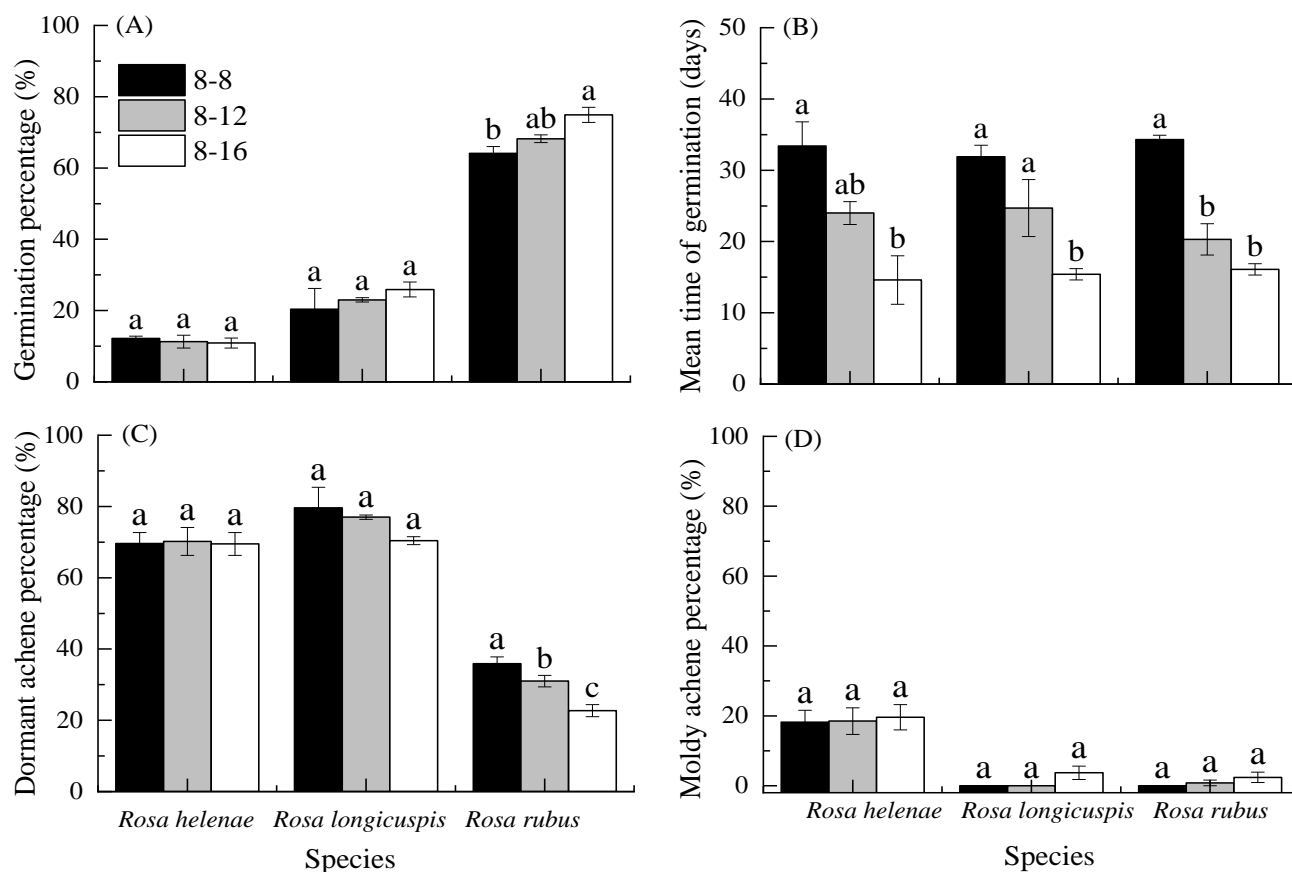
Fig. 5. Germination parameters (mean ± se) of rose achenes pretreated with warm plus cold stratification. Different letters within the same species indicate significant differences (LSD test, *p*<0.05).
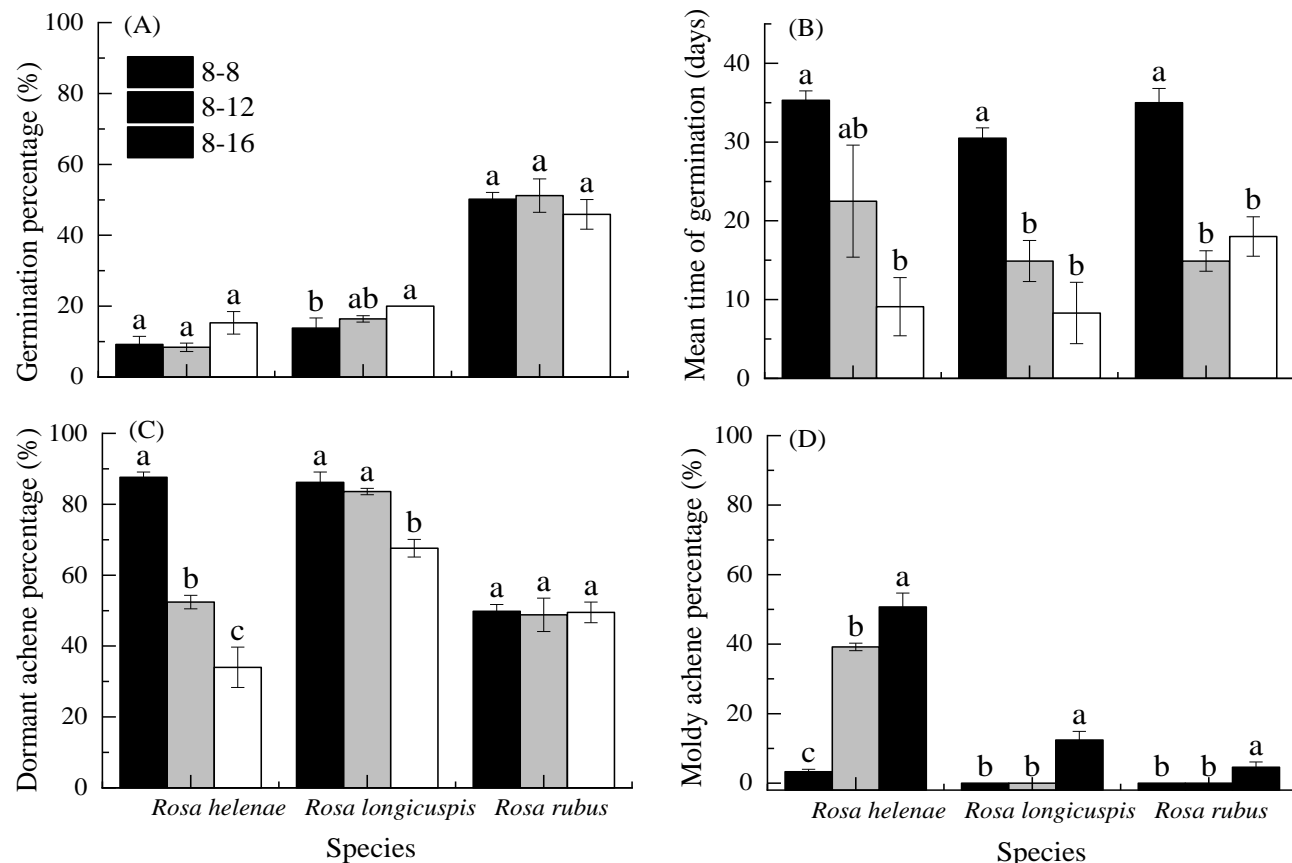

Fig. 6. Germination parameters (mean ± SE) of achenes pretreated with warm plus cold stratification with microbial nutrition. Different letters within the same species indicate significant differences (LSD test, *p*<0.05).

**Table 5. ANOVA table for germination parameters of *Rosa helenae*, *Rosa longicuspis* and *Rosa rubus*
exposed to different treatments.**

Treatment	Parameter	Source	df	Mean square	F	P
Cold stratification	GP	A: Species	2	1121.1	76.9	<0.001
		B: Period [a]	5	400.8	27.5	<0.001
		A * B	10	159.2	10.9	<0.001
	MGT	A: Species	2	2205.0	51.5	<0.001
		B: Period [a]	5	571.8	13.4	<0.001
		A * B	10	200.1	4.7	0.001
	DAP	A: Species	2	1338.1	75.9	<0.001
		B: Period [a]	5	3235.8	183.5	<0.001
		A * B	10	509.7	28.9	<0.001
	MAP	A: Species	2	729.5	100.2	<0.001
		B: Period [a]	5	2154.4	295.8	<0.001
		A * B	10	829.0	113.8	<0.001
H₂SO₄ scarification combined with cold stratification	GP	A: Species	2	7752.2	298.1	<0.001
		B: Period [a]	3	371.0	14.3	<0.001
		A * B	6	88.1	3.4	0.015
	MGT	A: Species	2	97.5	15.3	<0.001
		B: Period [a]	3	1945.3	305.9	<0.001
		A * B	6	34.2	5.4	0.001
	DAP	A: Species	2	14712.8	1021.4	<0.001
		B: Period [a]	3	2848.6	197.8	<0.001
		A * B	6	308.2	21.4	<0.001
	MAP	A: Species	2	2505.7	141.1	<0.001
		B: Period [a]	3	1515.0	85.3	<0.001
		A * B	6	230.5	13.0	<0.001
Warm plus cold stratification	GP	A: Species	2	8349.0	474.0	<0.001
		B: Regime [b]	2	57.1	3.2	0.063
		A * B	4	28.3	1.6	0.216
	MGT	A: Species	2	0.56	0.03	0.967
		B: Regime [b]	2	718.45	42.79	<0.001
		A * B	4	11.38	0.68	0.616
	DAP	A: Species	2	5588.2	212.5	<0.001
		B: Regime [b]	2	133.9	5.1	0.018
		A * B	4	34.4	1.3	0.305
	MAP	A: Species	2	932.7	61.2	<0.001
		B: Regime [b]	2	16.8	1.1	0.353
		A * B	4	1.7	0.1	0.978
Warm plus cold stratification with microbial nutrition	GP	A: Species	2	3806.0	165.1	<0.001
		B: Regime [b]	2	17.1	0.7	0.489
		A * B	4	39.6	1.7	0.190
	MGT	A: Species	2	63.3	1.9	0.179
		B: Regime [b]	2	1152.8	34.6	<0.001
		A * B	4	51.6	1.5	0.231
	DAP	A: Species	2	2114.0	71.8	<0.001
		B: Regime [b]	2	1318.8	44.8	<0.001
		A * B	4	605.4	20.5	<0.001
	MAP	A: Species	2	2406.1	276.0	<0.001
		B: Regime [b]	2	1041.5	119.5	<0.001
		A * B	4	484.5	55.6	<0.001
H₂SO₄ scarification combined with warm plus cold stratification	GP	A: Species	4	4160.7	113.4	<0.001
		B: Regime [b]	2	4.6	0.1	0.883
		A * B	2	105.3	2.9	0.053
	MGT	A: Species	4	56.2	2.8	0.085
		B: Regime [b]	2	364.2	18.4	<0.001
		A * B	2	31.3	1.6	0.223
	DAP	A: Species	4	1405.9	186.3	<0.001
		B: Regime [b]	2	4024.0	533.3	<0.001
		A * B	2	1354.6	179.5	<0.001
	MAP	A: Species	4	1353.2	34.8	<0.001
		B: Regime [b]	2	3757.5	96.7	<0.001
		A * B	2	1670.3	43.0	<0.001

Note: "Period" means period of cold stratification in treatment of cold stratification: 12, 16, 20, 24 and 28 weeks and in treatment of H₂SO₄ scarification combined with cold stratification: 8, 12 and16 weeks; b "Regime" means patterns of warm plus cold stratification
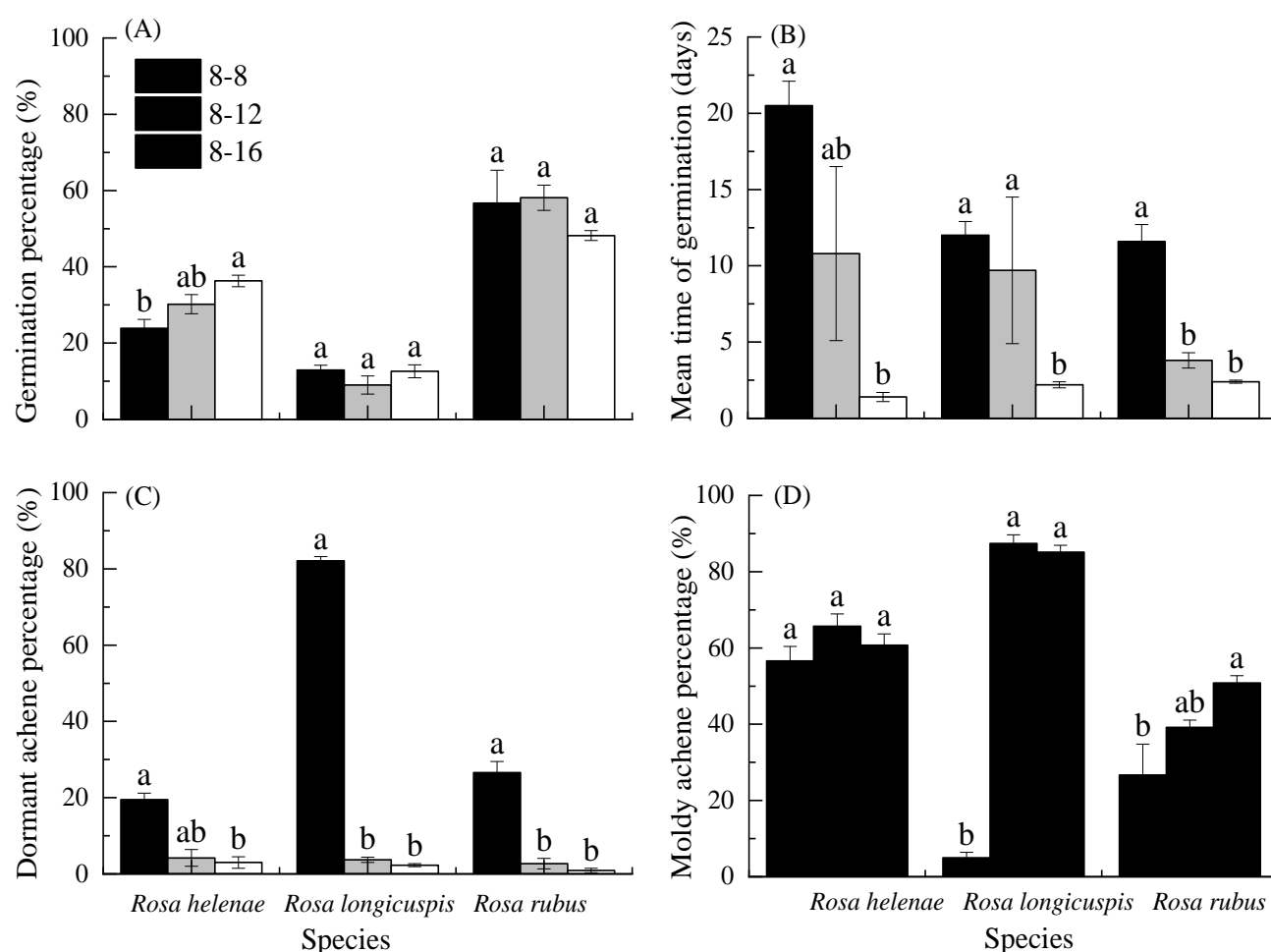
Fig. 7. Germination parameters (mean ± SE) of rose achenes pretreated with H_2SO_4 scarification combined with warm plus cold stratification. Different letters within the same species indicate significant differences (LSD test, $p<0.05$).

Pericarp plays an important role in regulating rose achene dormancy for providing the mechanical constraint, and the prevention of imbibition of water or air (Zhou & Bao, 2011). The pericarps of rose achenes in our study were permeable based on the absorption curve of intact and scarified achenes (Fig. 1). Zhou *et al.*, (2009) for *Rosa multibracteata* and Zhu *et al.*, (2013) for *Rosa platyacantha* also found that intact rose achenes can imbibe water and the percentage increase of water in achene mass was greater than 20%. Both mechanical scarification and H_2SO_4 scarification significantly improved GP (Fig. 2), which indicates that the pericarp provided a mechanical barrier for embryo growth (Zhou & Bao, 2011; Zhu *et al.*, 2013). Moreover, pericarp thickness was closely related with the level of the achene dormancy. In the present study, *R. helenae* achenes with the thickest pericarp had the strongest dormancy, and *R. rubus* with thinnest pericarp had the weakest achene dormancy. Positive correlation of achene dormancy and pericarp thickness was observed in *Rosa multibracteata* achenes collected along an elevational gradient in the eastern Tibetan Plateau (Zhou & Bao, 2014).

However, pericarp was not the sole factor and other factors play important role in dormancy-release since achenes germinated less than 15% when treated with mechanical scarification or H_2SO_4 scarification (Fig. 2). The H_2SO_4 scarification has only improved germination of a few rose species (Bhanuprakash *et al.*, 2004; Zhou & He,

2020.), and it had no effect for most of rose species (Zhou & Bao, 2011; Conev & Sandhu, 2015). Furtherly, the greater efficiency of combination treatments in breaking dormancy (Figs. 4, 5, 7) indicates the key role of embryo in regulating dormancy break and persistence for rose achenes.

Conclusion

Rose achenes of the examined three species had physiological dormancy. *R. rubus* likely has intermediate physiological dormancy and *R. helenae* and *R. longicuspis* have deep physiological dormancy. The most effective treatment for promoting germination for *R. rubus* (GP=74.9±2.1%) was 8-week warm stratification followed by 16-week cold stratification. Best treatment for *R. helenae* (GP=36.3±1.5%) was H_2SO_4 scarification combined with 8-week warm stratification and 16-week cold stratification; and for *R. longicuspis* (GP = 28.6±2.4%), it was 24-week cold stratification. The physiological dormancy of rose achenes was caused jointly by inhibitory substances in pulp and achenes, mechanical barrier of thick pericarp and physiological dormancy in embryo. Our findings will enable horticulturalists and seed ecologists to obtain rose seedlings effectively and thus provide a useful reference to horticultural industry and revegetation of degraded ecosystem using native species.

Acknowledgements

References

Anonymous. 2010. In: Miller, A.L. (Ed.), *Tetrazolium Testing Handbook*. Association of official seed analysts, USA.

Baskin, C.C. and J.M. Baskin. 2014. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*. 2th ed. Academic Press, USA.

Bhanuprakash, K., Y. Tejaswini, H.S. Yogeesha and L.B. Naik. 2004. Effect of scarification and gibberellic acid on breaking dormancy of rose seeds. *Seed Res.*, 32: 105-107.

Bo, J., H. Dong and X. Yang. 1995. Shortening hybridization breeding cycle of rose-a study on mechanisms controlling achene dormancy. *Acta Hort.*, 404: 40-47.

Chen, R., H. Wu, Y. Zhou, Q. Ren, X. Wang and H. Zhou. 2013. The research on germination test of 7 kinds of *Rosa* seeds. *Seed*, 32(03): 72-74.

Conev, R. and P. Sandhu. 2015. Effect of achene coat scarification, maceration and stratification on seed germination in cold hardy garden roses. *Act. Hort.*, 1064: 247-251.

Fatemeh, A., T. Masoud, A. Pejman and H. Aidin. 2014. Effect of plant growth promoting rhizobacteria (PGPRs) and stratification on germination traits of *Crataegus pseudoheterophylla* Pojark seeds. *Sci. Hort.*, 172: 61-67.

Han, Q., Z. Wang, Y. Zheng, H. Yang, F. Zeng, Y. Li, H.Zhao, S. Zhao and H. Liu. 2012. Effects of microbial nutrition on seed germination of *Rosa omeienisis*. *J. Northeast For. Univ.*, 40(11): 53-55.

Kazaz, S., S. Erba and H. Baydar. 2010. Breaking seed dormancy in oil rose (*Rosa damascena* Mill.) by microbial inoculation. *Afr. J. Biotechnol.*, 9(39): 6503-6508.

Kim, D.H. 2019. Practical methods for rapid seed germination from seed coat-imposed dormancy of *Prunus yedoensis*. *Sci. Hort.*, 243: 451-456.

Li, T. and X. Min. 2020. Dormancy characteristics and germination requirements of *Phoebe bournei* seed. *Sci. Hort.*, 260. doi:10.1016/j.scienta.2019.108903.

Morpeth, D.R. and A.M. Hall. 2000. Microbial enhancement of seed germination in *Rosa corymbifera* 'Laxa'. *Seed Sci. Res.*, 10(4): 489-494.

Pati, P.K., S.P. Rath, M. Sharma, A. Sood and P.S. Ahuja. 2006. *In vitro* propagation of rose—a review. *Biotechnol. Adv.*, 24: 94-114.

Rizwan, S. and F. Aftab. 2018. Effect of different pretreatments on breaking seed dormancy and *In vitro* germination in *Jatropha curcas* L.. *Pak. J. Bot.*, 50(2): 605-608.

Sandhu, P. and R. Conev. 2016. Effect of various stratification regimes on seed germination in cold-hardy garden roses. *Act. Hort.*, 1127: 231-236.

Tang, Y., K. Zhang, Y. Zhang and J. Tao. 2019. Dormancy-breaking and germination requirements for seeds of *Sorbus alnifolia* (Siebold & Zucc.) K.Koch (Rosaceae), a mesic forest tree with high ornamental potential. *Forests*, 10: 1-12.

Wawrzyniak, M.K., M. Michalak and P. Chmielarz. 2020. Effect of different conditions of storage on seed viability and seedling growth of six European wild fruit woody plants. *Ann. For. Sci.*, 77: DOI: 10.1007/s13595-020-00963-z

Wu, Z., P. Raven and D. Hong. 2005. *Flora of China*, Vol: 9. Science Press, China and Missouri Botanical Garden Press, USA.

Yang, F., F. Yang and R. Guo. 2017. Natural germination of eight Xinjiang wild rose seeds under introduction conditions. *Nort. Hort.*, 13: 108-112.

Yu, G.R. 2019. *Process and Dynamic of Forest Ecosystem*. Higher Education Press, China.

Zhou Z.Q. and Q.H. He. 2020. Geographic variation of seed dormancy and germination of *Rosa soulieana* in the dry valleys of the Hengduan mountain region. *Acta Ecol. Sin.* 40(17): 6037-6045.

Zhou, Z. and W. Bao. 2014. Changes in seed dormancy of *Rosa multibracteata* Hemsl. & E. H. Wilson with increasing elevation in an arid valley in the eastern Tibetan Plateau. *Ecol. Res.*, 29: 693-700.

Zhou, Z.Q. and W.K. Bao. 2009. Dormancy and breaking dormancy methods of rose seeds. *J. Trop. Subt. Bot.*, 17(06): 621-628.

Zhou, Z.Q. and W.K. Bao. 2011. Levels of physiological dormancy and methods for improving seed germination of four rose species. *Sci. Hort.*, 129(4): 818-824.

Zhou, Z.Q., N. Wu, W.K. Bao and P.F. Qiu. 2008. Post-dispersal factors regulating dormancy and germination of *Rosa soulieana* seeds. *Belg. J. Bo.*, 141(1): 103-111.

Zhou, Z.Q., W.K. Bao and N. Wu. 2009. Dormancy and germination in *Rosa multibracteata* Hemsl. & E. H. Wilson. *Sci. Hort.*, 119(4): 434-441.

Zhu, X.H., Q. Wang and S.P. Cheng. 2013. Study on seed dormancy mechanism of *Rosa multiflora*. *Shangdong Agri. Sci.*, 45(10): 59-62.

Zlesak, D.C. 2005. The effects of short-term drying on seed germination in *Rosa*. *HortScience*, 40(6): 1931-1932.