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Methods for breaking the dormancy of eastern redbud (*Cercis canadensis*) seeds

S. LI, T. SHI, F. KONG, Q. MA, Y. MAO AND T. YIN*

The National Southern Tree Seeds Inspection Center, Nanjing Forestry University, 159# Longpan Road, Nanjing, 210037, China (E-mail: tmyin@njfu.com.cn)

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Summary

Cercis canadensis is best propagated by using seeds, but the seed dormancy is hard to break. In this paper, we studied the physical and physiological barriers responsible for dormancy of *C. canadensis* seeds. Results showed that both seed coat and inhibiting substances in the endosperm played a role in dormancy of *C. canadensis* seeds. Consequently, we evaluated the effects of thermal and chemical scarification, cold and warm stratification, splitting the cotyledon end of the testa, and soaking in gibberellic acid in breaking seed dormancy. All the treatments had a positive effect on seed germination. The best method to break seed dormancy was to immerse seeds in sulphuric acid (98%) for 11-13 minutes, following cold stratification at 4°C for 14 days and soaking in 500 mg l^{-1} GA_3 for 24 hours.

Introduction

Eastern redbud (*Cercis canadensis* L.), a member of the *Caesalpinioideae* family, is native to the east of North America, distributed from southern Ontario to northern Florida in the United States, and in the south of Canada (Flint, 1997). *C. canadensis* is widely used in landscaping because of the showy flowers in the early spring and the beautiful heart-shape leaves (Young and Young, 1992). New leaves of *C. canadensis* are light green, which darken with time and finally turn yellow in autumn (Edward and Dennis, 2011). Besides its ornamental role, its flower is edible and it is also an important nectar plant. Furthermore, its bark has been used as an astringent in the treatment of dysentery (Hamel and Chiltoskey, 1975).

Recently, *C. canadensis* has been widely introduced and planted in other countries. It is best propagated with seeds, but seed dormancy causes problems: long germination duration, low germination percentage and poor germination uniformity. Hamilton and Carpenter (1975), Hu *et al*. (2009) and Merou *et al*. (2011) reported that seed dormancy of *C. canadensis* mainly results from the hard seed coat that prevents water absorption. *C. canadensis* seeds also exhibit physiological dormancy (Afanasiev, 1944; Frett and Dirr, 1979; Murat *et al.*, 2010). However, the underlying causes remain debated.

* Author for correspondence

In this study, our objectives were to explore the causes for seed dormancy in *C. canadensis* and to establish appropriate methods to break dormancy.

Materials and methods

Source of seeds
Seeds of *C. canadensis* were imported by Lovelace Seed Company in March, 2012. Seeds were collected in November 2011 from Elsberry, Missouri, USA.

Exploring the causes of seed dormancy
Two experiments were made to determine the causes of seed dormancy. Experiment I was done using excised embryos. First, seeds were split with a scalpel at the cotyledon end and placed on wet cotton to absorb water for 30 hours. After that, seed coats and endosperms were removed completely and embryos were put on wet absorbent cotton at 25°C with an 8-hour light period. During the culturing time, seeds were watered every two days to maintain the moisture level. Germination was monitored every other day for 12 days. Finally, germination percentage of the excised embryos was calculated. Experiment II was carried out with coatless seeds and the germination conditions were the same as described above. In both experiments there were four replicates of 50 seeds each.

Giberellin treatment after thermal scarification
In this experiment, we tried the following thermal scarification pre-treatments. Treatment 1: seeds were immersed in water at an initial temperature of 80°C and allowed to cool gradually at room temperature for 12 hours; this was repeated for the remaining hard seeds until all the seeds were imbibed. The imbibed seeds were then immersed in 500 mg l^{-1} or 800 mg l^{-1} gibberellic acid (GA_3) for 12 or 24 hours at room temperature. A control treatment comprised imbibed seeds without soaking in GA_3. Treatment 2: seeds were treated as described above, except that the remaining hard seeds were immersed in hot water with initial temperature of 70°C. Treatment 3: seeds were soaked twice in water with initial temperature of 40°C cooling gradually at room temperature for 12 hours, and then stratified at 4°C (cold stratification) or 25°C (warm stratification) for 14 or 21 days. After stratification, seeds were immersed in 500 mg l^{-1} GA_3 for 12 or 24 hours.

Splitting the testa and giberellin treatment
Seed testas were split at the cotyledon ends and then placed on wet cotton to absorb water for 30 hours. After that, seeds were immersed in 500 mg l^{-1} GA_3 for 12 or 24 hours. A control test was conducted without GA_3 immersion.

Sulphuric acid treatment, cold stratification and soaking in giberellin
Seeds were soaked in concentrated sulphuric acid (98%) for 7, 9, 11, 13 and 15 minutes, periodically stirring the solutions with a glass rod during the treatment. After chemical scarification, seeds were washed in running water for 24 hours. Seeds were then sown into plastic boxes with moistened sand and stored in a refrigerator at 4°C for 7 or 14 days.

After cold stratification, seeds were removed from the sand and soaked in 500 mg l^{-1} GA_3 for 24 hours.

Seed germination

Germination tests were carried out with four replicates of 50 seeds each. Seeds were germinated in moistened sand at 25°C with eight hours light per day. Germinated seeds were counted every other day for 20 days. During the germination test, water was added to keep the seeds moist. Normal seedlings, abnormal seedlings (seedlings with missing parts, lesions or other damage, and which will not develop into a complete plant under normal conditions), hard seeds (seeds that are not able to imbibe water under the suitable conditions), fresh seeds (seeds that are able to imbibe water and appear fresh at the end of the germination test) and dead seeds (seeds that are usually soft, discoloured, frequently mouldy and show no sign of seedling development at the end of the germination test) were evaluated following the rules of International Seed Testing Association (ISTA, 2012 edition, https://www.seedtest.org/.../OM11-05ProposedChangestotheISTAInternationalRulesforSeedTesting2012Edition).

Statistical analysis

A completely randomised design (Dimas *et al*., 2010) was used in all experiments. The effect of the treatments was assessed by the final germination for each trial. The significance of mean differences was determined using the least significant difference (LSD) test with SPSS v19.0 (http://support.spss.com/). Germination percentage was calculated as number of normal seedlings as a proportion of total number of seeds sown.

Results

Analysing causes for seed dormancy

After the excised embryos were cultured for three days, the cotyledons started to grow and turn green. Seven days later, the cotyledons were fully spread, dark green with elongated hypocotyls (normal seedlings). At 12 days, 93% of excised embryos had germinated (data not shown). Therefore, there was no dormancy for the excised embryos. By contrast, only 37.0% of the de-coated seeds germinated after the same length of time. The de-coated seeds began to germinate after eight days (five days later than the excised embryos) and germination was also less uniform.

Effects of thermal scarification and giberellin treatment

Soaking seeds in hot water resulted in imbibition. However, without GA_3, only 9.5% germination was reached (table 1), which is far lower than that of excised embryos (93.0%). By contrast, germination was up to 70.5% after soaking in GA_3 (treatment 2). When treated with the same GA_3 concentration for longer, germination improved a little, but not significantly. It is noticeable that germination of seeds treated with 500 mg l^{-1} GA_3 was significantly higher than that of seeds treated with 800 mg l^{-1} GA_3 (table 1). Therefore, in the following series of experiments, seeds were only treated with 500 mg l^{-1} GA_3.

Table 1. Effects of thermal scarification, stratification and gibberellin treatment on dormancy break of *Cercis canadensis* seeds.

Treatment	Thermal scarification	Stratification	GA_3 concentration (mg l^{-1})	Duration of GA_3 treatment (hours)	Germination (%)	Fresh ungerminated seeds (%)	Hard seeds (%)	Viability (%)
			0	0	9.5e	65.3a	0	74.8
1	80°C + cooled, repeated until imbibed	No	500	12	55.5c	17.1bc	0	72.6
				24	58.2bc	14.8bc	0	73.0
			800	12	44.9d	20.0b	0	64.9
				24	49.8cd	17.0bc	0	66.8
2	80°C + cooled, then 70°C + cooled until imbibed	No	500	12	66.4ab	14.1bc	0	80.5
				24	70.5a	13.4c	0	83.9
3	40°C, following 40°C	0 days	500	24	5.2d	1.3d	91.3a	97.8
		4°C 14 days	500	12	16.0bc	10.3bc	65.0b	91.3
			500	24	19.5b	12.1abc	61.6cd	93.2
		4°C 21 days	500	12	25.9a	13.2ab	42.4e	81.5
			500	24	26.8a	7.3cd	50.7ef	84.8
		25°C 14 days	500	12	12.7cd	10.3bc	59.2cd	82.2
			500	24	13.1cd	9.1bc	56.6de	78.8
		25°C 21 days	500	12	14.9bcd	9.2bc	50.8ef	74.9
			500	24	17.6bc	16.1a	48.7f	82.4

The letters following the numbers indicate whether there is significant difference by the LSD test. Numbers followed by different letters refer to significant difference at $P = 0.05$.

Comparing the results of treatments 1 and 2, germination was higher in treatment 2 than in treatment 1 and the dead seeds percentage was significantly lower in treatment 2 than in treatment 1 (table 1). Furthermore, all seed viabilities were lower than that of the excised embryos. Soaking seeds at lower temperature (40°C) did not make seeds permeable and relatively low germination was obtained (the highest mean germination is 26.8%; table 1).

By soaking seeds in water at 40°C for 24 hours, coupled with stratification and GA_3 treatment, the highest germination (26.8%) was obtained by cold stratification for 21 days and soaking in 500 mg l^{-1} GA_3 for 24 hours. By contrast, this value was only 5.2% for the control seeds that did not undergo any stratification treatment. Stratification was found to significantly reduce the percentage of hard seed, from 91.3% (control seeds that did not undergo any stratification treatment) to 42.4% (cold stratification for 21 days). Because stratification has negative effects on seed viability (dead seed percentage is 2.2% for seeds without stratification treatment, while it is 25.1% after 21 days warm stratification), the prolonged stratification (more than 21 days) did not proceed in this study.

Effect of splitting the seed at the cotyledon end followed by gibberellin treatment

Germination was only 14.5% for split seeds of *C. canadensis* without GA_3 treatment. By contrast, seed germination after GA_3 treatment increased significantly (up to 83.1%). In the aforementioned tests, results showed that germination was higher for seeds soaked in GA_3 for 24 hours than those soaked in GA_3 for 12 hours. So duration of gibberellins treatment was adjusted to 24 hours in the following experiments.

Effect of sulphuric scarification combined with cold stratification and gibberellin treatment

Sulphuric acid treatment, stratification and gibberellins soaking greatly affected the germination rate (table 2). After treatment with sulphuric acid for at least nine minutes, nearly all the seeds were able to imbibe and germinate. The viability of seeds exposed to sulphuric acid no longer than 13 minutes was nearly the same as that of the isolated embryos. Mildew appeared in the sand substrate during seed germination when seeds were immersed in acid for more than 15 minutes. This may be caused by acid damage to seeds.

Longer stratification in sulphuric acid improved germination a little, but not significantly. Maximum germination was about 77%, which was attained by immersing seeds in sulphuric acid for 13 minutes following cold stratification for seven days and soaking in 500 mg l^{-1} GA_3 for 24 hours, or immersing seeds in sulphuric acid for 11-13 minutes following cold stratification for 14 days and soaking in 500 mg l^{-1} GA_3 for 24 hours. When seeds were treated with the above protocol, nearly all the hard seededness was broken (remaining portion was less than 4.7%) and viability was maintained over 87.5%, which is very close to that of the isolated embryos (93.0%).

Table 2. Effect of sulphuric acid treatment combined with cold stratification and gibberellins soaking on seed germination.

Stratification duration (days)	Scarification with H_2SO_4 (minutes)	Germination (%)	Fresh ungerminated seeds (%)	Hard seeds (%)	Viability (%)
7	7	48.8e	11.5bc	32.0a	92.3
	9	69.6bcd	7.7cd	15.0c	92.3
	11	71.1abc	15.0ab	5.7d	91.8
	13	77.0ab	9.8c	3.6de	90.3
	15	62.4c	12.1bc	2.5de	76.9
14	7	51.0 e	16.9a	26.1b	94.0
	9	70.8abc	12.3abc	6.1d	89.2
	11	77.2ab	8.8cd	4.7d	90.7
	13	77.7a	8.8cd	1.0e	87.5
	15	64.3dc	4.5d	1.0e	69.7

The letters following the numbers indicate whether there is significant difference by the LSD test. Numbers followed by different letters refer to significant difference at $P = 0.05$.

Discussion

The causes of C. canadensis seed dormancy

Previous studies have revealed that the seed coat of legumes plays a major role in inhibiting seed germination. For seed of *Bauhinia racemosa* Lam., seed coat was the mechanical barrier to radicle emergence, and when seed coats were treated to be permeable, seeds germinated (Prasad and Nautiyal, 1996). In our study, de-coated seeds of *C. canadensis* still gave relatively low germination percentage. However, when endosperms were removed or treated with GA_3, seeds could achieve high germination percentage. These results indicate that there are inhibiting substances in the endosperm. Therefore, seeds of *C. canadensis* fall into the combinational dormancy category according to the seed dormancy classification of Baskin and Baskin (2004). Seeds of *C. canadensis* showed both physical and physiological dormancy. The conclusion is in agreement with studies by Geneve (1991) and Jones and Geneve (1995). However, in contrast with the results reported in those publications, no dormancy was found in the excised embryos in our study, while Hamilton and Carpenter (1975) reported similar findings to ours regarding excised embryos. The observed differences might be due to different seed sources but the precise causes remain unclear and further study is needed.

Methods to break seed dormancy

Thermal scarification is a good way to break hard seed dormancy (Fu *et al.*, 2001; Choudhury *et al*, 2009; Merou *et al.*, 2011). In this study, results showed that thermal

scarification also worked for seeds of *C. canadensis*. However, it would consequently lead to a high proportion of seed death (more than 16.1% at the last day of germination; table 1). This problem can be partially resolved by decreasing the starting temperature in the succeeding cycle of hot water treatment.

Stratification is also an effective way to increase permeability and it is regarded as an important method to break physical dormancy (Roy, 1974; Murat *et al.*, 2010; Ertekin, 2010). In this study, both warm and cold stratification were conducted with seeds of *C. canadensis;* cold stratification was found to be better than warm stratification. With different stratification treatments, the highest germination was only 26.8%, and there remained a high proportion of hard seeds (≥ 42.4%; table 1). Therefore, our study showed that stratification alone could not effectively break the seed dormancy. In our study, it was found that percentage of dead seeds would increase significantly if the stratification duration was prolonged. This result differed from studies by Afanasiev (1944), Frett and Dirr (1979) and Geneve (1991). Those authors reported that seeds would germinate completely without loss of viability after sufficient duration of cold stratification. The observed difference might also relate to the difference in seed sources. *C. canadensis* has a wide geographic distribution and it is suggested that seed dormancy-breaking requirements may vary among different seed provenances (Geneve, 1991).

Acid scarification is very effective in softening impermeable seeds (Fu *et al.*, 1996; Murat *et al.*, 2010). Our study showed that treatments involving soaking in acid for 11 to 13 minutes followed by 7 to 14 days stratification were effective in breaking hard seeds of *C. canadensis.* It has been recommended to scarify *C. canadensis* seeds with sulphuric acid for 30 minutes (Roy, 1974; Geneve, 1991), much longer than we attempted in our study; according to our results, acid treatment should be less than 15 minutes. Again the optimum duration for acid treatment may vary among different seed sources.

Splitting the cotyledon end of the testa can be used to make seeds permeable for experimental purposes (Geneve, 1991; Fu *et al.*, 2001). In this study, hard seeds decreased to zero by this method. Combined with soaking in GA_3, seed germination is higher than all the aforementioned methods. Thus, this method combined with soaking in GA_3 is suitable for laboratory seed quality inspection.

To conclude, it is necessary to make the seed coat permeable to break the seed dormancy of *C. canadensis*. Meanwhile, GA_3 treatment is also required to overcome the endo-dormancy. Although we established methods to attain high seed germination percentage, they all have a negative effect on viability. For a particular seed lot, thickness of seed coats varies among the seeds. All measures to make seed coats permeable would cause death to some of the seeds. There is a delicate balance between viability and germination percentage when taking measures to break *C. canadensis* seed dormancy. According to ISTA rules (2012 edition, https://www.seedtest.org/.../OM11-05ProposedChangestotheISTAInternationalRulesforSeedTesting2012Edition), fresh seed at the end of a germination test should not exceed 5%. This criterion was not met in this study; further work is still required. Nevertheless, this study provided desirable information for better understanding the causes of seed dormancy and the requirements for dormancy breaking of *C. canadensis* seeds.

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