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Seed Dormancy in Eastern Redbud (Cercis *canadensis)*

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Abstract. **Seed dormancy in Eastern redbud *(Cercis canadensis* var. canadensis L.) can be overcome by seedcoat scarification to allow water imbibition, followed by chilling stratification to permit germination. During chilling stratification, there was an increase in the growth potential of the embryo as indicated by the ability of the isolated embryo to germinate in osmotic solutions. Penetration resistance of the testa also decreased after chilling stratification. The combination of seedcoat alteration and the increase in embryonic growth potential was associated with overcoming dormancy in redbud seed. GA_3 or ethephon (50 µM) stimulated germination (28% and 60%, respectively) and increased the growth potential of treated embryos. Chemical names used: gibberellic acid (GA_3), (2-chloroethyl) phosphoric acid (ethephon).**

Eastern redbud is a small tree native to eastern North America. It is propagated from seed by the nursery industry and cultivar selections are commonly grafted. Redbud is a common landscape tree selected for the attractive flowers that appear before the foliage emerges in the spring. Redbud is a woody legume with a hard seedcoat that is impermeable to water. However, unlike most other temperate woody legume species, redbud seed appears to require a cold stratification period to satisfy dormancy conditions. Roy (1974) suggested that seeds of redbud have an internal dormancy requiring 60 days of stratification at 5C for germination. It is generally recommended that seed should be treated with. concentrated H_2SO_4 for 30 rein, followed by moist, cold stratification for 6 to 8 weeks (Dirr and Heuser, 1989; Roy, 1974).

Experimental evidence to support these recommendations were provided by Afanasiev (1944) and reaffirmed by Frett and Dirr (1979). Both studies showed that germination percent increased with increased duration of cold stratification. In contrast, a study by-Hamilton and Carpenter (1975) provided evidence that seed dormancy in redbud was only controlled by the hard tests. In their study, germination proceeded after scarification with no requirement for cold stratification. They also found no naturally occurring germination inhibitors or promoters in either the testa or embryo. Redbud has a wide geographic distribution in eastern North America and several authors have suggested that dormancy-breaking requirements vary between seed sources (Fordham, 1967; Heit, 1967). Afanasiev (1944) showed that the response of redbud seed to stratification varied between seedlots, but most of the seedlots tested showed > 90% germination after 5 to 7 weeks of chilling stratification.

This study was initiated tore-investigate the mechanisms controlling seed dormancy in redbud. This study identifies a requirement for cold stratification for redbud seed germination and reports on the physical changes that occur in the testa and embryo during cold or warm stratification that is related to dormancy release in redbud seed.

Materials and Methods

Seed of redbud were collected from a single tree (≈15 years old) on the Univ. of Kentucky, Lexington campus. Seeds were stored dry in a closed Mason jar at 5C before use. All seeds were scarified with concentrated H_2SO_4 for 30 min, rinsed in double-distilled water, and then allowed to imbibe water or growth regulator solutions of GA_3, ethephon, or abscisic acid (ABA) at 50 µM for 24 h at room temperature. After imbibition, the seeds were surface-disinfected with 0.5% sodium hypochlorite and 0.1 % Alconox detergent for 10 min with agitation followed by three rinses in autoclave deionized water. All subsequent procedures occurred under aseptic conditions. Ten seeds or excised embryos were placed in 10 cm disposable petri dishes on two pieces of Whatman #1 filter paper wetted with 4 ml autoclave deionized water. Each petri dish was sealed with parafilm. Stratification was at 5 or 25C in the dark, germination at 25 ± 3C in the dark. Data are presented as the means of 60 seeds or 30 embryos (10 embryos per petri dish) and each experiment was run at least twice.

Surgical treatments were performed on seeds imbibed in water for 24 h and consisted of fully excised embryos, seeds split along the embryonic axis at the cotyledon or radicle end, and seeds with half the tests removed. Seeds were split with a sharp scalpel blade through the seedcoat and endosperm. To remove half the testa, seeds were split at the cotyledon end and the tests pulled open with forceps along the flat edge of the seed. Twenty seeds were placed in each petri dish with the testa in contact with the moist filter paper or they were inverted with the exposed embryo in contact with the filter paper.

The force required to penetrate the testa was measured using an Instron Universal Testing Machine model 1122 (Instron, Boston). A 5-ml disposable plastic pipette was modified to hold a half-seed by making a tapering incision across the tip of the pipette. The half-seed was prepared by cutting the seed in half along an axis perpendicular to the embryo and the embryo was removed by applying gentle pressure to the base of the seed. A sewing needle was filed to a blunt tip approximating the diameter of the embryo radicle and inserted into a threaded 2.5-cm pipe fitted for the Instron. The Instron measured the force required for the needle to penetrate the radicle end of the testa with the load cell at 10-N full-scale load and a crosshead speed of 10 $cm \cdot min^{-1}$.

Growth potential, expressed as radicle length, was measured in isolated embryos pretreated with filter-sterilized water and 50 µM GA_3, ethephon, or ABA. Embryos were aseptically removed from the testa by splitting the seed at the cotyledon end with a scalpel, and pressure was gently applied to the base of the seed with forceps until the embryo was removed. Embryos

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Table 1. Radicle length (mm) and, in parentheses, percent germination of embryos isolated from redbud seed stratified for 60 days at 5 or 25C.

Stratification	No. days of germination[z] 1	2	3	4	Regression equation[y]	R^2
Control[x]	1.5 ± 0.1 (0)	1.8 ± 0.1 (0)	2.9 ± 0.5 (50)	4.5 ± 0.9 (70)	y = 0.15 + 1.01x	0.92
5C	1.0 ± 0.1 (55)	5.3 ± 0.6 (100)	8.7 ± 1.0 (100)	---	y = −1.37 + 3.35x	0.99
25C	1.6 ± 0.1 (0)	1.8 ± 0.1 (0)	2.0 ± 0.4 (20)	3.0 ± 0.8 (75)	y = 1.0 + 0.44x	0.83

[z]Values are the mean length of the radicle (mm) ±95% confidence interval followed by the germination percent in parentheses.
[y]y = Radicle length; x = days.
[x]Control embryos were isolated from seed imbibed for 48 h and that had not been stratified.

Table 2. Growth potential measured as radicle length (mm) and, in parentheses, percent germination of isolated embryos excised from chilling-stratified redbud seed.

PEG 4000 Molal	PEG 4000 MPa[y]	No. days of chilling stratification[z] 0	30	60
0	0	12.1 ± 1.2 (100)	14.5 ± 1.8 (100)	16.8 ± 1.0 (100)
0.02	−0.3	10.2 ± 1.2 (100)	12.2 ± 0.8 (100)	12.9 ± 1.3 (100)
0.04	−0.6	6.7 ± 0.6 (100)	8.4 ± 0.8 (100)	10.1 ± 0.8 (100)
0.06	−1.0	3.6 ± 0.4 (90)	4.6 ± 0.3 (100)	6.0 ± 0.6 (100)
0.08	−1.3	1.3 ± 0.09 (0)	1.9 ± 0.1 (65)	2.7 ± 0.2 (100)
0.10	−1.8	1.4 ± 0.09 (0)	1.3 ± 0.08 (0)	1.9 ± 0.05 (50)

	ANOVA F value	ANOVA Significance	Trend analysis Linear	Trend analysis Quadratic
Treatment	116.05	**		
PEG	374.21	**	**	NS
Stratification	35.58	**	**	NS
PEG × stratification	2.46	*		

[z]Values are the mean length of the radicle (mm) ± 95% confidence interval followed by the germination percent in parentheses after 4 days.
[y]MPa calculated from vapor-pressure deficit (Steuter et al., 1981).
[NS],*,**Nonsignificant or significant at $P = 0.05$ and 0.01, respectively.

were pretreated by splitting the cotyledon end of the testa from seeds imbibed in autoclave water for 24 h. Split seeds were then moved to filter-sterilized growth substance solutions and held there for an additional 24 h. Isolated embryos were placed in petri dishes with the filter paper moistened with 4 ml of autoclave deionized water, or polyethylene glycol (PEG 4000). Germination, defined as radicle growth >2 mm, and mean radicle length were measured after 4 days for isolated embryos or 7 days for intact seed.

Results and Discussion

Germination was compared in isolated embryos from seeds receiving no stratification or 60 days stratification at 5 or 25C (Table 1). The number of days to germination was less and radicle length was greater in the embryos from chilled seed. The release from dormancy in redbud seed increased the germination percentage and the growth potential of the embryonic axis (Tables 1 and 2). Growth potential represents the germinating force of an embryo as measured by radicle elongation in osmotic solutions (Baskin and Baskin, 1971; Junttila, 1973). Redbud embryos consistently showed increased growth potential after stratification at 5C, as indicated by increase in percent germination and mean radicle length on solutions of progressively more negative osmotic potential (Tables 1 and 2). The lowest growth potential was observed in the embryos from seed receiving warm (25C) stratification (Table 1). Embryos from warm-stratified seed remained viable for 1 year and continued to germinate after removal from the testa, but never germinated in intact seed (data not presented).

Embryo growth on solutions with progressively more negative water potentials suggests that embryos of redbud undergo osmotic adjustment during chilling stratification. Lower osmotic potential in the embryo would allow the axis to imbibe more water, generating a greater turgor pressure for cell expansion and radicle elongation. Embryos placed on a 0.08 -molal solution of PEG failed to germinate (radicle length < 2 mm) without chilling stratification, but exhibited 100% germination after 60 days of chilling (Table 2).

Osmotic adjustment could be the result of storage macromolecule metabolism to more osmotically active solutes. Afanasiev (1944) measured storage materials in the axis and testa of redbud and observed a marked decrease in storage fats and protein during chilling stratification, while embryo reducing sugars increased. These metabolic changes could account for the change in the osmotic potential observed in the present study.

Intact scarified seed without chilling stratification did not germinate. However, a high percentage of embryos isolated from the testa or seed with the testa split at the radicle end germinated (Table 3). This result suggests that the testa provided a physical barrier that the unchilled embryonic axis was unable to penetrate, thus preventing germination. Isolation of the embryo from the testa or reducing the resistance of the testa by splitting the testa at the radicle end released the axis for germination (Table 3). This process appears to be a physical release of the radicle

Table 3. Tests and growth regulator treatment relationships on germination of nonstratified redbud seed after 7 days.

Seedcoat treatment	Growth regulator		
	None	GA_3 (50 μM)	Ethephon (50 μM)
	Germination (%)[z]		
Intact seed	2 ± 1.7	28 ± 4.8	60 ± 4.5
Split at cotyledon	20 ± 2.6	50 ± 4.5	55 ± 2.2
Split at radicle	70 ± 5.2	61 ± 6.0	68 ± 6.0
Excised embryo	100	100	100

[z]Values are the mean germination percent ± SE.

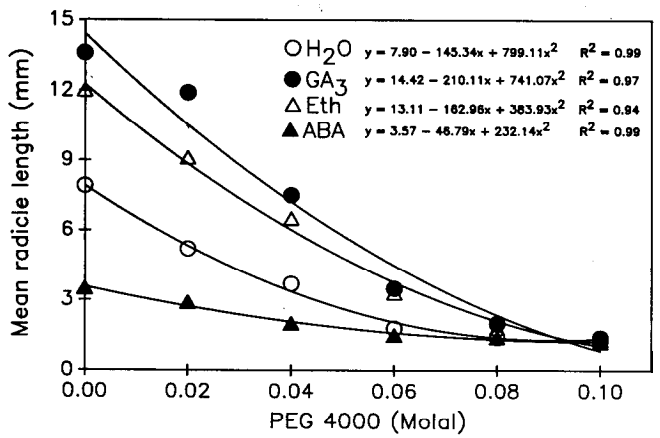

Fig. 1. Growth potential, expressed as radicle length, in embryos treated for 24 h with 50 μM GA_3, ethephon (ETH), or ABA before germination.

for germination and not merely a means allowing leakage of a germination inhibitor or increased opportunity for water uptake. This conclusion was based on the germination differences among intact seed and seeds split at the cotyledon or radicle end of the testa (Table 3). Also in support of this conclusion, the germination percentage was similar for seeds that had half the testa removed before having the exposed embryo (95% germination) or the testa (98% germination) placed in contact with the aqueous solution. In addition, bulk aqueous extraction of the testa or embryos failed to indicate the presence of an inhibitor, as indicated by germination bioassays with lettuce seed and isolated redbud embryos (data not presented).

Exogenous application of GA_3 or ethephon increased germination in nonchilled intact seed of redbud (Table 3). Ethephon has been previously reported to increase germination in dormant redbud seed (Hamilton, 1972). GA_3 and ethephon increased the growth potential of isolated embryos compared to untreated embryos (Fig. 1). The data suggest that the promotion of germination by GA_3 and ethephon in dormant redbud seed was related, at least in part, to a change in growth potential.

In contrast, ABA-treated embryos had a very low growth potential (Fig. 1). Endogenous ABA has been shown to affect water relations in *Brassica* seeds (Schopfer and Plachy, 1985). Endogermus levels or sensitivity to GA_3 and ABA could be involved in the change in growth potential associated with dormancy release in redbud seed.

The strength of the testa did not change in response to the duration of stratification (Table 4). However, the force required to penetrate the testa was lower in chilled than in nonchilled seed, possibly contributing to the release from dormancy of chilling-stratified seed. There was no difference in testa strength whether 3% or 95% of the seed germinated (Table 4). The physical resistance of the endosperm of pepper seed was directly related to the ability of the seed to germinate at various temperatures (Watkins and Cantliffe, 1983). Mechanical resistance of the endosperm in lilac seeds (Junttila, 1973) and the integuments of iris seed (Blumenthal et al., 1986) were considered a major factor in the maintenance of dormancy in these species.

The results of this study support previous observations (Afanasiev, 1944; Frett and Dirr, 1979; Hamilton, 1972) that redbud has a doubly dormant seed that requires chilling stratification to allow germination. However, the results contrast with those of Hamilton and Carpenter (1975), who concluded that redbud seed dormancy was imposed only by the hard testa and that chilling stratification was unnecessary for germination. They suggested that the double dormancy reported for redbud seed by other researchers was simply the effect of low temperature and water

Table 4. Effect of temperature and duration of stratification on force required to penetrate radicle end of the testa of redbud.

Stratification temp (°C)	No. days of stratification				
	0	14	28	42	56
	Penetration force (N)[z]				
25	2.63 ± 0.24	2.57 ± 0.18	2.46 ± 0.18	2.49 ± 0.09	2.71 ± 0.12
5	2.63 ± 0.24	2.46 ± 0.12	2.27 ± 0.13	2.19 ± 0.10	2.35 ± 0.11
	Trend analysis[y]		Orthogonal comparisons[x]		
	Linear	Quadratic			
	F value		F value		
25	0.18[NS]	5.95**	0.62[NS]		
5	17.02**	8.06**	17.77**		
	Germination (%)				
25	0	0	0	0	0
5	0	3	55	73	95

[z]Mean penetration force ± 95% confidence interval.
[y]Trend analysis for penetration force for stratification at 25 or 5C.
[x]Orthogonal comparison for penetration force of untreated seeds (0 days) compared with all stratification treatments.
NS,**Nonsignificant or significant at $P = 0.01$.

on weakening the testa to permit eventual germination. They felt that conditions within the embryo were not responsible for seed dormancy because embryos excised from the testa germinated promptly. The present study indicates that chilling stratification was necessary for germination of intact redbud seed. Chilling stratification increased the growth potential of the embryo, permitting the axis to penetrate the testa. The testa strength was also reduced after chilling stratification and may contribute to the release from seed dormancy. Growth regulator treatments that overcame dormancy in nonchilled dormant seed also increased the growth potential of embryos.

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