

Combinational dormancy in seeds of *Sicyos angulatus* (Cucurbitaceae, tribe Sicyeae)

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Abstract

Seeds with a water-impermeable seed coat and a physiologically dormant embryo are classified as having combinational dormancy. Seeds of *Sicyos angulatus* (burcucumber) have been clearly shown to have a water-impermeable seed coat (physical dormancy [PY]). The primary aim of the present study was to confirm (or not) that physiological dormancy (PD) is also present in seeds of *S. angulatus*. The highest germination of scarified fresh (38%) and 3-month dry-stored (36%) seeds occurred at 35/20°C. The rate (speed) of germination was faster in scarified dry-stored seeds than in scarified fresh seeds. Removal of the seed coat, but leaving the membrane surrounding the embryo intact, increased germination of both fresh and dry-stored seeds to >85% at 35/20°C. Germination (80–100%) of excised embryos (both seed coat and membrane removed) occurred at 15/6, 25/15 and 35/20°C and reached 95–100% after 4 days of incubation at 25/15 and 35/20°C. Dry storage (after-ripening) caused an increase in the germination percentage of scarified and of decoated seeds at 25/15°C and in both germination percentage and rate of excised embryos at 15/6°C. Eight weeks of cold stratification resulted in a significant increase in the germination of scarified seeds at 25/15 and 35/20°C and of decoated seeds at 15/6 and 25/15°C. Based on the results of our study and on information reported in the literature, we conclude that seeds of *S. angulatus* not only have PY, but also non-deep PD, that is, combinational dormancy (PY + PD).

Keywords: combinational dormancy, physical dormancy, physiological dormancy, seed dormancy, *Sicyos angulatus*.

Received 15 November 2010; revision received 15 February 2011; accepted 5 March 2011

Introduction

The Cucurbitaceae is one of the 17 angiosperm families reported to produce seeds with a water-impermeable seed (or fruit) coat that prevents water uptake (imbibition), that is, physical dormancy (PY) (Baskin *et al.* 2000, 2006; Baskin & Baskin 2003). However, as in several of the other 16 families with PY not all Cucurbitaceae species have water-impermeable seed dormancy. In fact, a preliminary review of the literature suggests that seeds of most species in this family have physiological dormancy (PD; growth potential of the hydrated embryo is too low to overcome mechanical restraint of its covering layers such as the seed coat) or they are non-dormant (X. Qu, C. Baskin, J. Baskin,

unpubl. data, 2007). Furthermore, a water-impermeable seed coat has been reported in only one genus, *Sicyos*, in this family, which consists of 122 genera and 940 species (Mabberley 2008): *S. angulatus* (Jones 1971; Walker 1973; Mann *et al.* 1981; Lim *et al.* 1994; Qu *et al.* 2010) and *S. deppei* (Brecht-Franco *et al.* 1992; Orozco-Segovia *et al.* 2000; Osuna-Fernández *et al.* 2000). Seeds of *S. deppei* have also been shown to clearly have physiological dormancy, thus combinational dormancy (PY + PD).

The purpose of the present study was to test the effect of dry storage (after-ripening) on germination of scarified seeds, decoated seeds and excised embryos and of cold stratification on germination of scarified and decoated seeds of *S. angulatus*. An increase in the percentage and/or rate of germination following after-ripening and/or cold stratification would indicate PD (Baskin & Baskin 1998). The fruit coat of *S. angulatus* is a very thin,

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papery layer that covers the water-impermeable seed coat, and Jones (1971) reported that germination of scarified fruits and scarified seeds was the same. Furthermore, there was no germination inhibitor or stimulator present in the fruit coat, and removal of the fruit coat did not affect germination (Jones 1971). Thus, in our research we tested germination of seed-coat scarified seeds, decoated seeds and excised embryos.

Materials and methods

Sicyos angulatus is a monoecious summer annual vine of 'damp soil' habitats that is native to eastern North American (Fernald 1950; Gleason & Cronquist 1991), but is now an invasive agricultural weed in Canada, Mexico, USA, Europe and Asia (European and Mediterranean Plant Protection Organization [no date]; Tzonev 2005; Kurokawa *et al.* 2009). The fruit is a one-seeded dry berry and the embryo is surrounded by three layers (see fig. 1 in Qu *et al.* 2010). The green inner membrane tightly surrounds the embryo, and the hard (water-impermeable) seed coat, which has a distinct palisade layer, covers the embryo and the inner membrane. The outermost layer is the thin papery fruit coat.

Seeds in the present study were produced by plants of *S. angulatus* grown on the campus of the University of Kentucky in Lexington in summer 2007 from seeds collected on the floodplain of the South Fork of the Kentucky River at Oneida, Clay County, Kentucky, in November 2006, as described in Qu *et al.* (2010). Seed coats of fresh seeds were water-impermeable. The mass of mechanically scarified seeds increased by 80% (fresh weight basis) in 72 h on wet filter paper under laboratory conditions (22–24°C, 50–60% relative humidity), whereas that of non-scarified seeds increased by only 1%. The three germination units used in the present study were scarified seeds, decoated seeds and excised embryos. A scarified seed had a small hole cut in the seed coat at the cotyledon end of the seed with a razor blade, a decoated seed had the entire seed coat removed and an excised embryo had both the seed coat and inner membrane removed (Jones 1971).

Three replicates each of 15 fresh (≤ 1 week old) and of 3-month-old dry-stored (22–24°C, 50–60% relative humidity) scarified seeds, decoated seeds and excised embryos were incubated at 15/6, 25/15 and 35/20°C under light/dark conditions (14/10 h; approximately 40 $\mu\text{mol}/\text{m}^2/\text{s}$, 400–700 nm, cool white fluorescent light). Germination was monitored every 4 days for 4 weeks. An emerged radicle was the criterion for germination of scarified and decoated seeds and radicle growth and geotropic curvature was the criterion for excised embryos.

Eighteen dishes of freshly matured scarified seeds were moist cold stratified at 5°C for 8 weeks. After cold strati-

fication, three dishes each were incubated directly at 15/6, 25/15 and 35/20°C under light/dark conditions for 28 days. The other half of the seeds was decoated and three dishes each incubated at 15/6, 25/15 and 35/20°C under light/dark conditions for 28 days. Eight-week-old laboratory stored seeds were used as controls in this experiment to test the effect of cold stratification on dormancy break. Thus, seeds of the same age that were not cold stratified (0 wk) were compared with those cold stratified for 8 weeks (8 wks). Three replicates of 15 seeds were used for each test condition. Final germination percentages of the cold stratified and of the non-stratified seeds were compared to determine the effect of cold stratification on breaking PD.

Data on the effect of cold stratification on breaking PD were analyzed by ANOVA using the GLM procedure in SAS software (SAS Institute, Cary, NC, USA). Data were arcsine transformed before analysis to normalize them, but non-transformed data are presented in Figure 2. Duncan's and Dunnett's mean separation procedures were used to determine differences between treatments (Scot *et al.* 1984).

Results

Three months of dry storage caused an increase in the germination percentage and/or rate of scarified seeds, decoated seeds and excised embryos (Fig. 1a–c). Whereas fresh, scarified seeds germinated only at 35/20°C, seeds stored dry for 3 months also germinated at 25/15°C. Although the final germination percentage of fresh, scarified seeds was similar to that of the 3-month dry-stored ones at 35/20°C, the germination rate of after-ripened seeds was higher than that of fresh ones (Fig. 1a). After dry storage, the germination percentages and rates of decoated seeds were also higher than those of fresh seeds (Fig. 1b). Excised embryos from both fresh seeds and 3-month dry-stored seeds had germinated to >95% after 4 days of incubation at 25/15 and 35/20°C, but to only 0 and 36%, respectively, at 15/6°C after 8 days (Fig. 1c). At 15/6°C, both the germination percentage and rate were higher in excised embryos of dry-stored seeds than they were in those of freshly matured seeds (Fig. 1c).

Eight weeks of cold stratification resulted in a significant increase in the germination of scarified seeds at 25/15 and 35/20°C and of decoated seeds at 15/6 and 25/15°C (Fig. 2). The germination percentages of both freshly harvested and cold stratified seeds increased significantly with an increase in temperature.

Discussion

Freshly matured and dry-stored scarified seeds of *S. angulatus* germinated to approximately 38% and 36%, respectively, at 35/20°C, and to 0% and 20%, respectively,

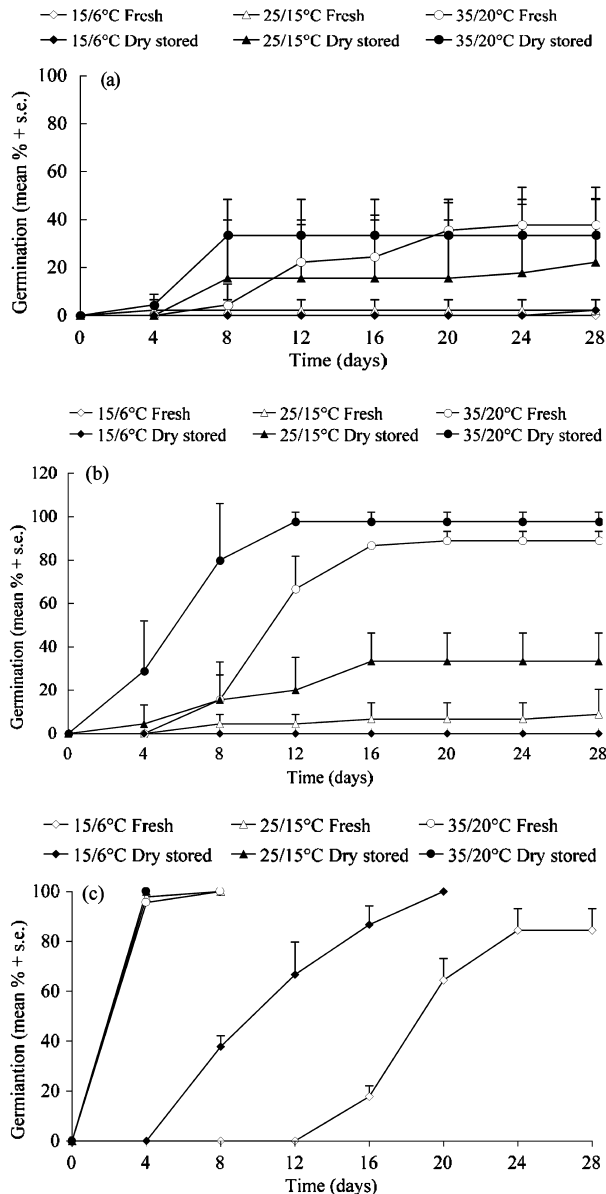


Fig. 1 Effect of 3 months of dry storage (after-ripening) on the germination percentage and rate of (a) scarified seeds, (b) decoated seeds and (c) excised embryos of *Sicyos angulatus* at three temperature regimes. Error bars indicate one standard error (s.e.).

at 25/15°C in 4 weeks. No seeds germinated at 15/6°C. Jones (1971) reported that the germination percentage of scarified seeds of *S. angulatus* on silt loam soil after 3 weeks of incubation in a greenhouse at 24/18°C and with a 16 h photoperiod (4600 lux) was 58%, but only approximately 37% germinated in Petri dishes on filter paper moistened with deionized water at 30°C. After removing the seed coat, decoated seeds in our study germinated to a higher percentage and higher rate than scarified seeds.

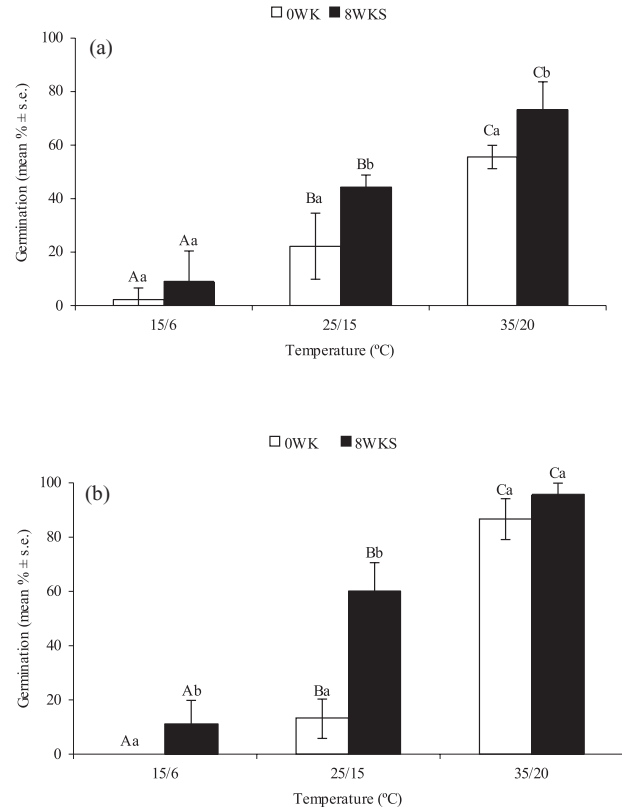


Fig. 2 Effect of cold stratification on the germination percentage of (a) scarified seeds and (b) decoated seeds of *Sicyos angulatus* at three temperature regimes. Different uppercase letters indicate significant differences in the germination percentages of seeds under the same treatment (cold stratified or non-stratified) across all temperature regimes, and different lowercase letters indicate significant differences in the germination percentages of cold stratified and of non-stratified seeds at the same temperature regime. 8 wks, 8 weeks of cold stratification at 5°C, starting with fresh seeds; 0 wk, zero weeks of cold stratification of seeds stored in the laboratory for 8 weeks. Error bars indicate one standard error (s.e.).

After the inner membrane was removed, approximately 80–100% of the excised embryos germinated at all three temperatures. Jones (1971) showed that there was no chemical inhibitor in the seed coat of *S. angulatus* and that the inner membrane slows (but does not prevent) water uptake. This evidence suggests that PD in *S. angulatus* is caused by mechanical restraint of the embryo covering layers: inner membrane and seed coat. This theory was supported in Jones' (1971) study by removing all or part of the seed coat and all or part of the inner membrane of decoated seeds before the seeds were germinated on moist filter paper. In his study, the germination percentage after 7 days at 30°C was as follows: (excised [naked] embryos) = (embryos with portion of inner membrane that covers radicle removed) > (decoated seeds [embryo

entirely covered by membrane)] > (seeds with half of the seed coat [below the embryo] left on) > (seeds with 80–90% of seed coat cracked) > (seeds with entire coat left on, but the hilum scarified) > (seeds with half of the coat covering the radicle end). However, if the radicle end was covered, removing other parts of the inner membrane resulted in less and slower germination than that of the excised embryos and than that of the ones with membrane covering the radicle cap removed (Jones 1971).

Germination of fresh and 3-month dry-stored decoated seeds was lower than that of excised embryos, which also shows that the inner membrane is a barrier for seed germination of *S. angulatus*. However, excised embryos of dry-stored seeds germinated at a higher rate at 15/6°C than fresh ones. This indicates that low embryo growth potential is also a cause for the non-deep PD and that PD can be broken by an increase in embryo growth potential during after-ripening. Jones' (1971) study also suggests that the embryo has some PD that is broken by after-ripening: aged decoated seeds germinated faster than fresh decoated seeds. In our study, dry storage not only increased the germination rate of the excised embryos at 15/6°C, but also promoted germination of decoated seeds and to a much lesser extent germination of scarified seeds. Furthermore, a short period (8 weeks) of cold stratification increased the germination of scarified and decoated seeds.

Further support for PD in seeds of *S. angulatus* is shown by studies on seed dormancy and germination in seeds of this species collected in South Korea. Lim *et al.* (1994) found that 1-year-old scarified seeds germinated to a much higher percentage (87%) than fresh scarified ones. Kang *et al.* (2003) showed that 1, 2 and 3 weeks of cold stratification at 3°C increased germination (>80%) over the non-stratified control (~10%) of scarified seeds of *S. angulatus*. These two studies support our findings that PD in seeds of *S. angulatus* can be broken by both after-ripening and cold stratification.

An increase in embryo growth potential or a combination of weakening of the membrane and/or seed coat and increase in embryo growth potential would allow water-permeable seeds to germinate under appropriate temperature/moisture conditions. Whether the inner membrane and/or seed coat is weakened during after-ripening or during cold stratification is not known. Welbaum *et al.* (1995) suspected that the perisperm–endosperm and seed coat of muskmelon (Cucurbitaceae) might weaken during breaking of PD. It seems possible that both an increase in embryo growth potential and weakening of the covering layers (inner membrane, seed coat) may occur during breaking of the non-deep PD in *S. angulatus* seeds.

Under natural conditions, seeds in a seed lot of *S. angulatus* may germinate throughout the growing season

(April–October) over a 5–6 year period following dispersal in autumn/winter of the year in which they are produced (C. Baskin and J. Baskin, unpubl. data, 2009). Under laboratory conditions, mechanical scarification or scarification with concentrated sulfuric acid (Jones 1971) makes the seed coat permeable. Soaking seeds in concentrated sulfuric acid for 15, 30, 45, 60 and 120 min caused 7, 67, 95 and 100% of them to imbibe, respectively. Seeds not acid-soaked did not imbibe (Jones 1971). However, the natural factor(s) that break(s) PY, which appears to involve opening of the hilar slit (X. Qu, unpubl. data, 2007), in seeds of *S. angulatus* has(have) not been identified, but may include fluctuating temperatures in spring (Van Assche *et al.* 2003) and throughout the rest of the growing season (Baskin & Baskin 1998). In nature, PD could be overcome by after-ripening before the seed coat becomes water-permeable or by cold stratification after it becomes water-permeable and imbibes water, as shown in the present study.

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