Seed Science Research (2005) **15**, 357–360 DOI: 10.1079/SSR2005224

SHORT COMMUNICATION

Underdeveloped embryos in dwarf seeds and implications for assignment to dormancy class

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Abstract

Studies were conducted to determine if small embryos (i.e. low embryo length:seed length ratio) in mature dwarf seeds (0.2–2 mm) are underdeveloped. In this case, they would grow (inside the seed) prior to germination, and seeds would have morphological or morphophysiological dormancy. Prior to radicle emergence, embryo length in seeds of *Drosera anglica* (*Droseraceae*), *Campanula americana*, *Lobelia appendiculata*, *L. spicata* (*Campanulaceae*) and *Sabatia angularis* (*Gentianaceae*) increased 0, 103, 182, 83 and 57%, respectively. Since embryo growth did not occur in seeds of *D. anglica* prior to germination, embryos, although small, are fully developed; seeds have only physiological dormancy. The underdeveloped embryo in seeds of *C. americana* has little or no physiological dormancy; thus, seeds have morphological dormancy. On the other hand, underdeveloped embryos in seeds of *L. appendiculata*, *L. spicata* and *S. angularis* are physiologically dormant, and seeds have morphophysiological dormancy. Therefore, since small embryos in dwarf seeds may or may not be underdeveloped, assignment of seeds to a dormancy class requires that studies be done to determine if embryos grow inside the seed before germination can occur. Such information is important in understanding the evolutionary relationship of the different kinds of seed dormancy.

Keywords: evolution of seed dormancy, *Drosera anglica*, *Campanula americana*, *Lobelia appendiculata*, *Lobelia spicata*, *Sabatia angularis*, morphophysiological dormancy

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Introduction

Martin (1946) described ten types of seeds based on characters of the endosperm (starchy versus non-starchy); shape, size and position of the embryo; erectness (versus bent) of the embryo; expansion (or not) of cotyledons; and degree of covering of the radicle by cotyledons. In addition, he described two types of seeds with non-starchy endosperm based on size of the seed interior: (1) dwarf, 0.2–2 mm long; and (2) micro, <0.2 mm long. A further type of seed was described by Martin as being medium to large in size and having a rudimentary embryo. Seeds with a rudimentary embryo are present in various primitive angiosperms, including the *Amborellaceae*, *Austrobaileyaceae*, *Chloranthaceae*, *Illiciaceae* and *Magnoliaceae* (Bailey and Swamy, 1948; Grushvitzky, 1967). Martin (1946) placed seeds with a rudimentary embryo at the base of his family tree of seed phylogeny and above it seeds with small, linear embryos. In seeds with rudimentary or small, linear embryos, the embryo must grow (inside the seed) before germination occurs; thus, both kinds of seeds generally are referred to as having underdeveloped embryos (Baskin and Baskin, 1998).

Martin's (1946) paper contains drawings of seeds with what appears to be underdeveloped embryos in families not reported to have such embryos. Further, these underdeveloped-looking embryos are in seeds that Martin classified as dwarf. The families (*sensu* APG; Angiosperm Phylogeny Group, 2003) listed by Martin as having dwarf seeds are the *Campanulaceae*, *Cephalotaceae*, *Clethraceae*, *Crassulaceae*, *Datiscaceae*, *Droseraceae*, *Elatinaceae*, *Ericaceae*, *Gentianaceae*, *Hypericaceae*, *Loganiaceae*, *Melastomataceae*, *Penthoraceae*, *Podostemaceae*, *Saxifragaceae* and *Scrophulariaceae*. In addition, one to four genera with dwarf seeds were described in the non-dwarf families *Hydrophyllaceae* (= *Boraginaceae*, *sensu* APG; Angiosperm Phylogeny

Group, 2003), *Polemoniaceae*, *Primulaceae*, *Rubiaceae* and *Solanaceae* (Martin, 1946).

A close examination of Martin's (1946) drawings reveals that although some dwarf seeds have embryos that extend the full length (or nearly so) of the seed interior, others have a small embryo in relation to the size of the endosperm. In the case of the *Campanulaceae* and *Gentianaceae*, both sizes of embryos in relation to the endosperm occur in the same family. Thus, if one looks at Martin's drawings and pays no attention to seed size, it would be easy to conclude that some members of the *Gentianaceae*, *Campanulaceae* and *Droseraceae* had underdeveloped embryos. In fact, Nikolaeva (2004) commented that some of Martin's dwarf seeds are very similar to his medium-sized seeds with rudimentary embryos, except for the overall length of the seed.

The purpose of our investigation was to determine if embryo growth occurs in dwarf seeds of the *Campanulaceae*, *Gentianaceae* and *Droseraceae* prior to germination, and thus if seeds have underdeveloped or fully developed embryos. Seeds of *Campanula americana* L., *Lobelia appendiculata* A. DC. var. *gattingeri* (Gray) McVaugh [= *L. gattingeri* A. Gray and hereafter referred to as *L. appendiculata*] and *L. spicata* Lam. in the *Campanulaceae*, *Sabatia angularis* (L.) Pursh in the *Gentianaceae* and *Drosera anglica* (Huds.) LePage & W. Bldw. in the *Droseraceae* were studied. The presence of underdeveloped embryos would mean that seeds have either morphological or morphophysiological dormancy, depending on whether physiological dormancy occurs in the embryo (Nikolaeva, 1969; Baskin and Baskin, 2004).

Materials and methods

Dry seeds of *C. americana*, *L. spicata*, *S. angularis* and *D. anglica* were placed on moist filter paper in darkness at room temperature for 24 h. Fifteen fully imbibed seeds of each species were cut open lengthwise with a razor blade. The maximum internal length of each seed, which was filled with endosperm, and the excised embryo were measured using a dissecting microscope equipped with a micrometer.

Fresh seeds of *C. americana* germinate to ≥60% in light at relatively high temperatures within about 10 d without any dormancy-breaking treatments (Baskin and Baskin, 1984). Thus, seeds were incubated in light at 25/15°C without first giving them a dormancy-breaking treatment.

Seeds of *L. spicata* (Baskin and Baskin, unpublished data), *S. angularis* (Baskin and Baskin, 1998) and *D. anglica* (Baskin *et al.*, 2001) require cold stratification to germinate. Seeds of these three species were placed on moist filter paper and cold stratified in light (12-h daily photoperiod) at a 12/12 h daily temperature regime of 5/1°C for 12 weeks, after which they were incubated in light (14-h daily photoperiod) at 25/15°C (*L. spicata* and *D. anglica*) and 20/10°C (*S. angularis*). Embryos were measured in 15 seeds of each species at the end of the cold stratification period. During the incubation period at 25/15 or 20/10°C, seeds were examined daily under the dissecting microscope. As soon as the seed coat began to split, i.e. when the embryo had grown to its critical length for germination (radicle emergence), the embryo was excised and measured; 15 embryos were measured for each species. Care was taken not to select any seeds with visible signs of radicle emergence.

Seeds of *L. appendiculata* are also dormant at maturity and will germinate to high percentages following cold stratification (Baskin and Baskin, 1979). Seeds that had been buried in soil under natural temperature regimes since 1990 were exhumed in March 2005, after receiving a winter cold-stratification treatment. Immediately after seeds of *L. appendiculata* were exhumed, seed interior and embryo measurements were made, as described above. Then some seeds were placed on moist filter paper in light at 25/15°C and checked daily for splitting of the seed coat. When a seed with a split seed coat was found, the embryo was excised and measured.

Results

Embryos grew prior to germination in seeds of *C. americana*, *L. appendiculata*, *L. spicata* and *S. angularis*, with the greatest percent increase in length occurring in *L. appendiculata* and the smallest in *S. angularis* (Table 1). No embryo growth occurred while *S. angularis* seeds were being cold stratified in light at 5/1°C; mean (±SE) embryo length before and after 12 weeks of cold stratification at 5/1°C was 0.23 ± 0.01 and 0.22 ± 0.01 mm, respectively. However, mean embryo length for *L. spicata* seeds increased from 0.29 ± 0.01 to 0.34 ± 0.02 mm during cold stratification; embryo growth was completed at 25/15°C. No embryo growth occurred in seeds of *D. anglica* before they germinated.

Discussion

Since considerable embryo growth occurred in seeds of *C. americana*, *L. appendiculata*, *L. spicata* and *S. angularis* before the radicle emerged, the seeds have underdeveloped embryos. Thus, seeds of these four species have either morphological or morphophysiological dormancy. Fresh seeds of *C. americana* germinate to about 90% in light at 15/6, 20/10 and 25/15°C within 30 d (Baskin and Baskin, 1984), indicating that they have little or no physiological

Table 1. Initial length of embryo and endosperm (i.e. full length of the interior of the seed), length of embryo at the time the seed coat splits and percentage increase in embryo length before germination occurred. Seeds of all species were incubated in light at 25/15°C, except those of *Sabatia angularis* which were incubated in light at 20/10°C

Species	Mean (± SE) initial length (mm)		Mean (± SE) embryo length (mm) when seed coat splits	% Increase in embryo length before germination
	Endosperm	Embryo		
Campanulaceae				
Campanula americana	0.69 ± 0.01	0.34 ± 0.01	0.69 ± 0.02	103
Lobelia appendiculata	0.36 ± 0.01	0.11 ± 0.01	0.31 ± 0.01	182
L. spicata	0.54 ± 0.01	0.29 ± 0.01	0.53 ± 0.02	83
Droseraceae				
Drosera anglica	0.51 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0
Gentianaceae				
Sabatia angularis	0.43 ± 0.01	0.23 ± 0.01	0.36 ± 0.01	57

dormancy. Consequently, morphological dormancy is the class of dormancy in seeds of *C. americana*.

Seeds of *L. appendiculata*, *L. spicata* and *S. angularis* have morphological dormancy in addition to physiological dormancy; thus, they have morphophysiological dormancy. Embryos did not grow in *S. angularis* seeds during cold stratification at 5/1°C, although seeds were in light. Further, embryos grew rapidly and seeds germinated after they were transferred from 5/1 to 25/15°C. Thus, seeds of *S. angularis* have non-deep simple MPD (*sensu* Baskin and Baskin, 1998). On the other hand, some embryo growth occurred in about half the seeds of *L. spicata* during cold stratification, indicating that they have deep complex MPD (*sensu* Baskin and Baskin, 1998). It is possible that seeds of *L. appendiculata* also had non-deep simple MPD, but more research is required to verify this. Since seeds of *L. appendiculata* were buried in soil, lack of light rather than low temperatures during winter may have inhibited embryo growth.

Light is required for germination of *L. appendiculata*, *L. spicata* and *S. angularis* seeds, and presumably it triggers embryo growth. Light was required for embryo growth and thus germination in seeds of the winter annual *Chaerophyllum tainturieri* (*Apiaceae*), which have non-deep simple MPD (Baskin and Baskin, 1990). Physiological dormancy in seeds of *C. tainturieri* is broken during summer, and embryo growth (followed by germination) occurs in autumn if seeds are exposed to light. Further, if seeds were exposed to light for 8–14 d in mid-summer (when temperatures were too high for germination and before physiological dormancy was completely broken) and then buried in soil, 62–90% of them germinated in darkness in autumn. Thus, exposure of seeds to light, even when conditions were not favourable for germination, resulted in germination in darkness. Such studies need to be done on seeds of *L. appendiculata*, *L. spicata* and *S. angularis*.

Embryos did not grow in seeds of *D. anglica* prior to emergence of the radicle; thus, seeds have physiological dormancy only. Even after radicle emergence from *D. anglica* seeds, cotyledons remained inside the seed but did not elongate. According to Boesewinkel and Bouman (1995), 'In *Drosera* the haustorial tips of the cotyledons remain longer in the seed to absorb the food reserves in the endosperm' (p. 19).

In conclusion, the class of dormancy cannot be confidently assigned to dwarf seeds with a small embryo (low embryo length/seed length ratio), unless information is obtained as to whether embryo growth is a prerequisite for germination. For example, without knowledge of the embryo growth requirement for germination, one would conclude that the morphologically dormant seeds of *C. americana* were non-dormant. Further, it has been assumed that seeds of *L. spicata*, *L. appendiculata* and *S. angularis* have only physiological dormancy, whereas they actually have morphophysiological dormancy. To our knowledge, this is the first report of underdeveloped embryos and of either morphological or morphophysiological dormancy in seeds of *Campanulaceae* and of morphophysiological dormancy in those of *Gentianaceae*. However, as seen in the dwarf seeds of *D. anglica*, the presence of an embryo that is small in relation to the endosperm does not necessarily mean that the embryo is underdeveloped. Before assigning seeds with a small embryo to a dormancy class, measurements need to be made to determine whether the embryo grows prior to germination.

Information on the presence (or not) of underdeveloped embryos helps us better understand the evolutionary relationship of families within an order with respect to seed dormancy. Underdeveloped embryos are not restricted to primitive angiosperms, and they occur in some families in advanced orders (*sensu* APG; Angiosperm Phylogeny Group, 2003),

https://doi.org/10.1079/SSR2005224 Published online by Cambridge University Press

including the *Aquifoliales*, *Apiales*, *Asterales*, *Dipsacales* and *Gentianales* (Martin, 1946). In the *Gentianales*, underdeveloped embryos have been reported in the *Rubiaceae* (Martin, 1946) and *Gentianaceae* (Table 1), and there is a possibility that the small embryos in dwarf seeds in the *Loganiaceae* are underdeveloped. In the *Asterales*, underdeveloped embryos have been reported in seeds of *Menyanthaceae* (Martin, 1946), and *Campanulaceae* (Table 1). However, additional studies are needed on other members of the *Asterales*, e.g. to determine if the small embryos in seeds of *Pentaphragmataceae* and *Stylidiaceae* are underdeveloped and the kind of embryo in seeds of the *Alseuosmiaceae*, *Argophyllaceae* and *Phellinaceae*.

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Received 5 July 2005
accepted after revision 20 August 2005

https://doi.org/10.1079/SSR2005224 Published online by Cambridge University Press