Seed dormancy in the early diverging eudicot *Trochodendron aralioides* (*Trochodendraceae*)

Jerry M. Baskin[1*], Carol C. Baskin[1,2], Ching-Te Chien[3] and Shun-Ying Chen[4]

[1]Department of Biology, University of Kentucky, Lexington, KY 40506-0225, USA; [2]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546-0312, USA; [3]Division of Silviculture, Taiwan Forestry Research Institute, Taipei 100, Taiwan; [4]Division of Forest Biology, Taiwan Forestry Research Institute, Taipei 100, Taiwan

Abstract

The embryo length/seed length (E/S) ratio of the early diverging eudicot *Trochodendron aralioides* is 0.34. Embryos in fresh seeds were 0.36 ± 0.01 mm long, and they increased in length by about 250% (in 20 d) before radicle emergence (germination) occurred, demonstrating that the embryo is underdeveloped at seed maturity. Seeds germinated to 95–100% at 20/10, 25/15 and 30/15°C in light in ≤ 4 weeks, without any pretreatment, but no seeds germinated in darkness. Thus, seeds of *T. aralioides* have morphological dormancy (MD), which is considered to be the primitive condition in seed plants, and MD probably has existed in the genus *Trochodendron* since its origin in the early Tertiary.

Keywords: basal eudicot, fossil record of *Trochodendron*, morphological dormancy, primitive dormancy class, underdeveloped embryo

Introduction

In seeds of some species with a low embryo length/seed length (E/S) ratio, the embryo must grow inside the seed prior to radicle emergence, whereas in seeds of other species (with either a low or a high E/S ratio), embryo growth is not a prerequisite for radicle emergence (Baskin and Baskin, 2005). Embryos in which growth occurs prior to radicle emergence are underdeveloped (*sensu* Grushvitzky, 1967), which is believed to be the primitive condition in seed plants (Grushvitzky, 1967; Baskin and Baskin, 1998; Nikolaeva, 1999; Forbis *et al.*, 2002), while seeds that do not grow before the radicle emerges are fully developed. Seeds with an underdeveloped embryo have morphological or morphophysiological dormancy, whereas those with fully developed embryos have physiological, physical or physical + physiological (combinational) dormancy, or they may be non-dormant (*sensu* Baskin and Baskin, 2004a).

The early diverging eudicot family *Trochodendraceae* (*sensu* Angiosperm Phylogeny Group, 2003; Kim *et al.*, 2004) consists of only two extant species, *Trochodendron aralioides* Siebold & Zucc. and *Tetracentron sinensis* Oliver; the E/S ratio in both of them is low (Grushvitzky, 1967; Mohana Rao, 1981; Doweld, 1998). However, it is not known whether embryo growth occurs inside the seed prior to radicle emergence, in which case the seeds would have either morphological or morphophysiological dormancy, or if radicle emergence occurs without prior embryo growth (see Baskin and Baskin, 2005). In the latter case, the seeds would be non-dormant or have physiological dormancy, i.e. since the seed coat of *Trochodendraceae* is water permeable, the seeds cannot have either physical or combinational dormancy (Baskin and Baskin, 2004a).

The purpose of our study was to determine the embryo growth, dormancy-breaking and germination requirements, and thus, the class of seed dormancy in *T. aralioides*. This species occurs naturally in South Korea, Japan (Honshu and southward), Ryukyu Islands

*Correspondence
Fax: +1 859 257 1717
Email: jmbask0@uky.edu

and Taiwan (Ohwi, 1965; Li and Chaw, 1996; Mabberley, 1997; Huang *et al.*, 2004). In Taiwan, the species grows at elevations of 500–3000 m (Li and Chaw, 1996).

Materials and methods

Seeds

Ripe seeds were collected on 19 November 2004 from plants of *T. aralioides* growing in Yangmingsham, Taiwan (25°10′N, 120°31′E) at an elevation of 790 m in the subtropical evergreen broadleaved forest zone. After drying at room temperature for 1 week, seeds were air-mailed to the University of Kentucky, Lexington, USA, where the study was performed. Seeds were stored dry at room temperature until used in germination or embryo growth studies.

Germination tests

Two germination tests were conducted. In the first one, which began on 22 December 2004, seeds were incubated in light (14 h daily photoperiod of about 40 μmoles m^{-2}s^{-1}, 400–700 nm, cool white fluorescent light) at a constant temperature of 5°C and at 12/12 h daily alternating temperature regimes of 15/6, 20/10 and 25/15°C. At the alternating temperature regimes, the photoperiod extended from 1 h before to 1 h after the daily high temperature period. Fifty seeds were sown in each of 12 9.5-cm-diameter Petri dishes on sand moistened with distilled water, and three dishes were placed at each temperature regime. Dishes were wrapped with plastic film to reduce the rate of water loss. Seeds were checked after 3, 4, 6, 8, 10 and 12 weeks, at which time germinated seeds were counted and removed from the dishes.

The second germination test was initiated on 9 January 2005, at which time seeds were incubated in light (14 h daily photoperiod) and in continuous darkness at 12/12 h daily alternating temperature regimes of 15/6, 20/10, 25/15 and 30/15°C. Fifty seeds were sown in each of 24 9.5-cm-diameter Petri dishes on sand moistened with distilled water. Three dishes each were placed in light and in darkness at each temperature. All dishes were wrapped with plastic film, and those to be incubated in darkness were wrapped additionally with two layers of aluminium foil. Seeds incubated in light were checked for germination after 1, 2, 3, 4, 6, 8, 10 and 12 weeks, but those incubated in darkness were checked only after 4 weeks, at which time they were discarded.

Embryo growth studies

To determine embryo length in fresh seeds, dry seeds were placed on moist filter paper on 18 December 2004 and incubated in darkness at room temperature for 24 h, by which time seeds were imbibed and soft. Fifteen seeds were cut open lengthwise with a razor blade. The internal length of each seed and length of the excised embryo were measured using a dissecting microscope equipped with a micrometer.

To determine critical embryo length for germination, embryos in seeds with a split seed coat were excised and measured. Starting on 22 December, 50 seeds were incubated on moist filter paper in light at 15/6°C for 4 weeks, at which time seeds were examined under a microscope. A seed was selected for embryo excision and measurement if the seed coat was split, but neither radicle tip nor endosperm had emerged beyond the end of the seed. Fully elongated embryos were excised from 15 seeds and measured.

To determine rate of embryo growth, 15 dry seeds were sown on moist filter paper in each of five Petri dishes on 2 January 2005 and incubated in light at 20/10°C. After 1, 5, 10, 15 and 20 d, embryos were excised from 15 seeds and measured. On day 20, six of the seeds had already germinated; thus the critical embryo length for germination was recorded for these seeds.

Results

Germination tests

In the first test, 100% of the seeds germinated at 15/6, 20/10 and 25/15°C, but none germinated at 5°C (Table 1). The rate of germination was highest at 25/15°C. In the second test, seeds germinated to 100% at all four alternating temperature regimes in light, but none germinated in darkness at any of the regimes. Again, seeds germinated faster at 25/15°C than at the other regimes; however, after 8 weeks 100% of the seeds in light had germinated at all four temperature regimes.

Embryo growth studies

Embryos were linear in shape and about twice as long as wide. Except for the space occupied by the embryo, the interior of the seed was filled with endosperm. Mean (±SE) lengths of seeds and embryos were 1.20 ± 0.03 and 0.36 ± 0.01 mm, respectively. Thus, embryo-length/seed-length ratio was 0.34.

The length of embryos in seeds with a split seed coat was 1.20 ± 0.02 mm. Embryo lengths were 0.36 ± 0.01, 0.41 ± 0.01, 0.66 ± 0.02, 0.86 ± 0.03 and

Table 1. Germination percentages (mean ± SE) of *Trochodendron aralioides* seeds incubated in light and in darkness. Percentages are cumulative for seeds incubated in light. Germination of seeds incubated in darkness was checked only after 4 weeks

		Test temperatures (°C)				
Time (weeks)	Incubated in	5	15/6	20/10	25/15	30/15
First test						
3	Light	0	0	5 ± 1	100	–[a]
4	Light	0	3 ± 1	94 ± 3	100	–
6	Light	0	100	100	100	–
12	Light	0	100	100	100	–
Second test						
1	Light	–[a]	0	0	0	6 ± 1
2	Light	–	0	0	100	82 ± 7
3	Light	–	0	61 ± 5	100	98 ± 1
4	Light	–	44 ± 5	100	100	100
6	Light	–	97 ± 2	100	100	100
8	Light	–	100	100	100	100
4	Dark	–	0	0	0	0

[a] No data.

1.20 ± 0.04 mm, after 1, 5, 10, 15 and 20 d of incubation in light at 20/10°C, respectively.

Discussion

A small embryo, which is either undifferentiated or differentiated (sometimes barely so) into an epicotyl–hypocotyl axis, and has to grow (elongate)/differentiate inside the seed before the radicle can emerge, is said to be underdeveloped (Grushvitzky, 1967; Nikolaeva, 1969, 1977; Baskin and Baskin, 1998, 2004a; Kondo *et al.*, 2005). The embryo in freshly matured seeds of *T. aralioides* is underdeveloped, increasing in length by about 250% inside the mature seeds before the radicle emerged (cotyledons emerged 1 or 2 d after radicles emerged). Further, at seed maturity, cotyledon lobes and the radicle of the longer-than-broad embryo can be distinguished easily (Mohana Rao, 1981; Doweld, 1998; present study). According to Takhtajan (1997, p.130), the embryo in *Trochodendrales* is 'straight, small, differentiated into cotyledons, radicle, and plumule'. Thus, the embryo in freshly mature seeds is of the linear, under-developed, differentiated type.

Since the embryo of *T. aralioides* is underdeveloped, its seeds, by definition, have either morphological dormancy (MD) or morphophysiological dormancy (MPD) (Baskin and Baskin, 2004a). In their key to the classes of seed dormancy, Baskin and Baskin (2004b) distinguished between MD and MPD based on how long it takes the seeds to germinate under appropriate temperature/light conditions. Thus, fresh seeds that germinate in ≤30 d have MD, whereas those that require >30 d to germinate have MPD. Seeds with

MPD require a warm, cold, warm + cold or a cold + warm + cold stratification treatment, depending on the intensity of MPD (see key to levels of MPD in Baskin and Baskin, 2004b), to come out of dormancy. Nearly fresh seeds of *T. aralioides* germinated to 94–100% over the temperature range of 20/10 to 30/15°C in the light in 2–4 weeks, and to 97–100% at 15/6°C in light after 6 weeks, without any pretreatment. Further, embryos began to grow within 5 d in seeds incubated at 20/10°C, and after 20 d they were fully elongated. Thus, the embryo did not appear to exhibit any physiological dormancy, either when it was underdeveloped (0.36 mm long in fresh seeds) or after it became fully elongated (1.20 mm long, just prior to radicle emergence), unlike seeds with MPD (Baskin and Baskin, 1998, 2004a). Using these criteria, seeds of *T. aralioides* from Taiwan have MD. Yokoyama and Mukai (1988) reported that seeds of *T. aralioides* collected from a single tree, growing in an arboretum in Tsukuba, Japan, and incubated in light at 28 and 30/20°C without pretreatment, germinated to high percentages in <30 d; seeds did not germinate in darkness.

Based on ecological considerations, Baskin and Baskin (1998) and Forbis *et al.* (2002) argued that MD, and not MPD, is the most primitive kind of seed dormancy in both gymnosperms and angiosperms. If this is the case, then MD can be added to the other primitive attributes of *T. aralioides*, such as lack of vessels (Bailey and Nast, 1945; Cronquist, 1988; Takhtajan, 1997), which characterize this early diverging eudicot. Further, we speculate that seeds of *Tetracentron sinensis*, a close relative of *T. aralioides*, also would have MD (Compilation Committee, 2000). If this is the case, MD would be a characteristic of the entire order *Trochodendrales* (*sensu* Cronquist, 1988; Takhtajan, 1997), the family *Trochodendraceae* in order *Hamamelidales* (*sensu* Thorne, 2000), or in the (unplaced-in-order) *Trochodendraceae* (*sensu* Angiosperm Phylogeny Group, 2003; Kim *et al.*, 2004).

The oldest fossil records of *Trochodendron* are from the late–early Eocene (*c*. 49–52 Ma; see Fig. 2 in Greenwood *et al.*, 2005) floras of the Okanagan Highlands in the north-western USA (state of Washington) and southern British Columbia, Canada (Pigg *et al.*, 2001; Devore *et al.*, 2005; Dillhoff *et al.*, 2005). Fossils of this genus also are known from the Miocene of the north-western USA, Japan and Kamchatka (Manchester *et al.*, 1991; Manchester, 1999; Devore *et al.*, 2005). Both infructescences and fruits of these Tertiary fossils are very similar to each other and to those of the extant *T. aralioides* (Manchester *et al.*, 1991; Pigg *et al.*, 2001; Devore *et al.*, 2005; Dillhoff *et al.*, 2005). In fact, Manchester *et al.* (1991) state that fossil infructescences and fruits from the Miocene of

Idaho (north-western USA) and from the late Miocene of Iwate Prefecture, Japan '... appear to be indistinguishable from those of extant *T. aralioides*'.

Thus, as Manchester *et al.* (1991) and Pigg *et al.* (2001) discuss, it is evident that there has been morphological stasis in the infructescences and fruits of *Trochodendron* since the origin of this genus in the late–early Eocene (or earlier?). Further, a comparison of the Tertiary and Recent vegetation types in which *Trochodendron* grew (grows) suggests that the climatic (and thus physiological) requirements of the genus should not have changed drastically since the early Tertiary. Thus, we suggest that morphological dormancy has probably been a characteristic of seeds of *Trochodendron* throughout its long geological history.

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