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SHORT COMMUNICATION

Nymphaeaceae: a basal angiosperm family (ANITA grade) with a fully developed embryo

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Abstract

Rudimentary, broad and small linear embryos occur among members of the most primitive (basal) extant angiosperms, collectively called the ANITA grade (i.e. *Amborella*, *Nymphaeales* and *Austrobaileyales*). *Amborella* (rudimentary) and *Austrobaileyales* (rudimentary in *Austrobaileyaceae*, *Illiciaceae* and *Schisandraceae* and small linear in *Trimeniaceae*) have kinds of embryos that are known to be underdeveloped; consequently, they must grow inside the seed prior to radicle emergence (germination). On the other hand, it is not known if broad embryos need to grow before radicles can emerge, and whether they are underdeveloped or fully developed. Thus, we addressed the question: 'Is the broad embryo of *Nymphaeales* also underdeveloped?'. Although the embryo length : seed length ratios in *Nymphaea* Albert Greenburg, *N. capensis* var. *zanzibariensis* and *N. immutabilis* were 0.311, 0.349 and 0.234, respectively, embryos did not grow prior to radicle emergence. Thus, they are fully developed at seed maturity. If *Amborella* and *Nymphaeales* are equally the most basal angiosperms, as some molecular phylogenetic studies indicate, then we must conclude that the broad and rudimentary embryos are equally primitive.

Keywords: angiosperm evolution, ANITA grade, broad embryo, *Nymphaeales*, underdeveloped embryo

Introduction

An underdeveloped embryo is one that must grow inside the seed before the radicle can emerge (Grushvitzky, 1967; Baskin and Baskin, 2005). That is, the embryo completes growth inside the seed between seed maturity and germination (*sensu* Nikolaeva, 1969). If embryo growth is the only prerequisite for germination, seeds have morphological dormancy. However, if underdeveloped embryos also have physiological dormancy and require a dormancy-breaking pretreatment before or after they grow inside the seeds, then seeds have morphophysiological dormancy (Baskin and Baskin, 2004). Embryo growth prior to radicle emergence is known to occur in seeds with rudimentary, small linear and small spatulate embryos; the latter two are referred to as linear-underdeveloped and spatulate-underdeveloped embryos, respectively (Baskin and Baskin, 2007).

Seed biologists generally agree that underdeveloped embryos are the primitive condition in seed plants (Martin, 1946; Grushvitzky, 1967; Baskin and Baskin, 1998; Nikolaeva, 1999; Forbis *et al.*, 2002; Finch-Savage and Leubner-Metzger, 2006), but it is not known if underdeveloped embryos occur in all of the basal angiosperms. Most molecular phylogeny studies have identified *Amborella* as the basal angiosperm; *Amborella*, along with *Nymphaeales* and *Austrobaileyales*, which consists of *Illiciaceae*, *Trimeniaceae* and *Austrobaileyaceae*, constitute the ANITA grade, or basal angiosperms (Qiu *et al.*, 1999). However, some studies retrieved a clade consisting of *Amborella* plus *Nymphaeales* as sister to all other angiosperms (see discussion in Qiu *et al.*, 2005, 2006). Thus, the question of whether *Amborella* alone or together with *Nymphaeales* represents the basal-most clade of extant angiosperms is not completely resolved.

A survey of the kind(s) of embryo(s) in families of the basal angiosperms (Fig. 1) reveals that all families except those in the *Nymphaeales* (*Cabombaceae* and *Nymphaeaceae*) have a small embryo of a type known to grow inside the seed before radicle emergence occurs. The question is: 'Do the broad embryos in seeds of the *Nymphaeales* grow inside the seed prior to radicle emergence, i.e. are the broad embryos

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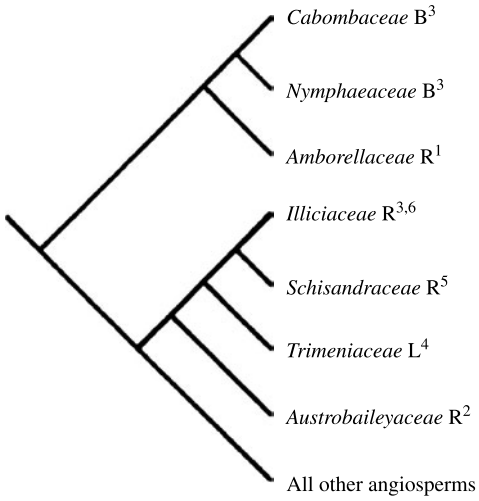

Figure 1. Types of embryos in extant basal (ANITA grade) angiosperm families. B, broad; L, small linear; R, rudimentary; [1]Bailey and Swamy (1948); [2]Endress (1980); [3]Martin (1946); [4]Morat and MacKee (1977); [5]Saunders (1998); [6]Takhtajan (1988). Cladogram adapted from Friedman (2001), reproduced with permission of Elsevier.

underdeveloped?'. According to Martin (1946), in dicots a broad embryo is found only in *Nymphaeales* and *Saururaceae* (*Piperales*). Various families of monocots, including *Restionaceae* (Takhtajan, 1985), *Eriocaulaceae*, *Juncaceae*, *Mayacaceae* and *Xyridaceae*, as well as *Eriophorum*, *Hemicarpha*, *Lipocarpha* and *Mariscus* in the *Cyperaceae* and *Aneilema* in the *Commelinaceae* (Martin, 1946), also have a broad embryo.

A broad embryo is relatively small in relation to the endosperm in the seed, and it is located at the base (lower edge) of the seed (Martin, 1946). However, the fact that the embryo is small, i.e. low embryo length : seed length (E : S) ratio, in relation to the endosperm does not necessarily indicate that it is underdeveloped. For example, the E : S ratio in the carnivorous plant *Drosera anglica* (*Droseraceae*) seeds is 0.54, but embryos do not grow prior to radicle emergence (Baskin and Baskin, 2005). In their survey of underdeveloped embryos in angiosperms, Forbis *et al.* (2002) assumed that the broad embryo in *Nymphaeaceae* was underdeveloped. However, we know of no studies that have been done on seeds with broad embryos to determine if they are underdeveloped. The purpose of this research was to determine if embryo growth occurs in seeds of *Nymphaea* prior to radicle emergence, i.e. is the embryo underdeveloped or fully developed at seed maturity?

Materials and methods

Mature fruits of five taxa of *Nymphaea* (Table 1) were collected from plants growing at the Nelson Water Garden in Texas (USA), placed in water in plastic bags and immediately shipped to the University of Kentucky. Fruits were placed in pans of water at room temperature, and seeds were allowed to fall from the fruits naturally. After about 1 week, seeds were removed from the pans of water and studies were initiated; seeds were never allowed to dry.

Table 1. Embryo length : seed length ratio (E : S) in fresh seeds and embryo length in germinating seeds of *Nymphaea*

		Embryo length (mm, mean ± SE)	
Nymphaea taxon	E : S	Fresh	Seed coat split
N. Albert Greenburg[a]	0.311	0.49 ± 0.04	0.51 ± 0.04
N. capensis var. *zanzibariensis*	0.349	0.44 ± 0.01	0.50 ± 0.03
N. immutabilis	0.234	1.00 ± 0.02	1.03 ± 0.05
N. mexicana	0.168	0.69 ± 0.02	–[b]
N. micrantha	0.342	0.47 ± 0.02	–[b]

[a] Cultivar.
[b] No data.

Embryo length : seed length (E : S) ratio was determined in fresh seeds of the five taxa of *Nymphaea*. Fifteen fully imbibed seeds of each taxon were cut open lengthwise with a razor blade. The maximum internal length of each seed, which was filled with perisperm, and the excised embryo were measured using a dissecting microscope equipped with a micrometer. To determine the length of the embryo at the time of germination, seeds of each taxon were placed in jars of water under cool white fluorescent light (14 h daily photoperiod) at 12/12 h daily alternating temperature regimes of 28/20°C and 25/15°C in incubators. Seeds in both incubators were examined each day under a dissecting microscope, and any of them with a split seed coat (indicating that the radicle was about to emerge) were removed from the jars. Care was taken not to select any seeds with the radicle protruding from the perisperm. For each taxon, the embryo was removed from 15 seeds with a split seed coat. However, seeds of two of the taxa were dormant, and no seeds with a split seed coat were ever found. It should be noted that after a seed coat split, the radicle emerged within 1–2 d.

Results

There was essentially no embryo growth prior to the time of radicle emergence in seeds of any of the three *Nymphaea* taxa that germinated (Table 1).

Discussion

Depending on the species, length of rudimentary (Baskin and Baskin, 1984, 1985, 1989), linear-underdeveloped

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(Baskin *et al.*, 2005; Kondo *et al.*, 2005) and spatulate-underdeveloped (Hidayati *et al.*, 2000a, b, c) embryos may increase 350 to >1000, 50 to >1000 and 70 to 200%, respectively, before seeds germinate. Thus, the broad embryos in seeds of the three species of *Nymphaea* are not underdeveloped; consequently, seeds do not have either morphological or morphophysiological dormancy. Further, since the seed coat is water-permeable (i.e. seeds were soft when cut open), fresh seeds of *Nymphaea* can only be non-dormant or have physiological dormancy (Baskin and Baskin, 2004, 2006).

From a phylogenetic perspective, the absence of morphological or morphophysiological dormancy in seeds of *Nymphaeales* is rather surprising, and it means that this is the only member of the ANITA grade with a fully developed embryo. However, a number of other morphological characters differ among members of the grade:

(1) *Amborella* has a nine-nucleate embryo sac (Friedman, 2006), *Nymphaeales* and some *Austrobaileyales* (*Illiciaceae* and *Schisandraceae*) have a four-nucleate embryo sac (and diploid endosperm) and other basal angiosperms have an eight-nucleate embryo sac (Williams and Friedman, 2002; Friedman and Williams, 2003).
(2) Pollen is somewhat diverse among basal angiosperms, especially in the ANITA grade, but monoaperturate pollen is predominant (Endress, 2004). *Austrobaileyaceae* and *Nymphaeales* have monoaperturate pollen with special patterns near the aperture (Wiersema, 1987).
(3) Although most *Nymphaeales* have whorled floral phyllotaxis (Endress, 2004), *Nuphar* (the basal genus of *Nymphaeaceae*), *Amborella* and all *Austrobaileyales* have spirally arranged sepals (Endress, 2001; Schneider *et al.*, 2003).

The fossil record of angiosperm flowers shows that members of the ANITA grade and *Chloranthaceae*, as well as other magnoliids, early monocots and early eudicots, had differentiated by the Early Cretaceous (Friis *et al.*, 2006). A fossil flower of a plant thought to be closely related to extant *Nymphaeaceae* was collected from the Early Cretaceous (Late Aptian or Early Albian) (Friis *et al.*, 2006); thus, the broad embryo probably has been present in dicots since at least the Early Cretaceous. Recently discovered fossil seeds of *Susiea newsalemae* (*Nymphaeaceae*) from the Late Palaeocene contained a 'broad-shaped' embryo cavity, indicating that they had a broad embryo (Taylor *et al.*, 2006). The other extant dicot family with a broad embryo is *Saururaceae* (Martin, 1946), which belongs to the *Piperales* in the magnoliids. The *Piperales* also have a fossil (flower) record from the Early Cretaceous (Friis *et al.*, 2006).

Finally, if *Amborella* and *Nymphaeales* are equally the most basal angiosperms, as some molecular phylogenetic studies indicate (Qiu *et al.*, 2005, 2006), then we must conclude that the broad and rudimentary embryos are equally primitive.

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