

4 Transfer Cells in the Seeds of Boraginales³

Abstract

The presence of transfer cells in the seeds of Boraginales (Boraginaceae *s.str.*, Hydrophyllaceae *s.str.*, Heliotropiaceae, Cordiaceae, Ehretiaceae, and Lennoaceae) has been reported but has not hitherto been studied systematically. This study, surveying the seed anatomy of 50 species of Boraginales, demonstrates that in Heliotropiaceae, Cordiaceae, Ehretiaceae, and Lennoaceae transfer cells are found in an uninterrupted course from the placenta *via* the funicle to the seed coat. These taxa are characterised by indehiscent fruits with a protective endocarp. Transfer cells may act as a sponge, thus promoting rapid germination when sufficient water is available. In Hydrophyllaceae *s.str.*, which have capsular fruits, transfer cells are often found, but have a different structure and are restricted to the seed coat. In Boraginaceae *s.str.* on the other hand, transfer cells are completely lacking. The presence of transfer cells in seeds is considered as an apomorphic trait and appears to be phylogenetically informative.

4.1 Introduction

The potential to develop transfer cells (TCs) is expressed in all major taxa of multicellular plants. Since the first systematic investigation by GUNNING & PATE (1969) the occurrence, location, structure, and function of TCs have been repeatedly reported (e.g., PATE *et al.* 1970, PATE & GUNNING 1972, BONNEMAIN *et al.* 1991, THOMPSON *et al.* 2001). TCs are characterised by the presence of irregular internal cell wall ingrowths and are generally easily recognisable. The protuberances are covered by the plasmalemma, thereby considerably increasing the internal surface of the cell and facilitating water and solute transfer between tissue compartments (GUNNING & PATE 1969). TCs are mostly found where improved transport is necessary in, e.g., the endosperm of maize (*Zea mays*) and in the seed coat and the cotyledons of field bean (*Vicia faba*) and pea (*Pisum sativum*, THOMPSON *et al.* 2001). The ingrowths develop late in cell ontogeny (PATE & GUNNING 1972).

³ Published as DIANE N, HH HILGER & M GOTTSCHLING (2002b): Transfer cells in the seeds of Boraginales. Bot J Linn Soc **140**: 155–164.

Own contributions: Collecting plants (partly), investigation of Cordiaceae, Ehretiaceae, and Lennoaceae, discussion on the manuscript, drawings, arranging the plates.

In their current circumscription, Boraginales comprise Boraginaceae *s.str.* (= Boraginoideae *sensu* GÜRKE 1893), Hydrophyllaceae *s.str.*, Heliotropiaceae, Cordiaceae, Ehretiaceae, and Lennoaceae (GOTTSCHLING *et al.* 2001). The fruits of Boraginaceae *s.str.* are one-seeded mericarpids ('nutlets') with a sclerified exocarp protecting the seeds. Hydrophyllaceae *s.str.* have capsules with few to many seeds. The remaining taxa have drupes, schizocarps, or fleshy capsules (Lennoaceae) with a thick and lignified endocarp, which encloses and protects the seeds. These so-called endocarpids ('stones', 'pyrenes') enclose one, two, or four seeds, each in a separate locule. Therefore, the seed coat has no protective function.

In Boraginales, TCs in seeds have not been studied systematically. They have been described previously as "... cells with thickened, verrucose, inner tangential walls ..." (GUIGNARD 1893: 146, cited by SVENSSON 1925), "... an intracellular deposition of wall material ..." (CHANCE & BACON 1984: 830), "... U-shaped cell wall thickenings ..." (HILGER 1987: 302), or they have been illustrated but not described (e.g., BACON & BRAGG 1986: 274, 276–277). The circumstantial observation of TCs in seeds of Boraginales species led us to a more thorough and systematic survey of the presence or absence of these cells in the taxon.

In this study, we examine the presence and distribution of TCs in the seeds of 50 species of 18 genera of the six families, thus including all the major taxa of Boraginales. We compare the results with a molecular tree of Boraginales (GOTTSCHLING *et al.* 2001) to elucidate the evolution of a presumed systematic character in the group.

4.2 Materials and Methods

A list of the species investigated in this survey, including the location of voucher specimen, appears in Table 2 (appendix).

Fruits were fixed with AFE (acetic acid-formalin-ethanol). For light microscopy, the fruits were dehydrated with FDA (formaldehyde-dimethyl-acetale, GERSTBERGER & LEINS 1978) and embedded in paraplast *via* tertiary butanol. Photographs of safranin-astra blue-stained serial sections (10 μ m) were taken with a Leitz Dialux 20 microscope and a Canon EOS D30 digital camera.

For scanning electron microscope (SEM) studies, the material was dehydrated in a graded ethanol series, critical point-dried with CO₂, sputtered with gold, and analysed with a LEO 430 SEM. For transmission electron microscopy (TEM), fruit parts of *Heliotropium humifusum* were fixed in 3% glutaraldehyde (0.05 M phosphate buffer, pH 7.4), postfixed with OsO₄ (2%), dehydrated in a graded ethanol series, and embedded in SPURR's epoxy resin (SPURR 1969). Ultra-thin sections were contrasted with lead citrate (2%) plus 2% aqueous uranyl acetate, and examined using a Zeiss EM 109 transmission electron microscope.

To demonstrate water transfer *via* TCs, mericarpids of *Heliotropium erosum* were incubated for 2 d in an aqueous solution of astra blue (1 g / 250 ml). Afterwards, the pericarp was removed to observe the staining of the testa. To prove the passive water transport in TCs, some seeds were treated by heating in a microwave oven prior to soaking and staining.

4.3 Results

Taxa are abbreviated as follows: BOR (Boraginaceae *s.str.*), COR (Cordiaceae), EHR (Ehretiaceae), HEL (Heliotropiaceae), HYD (Hydrophyllaceae *s.str.*), LEN (Lennoaceae).

Position of seeds in the gynoecium. The four seeds in the indehiscent fruits of Boraginales (except HYD) are separated from the placenta by sclerenchymatous tissue. The funicle passes through the major protective layer (in BOR the exocarp, in COR, EHR, HEL, and LEN the endocarp) and joins at the placenta outside the locule. The direction of the funicle and the funicular canal therefore depends on the position of the placenta. It may be orientated horizontally, ascending, or pendulous. Ovules may be anatropous (epitropous, hanging, AGARDH 1858, GUSULEAC 1937) or apotropous (only COR *p.p.*, KHALEEL 1985). At fruit maturity, the seeds themselves fill the locule completely, and the testa is often pressed against the inner epidermis of the endocarp.

Structure and occurrence of transfer cells. TCs are found in various parts of the seeds (seed coat, funicle, placenta) of all Boraginales (Figs. 4-1 to 4-21), with the only exception of BOR, in which they are noticeably absent. Potential locations are indicated by the red dotted areas in Figure 4-1, a schematic representation of a typical Boraginales seed. In general (all COR, EHR, HEL, LEN, some HYD), they form a uniform, homogeneous, labyrinth of finger-like, branched and anastomosing ingrowths of the cell wall; they have a porous, honeycombed appearance (Figs. 4-2, 4-6 to 4-10, 4-15 to 4-21). The protuberances fill half through two-third of the cell lumen. Within HYD additional types of TCs occur (Figs. 4-3 to 4-5): *Phacelia tanacetifolia* seeds are characterised by TCs of heterogeneous structure, ranging from dense spherical corpuscles to a fragile net with a spongiouse structure within one cell (Figs. 4-4 and 4-5). *Nama demissum* shows TCs with loose and irregularly spherical corpuscles (Fig. 4-3). Other HYD such as *Wigandia urens* and *Nemophila menziensis* lack TCs.

Seed coat. TCs are either restricted to the epidermis (EHR, HEL, HYD, LEN), in which the testa consists of a single layer (Figs. 4-3 to 4-7, 4-10, 4-17, 4-20), or is present in 3 to 4 subepidermal cell layers (only COR, Fig. 4-18). The TCs of the testa epidermis are characterised by protuberances only on the inner tangential and radial cell walls, the outer tangential cell walls remain without ingrowths. The protuberances of the subepidermal layers of COR are all around and fill up to a third of the lumen.

Funicle. In COR, EHR, HEL, and LEN, a distinct layer of perivascular tissue accompanies the funicular bundle from the chalaza to the placenta (Figs. 4-2, 4-7 to 4-10, 4-15), consisting of 1 to 3 layers of TCs. Protuberances are present on all parts of the cell walls. They fill at least half of the cell lumen, but sometimes fill it completely. In HYD the funicle lacks perivascular TCs. In HEL the endocarp in the transition region to the placenta also consists of TCs (Figs. 4-9 and 4-10).

Placenta. In mature fruits of COR, EHR, HEL, and LEN, parts of the placenta consist of TCs (Figs. 4-2, 4-7 to 4-10, 4-14 to 4-16), usually undergoing a transition to 1 to 3 layers of TCs (Fig. 4-7). In *Myriopus volubilis* (HEL, Figs. 4-14 to 4-16) the placenta consists of a thick tissue of TCs.

Summarising these results, the presence and distribution of TCs is associated with the systematic placement of the respective taxa in Boraginales as demonstrated in Table 4 (appendix).

Ontogeny. Ontogeny of TCs was investigated in HEL. The ingrowths are not found in young seeds developing just before maturity. At complete maturity, the protoplast degenerates, and the TCs die (Figs. 4-7, 4-9 and 4-10).

Inhibition experiments. Water uptake in TCs was investigated in HEL and is independent of viability. Treating the seeds in the microwave does not influence the amount of staining observed (Figs. 4-11 to 4-13).

4.4 Discussion

Structure and occurrence of transfer cells. We identified TCs in the seeds of all taxa of Boraginales except BOR. These TCs had been previously vaguely described as wall thickenings of differing structure depending on the method of observation (LM, SEM, TEM) and ontogenetic state of the seeds investigated (see below).

A testa consisting of cells with U-shaped cell wall thickenings has been reported for HEL (*Heliotropium europaeum*: GUIGNARD 1893, SVENSSON 1925; *Ixorhea tschudiana*: FULVIO 1978; *Heliotropium supinum*: HILGER 1987) and EHR (*Ehretia laevis*: JOHRI & VASIL 1956; *Ehretia acuminata*: KHALEEL 1977). Our own investigations confirm their presence in *Heliotropium*, *Ixorhea*, *Myriopus*, and *Tournefortia* (HEL) as well as *Bourreria* and some species of *Ehretia* and *Tiquilia* (EHR). We are not aware of reports of TCs for either COR or LEN, although we found them in *Cordia* and *Varronia* (COR) and *Pholisma* (LEN). This may be due to the assumed unimportance for systematic studies of the poorly developed seed coat.

It may also explain why the most detailed investigations of seed coat structures are available for HYD. Seeds from capsular fruits frequently display a distinct surface pattern, which has received attention in systematic studies (e.g., CHANCE & BACON 1984, CHUANG & CONSTANCE 1992). From the literature it is clear that the presence of TCs does not follow phylogenetic patterns in HYD, not even within a single genus such as *Nama*, which may be indicative of its polyphylety that could be demonstrated in a molecular study (FERGUSON 1999). CHANCE & BACON (1984) described a species group with ingrowths of the inner tangential and radial walls of the testa, remarking on their porous, honeycombed appearance. Subsequently, BACON & BRAGG (1986) and BACON (1987) showed that these structures are much less developed in other groups of *Nama*. A one-layered testa with wall ingrowths described as "... randomly interposed spherical

corpuscles ...” has been described and illustrated for *Phacelia tanacetifolia* (SERRATO-VALENTI *et al.* 2000: 626 with Figs. 1H and 3B), but without a discussion of the function of these structures. Seeds of *Emmenanthe penduliflora* germinate after exposure to fire, and EGERTON-WARBURTON (1998) showed that the subtesta is sensitive to smoke. As can be inferred from her figures (EGERTON-WARBURTON 1998: Figs. 4, 6), the overlaying testa epidermis consists of TCs with a spongiöse structure, but, again, their possible function has not been discussed. Our own observations of *Nama demissum*, *Phacelia tanacetifolia*, and *Emmenanthe penduliflora* confirm these reports. We have also found TCs absent in *Wigandia urens* and *Nemophila menziesii* (compare CHUANG & CONSTANCE 1992). For HYD, the circumstantial documentation of TCs may be due to the seeds being examined when they are too young. Our investigations on BOR (*Borago pygmaea*, *Anchusa strigosa*, *Echium vulgare*, and *Cynoglossum creticum*), covering the four major tribes of the taxon, show that the testa, funicle, and placenta lack TCs completely. This confirms previous studies on, e.g., Eritrichieae (HOFMANN 1994), Lithospermeae (SEIBERT 1978), Cynoglosseae (*Cynoglossum denticulatum*, KHALEEL 1974), and Boraginoideae (*Borago officinalis*, GUIGNARD 1893). HILGER (1985) did not mention any TCs in his overview of BOR fruit ontogeny.

Functional aspects. The widespread occurrence of TCs in the seed coat and the funicle up to the placenta in HEL, COR, EHR, and LEN can be easily interpreted as a consequence of their fruits being indehiscent. The seeds in all these taxa are protected by a thick, lignified endocarp. Exo- and mesocarp are either fleshy or dry. Many representatives of these taxa have drupes (Figs. 1-11 and 1-12), which are dispersed endozoochorously, usually by birds (RIDLEY 1930, SNOW 1981, SCOTT & MARTIN 1984). This requires an efficient protection of the embryo during passage through the gut. The development of schizocarps in many HEL may have evolved due to their differing ecology in, e.g., *Heliotropium* species, which prefer dry and disturbed areas.

Enclosing the seeds in impermeable stony endocarps makes germination more difficult. After removal of the exo- and mesocarp and separation of the endocarpids, the placenta is exposed, and the placental TCs may act as a sponge. The subsequent transport of the water to the embryo is facilitated by the funicular perivascular tissue acting as a wick, and the seed coat then distributes the water over the seed surface. The swelling of the seed ruptures the endocarp, and germination can take place. Water uptake and transport *via* the transfer cells appear to be a passive mechanism. Following this interpretation, HYD do not need placental and funicular TCs due to the free exposure of their seeds.

We cannot yet explain why BOR lack TCs in the corresponding parts of the fruit and seed. In contrast to all other taxa of Boraginales, the protective layer is the exocarp in BOR with incrustations of silica and calcium in a spongiöse matrix (HILGER *et al.* 1993).

Phylogenetic implications. The generalised occurrence of TCs in the seed coat of HYD, HEL, COR, EHR, and LEN is considered as an apomorphy of the corresponding group. In the

molecular tree calculated by GOTTSCHLING *et al.* (2001) this clade is well-supported (100% bootstrap support value). The uninterrupted course of TCs through the funicle to the seed coat, in correlation with a multilayered endocarp, is considered as an apomorphic trait of HEL, COR, EHR, and LEN. The corresponding clade has a bootstrap support value of 85%. The multilayered testa of COR can be regarded as apomorphy.

From a phylogenetic view, TCs in seeds may be interpreted as a useful supplementary structure in indehiscent fruits. The distribution and structure of TCs in HYD differs from those in the other taxa; the most noticeable feature is their absence in the funicular region. The complete lack of TCs in the testa of some representatives is either convergence or secondary reduction. To determine whether the presence or absence of TCs in HYD is of systematic information requires further research on this often overlooked feature.