4   Transfer cells in the seeds of Boraginales[3]

Abstract

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

4.1 Introduction

The potential to develop transfer cells (TCs) is present 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 characterized by the presence of irregular internal cell wall ingrowths and are generally easily recognizable. The protuberances are covered by the plasmalemma, thereby considerably increasing the internal

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Own contributions: investigation of Boraginaceae, Heliotropiaceae, Hydrophyllaceae, and Lennoaceae, photos writing manuscript.

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, e.g. in the endosperm of maize (*Zea mays* L.) or in the seed coat and the cotyledons of the field bean (*Vicia faba* L.), and pea (*Pisum sativum* L.) (THOMPSON *et al.* 2001). The ingrowths develop late in cell ontogeny (PATE & GUNNING 1972).

In its current circumscription, Boraginales includes the families Boraginaceae *s.str.* (syn. Boraginoideae *sensu* GÜRKE 1893), Hydrophyllaceae, Heliotropiaceae, Ehretiaceae, Cordiaceae, and Lennoaceae. The fruits of Boraginaceae *s.str.* are characterized by one-seeded mericarpids ('nutlets') with a sclerified exocarp protecting the seeds. Hydrophyllaceae have capsules with few to many seeds. The remaining families 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.

Within Boraginales, TCs in seeds have not been studied systematicallye. 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 accidental 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 the present study we examined the presence and distribution of TCs in the seed of 50 species of 18 genera of the six families, thus including all the major taxa. We compared the results with a molecular phylogenetic tree of Boraginales using the ITS1 secondary structure transcript (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 specimens, appears in Table **4-1** (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 safranine-astra blue-stained serial sections (10 μm) were taken with a Leitz Dialux 20 microscope and a Canon EOS D30 digital camera.

For SEM studies, the material was dehydrated in a graded ethanol series, critical point-dried with CO_2, sputtered with gold, and analysed with a LEO 430 Scanning Electron Microscope. For transmission electron microscopy (TEM), nutlet parts of *Heliotropium humifusum* were fixed in 3% glutaraldehyde (0.05 M phosphate buffer, pH 7.4), postfixed with OsO_4 (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 two days 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

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

Position of seeds in the gynoeceum—The four seeds in the indehiscent fruits of Boraginales (except HYD) are separated from the placenta by sclerenchymatic tissue. The funicle passes through the major protective layer (in the case of BOR the exocarp, and COR, EHR, HEL, LEN the endocarp) and joins the placenta outside the locule. The direction of the funicle and the funicular channel therefore depends on the position of the placenta. It may be orientated horizontally, ascending, or pendulous. Ovules may also be epitropous or apotropous (only COR). 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 seed transfer cells—TCs are found in various parts of the seeds (seed coat, funicle, placenta) of all Boraginales (Figs. **4-1** to **4-21**), except BOR, where they are noticeably absent. Potential locations are indicated by the lightly dotted areas in Fig. **4-1**, a schematic representation of a typical Boraginales seed. In general (all COR, EHR, HEL, LEN, some HYD), they form a uniform, homogenous, 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 to two-third of the cell lumen. Within HYD additional types of TCs occur (Figs. **4-3** to **4-5**): *Phacelia tanacetifolia* seeds are characterized by TCs of heterogeneous structure, ranging from dense spherical corpuscles to a fragile net, with a spongiose structure within one cell (Figs. **4-4**, **4-5**). *Nama demissum* shows TCs with loose and irregularly spherical corpuscles (Fig. **4-3**). Other HYD such as *Wigandia urens* and *Nemophila menziensii* lack TCs.

Seed coat—TCs are either restricted to the epidermis (EHR, HEL, HYD, LEN), where the testa consists of a single layer (Figs. **4-3** to **4-7**, **4-10**, **4-17**, **4-20**), or are present in 3-4 subepidermal cell layers (only COR) (Fig. **4-18**). The TCs of the testa epidermis are characterized 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 1-3 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 the case of HEL the endocarp in the transition region to the placenta also consists of TCs (Figs. **4-9**, **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 1-3 layers of TCs (Fig. **4-7**). In the case of *Myriopus volubilis* (HEL) (Figs. **4-14** to **4-16**) the placenta consist of a thick tissue of TCs.

Summarizing these results, the presence and distribution of TCs is associated with the systematic placement of the respective taxa in the Boraginales, as demonstrated in Table **4-2**.

	BOR	HYD	HEL	COR	EHR	LEN
common dispersal unit	'nutlet'	seed	drupe/ schizocarp	drupe	drupe/ exceptional nutlet	fleshy capsule
protective layer of the seed	exocarp	-	endocarp	endocarp	endocarp	endocarp
seed coat (layers)	-	+ (1) no overall occurrence	+ (1)	+ (3-4)	+ (1)	+ (1)
funicle	-	-	+	+	+	+
placenta	-	-	+	+	+	+

Table **4-2**. Presence and distribution of TCs in seeds of Boraginales. Families are abbreviated as follows: BOR (Boraginaceae *s.str.*), COR (Cordiaceae), EHR (Ehretiaceae), HEL (Heliotropiaceae), HYD (Hydrophyllaceae), LEN (Lennoaceae).

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

Inhibition experiments—The 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 seed transfer cells—We identified TCs in the seeds of all families 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 (citations see below).

A testa consisting of cells with U-shaped cell wall thickenings has been reported for HEL (*Heliotropium europaeum* L.: GUIGNARD 1893, SVENSSON 1925, *Ixorhea tschudiana* Fenzl: DIFULVIO 1978, *Heliotropium supinum* L.: HILGER 1987) and EHR (*Ehretia laevis* Roxb.: JOHRI & VASIL 1956, *Ehretia acuminata* R.Br.: KHALEEL 1977). Our investigations confirm their presence in *Heliotropium, Ixorhea, Myriopus*, and *Tournefortia* (HEL) and *Bourreria* (HER) as

well as in some species of *Ehretia* and *Tiquilia*. We are not aware of reports of TCs 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 cannot be generalized in HYD, not even in a single genus such as *Nama,* which may indicative of paraphyly (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 within *Nama*. A one-layered testa with ingrowths described as 'randomly interposed spherical corpuscles' has been described and illustrated for *Phacelia tanacetifolia* (SERRATO-VALENTI *et al*. 2000: 626, 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: 1323, fig. 4 and 1325, fig. 6), the overlying testa epidermis consists of TCs with a spongiose structure, but, again, their possible function has not been discussed. Our own observations of *Nama demissum*, *Phacelia tanacetifolia,* and *Emmenanthe penduliflora* confirm what these authors have reported. We have also found that TCs are absent in *Wigandia urens* (sometimes separated as tribe Wigandieae) and *Nemophila menziesii* (tribe Hydrophylleae, see also CHUANG & CONSTANCE 1992). Within HYD, the sporadic documentation of TCs may also be due to beeing examined when they are too young. Our investigations into BOR (*Borago pygmaea*, *Anchusa strigosa*, *Echium vulgare*, and *Cynoglossum creticum*) cover the four major tribes of the family; they show that the testa, funicle, and placenta completely lack TCs. This confirms previous studies on tribes Eritrichieae (HOFMANN 1994), Lithospermeae (SEIBERT 1978), Cynoglosseae (*Cynoglossum denticulatum* DC., KHALEEL 1974), and Boraginoideae (*Borago officinalis* L., GUIGNARD 1893). HILGER (1985) also 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 families are protected by a thick, lignified

endocarp; the exo- and mesocarp are either fleshy or dry. Many representatives of these families have drupes which are dispersed endozoochorously, usually by birds (RIDLEY 1930, SNOW 1981, SCOTT & MARTIN 1984). This requires an efficient protection of the embryo during the passage through the gut. The development of schizocarps in many HEL may have evolved due to their differing ecology, e.g. species of *Heliotropium* 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 water to the embryo is facilitated by the funicular perivascular tissue acting as a wick, and the seed coat then distributes it over the seed surface. The swelling of the seed ruptures the endocarp, and germination can take place. Water uptake and transport via the TCs appears to be a passive mechanism. Following this interpretation, HYD do not need placental and funicular TCs due to the free exposure of their seeds.

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

Phylogenetic implications—The generalized occurrence of TCs in the seed coats of HYD, HEL, COR, EHR, and LEN is considered to be a synapomorphy of the corresponding groups. In the molecular tree proposed by GOTTSCHLING *et al*. (2001) this clade is well supported (100% bootstrap value). The uninterrupted course of TCs through the funicle to the seed coat in correlation with a hard endocarp is considered a synapomorphic trait of HEL, COR, EHR, and LEN. The corresponding clade has a bootstrap support value of 85%. The multilayered testa of COR would then be an apomorphy.

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