Compartmentation of transport and transfer events in developing seeds

John W. Patrick and Christina E. Offler

School of Biological and Chemical Sciences, The University of Newcastle, NSW 2308, Australia

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Abstract

Developing seeds are net importers of organic and inorganic nutrients. Nutrients enter seeds through the maternal vascular system at relatively high concentrations in the phloem. They exit importing sieve elements via interconnecting plasmodesmata and, during subsequent symplasmic passage, are sequestered into labile storage pools (vacuoles; starch). Transporters function to retrieve nutrients leaked to the seed apoplastic during symplasmic passage. Maternal cells responsible for nutrient release to the seed apoplast are characteristically located at the maternal filial interface. Their plasma membranes are enriched in transport proteins and, in some species, these cells are modified to a transfer cell morphology. Aplastic volumes of seeds are relatively small, but contain high concentrations of sugars, potassium and a range of amino acids. Sucrose and amino acids are taken up from the seed apoplast by one to two cell layers of filial tissues that juxtapose the maternal tissues. The plasma membranes of the uptake cells are enriched in sucrose and amino acid H⁺-ATPases. In some species, these cells are modified to a transfer cell morphology. High densities of plasmodesmata support symplasmic delivery of accumulated nutrients to underlying storage cells where polymer formation (starch, protein) takes place. Hexoses, resulting from sucrose hydrolysis and leakage to the seed apoplast, are retrieved by hexose H⁺ symporters.

Key words: Transporters, membrane transport, apoplasma, plasmodesma, symplasm, metabolism, nutrients, developing seeds.

Introduction

In higher land plants, nutrients are commonly delivered to growth and storage organs (sinks) in the phloem (Patrick, 1997). The interconnected network of sieve elements forms a supracellular compartment for the transport of nutrients, phytohormones and macromolecules from sites of nutrient assimilation (sources) to sites of nutrient utilization (sinks: Lalone et al., 1999; Fig. 1). Partitioning of phloem-delivered nutrients between competing sinks is governed by their relative ability to unload major osmotic species from the importing phloem sieve elements (Patrick, 1997). This process depends upon a set of intercellular (post-sieve element) transport events which are integrated with growth or storage functions of the recipient sink tissues (Fig. 1).

The intimate anatomical connections between cells in most sinks presents considerable technical difficulties for unambiguous experimental investigation of post-sieve element transport. However, the morphology of developing seeds, and especially those of cereals and grain legumes (Fig. 2), obviates this experimental impasse. In particular, the absence of symplasmic linkage between maternal and filial generations permits independent investigation of nutrient transport and metabolism in these two tissues (Fisher, 1995; Patrick and Offler, 1995). Not surprisingly, therefore, nutrient transport and metabolism in developing seeds has attracted considerable interest (see reviews by Thorne, 1985; Murray, 1987; Jenner et al., 1991; Wolswinkel, 1992; Patrick and Offler, 1995; Fisher, 1995; Prioul, 1996; Weber et al., 1997b, 1998) as has the question of how these processes are developmentally programmed (Wang and Hedley, 1993; Liu et al., 1996; Wobus and Weber, 1999a, b).

The present review addresses intercellular compartmentation of transport events in seed sinks and how these events interface with metabolism of imported nutrients. Key issues are illustrated by drawing on a substantive
information base compiled for cereal and grain legume seeds, with most attention focused on the filling phase of seed development. During this phase, cell expansion and differentiation are completed early. Thereafter, developing seeds are physiologically and biochemically committed to storage of nutrients (principally as starch, protein, oil, and mineral elements). The precursors of starch, oil and protein are sucrose, amino acids and amides which, together with mineral elements, are imported into seeds through the maternal vascular system (Figs 1, 2).

Seed morphology defines tissue compartments

Angiosperm seeds are comprised of an embryo and endosperm resulting from double fertilization of the egg cell and two polar nuclei, respectively. These filial organs are enclosed by a maternal seed coat, derived from one or both ovular integuments (Fig. 2). The seed coat is comprised of a vascular compartment embedded in ground tissues. The latter serves as a symplasmic compartment to process and deliver imported nutrients to the underlying filial tissues (Murray, 1987). Nutrients are released from specialized maternal cells (Fig. 2B, C; Patrick and Offler, 1995) into the seed apoplasm. In certain species (e.g. wheat), nutrients are released into a specialized apoplasmic cavity derived from schizogenous activity (Fig. 2B).

The symplasmically-isolated filial tissues collectively function as a compartment committed to storage of nutrients released from the seed coat (Fig. 2C). Nutrients are retrieved from the seed apoplasm by specialized filial cells located at the maternal/filial interface and are moved symplasmically to the cellular sites of storage (Fig. 2C; Patrick and Offler, 1995). Storage products accumulate in either the embryo (Fig. 2A – principally the cotyledons) or endosperm (Fig. 2B). Where the endosperm acts as the principal storage organ, its outer layer develops into the aleurone (Fig. 2B) at the commencement of seed filling; the remaining endosperm is cellularized (e.g. cereals) or forms a liquid syncytium (e.g. coconut; Lopes and Larkins, 1993). In contrast, when cotyledons form the final storage organ, the endosperm is substantially degraded during cotyledon expansion (e.g. grain legumes; Fig. 2A). Cotyledons, are ensheathed by a dermal cell layer and are comprised largely of storage parenchyma cells with a provascular network located in their central plane (Offler et al., 1989).

Maternal seed tissues: vascular compartment

Phloem: a compartment under pressure

Interconnected sieve tubes form a continuous symplasmic compartment from the sites of loading through which photoassimilates (Offler et al., 1989; Ugalde and Jenner, 1990c), amino nitrogen (Pate, 1986; Ugalde and Jenner, 1990d) and mineral elements (Grusak, 1994; Pearson et al., 1995) are imported into developing seeds. The pressure flow hypothesis of phloem translocation predicts import rate \( (R_i) \) of a nutrient as:

\[
R_i = L_p (P_{source} - P_{sink}) AC
\]

This bulk flow occurs through a phloem path (Fig. 1) with an hydraulic conductivity \( (L_p) \) and cross-sectional area \( (A) \) at a sap concentration \( (C) \) and is driven by a difference in hydrostatic pressure \( (P) \) between the source (leaf minor veins) and sink ends of the path.

Hydraulic conductances \( (L_p \times A) \) of axial phloem paths do not limit rates of dry matter translocation (Wardlaw, 1990). Nutrient concentrations of phloem sap are set by phloem loading in source leaves (Lalonde et al., 1999) and along the axial path. For the axial path, loading can result from remobilization of storage reserves (Wardlaw, 1990), short-term buffering (Thorpe and Minchin, 1996) or xylem-phloem transfer (Pate, 1986). Indeed, import rates of minor osmotic species rely solely on sieve element loading as illustrated for amino nitrogen (Pate, 1986) and mineral elements (Grusak, 1994; Pearson et al., 1998).
Sap concentrations of nutrients also exert an osmotic influence that generates the hydrostatic pressure difference between source and sink ends of the phloem path (Thorpe and Minchin, 1996; Fig. 1; Equation 1). This particularly applies to sucrose which is loaded to high concentrations in leaf minor veins (Lalonde et al., 1999) to account for 50–70% of phloem sap osmolality (Pate et al., 1984; Fisher and Gifford, 1986). Potassium, as the other major osmotic species in phloem sap (Pate et al., 1984), interchanges with sucrose to sustain sap osmolality (Smith and Milburn, 1980). Since all seeds developing at a node receive their phloem-imported nutrients from common sources (Wardlaw, 1990), differences in volume flow rates imported by seeds are determined by variation in hydrostatic pressures at the seed-end of phloem pathways (Fig. 1).

Hydrostatic pressure within cells results from the separation of osmotically active nutrients across their plasma membranes between protoplasts and the surrounding cell wall (apoplast). Water in protoplasmic and apoplastic compartments in developing seeds is considered to be in quasi-equilibrium as their water flows are slow (Pate et al., 1985; Jenner and Jones, 1990) relative to water movement across membranes (Nobel, 1991). Hence water potential ($\Psi$) of sieve element sap (se) approximates that of the surrounding apoplasm (a) such that

$$P_{se} - \Pi_{se} = P_a - \Pi_a \quad (2)$$

and, as a consequence,

$$P_{se} = (\Pi_{se} - \Pi_a) + P_a \quad (3)$$

where $\Pi$ is sap osmotic pressure and $P$ hydrostatic pressure (Fig. 1).

In developing seeds, seed water potentials (Fisher, 1985) and turgor pressures (Shackel and Turner, 2000) are independent of the water relations of vegetative

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**Fig. 2.** Key structural features of (A, B), and generalized cellular pathways (C) followed by water and nutrients imported into developing seeds. Diagrammatic representation of (A) grain legume and (B) wheat seed structure. (C) Block diagram showing water (ndon) and nutrient (n) transport in a developing seed. Nutrients and water exit the importing phloem by bulk flow. Water returns to the parent plant in the xylem while nutrient loss is prevented by selectively-permeable apoplastic barriers (a) and xylem discontinuities (x). Nutrients move through extensive symplastic paths via interconnecting plasmodesmata (p) in maternal and filial tissues. Nutrient exchange to and from the seed apoplast, by cells specialized for eflux and influx, occurs at the maternal/filial interface. Finally, nutrients accumulate in filial cells supporting storage product biosynthesis.
organs, and hence are under seed control. \( P_a \) has not been measured in developing seeds. However, it is likely to exert minimal influence on \( P_{se} \) relative to \( \Pi_a \). This has been shown by reproducing seed import rates into attached seed coat halves with solution osmolarities (\( \Pi_a \)) that match those of seed apoplastic saps (Wolsink, 1992). Thus, \( P_{se} \) is determined by the difference between \( \Pi_a \) and \( \Pi_b \) (Fig. 1). Small gradients in \( \Pi_{se} \) along axial phloem paths (Fisher and Gifford, 1986) indicate that all vascularity interconnected seeds probably encounter the same \( \Pi_{se} \). Thus, variation in \( P_{se} \) is determined by \( \Pi_a \) of maternal seed tissues. This issue is explored later.

**Sieve-element unloading doubles as a pressure release valve for the phloem**

Sieve elements in seeds of grain legumes and cereals are symplysmically interconnected with the surrounding ground tissues (Fig. 2C; Wang and Fisher, 1994b; Wang et al., 1994; Patrick et al., 1995; Tegeder et al., 1999). Circumstantial evidence suggests that these sympysmatic routes allow sieve elements to unload water (Murphy, 1989; Wang and Fisher, 1994b), sucrose (Offer and Patrick, 1993; Wang and Fisher, 1994a), amino acids (Hsu et al., 1984; Fisher and Macnicol, 1986), certain mineral ions (e.g. iron: Marentes and Grusak, 1998), and macromolecules (Fisher et al., 1992).

Even release of photoassimilates along seed vascular systems (Offer et al., 1989; Ugalde and Jennner, 1990c, d) is consistent with phloem sap being unloaded under high pressures by bulk flow through a lateral path with an hydraulic conductance lower than that for axial flow. There is no direct demonstration of bulk flow through plasmodesmal pores, but their small dimensions would be expected to impose substantive drag forces (cf. Murphy, 1989). However, it is significant that the size exclusion limit of simple plasmodesmata in the post-sieve element path in sink leaves is up to 50 kDa with transport occurring through their cytoplasmic annulus (Oparka et al., 1999). Simple plasmodesmata are a common feature of the post-sieve element path in maternal seed tissues (Offer and Patrick, 1984, 1993; Wang et al., 1995a). Thus, given recent observations in sink leaves (Oparka et al., 1999), these could offer a pore geometry that optimizes their hydraulic conductivities and could serve as valves to regulate volume flow of phloem sap unloaded from the importing sieve elements (Fisher, 1995; Thomas et al., 2000; Equation 1; Fig. 2C). Plasmodesmata that impose most influence on transport from the sieve elements are likely to be those located at the sieve element/vascular parenchyma interface. Characteristics of the interface supporting this claim include (1) a marked drop in sucrose concentration of approximately 200–400 mM (Fisher and Wang, 1993, 1995); (2) the lowest total plasmodesmal cross-sectional area in the maternal sympysmatic pathway (Offer and Patrick, 1984, 1993; Wang et al., 1995a).

Unloading by bulk flow through a sympysmatic route carries a number of important consequences that deserve consideration. First, bulk flow avoids a build-up of hydrostatic pressure in sieve elements as water and solute removal are coupled (Murphy, 1989). Flow into the larger volume of the vascular parenchyma dissipates sap pressure and provides an increased membrane surface area for water exchange to the seed apoplasms (Murphy, 1989). The latter could occur through pressure-sensitive aquaporins (Chrispeels et al., 1999) conferring a capacity to regulate pressure differences between sieve elements and surrounding vascular parenchyma cells. Second, bulk flow renders import rates of nutrient species independent of their metabolism/compartmenntation in recipient ground tissues. This particularly applies to minor osmotic species in the phloem sap with a consequent requirement for their fluxes to be modulated commensurate with sink demand. Third, water flow into the maternal apoplasms has the potential to compromise nutrient delivery to the filial tissues. Under these circumstances, nutrients in the seed apoplasms could be re-exported in phloem water returning to the parent plant through the xylem (Fig. 2C; Pate et al., 1985). The potential loss of imported nutrients in the return water flow is attenuated as discussed below.

**Developing seeds are apoplasmically isolated from the parent plant**

Sites at which phloem-imported water is recycled and subsequent routes followed have not been resolved in developing seeds (Jenner and Jones, 1990, and earlier papers). However, it is clear that imported nutrients are not returned to the parent plant in recycled water or by any other means (Bennett et al., 1984). This outcome is considered to be achieved by strategically positioned semi-permeable apoplastic barriers in maternal seed tissues (Wang and Fisher, 1994b; Wang et al., 1995a) and/or efficient retrieval mechanisms located along xylem export routes (Fig. 2C). In the case of the latter, nutrient recapture in pedicles is optimized by structural modifications. Examples of these include diminished xylem paths (funicles of grain legumes: Hardham, 1976; Mawson et al., 1994), or complete discontinuities (pedicles of wheat grains: O’Brien et al., 1985), with closely juxtaposed xylem and phloem elements with (Hardham, 1976; O’Brien et al., 1985) or without (Mawson et al., 1994) intervening transfer cells. These structural modifications probably contribute to the independence of seed water relations from the vegetative plant body (Fisher, 1985; Shackel and Turner, 2000); a feature central to regulating phloem import (Equation 1).
Maternal seed tissues: non-vascular compartments

Post-sieve element transport: flow paths, conductances, driving forces and mechanisms

Phloem-imported nutrients are transported through maternal ground tissues along well-defined symplasmic paths that are species specific (Wang and Fisher, 1994b; Wang et al., 1994; Patrick et al., 1995; Tegeder et al., 1999). The restricted pathways result from selective plasmodesmal closure (cf. Offer and Patrick, 1984, 1993; Patrick et al., 1995).

Symplasmic transport is likely to be rate limited by the conductivities of interconnecting simple plasmodesmata (Offer and Patrick, 1984, 1993; Wang et al., 1995a) rather than the driving forces propelling passive transport through them (Thomas et al., 2000). Simple plasmodesma have large size exclusion limits (Wang and Fisher, 1994b). This property could account for their deduced high permeabilities (Fisher and Wang, 1995) and transport rates (Offer and Patrick, 1984, 1993; Wang et al., 1995a). The mechanism of nutrient transport through plasmodesmata is unknown. However, based on measured concentration gradients and estimated permeabilities, diffusion is considered to account for symplasmic transport along the post-sieve element pathway in developing wheat grains (Fisher and Wang, 1995, but cf. Darussalam et al., 1998).

Symplasmic transport: buffering from short-term storage pools and metabolic interconversions

Post-sieve element transport through a symplasmic compartment allows for imported nutrients to be sequestered into short-term storage pools and to undergo metabolic interconversion. The former functions to buffer variation in phloem import rates and the latter to modify imported nutrients to meet the nutritional requirements of filial tissues.

Buffering capacity depends upon post-phloem path volume. For instance, the small extra-phloem sucrose pool of developing wheat grains offers minimal buffering capacity (sucrose turnover time of 1.3 h: Fisher and Wang, 1993). This characteristic is associated with minimal vacuolation (Fig. 3E). However, some buffering does occur through remobilization from the pericarp, of microelements (Pearson et al., 1998) and photoassimilates (Ugalde and Jenner, 1990c, d). The more extensive post-sieve element pathways in grain legume seeds, which are comprised of highly vaculate cells (Fig. 3A, C), provide greater scope for temporary nutrient storage. Indeed, some 70–90% of amino acids (Lanfermeijer et al., 1992) and sucrose (Patrick et al., 1993b) are located in compartments with half-times for exchange consistent with vacuolar storage. The vacuolar nutrients are readily exchanged with cytoplasmic compartments for release to the seed apoplasm and their pool sizes are estimated to meet embryo demand for 4–12 h (Lanfermeijer et al., 1992; Patrick, 1994). Starch turnover also buffers sucrose levels (Rochat et al., 1995; Dejardin et al., 1997). However, relative to vacuolar sucrose, it contributes little to the overall sucrose flux (Fader and Koller, 1985; Patrick, 1993a). Furthermore, sucrose phosphatase and sucrose synthase in seed coats of grain legumes could regulate cytoplasmic levels of sucrose through futile cycling (Dejardin et al., 1997).

Bulk flow through the phloem means that developing seeds exert little control over rates at which minor osmotic species are imported. Thus, short-term storage of these nutrients, coupled with metabolic interconversion, is critical to meet nutritional requirements of the filial tissues. Amino nitrogen, imported principally as amides (Murray, 1987; Jenner et al., 1991), undergoes considerable metabolism prior to release to the seed apoplasm (Murray, 1987; Rochat and Boutin, 1991; Ugalde and Jenner, 1990b). In addition, nitrogen metabolism increases the molar ratio of amino nitrogen released to filial tissues (Murray, 1987; Jenner et al., 1991).

Nutrient release from maternal symplasm: transport mechanisms and location

Nutrient concentration differences at sites of symplasmic/apoplasmic exchange in maternal seed tissues (Fisher and Wang, 1995) provide a driving force for efflux by facilitated diffusion through carriers (Wang and Fisher, 1995) or non-selective pores (De Jong et al., 1996, 1997). However, in some grain legume species, sucrose transport rates are independent of symplasmic and apoplasmic sucrose concentrations (Fader and Koller, 1985; Thomas et al., 2000). This is consistent with an energy-dependent release mechanism that has been detected in vitro (Thorne, 1985; Minchin and Thorpe, 1990; Walker et al., 1995). Energy-dependent sucrose release appears to be coupled in antiport with protons (Walker et al., 1995). Depending upon the stage of seed development (grain legumes: Weber et al., 1995) or plant species (maize: Porter et al., 1985), the released sucrose may undergo hydrolysis catalysed by an extracellular invertase. Less is known about amino acid release. This appears to take place through non-selective pores in pea seed coats (De Jong et al., 1997). Potassium outward rectifiers have been detected in proplasts derived from release cells of seed coats. However, predicted current flow, at in vivo membrane potentials, indicates that these channels may not be the principal route for potassium release (Zhang et al., 1997).

Photoassimilate release to the seed apoplasm is considered to occur from cells located at, or near, the entire maternal/filial interface (e.g. grain legumes) or a specific
Fig. 3. Light and electron micrographs illustrating structural characteristics of release cells of developing seeds of grain legumes, *Vicia faba* (A, B) and *Phaseolus vulgaris* (C, D), and wheat (E, F). (A, C) Light micrographs of seed coats showing large vacuolated parenchyma cells with dense peripheral cytoplasm which act as sites of release. In (A) note wall ingrowths (darts) polarized to the inner periclinal wall of the thin-walled parenchyma transfer cells. No ingrowth walls are evident in the ground parenchyma cells of (C). (E) Light micrograph of the nucellar projection of wheat grain. Note progressive differentiation of cells to form transfer cells by the extent of the wall ingrowths (darts) and degradation of cells adjacent to the endosperm cavity. The cytoplasm is dense with some small vacuoles. (B, D, F) Electron micrographs of portions of the release cells illustrating their dense cytoplasm rich in mitochondria and rough endoplasmic reticulum. (A, C) Bar=10 μm; (B) bar=0.5 μm; (D, F) bar=1.0 μm; (E) bar=50 μm. bp, branch parenchyma; ctc, crushed transfer cell; dtc, degraded transfer cell; ec, endosperm cavity; gp, ground parenchyma; m, mitochondrion; n, nucleus; p, pericarp; pc, peripheral cytoplasm; rer, rough endoplasmic reticulum; tc; thin-walled parenchyma transfer cells; v, vacuole; vb, vascular bundle; wi, wall ingrowth; → pericarp cuticle.
portion thereof (e.g. wheat; maize: Patrick and Offler, 1995). Nucellar projection transfer cells in the nucellar projection of developing wheat grains have been identified definitively as the maternal cells responsible for nutrient release (Wang and Fisher, 1994b; Wang et al., 1994, 1995a). Identification of release cells in grain legumes has relied on indirect evidence derived from membrane surface areas (Offler and Patrick, 1984, 1993) and transport properties of sucrose release (Wang et al., 1995c; Harrington et al., 1997a). The release cells of grain legumes and wheat have a strikingly similar ultrastructure (Fig. 3). In all cases, a dense cytoplasm is dominated by mitochondria often aligned to the plasma membrane and an extensive network of rough endoplasmic reticulum. However, different strategies are exhibited to generate the plasma membrane surface area required for nutrient exchange. In broad bean and wheat, plasma membrane surface area is amplified as a consequence of development of extensive wall ingrowth labyrinths (Fig. 3B, F). In contrast, release cells of French bean do not develop wall ingrowths (Fig. 3D).

**Filial compartments**

To reach filial storage sites, nutrients released from maternal seed tissues are accumulated from the seed apoplasm. Hence, membrane transport is an integral component of nutrient storage in filial tissues.

**Plasma membrane transport mechanisms: gateways to the filial symplasm**

During storage product accumulation, sucrose is taken up from the seed apoplasm of grain legumes and temperate cereals (Patrick and Offler, 1995). In tropical cereals, it undergoes extracellular hydrolysis and is accumulated as free hexoses (Porter et al., 1985, but cf. Gouger Schmalstig and Hitz, 1987). Sucrose influx exhibits a saturable component at low concentrations (less than 50 mM) and a linear component dominates at higher concentrations (Patrick and Offler, 1995). The saturable component has biochemical and biophysical characteristics consistent with sucrose:H\(^+\) symporter in seeds of grain legumes and temperate cereals (Patrick and Offler, 1995). The presence of sucrose:H\(^+\) symporters has been confirmed by isolating SUT genes (VfSUT1: Weber et al., 1997a; PsSUT1: Tegeder et al., 1999; OsSUT1: Hirose et al., 1997) or detecting their transcripts (Harrington et al., 1997a, b; Tegeder et al., 2000b; Bagnall et al., 2000). The mechanism of sugar uptake by tropical cereals is less certain, but may involve hexose:H\(^+\) symport (Thomas et al., 1992). A sucrose binding protein is located in plasma membranes of grain legume cotyledons (Ripp et al., 1988; Harrington et al., 1997a; Tegeder et al., 2000b). However, its transport function is unclear (Tegeder et al., 2000b). A key role for sucrose:H\(^+\) symport in storage product accumulation by grain legumes is suggested by a strong correlation between its maximal transport activity per cotyledon and variations in rates of dry matter gain across seed development (Harrington et al., 1997b) and between cultivars (Tegeder et al., 2000b).

At early stages of storage product accumulation in grain legume cotyledons, amide release from their seed coats enriches apoplasmic sap in amino nitrogen (Rainbird et al., 1984). Here, cotyledon uptake of amino acids occurs by facilitated diffusion (Bennett and Spanswick, 1983; Rainbird et al., 1984; Lanfermeijer et al., 1990). As storage product accumulation progresses, a saturable low affinity (K\(_m\)=5 mM) and high capacity uptake system becomes apparent (Lanfermeijer et al., 1990). This coincides with the expression of an uncharacterized amino acid transporter gene (Weber et al., 1998). In this context, an amino acid transporter, PsAAP1, has been cloned from pea cotyledons (Tegeder et al., 2000a). The AAP family encode amino acid transporters of low affinity and broad specificity (Fischer et al., 1998). These are well suited for transporting the wide range of amino acids and amides released from seed coats (Murray, 1987; Ugalde and Jenner, 1990b).

**Cellular sites of nutrient uptake**

The entire apoplasm of developing grain legume cotyledons is accessible to nutrients released from seed coats (McDonald et al., 1995), but has an overall low conductance (Gifford and Thorne, 1985). As a result, relatively steep inward-directed concentration gradients of nutrients could develop in the cotyledon apoplasm. This proposition is consistent with sucrose transporter activity being restricted to cell layers proximal to adaxial cotyledon surfaces as found for pea (Tegeder et al., 1999), broad bean (McDonald et al., 1995, 1996a, b; Harrington et al., 1997a, b; Weber et al., 1997a) and French bean (Tegeder et al., 2000b). In cotyledons of broad and French bean, transporter activity is localized to their dermal cell complexes (McDonald et al., 1996a, b; Harrington et al., 1997a, b; Weber et al., 1997a, b). In contrast, for pea, SUT expression and symporter activity declines radially inward from the dermal cell complexes through several layers of storage parenchyma cells (Tegeder et al., 1999). Expression of an amino acid:H\(^+\) symporter, PsAAP1, exhibits a similar cellular distribution to expression of PsSUT1 in developing pea cotyledons (Tegeder et al., 2000a). This suggests a common pattern of cellular localization for transporters of major nutrients imported into grain legume cotyledons.

Similar conclusions are drawn for developing cereal grains. In wheat caryopses, a low conductance
(Wang et al., 1994) cell wall encrustation (Wang et al., 1995b) is deposited at the sub-aleurone/starchy endosperm interface (Fig. 2C). This apoplastic barrier points to the modified aleurone/sub-aleurone cell layers as the principal sites for nutrient uptake from the adjoining endosperm cavity. Indeed, SUT expression (Bagnall et al., 2000) and transport activity (Niemetz and Jenner, 1993; Wang et al., 1995b) is localized to these cells and is greatly diminished in starchy endosperm cells (Niemetz and Jenner, 1993; Darussalam et al., 1998).

In addition to high densities of transporter proteins in their plasma membranes, the outermost cell layers of filial tissues exhibit ultrastructural characteristics consistent with a transport function. For instance, in seeds of many species, these cells develop secondary wall ingrowths amplifying their plasma membrane surface areas (Fig. 4A, B, E; Patrick and Offer, 1995). However, as demonstrated for cotyledons of French bean (Fig. 4C) and the modified aleurone cell layer of wheat grains (Fig. 4D), a transfer cell morphology is not of universal occurrence for cells dedicated to nutrient uptake. Irrespective, uptake cells are cytoplasmically dense with prominent nuclei, extensive networks of endoplasmic reticulum and abundant mitochondria aligned adjacent to the periclinal plasma membrane proximal to the maternal tissue (Fig. 4B, C, D). Commonly, two cell layers exhibit these features to form transport complexes (McDonald et al., 1995; Harrington et al., 1997a; Bagnall et al., 2000). In addition, plastids, protein bodies and vacuoles are diminutive or absent (Fig. 4B, D, F) suggesting that these cell complexes are primarily committed to a transport function.

Symp laminic transport between uptake and storage compartments

Spatial separation of nutrient uptake and storage compartments in filial tissues necessitates a symplasmic transport step prior to nutrient storage. These compartments are interconnected by high densities of simple plasmodesmata with a potential conductance to support nutrient transport at observed rates (Wang et al., 1995b; Table 1). That this conductive potential is realized rests on indirect structural and functional evidence (Wang et al., 1994, 1995b,c; McDonald et al., 1995; Tegeder et al., 1999; Table 1). The symplasmic path and storage compartments serve as sites of short-term storage of nutrients that buffer variations in their supply from maternal tissues (Ugalde and Jenner, 1990a, b; Fader and Koller, 1985).

During peak dry matter accumulation by developing wheat grains, all storage cells are engaged concurrently in storage product accumulation (cf. Ugalde and Jenner, 1990a, b). This raises the question of whether precursors are transported in a symplasmic compartment protected from metabolism or whether precursor substrate levels saturate the biosynthetic enzymes. The former possibility reconciles the absence of sucrose retrieval mechanisms from these symplasmic pathways (Wang et al., 1995b; McDonald et al., 1996a; Harrington et al., 1997a). A low hexose/H+ symport capacity (Harrington, 2000, but cf. Weber et al., 1997a) acts to retrieve leaked hexoses resulting from hydrolysis of sucrose entering metabolic compartments in storage cells.

Seed apoplastic compartment: go-between or unifier

The seed apoplast may be continuous between maternal and filial seed organs or be divided into a number of compartments by apoplastic barriers (Fig. 2C). Irrespective, seed apoplastic volumes are small and represent less than 4% of the tissue volume (Hsu et al., 1984; Ho and Gifford, 1984). Confidence can be placed in estimates of nutrient concentrations in apoplastic saps collected directly from wheat endosperm cavities (Fisher and Gifford, 1986) and embryo sac fluid of grain legume seeds (Murray, 1987). This level of confidence does not extend to concentration estimates of apoplastic saps collected from seeds with morphologies of closely appressed maternal and filial organs. In these circumstances, sampling procedures to collect apoplastic saps are by necessity indirect. Not surprisingly therefore, wide ranges of nutrient concentration have been reported. For example, estimates of apoplastic sucrose levels in soybean seeds range from 20 mM (Hsu et al., 1984) to 200 mM (Gifford and Thorne, 1985). In these cases, gentle centrifugation was found to be the most straightforward and reliable method to collect uncontaminated samples of seed apoplastic saps (Zhang et al., 1996). A sufficient number of independent observations are now available to conclude that apoplastic saps contain relatively high concentrations (30–120 mM) of sugars (principally sucrose; except for tropical cereals where hexoses predominate: Porter et al., 1985), amino nitrogen and potassium. Sap osmolalities are correspondingly high and range from 250–400 mOs mol during the linear phase of seed fill (Fisher and Gifford, 1985; Zhang et al., 1996).

Seed apoplasm: a purveyor of developmental signals

Maternal tissues can exert profound effects on filial development (Wang and Hedley, 1993; Cheng and Chourcy, 1999). The molecular basis of maternal control has been explored in an elegant series of studies by the Gatersleben group (for review, see Wobus and Weber, 1999b) in which the seed apoplasm conveys developmental signals.
Fig. 4. Light and electron micrographs illustrating structural characteristics of uptake cells of developing seeds of the grain legumes _Vicia faba_ (A, B) and _Phaseolus vulgaris_ (C, D) and wheat (E, F). (A, C) Light micrographs of cotyledons showing dermal cell complexes which act as uptake sites. In (A), epidermal cells have a polarized ingrowth wall (darts) characteristic of transfer cells and ingrowth wall deposition also occurs in sub-epidermal cells on walls abutting intercellular spaces (darts). No ingrowth walls are evident in the dermal complex of (C). All cells of these complexes are densely cytoplasmic with a minimum of storage product organelles. The epidermal cells have prominent nuclei and large numbers of vesicles. (E) Light micrograph of the modified aleurone and sub-aleurone cell complex bordering the endosperm cavity of a wheat grain. The modified aleurone cells have no wall ingrowths, but exhibit the same cytoplasmic features as the epidermal cells of the dermal complexes of grain legumes. The sub-aleurone cells have wall ingrowths polarized to their outer pericultural wall (darts). (B, D, F) Electron micrographs of portions of the epidermal (B, D) and modified aleurone (F) cells illustrating their dense cytoplasm rich in mitochondria and rough endoplasmic reticulum and vesicles. Note that mitochondria are closely associated with the plasma membrane. (A) Bar=25 μm; (B, D) bar=0.5 μm; (C) bar=10 μm; (E) bar=5 μm; (F) bar=1.0 μm. cw, cell wall; cwm, cell wall material; ec, endosperm cavity; epc, epidermal cell; etc, epidermal transfer cell; m, mitochondrion; ma, modified aleurone; n, nucleus; rer, rough endoplasmic reticulum; sa, sub-aleurone transfer cell; sec, sub-epidermal cell; sp, storage parenchyma cell; ve, vesicle; wi, wall ingrowth.
Table 1. Quantitative characteristics of plasmodesma interconnecting cells of developing cotyledons of Vicia faba L. and estimated sucrose fluxes

<table>
<thead>
<tr>
<th>Interconnected cell type</th>
<th>Density' (no. μm⁻¹ wall)</th>
<th>Frequency (no. μm⁻² wall)</th>
<th>Internal diameter (nm)</th>
<th>Estimated sucrose flux² (× 10⁻⁴ mol m⁻² plasma s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal transfer cell/sub-epidermal cell</td>
<td>0.47</td>
<td>2.70</td>
<td>56 ± 2d</td>
<td>2.9</td>
</tr>
<tr>
<td>Sub-epidermal cell/storage parenchyma</td>
<td>0.80</td>
<td>7.12</td>
<td>42 ± 1</td>
<td>2.2</td>
</tr>
<tr>
<td>Storage parenchyma/storage parenchyma</td>
<td>0.57</td>
<td>2.77</td>
<td>80 ± 3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Reported values for transport through plasmodesma: 1.5 × 10⁻⁴ mol m⁻² plasma s⁻¹ to 84 × 10⁻⁴ mol m⁻² plasma s⁻¹ (Offer and Patrick 1993).

'Flux computed assuming total observed rate of sucrose delivery to cotyledons of 8.8 × 10⁻¹⁰ mol s⁻¹ was transferred exclusively through the available plasmodesmatal cross-sectional area: epidermal transfer cell/sub-epidermal cell – 4.58; sub-epidermal cell/storage parenchyma cell – 4.06; storage parenchyma cell/storage parenchyma cell – 2.18.


dDensity values computed from total plasmodesma and wall lengths scored from at least three replicate cotyledons. Wall lengths (μm) scored: epidermal transfer cell/sub-epidermal cell – 645; sub-epidermal cell/storage parenchyma cell – 297; storage parenchyma cell/storage parenchyma cell – 317.

Mean ± se of mean data for at least three replicate cotyledons. Total number of plasmodesma scored: epidermal transfer cell/sub-epidermal cell – 189; sub-epidermal cell/storage parenchyma cell – 90; storage parenchyma cell/storage parenchyma cell – 117.

Seed apoplasma: a key element in co-ordinating maternal nutrient supply with filial demand

Nutrient import rates depend upon hydraulic conductivities of, and pressures in, seed transport pathways (see Equation 1 and associated text). Pathway pressures are determined by releasing major osmotic species into seed apoplastic compartments (Equation 3 and associated text). Therefore, a key issue to address is the relationship between post-sieve element transport events and final sequestration of major osmotic species. These processes are integrated to sustain nutrient pool sizes at sequestration sites as illustrated by stable sucrose concentrations in maternal, apoplastic and filial compartments across a range of dry matter import rates (Fader and Koller, 1985; Patrick, 1994b; Thomas et al., 2000). Integration could be achieved by feedback-forward and feedback signals operating along the post-sieve element transport pathway. The nature of such signals and how they are transduced is fragmentary.

Substrate feedback de-repression of amino acid transporters in grain legume cotyledons (Bennett and Spanswick, 1983) coincides with peaking of storage protein biosynthesis during later stages of cotyledon development (Bennett and Spanswick, 1983; Lanfermeijer et al., 1990). Similar co-ordination may exist for dermal cell sucrose/H⁺ symporters, the activity of which increases on reducing sucrose supplies to in vitro cultured cotyledons (Patrick and Offer, 1995). This could result from de-repression of SUT expression (Weber et al., 1997a). Feed-forward control of nutrient sequestration could be mediated by altering nutrient supplies to substrate-limited biosynthetic reactions (Jenner et al., 1991) or by nutrients (sugar; nitrogen) acting as signals to regulate gene expression of metabolic machinery (Wobus and Weber, 1999a). For example, when wheat plants were subjected to pharmacological pretreatments with auxin, sucrose/H⁺ symporter activity in the modified aleurone/sub-aleurone layers of developing grains influenced rates of starch biosynthesis by elevating endosperm levels of sucrose (Darussalam et al., 1998). However, alterations of nutrient fluxes in the filial generation of developing seeds do not occur in isolation. They depend upon co-ordination with phloem import into, and nutrient release from, maternal tissues. The latter process includes symplasmic transport through an extensive path length and ultimately efflux across plasma membranes to the seed apoplasma.

Under conditions of source-limited growth, feed-forward control by maternal tissues is readily envisioned (Fader and Koller, 1985). However, for sink-limited seed growth, it is more difficult to reconcile how primary control of dry matter accumulation could be exercised by maternal transport events. Feedback control driven by filial demand for nutrients is a more likely option. Indeed, tight homeostatic control is indicated by maintenance of sucrose levels and total osmolality in seed apoplastic fluid independent of changes in dry matter fluxes (Patrick, 1994b; Thomas et al., 2000). A number of models could account for this behaviour. All of these depend upon two important characteristics of the apoplastic nutrient pool. First, the apoplastic pool is small with a high turnover rate (< 1 h; Fisher and Gifford, 1987; Patrick, 1994b). Consequently, changes in rates of nutrient withdrawal from this pool by filial tissues have an immediate and profound effect on apoplastic nutrient levels (Patrick, 1994b). Second, the transmembrane differences in nutrient concentrations are small (Patrick, 1994b; Fisher...
and Wang, 1995). As a result, a minor change in the apoplastic pool size has a large absolute impact on transmembrane concentration (and osmotic) differences.

Release by facilitated diffusion from maternal seed tissues (Wang and Fisher, 1995; De Jong et al., 1996, 1997) provides a mechanism specific for each nutrient to respond to filial demand. However, if the filial demand is for major transported nutrients, then a decline in their apoplastic pool sizes translates into an osmotic change. This change will cause turgor pressures of maternal cells to rise (Equation 3). If the maternal apoplasms is continuous (see Fig. 2C), phloem pressure will also rise bringing about an untenable situation in which increased nutrient demand would result in slowing phloem import (Equation 1).

The above situation is averted in temperate cereals by an apoplastic barrier in their maternal apoplasms separating the endosperm cavity from the crease phloem (Fig. 2C). Under these conditions, given that nutrients move along the post-sieve element pathway by diffusion (Fisher and Wang, 1995), turgor alterations in the nucellar projection transfer cells will not influence pressures in the crease phloem. This is consistent with phloem import being unaffected when endosperm cavities of attached wheat grains were perfused with solutions of differing osmolalities and sucrose concentrations (Wang and Fisher, 1994a). These, and other observations (Fisher and Gifford, 1986; Fisher and Wang, 1995), point to symplastic unloading from the post-sieve elements being a primary control point (Fisher, 1995). An alternative strategy is based around turgor-dependent release of nutrients from maternal tissues of tropical cereals (Porter et al., 1987) and grain legumes (Wolsinkwel, 1992; Patrick and Offner, 1995). This phenomenon underpins the capacity of maternal cells to turgor regulate (Zhang et al., 1996, but cf. Shackel and Turner, 1998) and hence not compromise phloem import rates (Westgate et al., 1989). In this model, it is envisioned that sink demand for photoassimilates is communicated as a turgor signal that integrates nutrient release and phloem import (Patrick, 1994b). These turgor signals are not distorted by shifts in whole plant water relations since developing seeds are hydraulically isolated from the parent plant (Fisher, 1985; Shackel and Turner, 2000).

Concluding comments and future prospects

Developing seeds, and especially those of grain legumes and wheat, are proven experimental models to examine the compartmentation of post-sieve element transport and transfer events in sink regions. A broad understanding of nutrient flows in developing seeds has emerged. Movement occurs through extensive symplasmic compartments in both maternal and filial tissues with nutrient exchange between them localized to the maternal/filial interface. The mechanism of symplasmic transport is yet to be resolved and, as shown by Fisher, particularly merits attention for seine element unloading (Fisher, 1995). Transport and transfer processes in the maternal symplasm both quantitatively and qualitatively modulate nutrient release to the filial tissues. The dynamics of buffering nutrient supplies from short-term storage compartments awaits resolution. However, some progress is being made in identifying cellular compartments in which amino N metabolism occurs (Walker et al., 1999). Unequivocal elucidation of transport mechanisms responsible for nutrient release to the seed apoplasms and their physiological significance will rely on innovative physiological and molecular approaches. Cloning amino acid and sucrose transporter genes (Weber et al., 1997a; Hirose et al., 1997; Tegeder et al., 1999, 2000a) now provides opportunities to evaluate their role in regulating nutrient flows into filial seed tissues (cf. Tegeder et al., 2000b). Ultimately, the above information will provide a platform for discovering how post-sieve element transport and transfer events are co-ordinated and maintain the observed steady rate of storage product accumulation by developing seeds. Early evidence indicates that regulation of nutrient flows is not driven by mass action effects, but rather by finely-tuned mechanisms that sense and respond to alterations in nutrient supply and demand (Patrick, 1994b; Fisher, 1995).

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