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REVIEW PAPER

Mechanisms for cellular transport and release of allelochemicals from plant roots into the rhizosphere

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Abstract

Allelochemicals and other metabolites released by plant roots play important roles in rhizosphere signalling, plant defence and responses to abiotic stresses. Plants use a variety of sequestration and transport mechanisms to move and export bioactive products safely into the rhizosphere. The use of mutants and molecular tools to study gene expression has revealed new information regarding the diverse group of transport proteins and conjugation processes employed by higher plants. Transport systems used for moving secondary products into and out of root cells are similar to those used elsewhere in the plant but are closely linked to soil environmental conditions and local root health. Root cells can rapidly generate and release large quantities of allelochemicals in response to stress or local rhizosphere conditions, so the production and transport of these compounds in cells are often closely linked. Plants need to manage the potentially toxic allelochemicals and metabolites they produce by sequestering them to the vacuole or other membrane-bound vesicles. These compartments provide secure storage areas and systems for safely moving bioactive chemicals throughout the cytosol. Release into the apoplast occurs either by exocytosis or through membrane-bound transport proteins. This review discusses the possible transport mechanisms involved in releasing specific root-produced allelochemicals by combining microscopic observations of the specialized root cells with the physical and chemical properties of the exudates.

Key words: allelochemicals, cellular transport, exocytosis, plant roots, rhizosphere, root exudates, secondary products, transport proteins.

Introduction: exudation and rhizodeposition in plant roots

In ecological terms, the rooting zone and rhizosphere is a very competitive environment where roots of neighbouring species and microorganisms compete for space, water, nutrients, and gases. In addition to providing mechanical support, water, and nutrients, roots also perform several more specialized roles in the rhizosphere, which rely on the synthesis and exudation of metabolites (Brigham *et al.*, 1999; Bertin *et al.*, 2003; Walker *et al.*, 2003). The rhizosphere is specifically defined as a narrow region of soil surrounding the living root that is influenced by root secretions and contains associated soil microorganisms. Many bacteria thrive in this location because of the physical environment in this zone (e.g. pH and moisture) and because they can feed on sloughed-off decomposing root cells and metabolites released by living plant roots. The process whereby sloughed-off root cells are deposited in close proximity to living roots is referred to as rhizodeposition. Other soil microbes, protozoa, nematodes, and fungi also contribute to the significant nutrient cycling occurring in the rhizosphere.

Although our knowledge of root function has improved significantly over the last decade, the complex interactions occurring at the root–soil interface, including the exudation of compounds, have not been well described. We now understand that root exudation and rhizodeposition are critical

Abbreviations: ABC, ATP-binding cassette; ALMT, aluminium-activate malate transporter; ER, endoplasmic reticulum; MFS, major facilitator superfamily.

for plant responses to biotic and abiotic stresses, as well as influencing microbial dynamics in the soil. As a result, exudates can repel herbivores and microbes, stimulate symbiotic relationships, alter soil properties, and inhibit the growth of competing species (Nardi *et al.*, 2000; Watt and Weston, 2009; Mathesius and Watt, 2011).

Roots of higher plants are estimated to release between 5 and 21% of all photosynthetically fixed carbon (Marschner, 1995). This loss would appear to represent a significant carbon cost to plants, but relatively little is known about the processes controlling this function (Bias *et al.*, 2004; Mathesius and Watt, 2011). Low-molecular-mass constituents such as amino acids, organic acids, sugars, phenolics, and other secondary metabolites comprise the majority of root secretions (Bertin *et al.*, 2003). However, exudates can also include high-molecular-mass root constituents, which consist primarily of mucilage (high-molecular-mass polysaccharides) and proteins (Walker *et al.*, 2003). Soil macro- and microbiota also compete for these organic materials, many of which serve as metabolic substrates (Ryan *et al.*, 2001; McCully, 2005).

Certain root exudates and rhizodeposits are important messengers for chemical communication systems that help regulate the interactions between roots and soil organisms, and mediate processes in response to environmental stressors (Uren, 2000). When roots are under stress or encounter challenges in the rhizosphere, they often react by releasing low-molecular-mass compounds, including amino acids, organic acids, and phenolics as well as proteins. These secretions can protect the plant, thus initiating a negative form of communication in the rhizosphere (Bertin *et al.*, 2003). Alternatively, these exudates can elicit symbiotic responses that initiate legume *Rhizobium* nitrogen fixation or attract common soil microbes, which are positive forms of communication (Peters *et al.*, 1986; Mathesius and Watt, 2011; Shi *et al.*, 2011).

Limited information currently exists with respect to the spatial localization of the root exudation process. Although aged roots generally exhibit less metabolic activity than younger, actively growing roots, it is recognized that variation in metabolic activity exists along the root axis (Eshel and Waisel, 1996). The limited information available also suggests that root exudation is variable along the root axis (Marschner *et al.*, 1987; Walker *et al.*, 2003). Recently, investigators found that detached border cells that exist in close association with the root cap are the site of significant metabolic activity associated with the production of diverse proteins and currently unidentified small organic molecules. Pea (*Pisum sativum*) is the primary experimental model for border-cell function because it is the only species among many tested in which one can reliably obtain roots whose root caps are free of culturable microbial species (Wen *et al.*, 2007). The root cap and associated border cells of certain dicots such as pea have been shown to secrete a complex mixture of proteins into the rhizosphere that appear to function in protection of the root tip from infection.

In comparison with the symbiotic associations studied, the negative forms of signal communication have received less attention in recent years, largely due to the difficulty in studying complex physiochemical interactions in a diverse soil matrix (Weston and Duke, 2003; Inderjit *et al.*, 2007). Recent opinions suggest that the chemical diversity of plant-derived natural products could be the result of adaptive evolution or niche colonization through defence-related signalling processes (Bednarek and Osbourne, 2009). Given the diverse roles of plant natural products, some have argued that a broader view be taken of the mechanisms driving chemical diversification in plants. To date, the specific role(s) of many secondary products in plant defence remain unknown, but studies using available mutants and gene silencing have provided further elucidation (Bednarek and Osbourne, 2009).

Role of allelochemicals in the rhizosphere

Plant-derived natural products associated with plant defence are commonly referred to as allelochemicals, and allelopathy is defined as the process mediated by the production and release of bioactive secondary products by plant parts that impacts on the establishment of neighbouring plant species (Rice, 1984). Allelopathy has been studied most recently in the context of its effects on agricultural systems (Weston, 2005), and its effects can be positive or negative in terms of crop establishment and performance (Weston and Duke, 2003). Allelopathic crops may be used to effectively suppress weeds, and invasive weeds could rely, in part, on allelopathic interactions to facilitate their establishment and wider distribution (Inderjit *et al.*, 2007). However, allelopathic interactions in the rhizosphere are less well characterized than above-ground interactions (Inderjit *et al.*, 2007). Root-produced allelochemicals are generally associated with the reduction in neighbouring plant growth, and resistance to or suppression of plant pathogens, soil microbes, and other herbivores. We now have the capacity to characterize the production and release of minute quantities of bioactive secondary products in the rhizosphere, and to study their release and metabolism in soils (Mohney *et al.*, 2009).

Exudates from some plants contain many different constituents, while in others larger quantities of specific allelochemicals occur (Watt and Weston, 2009). Plants that produce copious quantities of phytotoxins or allelochemicals often employ mechanisms to prevent autotoxicity. It has been estimated that higher plants can produce over 10,000 different allelochemicals, which vary in their activity and mode of action in receptor plants. An exhaustive list of these compounds is not presented here, but this subject has been well reviewed (Rice, 1984; Quasem and Foy, 2001; Weston and Duke, 2003). The protective mechanisms employed by higher plants have been examined in some living plant cells and root cell suspension cultures (Yazaki, 2005; Yazaki *et al.*, 2008), but they have not been as well characterized in living root systems. However, we now understand that plant cells that produce bioactive secondary products typically have specific transport mechanisms within specialized plant cells to move these compounds around and out of the cell (Grotewold, 2004). Molecular approaches are

currently being utilized to study the mechanisms transporting secondary plant products.

This review describes the identified transport mechanisms involved in the exudation of selected allelochemicals and metabolites from higher plant roots. It provides examples of the specialized root cells involved with allelochemical production and examines the immediate fate of the chemicals released into the rhizosphere.

Transport of secondary metabolites in plants and plant roots

More is known about the synthesis of most secondary metabolites and their function in the rhizosphere than about their export from roots. To be released from cells, a compound needs to cross at least one membrane and to permeate the cell wall. Effective pore size in plant cell walls is approximately 2 nm (Berestovsky *et al.*, 2001), indicating that the release of most secondary metabolites should not be restricted by the cell wall. The passage of larger metabolites and proteins through the cell wall could be impeded by pore size. The endodermis (located between the cortex and stele) and hypodermis (located beneath the epidermis) will prevent diffusion of substances into the rhizosphere if they are released into the apoplast behind these apoplastic barriers. The lipid solubility of charged or polar solutes is generally very low and prevents them from crossing biological membranes unassisted. Highly lipophilic compounds might be able to diffuse directly across the lipid bilayer, but most secondary metabolites remain too polar to simply diffuse through membranes, especially if modified by glycosylation, acylation, or hydroxylation reactions (Sirikantaramas *et al.*, 2008; Dixon *et al.*, 2010). Other transport pathways are required to move these compounds across membranes.

The release of volatile compounds from plants may also be regulated by specific transport processes (Dudareva *et al.*, 2004). For instance, the emission of volatile monoterpenes from peppermint (*Mentha×piperita* L.) is inconsistent with simple concentration-dependent diffusion out of the cells (Gershenzon *et al.*, 2000). This may partly be explained by the sequestration of the compounds in storage compartments or by the specific physicochemical properties of the compounds (Walker *et al.*, 2003; Niinemets *et al.*, 2004). It could also be attributed to the involvement of transport processes such as subcellular vesicles, which diffuse to the cell periphery and fuse with the plasma membrane to release their contents (exocytosis) or specific membrane-bound transport proteins embedded in the plasma membrane (Walker *et al.*, 2003). The following discussion provides examples of some of the mechanisms involved with transport of secondary metabolites around the cytosol and into the apoplast.

Vesicular transport

Indications that vesicles are involved in secondary metabolite transport arose from reports of small coloured bodies in the cytoplasm and the multiplication of these bodies in plant cells challenged with pathogens (Snyder and Nicholson, 1990; Ju *et al.*, 2005). Current models predict that vesicles or specialized organelles transport newly synthesized secondary metabolites to other storage compartments or to the plasma membrane for efflux (Grotewold, 2001; Robatzek 2007) (Fig. 1). Specific examples for the involvement of exocytosis in rhizodeposition are presented later. As mentioned above, many allelopathic compounds are also cytotoxic to the host cells, and so their separation from the cytosol by membrane-bound vesicles is one solution for safely transporting these compounds around the cytoplasm. For example, toxic flavonoids are synthesized in the same region of the endoplasmic reticulum (ER) from which vesicles originate, so the

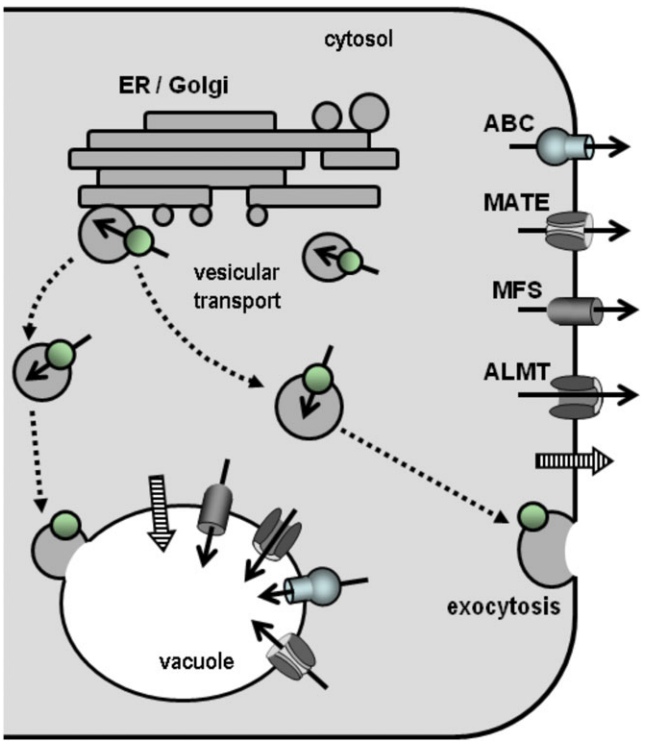

Fig. 1. Diagram of a root cell showing some of the pathways and proteins involved in transporting certain organic compounds and specialized metabolites around the cytosol and exporting them to the rhizosphere. Vesicles budding off from the endoplasmic reticulum and Golgi can be loaded with specialized metabolites, and these are directed either to the tonoplast or to the plasma membrane where they fuse with these membranes and release their contents into the vacuole or into the extracellular space (exocytosis). The round symbol depicts a generic transporter loading compounds into the vesicles. The membrane-bound transport proteins known to facilitate the transport of compounds across membranes include the ATP binding cassette family (ABC), the multidrug and toxic compound extrusion family (MATE), the major facilitator superfamily (MFS) and the aluminium-activated malate transporter family (ALMT). The striped arrow indicates the possible diffusion pathway of highly hydrophobic compounds across the lipid bilayers. The other arrows show the direction of substrate movement. Specific examples for each of these protein families are provided in the text.

loading of these vesicles with phytochemicals and their separation from the ER is likely to be well coordinated (Grotewold, 2001). Vesicular trafficking and exocytosis are known to be involved in combating pathogen attack in leaves because membrane-bound vesicles and other organelles (e.g. Golgi, ER, and peroxisomes) accumulate adjacent to the appressoria during the early stages of infection. The importance of these vesicles is highlighted by an *Arabidopsis* mutant called *pen1*, which shows enhanced susceptibility to powdery mildew invasion (*Blumeria graminis*). *PEN1* (penetration resistance1) encodes a syntaxin protein that forms part of the t-SNARE complex required for the docking and fusion of vesicles to the plasma membrane, and mutants show altered syntaxin activity (Robatzek, 2007; Kwon *et al.*, 2008). The content of these vesicles is unclear but could include antimicrobial compounds or material required for cell-wall repair. Similar pathways may defend roots from pathogen infection.

Membrane-bound transport proteins

Membrane-bound proteins provide an alternative pathway for metabolite release from cells, and several families of proteins have been implicated in the transport of secondary compounds across plant membranes. Some localize to the plasma membrane where they can directly export compounds from the cell. Others localize to internal membranes where they can help sequester compounds within subcellular compartments or perhaps load vesicles ready for exocytosis. These proteins include the ABC (ATP-binding cassette) family, the MATE (multidrug and toxic compound extrusion) family, the major facilitator superfamily (MFS), and the ALMT (aluminium-activate malate transporter) family of transport proteins (Fig. 1).

ABC proteins

The ABC proteins comprise a large and ancient family of proteins that occur in all phyla. They drive the transport of a very broad range of substrates (metabolic products, ions, lipids, and xenobiotics) using the energy from ATP hydrolysis. Therefore, these are primary active transporters, which have the potential to transport substrates against their electrochemical gradient. In eukaryotic cells, ABCs export substrates from the cytosol to the apoplast across the plasma membrane or from the cytosol to other internal organelles such as the vacuole. There are >100 putative ABCs in the genomes of rice and *Arabidopsis*, some of which have been implicated in the transport of glutathione conjugates (Martinoia *et al.*, 2002), chlorophyll catabolites (Tommasini *et al.*, 1998), auxins (Noh *et al.*, 2001; Geisler and Murphy, 2006), anthocyanins (Goodman *et al.*, 2004), and antifungal compounds, among others. For example, ABCs are thought to export the antifungal diterpene sclareol from *Nicotiana plumbaginifolia* leaves (Jasinski *et al.* 2001) and the isoflavone genistein from soybean roots (Sugiyama *et al.*, 2007). Genistein is a signalling molecule that is released from the roots of many legume species to stimulate nodulation. It may also function as a phytoalexin due to its slight antimicrobial activity (Geibel, 1994). Another gene required for powdery mildew resistance in *Arabidopsis* encodes an ABC transporter called PEN3. PEN3 localizes to the plasma membrane, and its recruitment to sites of pathogen attack on the epidermal cells and root hairs is triggered by pathogen-associated molecular patterns such as flagellin and chitin (Stein *et al.*, 2006). PEN3 could be releasing antimicrobial compounds, including glucosinolate derivatives into the apoplast adjacent to the appressoria to restrict pathogen invasion. Interestingly, PEN3 (=AtPDR8) also prevents heavy-metal toxicity by exporting cadmium ions or cadmium conjugates from root cells (Kim *et al.*, 2007), indicating that the same transporter performs different functions in different tissues. Another pathogen-related example from leaves is *Lr34*, one of the most important disease-resistance genes in wheat. Lr34 is a full-sized ABC transporter expressed in leaves that is necessary for durable rust resistance in wheat around the world (Krattinger *et al.*, 2009). The function of Lr34 remains unclear, but one model proposes that Lr34 protects infected cells by exporting antimicrobial compounds into the apoplast in response to pathogen attack. Badri *et al.* (2008) identified 25 ABC transporter genes highly expressed in *Arabidopsis* root cells that are likely to be involved in secretion processes. Plants with knockout mutations in these genes were obtained, and the exudates collected from wild-type plants and mutant lines were compared. They found that exudate composition differed between the wild-type and mutant plants and that more than one ABC transporter could be involved in the secretion of a specific phytochemical.

MATE proteins

MATE genes encode transporters that also export a wide range of substrates including secondary metabolites. The family occurs in eukaryotes and prokaryotes (Hvorup *et al.*, 2003; Magalhaes, 2010), and certain members in bacteria and mammals are responsible for multidrug resistance. While the details of their function are not well understood, MATEs appear to act as secondary active transporters that utilize the electrochemical gradient of other ions (sodium or protons) to drive substrate movement across membranes. *Arabidopsis* has 58 *MATE* genes in its genome, and *ALF5* is one of the few that have been characterized in detail. This gene is expressed in the epidermis and cortex of roots and is proposed to protect plants from xenobiotic compounds in the environment by exporting them from the root cells (Diener *et al.*, 2001). Another *Arabidopsis* gene named *AtDTX1* encodes a plasma membrane-localized protein that facilitates the efflux of plant-derived alkaloids, antibiotics, and other toxic compounds from roots (Li *et al.*, 2002). Other *MATE* genes in sorghum (*SbMATE1*), barley (*HvAACT1*), and *Arabidopsis* (*AtMATE1*) confer aluminium resistance by facilitating an aluminium-activated efflux of citrate anions from root apices (Furukawa *et al.*, 2007; Magalhaes *et al.*, 2007; Liu *et al.*, 2009). Citrate released from the roots is thought to chelate the toxic Al^{3+} cations in

the apoplast and prevent damage occurring to the rapidly dividing and elongating cells in the apices (Ma *et al.*, 2001; Delhaize *et al.*, 2007). More recently a MATE protein in rice, named PEZ1, was shown to export phenolic compounds into the xylem (Ishimaru *et al.*, 2011) and the authors speculated that similar proteins might also be facilitating the transport of phenolic compounds into the rhizosphere.

MFS proteins

The release of a special class of secondary metabolites called phytosiderophores is important for iron nutrition in grasses (Marschner *et al.*, 1987). These phytosiderophores chelate ferric iron in the rhizosphere and the entire complex is then transported into the root cells by proton-coupled transporters belonging to the oligopeptide transporter family (Kim and Guerinot, 2007). However the initial export of these compounds from roots involves the MFS proteins, one of the largest and most diverse group of transport proteins in biology. Individual members of this family can function as uniporters, co-transporters, or antiporters. One member in rice, named TOM1 (transporter of mugineic acid family phytosiderophores1), releases deoxymugineic and avenic acids from the roots of iron-deficient rice plants (Nozoye *et al.*, 2011). The expression of *TOM1* is induced when the iron supply is restricted, and transgenic plants overexpressing *TOM1* show enhanced deoxymugineic acid release and greater tolerance to a restricted iron supply.

ALMT proteins

Resistance to aluminium toxicity in many plant species, in both monocotyledons and dicotyledons, relies on the efflux of malate anions from roots in much the same way that citrate efflux contributes to the resistance of sorghum, barley, and *Arabidopsis* (see above; Ryan *et al.*, 2011). Members of the *ALMT* gene family facilitate malate efflux from these plants. *ALMT* genes occur in plants but not in animals or bacteria, and they encode anion channels that form selective pores in membranes and allow substrates to move across the membrane in a passive manner (down their electrochemical gradients). This means that, at the normal resting membrane potential difference across the plasma membrane (–100 to –200 mV, cytosol negative) or across the tonoplast (–10 to –50 mV, cytosol negative), ion channels would usually facilitate anion efflux from the cytosol or cation influx. The ALMT proteins involved in aluminium resistance are located on the plasma membrane of root cells, and aluminium ions are required to activate their function. Heterologous expression of the wheat gene *TaALMT1* in tobacco-suspension cells (*Nicotiana tabaccum* L.), barley, wheat, and *Arabidopsis* confers an aluminium-activated malate efflux and enhanced resistance to Al^{3+} stress (Delhaize *et al.*, 2004; Sasaki *et al.*, 2004; Pereira *et al.*, 2010; Ryan *et al.*, 2011). The relative expression of *TaALMT* in these systems is strongly correlated with malate efflux, so the transport step appears to be the major rate-limiting step in this process.

Allelochemical production and release from roots: selected examples

Exudation from root hairs

Sorghum spp. including Johnson grass (*Sorghum halpense* L. Pers.) and sorghum sudangrass hybrid (*Sorghum bicolour*×*Sorghum sudanese*) produce copious quantities of root exudates containing potent allelochemicals. Chemical characterization has shown that sorghum exudates contain several related long-chain hydroquinones, primarily sorgoleone and its resorcinol analogue, which inhibit the growth of nearby plants by a variety of mechanisms including the inhibition of photosynthesis and respiration (Czarnota *et al.*, 2001; Dayan *et al.*, 2009). To localize the sites of exudate production and secretion, roots of sorghum species were investigated microscopically using light, cryoscanning electron and transmission electron microscopy (see Figs 2A–C and 3 for examples). Sorgoleone, its resorcinol analogue, and other related hydroquinones are produced solely by living root hairs and are exuded as golden-coloured droplets from the tips of root hairs (Czarnota *et al.*, 2003; Dayan *et al.*, 2009; Fig. 2A, B). Interestingly, key biosynthetic genes responsible for the production of sorgoleone, specifically *SOR1*, which encodes a desaturase enzyme, are also only expressed in root hairs (Yang *et al.*, 2004). The droplets are clearly visible at the ends of hairs in humid or wet environments (Fig 3A), including in soil pores, which maintain a high relative humidity (Fig. 3B). Small globules of cytoplasmic exudate (Fig. 2C) are thought to be transported across the cell and deposited between the cell wall and the plasma membrane. They merge to form larger globules, which pass through the cell wall and appear as droplets on or near the tip of living root hairs. Microscopic studies using light, scanning and transmission electron microscopy techniques suggest that large quantities of these globules are deposited in the apoplast (Czarnota *et al.*, 2001, 2003; Fig. 3D). The production of a sorgoleone-containing droplet from the root hair tip appears to be associated with a feedback mechanism that allows increased production and exudation of sorgoleone, if droplet removal occurs (Dayan *et al.*, 2009). This feedback mechanism may occur in the rhizosphere where hairs contact soil particles, as exudate is continually wicked away from the hair (Fig. 3E). Interestingly, droplets of any description are observed much less frequently at the ends of root hairs of other graminaceous plants such as wheat (compare Fig. 3B and C).

Sorgoleone and other long-chain hydroquinones are cytotoxic due to their capacity to inhibit cellular electron transport processes such as respiration, and so their separation from the cytosol in membrane-bound vesicles allows safe transport around the cell (Bertin *et al.*, 2003). Czarnota *et al.* (2001, 2003) showed that sorgoleone synthesis is associated with the smooth endoplasmic reticulum and possibly with Golgi bodies. Similar to the toxic flavonoids produced by some roots (Grotewold, 2001), sorgoleone is synthesized in the same region from which vesicles originate in the cell, so careful coordination of synthesis and loading into vesicles

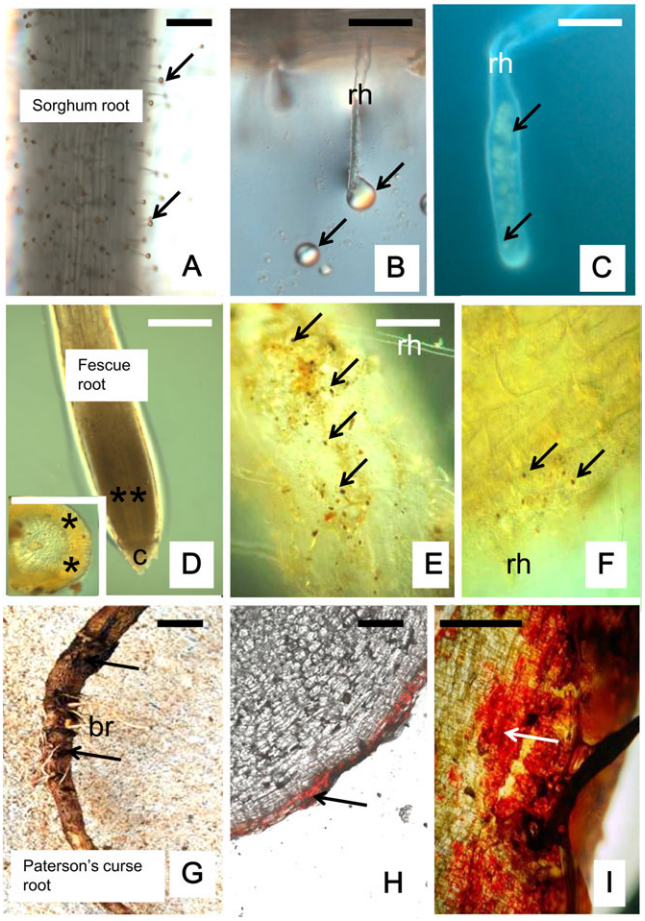

Fig. 2. Three examples of roots with allelochemical exudates. A–C, sorghum; D–F, fine fescue; G–I, Paterson's curse. All roots and cells were viewed unstained with bright light, except for (C), which was viewed unstained with UV optics, and (E) and (F), which were stained with Millon's reagent and viewed with bright-field optics. (A) Root hairs (rh) extending into humid air, all with droplets of pigmented, yellow exudate containing long-chain hydroquinones at their tips (arrows). Bar, 1 mm. (B) Sorghum root hair in water with a droplet of exudate at the tip (upper arrow). A droplet is floating unattached, indicating that long-chain hydroquinones are not soluble in water (lower arrow). Bar, 500 μm. (C) Sorghum root hair mounted in water and viewed with UV optics with yellow vesicles inside (arrows). Bar, 20 μm. (D) Fine fescue root with cells containing *m*-tyrosine within the meristem and elongation zone (asterisks) behind the cap (c). Bar, 200 μm. The inset shows a cross-section through the tip. The outer cells (asterisks) are yellow with *m*-tyrosine compared with the clear, inner cells. (E, F) Thick sections of fine fescue roots treated with a tyrosine-specific stain, Millon's reagent, and viewed with bright-field optics under a compound microscope showing dark yellow deposits of tyrosine-containing compounds located in the surface layers of the root tips (arrows). A high *m*-tyrosine-producing cultivar is shown in (E) and a low *m*-tyrosine-producing cultivar in (F). Bar, 50 μm (E, F). (G) Paterson's curse root with fine branch roots (br). The arrows indicate the blackened peridermal surface. Bar, 5 mm. (H) Cross-section through the root shown in (G). The outer periderm cells have a red pigment (arrow). Bar, 50 μm. (I) Higher magnification of the outer peridermal surface shown in (H), indicating cells with red-coloured pigments including napthoquinones (arrow). Bar, 50 μm.

could be occurring. Therefore, current models propose that, in *Sorghum* spp., small organelles or vesicles such as those shown in Fig. 2C and Fig. 3D transport newly synthesized sorgoleone to other storage compartments or to the plasma membrane for efflux (Battey and Blackbourn, 1993; Grotewold, 2001).

As sorgoleone and related hydroquinones are highly hydrophobic (see the intact drop in water in Fig. 2B), some partitioning into and perhaps across lipid bilayers is possible. Root hairs typically exude these droplets throughout their lifetime, which generally consists of several days to several weeks. Exudation has been observed as early as 3–4 h following seed germination and radical elongation in sorghum seedlings (Czarnota *et al.*, 2003), but the rate of exudation is dependent on environmental factors including plant stressors. The sheer volume of sorgoleone produced by living root hairs may result in continual exudation in functional root hairs, and dispersal may be facilitated by rapid partitioning across lipid bilayers.

Exocytosis and root exudation

Fine leaf fescues (*Festuca rubra* subsp. *commutata*) have been shown to produce large quantities of more polar root exudates, which originate from fibrous secondary root tips (Bertin *et al.*, 2003, 2007; Fig. 2D). Fibrous living fescue roots have been studied microscopically to evaluate site of production and localization of water-soluble bioactive exudates in root cells. Bertin *et al.* (2003, 2007) discovered that the outer layers of root cells synthesize and release phytotoxic exudates (Fig. 2D). Transmission electron microscopy indicated large numbers of small osmiophilic inclusions or vesicles, which were located throughout layers of epidermal cells in the root tips (Bertin *et al.*, 2003). Further chemical characterization studies indicated that the major bioactive constituent of fine fescue exudates was *m*-tyrosine, a potent inhibitor of radical elongation and seedling growth in many species (Bertin *et al.*, 2007). Light microscopy studies with the tyrosine-specific stain Millon's reagent showed that distinctive yellow deposits of tyrosine-containing compounds were located in the outer layers of root tips and on tip surfaces but not generally elsewhere in the living root system. Certain cultivars produced greater levels of *m*-tyrosine than others, and this was also visualized by tyrosine-specific staining in root tip epidermal cells (see Figs 2E and F, showing a high and a low *m*-tyrosine producer). Water stress applied to young fescue roots also resulted in greater levels of exudation of secondary products (Bertin *et al.*, 2003, 2009).

Over time, certain weed-suppressive fescue cultivars were observed to produce roots that generated large quantities of coloured constituents from the root tips. The root tips of these plants were brownish-gold in colour and the root exudates, when collected by extraction, were a similar colour (Fig. 2D). Given that *m*-tyrosine is water soluble and extremely toxic to developing roots, exocytosis may facilitate its release, but the involvement of other transporters cannot be dismissed. It is even possible that more than one mechanism is utilized to release large quantities of toxic secondary

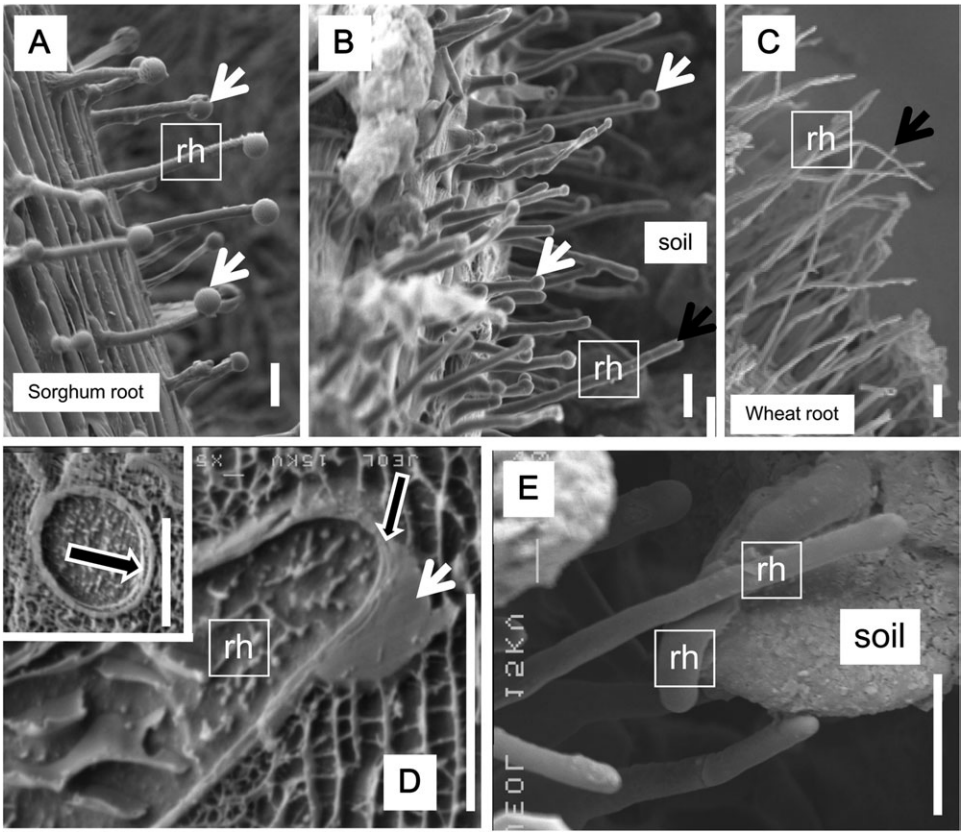

Fig. 3. Sorghum root hairs (except for C, which is wheat root hairs), frozen in liquid nitrogen and viewed frozen using a cryo-scanning electron microscope. (A) to (D) are viewed as a whole mount, while the root hairs in (E) were planed with a cryo-microtome. (A) Sorghum seedling grown on filter paper. The droplets at the ends of the root hairs contain long-chain hydroquinones, including sorgoleone (compare with Fig. 2A). (B) Sorghum grown in sand. The root hairs extend into a pore space. Most have droplets of long-chain hydroquinones. (C) Wheat root from the field with root hairs extending into a pore. No droplets were observed at the tips of the root hairs. (D) The root hair (rh) in the main image was planed longitudinally, revealing the internal area of the root hair and a droplet of exudate outside the tip. The inset shows a transverse section with arrow indicating potential space for deposition of long-chain hydroquinones between wall and membrane. The white arrow indicates the tip of the root hairs with a droplet of long-chain hydroquinones; black arrows indicate the tip of the hair without an exuded droplet. (E) Sorghum root hairs in close proximity to a sand particle, with no droplets observed. Bars, 500 μm (A–C and D, main picture); 10 μm (E and inset in D).

products from living fescue roots. In addition, processes such as glycosylation or glutathione conjugation may also be used to transport the toxic constituents across the cell until deposition into larger storage vacuoles for protection of cellular organelles and processes (Yazaki, 2005). The role of ABC-type transporters has been indicated in the transport and storage of glutathione conjugates in plant cells (Martinoia *et al.*, 2002).

Rhizodeposition by periderm degradation

Paterson's curse (*Echium plantagineum*) is a highly invasive broad-leaf weed species that produces a number of interesting bioactive secondary products, including a suite of pyrrolizidine alkaloids in its shoots that render it toxic to numerous herbivores, and red- coloured napthoquinones in its roots that inhibit the growth of microorganisms and plants (Weston *et al.*, 2011, Figs. 2G–I). Napthoquinones are used commercially in dyes and medicines (Tabata and Fujita, 1985). These bioactive compounds are produced in large quantities specifically in the outer layer of the periderm in living roots. The periderm is the outer corky layer of woody stems and roots, consisting of cork cambium, phelloderm, and cork. The bioactive compound production is strongly dependent on the immediate soil environment and especially on plant injury, drought or herbivory. Young roots are often red due to the accumulation of napthoquinones in the periderm (Fig. 2H), and red vacuoles and vesicles are clearly visible throughout these cells using light and fluorescence microscopy, performed without additional staining (Fig. 2I). Napthoquinones in related species are also assumed to be contained in vesicles (Brigham *et al.*, 1999).

The napthoquinones present in Paterson's curse roots include shikonin derivatives, specifically 1,8 dihydroxy-3-methylanthroquinone and acetyl shikonin, among others (Weston *et al.*, 2011). These compounds are soluble in ethanol and methanol, indicating that they are moderately polar. The periderm tissues are regularly sloughed off the roots of Paterson's curse due to natural degradative processes, and as this occurs, napthoquinones are released into the rhizosphere

(Weston *et al.*, 2011). Other transport proteins may be required to load napthoquinones into the vesicles. Research performed with related species in the Boragineaceae (*Lithospermum erythrorhizon*) noted that shikonin derivatives were produced in response to pathogen elicitors, and served an important role in protection of the plant against root pathogens such as *Rhizoctonia*. For example, shikonin content increased 30-fold in 'hairy' root cultures challenged by *Rhizoctonia solani* elicitors (Brigham *et al.*, 1999). Plants in the borage family are capable of producing large quantities of these products very quickly, so mechanisms must be available for the safe storage of these products in vacuoles and vesicles, or for their rapid release to the rhizosphere, either by tissue degradation/sloughing or by other membrane-bound transport proteins.

Conclusions

The transport systems deployed for movement of secondary plant products in roots are probably similar to those used elsewhere in the plant, but much larger quantities of secondary products are typically released from roots than from leaves. A variety of transport mechanisms are employed to move bioactive secondary products around root cells and into the rhizosphere, and these fluxes are often highly dependent on the local soil environment and root health. This review has highlighted the two likely pathways for the safe storage and exudation of secondary metabolites, including potentially harmful ones. One involves subcellular vesicles, which move to the plasma membrane and release their contents into the apoplast, while the other relies on specific transport proteins embedded in the plasma membrane. In living plant cells, the involvement of vesicular pathways is important for the prevention of autotoxicity. Although we do not yet understand all the details of secondary product synthesis and transport by cells, we are beginning to understand the conditions that affect production and to predict the transport mechanisms involved based on microscopic studies and the physical and chemical properties of the bioactive constituents in question. Molecular techniques can then be used to target candidate genes based on these observations, or to search for novel genes through transcriptomic and physiological approaches. The transport proteins identified to date already form a diverse group, but more are likely to be discovered as the research continues. Importantly, the transport of phytochemicals across membranes may be as critical for their biological function as the pathways regulating their synthesis. The further manipulation of these transport pathways in transgenic plants should provide useful strategies for studying the complex interactions occurring in the rhizosphere.

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