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# Root exudates as mediators of mineral acquisition in low-nutrient environments

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**Abstract**

Plant developmental processes are controlled by internal signals that depend on the adequate supply of mineral nutrients by soil to roots. Thus, the availability of nutrient elements can be a major constraint to plant growth in many environments of the world, especially the tropics where soils are extremely low in nutrients. Plants take up most mineral nutrients through the rhizosphere where micro-organisms interact with plant products in root exudates. Plant root exudates consist of a complex mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions (e.g. $HCO_3^-$, $OH^-$, $H^+$), gaseous molecules ($CO_2$, $H_2$), enzymes and root border cells which have major direct or indirect effects on the acquisition of mineral nutrients required for plant growth. Phenolics and aldonic acids exuded directly by roots of $N_2$-fixing legumes serve as major signals to Rhizobiaceae bacteria which form root nodules where $N_2$ is reduced to ammonia. Some of the same compounds affect development of mycorrhizal fungi that are crucial for phosphate uptake. Plants growing in low-nutrient environments also employ root exudates in ways other than as symbiotic signals to soil microbes involved in nutrient procurement. Extracellular enzymes release P from organic compounds, and several types of molecules increase iron availability through chelation. Organic acids from root exudates can solubilize unavailable soil Ca, Fe and Al phosphates. Plants growing on nitrate generally maintain electronic neutrality by releasing an excess of anions, including hydroxyl ions. Legumes, which can grow well without nitrate through the benefits of $N_2$ reduction in the root nodules, must release a net excess of protons. These protons can markedly lower rhizosphere pH and decrease the availability of some mineral nutrients as well as the effective functioning of some soil bacteria, such as the rhizobial bacteria themselves. Thus, environments which are naturally very acidic can pose a challenge to nutrient acquisition by plant roots, and threaten the survival of many beneficial microbes including the roots themselves. A few plants such as Rooibos tea (*Aspalathus linearis* L.) actively modify their rhizosphere pH by extruding $OH^-$ and $HCO_3^-$ to facilitate growth in low pH soils (pH 3 – 5). Our current understanding of how plants use root exudates to modify rhizosphere pH and the potential benefits associated with such processes are assessed in this review.

## Introduction

Plant developmental processes are controlled by internal molecular signals that depend on the adequate supply of mineral nutrients by soil to roots. Changes in the levels of certain nutrients are reported to alter tissue concentrations of cytokinins and abscisic acid (Moorby and Besford, 1983), two major signals controlling plant growth. Consequently, seedling development has a high nutrient demand not only for growth of individual organs, synthesis of new cytoplasm as well as sub-cellular organelles and cell walls, but also for both cell division and expansion (Moorby and Besford, 1983). Nitrogen, a major component

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of DNA and proteins in all cells, is together with Mg also the key elemental constituent of chlorophyll, the light-harvesting pigment of photosynthetic plants (Lehninger, 1970). In addition, specific concentrations of N and other nutrient elements (P, S, Ca, Mg, Fe and Cu) elicit the production of isoflavonoids in plants, and these molecules function as signals to mutualistic soil microbes and/or phytoalexins against infecting pathogens (Dakora and Phillips, 1996). Calcium is involved in the closure of guard cells and also mediates the response of plants to ethylene, a gaseous plant hormone that controls important physiological processes including seedling development, flowering, fruit ripening and senescence (Raz and Fluhr, 1992). Besides their involvement in signalling, some elements also play crucial physiological roles in plant life. Low concentrations of nutrients such as $K^+$, $Na^+$ and $Mg^{++}$ also readily stimulate the activity of major enzymes of the glycolytic pathway, namely phosphofructokinase and pyruvate kinase, which together regulate glycolysis in plant cells (Plaxton, 1996). Osmoregulation, the opening and closure of stomatal guard cells, and daily changes in leaf orientation are controlled by $K^+$ mobility in plant cells (Hsiao and Lauchli, 1986). Individual micronutrients are similarly important components of major enzymes, which regulate all biological processes in plants.

It is clear from these considerations that low nutrient availability can constrain plant growth in many environments of the world, especially the tropics where soils are extremely deficient in nutrients. Yet, the tendency in modern agriculture has been to select crop species for high soil fertility. This has resulted in the wide use of crop varieties that require high doses of applied fertilizer in order to meet optimal plant growth and grain yield. Plant species growing in naturally fertile soils also tend to respond to nutrient supply in a manner similar to agricultural cultivars. Because of the high concentrations of plant-available nutrients in fertilized or naturally fertile soils, root uptake rates are also high. This is in sharp contrast to nutrient-poor sites where root uptake rates are usually low due to low or poor nutrient availability (Marschner, 1995).

Plant uptake of nutrients from soil is more marked in the 'rhizosphere' surrounding the root than outside this zone (Darrah, 1993). Root exudation of various chemical molecules into the rhizosphere is largely dependent on the nutritional status of the plant, with some species exuding organic acid anions in response to P and Fe deficiency or phytosiderophores due to Fe and Zn defiency (Haynes, 1990; Jones and Darrah, 1994). Consequently, the released compounds can cause some nutrient elements to be relatively more available for uptake by plants. The rate of exudation itself is increased by the presence of microbes in the rhizosphere (Gardner et al., 1983) and promoted by the uptake and assimilation of certain nutrient elements. As a result, the composition of root exudates can be complex, and often ranges from mucilage, root border cells, extracellular enzymes, simple and complex sugars, phenolics, amino acids, vitamins, organic acids, nitrogenous macromolecules such as purines and nucleosides to inorganic or gaseous molecules such as $HCO_3^-$, $OH^-$, $H^+$, $CO_2$ and $H_2$ (Marschner, 1995; Rovira, 1969; Uren and Reisennuer, 1988; also see Table 1). Many of these organic substrates excreted into the rhizosphere, particularly amino acids, organic acids, proteins, carbohydrates and vitamins, promote microbial biosynthesis of ethylene (Arshad and Frankenberger, 1990), a powerful plant signal controlling development. That apart, these components all play different roles that ultimately affect nutrient acquisition by plants.

## Enhancement of nutrient supply by root exudate effects on symbiotic microbes

The components of plant root exudates are many and complex (Table 1), and serve not only as a source of carbon substrate for microbial growth, but also contain chemical molecules that promote chemotaxis of soil microbes to the rhizosphere. Although the root exudates of $N_2$-fixing legumes are known generally for their capacity to attract rhizobia to root hairs, it is in fact their individual chemical components such as flavonoids (Caetano-Anolles et al., 1988), aromatic acids (Parke et al., 1985), amino acids and dicarboxylic acids (Barbour et al., 1991) that function as specific chemoattractants for micro-organisms (Table 2). Once in the rhizosphere, many bacteria may multiply rapidly in response to growth stimulation by quercetin or other flavonoid molecules released by plants (Hartwig et al., 1991), and in turn promote further exudation of new or existing flavonoids into the rhizosphere (Dakora et al., 1993a,b; Recourt et al., 1991). Besides their direct effects on rhizobia, root exudates can also attract pathogenic microbes (Morris and Ward, 1992) and promote the growth of plants, mutualistic fungi (Siqueira et al., 1991) and rhizobacteria antagonistic to these pathogenic micro-organisms (Cook et al., 1995). In nutrient poor soils, such rapidly

*Table 1.* Organic compounds and enzymes identified in root exudates of different plant species[a]

| Amino acids | Organic acids | Sugars | Vitamins | Purines/nucleosides | Enzymes | Inorganic ions and gaseous molecules |
|---|---|---|---|---|---|---|
| $\alpha$-alanine | citric | glucose | biotin | adenine | acid/alkaline- | $HCO_3^-$ |
| $\beta$-alanine | oxalic | fructose | thiamin | guanine | phosphatase | $OH^-$ |
| asparagine | malic | galactose | niacin | cytidine | invertase | $H^+$ |
| aspartate | fumaric | maltose | pantothenate | uridine | amylase | $CO_2$ |
| cystein | succinic | ribose | rhiboflavin | | protease | $H_2$ |
| cystine | acetic | xylose | | | | |
| glutamate | butyric | rhamnose | | | | |
| glycine | valeric | arabinose | | | | |
| isoleucine | glycolic | raffinose | | | | |
| leucine | piscidic | desoxyribose | | | | |
| lysine | formic | oligosaccharides | | | | |
| methionine | aconitic | | | | | |
| serine | lactic | | | | | |
| threonine | pyruvic | | | | | |
| proline | glutaric | | | | | |
| valine | malonic | | | | | |
| tryptophan | aldonic | | | | | |
| ornithine | erythronic | | | | | |
| histidine | tetronic | | | | | |
| arginine | | | | | | |
| homoserine | | | | | | |
| phenylalanine | | | | | | |
| $\gamma$-Aminobutyric acid | | | | | | |
| $\alpha$-Aminoadipic acid | | | | | | |

[a] Compiled from West (1939), Fries and Forsman (1951), Rovira and Harris (1961), Vancura (1964), Vancura and Hovadik (1965), Boutler et al. (1966), Rovira (1969), Gardner et al. (1983), Lipton et al. (1987), Fox and Comerford (1990), Ae et al. (1990), Ohwaki and Hirata (1992), Hoffland et al. (1992) and Gagnon and Ibrahim (1998). The root exudates of plants studied include Bison and Novelty flax, barley, wheat, oat, cucumber, tomato, red pepper, turnip cabbage, pea, soybean, chickpea, peanut, lupin, alfalfa, slash pine, pigeon pea and rape.

intense colonization of the rhizosphere can itself lead to stiff competition between microbes and the plant for nutrient resources.

In addition to serving as chemotactic signals and growth promoters of rhizosphere bacteria (Table 2), root exudates also control the N nutrition of symbiotic legumes. These nodulating plants routinely use flavonoid molecules in root exudates to induce transcription of nodulation (*nod*) genes in rhizobia, leading to nodule formation and $N_2$ fixation. The compounds involved are typically flavonoid in nature, although roles for betaines (Phillips et al., 1992) and aldonic acids (Gagnon and Ibrahim, 1998) have also been reported (Table 3). The flavonoid *nod* gene inducers are either exuded directly by roots of $N_2$-fixing legumes (Phillips, 1992) or by root border cells present in exudates (Zhu et al., 1998). It has been shown that root exudates of legumes growing in an extremely acidic medium cause reduced induction of *nod* genes (Richardson et al., 1988), clearly indicating that the quality of root exudate determines the level of *nod* gene transcription. In fact, legumes which are inhibited in the biosynthesis and exudation of phenolic *nod*-gene inducers, exhibit reduced nodulation and $N_2$ fixation for their N nutrition (Phillips et al., 1994). However, because high soil N inhibits nodulation and $N_2$ fixation, and low concentrations of certain nutrients stimulate the biosynthesis of isoflavone *nod* gene inducers in symbiotic legumes (Dakora and Phillips, 1996), low-nutrient soils would seem to be the ideal for stimulating $N_2$ fixation and increased N nutrition in nodulated legumes. Whatever the case, root exudates contain the signals for transcription of *nod* genes in symbiotic rhizobia, and therefore control the extent of legume dependence on $N_2$ fixation for its N nutrition.

An important consideration is the persistence of these signals in soil. Depending on their stability, the activity of individual molecules could be short-lived

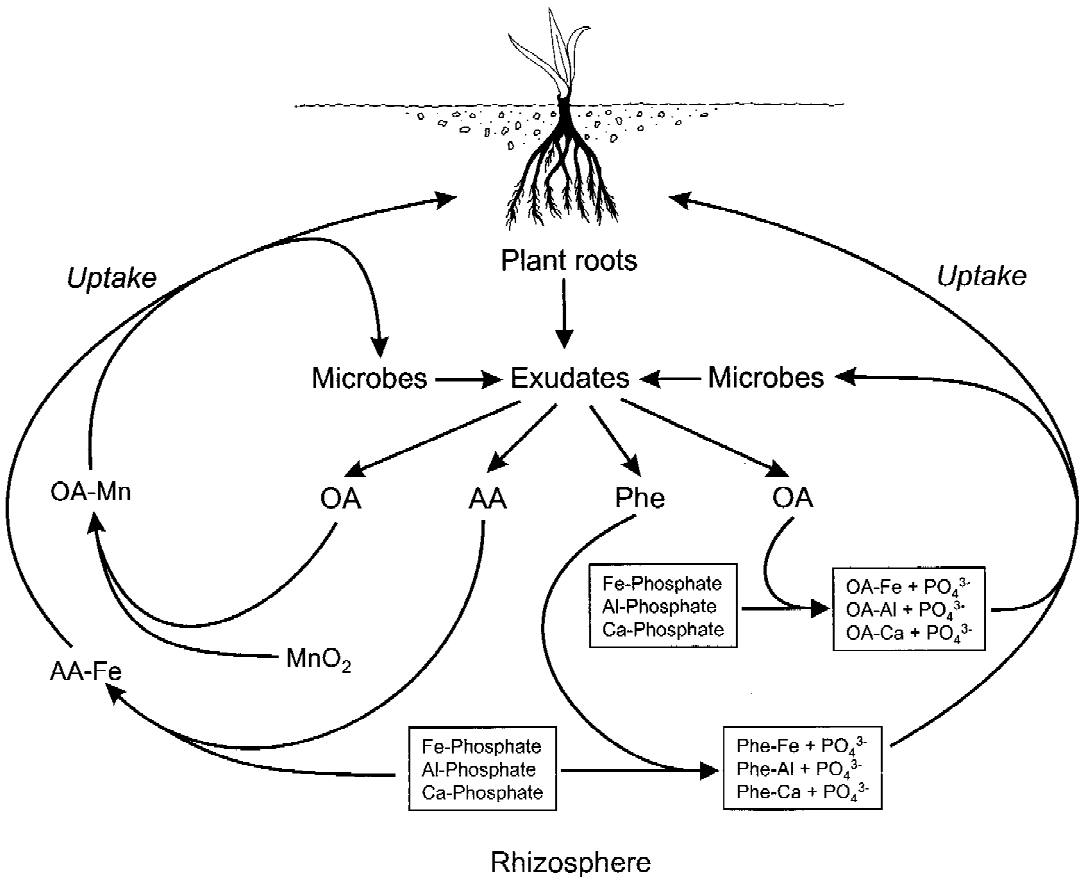


*Figure 1.* Effects of root exudate components on nutrient availability and uptake by plants and rhizosphere microbes. OA = organic acids; AA = amino acids including phytosiderophores, Phe = phenolic compounds.

in the rhizosphere due to rapid utilization as substrates by microbes (Barz, 1970). Also, the efficacy of both aglycones and their conjugates could be altered either following hydrolysis or chemical modification in the rhizosphere (Rao et al., 1991). For example, bacterial hydrolysis of the inactive compound luteolin-7-O-glucoside can produce the active *nod* gene inducer luteolin (Hartwig and Phillips, 1991). However, the compounds released from decomposition of organic matter such as dead roots and nodules or fallen leaves could potentially supplement root exudates in maintaining a steady concentration of flavonoids and mineral nutrients in the rhizosphere.

Root exudates also contain chemical molecules that govern the development of plant-fungal symbiosis (Table 3), as they provide powerful signals that alert the mycorrhizal fungi to the presence of a host plant. The molecules involved are the same group of flavonoid compounds used for signalling rhizobia. They induce spore germination and/or hyphal growth in vesicular-arbuscular fungi (Becard et al., 1992; Gianinazzi-Pearson et al., 1989). In alfalfa root exudates, quercetin and 4′,7-dihydroxyflavone are the specific molecules used to stimulate spore germination and fungal growth in *Glomus* and *Gigaspora* species (Tsai and Phillips, 1991), two mycorrhizal symbionts of this legume. Because of the increased growth of fungal hyphae which extend outwards into the soil environment and the network of plant and fungal nutrient-specific transporters, mycorrhizae promote increased acquisition of water and nutrients, especially P and N, by the host plant (Harrison, 1999). However, its efficacy in supplying nutrients, especially P, to the plant is more marked under nutrient-poor conditions than in fertile soils. The fynbos biome of the Western Cape in South Africa is characterized by P-deficient soils, consequently the dominant field legumes are infected with mychorrizae to enhance P nutrition (Allsop, 1992).

*Table 2.* Functional role of root exudate components in the rhizosphere

| Component | Rhizosphere function |
|---|---|
| Phenolics | nutrient source<br>chemoattractant signals to microbes<br>microbial growth promoters<br>*nod* gene inducers in rhizobia<br>*nod* gene inhibitors in rhizobia<br>resistance inducers against phytoalexins<br>chelators of poorly soluble mineral nutrients<br>detoxifiers of Al<br>phytoalexins against soil pathogens |
| Organic acids | nutrient source<br>chemoattractant signals to microbes<br>chelators of poorly soluble mineral nutrients<br>acidifiers of soil<br>detoxifiers of Al<br>*nod* gene inducers |
| Amino acids and phytosiderophores | nutrient source<br>chelators of poorly soluble mineral nutrients<br>chemoattractant signals to microbes |
| Vitamins | promoters of plant and microbial growth<br>nutrient source |
| Purines | nutrient source |
| Enzymes | catalysts for P release from organic molecules<br>biocatalysts for organic matter transformation in soil |
| Root border cells | produce signals that control mitosis<br>produce signals controlling gene expression<br>stimulate microbial growth<br>release chemoattractants<br>synthesize defense molecules for the rhizosphere<br>act as decoys that keep root cap infection-free<br>release mucilage and proteins |

Compiled from Hawes et al. (1998) and Dakora and Phillips (1996).

## Solubilization of nutrients by root exudate enzymes and cells

Plant species growing in low-nutrient environments also employ root exudates in ways other than as symbiotic signals to soil microbes involved in nutrient procurement. The activity of extracellular enzymes is one source of available P. Low P concentration in roots as a result of P deficiency induces *de novo* synthesis of extracellular and intracellular acid phosphatases (Duff et al., 1994), followed by an increase in the release of the extracellular phosphatases into root exudates (Dinkelaker and Marschner, 1992; Goldstein et al., 1988; Lee, 1988). These ectoenzymes of root exudates include root-borne acid phosphatase, fungal acid/alkaline phosphatase, and bacterial alkaline phosphatase (Table 1), and they function by hydrolyzing and mobilizing inorganic P from monoester soil organic phosphates (Table 2; Duff et al., 1994), which are estimated to account for about 30–80% of total P in agricultural soils (Gilbert et al., 1999). The role of these phosphatases in P nutrition is evidenced by the fact that P-stressed lupin plants secrete about 20-fold more acid phosphatases from roots compared to P-sufficient control plants (Tadano and Sakai, 1991).

A recent study (Gilbert et al., 1999) with white lupin has also shown that low P concentration in tissues elicits the synthesis and exudation into the rhizosphere of a novel acid phosphatase associated with cluster roots of minus-P plants. This suggests that, in addition to normal acid phosphatases, plants with the ability to form cluster roots release novel acid phosphatases for hydrolyzing P in the vicinity of the proteoid root hairs. Apyrases (enzymes that cleave $\gamma$- and $\beta$-phosphates on ATP) are another group of new enzymes involved in P nutrition of plants. Apyrases from pea are reported to improve P nutrition of this species through P release from extracellular ATP and other organic phosphate molecules (Thomas et al., 1999) commonly present in the rhizosphere.

In germinating seedlings, phytases hydrolyze phytic acid to release inorganic P for plant utilization (Duff et al., 1994). A recent study (Li et al., 1997) showed a strong correlation between P-deficiency and release of phytases by several plant species. Many other intracellular acid phosphatases exist in plants, with specific and non-specific substrates, but whether these hydrolases are released into root exudates has not yet been reported. The discovery that root border cells release extracellular enzymes, *nod*-gene-inducing compounds, signals controlling gene expression and substrate for mycorrhizal development (Hawes et al., 1998; Zhu et al., 1998) clearly suggest that they may also play a major role in nutrient acquisition in the rhizosphere. However, experiments involving the incubation of root border cells with poorly soluble nutrient compounds are needed to provide direct evidence of this function.

## Mobilization of nutrients by root exudate organic compounds

Phenolics are chemical components of root exudates (D'Arcy, 1982; D'Arcy-Lameta, 1986) that solubilize Fe, P and other nutrients from unavailable sources for

*Table 3.* Legume root exudate compounds that induce rhizobial *nod* genes and/or VA fungal development

| Legume species | Inducer molecule | Reference |
|---|---|---|
| **Rhizobium symbiosis** | | |
| Alfalfa | 4,4′-dihydroxy-2′-methoxychalcone | Maxwell et al. (1989) |
| | 4′-7-dihydroxyflavone | |
| | liquiritigenin | |
| Cowpea | daidzein | Dakora (2000) |
| | genistein | |
| | coumestrol | |
| Common bean | genistein | Dakora et al. (1993) |
| | genistein-3-Oglucoside | Hungria et al. (1991) |
| | eriodictyol | |
| | naringenin | |
| | daidzein | Dakora et al. (1993) |
| | coumestrol | |
| Kersting's bean | daidzein | Dakora (2000) |
| | genistein | |
| | coumestrol | |
| Soybean | isoliquiritigenin | Kape et al. (1992) |
| | genistein | Smit et al. (1992) |
| | genistein-7-O-glucoside | |
| | genistein-7-O-(6′′-O-malonylglucoside) | |
| | daidzein | |
| | daidzein-7-O-(6′′-O-malonylglucoside) | |
| Vetch | 3,5,7,3′-tetrahydroxy-4′-methoxyflavanone | Recourt et al. (1991) |
| | 7,3′-dihydroxy-4′-methoxyflavanone | |
| | 2′,4′,4-trihydroxychalcone | |
| | 4′,4-dihydroxy-2′-methoxychalcone | |
| | naringenin | |
| | liquiritigenin | |
| | 7,4′-dihydroxy-3′-methoxyflavanone | |
| | 5,7,4′-trihydroxy-3′-methoxyflavanone | |
| | 5,7,3′-trihydroxy-4′-methoxyflavanone | |
| White clover | 7,4′-dihydroxyflavone | Djordjevic et al. (1987) |
| | umbelliferone[a] | |
| | formononetin[a] | |
| Bambara groundnut | daidzein | Dakora and Muofhe (1996) |
| | genistein | |
| | coumestrol | |
| Sesbania | liquiritigenin | Messens et al. (1991) |
| Lupin | erythronic acid | Gagnon and Ibrahim (1998) |
| | tetronic acid | |
| **VA fungal symbiosis** | | |
| Alfalfa | 4′,7-dihydroxyflavone | Tsai and Phillips (1991) |
| | 4′,7-dihydroxyflavanone | |

[a] *nod* gene inhibitors.

uptake by plants (Table 2). When challenged with Fe deficiency, plants, especially dicots, release phenolic molecules that influence Fe and P mobility (Römheld, 1987). An example is the release by Fe-deficient alfalfa plants of the isoflavonoid phytoalexin [2-(3′,5′-dihydroxyphenyl)-5,6-dihydroxyben zofuran], which intensely dissolves ferric phosphate, thus making Fe and P available for plant utilization, and also serves as phytoalexin against infecting pathogens (Masaoka et al., 1993). Interestingly, Fe-sufficient plants produced root exudates that were limited in ferric phosphate-dissolving capacity. Fe-deficient tomato plants also exude caffeic acid for solubilizing Fe from insoluble Fe sources (Olsen et al., 1981). These phenolics make Fe and P available by forming relatively stable chelates with Fe and Al present in insoluble Fe- and Al-phosphates thereby increasing the solubility of Fe and P for plant uptake (Figure 1).

A more effective strategy used by Fe-deficient grasses and cereals is the release of phytosiderophores (Table 2), a group of hydroxy- and amino-substituted iminocarboxylic acids, with an ability to solubilize ferric compounds for uptake by roots (Römheld, 1987). Phytosiderophores also solubilize other micronutrients such as Mn, Cu and Zn (Treeby et al., 1989), indicating that phytosiderophore release induced by Fe-deficiency can increase micronutrient concentrations in the rhizosphere. The rate of phytosiderophore exudation can increase by about 20 times in cereal plants confronted with iron deficiency in contrast to an increase in uptake rate of only 5 times (Römheld and Marschner, 1990), suggesting that the release and uptake systems are under different genetic control. Also, there are cultivar, species and genotypic differences in response to iron and micronutrient deficiencies (Römheld and Marschner, 1990), in terms of rates of exudation, chemical nature of phytosiderophores produced, and the size of microbial populations at the apical root zone (Von Wirén et al., 1993). According to Römheld (1991), Fe or Zn deficiency in soil induces *de novo* synthesis of phytosiderophore in the cytoplasm, followed by enhanced release across the plasma membrane into the rhizosphere, where the phytosiderophore forms a complex with the insoluble ferric compound and the entire complex transported into the cytoplasm via a specific translocator in the membrane. $H^+$ extrusion into the rhizosphere promotes ferric reductase enzyme activity in the plasma membrane, leading to increased reduction of ferric to ferrous ion, which is preferentially absorbed by roots (Römheld, 1987). Apparently, microbial siderophores are also capable of solubilizing ferric compounds in the rhizosphere (Jurkevitch et al., 1986), although they are the least preferred in uptake. Iron is toxic when in excess inside cells. However, unlike in microbes, its regulation in plants still remains unknown. Microbial uptake of Fe is regulated by a chromosomal repressor, which shuts down expression of all components of the siderophore biosynthetic pathway, thus restricting further uptake of Fe into the cell (Neilands, 1987). Given that mainly grasses and cereal crops exude phytosiderophores, different plant species must have different ways of regulating internal Fe concentration than that observed in microbes.

Such Krebs Cycle intermediates as citric, malic, succinic, fumaric, and aconitic acids accumulate markedly in root exudates of different plant species (Table 1) suffering from nutrient starvation (Gardner et al., 1983; Hoffland et al., 1992; Jones, 1998; Lipton et al., 1987; Ohwaki and Hirata, 1992). But acetic acid, glycolic, malonic, oxalic, formic, and piscidic acid have also been identified in root exudates of a number of plants (Ae et al., 1990; Fox and Comerford, 1990; Smith, 1969, 1976; Vancura, 1965). These acids play a crucial role in nutrient acquisition (P, Fe and Mn) by plants growing in low nutrient soils (Table 2; Figure 1), and their release in response to nutrient starvation differs between plant species (Table 4). In one study (Lipton et al., 1987), symptons of P-starvation was exemplified by 82% increase in citrate exudation. However, not all plant species respond the same way to P deficiency (Table 4). While P-deficient rape typically releases malic acid near its root tips or at sites in contact with insoluble rock phosphate, P-starved hedge mustard does not secrete organic acids (Hoffland et al., 1992), possibly due to problems associated with synthesis and transport across membranes. Legume species also respond differently to P deficiency, in terms of exudation rates and the spectrum of organic acids released. For example, chickpea produced 11 and 24 times more root exudates than pigeon pea and soybean, respectively, while peanut produced only 8 and 17 times more than the two species (Ohwaki and Hirata, 1992). Although the concentrations of fumaric, malic and citric acids were also lower in exudates of soybean and pigeon pea (Ohwaki and Hirata. 1992), the latter is very efficient at P acquisition in acidic soils and apparently uses piscidic acid, a strong chelator of iron, to mobilize P (Ae et al., 1990). The organic acid anions in root exudates can also chelate Fe and Mn in iron and manganese oxides (i.e. $Fe_2O_3$ and $MnO_2$), thus making

*Table 4.* Concentration of citric and malic acids in root exudates of P-deficient or P-sufficient plant species. Wheat, tomatoe and chickpea initially received 0.25 mm P, and root exudates collected following a period of P deprivation. For these three species, 0.0 acid concentration ranges from actual zero to negative values relative to the control. Values followed by different letters for *P* treatments of each plant species are significant different at $P < 0.05$. The other values had standard deviations ranging from 0 to 25%

| Plant Species | P supply (mol $m^{-3}$) | Organic acid ($\mu$mol $h^{-1}$ $g^{-1}$ fwt) | | Reference |
|---|---|---|---|---|
| | | Citrate | Malate | |
| White lupin | 1.0 | 0.02a | 0.03a | Hohnson et al. (1996) |
| | 0.0 | 0.57b | 0.51b | |
| White lupin | 0.25 | 0.02a | 0.08a | Neumann et al. (1999) |
| | 0.0 | 0.09b | 0.61a | |
| Wheat | 0.25 | 0.0 | 0.25 | Neumann and Römheld (1999) |
| | 0.0 | 0.0 | 0.0 | |
| Tomato | 0.25 | 0.13 | 0.14 | Neumann and Römheld (1999) |
| | 0.0 | 0.0 | 0.0 | |
| Chickpea | 0.25 | 0.001 | 0.001 | Neumann and Römheld (1999) |
| | 0.0 | 0.0 | 0.002 | |

them available for plant uptake (Figure 1; Marschner, 1995). But whether the chelated metal is taken up is still open to debate. Similarly, these acid anions form complexes with Ca, Al and Fe present in soil as insoluble phosphates of calcium, iron and aluminium, and liberate P for uptake by roots (Figure 1; Marschner, 1995). Additionally, these acids can desorb P from sesquioxide surfaces by anion exchange (Bolan et al., 1994; Jones, 1998; Jones and Darrah, 1994; Parfitt, 1979), and also maintain sulphate mobility in rhizosphere soil through competitive displacement from adsorption sites (Evans and Anderson, 1990).

In some plants, the synthesis and release of organic acids represents a mechanism for detoxifying excess Al internally and externally via chelation of the active Al into a non-phytotoxic form. A number of studies show that in response to Al stress: (1) roots of Al-resistant snapbean release higher levels of citric acid into the rhizosphere than the Al-sensitive cultivar (Miyasaka et al., 1991), (2) Al-resistant wheat exuded 5–10-fold more malic acid than Al-sensitive genotypes (Delhaize et al., 1993), (3) citric acid exudation was enhanced in Al-resistant maize (Pellet et al., 1995) and Al-resistant buckwheat (Ma et al., 1998; Zheng et al., 1998). In these Al-resistant plants, there was a correlation between organic acid release and resistance to Al, and substituting other elements for Al did not elicit organic acid exudation (Delhaize et al., 1993). In buckwheat, oxalic acid forms an Al-oxalate complex, which renders the Al non-phytotoxic to the plant (Ma et al., 1998), suggesting that this might be the mechanism employed by plants to detoxify active Al species.

## Root exudate effects on rhizosphere pH and nutrient availability

Another nutritional effect that organic acids have in root exudates is acidification of the rhizosphere (Table 2). Root exudation of high concentrations of organic acid anions as a result of P deficiency (Hoffland et al., 1989) does lower rhizosphere pH, making P (Haynes, 1990; Jones and Darrah, 1994) and micronutrients such as Mn, Fe and Zn to be more available in calcareous soils (Dinkelaker et al., 1989). However, the relationship between organic acid exudation and rhizosphere acidification is not that simple as the extrusion of $H^+$ would depend on the amounts of anions absorbed by roots relative to cations (Haynes, 1990; Jones and Darrah, 1994). Whatever the case, acidification below pH 5.5 can cause even major macronutrients to become limiting. Because micronutrients such as Mn, Fe and Al occur in high concentrations below pH 5.5 (Brady, 1990), any further acidification

by organic acids below this level can result in phytotoxic effects on plant roots and beneficial microbes. Intriguingly, the white lupin can mobilize P from both acid and alkaline soils by using citric acid in its proteoid root exudates to acidify even the alkaline soil, and thus solubilize P as well as Fe, Mn, Cu and Zn for uptake by roots (Gardner et al., 1983).

Root excretion of inorganic ions (e.g. $HCO_3^-$, $OH^-$, $H^+$) is also important in the mineral nutrition of plants. Plant uptake of anions in excess of cations often causes the roots to secrete $HCO_3^-$ in order to maintain electrical neutrality, a process that leads to increased rhizosphere pH. Conversely, the uptake of cations in excess of anions can cause roots to exude $H^+$ and lower the rhizosphere pH. The change in pH with $HCO_3^-$ extrusion tends to increase nutrient supply in acidic soils, as happens with $H^+$ exudation in calcareous soils.

$H^+$ extrusion is largely governed by cation/anion balance and is thus very much influenced by the N source. Plants growing on nitrate generally maintain electronic neutrality by releasing excess anions, including $OH^-$, which cause an increase in rhizosphere pH and an enhanced nutrient (P, Mo, etc.) availability in acidic soils (Marschner, 1995). An exception is the study by Gahoonia et al. (1992) which showed no effect on P mobilization in a luvisol soil following nitrate-induced increase in rhizosphere pH. However, when ammonium is supplied, there is a large excess of cations and a resulting enhanced $H^+$ extrusion and rhizosphere acidification, thereby making P, Mn, Fe, Cu and Zn more toxic in the acidic range, or readily available in the alkaline range (Aguilar and Van Diest, 1981; Gahoonia et al., 1992; Gardner et al., 1983; Gillespie and Pope, 1990; Runge and Rode, 1991). Sometimes, however, acidification from ammonium nutrition does not result in increased P mobilization, especially in acidic oxisols (Gahoonia et al., 1992). Furthermore, $H^+$ extrusion occurs during $N_2$ fixation by symbiotic legumes (Raven et al., 1990), and this can lead to rhizosphere acidification and increased availability of limiting nutrient elements like P, Mo and Fe (Aguilar and Van Diest, 1981; Gahoonia et al., 1992; Gillespie and Pope, 1990; Runge and Rode, 1991) which are much needed in diazotrophy. Additionally, there are many reports of enhanced $H^+$ extrusion under Fe defiency and P deficiency, both leading to acidification of localized areas around the root tips (Bienfait, 1985, 1988; Gardner et al., 1983; Hoffland et al., 1989; Römheld and Marschner, 1986) and a consequent improvement in the availability of these nutrients. Higher cation/anion uptake ratios as occurs during Zn deficiency (Cakmak and Marschner, 1990) can also acidifiy the rhizosphere and influence nutrient uptake by plants.

Environments which are naturally very acidic can pose a challenge to nutrient acquisition by plant roots, and threaten the survival of many beneficial microbes and that of the roots themselves (Runge and Rode, 1991). Under those conditions, rhizobia develop tolerance of low pH through expression of acid tolerance genes (Tiwari et al., 1996). As found with a transgenic plant (Degenhardt et al., 1998), a few species such as the Rooibos tea plant (*Aspalathus linearis*), actively modify their rhizosphere pH by extruding $OH^-$ and $HCO_3^-$ (Muofhe and Dakora, 2000) to facilitate growth in low pH soils (pH 3–5). By raising the pH of the rhizosphere, major nutrients such as K, Ca, Mg, P, S and Mo that are low in Cederberg soils (Muofhe, 1997), become readily available for uptake by plant roots. With a favourable pH, soil N levels can also rise from increased activity of nitrifying bacteria. However, an elevation in rhizosphere pH caused by *Aspalathus linearis* plants growing in very acidic soils can also be a strategy for reducing the toxicity of Al, Mn and Fe (Runge and Rode, 1991). Whereas net alkalinization of the rhizosphere by nitrate assimilation in plants is understood, the mechanisms underlying rhizosphere alkalinization by root exudates of *Aspalathus linearis* are only now being clarified.

Increased soil acidification is generally accompanied by Al toxicity as both the concentration and activity of this element are increased with a lowering of pH in the rhizosphere (Gahoonia, 1993) often resulting in reduced Ca and Mg uptake and root growth (Kochian, 1995; Ryan et al., 1992 ). One of the ways for reducing Al toxicity is to increase rhizosphere pH. The findings of a recent study (Degenhardt et al., 1998) involving the use of transgenic plants revealed a twofold increase in $H^+$ influx, which resulted in increased rhizosphere pH. So, in addition to internal or external Al detoxification by organic acids (Larsen et al., 1998; Zheng et al., 1998), an increase in rhizosphere pH can also reduce Al toxicity. Plant genotypes however differ in their handling of excess Al. Calba and Jaillard (1997) showed that a maize genotype that was most tolerant of Al toxicity caused a lesser rhizosphere acidification because $NO_3^-$ uptake was less affected than for the Al-sensitive genotype.

### Gaseous root exudates affecting nutrient supply

Root excretion of gaseous molecules (e.g. $CO_2$, $H_2$) is also important in the mineral nutrition of plants. Actively-growing young roots release a lot of $CO_2$ into the soil environment from carbohydrate respiration (Zwarun, 1972) and stimulation by lumichrome, a plant and bacterial exudate molecule (Phillips et al., 1999). If its escape is prevented by high soil water, $CO_2$ can accumulate up to 17.5% in the root zone (Jackson, 1979). Such increased levels of $CO_2$ (and hence of carbonic acid which dissociates to form $H^+$ at neutral to alkaline pH) can enhance the dissolution of soil $CaCO_3$ to produce $Ca^{2+}$ for plant uptake. Similarly, $HCO_3^-$ excreted directly by plant roots into soil can aid the dissolution of calcite to yield soluble supplies of $Ca^{2+}$ for plant nutrition (Rendig and Taylor, 1989). $CO_2$ release by roots can also have other indirect effects on N and P nutrition of plants that form symbiosis with *Rhizobium* bacteria and VA fungi. An exogenous supply of $CO_2$ is required for better growth of rhizobia and VA fungi in the rhizosphere (Becard et al., 1992; Lowe and Evans, 1962); the increase in microsymbiont population should have the potential to provide adequate symbiotic N and P for the host plant.

Hydrogen gas ($H_2$) is a major byproduct of $N_2$ fixation in legumes, and its production consumes about 5% of net photosynthesis. In rhizobia with uptake hydrogenase ($HUP^+$) system, $H_2$ produced by nitrogenase is oxidised to yield more energy. However, with the $HUP^-$ strains, the $H_2$ is evolved as a gas into the rhizosphere environment. Although this was previously thought to be wasteful of C, a recent study (Kettlewell et al., 2000) has shown that pre-treating soil with $H_2$ can stimulate growth of agricultural crops by 10–30%. In the $H_2$-treated soils, 60% of the reducing power was found to flow to $O_2$ and 40% to $CO_2$, resulting in net chemoautrotrophic $CO_2$ fixation. Although the specific elicitor of plant growth promotion remains unknown, nodule release of $H_2$ does affect the growth and nutrition of symbiotic legumes.

Beyond their role in mineral acquisition, root exudates also appear to have potential in phytoremediation. A number of studies (Banuelos et al., 1993; De Souzaet al., 1999; Mench and Martin, 1991; Nanda Kumar et al., 1995; Terry et al., 1992; Zayed et al., 1998) have shown the ability of certain plant species to accumulate and release heavy metals as volatile root exudates, thus reducing their concentrations in the soil environment.

### Conclusions

Through diverse mechanisms, root exudates play a fundamental role in the mineral nutrition of plants. They either contain signals that act as regulators of microbial growth and function, or they possess molecules which directly control the rhizosphere processes that enhance nutrient uptake and assimilation (Figure 1). No doubt, manipulating some or all of these rhizosphere processes is likely to hold the key to improved plant nutrition, and hopefully increased crop yields in the next millenium.

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