



## 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.  $\text{HCO}_3^-$ ,  $\text{OH}^-$ ,  $\text{H}^+$ ), gaseous molecules ( $\text{CO}_2$ ,  $\text{H}_2$ ), 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  $\text{N}_2$ -fixing legumes serve as major signals to Rhizobiaceae bacteria which form root nodules where  $\text{N}_2$  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  $\text{N}_2$  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  $\text{OH}^-$  and  $\text{HCO}_3^-$  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 al-

ter 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 deficiency (Haynes, 1990; Jones and Dar-

rah, 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 Reissenauer, 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<sup>a</sup>

Amino acids	Organic acids	Sugars	Vitamins	Purines/nucleosides	Enzymes	Inorganic ions and gaseous molecules
$\alpha$ -alanine	citric	glucose	biotin	adenine	acid/alkaline-	HCO <sub>3</sub> <sup>-</sup>
$\beta$ -alanine	oxalic	fructose	thiamin	guanine	phosphatase	OH <sup>-</sup>
asparagine	malic	galactose	niacin	cytidine	invertase	H <sup>+</sup>
aspartate	fumaric	maltose	pantothenate	uridine	amylase	CO <sub>2</sub>
cystein	succinic	ribose	riboflavin		protease	H <sub>2</sub>
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						

<sup>a</sup>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<sub>2</sub> 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<sub>2</sub>-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<sub>2</sub> fixation for their N nutrition (Phillips et al., 1994). However, because high soil N inhibits nodulation and N<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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

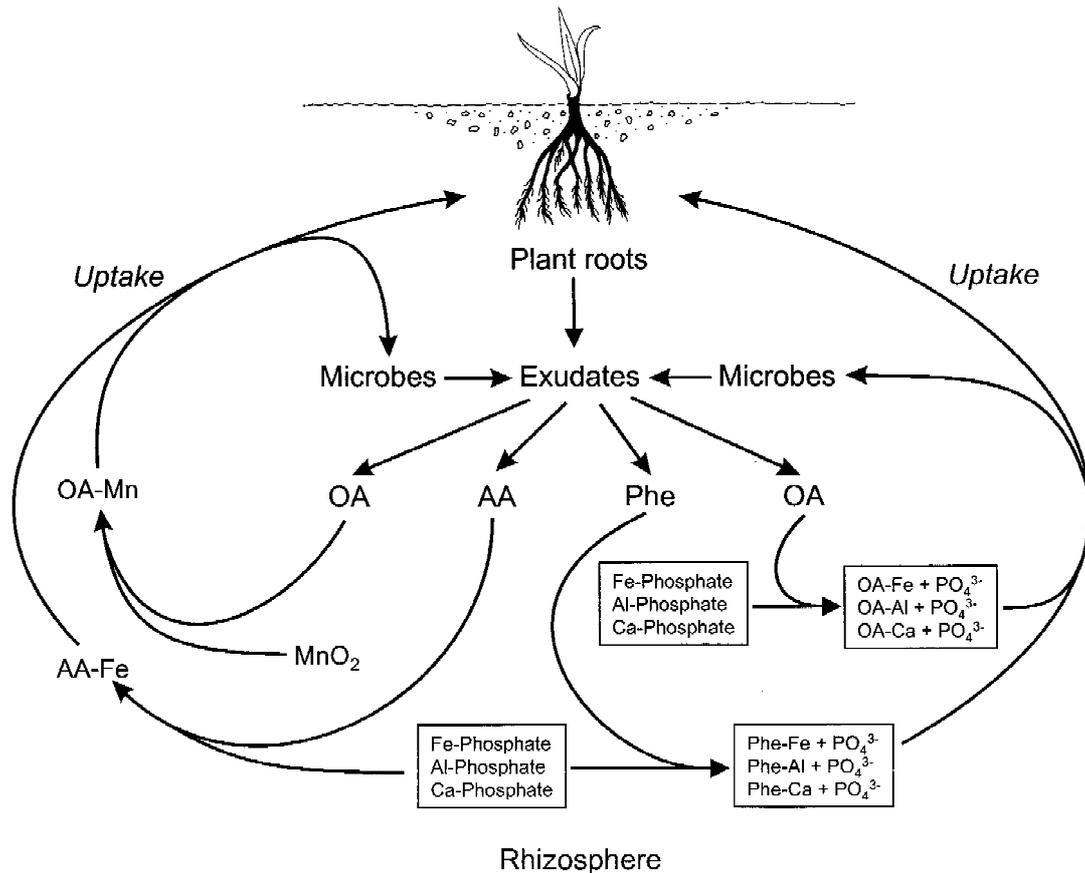


Figure 1. Effects of root exudate components on nutrient availability and uptake by plants and rhizosphere microbes. OA = organic acids; AA = amino acids including phytosiderophores, Phe = phenolic compounds.

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 mycorrhizae to enhance P nutrition (Allsop, 1992).

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

Component	Rhizosphere function
Phenolics	nutrient source chemoattractant signals to microbes microbial growth promoters <i>nod</i> gene inducers in rhizobia <i>nod</i> gene inhibitors in rhizobia resistance inducers against phytoalexins chelators of poorly soluble mineral nutrients detoxifiers of Al phytoalexins against soil pathogens
Organic acids	nutrient source chemoattractant signals to microbes chelators of poorly soluble mineral nutrients acidifiers of soil detoxifiers of Al <i>nod</i> gene inducers
Amino acids and phytosiderophores	nutrient source chelators of poorly soluble mineral nutrients chemoattractant signals to microbes
Vitamins	promoters of plant and microbial growth nutrient source
Purines	nutrient source
Enzymes	catalysts for P release from organic molecules biocatalysts for organic matter transformation in soil
Root border cells	produce signals that control mitosis produce signals controlling gene expression stimulate microbial growth release chemoattractants synthesize defense molecules for the rhizosphere act as decoys that keep root cap infection-free 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
<b>Rhizobium symbiosis</b>		
Alfalfa	4,4'-dihydroxy-2'-methoxychalcone 4'-7-dihydroxyflavone liquiritigenin	Maxwell et al. (1989)
Cowpea	daidzein genistein coumestrol	Dakora (2000)
Common bean	genistein genistein-3-O-glucoside eriodictyol naringenin daidzein coumestrol	Dakora et al. (1993) Hungria et al. (1991)  Dakora et al. (1993)
Kersting's bean	daidzein genistein coumestrol	Dakora (2000)
Soybean	isoliquiritigenin genistein genistein-7-O-glucoside genistein-7-O-(6''-O-malonylglucoside) daidzein daidzein-7-O-(6''-O-malonylglucoside)	Kape et al. (1992) Smit et al. (1992)
Vetch	3,5,7,3'-tetrahydroxy-4'-methoxyflavanone 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	Recourt et al. (1991)
White clover	7,4'-dihydroxyflavone umbelliferone <sup>a</sup> formononetin <sup>a</sup>	Djordjevic et al. (1987)
Bambara groundnut	daidzein genistein coumestrol	Dakora and Muofhe (1996)
Sesbania	liquiritigenin	Messens et al. (1991)
Lupin	erythronic acid tetronic acid	Gagnon and Ibrahim (1998)
<b>VA fungal symbiosis</b>		
Alfalfa	4',7-dihydroxyflavone 4',7-dihydroxyflavanone	Tsai and Phillips (1991)

<sup>a</sup>*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-dihydroxybenzofuran], 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), symptoms 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, tomato 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 <sup>-3</sup> )	Organic acid (μmol h <sup>-1</sup> g <sup>-1</sup> 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<sup>+</sup> 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.  $\text{HCO}_3^-$ ,  $\text{OH}^-$ ,  $\text{H}^+$ ) is also important in the mineral nutrition of plants. Plant uptake of anions in excess of cations often causes the roots to secrete  $\text{HCO}_3^-$  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  $\text{H}^+$  and lower the rhizosphere pH. The change in pH with  $\text{HCO}_3^-$  extrusion tends to increase nutrient supply in acidic soils, as happens with  $\text{H}^+$  exudation in calcareous soils.

$\text{H}^+$  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  $\text{OH}^-$ , 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  $\text{H}^+$  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,  $\text{H}^+$  extrusion occurs during  $\text{N}_2$  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  $\text{H}^+$  extrusion under Fe deficiency 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; Römhild 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 acidify 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  $\text{OH}^-$  and  $\text{HCO}_3^-$  (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 alkalization of the rhizosphere by nitrate assimilation in plants is understood, the mechanisms underlying rhizosphere alkalization 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 two-fold increase in  $\text{H}^+$  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  $\text{NO}_3^-$  uptake was less affected than for the Al-sensitive genotype.

### Gaseous root exudates affecting nutrient supply

Root excretion of gaseous molecules (e.g. CO<sub>2</sub>, H<sub>2</sub>) is also important in the mineral nutrition of plants. Actively-growing young roots release a lot of CO<sub>2</sub> 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<sub>2</sub> can accumulate up to 17.5% in the root zone (Jackson, 1979). Such increased levels of CO<sub>2</sub> (and hence of carbonic acid which dissociates to form H<sup>+</sup> at neutral to alkaline pH) can enhance the dissolution of soil CaCO<sub>3</sub> to produce Ca<sup>2+</sup> for plant uptake. Similarly, HCO<sub>3</sub><sup>-</sup> excreted directly by plant roots into soil can aid the dissolution of calcite to yield soluble supplies of Ca<sup>2+</sup> for plant nutrition (Rendig and Taylor, 1989). CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>) is a major byproduct of N<sub>2</sub> fixation in legumes, and its production consumes about 5% of net photosynthesis. In rhizobia with uptake hydrogenase (HUP<sup>+</sup>) system, H<sub>2</sub> produced by nitrogenase is oxidised to yield more energy. However, with the HUP<sup>-</sup> strains, the H<sub>2</sub> 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<sub>2</sub> can stimulate growth of agricultural crops by 10–30%. In the H<sub>2</sub>-treated soils, 60% of the reducing power was found to flow to O<sub>2</sub> and 40% to CO<sub>2</sub>, resulting in net chemoautotrophic CO<sub>2</sub> fixation. Although the specific elicitor of plant growth promotion remains unknown, nodule release of H<sub>2</sub> 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 Souza et 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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### References

- Ae N, Arihara J, Okada K, Yoshihara T and Johansen C 1990 Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. *Science* 248, 477–480.
- Aguilar A S and Van Diest A 1981 Rock-phosphate mobilization induced by the alkaline uptake pattern of legumes utilizing symbiotically fixed nitrogen. *Plant Soil* 61, 27–42.
- Allsop N 1992 The Occurrence and Ecophysiology of Vesicular-Arbuscular Mycorrhizal Plants in the Cape Floristic Region. PhD Thesis, Botany Department, University of Cape Town.
- Arshad M and Frankenberger W T Jr. 1990 Ethylene accumulation in soil in response to organic amendments. *Soil Sci. Soc. Am. J.* 54, 1026–1031.
- Banuelos G S, Cardon G, Mackey B, Ben-Asher J, Wu L, Beuselinck P, Akohouse S and Zambrzuski S 1993 Boron and selenium removal in boron laden soils by four sprinkler irrigated plant species. *J. Environ. Qual.* 22, 786–792.
- Barbour W M, Hattermann D R and Stacey G 1991 Chemotaxis of *Bradyrhizobium japonicum* to soybean exudates. *Appl. Environ. Microbiol.* 57, 2635–2639.
- Barz W 1970 Isolation of rhizosphere bacterium capable of degrading flavonoids. *Phytochemistry* 9, 1745–1749.
- Becard G, Douds D D and Pfeffer P E 1992 Extensive *in vitro* hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO<sub>2</sub> and flavonols. *Appl. Environ. Microbiol.* 58, 821–825.
- Bienfait H F 1985 Regulated redox processes at the plasmalemma of plant root cells and their function in iron uptake. *J. Bioenerg. Biomembr.* 14, 191–205.
- Bienfait H F 1988 Mechanisms in Fe efficiency reactions of higher plants. *J. Plant Nutr.* 11, 605–629.

- Bolan N S, Naidu R, Mahimairaja S and Baskaran S 1994 Influence of low-molecular-weight organic acids on the solubilization of phosphates. *Biol. Fert. Soils* 18, 311–319.
- Boutler D, Jeremy J J and Wilding M 1966 Amino acids liberated into the culture medium by pea seedling roots. *Plant Soil* 24, 121–127.
- Brady N C 1990 *The Nature and Properties of Soils*, Tenth Edition. Macmillan Publishing Company, New York. 621 p.
- Caetano-Anolles G, Crist-Estes D K and Bauer D W 1988 Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. *J. Bacteriol.* 170, 3164–3169.
- Cakmak I and Marschner H 1990 Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower and buckwheat plants. *Plant Soil* 129, 261–268.
- Calba H and Jaillard B 1997 Effect of aluminium on ion uptake and H<sup>+</sup> release by maize. *New Phytol.* 137, 607–616.
- Cook R J, Thomashow L S, Weller D M, Fujimoto D, Mazzola M, Bangera G and Kim D 1995 Molecular mechanisms of defence by rhizobacteria against root disease. *Proc. Natl. Acad. Sci. USA* 92, 4197–4201.
- Dakora F D 2000 Commonality of root nodulation signals and nitrogen assimilation in tropical grain legumes belonging to the tribe Phaseoleae. *Aust. J. Plant Physiol.* 27, 885–892.
- Dakora F D, Joseph C M and Phillips D A 1993 Common bean root exudates contain elevated levels of daidzein and coumestrol in response to *Rhizobium* inoculation. *Mol. Plant-Microbe Interact.* 6, 665–668.
- Dakora F D and Phillips D A 1996 Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. *Physiol. Mol. Plant Pathol.* 49, 1–20.
- Dakora F D and Muofhe M L 1996 Molecular signals involved in nodulation of the African Bambara groundnut (*Vigna subterranea* L. Verdc.). In *Proceedings of the International Bambara groundnut Symposium*, University of Nottingham, UK, 23–25 July 1996. pp. 171–179.
- Darrah P R 1993 The rhizosphere and plant nutrition: A quantitative approach. *Plant Soil* 155/156, 1–20.
- D'Arcy A L 1982 Study of soja and lentil exudates I. Kinetics of exudation of phenolic compounds, amino acids and sugars in the first days of plant growth. *Plant Soil* 68, 399–403.
- D'Arcy-Lameta A 1986 Study of soybean and lentil root exudates. II. Identification of some polyphenolic compounds, relation with plantlet physiology. *Plant Soil* 92, 113–123.
- Degenhardt J, Larsen P B, Howell S H and Kochian L V 1998 Aluminium resistance in the *Arabidopsis* mutant *atr-104* is caused by an aluminium-induced increase in pH. *Plant Physiol.* 117, 19–27.
- Delhaize E, Ryan P R and Randall P J 1993 Aluminium tolerance in wheat (*Triticum aestivum* L.) II. Aluminium stimulated excretion of malic acid from root apices. *Plant Physiol.* 103, 695–702.
- De Souza M P, Chu D, Zhao M, Zayed A M, Ruzin S E, Schichnes D and Terry N 1999 Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. *Plant Physiol.* 119, 565–573.
- Dinkelaker B, Römheld V and Marschner H 1989 Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). *Plant Cell Physiol.* 12, 285–292.
- Dinkelaker B and Marschner H 1992 *In vivo* demonstration of acid phosphatase activity in the rhizosphere of soil-grown plants. *Plant Soil* 144, 199–205.
- Djordjevic M A, Redmond J W, Batley M and Rolfe B G 1987 Clovers secrete specific phenolic compounds which either stimulate or repress *nod* gene expression in *Rhizobium trifolii*. *EMBO J.* 6, 1173–1179.
- Duff S M G, Sarath G and Plaxton W C 1994 The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant.* 90, 791–800.
- Evans A Jr. and Anderson T J 1990 Aliphatic acids: influence on sulphate mobility in a forested cecil soil. *Soil Sci. Soc. Am. J.* 54, 1136–1139.
- Fox T R and Comerford N B 1990 Low-molecular weight organic acids in selected forest soils of the southeastern USA. *Soil Sci. Soc. Am. J.* 54, 1139–1144.
- Fries N and Forsman B 1951 Quantitative determination of certain nucleic acid derivatives in pea root exudate. *Physiol. Plant.* 4, 210–234.
- Gagnon H and Ibrahim R K 1998 Aldonic acids: A novel family of *nod* gene inducers of *Mesorhizobium loti*, *Rhizobium lupini* and *Sinorhizobium meliloti*. *Mol. Plant-Microbe Interact.* 11, 988–998.
- Gahoonia T S 1993 Influence of root-induced pH on the solubility of soil aluminium in the rhizosphere. *Plant Soil* 149, 289–291.
- Gahoonia T S, Claassen N and Jungk A 1992 Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. *Plant Soil* 140, 241–248.
- Gardner W K, Barber D A and Parbery D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant Soil* 70, 107–124.
- Gianinazzi-Pearson V, Branzanti B and Gianinazzi S 1989 *In vitro* enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. *Symbiosis* 7, 243–255.
- Gilbert G A, Knight J D, Allan D L and Vance C P 1999 Acid phosphatase activity in phosphorus-deficient white lupin roots. *Plant Cell Environ.* 22, 801–810.
- Gillespie A R and Pope P E 1990 Rhizosphere acidification increases phosphorus recovery of Black locust: I. Induced acidification and soil response. *Soil Sci. Soc. Am. J.* 54, 533–537.
- Goldstein A H, Baertlein D A and McDaniel R G 1988 Phosphate starvation inducible metabolism in *Lycopersicon esculentum*. I. Excretion of acid phosphatase by tomato plants and suspension-cultured cells. *Plant Physiol.* 87, 711–715.
- Harrison M J 1999 Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 361–389.
- Hartwig U A, Joseph C M and Phillips D A 1991 Flavonoids released naturally from alfalfa seeds enhance growth of *Rhizobium meliloti*. *Plant Physiol.* 95, 797–803.
- Hartwig U A and Phillips D A 1991 Release and modification of *nod*-gene-inducing flavonoids from alfalfa seeds. *Plant Physiol.* 95, 804–807.
- Hawes M C, Brigham L A, Wen F, Woo H H and Zhu Y 1998 Function of root border cells in plant health: Pioneers in the rhizosphere. *Annu. Rev. Phytopathol.* 36, 311–327.
- Haynes R J 1990 Active ion uptake and maintenance of cation-anion balance: A critical examination of their role in regulating rhizosphere pH. *Plant Soil* 126, 247–264.
- Hoffland E, Findenegg G R and Nelemans J A 1989 Solubilization of rock phosphate by rape. II Local root exudation of organic acids as a response to P-starvation. *Plant Soil* 113, 161–165.
- Hoffland E, Van Den Boogaard R, Nelemans J and Findenegg G 1992 Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants. *New Phytol.* 122, 675–680.
- Hungria M, Joseph C M and Phillips D A 1991 *Rhizobium nod*-gene inducers exuded naturally from roots of common bean (*Phaseolus vulgaris* L.). *Plant Physiol.* 97, 759–764.

- Hsiao T C and Lauchli A 1986 Role of potassium in plant-water relations. *In* Advances in Plant Nutrition Volume 2. Eds. B Tinker and A Lauchli. pp. 281–312. Praeger Publishers, New York, USA.
- Jackson M B 1979 Rapid injury to peas by soil waterlogging. *J. Sci. Food Agric.* 30, 143–152.
- Jones D L 1998 Organic acids in the rhizosphere – a critical review. *Plant Soil* 205, 25–44.
- Jones D L and Darrah P R 1994 Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* 166, 247–257.
- Jurkevitch E, Hadar Y and Chen Y 1986 The remedy of lime-induced chlorosis in peanuts by *Pseudomonas* sp. siderophores. *J. Plant Nutr.* 9, 535–545.
- Kettlewell B, Dong Z, Wu L and Layzell D 2000 Why do legume nodules evolve H<sub>2</sub> gas? Abstract C10, 17th North American Conference on Symbiotic Nitrogen Fixation, 23–28 July 2000, Quebec City, Canada.
- Kochian L V 1995 Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 237–260.
- Larsen P B, Degenhardt J, Tai C-Y, Stenzler L M, Howell S H and Kochian L V 1998 Aluminium-resistant Arabidopsis mutants that exhibit altered patterns of aluminium accumulation and organic acid release from roots. *Plant Physiol.* 117, 9–18.
- Lee R B 1988 Phosphate influx and extracellular phosphatase activity in barley roots and rose cells. *New Phytol.* 109, 141–148.
- Lehninger A L 1970 Biochemistry. Worth Publishers, 70 Fifth Avenue, New York.
- Li M, Osaki M, Rao I M and Tadano T 1997 Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions. *Plant Soil* 195, 161–169.
- Lipton D S, Blanchar R W and Blevins D G 1987 Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. *Plant Physiol.* 85, 315–317.
- Lowe R H and Evans H J 1962 Carbon dioxide requirement for growth of legume nodule bacteria. *Soil Sci* 94, 351–356.
- Ma J F, Hiradate S and Matsumoto 1998 High aluminium resistance in buckwheat. II. Oxalic acid detoxifies aluminium internally. *Plant Physiol.* 117, 753–759.
- Masaoka Y, Kojima M, Sugihara S, Yoshihara T, Koshino M and Ichihara A 1993 Dissolution of ferric phosphate by alfalfa (*Medicago sativa* L.) root exudates. *Plant Soil* 155/156, 75–78.
- Marschner H 1995 Mineral Nutrition of Higher Plants. 2nd Edn. Academic Press, London. 889 pp.
- Maxwell C A, Hartwig U A, Joseph C M and Phillips D A 1989 A chalcone and two related flavonoids released from alfalfa roots induce *nod* genes in *Rhizobium meliloti*. *Plant Physiol.* 91, 842–847.
- Mench M and Martin E 1991 Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L. and *Nicotiana rustica* L. *Plant Soil* 132, 187–196.
- Messens E, Geelen D, Montagu M V and Holsters M 1991 7,4'-dihydroxyflavanone is the major *Azorhizobium nod* gene-inducing factor in *Sesbania rostrata* seedling exudate. *Mol. Plant-Microbe Interact.* 4, 262–267.
- Miyasaka S C, Buta J G, Howell R K and Foy C D 1991 Mechanisms of aluminium tolerance in snapbeans. Root exudation of citric acid. *Plant Physiol.* 96, 737–743.
- Moorby J and Besford R T 1983 Mineral nutrition and growth. *In* Encyclopedia of Plant Physiology New Series Vol. 15 B: Inorganic Plant Nutrition. Eds. A Läuchli and RL Bielecki. pp. 481–515. Springer-Verlag, Berlin.
- Morris P F and Ward E W B 1992 Chemoattraction of zoospores of the soybean pathogen, *Phytophthora sojae*, by isoflavones. *Physiol. Mol. Plant Pathol.* 40, 17–22.
- Muofhe M L 1997 N<sub>2</sub> Fixation and Rhizosphere Ecology of *Aspalathus linearis* subsp. *linearis* (Rooibos tea). MSc Thesis, Botany Department, University of Cape Town.
- Muofhe M L and Dakora F D 2000 Modification of rhizosphere pH by the symbiotic legume *Aspalathus linearis* growing in a sandy acidic soil. *Aust. J. Plant Physiol.* 27, 1169–1173.
- Nanda Kumar P B A, Dushenkov V, Motto H and Raskin I 1995 Phytoextraction: The use of plants to remove heavy metals from soils. *Environ. Sci. Technol.* 29, 1232–1238.
- Neilands J B 1986 Biochemistry and genetics of microbial iron assimilation. *In* Biotechnology for Solving Agricultural Problems. Eds. PC Augustine, HD Danforth and MR Bakst. pp. 195–206. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
- Neumann G and Römheld V 1999 Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil* 211, 121–130.
- Neumann G, Massonneau A, Martinoia E and Römheld V 1999 Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. *Planta* 208, 373–382.
- Ohwaki Y and Hirata H 1992 Differences in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid level in roots. *Soil Sci. Plant Nutr.* 38, 235–243.
- Olsen R A, Bennett J H, Blume D and Brown J C 1981 Chemical aspects of the Fe stress response mechanism in tomatoes. *J. Plant Nutr.* 3, 905–921.
- Parfitt R L 1979 The availability of P from phosphate-geothite bridging complexes. Description and uptake by ryegrass. *Plant Soil* 53, 55–65.
- Parke D, Rivelli M and Ornston 1985 Chemotaxis to aromatic and hydroaromatic acids: Comparison of *Bradyrhizobium japonicum* and *Rhizobium trifolii*. *J. Bacteriol.* 163, 417–422.
- Pellet D M, Grunes D L and Kochian L V 1995 Organic acid exudation as an aluminium tolerance mechanism in maize (*Zea mays* L.). *Planta* 196, 788–795.
- Phillips D A 1992 Flavonoids: Plant signals to soil microbes. *In* Phenolic Metabolism in Plants. Eds. HA Stafford and RK Ibrahim. 26, 201–231. Plenum Press, New York. Rec. Adv. Phytochem.
- Phillips D A, Joseph C M and Maxwell C A 1992 Trigonelline and stachydrine released from alfalfa seeds activate NodD2 protein in *Rhizobium meliloti*. *Plant Physiol.* 99, 1526–1531.
- Phillips D A, Dakora F D, Sande E, Joseph C M and Zon J 1994 Synthesis, release and transmission of alfalfa signals to rhizobial symbionts. *Plant Soil* 161, 69–80.
- Phillips D A, Joseph C M, Yang G-P, Martinez-Romero E, Sanborn J R and Volpin H 1999 Identification of lumichrome as a *Sinorhizobium* enhancer of alfalfa root respiration and shoot growth. *Proc. Natl. Acad. Sci. (USA)* 22, 12275–12280.
- Plaxton W C 1996 The organization and regulation of plant glycolysis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47, 185–214.
- Rao J R, Sharma N D, Hamilton J T G, Boyd D R and Cooper J E 1991 Biotransformation of the pentahydroxy flavone quercetin by *Rhizobium loti* and *Bradyrhizobium* strains (*Lotus*). *Appl. Environ. Microbiol.* 57, 1563–1565.
- Raven J A, Franco A A, De Jesus E L and Jacob-Neto J 1990 H<sup>+</sup> extrusion and organic-acid synthesis in N<sub>2</sub>-fixing symbioses involving vascular plants. *New Phytol.* 114, 369–389.
- Raz V and Fluhr R 1992 Calcium requirement for ethylene-dependent responses. *Plant Cell* 4, 1123–1130.

- Recourt K, Schripsema J, Kijne J W, Van Brussel A A N and Lugtenberg B J J 1991 Inoculation of *Vicia sativa* subsp. *nigra* roots with *Rhizobium leguminosarum* biovar *viciae* results in release of *nod* gene activating flavanones and chalcones. *Plant Mol. Biol.* 16, 841–852.
- Rendig V V and Taylor H M 1989 Principles of Soil-Plant Interrelationships. McGraw-Hill Publishing Company, New York, USA.
- Richardson A E, Djordjevic M A, Rolfe B G and Simpson R J 1988 Effects of pH, Ca and Al on the exudation from clover seedlings of compounds that induce the expression of nodulation genes in *Rhizobium trifolii*. *Plant Soil* 109, 37–47.
- Römheld V 1987 Different strategies for iron acquisition in higher plants. *Physiol. Plant.* 70, 231–234.
- Römheld V 1991 The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: An ecological approach. *Plant Soil* 130, 127–134.
- Römheld V and Marschner H 1986 Mobilization of iron in rhizosphere of different plant species. In *Advances in Plant Nutrition*, Volume 2. Eds. PBH Tinker and A Läuchli. pp. 155–204. Praeger Publishers, New York, USA.
- Römheld V and Marschner H 1990 Genotypical differences among graminaceous species in release of phytosiderophores and uptake of iron phytosiderophores. *Plant Soil* 123, 147–153.
- Rovira A D 1969 Plant root exudates. *Bot. Rev.* 35, 35–57.
- Rovira A D and Harris J R 1961 Plant root excretions in relation to the rhizosphere effect. V. The exudation of B-group vitamins. *Plant Soil* 14, 199–214.
- Runge M and Rode M W 1991 Effects of soil acidity on plant associations. In *Soil Acidity*. Eds. B Ulrich and ME Sumner. pp. 183–202. Springer-Verlag, Berlin.
- Ryan P R, Shaff J E and Kochian L V 1992 Aluminium toxicity in roots. Correlation among ionic currents, ion fluxes and root elongation in aluminium-sensitive and aluminium-tolerant wheat cultivars. *Plant Physiol.* 99, 1193–1200.
- Siqueira J O, Safir G R and Nair M G 1991 Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. *New Phytol.* 118, 87–93.
- Smit G, Puvanesarajah V, Carlson R W, Barbour M and Stacey G 1992 *Bradyrhizobium japonicum nod D1* can be specifically induced by soybean flavonoids that do not induce the *nodYABCSUIJ* operon. *J. Biol. Chem.* 267, 310–318.
- Tadano T and Sakai H 1991 Secretion of acid phosphatase by the roots of several crop species under phosphorus-deficient conditions. *Soil Sci. Plant Nutr.* 37, 129–140.
- Terry N, Carlson C, Raab T K and Zayed A M 1992 Rates of selenium volatilization among crop species. *J. Environ. Qual.* 21, 341–344.
- Thomas C, Sun Y, Naus K, Lloyd A and Roux S 1999 Apyrase functions in plant phosphate nutrition and mobilizes phosphate from extracellular ATP. *Plant Physiol.* 119, 543–551.
- Tiwari R P, Reeve W G, Dilworth M J and Glenn A R 1996 An essential role for *actA* in acid tolerance of *Rhizobium meliloti*. *Microbiology* 142, 601–610.
- Treeby M, Marschner H and Römheld V 1989 Mobilization of iron and other micronutrient cations from calcareous soil by plant-borne, microbial and synthetic metal chelators. *Plant Soil* 114, 217–226.
- Tsai S M and Phillips D A 1991 Flavonoids released naturally from alfalfa promote development of symbiotic *Glomus* spores *in vitro*. *Appl. Environ. Microbiol.* 57, 1485–1488.
- Uren N C and Reisenauer H M 1988 The role of root exudates in nutrient acquisition. *Adv Plant Nutr.* 3, 79–114.
- Vancura V and Hovadik A 1965 Root exudates of plants. II. Composition of root exudates of some vegetables. *Plant Soil* 22, 21–32.
- Vancura V 1964 Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. *Plant Soil* 21, 231–248.
- Von Wirén N, Römheld V, Morel J L and Guckert A 1993 Influence of micro-organisms on iron acquisition in maize. *Soil Biol. Biochem.* 25, 371–376.
- West P M 1939 Excretion of thiamin and biotin by the roots of higher plants. *Nature* 144, 1050–1051.
- Zayed A, Lytle C M and Terry N 1998 Accumulation and volatilization of different chemical species of selenium by plants. *Planta* 206, 284–292.
- Zheng S J, Ma J F and Matsumoto H 1998 High aluminium resistance in buckwheat. I. Al-induced specific secretion of oxalic acid from root tips. *Plant Physiol.* 117, 745–751.
- Zhu Y, Pierson L S III and Hawes M C 1998 Release of nodulation gene inducing chemicals from root border cells. *Plant Physiol.* 115, 1691–1698.
- Zwarun A A 1972 Microbial competition for glucose in excised root experiments. *Soil Sci. Soc. Am. Proc.* 36, 968–969.