



THE ROLE OF ORGANIC ACIDS EXUDED FROM ROOTS IN PHOSPHORUS NUTRITION AND ALUMINIUM TOLERANCE IN ACIDIC SOILS

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Abstract

Soil acidity is a major problem of large areas of arable land on a global scale. Many acid soils are low in plant-available phosphorus (P) or are highly P-fixing, resulting in poor plant growth. In addition, aluminium (Al) is soluble in acid soils in the toxic Al^{3+} form, which also reduces plant growth. There is considerable evidence that both P deficiency and exposure to Al^{3+} stimulate the efflux of organic acids from roots of a range of species. Organic acids such as citrate, malate and oxalate are able to desorb or solubilise fixed soil P, making it available for plant uptake. Organic acids also chelate Al^{3+} to render it non-toxic, and are, therefore, involved in Al tolerance mechanisms. In this review, we discuss the literature on the role of organic acids exuded from roots in improving plant P uptake and Al-tolerance in acid soils. Research is now attempting to understand how P deficiency or exposure to Al^{3+} activates or induces organic acid efflux at the molecular level, with the aim of improving P acquisition and Al tolerance by conventional plant breeding and by genetic engineering. At the agronomic level, it is desirable that existing crop and pasture plants with enhanced soil-P uptake and tolerance to Al due to organic acid exudation are integrated into farming systems.

1. INTRODUCTION

Soils that are naturally acid or have become acid through agricultural activities comprise large areas of arable land on a global scale [1]. Many are either naturally low in phosphorus (P) and require applications of P fertiliser to achieve economic yields, or are highly P-fixing so fertiliser P is "locked up" in the soil and unavailable to agricultural plants. Acid soils of notoriously high P-fixation capacity are the Alfisols, Andosols, Oxisols and Ultisols [2]. A further problem of acid soils is that the solubilisation of aluminium (Al) and manganese (Mn) into the Al^{3+} and Mn^{2+} forms can result in poor plant growth. Aluminium is much more detrimental than is Mn because it kills root tips, resulting in a stunted root system and greatly reduced uptake of water and nutrients [3, 4]. A combination of high P fixation and Al toxicity in acid soils can devastate crop production. The management of acid P-fixing soils in agriculture involves the application of P fertiliser, liming to raise the soil pH, implementation of practices that reduce inputs of acid, and the use of Al-tolerant plants. In many countries, however, the application of even moderate rates of P fertiliser and lime to acid P-fixing soils is not economical because of the large areas involved and/or the low input nature of agricultural production. Consequently, the inclusion of plants that can access poorly available soil P and that are Al-tolerant has an important role in sustaining agricultural production on acid soils.

Plant roots exude many compounds into the rhizosphere, including sugars, amino acids, phenolics, and organic acids [5]. Organic acids such as malate, citrate and oxalate are implicated in a variety of processes including nutrient acquisition and metal detoxification [6]. Evidence for a direct role of organic acids in plant nutrition is available only for P, Al, iron (Fe) and Mn. This review will concentrate on P and Al, as Fe is normally not limiting in acidic soils and Mn toxicity is insignificant compared to Al toxicity. We discuss how organic acids exuded from roots affect the P nutrition of plants and help to detoxify Al^{3+} , and conclude by outlining molecular approaches for developing plants with an improved ability to access P from soils and fertilisers, and for greater tolerance of Al in acid soils.

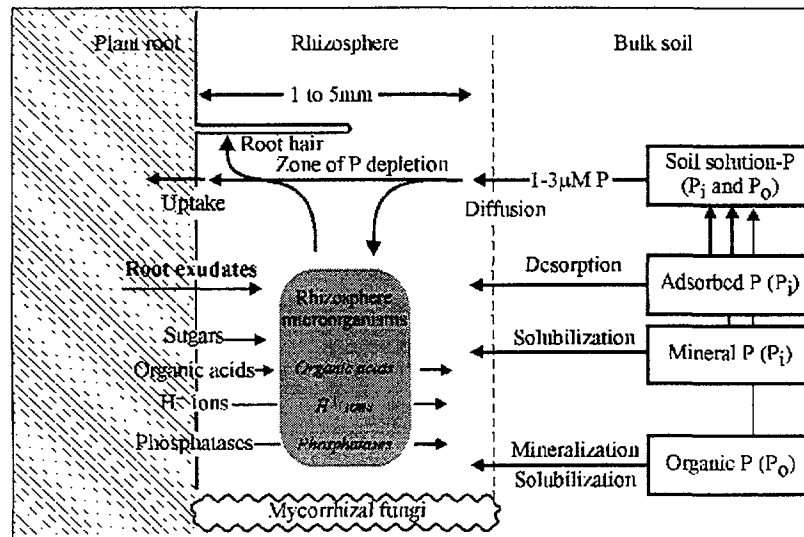


FIG. 1. Outline of the major processes affecting P availability in the rhizosphere and P supply to plant roots. Roots take up inorganic P (P_i) from the soil solution, which is replenished from (P_i) and organic P (P_o) in the solid phase. Root exudates, particularly organic acids, increase the rates of solubilisation, desorption and mineralisation, either directly or via effects on microorganisms. Mycorrhizas increase the effective absorbing area of the root and extend the volume of soil explored. (Reproduced from [8] with kind permission of Springer-Verlag Tokyo.)

2. PHOSPHORUS

2.1. Phosphorus in soils and uptake by plants

Phosphorus plays a major role in agricultural production because of its influence on plant growth and development. However, the supply of plant-available P is inadequate in most acid soils, even though the total amount of P may greatly exceed crop or pasture requirements [7]. Only a small fraction of the total P in soils is in solution in the inorganic (P_i) form (Fig. 1). This is important, as only P_i in the soil solution is directly available for uptake by roots. Because of the low P concentration in the soil solution, mass flow is relatively unimportant in supplying P to roots, and, therefore, diffusion is the major process influencing P uptake by plants. As plants deplete P_i in the soil solution, it is replenished by desorption from charged surfaces, solubilisation of P-containing minerals, and the hydrolysis of organic P compounds (Fig. 1). Despite this, rates of diffusion of P_i in soils are low ($\sim 0.13 \text{ mm day}^{-1}$) and generally insufficient to match rates of uptake by roots [9]. Consequently, it is necessary to apply fertiliser P to most agricultural soils to maintain a source of soluble P_i close to the roots to meet crop requirements. However, crops take up only about 10 to 20% of the fertiliser P in the season of application, even after many years of applying it [10, 11]. Most of the fertiliser P is either immobilised in soil organic matter, or sorbed on soil colloids or, particularly in acid soils, fixed by precipitation as Al or Fe phosphates. Soil P accumulated in fixed forms from past P applications represents a major investment by farmers, so improving the ability of plants to access it is highly desirable. In this context, plants capable of secreting large amounts of organic acids from their roots play an important role, as solubilisation of fixed P due to organic acids occurs in the rhizosphere, so that the P released is directly available to plants.

3. ROLE OF ORGANIC ACIDS IN P UPTAKE BY PLANTS

In the early 1980s, Gardner et al. [12–14] provided the first evidence linking organic acid exudation from roots to solubilisation of poorly available soil P and enhanced uptake of P. Specialized proteoid roots of white lupin (*Lupinus albus*) were shown to exude citric acid, and it was proposed that citrate improved the P nutrition of the plant by forming a ferric-hydroxy-phosphate polymer in the rhizosphere that diffused to the root, and there released the P after reduction [14]. Since then, there has been worldwide interest in the role of organic acids in enhancing nutrient acquisition by plants [15–19].

TABLE I. ORGANIC ACIDS EXUDED FROM ROOTS OF SELECTED SPECIES [8]

Species	Growth conditions	Organic acid released from roots (whole root systems; mmol h ⁻¹ g ⁻¹ dry wt. ^a)				Ref.
		Citric	Malic	Malonic	Other	
White lupin	P-deficient solution	11.0	8.0			[20]
Rice	Soil, low P	2.3			Traces of oxalic, malic, lactic, fumaric.	[21]
Maize	Nutrient-deficient	1.3	6.0			[22]
Narrow-leaved lupin	solution with nitrate as N source	1.2				[23]
Pigeon pea	Full nutrient solution	0.002	Trace	0.58	Oxalic (0.19) piscidic (0.09) tartaric (0.001)	[24]
Chickpea	Fe-deficient solution	0.001	Trace	0.006	Tartaric (0.002) fumaric (0.001)	[25]

^aAssumes dry weight is 7% of fresh weight where conversion required.

3.1. Mobilisation of P in the rhizosphere

Many studies have shown that plants exude organic acids from their roots. Citric, malic, oxalic and malonic acids are commonly found, and succinic, tartaric, piscidic, aconitic and fumaric acids have been reported also (Table I). The effectiveness of an organic acid in mobilising fixed soil P depends on its ability to complex metal ions such as Al and Fe, and displace P from charged surfaces. Organic acids desorb P in soils in the order tricarboxylic > dicarboxylic > monocarboxylic acids, and the amount of P released is proportional to the propensity to complex Al and Fe [15, 26]. Citric, a tricarboxylic acid, binds more strongly than do the di- and mono-carboxylic acids to metals such as Al and Fe that are important in the P chemistry of acid soils [15, 27]. Citric acid is effective also at releasing P in soils containing calcium (Ca) phosphates or rock phosphate fertiliser [22]; it is exuded in considerable quantities by some species, such as white lupin (Table I), especially from recently developed portions of proteoid roots [28, 29]. Significant quantities of citric acid have been detected in the rhizosphere soil of proteoid roots [30–33].

Organic acids exuded from roots can modify the chemistry of the rhizosphere, and thus alter the availability of P compounds. This occurs indirectly through promoting the growth of soil micro-organisms that mineralise P [34], or directly by:

- Changing conditions in the soil solution (e.g. pH), thus increasing the dissolution of sparingly soluble P minerals,
- Altering the surface characteristics of soil particles,
- Competing with phosphate ions for adsorption sites,
- Complexing cations that are bound to P [15].

The importance of each of these factors depends on the soil type and the forms of P present. For example, an increase in organic acid exudation can increase soil-solution P by solubilising Ca phosphates (such as from rock phosphate fertiliser) due to a decrease in pH in the rhizosphere, or by

phosphates (such as from rock phosphate fertiliser) due to a decrease in pH in the rhizosphere, or by desorption reactions in acidic soils where P solubility is controlled by ion-exchange equilibria involving charged clay minerals and organic matter [35].

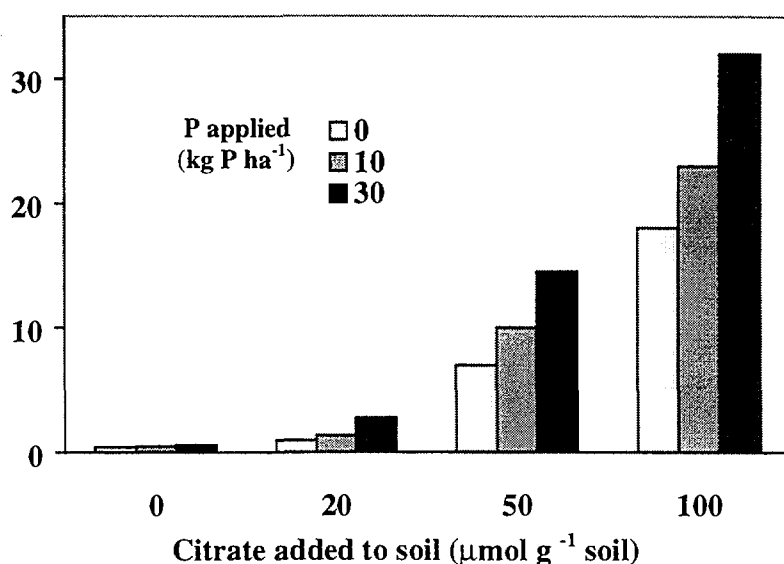


FIG. 2. Effect of eluting an Oxisol with various concentrations of citrate on the amount of P extracted. A level of 50 µmol citrate g⁻¹ soil is similar to the concentration of citrate found in the rhizosphere of white lupin. Note that citrate increased the extraction of both native and fertiliser P (G. Keerthisinghe, unpublished).

3.2. Exudation of organic acids

There is considerable evidence for the importance of organic acid exudation from roots in the acquisition of soil and fertiliser P by plants. The addition of organic acids, particularly citrate, to soils can solubilise significant quantities of fixed P [15, 36] (Fig. 2), or reduce the sorption of newly applied fertiliser P [22, 26]. A number of species respond to P deficiency by increased rates of organic acid exudation from their roots, which may be beneficial under P-limiting conditions. For example, root exudates containing citrate collected from P-stressed white lupin solubilised P bound to Fe [14]. Rhizosphere soil of proteoid roots of white lupin had elevated levels of citrate, and soluble P, Al, Mn and Fe [13, 31]. Similarly, exudates from pigeon pea (*Cajanus cajan*) roots containing malonic and piscidic acids solubilised P bound to Fe or Al in an acid soil, and the Fe-P solubilising activity of the exudates increased with increasing P stress [37]. The P mobilised by citrate exuded from proteoid roots of white lupin may persist in the soil for more than 10 weeks, although the extent to which this P is available to plants is uncertain [38]. However, wheat (*Triticum aestivum*) following white lupin had better growth and P nutrition than after wheat [39] (Table II), suggesting some carry-over benefit.

There is evidence also that organic acids exuded from roots enhance the availability of P from organic P substrates such as phytate (inositol hexaphosphate). Organic acids can free phytate that is complexed with metal ions in acid soils, making it available for breakdown by extracellular phytase enzymes [6]. However, further studies are required to establish the role of organic acids in relation to improving the capacity of agricultural plants to obtain P from phytate.

3.3. Organic acid exudation and plant access to different pools of P in the soil

Although it is clear that species differ in the amounts of P they obtain from a soil [40], it is difficult to determine if they access different P pools, or the same pools but at different rates. Studies comparing the capacity of pasture [41] and crop [42] species to obtain P, using the ³²P-dilution (L-

access to the same pools of P but with different rates of uptake. However, the species used in these studies are not known to exude organic acids.

In contrast, studies including species known to exude organic acids showed inter-specific variation in capacity to access P in different pools in the soil. For example, maize (*Zea mays*), sorghum (*Sorghum bicolor*) and chickpea (*Cicer arietinum*) obtained more P from Ca phosphate than from phosphates of Fe or Al, whereas pigeon pea took up P equally well from Ca- and Fe-bound P [43, 44]. Braum and Helmke [45] and Hocking et al. [46] used the ^{32}P -dilution technique to show that white lupin accessed soil P that was not available to soybean (*Glycine max*). Hocking et al. [46] grew seven species, including white lupin and pigeon pea, in an acidic highly P-fixing Oxisol labelled with ^{32}P . After 5 weeks of growth, the L-values indicated that the pool of soil P available to white lupin was substantially larger than that available to the other species (Fig. 3), probably due to high rates of citrate exudation from proteoid roots of white lupin. The L-value for pigeon pea, although lower than that of white lupin, was higher than values for narrow-leaved lupin (*Lupinus angustifolius*), soybean, oilseed rape (*Brassica napus*), wheat and sunflower (*Helianthus annuus*). The lower L-value for pigeon pea may be due to malonic and pascidic acids being less effective than citrate in freeing fixed P, or to lower rates of organic acid exudation than from white lupin [24]. Oilseed rape was ineffective at obtaining P from poorly available sources in this acidic Oxisol (Fe- and Al-bound P) (Fig. 3), although it can solubilise some P from Ca-bound P in rock phosphates [47, 48]. The inability of P-deficient rapeseed to access P from the Oxisol may have been due to the low rates of organic acid exudation, particularly of citrate, from its roots (3% of the rate from proteoid roots of white lupin [8]), and the rapid sorption of the organic acids exuded from roots by the Oxisol [6].

TABLE II. EFFECT OF PREVIOUS CROP ON GROWTH AND P UPTAKE BY WHEAT [39]

Previous crop	Dry matter (g plant ⁻¹)	P concentration (mg g ⁻¹)	P uptake (mg plant ⁻¹)
Wheat	0.36	2.06	0.74
White lupin	0.69	1.25	0.86

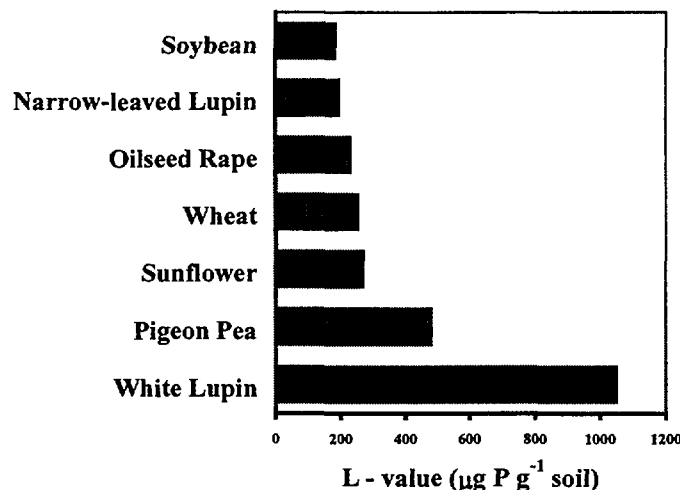


FIG. 3. Estimates of soil P available to crop species grown in an Oxisol with 1.8 mg kg⁻¹ Bray-1 extractable P and 3.1 g kg⁻¹ total P. The soil was thoroughly mixed with ^{32}P to label the isotopically-exchangeable P pool. L-values are a measure of the availability: the larger the value, the more P is available to the plant [46].

4. INCREASING THE ABILITY OF PLANTS TO ACQUIRE P

Although there is evidence that species that exude organic acids differ are superior in their ability to access different pools of soil P, there is very limited information on intra-specific variation in organic acid exudation from roots. Experimentally, it is difficult to show consistent differences between genotypes because exudation rates are altered by plant P status, root age and environmental factors. Recent work with pigeon pea cultivars did not directly measure organic acid exudation, but instead measured the ability of root exudates to solubilise soil P bound to Fe or Al [37]. Phosphorus uptake by pigeon pea from Fe-P in vermiculite culture was measured also [49]. Differences between cultivars were reasonably consistent using the two approaches. Such approaches are promising and offer hope for the development of a screening procedure to select for genotypes with enhanced organic acid exudation.

The only reported attempt to increase organic acid exudation by genetic engineering involved the enzyme citrate synthase. Tobacco transformed with a bacterial citrate synthase gene had both increased internal concentration and exudation of citrate in and from its roots [50]. Although, the significance of these changes for P nutrition has yet to be evaluated, it demonstrates the potential of a molecular approach. The next step is to characterize genes controlling the efflux of organic acids from roots so that the interaction between the biosynthesis and efflux of organic acids can be optimised.

5. ALUMINIUM TOLERANCE

Aluminium-tolerance mechanisms can be grouped into those that keep Al^{3+} out of root cells, and those that detoxify Al^{3+} internally. Recent research has focussed on the exudation of organic acids that chelate Al^{3+} in the rhizosphere and render it non-toxic. However, the ability of organic acids to chelate Al varies considerably. For example, citric, oxalic and tartaric acids were the most effective in protecting cotton (*Gossypium hirsutum*) roots from Al^{3+} toxicity, whereas malic, malonic, and salicylic acids were of moderate effectiveness, and succinic, lactic, formic, acetic, and phthalic acids provided little protection [27]. The effectiveness of organic acids in protecting roots against Al^{3+} toxicity is related to the relative positions of OH/COOH groups on the main carbon chain. Organic acids able to form stable 5- or 6-bond ring structures with Al^{3+} provide the best protection. In this review, we consider Al tolerance mechanisms based on the organic acid exuded.

5.1. Malate

Initial evidence of a role for organic acids exuded by roots in Al tolerance came from Christiansen-Weniger et al. [51] who found that an Al-tolerant wheat cultivar exuded more malate than did a sensitive cultivar. Subsequently, it was shown that for two near-isogenic wheat lines, Al^{3+} stimulated a 5- to 10-fold greater efflux of malate from roots of the Al-tolerant genotype than from the sensitive genotype [52] (Fig. 4). The malate was exuded mainly from the terminal 3 mm of the root, which is the part most susceptible to Al toxicity [53, 54]. This has since been confirmed for other wheat cultivars differing in Al tolerance [55, 56]. A strong correlation was found between malate efflux and Al tolerance among thirty wheat cultivars from diverse sources that covered a range of Al tolerances, suggesting that genes encoding for malate efflux account for a large proportion of the Al tolerance found in hexaploid wheat [57].

There is evidence that Al^{3+} activates a pre-existing mechanism for the transport of malate through a malate-permeable anion channel in Al-tolerant wheat, as there was no appreciable lag phase in malate secretion after adding Al^{3+} to the external medium [53] (Fig. 4). While the activities of key enzymes showed that both Al-tolerant and Al-sensitive genotypes had equal capacity for malate synthesis, the tolerance to Al^{3+} seemed to be related to the transport of malate out of root cells through Al-activated ion channels [53].

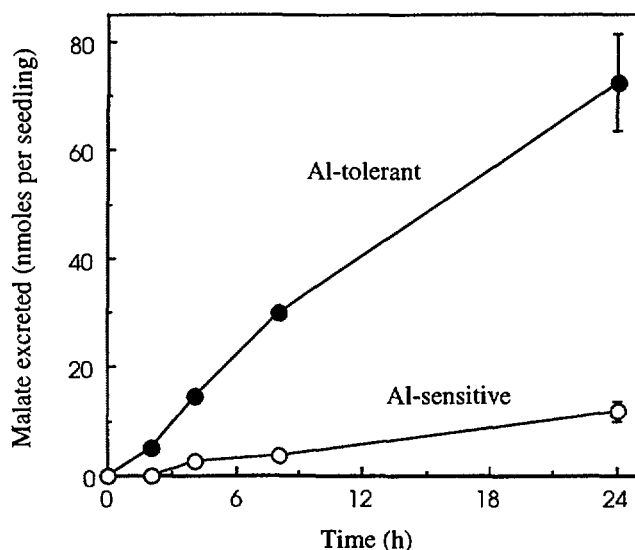


FIG. 4. The effect of exposure to $50 \text{ mmol m}^{-3} \text{ Al}^{3+}$ on the exudation of malate from roots of Al-tolerant and Al-sensitive wheat seedlings. In the absence of Al^{3+} , both genotypes secreted similar low amounts of malate. Vertical bars denote \pm range, $n=2$. (Reproduced from [52] with kind permission of Plant Physiology.)

5.2. Citrate

Citrate is probably the most effective chelator of Al, and a role for its exudation from roots in Al tolerance was initially proposed after it was found that an Al-tolerant snapbean (*Phaseolus vulgaris*) cultivar secreted 10-fold more citrate than one that was Al-sensitive [58]. Subsequently, it was shown that an Al-tolerant maize cultivar also secreted 10-fold more citrate than a sensitive one [59]. Although the efflux of citrate occurred primarily from the root apex, there was a lag phase before maximum efflux occurred, unlike the immediate response for malate efflux from wheat. This suggests that Al induces the *de-novo* synthesis of proteins involved in citrate biosynthesis and/or its transport out of maize roots. Citrate efflux from root tips of Al-tolerant maize was subsequently confirmed using another pair of cultivars differing in Al tolerance, and it was shown that citrate chelated Al^{3+} and reduced its accumulation in the root apex of the tolerant cultivar [60].

Ma et al. [61, 62] showed that Al^{3+} resulted in 2.5- to 3-fold more citrate exudation from roots of the Al-tolerant shrub *Cassia tora* than from the Al-sensitive *C. occidentalis*, and provided further evidence for the induction of citrate exudation by Al^{3+} . There is evidence also that Al-tolerance mechanisms based on the exudation of organic acids occur in tree species. Aluminium increased the exudation of citrate from roots of three leguminous trees, and this was not related to P-deficiency stress [63]. The quantities of citrate exuded were correlated with the degree of Al tolerance amongst the three species examined. Tobacco transformed with a bacterial citrate synthase gene showed enhanced Al tolerance that was associated with a 10-fold greater internal citrate concentration in the transgenics than in wild-type plants [50]. Similar results were obtained when the citrate synthase gene was over-expressed in papaya (*Carica papaya*), suggesting that this gene may be used as a general method to enhance Al tolerance by genetic engineering [50].

5.3. Oxalate

The exudation of oxalate from roots has been implicated in the Al tolerance of buckwheat (*Fagopyrum esculentum*). Exposure of its roots to Al^{3+} elicited exudation of oxalate, with kinetics similar to those of Al-stimulated efflux of malate from wheat roots [64]. Unlike wheat, buckwheat accumulated high concentrations of Al in leaves, much of it complexed with oxalate, the mechanism of which is not known. The efflux of oxalate and the internal chelation of Al by oxalate are likely to represent two related mechanisms that confer Al tolerance on buckwheat.

6. CONCLUDING REMARKS

Initially, researchers looked for variation in P-uptake efficiency within agricultural species that might be exploited by breeding and selection. Work to understand mechanisms proceeded at the physiological level following the discovery of high rates of citric acid exudation by roots of white lupin [12]. This understanding at the physiological level laid the foundation for the current progress at the molecular level. There is convincing evidence that some species secrete organic acids either to increase P uptake or to protect their root tips from Al toxicity. The strongest evidence supports a role for organic acids such as citrate, malonate and oxalate in enhancing P uptake, and malate, citrate and oxalate in Al tolerance. However, more work is needed to assess the efficacy of organic acid exudation in improving plant access to fixed P in different soils and for different forms of P fertiliser. At the agronomic level, the role of plants that exude organic acids needs to be evaluated in the P economy of crop and pasture sequences. Further research is needed to identify the mechanisms involved in activating or inducing the efflux of specific organic acids out of root cells by P deficiency or exposure to Al³⁺, and to clone genes involved in these processes. The first report of genetic engineering to increase citrate synthase activity and citrate efflux from roots [50] opens up the possibility of enhancing the access of crop and pasture species to poorly available soil P and/or increasing their Al tolerance by genetic modification. The isolation of genes the products of which are involved in transport of key organic acids across the plasma membrane is likely to further enhance both conventional and genetic engineering approaches aimed at improving the P uptake and Al tolerance of agricultural plants on acid soils.

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