Soil Biology & Biochemistry 38 (2006) 683–692

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Differential mobilization of P in the maize rhizosphere by citric acid and potassium citrate

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Received 24 March 2005; received in revised form 15 June 2005; accepted 20 June 2005
Available online 26 July 2005

Abstract

The release of organic acid anions from plant roots into soil has been hypothesized to be a mechanism for enhancing phosphorus availability in the rhizosphere. Although these compounds are excreted from the cytoplasm as organic acid anions (e.g. citrate, malate), when the H^+-ATPase is also upregulated there is evidence to suggest that they enter the soil as organic acids (e.g. citric acid, malic acid). The aim of this study was to evaluate the role of citric acid (H-citrate) and potassium citrate (K-citrate) in the mobilization and plant uptake of P from two acid soils contrasting in their P availability. Our results indicated that the mobilization of P from a $KH_2^{33}PO_4$ labelled patch of soil was soil type dependent, was controlled by its intrinsic P status, and that more P was made available by K-citrate than H-citrate. Similarly, the uptake of ^{33}P from the rhizosphere by *Zea mays* L. was greatest in the presence of K-citrate in comparison to H-citrate. However, a significant increase in shoot ^{33}P content was only observed in the more acidic soil with high P sorption potential (Haplic podzol) while no significant increase was observed in the less acidic soil with low P sorption potential (Eutric cambisol). We conclude that the chemical form of organic acid anion excretion may have a significant impact on its P mobilization capability. The contrasting results with the two acid soils indicate that organic acids may not provide a universal mechanism for enhancing P uptake from soil.

Keywords: Nutrient mobilization; Maize; Mineralization; Organic acid; Organic anion; Phosphorus; Phosphate; Root exudation; Sorption

1. Introduction

Phosphorus is often sparingly soluble in soils and consequently P deficiency in plants represents a major constraint to world agricultural production (Buresh et al., 1997). Consequently much research has concentrated on the potential to manipulate plants either through conventional breeding or genetic engineering to enhance their capability to mobilize P in soil (Kochian et al., 2004). Bhadoria et al. (2001) showed that only by increasing the soil solution P concentration by a factor of 5–6 could the P uptake by maize or groundnut be explained. Plants possess many potential mechanisms to increase P uptake from soil including an upregulation of P membrane transport systems, the increased growth of root hairs, enhanced mycorrhizal association, the release of phosphatases, changes in root architecture and the release of organic acids (Barber, 1995; Föhse et al., 1991; Marschner, 1995; Tinker and Nye, 2000). In addition, many indirect mechanisms for enhancing P availability in the rhizosphere have also been documented (Vessey, 2003; Zahir et al., 2004). The role of organic acids in the enhancement of P acquisition by plant roots remains controversial as genetic manipulation has yielded conflicting results and direct evidence for its role in soil in many plants is lacking (Jones, 1998; Jones et al., 2003). One exception is in *Lupinus albus* and members of the Proteaceae family where there is clear evidence that root exuded organic acids are capable of mobilizing P (Vance et al., 2003). In this situation, organic acid anions such as malate and citrate are released from specially adapted regions of roots (cluster roots) in very large concentrations resulting in a saturation of soil anion sorption sites and mineral

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0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.06.019

dissolution both of which can result in P release into solution (Egle et al., 2003; Shen et al., 2003). Whilst the exudation of organic acids is enhanced from most plant roots under P deficiency, in many cases the rate of release is very low in comparison to *L. albus* and it is unclear whether this is a direct response to enhance soil P availability or an indirect response due to the increased membrane permeability experienced under P deficiency (Jones, 1998). When low concentrations of organic acids are released into soil their role in mineral weathering and mineral solubilization has been questioned (Drever and Stillings, 1997). When present at low concentrations organic acids are rapidly mineralized by the soil microbial community with a mean residence time in soil of between 0.5 and 2 h (Jones and Darrah, 1994; van Hees et al., 2003). In contrast, when high concentrations of organic acids are released into the soil it is hypothesized that saturation of microbial transport systems occurs at which point they can be expected to have a long residence time in soil and be involved in P mobilization (Jones and Darrah, 1994; van Hees et al., 2002). While the mechanism for organic anion release under Al toxicity has been well characterized at a physiological and molecular level (Ryan et al., 1995; Sasaki et al., 2004) comparatively less information exists on organic anion release from P deficient plants (Ryan et al., 2003; Kochian et al., 2004; Zhang et al., 2004). Organic acid anions such as malate and citrate are predicted to exist in the cytoplasm (pH 7.1–7.4) in a fully dissociated state (e.g. citrate^{3-}, malate^{2}) rather than in the acid form (e.g. H_3·citrate^0, H_2·malate^0; Ryan et al., 2003). Current evidence suggests that upon exposure to rhizotoxic levels of Al, *Triticum aestivum* releases malate^{2-} with the release of two K^+ counter ions for charge balance (Ryan et al., 2003). Under Al toxicity an upregulation of the plasma membrane H^+-ATPase is not apparent presumably as this would reduce soil pH and exacerbate the toxic effect of Al^{3+}. However, under P deficiency there is strong evidence to suggest that the H^+-ATPase is upregulated and that H^+ release and organic acid anion release are both spatially and temporally co-ordinated (Yan et al., 2002; Hinsinger et al., 2003; Tang et al., 2004). In a calcareous soil, we have shown previously that the simultaneous presence of organic acids and H^+ enhances the availability of P (Jones and Darrah, 1994). However, the impact of these two factors on plant P uptake were not quantified and previous experiments indicated that while citrate could mobilize P in batch extracts no appreciable increase in plant P uptake was apparent when organic acids were added to the rhizosphere (Ström et al., 2002). The aim of this study was therefore to evaluate the effectiveness of organic acids in mobilizing P in two soils of contrasting P status and to determine the relative efficiency of organic acids (H-citrate) and organic acid anions (K-citrate) in plant P acquisition.

2. Materials and methods

2.1. Soil

Soil was obtained from two contrasting temperate oceanic agricultural grasslands located in Abergwyngregyn, Gwynedd, North Wales (53°14′ N, 4°01′ W). Soil A (Eutric cambisol) was collected from the surface Ah horizon (5–20 cm) of a lowland (15 m altitude) freely draining barley (*Hordeum vulgare* L.) field which receives regular fertilization (120 kg N, 60 kg K and 10 kg P yr^{-1}). Soil B (Haplic podzol) was collected from the surface Ah horizon (5–20 cm) of an upland (200 m altitude) freely draining, heavily leached, lightly sheep-grazed grassland which receives no fertilization and supports a grassland sward consisting predominantly of sheep's fescue (*Festuca ovina* L. var. Ovina) and common bentgrass (*Agrostis capillaris* L.). The mean annual soil surface temperature at 10 cm varies from 8 to 10 °C and the annual rainfall at the lowland site is 1250 mm and at the upland site 1700 mm.

Soil was removed using a spade and stored in CO_2 permeable polypropylene bags for immediate transport back to the laboratory. In the laboratory, the soil was sieved (<10 mm) and then stored field-moist at 3 °C in the same bags. Earthworms, above-ground vegetation and large masses of roots were removed by sieving.

Properties of the soils are listed in Table 1. Soil pH and electrical conductivity were determined in 1:1 (v/v) soil: H_2O extracts (Smith and Doran, 1996) and moisture by drying at 105 °C for 24 h. Total C and total N were

Table 1
Chemical and physical characteristics of the two soils used in the study

	Eutric cambisol	Haplic podzol
$EC_{1:1}$ (μS cm^{-1})	80±4	46±7
pH ($_{1:1}$, H_2O)	5.90±0.03	4.33±0.01
$CaCO_3$ (g kg^{-1})	0.11±0.02	<0.01
Water holding capacity (g kg^{-1})	520±20	690±40
Moisture content (g kg^{-1})	160±10	260±2
Organic C (g kg^{-1})	21±0.1	12±0.1
Total N (g kg^{-1}	0.16±0.01	0.8±0.01
C-to-N ratio	13.3±0.6	15.6±1.3
Soil solution NO_3^- (mg N l^{-1})	13.7±1.3	0.5±0.106
Soil solution NH_4^+ (mg N l^{-1})	1.40±0.04	1.13±0.09
Exchangeable cations		
Na (mg kg^{-1})	29±3	37±1
K (mg kg^{-1})	116±18	77±12
Ca (mg kg^{-1})	1595±217	89±8
Mg (mg kg^{-1})	89±19	15±2
Al (mg kg^{-1})	22±2	323±55
Extractable P (mg kg^{-1})	9.9±0.3	0.22±0.09
Root biomass (g m^{-3})	0.39±0.01	0.17±0.06
Soil respiration (g CO_2 m^{-2} h^{-1})	0.60±0.02	0.25±0.02

All values represent means±SEM (n=3).