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Low-P tolerance by maize (*Zea mays* L.) genotypes: Significance of root growth, and organic acids and acid phosphatase root exudation

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Abstract

We investigated some mechanisms, which allow maize genotypes to adapt to soils which are low in available P. Dry matter production, root/shoot-ratio, root length and root exudation of organic acids and acid phosphatase were investigated in four maize genotypes grown under P-deficient and P-sufficient conditions in sterile hydroponic culture. A low-P tolerant, an acid-tolerant and a low-P susceptible genotype of maize were compared with a Swiss commercial cultivar. The study found increased root development and increased exudation of acid phosphatase under P-deficient conditions in all maize genotypes, except for the Swiss cultivar. Effects on root formation and acid phosphatase were greater for the low-P tolerant than for the low-P susceptible, and the acid soil tolerant genotypes. Organic acid contents in root tissues were increased under P deficiency and related to increased PEPC activity. However, the increase in contents was associated with an increase in exudation for the low-P tolerant genotype only. The low-P susceptible genotype was characterized by high organic acid content in roots and low organic acid exudation. The organic acids content in the phloem exudates of shoots was related to root exudation under different P supply, to the difference between lines in organic acids root content, but not to the low-P tolerance or susceptibility of maize genotypes.

Introduction

Low-phosphorus (P) availability strongly limits plant productivity in tropical soils. Plant adaptations allowing for an improved growth in low P soils are related to the ability of a plant to take up more P from a deficient soil (higher P acquisition efficiency), or/and to its ability to produce more dry matter for a given quantity of P (higher P use efficiency) (Marschner, 1995; Raghothama, 1999). A higher P acquisition efficiency can be related (a) to the development of a more extensive root system, in association or not with mycorrhizal fungi, or specific specialized roots such as proteoid roots or root hairs (McCully, 1999; Raghothama, 1999), allowing the plant to explore a larger volume of soil, and (b) to changes in root physiology allowing the uptake of P at lower concentrations in the soil solution, and/or the uptake of P from insoluble inorganic or organic forms (Marschner, 1995).

Modification of root growth and architecture is a well-documented response to P starvation (Lynch, 1995; Mollier and Pellerin, 1999). Authors generally agree that P deficiency in maize leads to a higher root/shoot ratio (Anghinoni and Barber, 1980; Rosolem et al., 1994). Effects of P deficiency on root biomass and root length are more controversial. Anghinoni and Barber (1980), using a P starvation experiment, found increased root length and dry weight in 12 day-old maize plants. By contrast, Khamis et al. (1990) observed no effect of P deprivation on maize root biomass.

When phosphate is taken up by plants, it is transferred from the solid phase of the soil to the roots via the rhizosphere (soil/root interface), a zone where up to 30% of the plant photosynthetates may be exuded

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by the roots (Lynch and Whipps, 1989). Excretion of organic acids, enzymes and protons by roots may play a major role in the P nutrition of various crops (Raghothama, 1999; Uren and Reisenauer, 1988). The competition of phosphate and organic anions for similar adsorption sites can increase the concentration of P in the soil solution (Gerke, 1992; Staunton and Leprince, 1996). Nagarajah et al. (1970) and Jones and Darrah (1994b) showed that the efficiency with which organic acids desorb P from iron-oxide and clay minerals, or prevent the sorption of newly-added P, decreased in the order tri-, di- and monocarboxylic oganic acids. Root exudation of malic and succinic acids were increased in radish and rape plants under P starvation (Zhang et al., 1997). Increased exudation of citric acid by white lupin under P-deficient conditions (Johnson et al., 1996) led to a higher release of inorganic phosphate from phosphated ferric hydroxide (Gardner et al., 1983) and from a pool of soil phosphorus unavailable to soybean (Braum and Helmke, 1995). Gerke (1992) showed that citric acid also liberates P complexed by soil organic matter. In white lupin, Johnson et al. (1996) demonstrated that increased citric and malic acid secretion from proteoid roots under P deficiency was correlated with the increased activity of several enzymes involved in organic acid synthesis, including phospho*enol*pyruvate carboxylase (PEPC). While the secretion of carbohydrates and amino acids by maize roots increased under P-deficient conditions (Jones and Darrah, 1994a; Matsumoto et al., 1979), little is known about the influence of P nutrition on the release of organic acids from maize roots.

Tarafdar and Jungk (1987) measured acid phosphatase activity in the rhizosphere of wheat and clover corresponding to a depletion of soil organic phosphates across this zone. Their work suggests that rhizosphere phosphatases play an important role in the release of Pi from organic soil P, for subsequent uptake by plants. Enhancement of acid phosphatase activity with phosphate starvation has been demonstrated for maize (Helal and Sauerbeck, 1987; Kummerova, 1986).

This research was conducted to elucidate some of the putative mechanisms governing P acquisition efficiency in maize. To achieve this, four lines (a low-P tolerant line from Thailand, acid soil-tolerant and low-P susceptible lines from Colombia, and a Swiss cultivar bred in P-rich soils) were grown in hydroponic culture with the roots maintained under sterile conditions, and the effect of P starvation on plant growth, root growth and the exudation of organic acids and acid phosphatase by roots was studied.

Materials and methods

Plant material

Four genotypes of maize (*Zea mays* L.) were selected for the study. These were as follows:

*NST*90201 (S) CO-422-2-3-1-7. An inbred line derived from a triple hybrid developed by the Thai Department of Agriculture. This inbred has been selected as tolerant to low-P conditions.

SA3-C4HC (16×25)-2-4-9-7-B-B-B-B-1. An inbred line developed by CIMMYT-Colombia from the acid soil tolerant population SA3. This inbred is susceptible to low-P conditions.

ICA V-110 *Sikuani*. An open pollinated variety developed in Colombia by recombining selected acid soil-tolerant lines derived from Population SA3 (Friesen et al., 1997).

Corso. A swiss genotype selected in high P soils for fast development and high dry matter production in the first vegetative growth stages.

NST, SA3 and Sikuani were provided by the CIMMYT regional office at CIAT, Cali, Colombia. Corso was provided by UFA Seed Company, Switzerland.

Growth conditions

Seeds of 0.28–0.30 g weight were surface sterilized (30 s in 18 *m* H_2SO_4, 5 min in 95% ethanol, and 30 min in 10% H_2O_2, washing with sterile water after each treatment), germinated on agar plates (4 d in the dark at room temperature) and transferred to culture vessels under sterile conditions. A culture vessel consisted of a Pyrex tube (50 cm long, 5 cm diameter) with a rubber cap on the bottom and an aluminum cap on the top. One germinated seed was placed on a net of Teflon (0.4 cm diameter mesh), fixed 6 cm below the top end of the tube. Nutrient solution was contained in the tube up to 2.0 cm below the Teflon-net. Nutrient solution and culture vessel had been autoclaved separately before the seed was added. Nutrient solutions were modified from Hoagland and Arnon (1938) and consisted of $mgSO_4$ (1 m*M*); $Ca(NO_3)_2$ (2.5 m*M*); Fe-EDTA (0.1 m*M*); H_3BO_3 (0.01 m*M*); $MnSO_4$ (0.001 m*M*); $ZnSO_4$ (0.001 m*M*); $CuSO_4$ (0.0005 m*M*); Na_2MoO_4 (0.0005 m*M*). The + P treatment contained normal nutrient solution with 0.75 m*M* K_2SO_4 and 1 m*M* KH_2PO_4, while the – P treatment

was amended with 1.25 m*M* K_2SO_4only. The pH of the nutrient solution was adjusted to 5.5 and was not affected by plant growth as showed by Bertrand (1998). After 5 days of plant growth, when seedlings had been established, aluminum caps were removed and the Teflon-net was overlayered with 0.5–1.0 cm of sand (0.5–0.7 cm particle size) and a mixture of liquid paraffin (solid below 51–53°C, Merck) and Vaseline (Maino Pharm AG) leaving the roots in the sterile nutrient solution and the shoots exposed to the open air. Air was introduced into the nutrient solution at the bottom through a sterile filter (0.2 μm, Millipore) and released at the top through a silicone hose crossing the paraffin layer and a second sterile filter. Nutrient solution was changed every 3 d through the bottom of the tube. Each manipulation with the culture vessels was conducted using aseptic technique. Plants were grown in a controlled environment with a photosynthetic photon flux density of 250 μmol quanta $m^{-2}s^{-1}$ during a 16 h photoperiod and a day/night-temperature of 23/18 °C.

Determination of growth characteristics

Dry matter, P content in root and shoot and length of the 3 longest roots of each plant were determined in 18-day old seedlings. Dried plant parts were homogenized and samples (250 mg) were ignited at 540 °C in an oven for 5 h. Residues were extracted in 6.5 *N* HCl and analyzed for P according to John (1970).

Preparation of samples for analysis of organic acid contents and PEP Carboxylase activity

Root extracts

Root tips (segment A; 0–1.5 cm), and segments at 5 cm distance from root tips (segment B; 5–6.5 cm) were sampled. Twenty root fragments (50–150 mg fresh weight) for each root part were weighted and kept on ice, and ground with a pestle in a cold mortar, with 1.5 ml CO_2-free buffer (100 m*M* Tris, 10 m*M* $mgCl_2$, pH 8.0) and 0.5 g sea sand. After centrifugation (14 000 *g*, 2 min) organic acid content and PEPC activity were determined in the supernatant.

Exudation samples

Phloem sap was collected according to the modified method of King and Zeevaart (1974). Shoots were cut 0.5 cm above the basis and dipped in 1 m*M* Na-EDTA to discard xylem exudates. After 10 min, phloem sap was collected in 50 ml 1 m*M* Na-EDTA for 2 h.

To measure organic acids exuded by whole roots, samples of nutrient solution were collected 3 h after nutrient solution in the culture vessels were renewed.

The exudation by specific root parts was examined by transferring plants into a 5 cm high PVC box (25 cm × 15 cm × 5 cm) filled with nutrient solution. Specific parts of the intact root (segment A and B) were dipped for 3 h into a plastic cap (1.5 cm high and 4 cm ∅), filled with the specific nutrient solution and containing 0.25 mg benzylpenicillin potassium salt ml^{-1} (Fluka ref. 13750) to prevent bacterial growth.

The exudation samples were tested for sterility on agar plates at room temperature and 37 °C, and stored at −20 °C until required for analysis of organic acids.

Organic acid analyses

Organic acids were analyzed by ion chromatography using a Dionex DX500 system. An anion exclusion column Ion Pac ICE-AS6 was used in combination with an anion-ICE micromembrane suppressor. The eluent was 1 m*M* fluorobutyric acid and had a flow rate of 1 ml/min. The regenerant for the suppressor was 5 m*M* tetrabutylammonium hydroxide and had a flow rate of 4 ml/min. Suppressed conductivity was detected. Samples were acidified (100 μl 1 *N* HCl added to 10 ml sample) and purged with nitrogen (N_2) in order to lower carbonate concentration. Undiluted samples (50 μl) were injected and analyzed. This method was not appropriate for oxalic acid, which coeluted with inorganic anions, or for long-chain carbonic acids (butyric acid etc.), which were not eluted in this system. Samples were tested for oxalic acid using an anion-exchange column Ion Pac AS10 in combination with a suppressor ASRS II with 50 m*M* NaOH as eluent. Oxalic acid contents were very low and are not reported.

Determination of PEP carboxylase (PEPC) activity in roots

The PEPC assay was conducted in a spectrophotometer at 25 °C according to the NADH-linked method (Vance et al., 1983). The final volume of the reaction mixture was 1 ml and contained 25–100 μl root extract in CO_2-free extraction buffer, 2 m*M* phospho*enol*pyruvate (PEP), 0.14 m*M* nicotinamide adenine dinucleotide (NADH), and 5 Units malate dehydrogenase (MDH). The reaction was initiated by adding 5 m*M* HCO_3^- and the decrease in absorption at 340 nm was recorded.

Determination of acid phosphatase activity

Activity released into solution

Nutrient solution in the culture vessels was replaced by sterile distilled water containing 1 m*M* $CaCl_2$. The activity of acid phosphatase was assayed after 24 h of root exudation, using *p*-nitrophenyl phosphate (*p*NPP) as a substrate (Tabatabai, 1982). An aliquot of solution adjusted to a total volume of 5 ml with distilled water was added to 0.5 ml of the Modified Universal Buffer (pH 5.0) and 1 ml of 0.025 *m p*NPP in reagent tubes. Tubes were maintained at 37 °C for 1 h and the reaction was terminated by the addition of 20 ml of 0.5 *M* NaOH. The absorbance at 410 nm was measured to determine the amount of released *p*-nitrophenol (*p*NP). Phosphatase activity was expressed in terms of Units (U). One Unit of acid phosphatase is the amount of enzyme which hydrolyses 1.0 μmol of *p*-nitrophenyl phosphate per min at 37 °C.

Activity adhering to the roots

Roots were incubated for 30 min at 3–4 °C in sterile distilled water containing 100 m*M* NaCl, in order to collect the acid phosphatase adhering to the epidermal cell layers of the roots. The activity of acid phosphatase released into the solution was determined as described above.

Statistical background

Statistical analyses of data were carried out by ANOVA tests. Significance was assigned at $p < 0.05$ with Duncan's test. All the analyses were performed using the SYSTAT® statistical package (SYSTAT, 1994).

Results and discussion

Plant growth

Dry matter production and morphological traits

Plant dry matter production of 18 d old seedlings was significantly higher for the genotype NST than for the other three genotypes ($p < 0.001$) (Table 1). P deficiency significantly decreased total plant dry matter ($p < 0.001$) but not root dry matter and increased the root/shoot-ratio ($p < 0.001$). The effects of P deficiency were especially strong for NST: a considerable decrease in plant dry matter (27%) was associated with a strong increase in both root dry matter (56%) and average maximum root length (33%) suggesting increased assimilate allocation in the root system for nutrient uptake in this low-P tolerant genotype. Relatively small changes with P deficiency were found for the low-P susceptible SA3 and for Corso. The acid soil-tolerant Sikuani responded similarly to NST to P starvation (Table 1).

P deficiency increased the anthocyanin red coloration in the maize genotypes, whereas in the presence of P, leaves of the four genotypes remained green (Table 2). Coloration was especially strong in the low-P tolerant NST and the acid soil-tolerant Sikuani. Anthocyanin formation may be a protective mechanism against oxygen free radical stress induced in P-deficient illuminated leaves (Hrazdina and Zobel, 1991; Schopfer, 1984). It is suggested that anthocyanin formation may contribute to low-P tolerance of maize genotypes.

P content in seedlings

P contents in 18-d old maize seedlings grown in P-deficient nutrient solution were lower than the amount of P measured in seeds (1.14 ± 0.08 mg P/seed across the four genotypes), suggesting that part of the P initially present in seeds remained in the seed (Table 2). Under P starvation, the smallest difference between P content in seedlings and in initial seeds was found for the low-P tolerant NST. This suggests that this genotype mobilized most of the seed P during the early growth stages. Under P-deficient conditions, the highest P concentration in roots was observed for NST, suggesting a priority in the partitioning of P to root development in this genotype.

Organic acids exudation and synthesis

Organic acids in root exudates

Axenic conditions in the root compartment of the culture vessel were maintained until the end of the experiments (18 d). Organic compounds found in the solutions of the root compartment could, therefore, be assumed to be the original exudates and not metabolic products of micro-organisms. Exudates contained monocarboxylic organic acids (acetic, formic, glycolic and lactic acids), dicarboxylic organic acids (malic, oxalic and succinic acids) and tricarboxylic organic acids (citric and *trans*-aconitic acids). The composition compared well with previous studies of root exudates using maize (Jones and Darrah, 1995; Kraffczyk et al., 1984; Mench et al., 1988; Petersen and Böttger, 1991).

Anions of monocarboxylic acids are weak chelators of polyvalent metal cations such as Fe^{3+} and Ca^{2+}

Table 1. Dry matter production, root/shoot-ratio and maximum root length of 18-d old seedlings. + P = 1 m*M* P; - P = no P. $n = 6$. Within the same genotype and the same parameter values followed by the same capital letter are not statistically different at $p = 0.05$. Within the same P treatment and the same parameter values followed by the same small letter are not statistically different at $p = 0.05$

Genotype	P supply	Plant dry matter (g)	Root dry matter (g)	Root / shoot-ratio of dry matter	Average maximum root length (cm)
Corso	+ P	0.89 ^{Ab}	0.29 ^{Aa}	0.49 ^{Ba}	34.6 ^{Aa}
	− P	0.77 ^{Bb}	0.29 ^{Ab}	0.60 ^{Ab}	33.4 ^{Ac}
Sikuani	+ P	0.98 ^{Ab}	0.22 ^{Aa}	0.30 ^{Bb}	33.8 ^{Ba}
	− P	0.72 ^{Bb}	0.26 ^{Ab}	0.56 ^{Ab}	48.5 ^{Aa}
SA3	+ P	0.96 ^{Ab}	0.25 ^{Aa}	0.35 ^{Bb}	35.6 ^{Aa}
	− P	0.78 ^{Bb}	0.27 ^{Ab}	0.54 ^{Ab}	39.2 ^{Ab}
NST	+ P	1.28 ^{Aa}	0.25 ^{Ba}	0.24 ^{Bb}	34.2 ^{Ba}
	− P	0.93 ^{Ba}	0.39 ^{Aa}	0.73 ^{Aa}	45.4 ^{Aa}

and are considered to be inefficient in mobilizing of metal-bound P (Nagarajah, 1970). Monocarboxylic acids were therefore not specifically considered in our study. Oxalic acid occurred in very low concentrations and was also ignored. Therefore, the following data specifically concentrate on malic, succinic, citric and *trans*-aconitic acids as well as on the total amount of organic acids.

Exudation of organic acids from the whole root system

Release of organic acids from the different genotypes was generally higher under P starvation conditions than with 1 m*M* P treatment (Table 3). Between genotypes, differences in organic acids root exudation existed. The low-P tolerant genotype, NST, showed the highest increase in organic acids root exudation with P deficiency. Furthermore, under P-deficient conditions, the release of citric and malic acids, both determined in the literature as efficient in the P mobilization from soil (Hoffland et al., 1989; Jones and Darrah, 1994b; Nagarajah et al., 1970), was significantly higher for NST than for the other genotypes ($p < 0.001$). When expressed in nmol C g^{-1} plant dry weight, *trans*-aconitic acid was the predominant organic acid in the root exudates of the four maize genotypes (Table 3). The highest rate of exudation of *trans*-aconitic acid was observed for Corso. While the concentration of *trans*-aconitic acid in root exudates for NST, SA3 and Sikuani lines was lower than for Corso, the contribution of malic and citric acids in these genotypes was increased. Jones and Darrah (1995) suggested that aconitate may act as a charge-balancing anion in the absence of other organic acids. Succinic acid was only important in root exudates of the acid soil-tolerant line Sikuani. Hue et al. (1986) showed that succinic acid may contribute to Al detoxification by Al-resistant genotypes.

Exudation of organic acids from two different root segments

The rate of exudation was different for various root segments (Table 4). It was significantly higher for root tips (segment A) than for segments B, located at 5 cm distance from the tips ($p < 0.001$). Release of organic acids from the different root segments was significantly higher under P starvation conditions than with 1 m*M* P treatment ($p < 0.001$) (Table 4). When expressed in nmol C cm^{-1} fresh root, *trans*-aconitic acid was again the predominant organic acid in the root exudates of the four maize genotypes. For segments A and B, the total exudation of organic acids ($p < 0.001$), and the release of citric ($p = 0.004$) and malic acids ($p = 0.003$) in particular, were significantly higher for NST than for the other three genotypes. The exudation of succinic acid was only detected from the root tips of Sikuani under P-deficient conditions (Table 4).

Table 2. P content in 18 d old seedlings grown in P-deficient solution (- P) and in 1 m*M* P solution (+ P). $n = 6$. Within the same P treatment and the same parameter mean values followed by the same letter are not statistically different at $p = 0.05$. For each parameter and genotype, mean values from both P treatments were significantly different at $p = 0.05$

Genotype	P treatment	P content (mg/plant)	P in roots (mg/g dry weight)	P in shoot (mg/g dry weight)	Anthocyanin coloration
Corso		0.86 ^{b}	0.99 ^{c}	1.19 ^{a}	+ +
Sikuani		0.79 ^{b}	0.99 ^{c}	1.14 ^{a}	+ + + +
SA3	– P	0.81 ^{b}	1.14 ^{b}	0.99 ^{b}	+
NST		1.06 ^{a}	1.35 ^{a}	0.99 ^{b}	+ + +
Corso		9.02 ^{b}	9.57 ^{b}	10.47 ^{a}	–
Sikuani		7.98 ^{b}	8.94 ^{b}	7.89 ^{b}	–
SA3	+ P	8.89 ^{b}	9.99 ^{b}	8.99 ^{b}	–
NST		13.59 ^{a}	11.49 ^{a}	10.41 ^{a}	–

Table 3. Root exudation of organic acids. + P = 1 m*M* P; - P = no P. Mean values; n = 6. n.d.: not detected. Means with the same letter are not statistically different at $p = 0.05$. Capital letters refer to differences between P treatments (read horizontally). Small letters refer to differences between genotypes (read vertically)

Genotype	Organic acid	Root exudation	
		– P	+ P
		nmol C / g plant dry weight / h	
Corso	Malic	58.1 ^{Ac}	37.6 ^{Bc}
	Succinic	4.6 ^{Ab}	3.8 ^{Aa}
	Citric	43.0 ^{Ab}	22.9 ^{Bb}
	Trans-aconitic	243.8 ^{Aa}	252.6 ^{Aa}
	Total org. acids	395.8 ^{Ab}	357.4 ^{Ba}
Sikuani	Malic	98.8 ^{Ab}	59.6 ^{Bb}
	Succinic	18.8 ^{Aa}	2.4 ^{Bb}
	Citric	36.9 ^{Ac}	12.7 ^{Bc}
	Trans-aconitic	212.7 ^{Ab}	103.1 ^{Bb}
	Total org. acids	411.4 ^{Ab}	215.6 ^{Bb}
SA3	Malic	86.8 ^{Ab}	72.5 ^{Aa}
	Succinic	n.d.	2.2 ^{b}
	Citric	47.2 ^{Ab}	37.8 ^{Aa}
	Trans-aconitic	112.0 ^{Ac}	67.2 ^{Bc}
	Total org. acids	291.0 ^{Ac}	220.7 ^{Bb}
NST	Malic	133.3 ^{Aa}	52.3 ^{Bb}
	Succinic	n.d.	0.9 ^{c}
	Citric	69.7 ^{Aa}	22.2 ^{Bb}
	Trans-aconitic	199.4 ^{Ab}	90.0 ^{Bb}
	Total org. acids	451.2 ^{Aa}	208.9 ^{Bb}

Table 4. Organic acid exudation from two root segments. Segment A: root tip, length: 1.5 cm; Segment B: distance from root tip: 5 cm, length 1.5 cm. + P = 1 m*M* P; − P = no P. Mean values; n = 6. n.d.: not detected. Means with the same letter are not statistically different at $p = 0.05$. Capital letters refer to differences between P treatments (read horizontally). Small letters refer to differences between genotypes (read vertically). Roman numbers refer to differences between root segments (read horizontally)

Genotype	Organic acid	Root exudation			
		Segment A		Segment B	
		− P	+ P	− P	+ P
		nmol C / cm fresh root / h			
Corso	Malic	0.4 ^{AbI}	0.3 ^{Ab}	0.1 ^{bII}	n.d.
	Succinic	n.d.	n.d.	n.d.	n.d.
	Citric	0.3 ^{bI}	n.d.	0.1 ^{bI}	n.d.
	Trans-aconitic	2.5 ^{AbI}	2.3 ^{AbI}	0.7 ^{AbII}	0.4 ^{AbII}
	Total org. acids	3.6 ^{AbI}	3.2 ^{AbI}	1.1 ^{AbII}	0.5 ^{BbII}
Sikuani	Malic	0.7 ^{AbI}	0.6 ^{AbI}	0.3 ^{AbII}	0.2 ^{AbII}
	Succinic	0.3 ^{b}	n.d.	n.d.	n.d.
	Citric	0.3 ^{b}	n.d.	n.d.	n.d.
	Trans-aconitic	1.9 ^{AbI}	1.2 ^{BbI}	0.6 ^{AbII}	0.4 ^{AbII}
	Total org. acids	3.6 ^{AbI}	2.1 ^{BbI}	1.1 ^{AbII}	0.7 ^{BbII}
SA3	Malic	0.8 ^{AbI}	0.6 ^{AbI}	0.3 ^{AbII}	0.2 ^{AbII}
	Succinic	n.d.	n.d.	n.d.	n.d.
	Citric	0.5 ^{AbI}	0.1 ^{Bb}	0.1 ^{bII}	n.d.
	Trans-aconitic	0.8 ^{AbI}	0.6 ^{AbI}	0.3 ^{AbII}	0.2 ^{AbII}
	Total org. acids	2.5 ^{AbI}	1.4 ^{BbI}	0.8 ^{AbII}	0.5 ^{AbII}
NST	Malic	0.9 ^{AbI}	0.6 ^{AbI}	0.4 ^{AbII}	0.2 ^{AbII}
	Succinic	n.d.	n.d.	n.d.	n.d.
	Citric	0.6 ^{AbI}	n.d.	0.1 ^{bII}	n.d.
	Trans-aconitic	2.2 ^{AbI}	1.3 ^{BbI}	1.0 ^{AbII}	0.9 ^{AbI}
	Total org. acids	4.2 ^{AbI}	2.2 ^{BbI}	1.7 ^{AbII}	1.4 ^{AbII}

The root release of organic acids did not appear to be associated with the release of protons from maize roots (data not shown), as also showed by Bertrand (1998). This suggests that the mobilization of P in substrates of maize roots may be increased due to the anion exchange and metals complexation of released organic acid anions and not to acidification. Furthermore, as described by Jones and Darrah (1994b), organic acids are dissociated in root cells in the pH conditions of the cytoplasm, and so they should be released as organic anions and should not contribute *per se* to the acidification of the rhizosphere.

Organic acid content

Organic acid content in two different root segments

When expressed in μmol C per fresh weight, the total content of the organic acids in the root tips (segment A, 0–1.5 cm) and in the root segments B (5–6.5 cm) was higher when plant nutrition was P-deficient than when it was P-sufficient ($p < 0.001$) (Table 5). This increase can be related to an increased cation uptake rate, which could not be fully compensated by anion uptake in the absence of phosphate ions, and/or to an increase in NO_3^- uptake following the onset of P deficiency (Imas et al., 1997a, b). Organic acids in tissues are required for counter-balancing increased cation/anion-ratios. Nitrate nutrition leads to an in-

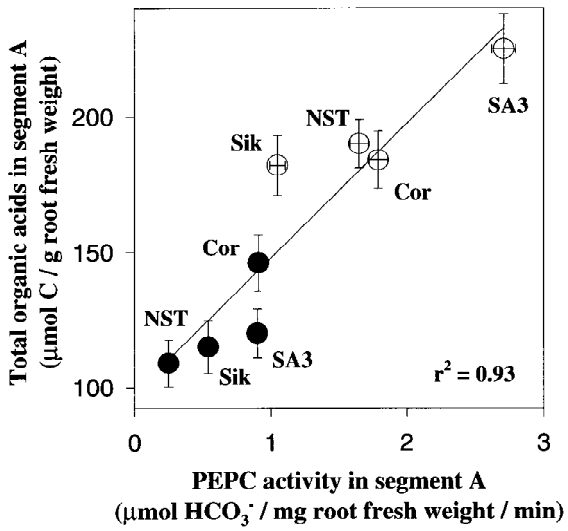

Figure 1. Total organic acids content as related to PEPC activity in root tips (1.5 cm). Closed symbols: P-sufficient conditions; open symbols: P-deficient conditions. Cor: Corso; Sik: Sikuani. Mean value ± SE. $n = 6$.

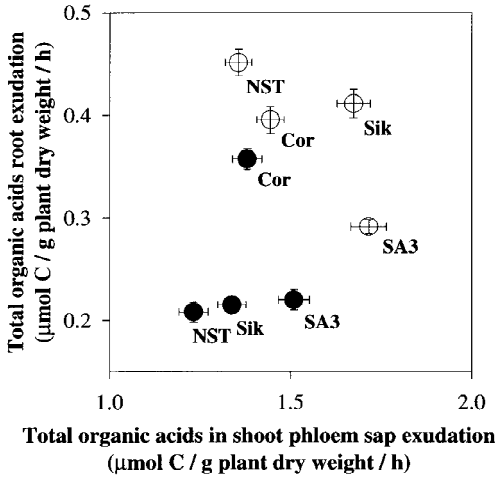

Figure 2. Total organic acids exudation from roots as related to phloem exudation from shoots. Closed symbols: P-sufficient conditions; open symbols: P-deficient conditions. Cor: Corso; Sik: Sikuani. Mean value ± SE. $n = 6$.

crease in cytoplasmic pH and the synthesis of organic anions serves as a compensation for the biochemical pH stat and to replace the negative charge lost when NO_3^- is reduced (Imas et al. 1997a, b; Kirkby and Knight, 1977, Marschner, 1995). Differences in the content of organic acids in segments A and B existed (Table 5). Under P-deficient conditions for the four maize lines, organic acids content was equal or lower in root tips than in root segments B. Differences were also noticed between maize genotypes. Total organic acids content, and the content of malic and citric acids in particular were higher in the low-P susceptible SA3 than in the three other genotypes ($p < 0.001$).

Organic acid contents in root tips as related to PEPC-activity

Organic acid content in the root tips (segment A) was correlated with PEPC activity (r^2=0.93; Figure 1), suggesting that organic acid content was at least partly controlled by PEPC activity.

Root exudation of organic acids from the whole root system and two different root segments as related to organic acid contents

Root exudation of organic acids from root segment A (tip, length 1.5 cm) and root segment B (5 cm from the tips, length 1.5 cm) increased with organic acid contents, when expressed both per cm root length and on a fresh weight basis respectively (Tables 4 and 5). This was especially true for the NST and Sikuani in the whole root system and segment A. However, root exudation by the lines Corso and SA3 was less affected by an increase in organic acid contents. Collectively, the organic acids measurements indicate that under P deficiency, a higher content in total organic acids, and malic and citric acids in particular, in segments A and B and a lower root exudation were found for SA3 than for the other lines. It is suggested that the lower rate of exudation of organic acids for SA3 was one reason for the low tolerance of this genotype to P deficiency.

Organic acids in phloem exudation

Trans-aconitic acid was the most abundant organic acid in the phloem exudates of the genotypes Corso, Sikuani and NST ($p < 0.001$) (Table 6). For SA3, however, malic acid was the most predominant organic acid ($p < 0.001$). As was suggested above within the root exudation, trans-aconitic may act as a charge-balancing anion in the absence of other organic acids. Under P deficiency, the concentration of organic acids significantly increased in the phloem exuded from the shoot ($p < 0.001$). NO_3^- reduction and assimilation in the shoots is very important for maize (Marschner, 1995; Pearson et al., 1981), suggesting that a high proportion of organic acids may be synthesized in the shoots and transported to the roots through phloem, where it contributes to root exudation. For rape, Hoffland et al. (1990) showed that the secretion of organic acids by the roots of P-deficient resulted

Table 5. Organic acid content of two root segments. Segment A: root tip, length: 1.5 cm; Segment B: distance from root tip: 5 cm, length 1.5 cm. + P = 1 m*M* P; − P = no P. Mean values; $n = 6$. n.d.: not detected. Means with the same letter are not statistically different at $p = 0.05$. Capital letters refer to differences between P treatments (read horizontally). Small letters refer to differences between genotypes (read vertically). Roman numbers refer to differences between root segments (read horizontally)

Genotype	Organic acid	Root content			
		Segment A		Segment B	
		− P	+ P	− P	+ P
		μmol C / g root fresh weight			
Corso	Malic	12.4 ^{AdI}	7.2 ^{BcI}	13.2 ^{AcI}	4.0 ^{BcII}
	Succinic	1.3 ^{AbI}	0.8 ^{AbI}	0.3 ^{AbII}	0.4 ^{AbI}
	Citric	11.5 ^{BaI}	21.7 ^{AbI}	7.8 ^{BbI}	13.8 ^{AcII}
	Trans-aconitic	133.9 ^{AaI}	99.3 ^{BaI}	143.7 ^{AbI}	89.8 ^{BaI}
	Total org. acids	184.9 ^{AbI}	146.7 ^{BaI}	191.0 ^{AcI}	120.9 ^{BbI}
Sikuani	Malic	39.8 ^{AcI}	29.1 ^{BbI}	35.6 ^{AbI}	24.0 ^{BbI}
	Succinic	7.6 ^{AaI}	4.8 ^{BaI}	6.8 ^{AaI}	4.4 ^{BaI}
	Citric	39.7 ^{AaI}	16.1 ^{BbI}	8.4 ^{AbII}	6.0 ^{BdII}
	Trans-aconitic	71.1 ^{AcII}	49.0 ^{BbII}	107.3 ^{AcI}	68.6 ^{BaI}
	Total org. acids	183.0 ^{AbI}	116.0 ^{BbI}	184.8 ^{AcI}	117.1 ^{BbI}
SA3	Malic	68.1 ^{AaI}	58.2 ^{AaI}	71.3 ^{AaI}	48.7 ^{BaI}
	Succinic	0.8 ^{AbI}	0.9 ^{AbI}	0.3 ^{AbII}	0.5 ^{AbI}
	Citric	38.4 ^{BaII}	47.0 ^{AaI}	112.0 ^{AaI}	24.0 ^{BbII}
	Trans-aconitic	87.8 ^{AbII}	33.2 ^{BcII}	187.0 ^{AaI}	80.9 ^{BaI}
	Total org. acids	225.8 ^{AaII}	120.6 ^{BbII}	315.7 ^{AaI}	169.9 ^{BaI}
NST	Malic	55.6 ^{AbI}	36.0 ^{BbI}	39.8 ^{AbII}	19.3 ^{BbII}
	Succinic	0.8 ^{AbI}	0.4 ^{AbI}	0.4 ^{AbI}	0.4 ^{AbI}
	Citric	14.4 ^{AaI}	3.6 ^{BcII}	7.7 ^{BbII}	31.3 ^{AaI}
	Trans-aconitic	93.2 ^{AbII}	46.0 ^{BbI}	167.1 ^{AaI}	29.5 ^{BbII}
	Total org. acids	190.6 ^{AbII}	109.6 ^{BbI}	245.8 ^{AbI}	78.0 ^{BcII}

from an increase in the PEP carboxylase activity in the shoot under P-deficient conditions, which also led to the accumulation of citrate in the shoot and a higher citrate/sugar ratio in the phloem.

Root exudation as related to phloem exudation of shoots

The organic acids content in the phloem exudates of shoots was related to root exudation (Figure 2). Under P deficiency, organic acids increased both in roots and phloem exudates, suggesting that the organic acids synthesized in shoots may be transferred to the roots and exuded.

Nevertheless, as also observed for the organic acids content in roots (Table 5), the total release of organic acids from phloem, malic and citric acids especially, was significantly higher for SA3 than for the other lines ($p < 0.001$) (Table 6). These results suggest that for maize the transfer of organic acids from the shoot (via the phloem) to the roots does not control the tolerance of some genotypes to low-P conditions.

Acid phosphatase

Acid phosphatase activity was measured on the root surface and was significantly lower in Corso than in the three other maize lines ($p < 0.001$) (Table 7). Activity increased strongly under P deficiency in the low-P tolerant NST line ($p = 0.002$). Only small effects of P deficiency were found for SA3 ($p = 0.36$) and Sikuani ($p = 0.13$). The results suggest that the high acid phosphatase activity in NST under P defi-

Table 6. Organic acids released in phloem from shoot. + P = 1 m*M* P; − P = no P. Mean values; $n = 6$. n.d.: not detected. Means with the same letter are not statistically different at $p = 0.05$. Capital letters refer to differences between P treatments (read horizontally). Small letters refer to differences between genotypes (read vertically)

Genotype	Organic acid	Phloem shoot exudation	
		− P	+ P
		nmol C / g plant dry weight / h	
Corso	Malic	174.5 ^{Ac}	188.9 ^{Ac}
	Succinic	14.3 ^{Ab}	12.5 ^{Aa}
	Citric	108.1 ^{Ad}	92.6 ^{Ac}
	Trans-aconitic	1005.7 ^{Aa}	1027.1 ^{Aa}
	Total org. acids	1444.6 ^{Ab}	1380.1 ^{Ab}
Sikuani	Malic	389.5 ^{Ab}	416.5 ^{Ab}
	Succinic	73.7 ^{Aa}	15.9 ^{Ba}
	Citric	153.4 ^{Ac}	88.2 ^{Bc}
	Trans-aconitic	887.4 ^{Ab}	711.7 ^{Bb}
	Total org. acids	1674.9 ^{Aa}	1338.3 ^{Bb}
SA3	Malic	667.1 ^{Aa}	575.4 ^{Ba}
	Succinic	n.d.	17.6 ^{a}
	Citric	289.4 ^{Aa}	275.5 ^{Aa}
	Trans-aconitic	566.6 ^{Ac}	488.5 ^{Bd}
	Total org. acids	1716.6 ^{Aa}	1509.2 ^{Ba}
NST	Malic	399.9 ^{Ab}	388.1 ^{Ab}
	Succinic	n.d.	6.2 ^{b}
	Citric	216.2 ^{Ab}	165.1 ^{Bb}
	Trans-aconitic	591.9 ^{Ac}	608.8 ^{Ac}
	Total org. acids	1356.7 ^{Ab}	1232.7 ^{Ac}

Table 7. Acid phosphatase activity as released into solution and as adhering to root surface of 18 days old seedlings. Mean value; $n = 6$. + P = 1 m*M* P; − P = no P; mU = nmol P / min. Means with the same letter are not statistically different at $p = 0.05$. Capital letters refer to differences between P treatments (read horizontally). Small letters refer to differences between genotypes (read vertically).

Genotype	Acid phosphatase activity			
	Released into solution		Adhering to root surface	
	− P	+ P	− P	+ P
	mU / g root dry weight / day		mU / g root dry weight	
Corso	11.9 ^{Ac}	11.4 ^{Ab}	203 ^{Ad}	212 ^{Ab}
Sikuani	15.8 ^{Ab}	13.7 ^{Ba}	319 ^{Ab}	294 ^{Aa}
SA3	9.2 ^{Ad}	7.7 ^{Bc}	281 ^{Ac}	274 ^{Aa}
NST	19.5 ^{Aa}	10.6 ^{Bb}	398 ^{Aa}	306 ^{Ba}

ciency may contribute to the low-P tolerance of this genotype. Released acid phosphatase activity significantly increased under P deficiency ($p < 0.001$) and was maximum for the low-P tolerant NST line. The acid phosphatase activity in the NaCl eluates was higher than in the nutrient solution and may have originated from acid phosphatase adhering to the root epidermal cell layers, as suggested by Tadano and Sakai (1991).

Conclusions

P deficiency in hydroponic culture resulted in decreased dry matter production of the four maize genotypes. The decrease was especially evident in the low-P tolerant NST and the acid-tolerant Sikuani, and was accompanied by increases in root/shoot-ratio and in average maximum root length. This response appeared to be related to an increased investment of plant resources in root growth to improve P acquisition.

Organic acids contents in root tips were increased under P deficiency and were closely related to PEPC activity. Differences in organic acids contents between genotypes were not related to their low-P tolerance. Root exudation of organic acids increased with root contents as P supply was varied for the genotypes NST and Sikuani. On the other hand, root exudation was not related to root content in the genotypes Corso and SA3. The low exudation of organic acids by SA3, despite high root contents, may contribute to the susceptibility of this maize genotype to low-P conditions.

Under P deficiency, organic acids content increased in phloem sap released from shoots, co-committant with root exudates. Shoot–root transport of organic acids may contribute to root exudation. Nevertheless, as this transfer of organic acids, malic and citric acids in particular, was higher for SA3 than for NST, the contribution of organic acids synthesized in the shoot might only partly explain the response of low-P tolerant maize genotypes to P deficiency. There was a difference between genotypes in the organic acids composition of roots and root exudates. *Trans*-aconitic acid and malic acid were predominant in phloem exudates from the shoot, in roots and in root exudates of the four maize genotypes.

Root acid phosphatase activity was higher in the lines NST and Sikuani than in the genotypes Corso and SA3 and may be important for low-P tolerance of these genotypes.

We propose that the higher tolerance of the genotype NST to low-P conditions may be related to 1. a high utilization of the seed P by the young seedling plant, 2. a relatively high root dry matter and greater root length, to explore a larger volume of soil, 3. a high anthocyanin coloration, as a protection against oxygen free radical stress, 4. exudation of large amounts of citric and malic acids, both known to be efficient for soil P mobilisation, and 5. a high acid phosphatase activity which may promote P acquisition from organically bound P.

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