Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency

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Abstract

Root exudates play a major role in the mobilization of sparingly soluble nutrients in the rhizosphere. Since the amount and composition of major metabolites in root exudates from one plant species have not yet been systematically compared under different nutrient deficiencies, relations between exudation patterns and the type of nutrient being deficient remain poorly understood. Comparing root exudates from axenically grown maize plants exposed to N, K, P, or Fe deficiency showed a higher release of glutamate, glucose, ribitol, and citrate from Fe-deficient plants, while P deficiency stimulated the release of γ-aminobutyric acid and carbohydrates. Potassium-starved plants released less sugars, in particular glycerol, ribitol, fructose, and maltose, while under N deficiency lower amounts of amino acids were found in root exudates. Principal-component analysis revealed a clear separation in the variation of the root-exudate composition between Fe or P deficiency *versus* N or K deficiency in the first principal component, which explained 46% of the variation in the data. In addition, a negative correlation was found between the amounts of sugars, organic and amino acids released under deficiency of a certain nutrient and the diffusion coefficient of the respective nutrient in soils. We thus hypothesize that the release of dominant root exudates such as sugars, amino acids, and organic acids by roots may reflect an ancient strategy to cope with limiting nutrient supply.

Key words: nutrient deficiency / root exudates / axenic culture / exudate composition / *Zea mays*

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1 Introduction

Plant roots release a wide range of carbon-containing compounds that are known collectively as rhizodeposits. Rhizodeposition is estimated to represent approximately 11% of net fixed carbon and 27% of carbon allocated to roots (*Jones* et al., 2009). However, carbon economies within plants vary considerably depending on experimental conditions (*Kuzyakov* and *Domanski*, 2000). Rhizodeposits include a wide variety of substances that originate from sloughed-off root cells and tissues, mucilages, volatiles, soluble lysates, and exudates that are released from damaged and intact cells, respectively (*Dakora* and *Phillips*, 2002). Root exudates comprise the largest fraction of nonvolatile rhizodeposits (*Meharg* and *Killham*, 1988) and are complex mixtures of carbon-containing compounds including carbohydrates, amino acids, organic acids, phenolic compounds, fatty acids, sterols, vitamins, enzymes, purines/nucleosides as well as inorganic molecules, such as HCO_3^-, OH^-, and H^+ (*Dakora* and *Phillips*, 2002). Among these components, sugars, amino acids, and organic acids are thought to be released in largest quantities (*Farrar* et al., 2003).

Current evidence suggests that certain components that are present in root exudates are involved in a variety of functions including the modulation of nutrient availabilities (*Cakmak* et al., 1998; *Wang* et al., 2008), increased tolerance to heavy metals (*Osawa* and *Kojima*, 2006), or attraction of rhizobacteria (*Bais* et al., 2004). Nevertheless, the quantity and composition of root exudates is influenced by many factors (*Jones* et al., 2004) including the soil structure (*Berg* and *Smalla*, 2009), the presence of microorganisms (*Groleau-Renaud* et al., 2000), the plant species (*Lesuffleur* et al., 2007) as well as their developmental stage (*Gransee* and *Wittenmayer*, 2000) and nutritional status (*Hinsinger*, 2001; *Marschner*, 1998).

Despite a wealth of literature reporting on the composition of root exudates from a wide range of plant species and growth conditions (*von Wirén* et al., 1994; *Yoneyama* et al., 2007), a systematic study comparing exudation profiles from a single plant species exposed to different nutrient deficiencies has not yet been reported. Therefore, it is not known whether

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there is a relationship between root-exudation pattern and the type of nutrient being limited in availability to the plant. In this study, we compared qualitative and quantitative changes in maize-root-exudate profiles collected from axenically grown plants exposed to four different nutrient deficiencies: nitrogen (N), potassium (K), phosphorus (P), and iron (Fe). A major goal was to investigate whether metabolite patterns may change in qualitative and/or quantitative terms under deficient provision of certain nutrients and whether there is a relation between metabolite exudation patterns and diffusion properties of these nutrients in soils.

2 Material and methods

2.1 Sterilization of corn seeds

Maize seeds (*Zea mays* L. var. Surprise) were shaken for 3 min in 96% ethanol, 30 min in 3% sodium hypochlorite solution, rinsed twice in sterile distilled water (SDW), and then left to soak in SDW for 4 h at 25°C. Sterility was confirmed by the absence of microbial growth in liquid Luria-Bertani (LB) and semisolid Tryptic Soy Agar (TCA, 0.3% Agar) media to which seeds had been added and incubated for 7 d at 37°C.

2.2 Plant-growth conditions

Surface-sterilized seeds were pregerminated on solid half-strength Murashige Skoog medium containing 1% sucrose and 0.7% agar (Difco, Becton Dickinson) maintained at 28°C in the dark. Seedlings were transferred to glass bottles designed to facilitate axenic hydroponic growth conditions (*von Wirén* et al., 1995). The hydroponic system was permanently aerated and maintained in a controlled-environment chamber at 60% humidity, 8 h darkness at 20°C, and 16 h light at 280 μmol photons m^{-2} s^{-1} and 25°C. The composition of the nutrient solution was as follows: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KCl, 0.1 mM KH_2PO_4, 1.0 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, and 100 μM Fe(III)-EDTA.

2.3 Root-exudate collection

In the N-, P-, Fe-, or K-deficiency treatments, the corresponding nutrient was omitted from the nutrient solution. To maintain the ion balance of the nutrient solution, $Ca(NO_3)_2$ was replaced by $CaCl_2$, KH_2PO_4 was replaced by $NaH_2PO_4 \cdot 2H_2O$, and K_2SO_4 by $MgSO_4$. The nutrient solution was replaced once during the first 7 d and, from then on, every day after root-exudate collection. During each nutrient-solution replacement, a 100 μL aliquot was removed and spread on a solid LB medium to check for sterility. Contaminated vessels were discarded.

As our aim was to identify primary responses of root exudates to individual nutrient deficiencies, nutrient-starvation periods were chosen according to the plant demand for each nutrient. The relative growth rate based on dry weight is considered a reliable measure of N stress (*Greenwood*, 1976) and is not altered significantly up to 3 d of N starvation (*Lee* and *Rudge*, 1986), even though N-deficiency responses such as an enhanced expression of ammonium and nitrate transporters are induced within the first 24 h of deficiency (*Ono* et al., 2000; *von Wirén* et al., 2000). Therefore, plants were subjected to 2 d of N deficiency. With respect to the relatively high plant demand for K and the rapid induction of K-deficiency responses (*Marschner*, 1995), plants were also subjected to 2 d of K deficiency. In agreement with the relatively lower plant demand for P and the slower induction of typical deficiency responses (*Marschner*, 1995; *Nagy* et al., 2006), the P-starvation period was set to 3 d. Plants were subjected to Fe deficiency for 6 d, which is a typical time period required to induce Fe-deficiency responses (*Meda* et al., 2007; *Schaaf* et al., 2004). To increase the likelihood of including the peak of exudate release, root exudates were collected over a period of 3 subsequent days, pooled, freeze-dried, and then stored at –20°C.

Root exudates were collected from all treatments 13, 14, and 15 d after germination (fourth-leaf stage). This ensured that carbon associated with the seed reserves was exhausted prior to root-exudate collection. Two hours after the onset of the light period, the nutrient solution was replaced with autoclaved ultrapure water in which root exudates were collected for 6 h. A period of 6 h was chosen because this time span allowed collecting sufficient quantities of root exudates for metabolite analysis, while longer time periods may less efficiently exclude secondary effects such as the release of cell contents from sloughed-off root cells. Moreover, other studies have shown that root exudation rates are still comparable after 0.5 or 6.0 h (*Shepherd* and *Davies*, 1994). The root system was aerated throughout the collection period.

2.4 Chemical analyses of root exudates

We focused our analyses on sugars, amino acids, and organic acids and omitted the analysis of root exudates that are specifically released under certain nutrient deficiencies, such as phytosiderophores released under Fe deficiency, to ensure comparability of exudate profiles.

Amino acids were determined using a Shimadzu HPLC system equipped with a fluorescence detector. For each sample, 40 μL were derivatized with 160 μL OPA (o-phthaldialdehyde) reagent and 20 μL of the resulting mixture were injected and separated on a GROM-SIL OPA-3 column (3 μm, 125 × 4.0 mm) using gradient elution by solvent A (25 mM phosphate buffer pH 7.2 with 0.75% tetrahydrofuran) and solvent B (methanol to acetonitrile to 25 mM phosphate buffer pH 7.2 [35 : 15 : 50 / v : v : v]). Gradient profile: 0–2 min, 0% B; 2–10 min, 0%–50% B; 10–15 min, 50%–60% B; 15–20 min, 60%–100% B; 20–25 min, 100% B; 25–26 min, 100%–0% B; 26–35 min, 0% B. The flow rate was 1 mL min^{-1}. Subsequent fluorescence detection of the derivatives was performed at an excitation wavelength of 330 nm and 450 nm for fluorescence emission.

Organic acids were determined by means of ion chromatography (Dionex, Idstein, Germany) equipped with conductivity detector and suppressor ASRS Ultra II. For each sample, a 20 μL volume was separated on the Dionex IonPac AS 11

HC column (2 × 250 mm) using gradient elution starting from 4 mM KOH (0–4 min), with a stepwise linear increase to 80 mM over 28 min (4–10 min, 4–15 mM; 10–14 min, 15–25 mM; 14–24 min, 25–80 mM; 24–28 min, 80 mM), followed by re-equilibration to 4 mM for 2 min and 10 min equilibration by 4 mM KOH. The flow rate was 0.2 mL min^{-1}. Organic acids were identified by comparison of retention time with known standards.

Sugars were determined by GC-TOF-MS (*Lisec* et al., 2006). A lyophilized 75 μL aliquot of root exudates was dissolved in 50 μL methoxyamine hydrochloride in dry pyridine and derivatized for 2 h at 37°C followed by 30 min treatment with 50 μL N-methyl-N-trifluoroacetamide at 37°C. A volume of 1 μL was injected into the GC column in a splitless mode.

2.5 Statistical analysis

The main effect of nutrient deficiencies on the quantity of root-exudate components was assessed using ANOVA, and differences between individual deficiency treatments were determined using Tukey's Honestly Significant Difference (HSD) test. All significant differences were considered at the 95%-confidence level. These analyses were implemented using the core functions within the R statistical environment (*R-Development-Core-Team,* 2005). To determine whether various nutrient deficiencies led to significantly different exudate profiles, data were first converted to z scores (the amount of a compound within a sample minus the mean and divided by the standard deviation of that compound over all samples) and then analyzed using ANOSIM with 999 random permutations (*Clarke*, 1993). This analysis was performed in the Primer 6 statistical software (Primer-E Ltd. Plymouth, UK). The structure and composition of exudate profiles (z score data) were analyzed using Principal-component analysis (PCA) based on the correlation matrix. To interpret principal-component (PC) axes, we calculated a matrix of Pearson's correlation coefficients and associated P values for the latent vectors (loadings) from the PCA analysis and the z scores for each exudate component. This facilitated interpretation based on significant relationships between exudate components and PC axes only. The abbreviations of chemical compounds were plotted in the graphs to further facilitate data interpretation. To investigate whether total exudation rates were related to the mobility of nutrients in soil, we performed linear regressions on log nutrient diffusion coefficients in soil taken from the literature (*Nielsen*, 2006) and our measured exudation rates for organic acids, amino acids, and carbohydrates. The models, PCA, correlation, and regression analyses were implemented using the GenStat statistical system (GenStat 11th edition, Lawes Agricultural Trust; VSN International, Hemel Hampstead, UK).

3 Results and discussion

3.1 Specific responses to nutritional deficiencies in root exudates

Even though root-exudation rates are likely to increase when plants are grown in solid substrates (*Neumann* and *Römheld*, 2000), a hydroponic system was chosen for the present study to facilitate the exchange of nutrient solutions and a complete recovery of root exudates. Relative to the control, increased concentrations of glutamate (Glu), citrate (Cit), ribitol (Rib), and glucose (Glc) were found in exudates collected from Fe-deficient plants (Fig. 1). A substantial increase in citrate and ribitol concentrations has also been observed in Fe-deficient sugarbeet roots (*Rellan-Alvarez* et al., 2010). It is likely that the exudation of phytosiderophores by maize roots was also increased in response to Fe deficiency (*von Wirén* et al., 1994). However, since these compounds are specific to Fe deficiency, they were not further considered in this study. An enhanced exudation of glutamate under Fe deficiency has also been observed for barley roots (*Fan* et al., 1997). Glutamate has been characterized as a strong bacterial attractant (*Barbour* et al., 1991), and ribitol and glucose are readily utilized C sources by most bacteria. As microbial siderophores may increase the mobility of Fe in the rhizosphere (*Hördt* et al., 2000), the enhanced release of glutamate, glucose, and ribitol may be a strategy to attract microorganisms and thus to cope with Fe deficiency. A higher efflux of citrate has previously been reported in nutrient-deficient maize plants (*Jones* and *Darrah*, 1995). Other monocots such as barley also exhibited higher exudation rates of organic acids under Fe deficiency, in particular malate (*Fan* et al., 1997). Ultimately, Fe mobility in soils is enhanced by the presence of organic acids either directly by the formation of Fe complexes that are suitable for Fe acquisition by plant roots (*Jones* et al., 1997) or indirectly by the formation of labile Fe^{III} complexes with organic acids that facilitate subsequent ligand exchange (*Kraemer* et al., 2006).

In exudates collected from P-deficient plants, higher concentrations of γ-aminobutyrate (GABA) and carbohydrates, such as inositol (Ino), erythritol (Ery), ribitol (Rib), fructose (Fru), glucose (Glc), and arabinose (Ara), were found (Fig. 1). Interestingly, in rice roots the expression of a glutamate decarboxylase (GAD) was also higher under P-deficient conditions (*Oh* et al., 2005). GADs catalyze the conversion of L-glutamate to GABA, which accumulates in various plant tissues under a variety of stress conditions (*Kinnersley* and *Turano*, 2000). Although the role of GABA in plants is still unclear, a stress-signaling function has been suggested (*Bouche* and *Fromm*, 2004). An increased exudation of sugars under P deficiency was also observed in other plant species, such as *Sorghum vulgare* and *Citrus aurantium* (*Ratnayake* et al., 1978; *Schwab* et al., 1983). This phenomenon has been associated with a decrease in phospholipid levels and a higher permeability of the cell membrane (*Graham* et al., 1981; *Ratnayake* et al., 1978). Since amino acids and organic acids are present as anions with low plasma-membrane permeability at typical cytosolic pH (7.1–7.4) (*Bertin* et al., 2003) and carbohydrates are known to accumulate in root tissues under P starvation (*Cakmak* et al., 1994b), sugars are very likely to be the most diffusible group of substances when the integrity of the membrane is affected. Consequently, the release of carbohydrates into the rhizosphere may stimulate germination and growth of mycorrhizal fungi, which are known to improve P acquisition (*Graham* et al., 1981; *Ratnayake* et al., 1978; *Schwab* et al., 1991). Qualitative differences in the exudation profile of sugars have also been reported in P-deficient

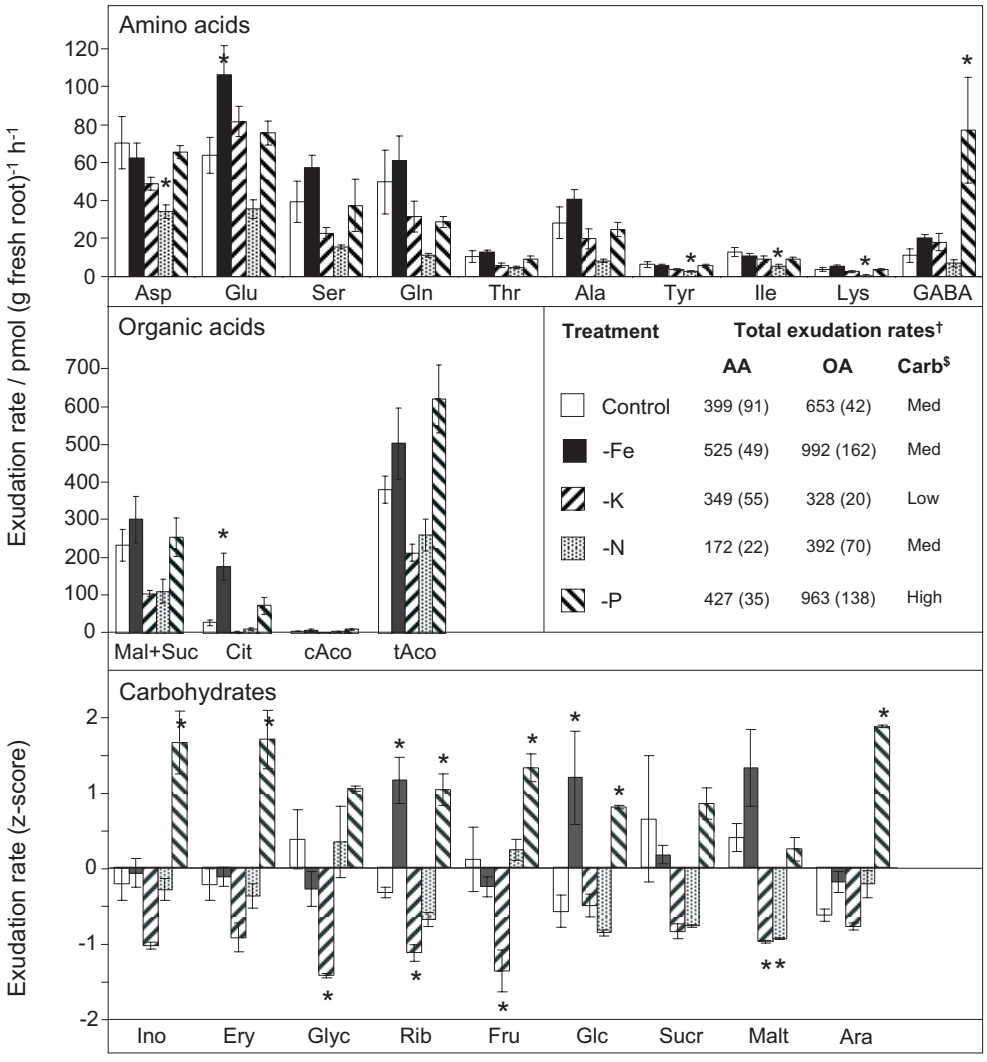

Figure 1: Exudation rates of amino acids, organic acids, and carbohydrates released by maize root under iron (–Fe), potassium (–K), nitrogen (–N), or phosphorus (–P) deficiency. For clarity Asn, His+Gly, Arg, Val, Phe, and Leu were not displayed because they did not differ between treatments; * denotes treatments that are significantly different to the control ($p < 5.0\%$ in Tukey HSD test). AA denotes amino acids; OA, organic acids; Carb, carbohydrates. † Total exudation rates are displayed in pmol (g fresh root)$^{-1}$ h^{-1}. ‡ Total exudation rates of carbohydrates are displayed as z scores. Bars represent means ± standard errors; $n = 4$.

plants. For instance, a greater proportion of pentoses relative to glucose and sucrose was released by *Zea mays, Brassica napus*, and *Pisum sativum* roots (*Schilling* et al., 1998). As a consequence, the mobilization of phosphate from $Ca_3(PO_4)_2$ by microorganisms such as *Pantoea agglomerans* may be increased. Our results also revealed a higher exudation of ribose under P deficiency but there was no significant difference in sucrose release compared to the control. Dicotyledonous plants generally respond to P deficiency by increasing root exudation of carboxylates (*Neumann* and *Römheld*, 2000), and this response is often observed at later stages of P deficiency (*Johnson* et al., 1996). An enhanced release of carboxylates was observed for white lupin and chickpea, but not for tomato and wheat (*Neumann* and *Römheld*, 1999). In our study, there was no significant difference in the exudation of organic acids by maize in the early stages of P deficiency. Nonetheless, relative to the controls the concentration of *cis*-aconitate was higher in root exudates of P-deficient plants. Interestingly, in a comparison of maize genotypes that differed in their tolerance to P deficiency, a higher organic acid exudation in P-starved plants was evidenced only in low-P-tolerant maize genotypes (*Gaume* et al., 2001; *Li* et al., 2008).

Relative to the control, lower concentrations of sugars, including glycerol, ribitol, fructose, and maltose, were measured in exudates collected from K-deficient plants (Fig. 1). The only study that has documented changes in root exudation by maize under K deficiency reported an increase in sugars, amino acids, and organic acids (*Kraffczyk* et al., 1984). In this case, however, the plants were exposed to K deficiency for 10 and 15 d, which is a much longer duration of starvation than we used in this study (2 d). It is therefore likely that the observations of *Kraffczyk* et al. (1984) represent secondary responses to K deficiency. Furthermore, the allocation of photosynthates to roots is inhibited under K-deficient conditions (*Cakmak* et al., 1994a, b) due to impaired phloem loading (*Marschner* et al., 1996). Therefore, given that the exudation of carbohydrates occurs mainly through passive diffusion (*Jones* et al., 2009), a lower amount of sugars in K-deficient root tissue as a consequence of impaired translocation might explain the low carbohydrate release observed under K-deficient growth conditions.

Relative to the control, a lower concentration of amino acids (particularly aspartate, tyrosine, isoleucine, and lysine) and maltose was found in exudates collected from N-deficient

plants (Fig. 1). A lower release of amino acids from N-depleted plants has also been reported for pine (*Bowen*, 1969) and bean (*Haase* et al., 2007). It is suggested that the lower amount of amino acids found in N-deficient root exudates is a direct consequence of the lower amount of amino acids being produced in N-deficient roots (*von Wirén* et al., 2000) rather than the retrieval of previously released amino acids under N deficiency (*Jones* et al., 2004).

3.2 General responses of root exudation to nutrient deficiencies

The relative proportion of sugars, organic acids, and amino acids differed among plants subjected to different nutritional deficiencies. A similarity analysis (ANOSIM) revealed that all treatments were different from each other ($p < 0.1\%$), except the control and the Fe-deficiency treatment ($p = 1.14\%$). The difference between control and K-deficient exudates was marginal ($p = 5.7\%$). The main trends in the variation of root-exudate compositions among treatments are summarized in Fig. 2.

The first three principal components accounted for 75.4% of the total variation in the dataset. The sequence of the nutrient-deficiency treatments along the principal component 1 (PC1) was mostly influenced by total exudation rates of all three metabolic groups, particularly amino acids. As expected, the low amino acid release from N-deficient roots was set apart from that of the other treatments. However, amino acid release was rather low under K deficiency, too, which might result from a lower assimilate translocation to the roots. A clear separation became apparent between the Fe- or P-deficiency *versus* N- or K-deficiency treatments, indicating that amino acid release was more prominent under deficiency of those two nutrients with a particular low solubility in soils. Total exudation rates, particularly those of amino acids (AA) and of organic acids (OA) (Fig. 1), support this observation.

The principal component 2 (PC2) was mostly affected by differences in the exudation of carbohydrates as well as of *cis*- and *trans*-aconitate with an accumulation of these carbon compounds in the range of negative values (Fig. 2). The Fe- and P-deficiency treatments led to higher exudation rates of sugars and the two mentioned organic acids than N or K deficiency. As mentioned previously for root exudation by P-deficient roots, the release of carbohydrates in the rhizosphere may be a strategy to stimulate the growth and activity of rhizosphere microorganisms, such as mycorrhizal fungi (*van Scholl* et al., 2006), phosphate-solubilizing (*Zaidi* et al.,

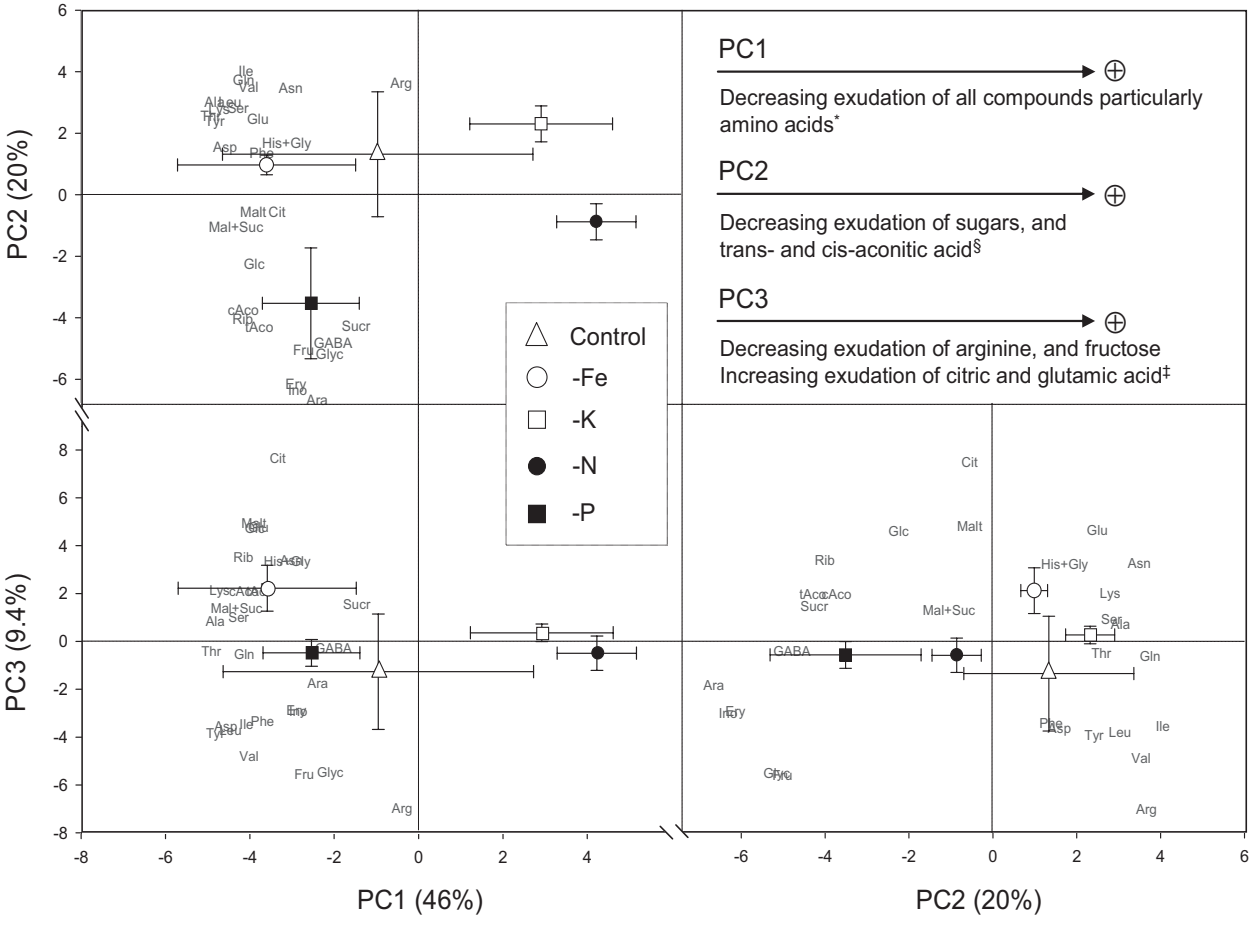

Figure 2: Principal-component analysis based on exudation rates of chemical compounds released by plants grown under different nutritional deficiencies. §Sugars negatively correlated with PC2 were: Ara, Ino, Ery, Fru, Sucr, Rib. Glu and Ile were positively correlated with PC2. Other compounds were not significantly correlated with PC2. ‡All other compounds were not significantly correlated with PC3.

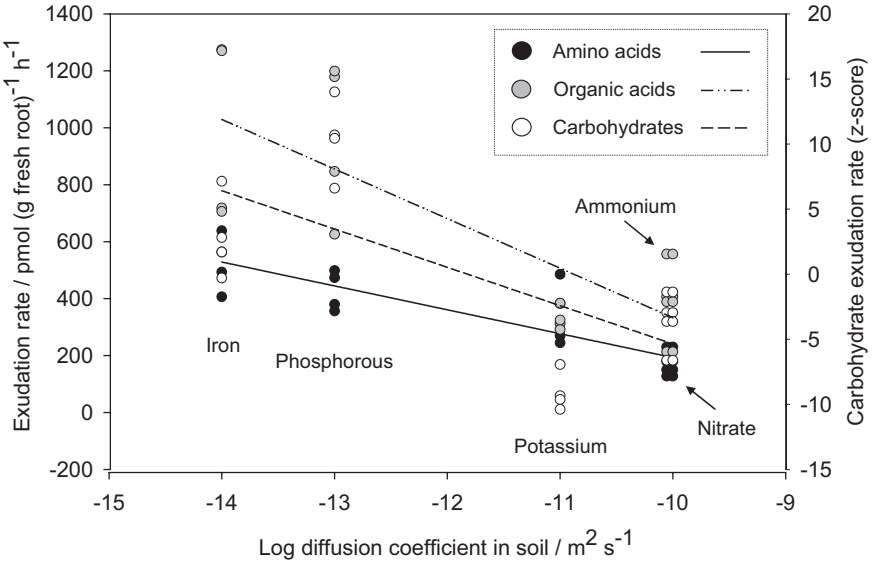

Figure 3: Regressions correlating Fe, P, K, and nitrate diffusion coefficients and exudation rates of total amino acids, organic acids, and carbohydrates. Effective diffusion coefficients for nutrients in soil were obtained from *Nielsen* (2006).

2009), associative N_2-fixing (*Perin* et al., 2006), and siderophore-releasing bacteria (*Guerinot*, 1994). The K-deficiency treatment was the one which resulted in the lowest exudation rates of sugars. *Trans*-, *cis*-aconitate, and GABA also had a significant influence on PC2, all being strongly associated with P limitation (Fig. 2). *Trans*-aconitate is the predominant organic acid in grasses (*Stout* et al., 1967), which is in agreement with our findings in maize (Fig. 1). The release of *trans*-aconitate was also important in P-deficient maize lines when grown on acid soils (*Gaume* et al., 2001). Moreover, GABA was linked with P starvation. Besides its role in stress signaling, the breakdown of GABA generates succinate. Since some microorganisms are able to produce enzymes involved in this reaction (*Priefer* et al., 2001), it is possible that GABA might be used by rhizosphere microorganisms as a precursor for the generation of organic acids, which improve P mobilization. However, additional data on the conversion of root-derived substances to organic acids by microbial activity are needed to prove this hypothesis.

Citrate and glutamate largely influenced the sequence of the treatments along principal component 3 (PC3) and separated Fe-deficient root exudates from the rest of the other treatments. Citrate has been reported to play a major role in solubilizing and translocating Fe in maize, and to be a more efficient Fe chelator than *trans*-aconitate (*Clark* et al., 1973). Additionally, arginine and fructose affected the separation of the treatments in PC3. With respect to any lacking significant differences among treatments in arginine exudation as revealed by ANOVA, the variation among treatments displayed by PC3 (9.4%) should be interpreted with caution.

3.3 Relationship between root exudation and the diffusion coefficient of nutrients in soils

To investigate a relation between the quantity of root exudates and the type of nutrient being deficient, exudation rates of amino acids, organic acids, and carbohydrates were plotted against the diffusion coefficients of the four nutrients in the soil (Fig. 3). Interestingly, exudation rates were inversely related to the diffusion coefficient of the growth-limiting nutrient. These negative correlations held true for each of the metabolic groups (amino acids, organic acids, and carbohydrates) and were in agreement with an increased exudation of organic acids as has been demonstrated in many investigations involving P- and Fe-limiting conditions (*Neumann* and *Römheld*, 1999; *Sas* et al., 2001), whereas a lower root exudation of carboxylates, sugars, and amino acids has been observed in N-deficient bean plants (*Haase* et al., 2007). With the exception of citrate and malate, this inverse correlation mainly relies on exudate components for which a positive effect on nutrient mobilization has not yet been demonstrated. Thus, the release of root exudates may reflect a non-specific mechanism to nutrient deficiencies with exudation rates increasing with decreasing nutrient solubility. We therefore propose the hypothesis that this negative correlation reflects an ancient adaptation strategy evolved before the release of specific nutrient-mobilizing exudates (*e.g.,* phytosiderophores), in which plant roots just released more exudates the lower the solubility of the required nutrient was. To precisely identify the maximum peaks of root exudation under any of the investigated nutrient deficiencies, a time-dependent analysis of root-exudate profiles would be required. For theat reason, the current study cannot yet prove, but rather provides experimental evidence for raising and further testing this hypothesis.

The molecular mechanism behind this strategy remains unclear, but plant-growth regulators, such as indol-acetic acid (IAA), zeatin, or kinetin, may play a role. There is a long series of studies showing that these and other phytohormones in roots change with the nutritional status of plants (*Argueso* et al., 2009; *Seguela* et al., 2008; *Lopez-Bucio* et al., 2002). In turn, phytohormones may affect ion leakage from cell cultures of winter wheat (*Filek* et al., 2004) or the membrane permeability of rice suspension cells (*Grossmann* et al., 1986). Assuming that the unspecific release of amino acids, organic acids, and carbohydrates is mainly mediated by pas-

sive diffusion (*Jones* et al., 2009), phytohormones may also influence the net release of exudates *via* their retrieval back into roots. Auxins enhance the H^+-ATPase activity at the plasma membrane (*Zandonadi* et al., 2007) and thereby increase the driving force for secondary active transport processes that may re-import sugars and organic and amino acids lost by leakage (*Mühling* et al., 1993; *Matzke* and *Mengel*, 1993; *Jones* and *Darrah*, 1992, 1995). A systemic comparison of the H^+-ATPase activities at the plasma membrane under different nutrient regimes may shed further light on the importance of retrieval mechanisms for net exudate release from roots.

4 Conclusions

The present results indicate that root exudation is strongly influenced by the nutritional status of a plant and that both, the quantity and composition of exudates, differ depending on the particular nutrient that is deficient. Our multivariate-analysis approach revealed that the majority of variation in exudate profiles among deficiency treatments can be explained in terms of the total quantity of exudates being released. Regression analysis further indicated a trend in which the quantity of exudates released was inversely related to the mobility of the growth-limiting nutrient in soils. We therefore propose the hypothesis that the release of nonspecific root exudates may represent a general strategy to cope with limiting nutrient supply that relies on prevailing benefits conferred by enhanced concentrations of plant-root metabolites in the rhizosphere. These may include their direct effect on nutrient solubility, such as previously demonstrated for organic acids, or an indirect effect such as the attraction of rhizosphere microorganisms that may assist in nutrient mobilization. Thus, even the nonspecific release of root exudates may have been advantageous for the acquisition of sparingly soluble nutrients until the evolution of specific root exudates conferred a more efficient nutrient acquisition. For instance, the evolution of phytosiderophores appeared relatively late in the evolution of higher plants (*Charlson* and *Shoemaker*, 2006). We urge that this hypothesis is tested in a wider range of growth conditions and plant species.

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