RHIZOSPHERE PROPERTIES OF MAIZE GENOTYPES WITH CONTRASTING PHOSPHORUS EFFICIENCY[1]

Edilson Carvalho Brasil[2], Vera Maria Carvalho Alves[3], Ivanildo Evódio Marriel[3], Gilson Villaça Exel Pitta[4] & Janice Guedes de Carvalho[5]

SUMMARY

An experiment was conducted in a growth chamber to evaluate characteristics of the rhizosphere of maize genotypes contrasting in P-use efficiency, by determining length and density of root hairs, the rhizosphere pH and the functional diversity of rhizosphere bacteria. A sample of a Red Oxisol was limed and fertilized with N, K and micronutrients. In the treatment with the highest P level, 174 mg kg^{-1} P was added. Each experimental unit corresponded to a PVC rhizobox filled with 2.2 dm^{-3} soil. The experiment was completely randomized with three replications in a 5 x 2 factorial design, corresponding to five genotypes (H1, H2 and H3 = P-efficient hybrids, H4 and H5 = P-inefficient hybrids) and two P levels (low = 3 mg dm^{-3}, high = 29 mg dm^{-3}). It was found that 18 days after transplanting, the nodal roots of the hybrids H3 and H2 had the longest root hairs. In general, the pH in the rhizosphere of the different genotypes was higher than in non-rhizosphere soil, irrespective of the P level. The pH was higher in the rhizosphere of lateral than of nodal roots. At low P levels, the pH variation of the hybrids H2, H4 and H5 was greater in rhizospheric than in non-rhizospheric soil. The functional microbial activity in the rhizosphere of the hybrids H3 and H5 was highest. At low soil P levels, the indices of microbial functional diversity were also higher. The microbial metabolic profile in the rhizosphere of hybrids H1, H2, H3, and H5 remained unaltered when the plants were grown at low P. The variations in the rhizosphere properties could not be related to patterns of P-use efficiency in the tested genotypes.

Index terms: P-acquisition, mechanisms, rizobox.

---

[1] Part of the Doctoral Thesis of the first author presented to the Federal University of Lavras, UFLA. Received for publication in October 2009 and approved in November 2010.

[2] Researcher of the Embrapa Eastern Amazon, . CEP 66095-100 Belém (PA). E-mail: brasil@cpatu.embrapa.br

[3] Researcher of the Embrapa Maize and Sorghum, CEP 35701-970 Sete Lagoas (MG). E-mail: vera@cnpms.embrapa.br; imarriel@cnpms.embrapa.br

[4] Researcher of the Embrapa Maize and Sorghum, CEP 35701-970 Sete Lagoas (MG). E-mail: gpitta@cnpms.embrapa.br

[5] Professor of the Soil Science Department, Federal University of Lavras, UFLA. E-mail: janicegc@ufla.br

RESUMO: *CARACTERÍSTICAS DA RIZOSFERA DE GENÓTIPOS DE MILHO CONTRASTANTES NA EFICIÊNCIA DE USO DE FÓSFORO*

Um experimento foi conduzido em câmara de crescimento da Embrapa Milho e Sorgo, MG, visando avaliar características da rizosfera de genótipos de milho contrastantes na eficiência de uso de P, por meio da determinação do comprimento e densidade de pelos radiculares, do pH da rizosfera e da diversidade funcional bacteriana associada à rizosfera. Utilizou-se amostra de um Latossolo Vermelho distrófico, que, após a correção da acidez, recebeu adubação com N, K e micronutrientes. No tratamento correspondente ao nível alto de P, adicionaram-se 174 mg kg^{-1} de P. Como unidade experimental, utilizou-se rizobox de PVC, que recebeu 2,2 dm^{-3} de terra. O delineamento experimental foi o inteiramente casualizado, com três repetições, em arranjo fatorial 5 x 2, correspondendo a cinco genótipos (H1, H2 e H3 = híbridos eficientes, H4 e H5 = híbridos ineficientes) e dois níveis de P (baixo = 3 mg dm^{-3}; alto = 29 mg dm^{-3}). Aos 18 dias do transplantio, verificou-se que as raízes nodais dos híbridos H3 e H2 apresentaram maiores comprimentos de pelos radiculares. Em geral, o pH da rizosfera dos diferentes genótipos foi maior do que o pH não rizosférico, independentemente do nível de P. O pH da rizosfera de raízes laterais foi superior ao das nodais. Sob baixo nível de P, os híbridos H2, H5 e H4 mostraram maior variação de pH rizosférico em relação ao não rizosférico. Os híbridos H5 e H3 apresentaram maior atividade funcional microbiana na rizosfera. Sob baixo nível de P no solo, os índices de diversidade funcional microbiana, também, apresentaram maiores valores. A rizosfera dos híbridos H1, H2, H3 e H5 apresentou o mesmo perfil metabólico microbiano quando as plantas foram cultivadas sob baixo nível de P. As variações nas características da rizosfera não permitiram identificar padrões relacionados com a eficiência de uso de P nos materiais genéticos utilizados.

Termos de indexação: P-aquisição, mecanismos, rizobox.

INTRODUCTION

In the natural ecosystems of tropical and subtropical regions with predominantly Oxisols, P is the most yield-limiting nutrient (Novais & Smyth, 1999). The high P sorption capacity, low nutrient levels and high acidity are predominant characteristics of the soils in this region.

Under adverse soil conditions, plants adapted to specific environments have developed several mechanisms that influence the conditions of the root-soil interface, which increases P availability in the rhizosphere (Hinsinger, 1998). However, the relative influence of these processes on P mobilization may differ according to plant species and cultivar (Gahoonia et al., 2000). Several morphological, physiological and biochemical mechanisms have already been suggested to explain genotypic differences in P use efficiency. The main mechanisms that determine the efficiency of soil P absorption can be grouped into: changes in morphological root properties, chemical modifications in the rhizosphere, changes in physiological characteristics of absorption kinetics, changes in biochemical processes, and interactions with microorganisms (Alves et al., 2002).

The formation of root hairs is an important means to improve P uptake from the soil. Root hairs are involved in numerous processes, including water and nutrient absorption, due to the increased root surface area. They are also preferential exudation sites of compounds (carboxylates and plant siderophores), which can be released, especially in the early growth stages, when cell walls are being formed and when the cell conductivity of water and soluble substances is high (Michael, 2001). Depending on the environmental and genetic factors, root hairs vary in number, length and longevity (Jungk, 2001). According to Föhse & Jungk (1983), the development of root hairs is strongly influenced by the supply of mineral nutrients, especially P. The authors found that at high P concentrations, root hairs were absent or only rudimentary in rape, spinach and tomato plants, but increased in number and length at low concentrations. In wheat and barley, differences in P uptake have been attributed to variations in length, diameter and density of root hairs (Gahoonia et al., 1997, 1999).

The rhizosphere soil has other properties than the surrounding soil mass, since it is a microenvironment characterized by dynamic changes that are continually renewed or affected by root growth and exudation or release of substances from the roots. Aside from acting as a nutrient and water sink, several other crucial processes of plant mineral nutrition occur in this root region (Marschner, 1995). Plant roots are responsible for considerable changes in rhizosphere pH, caused mainly by the release of H^+, OH^- or HCO_3^-, to balance the excess of cations or anions absorbed by the roots, respectively (Haynes, 1990). Some studies of calcareous soils have shown

that acidification of the rhizosphere by plant roots results in increased P bioavailability, probably due to the increased solubility of calcium phosphate (Grinsted et al., 1982; Hedley et al., 1982). However, in acid soils, there is little evidence that the increase in rhizosphere pH may promote increased availability of inorganic P. Gahoonia et al. (1992) showed that rye plants grown on Oxisols fertilized with N in the form of nitrate absorbed more P than when fertilized with N as ammonium. The authors explained this effect by an increase in the rhizosphere pH.

The ability of some cultivars to tolerate nutrient stress may be related to the occurrence of certain microorganisms in the plant rhizosphere (Marschner et al., 2006). Under P deficiency, plant roots may excrete functional substances, representing an important adaptation mechanism to P variations in the environment (Li et al., 2004; Marschner et al., 2006). The exudate composition varies with plant age, genetic material, metabolism, and nutritional status, among other factors (Barea et al., 2005; Richardson et al., 2009). The release of varying amounts of different C compounds from the roots of different genotypes has an important ecological significance for functional microbial activity in the rhizosphere, since root exudates are the principal energy source for microbial growth in soil (Bowen & Rovira, 1999). The bacterial community composition in the rhizosphere may affect P availability to plants (Marschner et al., 2006).

Knowledge of the mechanisms that influence P acquisition may favor the identification of desirable traits to be genetically transferred to plants in breeding programs, but also contributes to the establishment of more time-saving selection methods for the development of new cultivars. Despite the existence of information on rhizosphere properties of some cultivated plants, little is known yet about the changes in the P acquisition efficiency in the rhizosphere of maize plants. Thus, the objective was to evaluate the characteristics of the rhizosphere of maize genotypes contrasting in P-use efficiency in soil samples with different P levels, based on the length and density of root hairs, rhizosphere pH and the functional bacterial diversity in the rhizosphere.

MATERIAL AND METHODS

The experiment was conducted by Embrapa Maize and Sorghum, in Sete Lagoas, state of Minas Gerais, in a growth chamber with day/night temperatures of 28/20 ^{o}C, mean relative humidity of 70 %, photoperiod (day/night) 12 h, and light intensity of 540 mE s^{-1}.

A sample of the surface layer (0–20 cm) of an Red Oxisol was air-dried, crumbled and sieved (mesh 4 mm). The following properties were determined by chemical analysis: 3 and 60 mg dm^{-3} P and K (Mehlich-1), respectively, pH 5.2 (soil: water ratio 1:2.5), and 2.29, 0.36, 0.25, and 8.07 $cmol_c$ dm^{-3} of Ca, Mg, Al, and CEC (pH 7.0), respectively, and 29 g kg^{-1} organic matter.

To reduce the acidity of the soil sample, calcium and magnesium carbonate were used in a 3:1 molar ratio, in amounts calculated based on the criterion of base saturation, increasing the initial value to 50 %. The sample was incubated for 30 days at about 70 % of the maximum water retention capacity. After incubation, 80 and 60 mg kg^{-1} N and K, respectively, was base-applied in the form of NH_4NO_3 and KCl, plus 1 g kg^{-1} of FTE BR-12. The fertilizers N and K were dissolved in water and applied in installments; one third at the beginning of the experiment, one-third a week and the rest two weeks later. After fertilization, the soil samples were placed in PVC rhizoboxes (20 x 2.5 cm, height 50 cm), with a removable side to facilitate handling during the evaluations. Each box was filled with 2.2 dm^3 soil (experimental unit) and watered regularly, to maintain moisture at about 70 % of the maximum retention capacity, controlled by regular weighing of the boxes.

The experiment was completely randomized with three replications in a 5 x 2 factorial design, corresponding to five hybrids and two soil P levels (low = 3 mg dm^{-3}, high = 29 mg dm^{-3}). The maize hybrids were: H1 (E) and H3 (E), P-efficient single-cross hybrids, H2 (E), P-efficient triple-cross hybrid, H4 (I) and H5 (I), P-inefficient single-cross hybrids. The genotypes of the Embrapa Maize and Sorghum breeding program, previously selected based on results of field trials, were characterized for P-use efficiency, based on grain yield (Parentoni et al., 1999). In the treatment with the highest P content, 174 mg kg^{-1} P was applied (single superphosphate), in an amount corresponding to a fertilization of 800 kg ha^{-1} P_2O_5, defined on the basis of maximum soil P adsorption capacity determined in a previous laboratory study (see Table 1 for chemical soil properties after treatment application).

The hybrid seeds were disinfected with sodium hypochlorite (5 g L^{-1} for 10 min) and germinated in rolls of paper towels placed in containers containing deionized water under continuous aeration for four days. After germination, the remaining endosperm of the seeds was removed and uniform seedlings were transplanted. Three maize seedlings with approximately 5 cm long seminal roots were placed at equal distances at the top of each rhizobox. The rooting boxes were inclined to about 45^{o} slope, the removable side face-down, to stimulate root growth on the soil surface.

The mean length and density of root hairs, rhizosphere pH and the bacterial functional diversity in the rhizosphere were assessed 18 days after transplanting.

Table 1. Soil chemical properties after phosphorus treatments

Treatment	Chemical properties[1]										
	pH H_2O	P[2]	K[2]	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	T[3]	MO	m[4]	V[5]
		— mg dm^{-3} —		— $cmol_c$ dm^{-3} —					g kg^{-1}	— % —	
Low P level	5.4	3	175	3.26	0.92	0.00	3.64	8.27	30.3	0.00	55.95
High P level	5.4	29	175	5.02	0.90	0.00	3.65	10.01	29.8	0.00	63.53

[1] According to a method of Embrapa (1997). [2] Mehlich 1. [3] CEC at pH 7.0. [4] Al saturation. [5] Base saturation.

To evaluate the root hairs one plant per rooting box was selected, from which approximately 2 cm long segments of seminal, nodal and lateral roots were cut. Eight segments of each root type were collected, along with the surrounding soil (to avoid damage to root hairs) and transferred to a container with distilled water and maintained in the dark for a night at about 5 °C. The soil adhered to the hairs was removed carefully by washing with distilled water on a sieve and placed in ultrasound for 15 min at 120W and 47 kHz (Gahoonia et al., 1999). The segments were immersed in dye solution (violet blue) for 2 min and placed on a Petri dish with a small volume of water (10 mL), to quantify the mean length and density of root hairs. For this purpose, a digital image processing system (Kontron Imaging System KS-300, Carl Zeiss) was used, consisting of a hardware and software set for image detection and processing (Ferreira et al., 2001). Direct images were obtained by a video camera connected to a magnifying glass. The equipment was coupled with a hardware component with a graphics card that transforms the images (analog) into digital format. The root hair length was measured at 10 points along the root segment of each image captured. For hair density the number of root hairs per millimeter were counted.

The rhizosphere pH was obtained by a non-destructive method, described by Häussling et al. (1985), using a combined microelectrode, with a bulb (diameter 1.5 mm), connected to a portable pH meter. The pH readings were taken in the upper third of the rooting box, where root concentration was greater, introducing the tip of the microelectrode directly into the soil at a distance of about 2 mm from the nodal and lateral roots (rhizosphere soil). Points beyond the root influence region were also measured, to represent the non-rhizospheric soil.

The bacterial functional diversity in the rhizosphere was measured by a method described by Zak et al. (1994), based on the EcoPlate system (Biolog Inc., Hayward, CA, USA) that measures the intensity of bacterial use of different C sources and produces a metabolic pattern. Rhizosphere soil samples consisted of soil particles adhered to the roots of the two plants in each rooting box after removing excess soil by drying for 20 min on paper towel. Soil was also collected at some distance from the roots, representing the non-rhizosphere soil samples. The determination of functional diversity consisted of shaking 5 g of soil sample in 45 mL of saline solution (8.5 g L^{-1} NaCl) for 30 min in a horizontal shaker. The suspension extract (5 mL) was centrifuged at 4000 rpm for 15 min and 120 mL of supernatant was transferred to each well of the Ecoplates and incubated in the dark for five days at 25 °C. Each plate contained three sets of 31 different substrates (carboxylic acids, carbohydrates, polymers, amino acids, starches), plus a control (cavity without substrate). The degree of use of a specific substrate by the bacterial community (activity) in the rhizosphere extract was quantified by measuring the intensity of color change in each cavity of the plate, caused by the oxidation of the substrates due to microorganism respiration. After incubation, the activity on the plates was assessed using a spectrophotometer plate reader (Labsystems, MultSkan, MS, USA) at 405 nm, at intervals of 24, 48, 72, 96, and 120 h. A reading of 72 h was used to calculate the components of functional diversity represented by the average time between readings. The absorbance values of control wells (without carbon sources) were established as white. Values above zero were considered as positive reaction and called "substrate-use activity", whereas negative values indicated the absence of substrate use.

The functional diversity was assessed using the indices described by Garland & Mills (1991) and Zak et al. (1994), consisting of: development of color substrates (AWCD, average well color development), substrate richness (S, sum of C sources with positive reaction), substrate diversity (H, sum of the relations between the activities of individual and total substrate activities); equivalence of substrate (E, mean effective substrate activity). The AWCD was obtained by dividing the substrate use activity (reading of absorbance of the developed color) in each well, by the mean reading value of the entire board. The H index was calculated by the equation $H = -\Sigma\, p_i\, (\ln p_i)$, in which p_i is the relationship between the individual activity of each substrate and the sum of the activities of all substrates; ln is the neperian logarithm. Index E was computed by the equation $E = H/\ln (S)$.

At the end of the evaluations, the plants were harvested (shoot and root) and dried to constant weight in an oven with forced-air circulation. The P levels of the plant material were determined, according to a method described by Sarruge & Haag (1974).

The response variables were subjected to analysis of variance (F test) and according to the degree of significance, the treatment means were statistically compared by the Scott-Knott, at 5 %. The data of substrate use activity (S) and total activity (transformed) were used for cluster analysis using UPGA (Unweighted Pair-Group Average) and the Euclidean distance coefficient.

RESULTS AND DISCUSSION

P accumulated in plant

Total P accumulated in plant tissue (shoot and root) of maize genotypes was influenced by the interaction of P levels x genotypes. The hybrids grown at low soil P levels did not differ in terms of total P, but the total P amounts were higher than of the other hybrids (Table 2). In general, the amounts of P in plant tissue of genotypes grown in high-P were higher than in low-P soil, with exception of hybrid H5 (I), with no significant difference between soil P levels. These results suggest that P stress in the soil hindered the nutrient absorption to meet the nutritional plant requirements. Machado et al. (1999), assessing the variability of maize genotypes for P-use efficiency, found that the P shoot content did not differ significantly between the genotypes, at the lowest dose. Nevertheless, the authors found variations in rates of P-use efficiency, indicating that the P content alone may not be effective in the discrimination of genotypic variation for P-use efficiency in maize.

Root hairs

The mean root hair length was affected by genotypes and by root types, with no differences between the soil P levels. The hybrids H2 (E), H3 (E), H4 (I), and H5 (I) did not differ from each other in mean root hair length, but were significantly longer than H1 (Figure 1). This showed that despite the variation among genotypes, this characteristic may not be related to P-use efficiency Regardless of the genotype, the mean lengths of hair on nodal and seminal roots did not differ from each other but were longer than on lateral roots (Figure 2), indicating that plants may have a strategy to increase the surface area of these roots.

For total hair length, only the interaction genotype x root type was significant. There was no difference between hybrids in total hair length, neither in seminal nor in lateral roots. On nodal roots, the total hair length of H2 (E) and H3 (E) was longer than of the other genotypes, irrespective of soil P levels

Table 2. Total phosphorus accumulated in plant tissues (shoot and root) of maize hybrids grown in P-rich and P–poor soil

Hybrid	P total[(1)]	
	Low P	High P
	mg/plant	
H1 (E)	0.41 aB	0.87 aA
H2 (E)	0.45 aB	0.92 aA
H3 (E)	0.40 aB	0.72 bA
H4 (I)	0.34 aB	0.65 bA
H5 (I)	0.52 aA	0.85 aA

[(1)] Means followed by the same lower-case letters in the columns and capital letters in the rows did not differ significantly by the Scott-Knott test, at 5 %. I: inefficient, E: efficient.

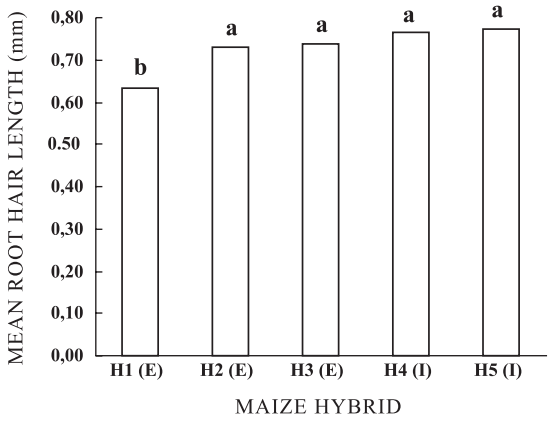

Figure 1. Mean root hair length of maize hybrids contrasting in P-use efficiency. Means followed by the same letter did not differ significantly by the Scott-Knott test, at 5%.

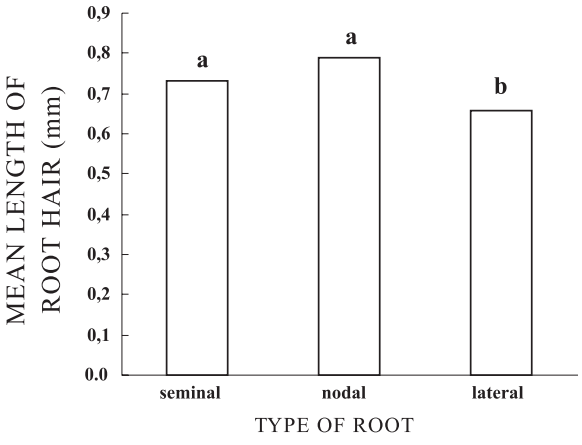

Figure 2. Mean length of root hair of seminal, nodal and lateral roots of maize hybrids contrasting in P-use efficiency. Means followed by the same letter did not differ significantly by the Scott-Knott test, at 5%.

Table 3. Total length and hair density of seminal, nodal and lateral roots of maize hybrids contrasting in P-use efficiency (mean of P level)

Hybrid	Root hair property[1]		
	Seminal root	Nodal root	Lateral root
	Total length (mm)		
H1 (E)	22.00 aA	21.50 bA	13.83 aB
H2 (E)	19.40 aB	30.50 aA	19.00 aB
H3 (E)	21.66 aB	36.66 aA	16.20 aB
H4 (I)	25.50 aA	24.33 bA	17.83 aB
H5 (I)	24.40 aA	25.80 bA	16.20 aB
	Density (number mm^{-1})		
H1 (E)	31 aA	33 cA	24 aB
H2 (E)	28 aB	36 bA	28 aB
H3 (E)	31 aB	41 aA	25 aC
H4 (I)	31 aA	30 c A	25 aB
H5 (I)	29 aA	32 c A	22 aB

[1] Means followed by the same lower-case letters in the columns and capital letters in the rows did not differ significantly by the Scott-Knott test, at 5 %. I: inefficient, E: efficient.

(Table 3). This indicates that the effect is related to genetic variability, although found only in P-efficient hybrids. In general, it was observed that the total hair length on nodal and seminal roots was longer than on lateral roots, a performance similar to mean hair length.

The density of root hairs of maize hybrids responded differently according to the root type, indicating variation in the genetic material only in the nodal roots (Table 3). In nodal roots, the hair density of hybrid H3 (E) was highest, followed by H2 (E) with slightly lower density. These results repeat the performance of both hybrids for total root hair length, showing a clear genotype-related pattern for hair formation. Similarly as observed for mean and total hair length, the hair density of nodal and seminal was higher than of lateral roots.

The results of mean and total hair length and density of root hairs show the variability in the tested genotypes. Variations among genotypes have been found by other authors for barley (Gahoonia & Nielsen, 1997), wheat (Gahoonia et al., 1997), and peanut (Wissuwa & Ae, 2001), which are generally associated with greater P absorption. Despite the variations in the genotypes used in this study, the differences in mean and total hair length and density of root hairs were not directly related to P stress conditions, since there was no difference among the treatments with varying P levels. Itoh & Barber (1983), using the technique of micrographs taken with a microscope, measured length and density of root hairs as related with P absorption. The results showed great variation in length (0.04 to 0.60 mm) and density (560 to 1800 hairs cm^{-1}) of root hairs of wheat, lettuce, tomato, onion, and carrot. According to the authors, the contribution of root hairs to P uptake was clearly confirmed for tomato, and to a lesser degree for lettuce, but not in the case of wheat.

The root hair density was also affected by the interaction between hybrids and P levels. Variation among genotypes was only observed when grown in nutrient-rich medium, where the hair density of hybrid H3 (E) was higher than of the others (Table 4). Comparing the P levels, it was observed that the nutrient concentration in the medium had no influence on most hybrids, except for H3 (E), for which hair density increased with increasing soil P levels. These results indicate a distinctive feature of this hybrid which is more clearly expressed in nutrient-richer environments.

Some studies show that the differences between genotypes in terms of root hairs are more significant under soil P deficiency (Föhse & Jungk, 1983; Gahoonia et al., 1999). This finding was not confirmed in this experiment, since the variations in length and density of root hairs were greatest in the treatment with high soil P content. According to Bates & Lynch (2000) the highest root hair density, observed under soil P deficiency in the medium, did not increase shoot dry matter production or even plant P uptake in *Arabidopsis thaliana* plants. Moreover, according to the authors, despite the differential growth of root hairs, it is possible that low P availability was a limiting factor for plant uptake and growth. Jungk (2001) mentioned that despite the contribution of root hairs to P acquisition and adaptive capacity to the varying conditions in nutrient availability, root hairs are not the only root characteristic that can increase nutrient absorption. The author claims that other strategies can be used by plants, such as increased root/shoot ratio, change in absorption kinetics, association with mycorrhizal fungi and exudation of substances by the roots.

Rhizosphere pH

The rhizosphere pH was influenced by soil P levels and root types. In plants grown at high soil P levels

Table 4. Root hair density of maize hybrids grown at low and high soil P levels

Hybrids	Root hair density[1]	
	Low P	High P
	number mm^{-1}	
H1 (E)	29 aA	29 bA
H2 (E)	30 aA	31 bA
H3 (E)	29 aB	37 aA
H4 (I)	30 aA	27 b A
H5 (I)	28 aA	27 b A
Mean	29	30

[1] Means followed by the same lower-case letters in the columns and capital letters in the rows did not differ significantly by the Scott-Knott test, at 5 %. I: inefficient, E: efficient.

the mean value of rhizosphere pH was slightly higher than in plants grown in low P soil, irrespective of the genotype (Table 5). The increase in rhizosphere pH in the treatment with high soil P level may be due to a greater anion release (OH^- or HCO_3^-) into the plant rhizosphere, to regulate the electrochemical cell equilibrium, due to higher P amounts absorbed by plants from this treatment. In general, a factor that causes major changes in the rhizosphere pH is an imbalance in the ratio of root-absorbed cations/anions (Haynes, 1990).

The rhizosphere pH of lateral roots of maize genotypes was significantly higher than that of nodal roots, regardless of soil P level and genotype used (Table 5). Furthermore, the lateral roots showed greater variations between rhizospheric and non-rhizospheric pH (ΔpH); increases of 0.36 and 0.43 pH unit, respectively, were observed for the low and high P levels. This may indicate a higher nutrient uptake of the lateral roots, resulting in greater excretion of anions in the rhizosphere, increasing the pH in this region of root influence.

Although the differences in rhizosphere pH between maize genotypes (Table 6) were not significant, the variation in rhizosphere pH between genotypes grown in low P soil (0.25 pH unit) was greater than the variation between genotypes grown in nutrient-rich soil (0.1 pH unit), showing a difference in the ability of genotypes to induce changes in rhizosphere pH and favor an increase in P absorption under nutrient stress.

In P-deficient soil, the rhizosphere pH of hybrid H2 (E) was highest of all genotypes, followed by H4 (I) and H5 (I) with slightly lower values than of the first, but higher than the others (Table 6). Under this condition, the variation between rhizospheric and non-rhizospheric pH (ΔpH) of different genotypes was great, with values in a range of 0.18 to 0.39 pH unit. It was also found that the hybrids H2 (E), H4 (I) and H5 (I) had very similar ΔpH values at both P levels, unlike the hybrids H1 (E) and H3 (E), with much lower ΔpH values when grown in low-P soil. These results suggest that the ΔpH would allow a more direct visualization of changes in the rhizosphere pH. Based on this index and the P content in plant tissue of the genotypes, higher values of P uptake were observed (Table 2) in tissues of the single-cross H5 (I) and triple-cross hybrid H2 (E) under P deficiency. This fact may be related to the increase in rhizosphere pH, suggesting a possible mechanism of P uptake efficiency, which was not observed for the single-cross hybrid H4 (I). Although this hypothesis was not consistent with prior field studies of genotype characterization in terms of P-use efficiency, the results may be due to the short experimental period (18 days). Several plant-induced factors may be responsible for changes in the rhizosphere pH of which the most important, according to Marschner (1998), are (a) imbalance in the ratio of cation/anion absorption, with corresponding differences in rates of H^+ and OH^-/HCO_3^- excretion; (b) exudation of organic acids by roots or produced by microorganisms stimulated by the release of organic carbon by the roots, (c) CO_2 production by roots and/or microorganisms from the rhizosphere.

Table 5. Mean rhizosphere pH values of lateral and nodal roots of maize genotypes grown at low and high soil P levels

Root type	Rhizosphere pH[1]				
	Low P		High P		Mean
	pH	(ΔpH) [2]	pH	(ΔpH)	
Lateral	5.47	(0.36)	5.57	(0.43)	5.52 a
Nodal	5.31	(0.20)	5.39	(0.25)	5.35 b
Mean	5.39 B	--	5.48 A	--	--

[1] Means followed by the same lower-case letters in the columns and capital letters in the rows did not differ significantly by the Scott-Knott test at 5 %. [2] Values in brackets indicate the difference between the mean pH in rhizospheric and non-rhizospheric soil in each treatment.

Table 6. Mean of rhizosphere pH values of roots maize genotypes grown at low and high soil P levels

Genotype	Rhizosphere pH[1]			
	Low P		High P	
	pH	(ΔpH) [2]	pH	(ΔpH)
H1 (E)	5.24	(0. 18)	5.47	(0.31)
H2 (E)	5.49	(0.37)	5.45	(0.36)
H3 (E)	5.36	(0.13)	5.43	(0.36)
H4 (I)	5.40	(0.35)	5.53	(0.32)
H5 (I)	5.40	(0.39)	5.52	(0.37)

[1] Means followed by the same lower-case letters in the columns did not differ significantly by the Scott-Knott test at 5 %. I: inefficient, E: efficient, ns: non-significant. [2] Values in brackets indicate the difference between mean pH of rhizospheric and non- rhizospheric soil of each treatment.

Although differences in rhizosphere pH values between genotypes were apparently small, the relevance of the value is expressed in terms of chemical changes in this region. Tyler et al. (1987) mentioned that in the pH range of 4.0–4.5, an increase of 0.2 pH unit can reduce Al concentration in soil solution two to three times. In addition, the non-destructive methodology used in this study enables a direct pH measurement in the rhizosphere region, which allows a highly detailed and accurate assessment, differing from the traditional soil pH assessments that use a diluted soil-water suspension.

To evaluate the influence of non-rhizosphere soil pH on the pH in the region of rhizosphere influence (rhizospheric pH) of maize plants, an orthogonal

contrast analysis was performed, comparing the mean values of rhizosphere and non-rhizosphere pH at low and high P levels. The non-rhizosphere pH was also compared of the rhizosphere of nodal and lateral roots, at both P levels.

The mean pH values of maize genotypes were significantly higher in the rhizospheric than non-rhizospheric soil, at both soil P levels (Figure 3), indicating that maize hybrids have the capacity of changing the soil pH in the rhizosphere, independent of the P concentration in the medium. The pH increase observed in the plant rhizosphere may be due to an increased excretion of OH^- or HCO_3^- by the roots, because at the beginning of the experiment NH_4NO_3 was applied as N source. According to Klotz & Horst, (1988), the preferential uptake of N-NO_3, along with a high nitrate reductase activity in the apical region of soybean roots were the two main factors responsible for pH increase in the rhizoplane and rhizosphere of acid-soil-tolerant plants. The authors also mentioned that in comparison with the pH of the basal area and even the non-rhizosphere pH, the rhizosphere pH in the apical root region may increase when NH_4NO_3 is used as N source.

The analysis of orthogonal contrasts also showed that the rhizosphere pH, of the nodal as well as lateral roots, was not higher than the pH in non-rhizospheric soil, irrespective of the P level in the medium (Figure 4). Furthermore, the rhizosphere pH of the lateral was higher than of the nodal roots, indicating a more intense anion flux in the cells of these roots.

Functional microbial diversity

The analysis of variance performed for the bacterial functional diversity indices, estimated after 72 h of sample incubation showed no significant difference among the treatments, for all indices estimated (Table 7). Although the applied statistical method could not clearly separate the treatment effects, a tendency was detected in the results of the indices.

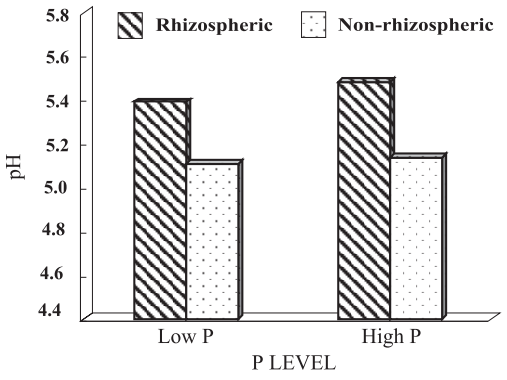

Figure 3. Mean pH values of rhizospheric and non-rhizospheric soil of maize genotypes grown at low and high P levels.

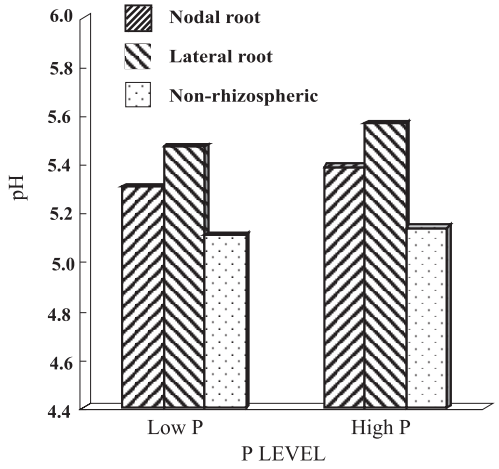

Figure 4. Mean pH values in rhizospheric and non-rhizospheric soil of nodal and lateral roots of maize genotypes grown at low and high P levels.

In general, higher values of functional diversity were observed in bacterial extract from the rhizosphere of hybrids grown in low-P soils, based on microbial catabolic activity expressed by the indices evaluated, than in plants grown in high-P soils (Table 7). These results show the changing profile of microbial metabolism in the rhizosphere of maize genotypes exposed to contrasting P levels, modifying the activity according to the C sources used by microorganisms. An important aspect is the growing period of plants of 18 days only and for which, in the case of P, a longer time of cultivation would be required to induce significant changes in the rhizosphere. According to Grayston et al. (1998), the abundance and activity of soil microorganisms are influenced by a series of environmental (soil type, nutritional status, pH, moisture, etc.) and plant factors (species, age, etc.). The wide range of organic compounds released by plants has been considered a major influence on microorganism diversity in the rhizosphere of different plant species (Bowen & Rovira, 1991; Bolton et al., 1992). This may encourage the development of certain microorganism communities possibly participating in processes linked to P use efficiency. Also using the Biolog system, Grayston et al. (1998) found a clear discrimination between the carbon sources used by microbial communities from the rhizosphere of different plant species. In a greenhouse study, Oliveira (2009) obtained similar results of microbial metabolic activity in the rhizosphere for these same genotypes, suggesting that this characteristic is inherent to the hybrids.

The compositional ratio of the metabolic profile of the plant rhizosphere in different treatments is shown in figure 5. As shown in the cluster analysis, two major groups of treatments with different metabolic profiles in the substrate use can be discriminated. One group contained the treatments involving the hybrids H1 (E), H3 (E), H2 (E), and H5 (I) at low P

Table 7. Mean values of microbial diversity indices in the rhizosphere of maize hybrids, determined in soil extract after 72 h of incubation in Biolog plate

P rate	Hybrid					
	H1(E)	H2(E)	H3(E)	H4(I)	H5(I)	Mean
			Mean substrate activity (AWCD)			
Low	0.26	0.19	0.24	0.22	0.29	0.24
High	0.19	0.12	0.29	0.14	0.19	0.19
Mean	0.22	0.16	0.27	0.18	0.24	--
			Substrate abundance (S)			
Low	26.33	26.67	23.00	29.67	30.00	27.13
High	27.00	27.00	26.67	27.00	25.33	26.60
Mean	26.67	26.83	24.83	28.33	27.67	--
			Substrate diversity (H)			
Low	2.80	2.80	2.74	2.90	2.82	2.81
High	2.75	2.75	2.85	2.78	2.55	2.74
Mean	2.78	2.77	2.80	2.84	2.68	--
			Substrate equivalence (E)			
Low	0.86	0.85	0.88	0.86	0.83	0.86
High	0.84	0.83	0.87	0.84	0.79	0.83
Mean	0.85	0.84	0.88	0.85	0.81	--

level and the other group the efficient hybrids H1 and H2 and inefficient hybrids H4 and H5, at high P. In the case of H3, the microbial activity in the rhizosphere was not affected by the soil P level. These results indicate that the activity of specific substrates was influenced by the P level in the rhizosphere of most tested maize genotypes. Garland (1996) observed different patterns of carbon source utilization in the rhizosphere of soybean, wheat, and sweet and white potato. Therefore, in the present study the functional structure of the rhizosphere bacterial population of maize genotypes was more influenced by the soil P concentration than the P-use efficiency of plants, previously characterized under field conditions.

Within each major group structural categories of substrates were identified that were used by the microbial community in all treatments of these groups. The first group comprised the structural categories of amino acids > carbohydrates > carboxylic acids > polymers. In the second large group the discrimination was based mainly on the categories of carbohydrates > amino acids > carboxylic acids. At low P levels, characterized in the first group, the rhizosphere microbial populations have a specific ability to metabolize preferentially amino acids. Although the rhizosphere microorganisms have access to a variety of rhizodepositions, the preferential use of amino acids may be due to the fact that these molecules represent the most abundant and readily available N source in the rhizosphere. Kraffczyk et al. (1984) and Merbach et al. (1999) most frequently observed high carbohydrate contents in the overall composition of exudates of maize, pea, and wheat grown in hydroponic culture, followed by carboxylic acids and a minority of amino acids, which is consistent with the results of these authors, since the preferred uptake/consumption source were carbohydrates.

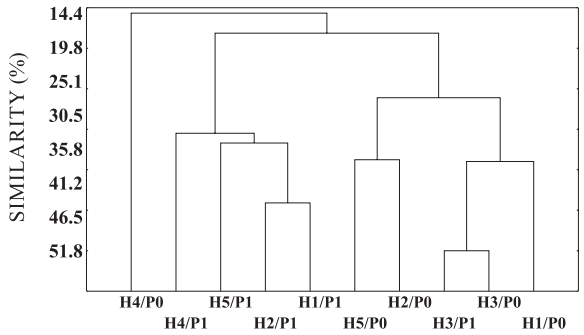

Figure 5. Cluster analysis based on the presence and absence of substrates (72 h of incubation) by the microbial extract from the rhizosphere of maize genotypes grown at two P levels (P0 = low and P1 = high). Utilizou-se a UPGMA cluster analysis and Euclidian distance.

CONCLUSIONS

1. At all soil P levels, the root hairs of the hybrids varied in length and total density and were more abundant on nodal and seminal than on lateral roots.

2. The hybrids differed in ability to change the rhizosphere pH, irrespective of the P concentration in the medium.

3. The hybrids influenced the catabolic activity of rhizosphere microbial communities and the structural categories of carbon sources differed depending on the soil P level.

4. No patterns were detected between the variations in the rhizosphere and the P-use efficiency in the tested genotypes.

LITERATURE CITED

ALVES, V.M.C.; PARENTONI, S.N.; VASCONCELLOS, C.A.; PITTA, E. & FRANÇA, C.C.M. Cinética de absorção de fósforo e crescimento do sistema radicular de genótipos de milho contrastantes para eficiência a fósforo. R. Bras. Milho Sorgo, 1:85-92, 2002.

BAREA, J.M.; POZO, M.J.; AZCÓN, R. & AZCÓN-AGUILAR, C. Microbial co-operation in the rhizosphere. J. Exper. Bot., 56:1761-1778, 2005.

BATES, T.R. & LYNCH, J.P. Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot., 87:958-963, 2000a.

BOLTON, H.; FREDERICKSON, J.K. & ELLIOT, L.F. Microbial ecology of the rhizosphere. In: METTING, F.B. Soil microbial ecology. New York, Marcel Dekker, 1992. p.27-36.

BOWEN, G.D. & ROVIRA, A.D. The rhizosphere and its management to improve plant growth. Adv. Agron., 66:1-102, 1999.

BOWEN, G.D. & ROVIRA, A.D. The rhizosphere, the hidden half of the hidden half. In: WAISEL, Y. & KAFKAFI, U., eds. Plant roots – The hidden half. New York, Marcel Dekker, 1991. p.641-649.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA. Centro Nacional de Pesquisa de Solos. Manual de métodos de análise de solo. 2.ed. Rio de Janeiro, 1997. 212p.

FERREIRA, M.E.; ANDRADE, L.R.M.; SANO, E.E.; CARVALHO, A.M. & JUNQUEIRA, N.T.V. Uso de imagens digitais na avaliação da cobertura do solo. Planaltina, Embrapa Cerrados, 2001. 50p. (Boletim de Pesquisa e Desenvolvimento. Embrapa Cerrado, 21).

FÖHSE, D. & JUNGK, A. Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. Plant Soil, 74:359-368, 1983.

GAHOONIA, T.S.; CLAASSEN, N. & JUNGK, A. Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. Plant Soil, 140:241-248, 1992.

GAHOONIA, T.S.; ASMAR, F.; GIESE, H.; GISSEL-NIELSEN, G. & NIELSEN, N.E. Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. Europ. J. Agron., 12:281-289, 2000.

GAHOONIA, T.S.; CARE, D. & NIELSEN, N.K. Root hairs and phosphorus acquisition of wheat and barley cultivars. Plant Soil, 191:181-189, 1997.

GAHOONIA, T.S. & NIELSEN, N.E. Variation in root hairs of barley cultivars doubled soil phosphorus uptake. Euphytica, 98:177-182, 1997.

GAHOONIA, T.S.; NIELSEN, N.E. & LYSHEDE, O.B. Phosphorus acquisition of cereal cultivars in the field at three levels of P fertilization. Plant Soil, 211:269-281, 1999.

GARLAND, J.L. Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem., 28:223-230, 1996.

GARLAND, J.L. & MILLS, A.L. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol., 57:2351-2359, 1991.

GRAYSTON, S.J.; WANG, S.; CAMPBELL, C.D. & EDWARDS, A.C. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem., 30:369-378, 1998.

GRINSTED, M.J.; HEDLEY, M.J.; WHITE, R.E. & NYE, P.H. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald). I. pH changes and the increase in P concentration in the soil solution. New Phytol., 91:19-29, 1982.

HÄUSSLING, M.; LEISEN, E.; MARSCHNER, H. & RÖMHELD, V. An improved method for non-destructive measurements of the pH at the root-plant interface (rhizosphere). J. Plant Physiol., 117:371-375, 1985.

HAYNES, R.J. Active ion uptake and maintenance of cation-anion balance: A critical examination of their role in regulating rhizosphere pH. Plant Soil, 126:247-264, 1990.

HEDLEY, M.J.; NYE, P.H. & WHITE, R.E. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. II. Origin in the pH change. New Phytol., 91:31-44, 1982.

HINSINGER, P. How do plant roots acquire mineral nutrient? Chemical processes involved in the rhizosphere. Adv. Agron., 64:225-265, 1998.

ITOH, S. & BARBER, S.A. Phosphorus uptake by six plant species as related to root hairs. Agron. J., 75:457-461, 1983.

JUNGK, A. Root hairs and the acquisition of plant nutrients from soil. J. Plant Nutr. Soil Sci., 164:121-129, 2001.

KLOTZ, F. & HORST, W.J. Genotypic differences in aluminium tolerance of soybean (*Glycine max* L.) as affected by ammonium and nitrate-nitrogen nutrition. J. Plant Physiol., 132:702-707, 1988.

KRAFFCZYK, I.; TROLLDENIER, G. & BERINGER, H. Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms, Soil Biol. Biochem., 16:315-322, 1984.

LI, Q.C.; ALLEN, H.L. & WOLLUM, A.G. Microbial biomass and bacterial functional diversity in forest soils: Effects of organic matter removal, compaction, and vegetation control. Soil Biol. Biochem., 36:571-579, 2004.

MACHADO, C.T.T.; GUERRA, J.G.M.; ALMEIDA, D.L. & MACHADO, A.T. Variabilidade entre genótipos de milho para eficiência em níveis de fósforo. Bragantia, 58:109-124, 1999.

MARSCHNER, H. Mineral nutrition in higher plants. San Diego, Academic Press, 1995.889p.

MARSCHNER, H. Rhizosphere pH effects on phosphorus nutrition. In: RÖMHELD, V. A interface solo-raiz (rizosfera) e relações com a disponibilidade de nutrientes, a nutrição e as doenças de plantas. Piracicaba, Potafos/ USP/CEA, 1998. p.107-115.

MARSCHNER, P.; SOLAIMAN, Z. & RENGEL, Z. Rhizosphere properties of Poaceae genotypes under P limiting conditions. Plant Soil, 283:11-24, 2006.

MERBACH, W.; MIRUS, E.; KNOF, G.; REMUS, R.; RUPPEL, S.; RUSSOW, R.; GRANSEE, A. & SCHULZE, J. Release of carbon and nitrogen compounds by plant roots and their possible ecological importance, J. Plant Nutr. Soil Sci., 162:373-383, 1999.

MICHAEL, G. The control of root hair formation: Suggested mechanisms. J. Plant Nutr. Soil Sci., 164:111-119, 2001.

NOVAIS, R.F. & SMYTH, T.J. Fósforo em solo e planta em condições tropicais. Viçosa, MG, Universidade Federal de Viçosa, 1999, 399p.

OLIVEIRA, C.A.; MARRIEL, I.E.; GOMES, E.A.; LANA, U.G.P.; SCOTTI, M.R. & ALVES, V.M.C. Diversidade bacteriana da rizosfera de genótipos de milho contrastantes na eficiência de uso de fósforo. Pesq. Agropec. Bras., 44:1473-1482, 2009.

PARENTONI, S.N.; GAMA, E.E.G.; SANTOS, M.X.; LOPES, M.A.; ALVES, V.M.C.; BAHIA FILHO, A.F.C.; VASCONCELLOS, C.A.; MAGNAVACA, R.; PACHECO, C.A.P.; MEIRELLES, W.F.; GUIMARÃES, P.E.O.; SILVA, A.E.; NASPOLINI FILHO, W.; MORO, J.R.; VIANA, R.T.; PITTA, G.V.E.; FRANCA, G.E.; PURCINO, A.A.C.; SOUZA, I.R.P.; ARRIEL, I.E.; MACHADO, A.T.; CORREA, L.A.; OLIVEIRA, A.C. & PAIVA, E. Adaptação de milho a solos ácidos: Tolerância à toxidez de alumínio e eficiência no uso de nutrientes no programa de pesquisa da EMBRAPA - Milho e Sorgo. In: REUNION LATINOAMERICANA DEL MAIZ, 18., Sete Lagoas, 1999. Memórias. Sete Lagoas: EMBRAPA Milho e Sorgo, México: CIMMYT, 1999. p.179-199.

RICHARDSON, A.E.; BAREA, J.M.; MCNEILL, A.M. & PRIGENT-COMBARET, C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil, 321:305-339, 2009.

SARRUGE, J.R. & HAAG, H.P. Análises químicas em plantas. Piracicaba, Escola Superior de Agricultura "Luiz de Queiroz", 1974. 56p.

WISSUWA, M. & AE, N. Genotypic differences in the presence of hair on roots and gynophores of peanuts (*Arachis hypogea* L.) and their significance for phosphorus uptake. J. Exper. Bot., 52:1703-1710, 2001.

ZAK, J.C.; WILLING, M.R.; MOOREHEAD, D.L. & WILDMAN, H.G. Functional diversity of microbial communities: a quantitative approach. Soil Biol. Biochem., 26:1101-1108, 1994.