Pedosphere **22**(6): 870–877, 2012
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Fine Root Patterning and Balanced Inorganic Phosphorus Distribution in the Soil Indicate Distinctive Adaptation of Maize Plants to Phosphorus Deficiency*[1]

ZHANG Yu, YU Peng, PENG Yun-Feng, LI Xue-Xian, CHEN Fan-Jun and LI Chun-Jian*[2]

Key Laboratory of Plant-Soil Interactions, Ministry of Education, Department of Plant Nutrition, China Agricultural University, Beijing 100193 (China)

(Received May 25, 2012; revised September 18, 2012)

ABSTRACT

Plants have diverse strategies to cope with phosphorus (P) deficiency. To better understand how maize responds to P deficiency, a field experiment with two P levels, 0 and 100 kg P_2O_5 ha^{-1} (P0 and P100, respectively), was carried out as a part of a long-term P-fertilizer field trial. Plant and soil analyses showed that P-deficient maize reduced its growth rate, increased P use efficiency, and formed more thin roots with the diameter less than 0.6 mm at jointing and silking stages, compared to the plants treated with P100. Further, there were no differences in major inorganic P fractions (Ca_2-P, Ca_8-P, Al-P, Fe-P, occluded P and Ca_{10}-P) between the rhizospheric and bulk soils at each harvest, even when soil Olsen-P was only 1.38 mg kg^{-1}. These results suggested that maize responded to P deficiency by reducing the internal P demand for growth and increasing P acquisition ability by favorable root morphological alteration at low carbon cost.

Key Words: inorganic phosphorus fractions, phosphorus starvation, rhizosphere, root length, root morphology

Citation: Zhang, Y., Yu, P., Peng, Y. F., Li, X. X., Chen, F. J. and Li, C. J. 2012. Fine root patterning and balanced inorganic phosphorus distribution in the soil indicate distinctive adaptation of maize plants to phosphorus deficiency. *Pedosphere*. **22**(6): 870–877.

INTRODUCTION

Phosphorus (P) availability in the soil is one of the most limiting factors for plant growth and productivity in natural and agricultural ecosystems (Lynch and Deikman, 1998). In response to limited P availability, plants have evolved a wide array of adaptive mechanisms, such as modification of root growth (Barber, 1995; Lynch, 1995; Marschner, 1995), acidification of rhizospheric soils (Neumann and Römheld, 1999; Hinsinger *et al.*, 2003), exudation of carboxylates (Dinkelaker *et al.*, 1989; Jones, 1998; Neumann and Römheld, 1999) and phosphatases (Helal and Dressler, 1989; Helal, 1990). Plant species such as *Lupin* and *Brassica* form characteristic cluster roots that efficiently increase P availability in the soil through proton and organic anion release under P deficiency (Gardner *et al.*, 1982; Neumann *et al.*, 1999; Neumann and Martinoia, 2002; Shane and Lambers, 2005). Interestingly, maize does not change their rhizospheric pH significantly under P deficiency (Neumann and Römheld, 1999; George *et al.*, 2002). Organic acid exudates even decrease under P deficiency (Neumann and Römheld, 1999; Liu *et al.*, 2004). A plausible hypothesis is that maize may undergo root growth re-patterning to explore larger soil volume for P acquisition under P deficiency, rather than exert chemical and/or biochemical alteration in the rhizosphere to increase the availability of sparingly soluble sources. It is known that three-dimensional root growth continuously increases rhizosphere periphery in the soil profiles, and the resulting root architecture determines the efficiency of P acquisition (Lynch, 1995; Linkohr *et al.*, 2002; Zhu *et al.*, 2005). Root length density and mycorrhizal fungus colonization are important for increasing uptake efficiency of nutrients such as poorly mobile P (Marschener, 1998; Linkohr *et al.*, 2002).

Indeed, maize plants alter root growth upon P starvation (Lynch, 1995; Mollier and Pellerin, 1999). Genotypes with more shallow roots show greater total P content, relative growth rates, and biomass accumu-

*[1]Supported by the National Basic Research Program (973 Program) of China (No. 2013CB127402), the Fundamental Research Funds for the Central Universities, China (No. 2012YJ054), and the Innovative Research Group Grant of the National Natural Science Foundation of China (No. 31121062).
*[2]Corresponding author: E-mail: lichj@cau.edu.cn.

lation than those with deeper root systems (Zhu *et al.*, 2005). Under low P stress, efficient maize genotypes display greater root:shoot ratio, higher lateral root number as well as longer total root length to explore new soil areas, allowing greater P acquisition and biomass accumulation (Zhu and Lynch, 2004; Zhu *et al.*, 2005; Li *et al.*, 2006). However, it remains unclear how maize plants modify fine root architecture to cope with P deficiency. Further, what are the effects of root patterning on inorganic P distribution in the rhizospheric and bulk soils? In the present study, a field experiment with extremely low P availabilities (Olsen-P < 5 mg kg^{-1}) on the basis of a long-term P-fertilizer field trial was carried out using maize as a model monocot, to examine P uptake, root distribution in 0–60 cm soil profiles, especially the P depletion and inorganic P fractions in the rhizospheric soil at different growth stages. The objective of this study was to verify the hypothesis that maize responds to P deficiency by modifying root morphology rather than by changing availability of sparingly soluble P in the rhizosphere. The aim of this study was to get a better understanding of the adaptive mechanisms of maize to P deficiency.

MATERIALS AND METHODS

Experimental design

The field experiment was carried out in long-term P-fertilizer field trials in the Shangzhuang Experimental Station of China Agriculture University in Beijing, China. Two P levels of 0 and 135 kg P_2O_5 ha^{-1} have been applied in the long-term P-fertilizer field trials since 1984. From then on, maize was continuously cultivated in each year. The soil type at the study site is a calcareous alluvial fluvo-aquic soil with a loamy and silt texture. The soil samples were obtained using the soil auger method and analyzed before fertilization. Some selected chemical properties of the 0–30 cm soil layer are shown in Table I. Maize genotype 478, provided by the maize breeding group of the Department of Plant Nutrition, China Agricultural University, was used in the experiment and sown on May 4, 2010. Flooding irrigation before sowing was used to keep the available soil water content above 75%. The field was plowed just before sowing. Maize was overseeded with hand planters and the plots were thinned at the seedling stage to a stand of 100 000 plants ha^{-1}. In this study two P treatments were applied at the rates of 0 and 100 kg P_2O_5 ha^{-1} (P0 and P100, respectively) to the corresponding fields receiving 0 and 135 kg P_2O_5 ha^{-1} since 1984. Phosphorus (as superphosphate) and potassium (150 kg K ha^{-1}, as potassium sulfate) were applied as base fertilizer. Urea was used as N fertilizer at a rate of 240 kg ha^{-1}, of which 30% was applied before sowing as base fertilizer and the rest was top-dressed at 12-leaf stage. Following a randomized block design, the plot size was 21 m^2 with three replicates. The distances between rows and plants were 50 and 20 cm, respectively. Border plots were included on the sides of the experimental field. Weed growth on plots was controlled by pre-emergence herbicides and cultivation.

TABLE I

Selected soil chemical properties (0–30 cm soil layer) of the long-term P-fertilizer field trials before P application in this study

Trial (P applied)	pH (H_2O)	Total nitrogen	Olsen-P	NH_4OAc-K	Organic matter
kg P_2O_5 ha^{-1}		g kg^{-1}	mg kg^{-1}		g kg^{-1}
0	8.2a[a)]	0.99a	1.38b	95.0a	5.93b
135	8.3a	0.87a	3.48a	94.0a	7.36a

[a)] Means followed by the same letter within each column are not significantly different at $P < 0.05$.

Sampling

Plants were harvested at jointing, silking and physiological maturity (50% of the plants showed black layer formation in the grains from the mid-portion of the ears). That was 55, 90 and 147 d after sowing, respectively. At each harvest, five consecutive plants in each plot were cut at the stem base, chopped to a fine consistency, killed at 105 °C for 30 min, dried to a constant weight at 75 °C and ground into powder. Appropriate amount of the ground plant material was digested by a modified Kjeldahl procedure using $H_2SO_4 + H_2O_2$ as catalyst, and the digests were analyzed for P concentration by automated colorimetry using the molybdovanadate method (Soon and Kalra, 1995).

After cutting off shoots at harvest, a soil volume of 50 cm × 20 cm × 60 cm was dug out in each plot of the two treatments and then divided into 6 soil blocks with each 50 cm × 20 cm × 10 cm. All visible roots in each soil block were picked out in the field by hand and placed in individual marked plastic bag. These roots were washed free of soil after transfer to the laboratory and then frozen at −20 °C until root length analysis as described by Niu *et al.* (2010). For rhizospheric and bulk soils collection, the whole roots of two other plants were separately dug out and each root was excavated with a soil volume of 50 cm × 20 cm × 40 cm. After carefully removing the loosely held soil, which was collected as bulk soil, the tightly held soil on the root

surface was gently shaken over a clean paper sheet and collected as rhizospheric soil. All fresh soil samples were crushed, sieved through a 2 mm sieve in the field and then air-dried in the laboratory. After collection of the rhizospheric and bulk soils, the two whole roots in each plot were washed free of soil, dried to a constant weight and used for root dry weight and P content measurement as mentioned above.

Analyses of Olsen-P and inorganic P fractions

The dry rhizospheric and bulk soils were extracted with 0.5 mol L^{-1} $NaHCO_3$ for 30 min, and Olsen-P was analyzed colorimetrically according to Murphy and Riley (1962). Inorganic P fractions were only measured with P0 soil. Inorganic P fractions were analyzed by using a fractionation system adapted from Gu and Jiang (1990). This method involved sequential extraction of soil (1 g from air-dry equivalent) with 0.25 mol L^{-1} $NaHCO_3$ (Ca_2-P), 0.5 mol L^{-1} NH_4OAc (Ca_8-P), 0.5 mol L^{-1} NH_4F (Al-P), 0.1 mol L^{-1} NaOH and Na_2CO_3 (Fe-P), 0.3 mol L^{-1} Na-citrate and 0.5 mol L^{-1} NaOH (occluded P), and 0.5 mol L^{-1} H_2SO_4 (Ca_{10}-P). The supernatant from each step was analyzed colorimetrically by molybdovanadate method (Soon and Kalra, 1995).

Root length analysis

Root samples harvested from different soil layers at each harvest were scanned with a scanner (Epson 1680, Indonesia). For scanning, the root sample was placed in a rectangular dish (300 mm × 200 mm) with a layer of water about 4–5 mm in depth to untangle the roots and minimize root overlap. When necessary, root systems were separated into subsamples until they could be fully spread into the rectangle dish. The images were analyzed using the software WinRHIZO (version 5.0, Canada). After calculation, the roots with different diameters and the total root length in each soil layers were obtained.

Statistical analyses

All data are presented as means±standard errors in figures. Some data were analyzed following analysis of variance by SAS package (SAS Institute, 1996) and the means were compared based on least significant difference at $P = 0.05$.

RESULTS

Plant growth and P uptake

Shoot dry weight and shoot P content of maize plants increased steadily with the prolonged growth period. The differences in shoot dry weight and shoot P content of the plants between two P levels increased after the elongation stage (Fig. 1). As a result, the P0 plants produced more biomass using less P compared with the P100 plants (Fig. 1). At each harvest, shoot P concentration did not change very much, and that of the P0 plants was significantly lower than that of the P100 plants (Fig. 1). Root dry weight reached its maximum at silking stage and then declined until maturity stage. The root dry weight of the P0 plants was lower than that of the P100 plants at silking and maturity stages (Fig. 1). The difference in grain yield between the treatments at maturity was significant (Fig. 1). The very low grain yield of the P0 plant was because of the high ratio of kernel abortion.

Root distribution in different soil layers

Total root length of maize plants in each soil layer increased dramatically from jointing to silking, because of the development of the brace roots after jointing; while the total root length decreased rapidly after silking, due to root mortality (Fig. 2). When focusing on the root length with different diameters in each soil layer, it became obvious that P0-treated maize plants had longer roots in the soil layers 40–60 cm at jointing and in the soil layers 30–50 cm at silking, especially for the fine roots with the diameter < 0.6 mm. At maturity, however, the root length of the P100 plants in the whole soil profile was longer than that of the P0 plants, regardless of the root diameter.

Olsen-P and inorganic P fractions in rhizospheric and bulk soils

P application obviously increased Olsen-P concentration in the rhizospheric and bulk soils. Unexpectedly, there was no Olsen-P depletion in the rhizospheric soil under both treatments. The difference in Olsen-P between the rhizospheric and bulk soils was not significant (Fig. 3). These results also indicated a surprising finding that there were no differences in inorganic P fractions between the rhizospheric and bulk soils in this 26-year P0 field (Fig. 4).

DISCUSSION

Increasing internal P use efficiency under P deficiency

P deficiency significantly reduces photosynthetic capacity (Foyer and Spencer, 1986; Fredeen *et al.*, 1989; Usuda and Shimogawara, 1991). It is not surprising that P deficiency reduces the leaf growth and dry matter accumulation in maize (Plénet *et al.*, 2000a,

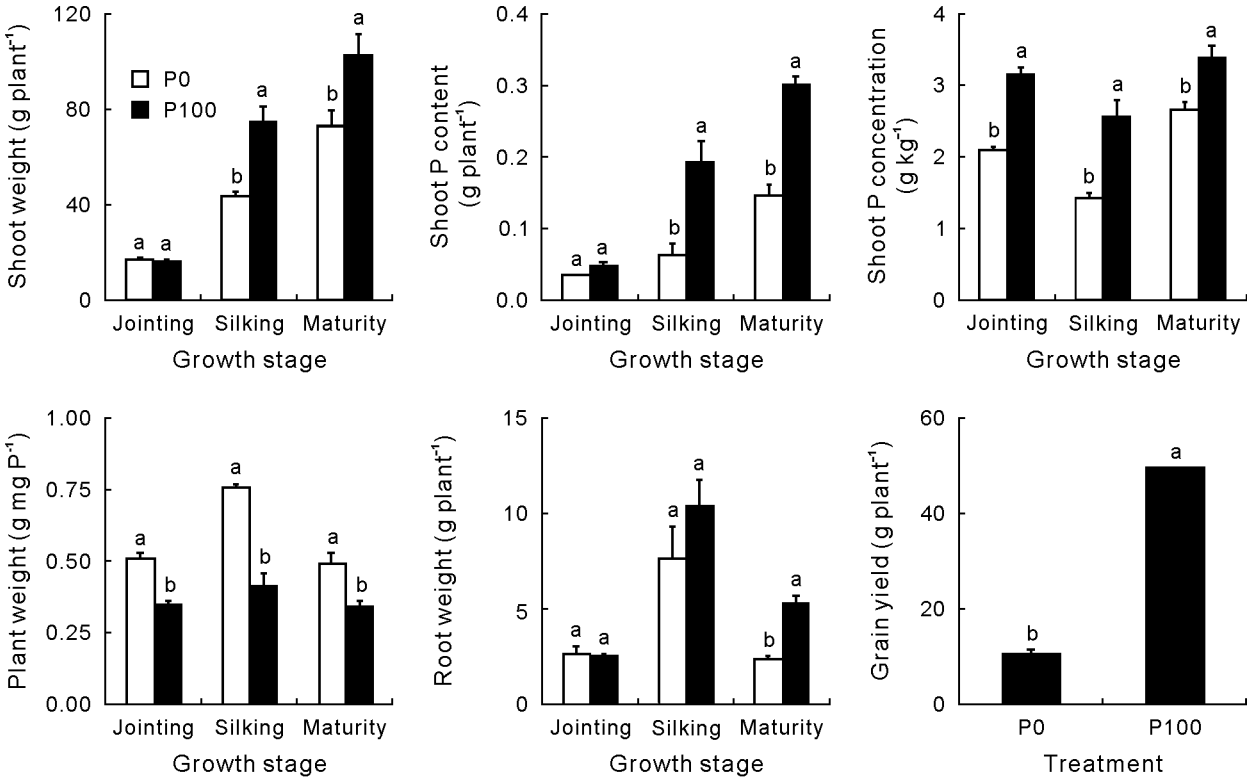

Fig. 1 Changes in shoot dry weight, shoot P content and concentration, plant dry weight production per unit P, root dry weight and grain yield of maize plants grown under the P application of 0 and 100 kg P_2O_5 ha^{-1} (P0 and P100, respectively) at different growth stages. Verticle bars indicate standard errors of the means ($n = 3$). Bars with the same letters at each growth stage are not significantly different at $P < 0.05$.

b). Plants develop diverse adaptive mechanisms to cope with P deficiency. One strategy is to reduce growth rate and increase internal P use efficiency, as shown in Fig. 1. Differing from the low mobility in the soil, P is a relatively mobile element in plants, moving easily between organs (Marschner, 1995). P deficiency alters P partitioning among organs; more preference is given to the reproductive organs at the expense of P contents of vegetative organs (Crafts-Brandner, 1992; Peng and Li, 2005). The difference in shoot dry weight between P0 and P100 plants was smaller than that in shoot P content, since the P0 plants had higher P use efficiency compared with the P100 plants (Fig. 1). The results indicated that one adaptive mechanism of maize plants in response to P deficiency was to increase internal P use efficiency and to reduce the P demand for growth.

Modification of root growth under P deficiency

In addition to the increased internal P use efficiency, plants develop many other adaptive mechanisms to enhance P acquisition from external environment. One such characteristic adaptation is root architecture modification (Lynch, 1995; Lynch and Brown, 2001). When root growth of maize seedling was restricted, the remaining roots underwent comprehensive morphological adaptation, including the increased dry weight, lateral root number, root density, and the decreased average root diameter (Yan *et al.*, 2011). The Olsen-P content in the P0 and P100 plots before fertilization was 1.38 and 3.48 mg kg^{-1}, respectively (Table I), and that in the P100 plot after fertilization was about 10 mg kg^{-1} (Fig. 3). Soil Olsen-P below 10 mg kg^{-1} is considered as P deficiency for agricultural production in China (Lu, 2003). The extremely low Olsen-P in the P0 soil severely inhibited root growth; therefore, the root dry weight of P0 plants was lower than that of P100 plants at later growth stage (Fig. 1). However, P0 plants formed more thin roots with the diameter less than 0.6 mm at jointing and silking stages (Fig. 2), indicating that P0 plants formed larger root surface area utilizing less carbon, which allowed plants to explore larger soil volume for P nutrients. The total root length of P0 and P100 plants decreased dramatically after silking. This can be attributed to the root mortality during the reproductive growth stage (Wells and Eissenstat, 2003; Niu *et al.*, 2010). In comparison, the root length of the P0 plants in the whole soil pro-

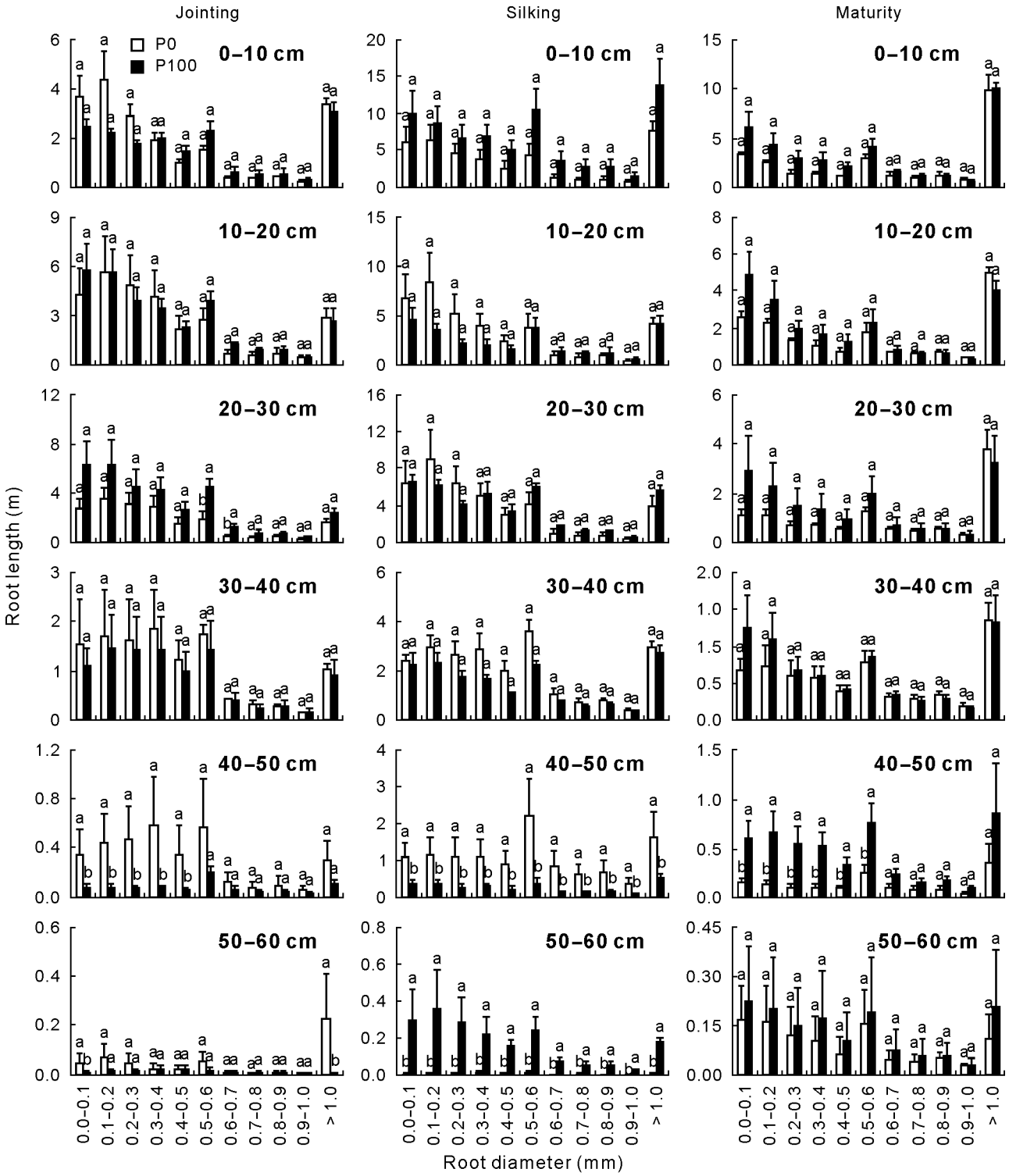

Fig. 2 Changes in root length with different diameters in 0–60 cm soil layers of maize plants grown under the P application of 0 and 100 kg P_2O_5 ha^{-1} (P0 and P100, respectively) at different growth stages. Verticle bars indicate standard errors of the means ($n = 3$). Bars with the same letters at each growth stage are not significantly different at $P < 0.05$.

file decreased faster than that of the P100 plants after silking, because of the extremely low Olsen-P in the P0 soil and the inhibited shoot growth, which provided less carbon to roots.

Balanced inorganic P distribution in rhizospheric and bulk soils

It is well known that there is a distinct P depletion

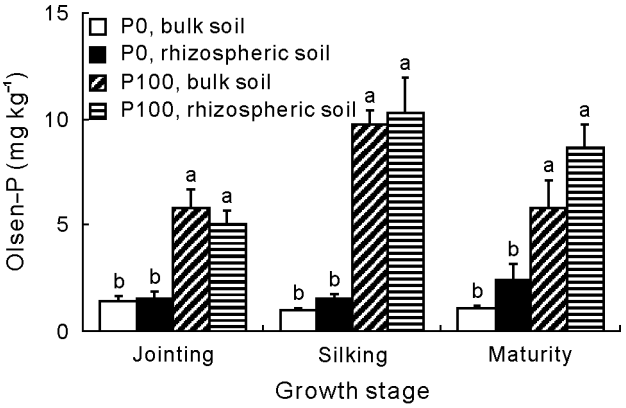

Fig. 3 Changes in Olsen-P in the rhizospheric and bulk soils of maize plants grown under the P application of 0 and 100 kg P_2O_5 ha^{-1} (P0 and P100, respectively) at different growth stages in 0–40 cm soil layer. Verticle bars indicate standard errors of the means ($n = 3$). Bars with the same letters at each growth stage are not significantly different at $P < 0.05$.

zone in the rhizosphere due to plant uptake (Lewis and Quirk, 1967; Barber, 1995; Bertrand *et al.*, 1999). This P depletion zone is closely related to the rhizobox approach. The present field study showed no Olsen-P depletion in the rhizospheric soil compared with the bulk soil even when soil Olsen-P was extremely low (Fig. 3). Similarly, Arienzo *et al.* (2004) observed increases in soluble and immaterially available P in the rhizosphere of field-grown maize due to ammonium fertilization and subsequent calcium phosphates solubilization. George *et al.* (2002) also found increased organic P pools (NaOH-Po) in the rhizosphere of maize grown in the rhizobox. It has been suggested that a stimulated microbial biomass in the rhizosphere may lead to P conversion into organic forms. The possible explanations for the failure of the determination of rhizospheric P depletion in the present study were that 1) the method used in the field study for collection of rhizospheric soil cannot be very accurate as used in the rhizobox study, and this might cause a portion of bulk soil to be mixed with the rhizospheric soil, thereby increase Olsen P of the rhizospheric soil; and 2) the rhizospheric soil was obtained from a whole root system while P depletion was occurred mainly in young and fine roots. Both of them might mask the expected Olsen P depletion in the rhizospheric soil of apical, active young roots.

An intriguing question was that whether maize could increase mobilization and exploitation of sparingly soluble P sources in soils by chemically modifying the rhizosphere as *Lupin* and *Brassica* do (Neumann and Römheld, 1999). The pH and the anions that compete with P ions for ligand exchange reactions are the major factors influencing bioavailability of soil ino-

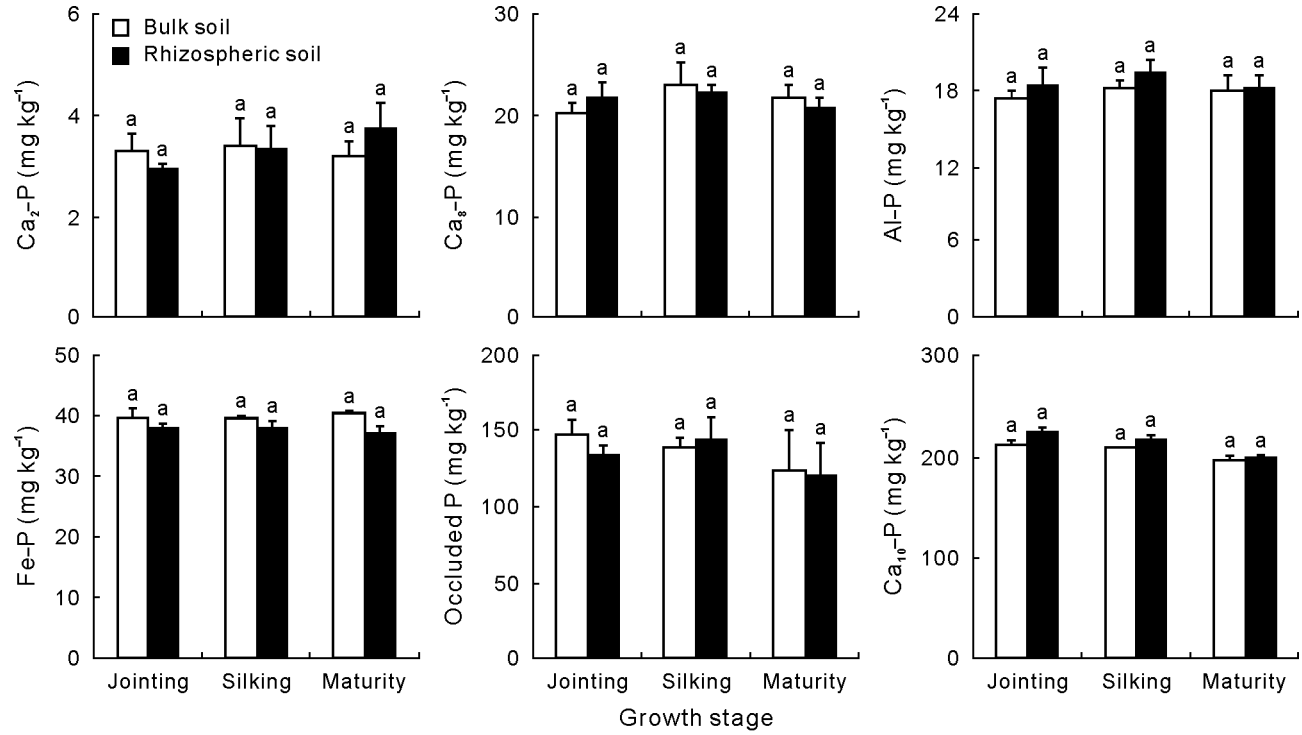

Fig. 4 Changes in inorganic P fractions in the rhizospheric and bulk soils of maize plants grown under the P application of 0 kg P_2O_5 ha^{-1} at different growth stages. Verticle bars indicate standard errors of the means ($n = 3$). Bars with the same letters at each growth stage are not significantly different at $P < 0.05$.

rganic P (Hinsinger, 2001). However, maize does not change its rhizospheric pH significantly (George*et al.*, 2002) and exudes less organic acids (Liu *et al.*, 2004) when grown under P deficiency. One reason for no significant pH change in maize rhizosphere under P deficiency can be explained by the fact that many monocotyledonous plants have a higher anion-cation uptake ratio (Marschner, 1995). In the present study, there were no differences in all of the analyzed inorganic fractions between the rhizospheric and bulk soils at each harvest, even when soil Olsen-P was extremely low (1.38 mg kg^{-1}) (Fig. 4). The results implied that mobilization of sparingly soluble P in soils might play no role or a very minor role in maize adaptation to P deficiency.

CONCLUSIONS

Plants have diverse strategies to cope with P deficiency. In the field, maize plants responded to P deficiency by reducing P demand through slowing growth rate and increasing internal P use efficiency, and increasing P acquisition ability by root morphological alteration, especially by significantly higher growth rate of fine roots at key growth stages. No differences in analyzed inorganic P fractions between the rhizospheric and bulk soils with extremely low Olsen-P (1.38 mg kg^{-1}) suggested that fine root patterning allowed maize to gain access to easily available P from soil solution and contributes to balanced inorganic P distribution in soil.

REFERENCES

Arienzo, M., Di Meo, V., Adamo, P. and Violante, P. 2004. Investigation by electro-ultrafiltration on N and P distribution in rhizosphere and bulk soil of field-grown corn. *Aust. J. Soil Res.* **42**: 49–57.

Barber, S. A. 1995. Soil Nutrient Bioavailability: A Mechanistic Approach. 2nd Edition. John Wiley & Sons, New York.

Bertrand, I., Hinsinger, P., Jaillard, B. and Arvieu, J. C. 1999. Dynamics of phosphorus in the rhizosphere of maize and rape grown on synthetic, phosphated calcite and goethite. *Plant Soil.* **211**: 111–119.

Crafts-Brandner, S. J. 1992. Significance of leaf phosphorus remobilization in yield production in soybean. *Crop Sci.* **32**: 420–424.

Dinkelaker, B., Römheld, V. and Marschner, H. 1989. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). *Plant Cell Environ.* **12**: 285–292.

Foyer, C. and Spencer, C. 1986. The relationship between phosphate status and photosynthesis in leaves. Effects on intracellular orthophosphate distribution, photosynthesis and assimilate partitioning. *Planta.* **167**: 369–375.

Fredeen, A. L., Madhusudana Rao, I. and Terry, N. 1989. Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. *Plant Physiol.* **89**: 225–230.

Gardner, W. K., Parbery, D. G. and Barber, D. A. 1982. The acquisition of phosphorus by *Lupinus albus* L. II. The effect of varying phosphorus supply and soil type on some characteristics of the soil/root interface. *Plant Soil.* **68**: 33–41.

George, T. S., Gregory, P. J., Robinson, J. S. and Buresh, R. J. 2002. Changes in phosphorus concentrations and pH in the rhizosphere of some agroforestry and crop species. *Plant Soil.* **246**: 65–73.

Gu, Y. C. and Jiang, B. F. 1990. The fraction method for determining soil inorganic P in calcareous soils. *Soils* (in Chinese). **22**: 101–102.

Helal, H. M. 1990. Varietal differences in root phosphatase activity as related to the utilization of organic phosphates. *Plant Soil.* **123**: 161–163.

Helal, H. M. and Dressler, A. 1989. Mobilisation and turnover of soil phosphorus in the rhizosphere. *Z. Pflanzenern. Bodenk.* **152**: 175–180.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil.* **237**: 173–195.

Hinsinger, P., Plassard, C., Tang, C. and Jaillard, B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant Soil.* **248**: 43–59.

Jones, D. L. 1998. Organic acids in the rhizosphere—a critical review. *Plant Soil.* **205**: 25–44.

Lewis, D. G. and Quirk, J. P. 1967. Phosphate diffusion in soil and uptake by plants. II. P^{31}-movement and uptake by plants as indicated by P^{32}-autoradiography. *Plant Soil.* **26**: 445–453.

Li, K. P., Xu, Z. P., Zhang, K. W., Yang, A. F. and Zhang, J. R. 2006. Efficient production and characterization for maize inbred lines with low-phosphorus tolerance. *Plant Sci.* **172**: 255–264.

Linkohr, B. I., Williamson, L. C., Fitter, A. H. and Leyser, H. M. O. 2002. Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. *Plant J.* **29**: 751–760.

Liu, Y., Mi, G. H., Chen, F. J., Zhang, J. H. and Zhang, F. S. 2004. Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Sci.* **167**: 217–223.

Lu, R. K. 2003. The phosphorus level of soil and environmental protection of water body. *Phosphate Comp. Fert.* (in Chinese). **18**: 4–8.

Lynch, J. P. 1995. Root architecture and plant productivity. *Plant Physiol.* **109**: 7–13.

Lynch, J. P. and Brown, K. M. 2001. Topsoil foraging—an architectural adaptation to low phosphorus availability. *Plant Soil.* **237**: 225–237.

Lynch, J. P. and Deikman, J. 1998. Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic, and ecosystem processes. *In* Lynch, J. P. and Deikman, J. (eds.) Current Topics in Plant Physiology: An American Society of Plant Physiologists Series. American Society of Plant Physiologists, Rockville. p. 19.

Marschener, H. 1998. Role of root growth, arbuscular mycorrhiza, and root exudates for the efficiency in nutrient acquisition. *Field Crop. Res.* **56**: 203–207.

Marschner, P. 1995. Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, London.

Mollier, A. and Pellerin, S. 1999. Maize root system growth and development as influenced by phosphorus deficiency. *J. Exp. Bot.* **50**: 487–497.

Murphy, J. and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* **27**: 31–36.

Neumann, G. and Martinoia, E. 2002. Cluster roots—an underground adaptation for survival in extreme environments. *Trends Plant Sci.* **7**: 162–167.

Neumann, G., Massonneau, A., Martinoia, E. and Römheld, V. 1999. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. *Planta.* **208**: 373–382.

Neumann, G. and Römheld, V. 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil.* **211**: 121–130.

Niu, J. F., Peng, Y. F., Li, C. J. and Zhang, F. S. 2010. Changes in root length at the reproductive stage of maize plants grown in the field and quartz sand. *J. Plant Nutr. Soil Sci.* **173**: 306–314.

Peng, Z. P. and Li, C. J. 2005. Transport and partitioning of phosphorus in wheat as affected by P withdrawal during flag-leaf expansion. *Plant Soil.* **268**: 1–11.

Plénet, D., Etchebest, S., Mollier, A. and Pellerin, S. 2000a. Growth analysis of maize field crops under phosphorus deficiency. I. Leaf growth. *Plant Soil.* **223**: 117–130.

Plénet, D., Mollier, A. and Pellerin, S. 2000b. Growth analysis of maize field crops under phosphorus deficiency. II. Radiation-use efficiency, biomass accumulation and yield components. *Plant Soil.* **224**: 259–272.

Shane, M. W. and Lambers, H. 2005. Cluster roots: a curiosity in context. *Plant Soil.* **274**: 101–125.

Soon, Y. K. and Kalra, Y. P. 1995. A comparison of plant tissue digestion methods for nitrogen and phosphorus analyses. *Can. J. Soil Sci.* **75**: 243–245.

Usuda, H. and Shimogawara, K. 1991. Phosphate deficiency in maize. I. Leaf phosphate status, growth, photosynthesis and carbon partitioning. *Plant Cell Physiol.* **32**: 497–504.

Wells, C. E. and Eissenstat, D. M. 2003. Beyond the roots of young seedlings: the influence of age and order on fine root physiology. *J. Plant Growth Regul.* **21**: 324–334.

Yan, H. F., Li, K., Ding, H., Liao, C. S., Li, X. X., Yuan, L. X. and Li, C. J. 2011. Root morphological and proteomic responses to growth restriction in maize plants supplied with sufficient N. *J. Plant Physiol.* **168**: 1067–1075.

Zhu, J. M., Kaeppler, S. M. and Lynch, J. P. 2005. Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). *Funct. Plant Biol.* **32**: 749–762.

Zhu, J. M. and Lynch, J. P. 2004. The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays*) seedlings. *Funct. Plant Biol.* **31**: 949–958.