Communications in Soil Science and Plant Analysis

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcss20

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Published online: 04 Sep 2007.

To cite this article: Guodong Liu , James Dunlop , Thai Phung & Yuncong Li (2007) Physiological Responses of Wheat Phosphorus-Efficient and -Inefficient Genotypes in Field and Effects of Mixing Other Nutrients on Mobilization of Insoluble Phosphates in Hydroponics, Communications in Soil Science and Plant Analysis, 38:15-16, 2239-2256, DOI: 10.1080/00103620701549249

To link to this article: http://dx.doi.org/10.1080/00103620701549249

Communications in Soil Science and Plant Analysis, 38: 2239–2256, 2007

ISSN 0010-3624 print/1532-2416 online
DOI: 10.1080/00103620701549249

Physiological Responses of Wheat Phosphorus-Efficient and -Inefficient Genotypes in Field and Effects of Mixing Other Nutrients on Mobilization of Insoluble Phosphates in Hydroponics

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Abstract: To elucidate the mechanism of mobilization of insoluble phosphates, a field trial and a split root experiment in a growth chamber were conducted. The results showed that a phosphorus (P)-efficient wheat genotype, Yanzhong 144 (YZ), transpired 50% less than a P-inefficient genotype, 80-55. At the grain-filling stage, the free water content in YZ's rhizosphere was 2.4 times of that of 80-55's. When either tricalcium phosphate [$Ca_3(PO_4)_2$] or ferric phosphate ($FePO_4$) were combined with the other nutrients in the same solution, total dry-matter production was 140% greater for $Ca_3(PO_4)_2$ and 60% greater for $FePO_4$ than when these P sources were each supplied alone to one half of the root system while the other nutrients were supplied to the other half. The excess absorption of cations over anions by the roots facilitated

Received 13 September 2006, Accepted 22 October 2006
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mobilization of insoluble phosphates. The P-efficient genotype was also water efficient, and the insoluble phosphates TCP and FP were phyto-available in water culture.

Keywords: Biomass, drought stress, low-P stress, P-efficient genotypes, *Triticum aestivum* L., water content in rhizosphere

INTRODUCTION

Worldwide, at least 45% of the total agricultural land area, some 5.8 billion hectares, suffers from phosphorus (P) deficiency (Batjes 1997; Gaume 2000; Liu et al. 2001). Phosphate fertilizer application remains the most effective way to increase crop productivity in soils with low levels of plant-bioavailable phosphates (Narang and Altmann 2001). Regrettably, utilization efficiencies of phosphorus are still very low in spite of decades of investigations into the physiology and molecular biology of mechanisms of P-uptake, concerted genetic analyses, breeding, and selection (Raghothama 1999). The mechanisms of efficient use of phosphates remain unclear.

Tragically, much of phosphate fertilizer not used by crop plants is borne into lakes and rivers to pollute these waters. China annually consumes an average of US$3.5 billion worth of phosphate fertilizers, of which US$1.0 billion has been imported each year since 1990 (Liu 2002). Because of inefficient phosphate uptake by crops and runoff caused by seasonal torrential rains, Tai Lake in Jiangsu Province and Dianchi Lake in Yunnan Province are severely eutrophicated. Residents in the vicinity of such polluted lakes rely on them for their supply of drinking water, which poses risks to human health. Moreover, at current rates of use, the known deposits of phosphate rock are projected to be sufficient for only another 60–90 years (Plaxton and Carswell 1999; Raghothama 1999).

Nevertheless, the majority of P-deficient soils contain large amounts of P that cannot be extracted from the soil by extant crop varieties to the extent required for vigorous growth. For instance, in the north of China, the total P concentrations in soils are as much as 200 to 500 times higher than the P concentrations considered to be bioavailable (Li et al. 1995). Because the monetary and environmental costs of phosphate-fertilization are rather high, increased effort has been devoted in recent decades to genotype screening and varietal improvement for enhanced P efficiency (Gerloff and Gabelman 1983; Narang and Altmann 2001).

Another huge long-standing challenge facing agriculture is water stress. Of China's farmlands, only 52.1 million hectares are irrigated, whereas 72.8 million hectares are rainfed (Shi and Lu 2001). China's per capita water resource is 2300 m^3, thus being only a quarter of the world level. China uses about 560 billion m^3 of water per year, of which about 70% is allocated to agriculture, still leaving it with a shortfall of at least 30 billion m^3 (Shan, Wu, and Kang 2004). This water stress serves to worsen the phosphate availability in

farmlands and compounds the severe limits on crop production imposed by P deficiency (Liu, Li, and Li 1997). The mobilization of scarcely soluble phosphates is of immense agricultural and economic significance.

To better understand the mechanisms of mobilization of the sparingly soluble phosphates and the relationship between phosphorus efficiency and water efficiency of wheat, *Triticum aestivum* L., the objectives of this research were (1) to quantify the differences in the transpiration capacities and in the water contents in the soils of the rhizospheres of both P-efficient and P-inefficient wheat genotypes, (2) to determine the effects of nutrient interactions on pH and on the modification of solubility of sparsely soluble phosphates in culture solutions using a typical P-efficient genotype, Yanzhong 144 (YZ), and (3) to elucidate the crop's underlying mechanisms of phosphorus efficiency.

MATERIALS AND METHODS

Wheat Genotypes

A typical P-efficient wheat genotype, Yanzhong 144 (YZ), and a P-inefficient one, 80−55, were utilized in this experiment. They had also been employed in previous studies (Liu 1995, Li et al. 1995).

Field Experiment

Both typical wheat genotypes were grown at the experiment farm of CAAS, Beijing, China. At the grain-filling stage, the rhizosphere soil of the two wheat genotypes was collected. Soil from the space surrounding the roots of the plant was removed. The fine soil in which the roots were growing was considered the rhizosphere soil of the genotypes. This rhizosphere soil was sampled and analyzed for free water content. The initial weight of each sample was determined. Each sample was dried at room temperature for 4 weeks, and each week the sample was weighed to determine the amount of water loss. After the third week, the samples did not lose any additional weight. At this time, the samples were put into an oven at 80°C for 48 h, and weighed once more. The loss in weight from oven-drying was considered to be the bound water content of the sample. The free water content of each sample was considered to be the difference between the total water content and the bound water content. The soil devoid of growing plants served as the control (CK) in the same plot.

Number of Open Stomatal Apertures

Five flag leaves of each wheat genotype were collected from our experimental farm. To restrict the influence of fluctuations of temperature, humidity, and

light on stomatal status, the leaves were sealed in Ziploc® bags and kept on ice or in a refrigerator until the numbers of open stomata had been determined. Twenty μl of anhydrous ethanol was delivered over the abaxial epidermis of the flag leaf under a light microscope (model BH2, Olympus Co., Tokyo, Japan). Three seconds later, the transparent spots were recorded as the number of open stomata per 40 × field in each of five such fields in each leaf (SAC and NWAC 1980). Stomata/mm^2 were calculated using calibration factors determined for the microscope (Malone et al. 1993).

Wax Contents

Five flag leaves of each wheat genotype were collected from the field of our experimental farm. The leaves were sealed in Ziploc® bags and kept on ice or in a refrigerator until examined. The wax contents of the flag leaves were analyzed using the method of Kumar and Sridhar (1987).

Split Root Experiment

Seeds of YZ were soaked for 24 h in MilliQ water in Petri® dishes (each 9 cm in diameter), germinated, and grown for 3 days at 25°C in a growth chamber, when the root length had attained about 5 cm. Thirty-six pots (each with a volume of 1200 mL) were assembled in 18 pairs. One seedling was planted in each pair of pots. For this purpose, 18 uniform and healthy seedlings were chosen, their primary roots were arranged into essentially two equal parts, and each part was inserted into one of a pair of pots. The seedlings were stabilized with plastic foam board, which covered the openings of the pair of pots. The pots in each pair were kept about 5 mm apart from each other to avoid contamination.

Each wheat seedling was grown hydroponically in 2400 mL of culture solution (1200 mL per pot) for 3 weeks, as previously reported (Liu et al., 1998). One mL of nutrient stock solution was added to the left-side pot of each pair of pots on day 1, 8, and 15. Sodium dihydrogen phosphate (SDP) was the soluble P source added to the leftside for the CK. Tricalcium phosphate (TCP) and ferric phosphate (FP) were the scarcely soluble phosphate compounds used for the treatments. One extra mM calcium (Ca^{2+}) as calcium chloride ($CaCl_2$) was always added with TCP to further reduce the solubility of TCP. There were six treatments including the CK as shown in Table 1. On day 21 all the seedlings were dried for biomass measurement and then submitted for P analysis.

Phosphorus and pH Analysis

Plant materials were digested using the wet method, and the P-level was measured colorimetrically (Shimogawara and Usuda 1995) with a

Table 1. Exposure of the two halves of the root system of a wheat seedling to nutrients, sources of *P*, or water in each treatment of the split-root experiment

Treatment	Left pot content(s)	Right pot content(s)
Control (CK)	CNS[a]	H_2O
TR1	CNS-P[b] + FP[c]	H_2O
TR2	CNS-P	H_2O + FP
TR3	CNS-P + TCP[d]	H_2O
TR4	CNS-P	H_2O + TCP
TR5	CNS-P	H_2O

[a]Complete nutrient solution.
[b]Complete nutrient solution except that P is lacking
[c]Ferric phosphate.
[d]Tri-calcium phosphate.

spectrophotometer (model DU 640, Beckman Instruments Inc. USA). The culture solution was sampled for P analysis on day 3 after the third addition of nutrient solution. The pH in the culture solutions was measured with Metrohm titroprocessor. 672 (Metrohm A6, Herisav, Switzerland) on day 21.

All the measurements were replicated three times unless stated otherwise.

Statistical Analysis

The Statistical Analysis System (SAS) package (version 9.1) developed by SAS Institute, Inc. (SAS 2006) was used to perform the statistical analyses of the data. The data were tested by Duncan's multiple range test (DMRT) with a statistical significance of $P \leq 0.05$. The critical range of $P \leq 0.05$ together with two means ($CR_{0.05,\ 2}$) are presented in Figures 1–8.

RESULTS

Differences in Rhizosphere Soil Moisture Levels Associated with the Two Wheat Genotypes

The free water (FW) and total water (TW) contents were significantly different in the rhizosphere soils of the plants at the grain-filling stage. The FW content of the soil devoid of plants in the same experimental plot was 93.4% two days after irrigation, whereas that in the rhizosphere of the P-efficient genotype, YZ, was 73.3%, but that in the rhizosphere of the P-inefficient genotype, 80-55, was only 31.0% (Figure 1). The FW content in the rhizosphere of YZ was as much as 236% of that in the rhizosphere of 80-55. Indeed the

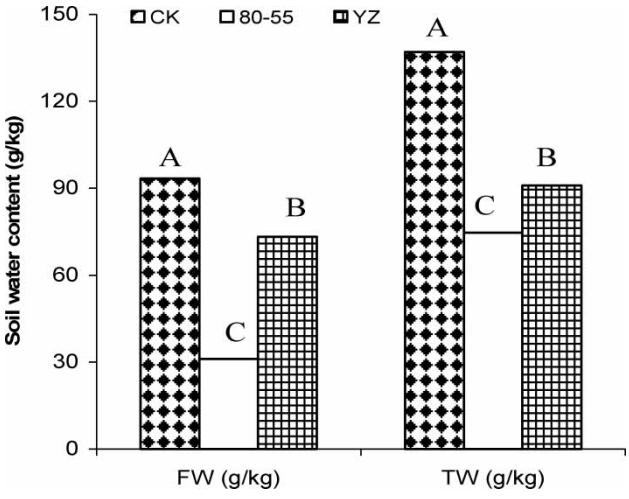

Figure 1. Free water (FW) and total water (TW) contents in the rhizospheres of Yanzhong 144 (YZ) and 80-55 at the grain-filling stage in the field. The CK was bare soil. Means (n = 3); vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 6.1 (g/kg) for free water content and 8.1 (g/kg) for total water content in rhizosphere soils.

rhizosphere soil of YZ was wet and could be manually rolled into balls, but that of 80-55 was dry and dusty. Clearly 80-55 was suffering from drought while growing in the same experimental plot beside YZ, which had ample moisture in its rhizosphere.

Transpiration rates (TR) of the tested seedlings were significantly different between the P-efficient and P-inefficient genotypes at the seedling stage at room temperature. The transpiration rate of 80-55 was 60% greater

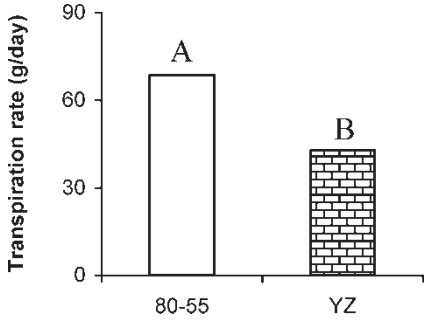

Figure 2. Genotypic differences in the transpiration rate (TR) at day 21 of wheat seedlings grown in hydroponics. Vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 21.8 (g/day).

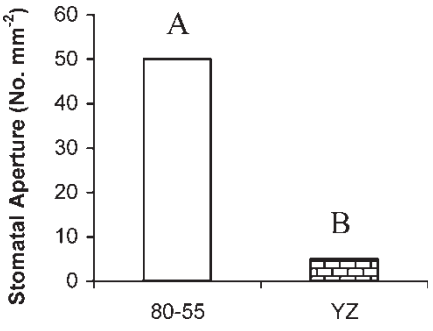

Figure 3. Genotypic differences in the stomata aperture on abaxial surface of flag leaves. Vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 2.3 (No. mm^{-2}).

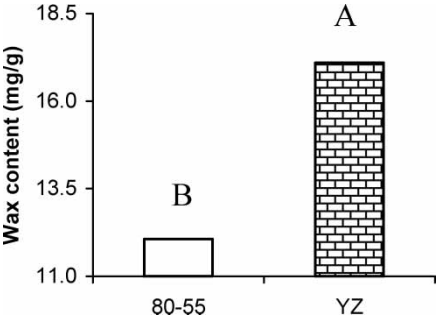

Figure 4. Genotypic difference in cuticular wax on the flag leaves of 80-55 and YZ. Vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 1.1 (mg/g).

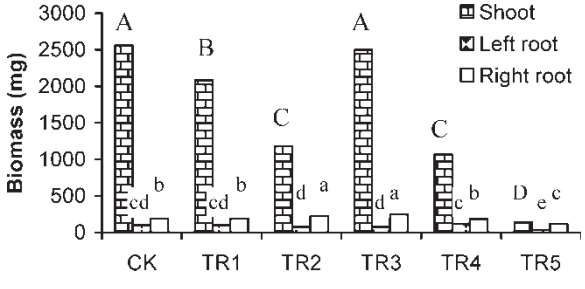

Figure 5. Biomass of the shoot and of the left and right halves of the root systems per single seedling at 21 days of hydroponic culture in a split-root experiment. Bars for shoots or roots having the same upper case for shoots or lower for roots case letter are not statistically significant at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 373.0 mg for shoots and 24.9 for roots, respectively.

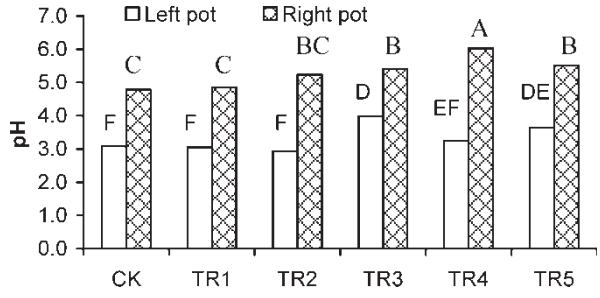

Figure 6. Differences in pH in the culture solutions of various treatments on day 21. Vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is pH 0.47.

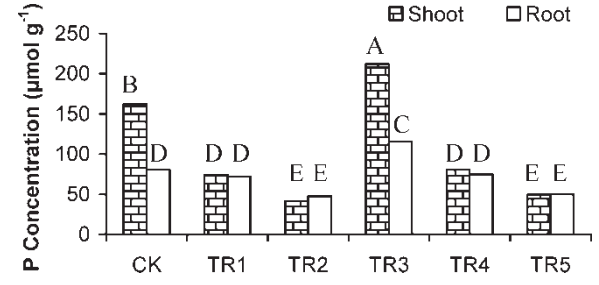

Figure 7. P content (μmol/g) in wheat shoots and roots. The roots used for P determination were from the half of the split root system, which had no P in the culture solution. Vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 16.7 μmol g^{-1}.

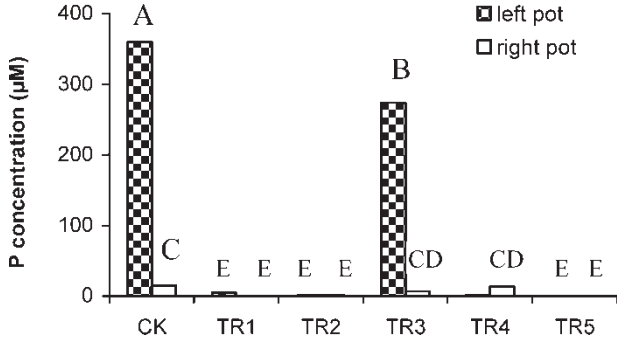

Figure 8. P concentration (μM) in the culture medium in each pot containing half of the split wheat root system in the various treatments. Vertical bars not followed by the same letter are significantly different at $P \leq 0.05$ by DMRT. $CR_{0.05,2}$ is 7.2 μm g^{-1}.

than that of YZ. A single 80-55 plant transpired about 26 g water per day more than an YZ plant (Figure 2). This excessive transpiration by 80-55 dried the soil and induced water-deficit stress. Under low-P stress, excessive transpiration might induce drought stress and thereby further exacerbate low-P stress. This genotypic difference could attribute to the characteristics of the leaves of each genotype such as stomatal aperture and wax contents on the leaves.

Because transpiration is done basically through the stomata and the cuticle, the density of the open stomata and the thickness of the cuticular wax layer determine the transpiration rate (Lambers, Chapin, and Pons 1998). The density of open stomata in 80-55 was 10-fold greater than in YZ (Figure 3), whereas the wax content of 80-55 leaves was 41% less than that of YZ (Figure 4). This showed that P-efficient genotype, YZ, was distinctly water efficient. The water efficiency was an intrinsic characteristic of YZ that ensured its high efficiency in P utilization. In contrast, 80-55 used water inefficiently and thereby also used P inefficiently.

Insoluble Phosphate is Phyto-available in Hydroponics

As shown in Figure 5, the amount of shoot biomass of the wheat seedling from TR5 was exceeded by that produced in the other treatments as follows: CK, 20-fold; TR3, 19.6-fold; TR1, 16.3-fold; TR4, 9.1-fold, and TR-2, 8.3-fold. These data indicate that the P from both of the sparingly soluble phosphate compounds is somewhat bioavailable to the plants when these compounds are mixed together with the other nutrients. Actually, TCP is sufficiently bioavailable to support plant growth and development in hydroponics. In this study, 1 mM of extra calcium as $CaCl_2$ was added to further decrease the solubility of TCP, but even so, the shoot biomass in TR3 was almost as great as that of the CK. However, based on the amount of shoot biomass produced in TR2 and TR4 compared to the CK, the bioavailability of both FP and TCP was suppressed between 44% and 58% when the phosphates were provided separately from the other nutrients. Even though exactly the same quantities of nutrients were supplied to the plant roots, the shoot biomass differed greatly depending on whether the scarcely soluble phosphate was mixed together with the other nutrients or provided separately from them. Thus the amount of shoot biomass in TR1 was 78.5% greater than in TR2, and the shoot biomass of TR3 was 136% greater than in TR4 (Figure 5).

Morphologically, the right halves of the root systems in the CK and all other treatments were always more developed than the left halves (Figure 5) because the right halves of the root systems were suffering from nutrient deficiencies. This implies that the two halves of the root systems did not share their information on nutrient status or that the plant was unable to respond effectively to such information. Also, TR5, which received absolutely no P, had the longest roots, which suggests that they were programmed to grow until they encountered an adequate source of bioavailable P and other nutrients.

When the scarcely soluble phosphate compounds were mixed together in the same pot with the other nutrient compounds, it appeared that the mixture facilitated the dissociation of P from the insoluble phosphate compound. The extent of such dissociation is determined by the magnitude of the imbalance between cations and anions derived from the nutrient salts. This was substantiated by the fact that the difference in pH between the two pots of a given pair differed by as much as 2.8 units, as occurred with TR4. Also in TR1 and TR3, the pots containing the scarcely soluble P compound together with all the other nutrients had a markedly lower pH than the pots containing only TCP (Figure 6). In fact, pH in the left pots was always significantly lower than that in the right pots because the left pots had all the other nutrients with either soluble or insoluble phosphate or without any phosphate, but the right pots had only water with or without insoluble phosphate. Plants usually take up three times more cations than anions from the growth medium (Liu 1990), and this differential uptake depresses the pH around roots by a few units (Figure 6).

Phosphorus Level in Plant Parts and Culture Media

Phosphorus analysis showed that greatest P contents in both shoots and roots occurred in TR3, in which TCP was mixed together with the other nutrients in the left pot (Figure 7). The P concentration in either shoots or roots grown in TR3 was significantly greater than that in either shoots or roots grown in the CK. This demonstrates that TCP could provide even more bioavailable P to the plants than the CNS used in the CK, because TCP was able to form a P-nutrient-buffer system when it was mixed with the nutrient solution. Yet, soluble phosphate as in the CK could not do that. Therefore, the P contents of wheat shoot and root tissues from TR3 were 2.3- to 4.3-fold greater than those from TR5, but the P contents from the CK were only 1.6- to 3.3-fold greater than those from TR5, even though the level of instantly bioavailable P in the CK culture solution was higher than in TR3 based on measurements made on the third day after the third addition of the nutrients. The P concentration in shoots was significantly greater than that in roots in both of the CK and TR3 in which the seedlings had sufficient bioavailable P. However, there was not any statistical difference in P concentration between the shoots and roots grown in TR1, TR2, TR4, and TR5 because the seedlings in those treatments were suffering from low-P stress (Figure 7). Additionally, the level of bioavailable P in the right pot of the CK was 15.1 μM (Figure 8), even though no phosphate had been supplied to that pot.

Similarly, 6.87 μM of P occurred in the right pot of TR3 (Figure 8), which initially contained only distilled water. This means that P was transferred from the roots in the left pot to those in the right pot. Such transfer is ecologically significant in crop production because it suggests a mechanism whereby intercropping or rotation of crops may be especially beneficial to agriculture in

low-P farmlands. Figure 8 illustrates how the ionic composition of the medium can drastically alter the amount of bioavailable P released from insoluble phosphates. Depending on the ionic composition of the medium containing the scarcely soluble phosphate, TCP, it could be induced to release up to 20-fold more bioavailable P in 21 days than that provided by the seeds in TR5, which lacked exogenous P. This is illustrated with the data obtained from TR3 and TR4 in which TCP was the scarcely soluble phosphate. On the other hand, when ferric phosphate served as the scarcely soluble phosphate, only 6-fold more bioavailable P was released than that in TR5, as can be seen by examining the results of TR1 and TR2. Consequently, the total biomass differed 140% between TR3 and TR4 when 1 mM of extra Ca^{2+} was added to the TCP in TR3 and TR4, but it differed only 60% between TR1 and TR2 when FP was added to TR1 and TR2.

DISCUSSION

Saving Water Saves P Fertilizers

Dilution, as mentioned later, is an important mechanism for mobilizing scarcely soluble phosphate. For instance, the K_{sp} of the scarcely soluble phosphate, TCP, is only 2.07×10^{-33}, at $pH = pK_{a2} = 7.21$, so the total amount of bioavailable P is 51.4 μM. This concentration is about 240-fold greater than the C_{min} of lettuce and 1280-fold greater than the C_{min} of soybean (Barber 1995). In contrast, 1 L of water can dissolve and release 4.38 mg of P_2O_5. This amount of bioavailable P can produce 365 mg of wheat grain (www.spur-ventures.com/fertilizerinfo31.html 2003). In fact, 1 L of water can release much more bioavailable phosphate than 4.38 mg because TCP is somewhat insoluble and can continue to release phosphate as the P and the Ca^{+2} are taken up by the plants.

Clearly, the amount of bioavailable P provided can be doubled if the bioavailability of water is also doubled. Actually, the bioavailability of P is tightly controlled, and this tends to maintain the water balance between crop plants and their environment. Crop plants can garner more bioavailable P from the soil if they can use the water sparingly and thereby maintain a moist rhizosphere as was done by YZ (Figure 1), but they will suffer both P deficiency and water stress if they transpire excessively and thereby dry the soil, as exemplified by 80-55 (Figure 1). Crop plants can extract sufficient bioavailable P if the amount of bioavailable water in the rhizosphere is sufficient. For example, hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$, one of the most insoluble phosphates, has a K_{sp} of merely 1.51×10^{-112} and releases 10^{-5} M of bioavailable P at pH 6.5 and at a calcium concentration of 10^{-3} M (Sample, Super, and Racz 1980). Nevertheless, this P concentration is 1 or 3 orders of magnitude greater than the C_{min} in soil or in hydroponics (Marschner 1986).

The FP is even more insoluble than TCP. Indeed, FP is considered to be insoluble because its K_{sp} is merely 1.3×10^{-22}. Thus, FP is generally thought not to be bioavailable to plants, but "insoluble" does not mean that the phosphate compound does not dissolve at all, and given the K_{sp}, the free concentrations of PO_4^{3-} and iron (Fe^{3+}) would be 1.14×10^{-11} M in solution. According to Barber (1995), the C_{min} for P is between 4×10^{-8} M for soybean and 2.4×10^{-7} M for lettuce. Although the free level of phosphate (PO_4^{3-}) is lower than the C_{min}, PO_4^{3-}, the compound will dissociate and equilibrate with HPO_4^{2-} and $H_2PO_4^-$ in a neutral solution with water containing protons. These two ions will be present at concentrations 5.66×10^5 times higher than the free PO_4^{3-} at $pK_{a2} = pH\ 7.21$. At this pH, both HPO_4^{2-} and $H_2PO_4^-$ concentrations are equal, and the ratio of H_3PO_4/PO_4^{3-} is 2.35 based on the following formulae (Hargis 1988):

$$\alpha = \frac{[PO_4^{3-}]_{free}}{[PO_4^{3-}]_{total}} = \frac{[PO_4^{3-}]_{free}}{\{[PO_4^{3-}]_{free} + [HPO_4^{2-}]_{free} + [H_2PO_4^-]_{free} + [H_3PO_4]_{free}\}}$$

$$\frac{1}{\alpha} = 1 + \frac{[H^+]}{k_{a3}} + \frac{[H^+]^2}{k_{a2}k_{a3}} + \frac{[H^+]^3}{k_{a1}k_{a2}k_{a3}}$$

where α is the fraction of free phosphate to total phosphate and k_{a1}, k_{a2}, and k_{a3} are the dissociation constants of orthophosphoric acid. This means that the total available phosphate can be 6.45×10^{-6} M, and that is about 30-fold higher than the P C_{min} of lettuce and 160-fold higher than the P C_{min} of soybean (Barber 1995). In reality, both the bioavailabilities of phosphate and water are very closely related as follows:

$$FePO_4(s) + H_2O(l) \underset{\text{Dehydration(e.g.transpiration)}}{\overset{\text{Hydration(e.g.rainfall)}}{\rightleftharpoons}} PO_4^{3-}(aq) + Fe^{3+}(aq) \qquad (1)$$

$$Fe^{3+}(aq) \underset{\text{Dehydration(e.g.transpiration)}}{\overset{\text{Hydration(e.g.rainfall)}}{\rightleftharpoons}} Fe^{2+}(aq) \qquad (2)$$

Therefore, crop plants should be able to utilize P from the soil solution where the P is in the form of $FePO_4$. The soil moisture content limits this salt's bioavailability. Irrigation or rainfall can shift the reaction in Eq. (1) to the right side, so that more phosphate is released. Free water also aids both crop plants and microorganisms in taking up more phosphate and ferrous ions. Moreover, free water facilitates the release of exudates from roots (Bar-Yosef 1996) and/or microorganisms, which, in turn, may improve the solubility of the insolvble phosphate. Because both irrigation and rainfall usually are quite limited, low transpiration by crops such as YZ would preserve high rhizosphere water concentrations and thereby increase rhizosphere P bioavailability from the insoluble mineral salts. In contrast, high transpiration (Figure 2) by crops, such as 80-55, that have a high density of open stomata and low wax content of leaves (Figures 3 and 4) would deplete the rhizosphere water concentrations and thereby limit the release of rhizosphere

P bioavailability from the insoluble mineral salts. The dearth of water limits not only the amount of P released from soil minerals but also the rate of P uptake from applied phosphate fertilizers by facilitating precipitation of P salts. Also, we must consider that $FePO_4$ is a salt with a variable valence cation. High moisture in soils favors reduction and hence the conversion of Fe^{3+} to Fe^{2+} [Eq. (2)], thereby favoring the continued solubilization of the $FePO_4$ salt [Eq. (1)]. Plants that consume less water (water-efficient varieties) would therefore be expected to maintain rhizosphere water levels that would also favor the solubilization of P salts in the soil. This scheme is supported by other experiments where we tested the bioavailability of P from insoluble P sources. We used unprocessed swine (*Sus scrofa* [L.]) bone as the only P source to grow a P-inefficient wheat, Jingsong 5, in hydroponics, in which it produced a slightly greater grain yield than the control (Liu 1995). This is despite the fact that the P in bone in different circumstances is basically nonavailable to plants. Actually, Ho and Lynch (2005) and Ho, McCannon, and Lynch (2004) reported that there is a close relationship between water and P acquisition.

Imbalance among Cations and Anions, pH, and P-Mobilizations

In chemical equilibria of insoluble phosphate compounds in water, either the K_{sp} (the solubility product is the ionic product when the system is at equilibrium) or the Q_{sp} (the ionic product is simply a measure of the ions present in the solvent) determines the extent of P mobilization in sparingly soluble phosphates or the extent of immobilization of soluble phosphates. Insoluble phosphates can be mobilized if $Q_{sp} < K_{sp}$, but soluble phosphates will be immobilized if $Q_{sp} > K_{sp}$. To mobilize insoluble phosphate, it is essential to reduce Q_{sp}. There are at least six ways to reduce Q_{sp}: (1) dilution by increasing the bioavailability of water through irrigation or rain, (2) acidification in calcareous soils by the application of physiological acid fertilizers such as ammonium sulfate $[(NH_4)_2SO_4]$ or potassium chloride (KCl) and organic compounds such as citric acid excreted from roots, because organic acids or even carbon dioxide (CO_2) can convert PO_4^{3-} to HPO_4^{2-}, and HPO_4^{-2} to $H_2PO_4^{1-}$; (3) reduction of companion cations such as Fe(III) reduced to Fe(II) [$FePO_4$ is insoluble with a K_{sp} of only 10^{-22} but $Fe_3(PO_4)_2$ is completely soluble]; (4) chelation of companion cations (e.g., citrate excreted from roots can chelate Fe^{3+} and thus PO_4^{-3} can be released as a free or soluble form; (5) formation of a more insoluble salt of the companion cations and some other anions, (e.g., the K_{sp} of $FePO_4$ is 10^{-22} but the K_{sp} of $Fe(OH)_3$ is only 10^{-39}) and therefore, the activity of Fe(III) can be reduced 1000-fold and that of P increased 1000-fold when the pH in acid soil is increased one unit by liming; and (6) consumption or uptake of phosphate anions and companion cations by plants or other organisms. Acidification is one of the primary mechanisms to mobilize the insoluble phosphates in soils, because

the lower the pH is depressed the more bioavailable P is released from sparingly soluble phosphate compounds.

The essential elements for plant growth and development include some in the form of cations and others as anions. Of the macroelements (nitrogen, P potassium, calcium, magnesium, and sulfur), the three metal nutrients, are cations. Phosphorus and sulfur are always anionic nutrients, but nitrogen is redox status dependent. Its reduced form, ammonium, is cationic, but its oxidized form, nitrate, is anionic. Hence, the form of nitrogen fertilizer determines the ratio between cations and anions taken up by the plants. Based on molarity, the amount of P a plant requires is only 48% as much as of calcium or only 75% as much as of magnesium, even though P is only slightly less important than nitrogen, which is the most important nutrient (Salisbury and Ross 1979). Sulfur uptake by plants is much less than that of potassium.

Because plants take up about 3-fold more cationic nutrients than anionic nutrients, the pH of a hydroponics medium will decline if the ratio of ammonium and nitrate is not suitable (Liu 1990). These imbalances between cations and anions could drive the pH value in nutrient solutions down to about pH 3 (Figure 7), and they are helpful in mobilizing insoluble phosphates. However, if TCP is the only P source in the solution, TCP could buffer the pH at about 6 to 7, because TCP can consume many protons and thereby prevent the pH of the medium from declining further.

Generally, ammonium nitrate is a favorite nitrogen source of growers because it provides equal amounts of nitrogen as cations and anions. However, equal amounts of ammonium and nitrate ions are not optimal for hydroponics or aeroponics because at this ratio the solution pH still trends downward. Consequently, both in hydroponics and aeroponics systems, the culture solutions have to be changed frequently to control the pH. Therefore, some researchers would use $CaCO_3$ (Wych and Rains 1979) or 4-morpholineethanesulfonic acid (MES) (Metwally et al. 2003) to buffer the solution pH for hydroponics. However, according to our understanding, it is unnecessary to use any special products to control the pH of the medium. The pH of a culture solution can be kept very stable if we supplement the ammonium nitrate with another nitrate such as sodium nitrate to increase the nitrate–ammonium ratio to more than 50%, for example, up to about 80% or to make a 4:1 ratio. With a more favorable ratio, there is no need to change solution at frequent intervals.

Obviously, the pH of a growth medium is vulnerable to decline when an imbalance exists between the cations and the anions. This study showed that the pH in the left pot was always lower than that in the right pots (Figure 6) because there was an imbalance between cations and anions from the culture solution. However, the pH will neither decline nor increase if there are not enough cationic nutrients to prevent the plants from depleting them in the growth medium (Liu, Dunlop, and Phung 2006). Depletion of cationic nutrients often happens in the farmlands of south of China because

these soils are not only deficient in phosphate but also in potassium, calcium, etc. Yet the insoluble phosphates in these soils are about 500-fold more concentrated than the bioavailable phosphate (Li et al. 1995). Hence, the application of cationic fertilizers such as lime or use of potash fertilizers can create a favorable imbalance between cations and anions and thereby serve to mobilize P from the abundant but scarcely soluble phosphates already present in the soil.

Phosphorus could Transfer and Release Cross Roots in Various Pots

Phosphorus does not move readily in soils. However, P can be absorbed by one root and transferred to another root and released into its surroundings of the latter. Indeed, this was demonstrated in the CK and TR3 (Figure 8) in which the left halves of the root systems were bathed in a P-rich medium, while the right halves were bathed in distilled water. In these cases, a considerable amount of P was transferred by the plant into the distilled water on the right side. This may be very significant in the ecology of crop production, because plants with some roots with access to sufficient bioavailable P would transfer some P to roots deprived of it and then release P into the P-deficient environment. This may mean that two different but related P-efficient genotypes or even different P-efficient species are able to share the bioavailable phosphate when they are intercropped. Actually, in another experiment, Dunlop and Phung (2000) found that rye, *Secale cereale* L ssp. *cereale*, grown together with white clover, *Trifolium repens* L., enabled the latter to take up more bioavailable P from environment than when the white clover was grown in a pure stand. This may in part explain why intercropping (Zhang et al. 2003) or crop rotation may result in greater harvest yields.

CONCLUSIONS

These experiments suggest that there is a strong correlation between P efficiency and water-use efficiency, at least in some wheat cultigens. As long as there are appropriate levels of water in the rhizosphere, P can be solublized and made bioavailable to plants from sources considered essentially unavailable to plants (such as TCP or FP). Therefore, water-use efficiency is a prerequisite of P-utilization efficiency. Also a P-efficient genotype, YZ, was water-use efficient, and this suggests that water-use efficiency and P efficiency may occur together in the same genotype. To enhance the utilization of scarce agricultural resources, emphasis should be placed on combining research on P efficiency and water-use efficiency, because this may lead to major advances in agricultural productivity. The split-root experiment showed a scarcity imbalances between cations and anions in the growth medium can suppress the mobilization of bioavailable P to such an extent that shoot biomass

produced is diminished by 44% to 58%. This experiment demonstrated that other nutrients such as potassium, calcium, and magnesium play important roles in the mobilization of insoluble phosphates. In addition, this experiment demonstrated that the bioavailable or mobilized P could be transferred from the roots with sufficient P to roots suffering low-P stress and then released from the latter to the low-P growth medium. This may be very significant in the ecology of crop production and explain how intercropping produces, yield increases.

ACKNOWLEDGMENTS

This research was financially supported by National Natural Science Foundation of China through grant numbers 30270785 and 39770479 and by 948 Project Office, Ministry of Agriculture, China, through grant number 201068. The authors cordially thank Professor Waldemar Klassen for his invaluable comments and suggestions.

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