Effects of soil water on maize root morphological and physiological responses to phosphorus supply

Ai Zhan1,2, Xinping Chen2, and Shiqing Li1*

1 State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi, 712100, China
2 College of Resource and Environment, China Agricultural University, Beijing, 100193, China

Abstract

Water shortage directly constrains plant growth and survival, and indirectly influences plant responses to soil mineral nutrients, especially low-mobility nutrients such as phosphorus (P). We examined the effects of soil water content on the responses of agronomic and root morphological and physiological traits of maize (Zea mays L. cv. NE15) to P supply. Seven P supply levels (0, 12.5, 25, 50, 75, 100, and 300 mg P kg−1 soil) and two water regimes (well watered, WW; water stress, WS) were employed. Shoot dry weight, root dry weight, and root length were enhanced with the increase of P supply rates, while the root : shoot ratio, specific root length, and transcription levels of four inorganic phosphate (Pi) transporter genes (Pht1;1–4) declined with the increase of P supply rates. Under WS conditions, root dry weight and root length decreased by 4–38% and 6–32%, respectively, compared to the WW treatment, whereas the root : shoot ratio and specific root length increased by 7–33% and 8–28%, respectively. P transporter gene transcription was up-regulated and that of the arbuscular-mycorrhizal-induced transporter (Pht1;6) gene was both up- and down-regulated in response to the WS treatment. Comprehensive analysis revealed that interaction between maize root morphological and physiological traits was stimulated as a strategy for efficient P acquisition under WS conditions. This study is the first to use both morphological and physiological traits to describe the impacts of soil water conditions on maize responses to P fertilization. Our results provide valuable information for optimization of P management under different soil water conditions.

Key words: phosphorus / root morphology / root physiology / water / Zea mays

Accepted March 07, 2019

1 Introduction

Phosphorus (P) is an essential macro-element required by plants. Despite its abundance in the environment, P is neither easily accessible nor evenly distributed in most soils (Hodge, 2004). Thus, insufficient P intake is the major nutritional disorder limiting crop growth (Vance et al., 2003). Water shortage is another major abiotic factor limiting global crop productivity (Gerke, 2015). Unlike other factors that may limit crop yield (e.g., soil acidity, alkalinity, or salinity), water availability is highly variable within the growth season and among years. The improvement of P and water management is therefore an important goal for sustainable agriculture.

Plants have developed multifaceted adaptive mechanisms to respond to persistent P deficiency, including morphological, physiological, biochemical, and molecular mechanisms to enhance P uptake and its utilization. These include: (1) the modification of root system architecture and establishment of symbiotic relationships with arbuscular-mycorrhizal (AM) fungi to increase the root-soil interface area (Lynch and Brown, 2008; Péret et al., 2011; Smith and Smith, 2011; Zhan et al., 2014), (2) increasing root exudates into rhizosphere soil to mobilize unavailable forms of P (Vance et al., 2003; Gerke, 2015), and (3) the activation of high-affinity inorganic phosphate (Pi) transporter gene expression, which enhances the capacity of plant cells to take up P effectively from soils with low-P availability (Vance et al., 2003; Hammond et al., 2004). Understanding these responses therefore provides useful information to increase the mobilization and acquisition of P by crops (Zhang et al., 2010; Li et al., 2011).

Water shortage directly constrains plant growth and survival, and indirectly influences plant responses to soil mineral nutrients, especially low-mobility nutrients such as P (Otsus and Zobel, 2004; Ma et al., 2009, 2011). Abundant evidence shows that drought stress reduces P uptake and its availability for growth (Misra and Tyler, 2000; Wittenmayer and Merbach, 2005; Zhan et al., 2014). Thus, plant responses to P can be significantly influenced by soil water conditions. Understanding the effects of soil water on plant growth responses to P supply is therefore necessary.

Maize is the most widely cultivated crop worldwide (FAO, 2016). It is an important food component in many developing countries and is among the crops with the highest biotechno-
logical potential for energy production (e.g., ethanol, biofuel) and other industrial applications in developed countries (McLaren, 2005). However, due to its wide cultivated range, water shortage and P deficiency are major limitations for maize growth (Campos et al., 2004; Farooq et al., 2009). To date, research in this field has focused mainly on the interactive effects of soil water and P on plant growth (Gutiérrez-Boem and Thomas, 1999; Song et al., 2010), the effects of soil water content and/or P supply on root morphology, and AM colonization (Kuchenbuch et al., 2006; Deng et al., 2014; Bera et al., 2018). However, it remains unclear how soil water conditions affect root morphological and physiological responses to P application. In this study, we examined root morphology, AM colonization, and Pi transporter gene transcription responses to P application under two water-supply regimes. Our hypothesis was that the responses of root morphological and physiological traits of maize to P supply can be affected by soil water conditions.

2 Material and methods

2.1 Plant and soil materials

Maize (Z. mays L. cv. NE15) was used in this study. We used a silt loam collected from the Shangzhuang Experimental Station at China Agricultural University (40°8′ N, 116°10′ E) in Beijing, China. Soil samples were collected at the beginning of the experiment; the main soil properties were 7.09 g kg⁻¹ organic matter, 0.51 g kg⁻¹ total nitrogen (N), 1.19 mg kg⁻¹ Olsen-P, 90 mg kg⁻¹ exchangeable potassium (K), and pHH₂O 8.4.

2.2 Experimental design

The experiment was conducted with seven P application rates (0, 12.5, 25, 50, 75, 100, and 300 mg P kg⁻¹ soil) and two water levels: well watered (WW, 75–85% of water-holding capacity), and water stress (WS, 60–70% of water-holding capacity). Each treatment was repeated six times, for a total of 84 pots. P was applied using Ca(H₂PO₄)₂. All pots (20 cm in diameter and 40 cm in depth) also received N and K, at rates (0, 12.5, 25, 50, 75, 100, and 300 mg P kg⁻¹ soil) and Olsen-P, 90 mg kg⁻¹ exchangeable potassium (K), and pHH₂O 8.4.

2.3 Sampling and analyses

Maize plants were harvested 8 weeks (sixth leaf stage, V6) after being transplanted. In each treatment, three pots were chosen for shoot, root, and soil sampling. Plants were separated into roots and shoots. Shoots were severed at the soil surface, oven-dried at 105°C to a constant weight, and ground for nutrient analysis. Roots were carefully picked out and thoroughly washed to remove soil. Soil was then sampled for nutrient analysis. Roots were examined using a scanner (EPSON V700 Photo, Seiko, EPSON Corp, Nagano, Japan). The scanned root images were analyzed to determine root length using the WinRhizo software (Regent Instruments Inc., Quebec, QC, Canada). Root dry mass was measured after the previously described measurements had been performed. Soil Olsen-P was determined using the molybdo-vanadophosphate method based on extraction of air-dried soil with 0.5 M NaHCO₃ at pH 8.5 (Olsen et al., 1954). Plant P concentration was measured using the Mo-Sb-Vc method after samples had been digested with concentrated H₂SO₄ and H₂O₂ (Barry and Miller, 1989).

The remaining three pots in all treatments were sampled for Pi transporter gene transcription analysis and AM colonization. Plant roots were removed and immediately washed clean and frozen with liquid nitrogen for RNA extraction, and then subsamples were collected to assess AM colonization. Total RNA in maize root samples was extracted using Trizol reagent (cat. no. 15596018, Invitrogen, USA), treated using the RNase-Free DNase Set (cat. no. 79254, Qiagen, Germany), and further purified using an RNeasy Plant Mini Kit (cat. no. 74904, Qiagen, Germany) based on the manufacturer’s protocol. First-strand cDNA was synthesized using the PrimeScript RT reagent Kit Perfect Real Time (cat. no. DRR037A, Takara, Dalian) according to the manufacturer’s protocol. Quantitative real-time polymerase chain reaction (PCR) was performed using a Mastercycler Realplex4 Real Time PCR System (Eppendorf, Germany) based on the SYBR Premix EX Taq (cat. no. DRR041A, Takara, Dalian) protocol. Using the UBQ2 gene (Carlos et al., 2009) as an internal control, transcription levels were measured with the 2⁻ΔΔCT method. For each gene, the lowest transcription relative to UBQ2 was set equal to 1.0. There were three technical replicates of PCR amplification for each sample. The gene-specific primer information for the five transporters is listed below (provided by L. Z. Long, China Agricultural University):

ZmPht1;1 primers:
5'-GACCAGATGGTGATAGAATGCACAT-3' and 5'-TCACCTTACTTTCCCGCCTATAACACACA-3'

ZmPht1;2 primers:
5'-GTCTGGTGAGGCTGAAGACTCAGAGG-3' and 5'-ACATGATAGCCCACCATGTGCAGTGC-3'

ZmPht1;3 primers:
5'-TGGTTTCCGTCTGCTGGTGGTGTG-3' and 5'-TCCCCACGGTGACCTCCGATTTA-3'

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ZmPht1:4 primers:
5'-GAGACCCAGATGGTGTAGAGAATCG-3' and 5'-CATCAAAAACACAGCCAGGTTGACT-3'

ZmPht1:6 primers:
5'-CGACGTGACAGGACTGACAA-3' and 5'-GGATTCCACACCCCTGTTAGT-3'

For AM colonization, a thoroughly mixed 0.5-g root subsample from each pot was cut into lengths of approximately 1 cm, cleared for 1 h at 90°C in 10% (w/v) KOH, neutralized in 1% (v/v) HCl, and stained with 0.05% (w/v) Trypan blue for 30 min (Feng et al., 2003). Colonization was determined from 30 randomly selected stained root segments by observation under a microscope (Trouvelot et al., 1986). We used AM colonization intensity (%) to represent AM root colonization (Covacevich et al., 2007).

2.4 Statistical analyses

We performed one- and two-way analyses of variance using the SAS statistical software (SAS Institute, Cary, NC, USA). Differences among treatment means were compared using the least significant difference method and significance was determined at a level of P < 5%. The linear-plus-plateau model was fitted to describe the relationship between shoot biomass and soil Olsen-P (Tab. 1). We illustrated the response patterns of root morphological traits, AM colonization, and P transporter gene transcription to P application using the SigmaPlot 10.0 statistical software (SigmaPlot 10.0, USA).

### Table 1: Influence of soil water conditions (WS, water stress; WW, well watered) and phosphorus (P) application rates on soil Olsen-P, shoot dry weight (SDW), shoot P concentration (SPC), and shoot P uptake (SPU). Each value is the mean of three replicates (SE). *, **, and *** denote significance at P levels of 5%, 1%, and 0.1%, respectively; NS: no significant difference.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Water level</th>
<th>P application rate (mg P kg⁻¹ soil)</th>
<th>Source of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olsen-P (mg kg⁻¹)</td>
<td>WS</td>
<td>1.08 (0.10) 1.57 (0.17) 2.27 (0.24) 3.29 (0.26) 6.83 (0.31) 8.98 (1.05) 31.55 (1.54)</td>
<td>W P P·W</td>
</tr>
<tr>
<td></td>
<td>WW</td>
<td>1.18 (0.02) 1.91 (0.19) 2.87 (0.16) 4.43 (0.45) 7.54 (0.24) 10.16 (0.70) 36.28 (1.12)</td>
<td>** *** *</td>
</tr>
<tr>
<td>SDW (g plant⁻¹)</td>
<td>WS</td>
<td>0.45 (0.03) 0.60 (0.03) 0.87 (0.08) 1.14 (0.06) 1.76 (0.05) 1.87 (0.06) 2.01 (0.08)</td>
<td>W P P·W</td>
</tr>
<tr>
<td></td>
<td>WW</td>
<td>0.81 (0.04) 0.99 (0.08) 1.37 (0.05) 2.32 (0.06) 2.46 (0.09) 2.50 (0.09) 2.42 (0.06)</td>
<td>*** *** ***</td>
</tr>
<tr>
<td>SPC (mg kg⁻¹)</td>
<td>WS</td>
<td>1.22 (0.19) 1.30 (0.10) 1.77 (0.08) 1.89 (0.04) 2.11 (0.04) 2.25 (0.09) 2.34 (0.06)</td>
<td>W P P·W</td>
</tr>
<tr>
<td></td>
<td>WW</td>
<td>0.95 (0.05) 1.32 (0.04) 1.89 (0.05) 2.98 (0.10) 3.09 (0.14) 3.14 (0.16) 3.29 (0.13)</td>
<td>*** *** ***</td>
</tr>
<tr>
<td>SPU (g plant⁻¹)</td>
<td>WS</td>
<td>0.55 (0.08) 0.79 (0.06) 1.55 (0.18) 2.15 (0.10) 3.71 (0.22) 4.20 (0.22) 4.73 (0.54)</td>
<td>W P P·W</td>
</tr>
<tr>
<td></td>
<td>WW</td>
<td>0.77 (0.04) 1.31 (0.13) 2.58 (0.15) 6.92 (0.30) 7.62 (0.47) 7.77 (0.42) 7.97 (0.40)</td>
<td>*** *** ***</td>
</tr>
</tbody>
</table>

3 Results

3.1 Maize growth and P uptake responses to P supply were influenced by soil water content

Phosphorus (P) application significantly affected the soil Olsen-P level (Tab. 1), which initially increased rapidly with P supply, and then increased more slowly until the maximum P supply rate was reached. Compared to the WW treatment, soil Olsen-P significantly decreased under the WS treatment. Soil water content and P supply exhibited significant positive interactions with soil Olsen-P. At all P supply rates, shoot dry weight (SDW) increased significantly as the P application rate increased. The average SDW with the WW treatment was about 50% greater than that with the WS treatment (Tab. 1). Soil water content and P supply interacted significantly with SDW. SDW initially increased rapidly until the P application rate reached 75 mg P kg⁻¹ soil in the WS treatment and 50 mg P kg⁻¹ in the WW treatment. Subsequently, SDW increased more slowly, although the P application rate increased to 300 mg P kg⁻¹ soil. This trend is illustrated in Fig. 1A. Regression analysis clearly shows that SDW was much greater with the WW treatment than with the WS treatment. The highest SDW values were observed when the soil Olsen-P was 7.38 mg kg⁻¹ in the WS treatment and 4.88 mg kg⁻¹ in the WW treatment. Subsequently, SDW showed no further improvement, despite an increase in soil Olsen-P.

Soil water content, P supply, and their interaction significantly influenced shoot P concentration (SPC) and shoot P uptake (SPU; Tab. 1). Across the seven P supply rates, SPC and SPU initially increased rapidly with P supply and subsequently increased much more slowly. Compared to the WW treatment, WS significantly decreased SPC and SPU. These
results are consistent with those of the regression analysis (Fig. 1B, C), which clearly shows that SPC and SPU values were lower with the WS treatment than with the WW treatment. The highest SPC and SPU values were found when soil Olsen-P values were 7.40 and 7.79 mg kg\(^{-1}\), respectively, with the WS treatment, and 4.79 and 5.13 mg kg\(^{-1}\), respectively, with the WW treatment. The SPC and SPU values did not increase with further increases in soil Olsen-P.

3.2 Root morphology and Pi transporter gene transcription responses to P supply were influenced by soil water content

We examined root dry weight (RDW), root length (RL), the root : shoot ratio (RSR), and specific root length (SRL) as root morphological traits. The average RDW and RL values were approximately 26% and 18% higher, respectively, with the WS treatment than with the WW treatment (Fig. 2A, B). Soil water conditions affected the responses of root morphological traits to P supply. RDW and RL values increased significantly with increased P application rate up to a rate of 50 mg P kg\(^{-1}\) soil with the WW treatment and a rate of 75 mg P kg\(^{-1}\) soil with the WS treatment, after which no significant additional increase occurred with further increases in the P supply rate (Fig. 2A, B). Unlike the RDW and RL values, the average values of the RSR and SRL were 21% and 16% lower in the WW treatment than in the WS treatment. Under WS conditions, the RSR and SRL decreased significantly with the increase of P application. Although the RSR did not differ significantly among P supply rates, with the WW treatment the RSR and SRL showed similar patterns with increasing P supply rate. Namely, the RSR and SRL gradually decreased with increased P application rate and reached a plateau after the P application rate exceeded 50 mg P kg\(^{-1}\) soil (Fig. 2C, D).

Figure 1: Maize shoot dry weight (A), shoot P concentration (B), and shoot P uptake (C) responses to soil Olsen-P levels under water stress (WS, filled symbols) and well watered (WW, empty symbols) conditions. Each point represents the mean of three replicates.

Figure 2: Effects of soil water conditions (WS, water stress; WW, well watered) on (A) root dry weight, (B) root length, (C) the root : shoot ratio, and (D) specific root length of maize in response to P application rates. Values are presented as means and bars indicate standard errors. Within each water treatment, means accompanied by different letters are significantly different at P < 5%.
The average relative transcription levels of the Pht1;1–4 genes were higher under WS conditions than under WW conditions (Fig. 3). The responses of Pht1;1–4 transcription to P supply were also affected by soil water conditions. The transcription levels remained low when the P supply rates decreased from 300 to 50 mg P kg\(^{-1}\) soil with the WW treatment and to 25 mg P kg\(^{-1}\) soil with the WS treatment, and then were steadily up-regulated when the P fertilizer rate decreased further to a deficient level (Fig. 3).

The AM colonization rate exhibited both increasing and decreasing trends as P supply increased, but the rates of increase were affected by the soil water conditions (Fig. 4A). The AM colonization rate initially increased significantly with increasing P, peaked at 50 mg P kg\(^{-1}\) soil with the WS treatment and at 25 mg P kg\(^{-1}\) soil with the WW treatment, and then decreased significantly as the P application rate increased to 300 mg P kg\(^{-1}\) soil. Under the WS treatment, Pht1;6 gene transcription was up-regulated when the P application rate was decreased from the sufficiency level to 50 mg P kg\(^{-1}\) soil.

Figure 3: Effects of soil water conditions (WS, water stress; WW, well watered) on Pht1;1–4 transporter gene transcription in response to P application rates. Values are presented as means and bars indicate standard errors. Within each water treatment, means indicated by different letters are significantly different at P < 0.05.

Figure 4: Effects of soil water conditions on root arbuscular mycorrhizal colonization (A) and Pht1;6 transporter gene transcription (B) in response to P application rates. Values are presented as means and bars indicate standard errors. Within each water treatment, means accompanied by different letters are significantly different at P < 5%.

3.3 AM colonization and AM-inducible transporter gene responses to P supply were influenced by soil water content

The AM colonization rate exhibited both increasing and decreasing trends as P supply increased, but the rates of increase were affected by the soil water conditions (Fig. 4A). The AM colonization rate initially increased significantly with increasing P, peaked at 50 mg P kg\(^{-1}\) soil with the WS treatment and at 25 mg P kg\(^{-1}\) soil with the WW treatment, and then decreased significantly as the P application rate increased to 300 mg P kg\(^{-1}\) soil. Under the WS treatment, Pht1;6 gene transcription was up-regulated when the P application rate was decreased from the sufficiency level to 50 mg P kg\(^{-1}\) soil.
P kg\(^{-1}\), and was then down-regulated when P application was further decreased to a deficiency level (Fig. 4B). In contrast, under WW treatment, up-regulation of Pht1;6 was found with the decreasing of P application rate.

4 Discussion

We examined maize root morphology (RDW, RL, RSR, and SRL), AM symbiosis, and the transcription of five Pht1 transporter genes (Pht1;1–4 and Pht1;6) to investigate the impacts of soil water content on maize responses to P supply. Our results show that soil water conditions and P supply rates affected maize root morphological and physiological traits, and soil water conditions also impacted the responses of maize root morphological and physiological traits to P supply.

Phosphorus is important for plant growth and development, but soils have been shown to be P deficient in both intensive and conventional agricultural contexts. Therefore, farmers apply large quantities of P fertilizer to ensure yield and quality (Zhan et al., 2015). However, yield does not continue to increase with increasing levels of P application (Mallarino and Blackmer, 1992). Previous studies have shown that crop yield has an exponential Mitscherlich relationship to the soil P-supply level (Johnston and Dawson, 2005; Howard, 2006; Kirkby and Johnston, 2008). The response of SDW to soil Olsen-P showed a similar trend in this study (Tab. 1, Fig. 1). However, the average SDW values were significantly lower under the WS treatment than under the WW treatment. These results may suggest that shoot growth is more sensitive to P deficiency under water shortage conditions than under sufficient water supply.

As P supply increased, both RDW and RL increased significantly, whereas the RSR and SRL values showed an overall decreasing trend (Fig. 2). These results are consistent with commonly observed responses of plants to P limitation, mainly because plants allocate more biomass to roots and produce more RL per unit of metabolic investment in root tissue to improve the capacity of roots to explore soil and increase P uptake under P-deficiency conditions (Lambers et al., 2006; Lynch and Brown, 2008). Thus, P does not increase root growth in proportion to shoot growth. Maize root growth is more sensitive than shoot growth to a reduction in soil P supply, and maize root morphology acclimates to a reduced P supply primarily by increasing root growth. At all P supply rates, RDW and RL were greater under the WW treatment than under the WS treatment, whereas the RSR and SRL were decreased by the WW treatment (Fig. 2). These results are consistent with those of previous studies with barley; under the same P application rate, reduced barley root biomass and an increased the RSR were observed under WS conditions (Jones et al., 2005). Increasing the RSR and SRL values would be expected to increase drought tolerance due to the improved ability of the plant to access soil water. However, in this study, the ability to increase water acquisition from soil through the stimulation of root growth was limited because root growth was inhibited under the WS treatment.

Nagy et al. (2006) identified six Pht1 genes (Pht1;1–6) encoding P\(_i\) transporters that contribute to P uptake and allocation in maize. Among these six genes, Pht1;1–4 are considered to be involved in the P uptake by roots and are strongly influenced by soil P availability (Nagy et al., 2006). Phylogenetic analysis has shown that Pht1;1, Pht1;2, and Pht1;4 are closely related to OsPT8 in rice, whereas Pht1;3 is closely related to OsPT6 in rice (Nagy et al., 2006). OsPT6 and OsPT8 have been identified as high-affinity P transporters (Nussaume et al., 2011). Thus, we speculated that the Pht1;1–4 genes may also encode high-affinity P transporters; a study of the expression of these four genes could demonstrate, at least partially, root responses to soil P supply. In this study, the up-regulation of Pht1;1–4 transcription began when the P application rates declined to 75 mg P kg\(^{-1}\) soil under WS conditions and 50 mg P kg\(^{-1}\) soil under WW conditions (Fig. 3). These findings illustrate the regulation of their transcription by P supply level, and are in accordance with previous work showing that the transcripts of these four genes are preferentially expressed under P-deficiency conditions (Nagy et al., 2006).

A greenhouse and field study of maize by Deng et al. (2014) also showed that Pht1;1–4 transcription levels increased rapidly as P supply decreased, and the highest transcription level was observed when no P was supplied. The transcription of the P-transporter genes was up-regulated as a strategy to take up as much available P as possible from contacted pools, mainly because of the reduced ability of roots to acquire P from soil under low-P conditions. However, P transport across the plasma membrane is an energy-mediated process (Schachtman et al., 1998; Raghothama, 1999; Mimura, 1999), and up-regulation of P\(_i\) transporter genes may increase the risk of P toxicity for plants at elevated P supply levels (Lambers et al., 2006). Therefore, with improved P availability, decreased expression can save carbon and protect plants from P toxicity. Furthermore, we found that the transcription levels of the P\(_i\) transporter genes exhibited positive responses to the WS treatment (Fig. 3). These results suggest that maize roots suffer more severe P deficiency under WS conditions than under WW conditions, which stimulates the physiological pathways, and up-regulates P\(_i\) transporter gene transcription to increase P uptake from the soil.

Two pathways for P uptake exist in terrestrial plants: the direct uptake pathway from the rhizosphere by root epidermal cells and root hairs, and the indirect uptake pathway via AM fungi (Nagy et al., 2006). The uptake of P is considered to be the key physiological process by which AM fungi stimulate host plant growth (Smith and Smith, 2011), mainly because the fungal mycelia can grow up to 100 times longer than root hairs and branches (Jakobsen et al., 1992; Bates and Lynch, 1996), providing an efficient nutrient-absorbing network beyond the P-depletion zone. However, the AM colonization rate is usually affected by soil water conditions and the P application rate (Augé et al., 1995).

In this study, we observed both positive and negative impacts of P supply on the AM colonization rate (Fig. 4A). The initial increase in the AM colonization rate with increasing P supply suggests that AM colonization was inhibited due to decreased SDW. However, after the AM colonization rate reached its maximum value, it declined as P supply decreased, possibly...
due to the inhibition of soil P supply levels (Deng et al., 2014). Soil water conditions also affected the AM colonization rate. Before the AM colonization rate reached a maximum, it was greater under the WW treatment than under the WS treatment (Fig. 4A), suggesting that WS limited AM colonization. This finding is consistent with those of previous studies (Michelsen and Rosendahl, 1990). We also found that the P application rate at which AM colonization reached a maximum was greater under the WS treatment than under the WW treatment (50 mg kg⁻¹ soil vs. 25 mg kg⁻¹ soil, respectively), suggesting that under WS conditions, the mycorrhizal pathway was enhanced to increase P acquisition.

AM-inducible P transporter genes have been found in many plant species (Maeda et al., 2006; Nagy et al., 2006; Glassop et al., 2007). In maize, Pht1;6 has been identified as an AM-inducible Pi transporter gene; its expression can be specifically activated in roots colonized by AM fungi and it is thought to be involved in the mycorrhizal P uptake pathway (Nagy et al., 2008). Coinciding with the response of root AM colonization, the transcription of Pht1;6 showed up-regulation and/or down-regulation with P application under the two water treatments (Fig. 4B). These results confirm that Pht1;6 expression can be induced by AM colonization. Plants acquire P from AM symbiosis by investment of carbon for exchange (Smith et al., 2009). Under the WS treatment, the low Pht1;6 transcription level when P application was relatively low (0–50 mg kg⁻¹ soil) possibly serves as a strategy to conserve carbon for efficient P acquisition under both water- and P-deficiency conditions. With improved P availability, the indirect P uptake pathway was stimulated and consequently enhanced the transcription of AM-inducible P transporter genes. With further increased P supply, plants were able to acquire enough P by the direct pathway and became less dependent on indirect P uptake, and the AM colonization rate decreased as a consequence. Coinciding with root AM colonization, Pht1;6 transcription was lower under the WW treatment than under the WS treatment, and the P application rate at which Pht1;6 transcription peaked was higher under the WS treatment than under the WW treatment (50 mg kg⁻¹ soil vs. 25 mg kg⁻¹ soil, respectively), suggesting that under the same P application rate, plants under WS conditions need more support from the mycorrhizal pathway to acquire P from the soil than do plants under WW conditions.

5 Conclusion
The results suggest that under water stress maize root growth was stimulated as a direct pathway for efficient P acquisition. Additionally, the P transporter genes were up-regulated and the AM-induced transporter gene was both up- and down-regulated under water stress, indicating that root physiology was enhanced as a strategy for efficient P acquisition under water stress.

Acknowledgments
The present research was supported by the National Natural Science Foundation of China (41601308), the China Postdoctoral Science Foundation (2016M591295), the Natural Science Foundation of State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (A314021402-1713, A314021402-1606), and the External Cooperation Program of Chinese Academy of Science (16146KYSB20170013). The authors also would like to thank Prof. Chunqin Zou from China Agricultural University, China, for the comments of the manuscript.

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