Alteration in root morphological and physiological traits of two maize cultivars in response to phosphorus deficiency

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ABSTRACT

Phosphorus-efficient cultivars have an increased capacity to exploit the soil and/or to convert non-available forms of phosphorus (P) into available ones for root uptake. Preliminary field observations showed that nitrogen (N) inefficient maize cultivar ZD958 exhibited high tolerance to low P soil in the shoot performance than N-efficient cultivar XY335. However, it remains unknown whether ZD958 had a higher potential in acquiring sparsely soil P than XY335. In this study, three separate glasshouse experiments were carried out to explore the capacity of both cultivars in accessing soil P. The two cultivars grew for 40 days to evaluate functional root traits in soil columns (Expt.1) and for 20 days to examine root hair length in soil filled rhizoboxes (Expt.3) in a calcareous loamy soil amended with P (high P) or background soil (low P). The third experiment (Expt.2) cultured hydroponically the both cultivars for 15 days to determine the correlation between P uptake rate and P supply intensity. Results from Expt.1 showed that shoot P content of ZD958 was significantly higher than XY335. Higher capacity of the ZD958 to access soil P were not correlated with biomass allocation and root morphological traits, since ZD958 had a lower root mass ratio at two P supplies and a lower degree of total root length and root surface area at high P than XY335. Results from Expt.3 indicated that average root hair length was not associated with the improved P uptake by ZD958, since their root hair length remained the same under low P or high P supply. Therefore, changes in root morphological traits did not explain the improved P acquisition by ZD958 roots. Rhizosphere pH, malic acid concentration and acid phosphatase activity were similar between the two cultivars, while citric acid concentration was obviously higher in ZD958 than that in XY335 (Expt.1). These results suggested that the higher capacity of P acquisition by ZD958 might be attributed to the release more citric acids from roots. P uptake rate of ZD958 was significantly higher than XY335 with the solution P ranging from 12.5 to 250 μM (Expt. 2), indicating that the changes in P uptake rate largely explained the greater P acquisition by ZD958 than XY335. Taken together, ZD958 was identified as a P-efficient cultivar, with a better adaptation to low P environment by altering the exudation of citric acid and P uptake rate. The research findings highlight the importance of plant strategies in altering the root morphological and physiological traits for a better adaptation to the low P environment, and enhance our understanding of physiological mechanisms of plant P acquisition.

1. Introduction

Phosphorus (P), the second most important nutrient element after nitrogen (N), is a significant factor which presents a hindrance in agricultural production in many regions of the world (Holford, 1997). In the soil, the element P often exists in unavailable forms (Schachtman et al., 1998). However, P is fixed to the oxides and hydroxides of Al and Fe in the acidic soil, and precipitates in the form of calcium phosphates in the calcareous soil (Hinsinger, 2001; Marschner, 1995). In view of the difficulty in P acquisition, a number of adaptive strategies

spontaneously appear for plants to acquire P in soil for further plant growth (Richardson et al., 2011; Vance et al., 2003), including: (i) Root-foraging strategies to facilitate P acquisition based on the increase of root/shoot ratio (Hermans et al., 2006), the modification of root architecture and morphology (Vance et al., 2003; Chen et al., 2018; Zhu et al., 2005), the enhancement of root hair length (RHL) (Zhu et al., 2010); (ii) P-mining strategies to enhance the desorption, solubilization or mineralization of P from sparingly-available sources in soil through root exudates (proton, organic anions, phosphatases) (Vance et al., 2003; Richardson et al., 2011; Gerke, 2015); (iii) Modification of

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carbon metabolism and alternative respiratory pathways by balancing metabolic costs and metabolic benefits for P acquisition improvement (Lynch and Ho, 2005); (iv) Promotion of physiological P-uptake capacity through activating expression of high-affinity phosphate transporters (such as PHT1) (Rausch and Bucher, 2002); (v) Extension of the soil exploration space beyond the roots by establishing the symbiotic association with arbuscular mycorrhizal (AM) fungi (Harrison et al., 2010). Furthermore, plant growth-promoting rhizobacteria (PGPR) around the roots also improve P acquisition due to P solubilization. The exploration of these strategies contributed to a more efficient absorption of soil P for crops with a decreasing demand of P fertilizers in the meanwhile (Lambers et al., 2006; Shen et al., 2011; Vance et al., 2003).

Plant species or cultivars differ in the capacity to convert the non-available forms of P into available forms for effective utilization (Rengel and Marschner, 2005). The capacity of P acquisition is closely related to the changes in root morphological traits (defined as the two-dimensional structure of root system, e.g. root length, fineness) and architectural traits (defined as the spatial configuration of root system, e.g. root branches, angle), which can make the plant anchored in a larger volume of soil, cause the alteration in root physiological traits (defined as the secreted compounds by roots and ability to absorb P from soil solution, eg. proton, organic acids, P uptake rates), and facilitate the uptake of P from insoluble inorganic or organic forms (Chen et al., 2013a, 2013b; Lambers et al., 2006; Vance et al., 2003). Previous studies have suggested that the higher tolerance of soybean and sunflower to low P stress than that of maize may be associated with a more favorable root morphology and architecture (Fernandez et al., 2009; Fernandez and Rubio, 2015). With the low P supply, P uptake of three herbaceous perennial legumes was closely correlated with the total root length, rhizospheric carboxylate concentration, and acid phosphatase activity (Pang et al., 2015). Barley cultivars with long root hair better adapted to the low-P soils than those with short hair (Gahoonia and Nielsen, 2004). The P uptake of *Brassica oleracea* cultivars was correlated with root surface area, lateral root length, lateral root growth rate and the number of lateral roots under low P environment (Hammond et al., 2009). These adaptive changes in root morphological/architectural and physiological traits in response to low P availability play an important role for the acquisition of sparingly soil P.

As a cereal crop, maize is widely cultivated in the world in a variety of agro-ecological environments. Maize cultivars have made a historic contribution to the agricultural Green Revolution and a steady increase is shown in grain yield due to the continuous release of new cultivars with the improved stress tolerance and nutrient efficiency (Duvick, 2001; Khush, 2001). Phosphorus deficiency is a major constraint for maize production in many low-input agro-ecosystems. Maize displays a variety of adaptations to cope with low P availability (Calderón-Vázquez et al., 2009). It has been shown that P deficiency affected the root morphology of maize mainly through its effect on the carbon budget (Mollier and Pellerin, 1999). Lateral rooting contributed to P acquisition when the metabolic costs from the production and maintenance of lateral roots were exceeded by those for enhancing P acquisition (Lynch and Ho, 2005). Maize cultivars differ in the capacity to acquire P from soil. P-efficient maize cultivars had a higher root-to-shoot ratio, root hair density and length of first-order lateral roots under P deficiency (Bayuelo-Jimenez et al., 2011). Maize cultivars with the enhanced or sustained lateral rooting had a greater P acquisition and biomass accumulation than those with the reduced lateral rooting at low P availability (Zhu and Lynch, 2004). Modeling results indicated that maize cultivars with more branches adapted to the lowP environments better while those with fewer lateral root branches tended to get accustomed to low-nitrate environments (Postma et al., 2014). Shallow-rooted maize cultivars had a significantly greater growth and P accumulation compared with deeper-rooted cultivars at low P availability, suggesting that the alterations in root architectural traits were important to improve P acquisition of maize (Zhu et al., 2005). RHL, to a large extent, varies with P availability, and cultivars with long root hairs under low P availability had a significantly greater plant growth, P uptake and lower metabolic cost-benefit ratios than short-haired cultivars (Bayuelo-Jimenez et al., 2011; Zhu et al., 2010). Low-P tolerant cultivars were characterized by a higher organic acid exudation than that in low-P susceptible cultivars (Gaume et al., 2001).

The two maize (*Zea mays* L.) cultivars, widely planted in the Northern China, ZD958 and XY335, have different genetic backgrounds. Cultivar XY335 released in 2000 has been identified as N efficiency under the low N environment in comparison with ZD958 released in 1996 (Han et al., 2015). Preliminary results from a field experiment showed that ZD958 exhibited a higher tolerance for low P in the shoot performance than XY335 when grown with the low-P soil, indicating a possible converse response to P deficit. However, it remains unknown whether the N-efficient XY335 cultivar had a higher potential in acquire sparingly soil P than ZD958. It was hypothesized that ZD958 had a superiority in the shoot growth and access to sparingly P from soil than XY335, and the root physiological traits were superior to root morphological traits in response to low P stress. To address these questions, three glasshouse experiments were carried out to investigate (1) the responses of shoot growth, root morphological and physiological traits to P deficiency and their contributions to P acquisition between the two cultivars (Expt. 1 – soil columns), (2) the correlation between P uptake rate and P supply intensity (Expt. 2 – hydroponics), and (3) the variation in average root hair length between the two cultivars at two different P levels (Expt. 3 – rhizoboxes).

2. Materials and methods

Three separate glasshouse experiments were conducted: Expt. 1 – soil columns, Expt. 2 – hydroponics, and Expt. 3 – rhizoboxes. Two maize (*Zea mays* L.) cultivars ZD958 (released in 1996) and XY335 (released in 2000), widely planted in Northern China, with different genetic backgrounds and contrasting N efficiency, were used in these experiments. Seeds were sterilized with 10% (v/v) H_2O_2 on surface for 20 min, washed five times in the deionized water, and then pre-germinated on the wet filter paper at 25 °C in the dark for 36 h before transferred as per relevant experiments. All experiments were conducted in a controlled growth chamber with the artificial light (day/night 16 h/8 h), temperature of 28/25 °C and light intensity of 300 μmol m^{-2} s^{-1}. The growth units were arranged in a randomized complete design and repositioned every week to minimize the adverse effect as a result of uneven environments.

2.1. Expt. 1 – soil columns

2.1.1. Experimental design and soil preparation

A complete randomized block design, consisting of two cultivars and two P levels was adopted with four replicates per treatment (three plants per column). The experiment selected the calcareous loamy soil collected from a farmland in Baoding city, Hebei Province, Northern China. The soil was mainly composed of the organic carbon of 8.42 g kg^{-1}, total N of 0.85 g kg^{-1}, total P of 0.58 g kg^{-1}, available P (Olsen-P) of 9.7 mg kg^{-1}, NaOH-extractable N of 65.74 mg kg^{-1}, ammonium acetate-exchangeable K of 89.5 mg kg^{-1}, and pH (in CaCl$_2$) of 8.02. The air-dried soil was sieved through a 2 mm mesh, thoroughly mixed and placed for about two days before the use. Basal nutrients were added to the dry soil at the following rates (in mg kg^{-1} soil): Ca (NO$_3$)$_2$·4H$_2$O 1686.67, K$_2$SO$_4$ 133.34, MgSO$_4$·7H$_2$O 43.34, CaCl$_2$·6H$_2$O 125.67, EDTA-FeNa 32.86, MnSO$_4$·4H$_2$O 6.67, ZnSO$_4$·7H$_2$O 10, CuSO$_4$·5H$_2$O 3.0, H$_3$BO$_3$ 0.67, (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O 0.13. Phosphorus was added to the soil in the form of KH$_2$PO$_4$ at a rate of 200 mg P kg^{-1} (High P). The background soil P (without adding external P) was regarded as low P treatment. Equivalent amount of K with KCl instead of KH$_2$PO$_4$ was added to the low-P treatment for K compensation.

2.1.2. Plant growth, maintenance and harvest

Five uniform germinated seeds were planted in each PVC column containing 4.0 kg of the air-dried soil, improved by basal nutrients, with (high P) or without (low P) P addition. Plants were thinned to three at the seeding stage. The columns were supplied with deionized water and soil moisture, weighted by water every two days, was maintained at 75% of the field capacity before planting and during the experiment.

The first and second harvests of maize plants were gathered after 7 and 40 days of shoot emergence respectively. At harvest time, shoots were cut at the soil surface level and roots were separated from the soil by gently shaking and sieving. Separated roots were sampled for rhizosphere exudation (see below), then washed with deionized water free of soil and kept in a sealed plastic bag at 4 °C before root scanning and analysis. Shoots and roots were dried at 70 °C for 3 d and weighed to determine dry matter. The whole-plant mass ratios were calculated based on the division of whole-plant mass in low P soil by whole-plant mass in high P soil.

2.1.3. Rhizosphere exudation collection and root analysis

At harvest, roots were shaken gently to remove the loosely adhering soil, immersed into a beaker containing 50 ml of 0.2 mM $CaCl_2$ solution. Then the tightly adhering soil was removed by shaking for 1 min (defined as rhizosphere extracts). Meanwhile, a comparable amount of bulk soil was also suspended in the same amount of $CaCl_2$ solution as described above. After kept for 20 min, 0.5 ml of rhizosphere extract was transferred to 2 ml Eppendorf reaction vials in order to determine the activity of acid phosphatase (APase), and 2 ml subsamples of the rhizosphere extracts were filtered through a 0.22 μm syringe filter into a 1-ml HPLC vial to determine the concentration of carboxylates. Each HPLC sample was acidified with a drop of concentrated orthophosphoric acid (H_3PO_4), and placed on ice for the transfer to a freezer at −20 °C (see below) (Chen et al., 2013a; Pearse et al., 2006b). The rest of rhizosphere extract was subsequently dried at 70 °C in an oven, and the residual soil were used as a reference base for APase and carboxylates.

Root samples were scanned with an Epson Perfection V750 PRO scanner. Root morphological traits, such as total root length (TRL), root surface area (RSA) and average root diameter (ARD) were acquired from the scanned root images in WinRHIZO (Regent Instructions, Quebec, Canada). The specific root length (SRL) was calculated based on the root length per unit root dry weight (m g^{-1}).

2.1.4. Rhizosphere extract analysis

Rhizosphere extracts were used to determine the variation in pH, activity of APase and carboxylates. In view of different maize cultivars or P treatments, obvious differences were shown in the content of rhizosphere soil, which exercised great influence on the pH of the rhizosphere extracts. Therefore, a modified pH method, derived from the pH of rhizosphere extracts, was used to denote the pH of the rhizosphere (Li et al., 2010). For the bulk soil, the pH was measured immediately after shaken in 50 ml 0.2 mM $CaCl_2$ solution for 30 min with a pH meter at the soil-to-water ratio of 1:5 (Sartorius PB-10, Germany).

The activity of APase in the rhizosphere was determined by the method of (Neumann, 2006). The methods were elaborated as follows: 0.5 ml of rhizosphere extract was transferred to 2 ml Eppendorf reaction vials with 0.4 ml Na–Ac buffer and 0.1 ml substrate (p-NPP) solution. After the incubation for 1 h at 25 °C, 0.4 ml of 0.5 M NaOH was added to terminate the reaction. The soil was removed by centrifuging at 4000 g for 10 min. The absorption of supernatants was measured at 405 nm on a spectrophotometer. The control groups were treated with the rhizosphere soil suspension in order to eliminate the color not produced in the hydrolysis of p-NPP. The enzyme activity was expressed as microgram of p-nitrophenol formed per gram of dry soil per hour (μg p-nitrophenol h^{-1} g^{-1} dry soil).

Malic and citric acids, two important carboxylates in the rhizosphere extracts, were analyzed according to the high-performance

liquid chromatography (HPLC) under the ion suppression mode. The separation was conducted on a 250 mm × 4.6 mm reversed-phase column (Alltima C_{18}, Alltech Associates, Deerfield, MA, USA). The mobile phase was 25 mM KH_2PO_4 (pH = 2.25), with a flow rate of 1 ml min^{-1} at 31 °C and UV detection at 214 nm. The sample injection volume was 20 μL. The identification of carboxylates was carried out by comparing the retention time and absorption spectra with those of known standards (Chen et al., 2013a; Pearse et al., 2006b).

2.1.5. Tissue P concentration and P uptake calculation

To determine P concentration in shoots and roots, the oven-dried shoot and root samples were ground to a fine powder in a coffee mixer. Approximately 0.3 g of ground subsamples were digested in the concentrated H_2O_2–H_2SO_4. Phosphorus concentrations were measured with the vanado-molybdate colorimetric reaction as described by Westerman (1990).

Specific P uptake was calculated as the total amount (shoot and root) of P taken up from soil per unit root length (μM P cm^{-1} root). Phosphorus uptake rate was expressed as the net amount of P taken up per unit root length per unit time (μM P cm^{-1} root d^{-1}). Assuming that plants have exponential root growth, the P uptake rate (P_u) was calculated based on the formula of (Williams, 1948) as:

$$P_u = \frac{U2 - U1}{RL2 - RL1} * \frac{\ln(RL2/RL1)}{t2 - t1}$$

where U is P content (mg plant^{-1}); RL is root length (cm); t is time of harvest (d); Number 1 and 2 refer to the first and second harvest, respectively.

2.2. Expt. 2 – hydroponics: determination of correlation between P uptake rate and P supply intensity

A hydroponic experiment with the same two maize cultivars was carried out to investigate the correlation between P supply intensity and P uptake rate. The experiment consisted of two cultivars (ZD958 and XY335) and 8 P levels (0, 12.5, 25, 50, 100, 150, 200 and 250 μM P) with four replicated pots per treatment (three plants per pot). Germinated seeds were transferred to porcelain pots containing 2 L of a continuously aerated nutrient solution (in μM): $Ca(NO_3)_2$ (2000), K_2SO_4 (750), $MgSO_4$ (650), KCl (100), H_3BO_3 (1), $ZnSO_4$ (1), $MnSO_4$ (1), $CuSO_4$ (0.1), $(NH_4)_6Mo_7O_{24}$ (0.05) and Fe-ethylene diamine tetraacetic acid (EDTA) (10). Phosphorus, as KH_2PO_4, was added to the nutrient at a rate of 5 μM during the growth of initial 12 days. The solution was renewed every 3 days. After the growth of 12 days, P, in the form of KH_2PO_4, was applied to the nutrient solution to give final concentrations of 0, 12.5, 25, 50, 100, 150, 200 and 250 μM P. Potassium was balanced by supplying appropriate concentrations of KCl to the P treatments. The pH of nutrient solution was modified to 5.8 with 0.05 M NaOH every day. Maize plants were harvested after 12 (before P treatment) and 15 days (3-day after P treatment) of transplanting. Root length, biomass, shoot P content and P uptake rate were measured as described in Expt. 1.

2.3. Expt. 3 – rhizoboxes

PVC rhizoboxes (20 cm × 1.5 cm, 35 cm deep) were used to determine the average root hair length (ARHL) according to the method of Zhu et al. (2010) with minor revision. Germinated seeds of two maize cultivars were cultivated in the rhizobox filled with the same soil as Expt. 1 under low P and high P for 20 days. At harvest, four first-order lateral roots were randomly selected from each plant and examined under a stereo microscope (Leica Z16 APO, Germany) equipped with DFC295 3mp digital camera. A section of root with the maximal root hair length (i.e. a zone with mature, fully elongated hairs) was selected for image capture on the lateral roots. The images were imported to Image J software (National Institutes of Health, USA) for the

quantitative analysis of the average root hair length (ARHL).

2.4. Data analysis

The data collected from each experiment were required for a two-way analysis of variance (ANOVA) in SPSS 18.0 (IBM Inc., USA) to examine the impacts of P and cultivars, as well as their interactions on maize growth, root morphological and physiological traits. With regard to the data for shoot P content, rhizosphere pH and carboxylate concentration (Expt. 1), the log transformation is performed to meet ANOVA assumptions. Comparisons between means were made according to Tukey HSD test. The means were presented with standard error (SE), and the significance was expressed at the 5% probability level ($P \leq 0.05$). For the hydroponic experiment (Expt. 2), P uptake rates were presented with the means of SE and their correlation with P supply intensity was plotted in SigmaPlot (v.12.0).

3. Results

3.1. Dry matter accumulation and allocation (Expt. 1)

Two-way ANOVA showed significant effects of the two cultivars and the two P treatments on shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and root-shoot mass ratio (RMR), but there was little interaction between the cultivars and P in these parameters except RDW (Table 1). Unlike under high P supply, under low P supply, TDW, SDW and RDW markedly reduced and RMR increased in the two maize cultivars (Table 2). At low P supply, ZD958 had a significantly higher TDW and SDW than XY335, whereas no significant difference was observed for RDW. However, at high P supply, TDW, SDW and RDW were significantly higher in ZD958 than in XY335. Regardless of P supply, XY335 had a significantly higher root mass ratio than ZD958 (Table 2). To evaluate the adaptability difference to low P between the two maize cultivars, whole-plant mass ratios were calculated. ZD958 had a significantly higher whole-plant mass ratio than XY335 (Student's $t = 2.521$, $P = 0.045$), indicating that ZD958 were under much higher degrees of P stress than XY335.

3.2. P content, specific P uptake and P uptake rate (Expt. 1)

Two-way ANOVA showed that cultivars, P and their interaction had a significant effect on shoot P content, root P content, specific P uptake and P uptake rate (Table 1). Compared with low P environment, high P supply significantly led to the increase of the P content of shoot and root, specific P uptake and P uptake rate in the two cultivars. At low P supply, shoot P content of ZD958 was significantly higher than that of XY335, although no significant difference was observed for root P content, specific P uptake and P uptake rate in the two cultivars. By comparison, at high P supply, ZD958 had a significantly higher shoot P content, specific P uptake and P uptake rate, yet lower root P content than XY335 (Table 2).

3.2.1. Root morphological traits (Expt. 1)

Two-way ANOVA showed a significant effect of cultivars and P on TRL and RSA, and of P on SRL as well as ARD, but no significant interaction between cultivar and P in SRL and ARD (Table 1). Low P supply significantly increased the TRL and RSA in ZD958, while decreased them in XY335 as compared with high P (Fig. 1a and b). Low P supply resulted in a significant increase of SRL (Fig. 1c), yet a significant decrease of ARD for both cultivars. At low P supply, no difference was found in TRL, RSA, SRL and ARD between the two cultivars. Comparatively, at high P supply, XY335 exhibited a significantly higher TRL and RSA than ZD958, whereas no significant difference was observed in SRL and ARD (Fig. 1d).

3.3. Root hair length (Expt. 3)

Two-way ANOVA showed that P had a significant effect on average root hair length (ARHL), but it had no significant interaction with cultivar in ARHL (Table 1). Under low P supply, ARHL of the two cultivars significantly increased (Fig. 2, Fig. 3). Under low P or high P supply, no significant difference was found in ARHL in the two cultivars.

3.3.1. Root physiological traits (Expt. 1)

Two-way ANOVA showed that cultivars had a significant effect on

Table 1
Two-way analysis of variance (ANOVA) on the effects of maize cultivar, P supply and their interaction on dry matter accumulation, P content, specific P uptake, root morphological and physiological traits.

Parameters	Source of variation					
	Cultivar		P treatments		Cultivar × P	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Shoot and root traits						
Total dry weight (TDW)	21.2	< 0.01	221.5	< 0.001	0.51	0.49
Shoot dry weight (SDW)	30.9	< 0.001	237.7	< 0.001	1.62	0.23
Root dry weight (RDW)	6.06	< 0.05	43.4	0.001	7.48	0.02
Root mass ratio (RMR)	39.8	< 0.001	24.1	< 0.001	2.17	0.17
Total root length (TRL)	29.8	< 0.001	3.74	< 0.05	54.1	< 0.001
Root surface area (RSA)	36.5	< 0.001	8.68	< 0.05	71.5	< 0.001
Specific root length (SRL)	0.044	0.837	79.8	< 0.001	1.12	0.31
Average root diameter (ARD)	1.18	0.298	84.4	< 0.001	1.62	0.23
Average root hair length (ARHL)	5.39	0.052	67.5	< 0.001	0.249	0.63
Plant physiological traits						
Shoot P content (SPC)	72.2	< 0.001	886.1	< 0.001	29.1	< 0.001
Root P content (RPC)	12.9	< 0.01	131.9	< 0.001	10.7	< 0.01
Specific P uptake (SPU)	126.5	< 0.001	715.8	< 0.001	94.8	< 0.001
P uptake rate (PUA)	154.8	< 0.001	598.1	< 0.001	111.9	< 0.001
Rhizosphere pH	0.004	0.987	129.9	0.001	23.3	< 0.001
Acid phosphatase activity (APase)	0.324	0.58	0.014	0.909	0.003	0.955
Citric acid concentration (CAC)	16.1	< 0.01	48.1	< 0.001	0.19	0.67
Malic acid concentration (MAC)	1.49	0.246	34.1	< 0.001	0.27	0.61

Specific root length was calculated as the root length per unit root dry weight (m g^{-1}); Specific P uptake was calculated as the total amount (shoot and root) of P taken up from soil per unit root length; P uptake rate was calculated as the net amount of P taken up per unit root length per unit time.

Table 2
Effect of P supply on the dry matter accumulation and allocation, shoot P content and P uptake rate in two maize cultivars. The values are mean ± SE (n = 4). Means for each parameter with different letters denote significant differences ($P \leq 0.05$) across cultivars and P treatments.

Parameters	ZD958		XY335	
	High P	Low P	High P	Low P
Dry matter				
Shoot dry weight (g plant^{-1})	2.16 ± 0.10a	1.14 ± 0.03c	1.74 ± 0.07 b	0.88 ± 0.02 d
Total dry weight (g plant^{-1})	2.35 ± 0.11a	1.29 ± 0.02c	1.99 ± 0.07 b	1.02 ± 0.02 d
Root dry weight (g plant^{-1})	0.19 ± 0.02 b	0.15 ± 0.01c	0.25 ± 0.01a	0.15 ± 0.01c
Root mass ratio (%)	8.10 ± 0.31c	11.68 ± 0.80 b	12.47 ± 0.50 b	14.40 ± 0.53a
Whole-plant mass ratio	0.55 ± 0.01a		0.51 ± 0.01 b	
Shoot P uptake				
Shoot P content (mg plant^{-1})	5.36 ± 0.15a	1.50 ± 0.05c	3.83 ± 0.11 b	1.16 ± 0.11 d
Root P content (mg plant^{-1})	0.64 ± 0.06 b	0.30 ± 0.03c	0.92 ± 0.04a	0.32 ± 0.03c
Specific P uptake (mg m^{-1})	0.38 ± 0.01a	0.09 ± 0.003c	0.21 ± 0.01 b	0.08 ± 0.005c
P uptake rate (μg cm^{-1}d^{-1})	0.70 ± 0.02a	0.12 ± 0.006c	0.32 ± 0.02 b	0.10 ± 0.01c

Whole-plant mass ratios were calculated as whole-plant mass in low P soil divided by whole-plant mass in high P soil; Specific P uptake was calculated as the total amount (shoot and root) of P taken up from soil per unit root length; P uptake rate was calculated as the net amount of P taken up per unit root length per unit time.
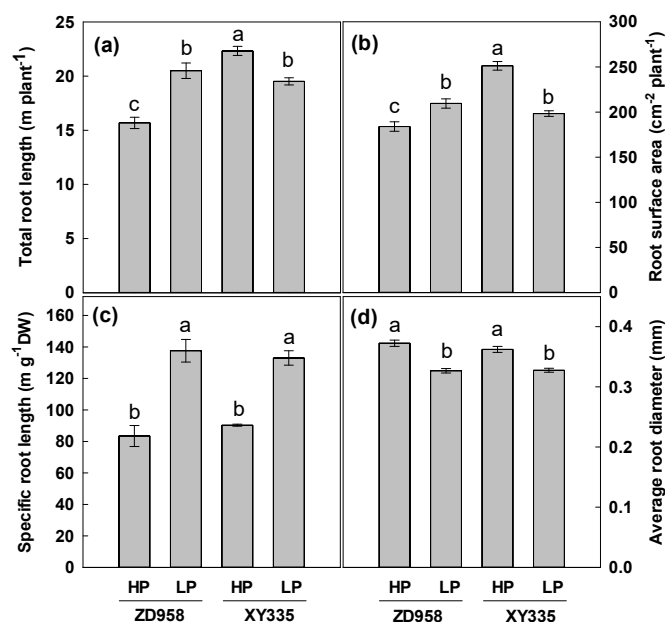

Fig. 1. Total root length (a), specific root length (b), root surface area (c) and average root diameter (d) of two maize cultivars (ZD958 and XY335) grown at low P (LP) and high P (HP) soils. Data were the mean ± SE (*n* = 4). For each trait, bars with the same letter are not significantly different.
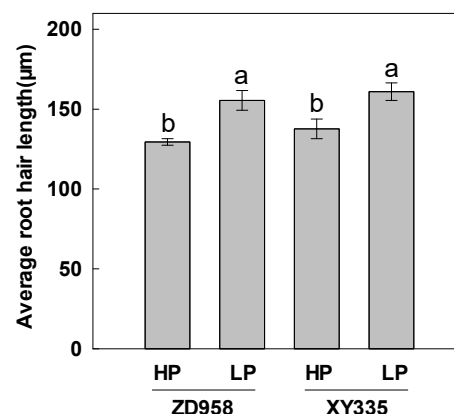

Fig. 3. Average root hair length for two maize cultivars, ZD958 and XY335, under low (LP) and high phosphorus (HP) soil. Data were the mean ± SE (*n* = 4). Bars with the same letter are not significantly different.

citric acid concentration and P on rhizosphere pH, citric and malic acid concentration, but there was no significant interaction between cultivar and P in citric and malic acid concentration except rhizosphere pH (Table 1). The bulk soil extracts had an average pH of 7.97, ranging from 8.04 to 7.89, higher than rhizosphere pH. Under low P supply, rhizosphere pH of the two cultivars significantly reduced. ZD958 had an average rhizosphere pH of 7.19 at high P supply and 7.07 at low P supply. Comparatively, XY335 had an average rhizosphere pH of 7.24 at high P supply and 7.04 at low P supply (Table 3). The APase
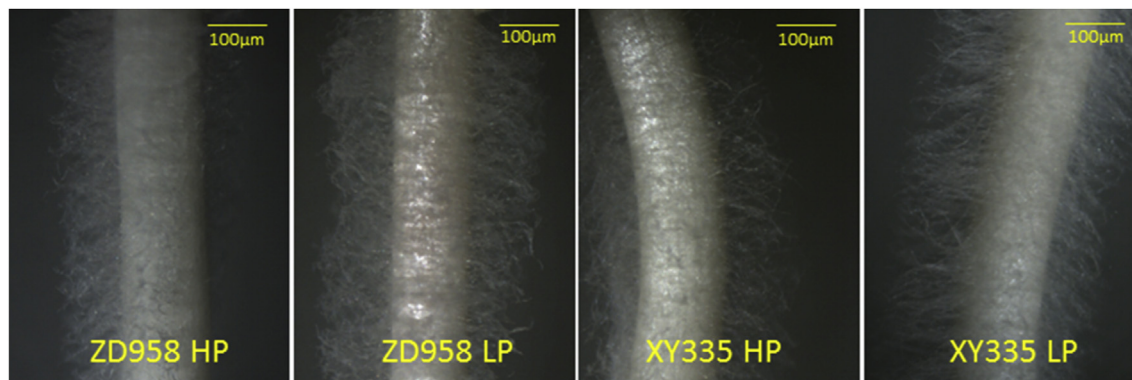

Fig. 2. Root hair images of two maize cultivars, ZD958 and XY335, under low (LP) and high phosphorus (HP) soil. A section of lateral root with maximal root hair length (i.e. a zone with mature, fully elongated hairs) was selected for image capture under a stereo microscope (Leica Z16 APO, Germany) equipped with DFC295 3mp digital camera.

Table 3
Effect of P supply on rhizosphere pH, acid phosphatase (Apase) activity, carboxylates concentration in two maize cultivars. The values are mean ± SE (n = 4). Different letters within each column denote significant differences ($P \leq 0.05$) between treatments.

Maize cultivars	P supply	Rhizosphere pH [a]	Acid phosphatase activity (µg p-nitrophenol h^{-1} g^{-1} dry soil)	Citric acid concentration (µmol g^{-1}dry soil)	Malic acid concentration (µmol g^{-1}dry soil)
ZD958	HP	7.19 ± 0.02a	370 ± 27.6a	1.28 ± 0.05 b	1.57 ± 0.10 b
	LP	7.07 ± 0.02 b	372 ± 14.9a	2.23 ± 0.27a	2.92 ± 0.48a
XY335	HP	7.24 ± 0.01a	384 ± 27.3a	0.63 ± 0.06c	1.12 ± 0.02 b
	LP	7.04 ± 0.01 b	389 ± 34.8a	1.71 ± 0.10 b	2.74 ± 0.18a

[a] Note: bulk soil pH: 7.97 ± 0.03.

activity ranged from 367 to 389 µg p-nitrophenol h^{-1} g^{-1} dry soil. No significant difference was observed for APase activity between the two P treatments and the two cultivars. The concentration of citric and malic acid was markedly enhanced under low P supply, but the two maize cultivars presented different patterns (Table 3). The concentration of citric acid was significantly higher in ZD958 than in XY335 regardless of the P supply. By contrast, the concentration of malic acid showed little difference in the two cultivars.

3.4. Correlation between P uptake rate and P supply intensity (Expt. 2)

The hydroponic experiment showed that the difference of P uptake rate between the two maize cultivars became progressively enlarged with the increase of P supply intensity (Fig. 4). ZD958 had a significantly higher P uptake rate than XY335, until P supply intensity was over 12.5 µM.

4. Discussion

This study has identified ZD958 as a P-efficient cultivar and XY335 as a P-inefficient cultivar, and has explored the factors responsible for higher acquisition of soil P in ZD958 than in XY335 by comparing root morphological and physiological adaptation to soil P deficit. The results in this study will be useful for early selection strategies in P efficiency in maize.

In the present study, shoot P content of ZD958 was significantly higher than that of XY335 under low- and high-P supplies, suggesting that ZD958 could access more P from soil than XY335. The difference in soil P acquisition is associated with the changes in biomass allocation, root morphology, root exudation, root physiological uptake capacity (Chen et al., 2013b; Lambers et al., 2006; Pearse et al., 2006a; Vance et al., 2003). The patterns of biomass allocation are thought to assist P

Fig. 4. Effects of P supply intensity on P uptake rate in two maize cultivars (ZD958 and XY335). Data were the mean ± SE ($n = 4$). Significant differences between the two cultivars for a given P supply: *, $P < 0.05$; **, $P < 0.01$.

acquisition by enhancing the capacity of root foraging and uptake (Hermans et al., 2006; Richardson et al., 2011). When P is in short supply, plants tend to allocate a greater proportion of biomass to the root system (Hermans et al., 2006). This study suggested that low P supply resulted in a greater biomass allocation to roots for the two cultivars, which confirmed the previous reports (Mollier and Pellerin, 1999; Pang et al., 2015; Pearse et al., 2006b). However, the two maize cultivars differed in biomass allocation, as XY335 allocated a greater ratio of biomass to the roots (higher RMR) than ZD958 (lower RMR) regardless of the P supply (Table 2). Since P is relatively immobile in soil, increased biomass allocation to roots is thought to be beneficial for P acquisition of maize (Mollier and Pellerin, 1999). Interestingly, RMR does not closely correlate with shoot P content. XY335 with a higher RMR produced a less shoot P (P concentration and P content), whereas ZD958 with a lower RMR produced a higher shoot P. A detailed explanation about the relationship between root morphological and physiological traits with the acquisition of soil P is further discussed below.

Root morphological traits played an essential role in the acquisition of soil P. The increase in TRL and RSA allowed the plant to explore a larger volume of soil for the acquisition of soil P. When P was in short supply, there appeared a positive response of TRL and RSA for the acquisition of soil P. The results presented here revealed two different responses of maize roots to soil P shortage. Low P supply led to a significant increase of TRL and RSA in ZD958 while an obvious decrease in XY335, suggesting that XY335 was more sensitive in response to soil P shortage and inferior in the acquisition of soil P than ZD958. Maize has long been considered as a species with strong root morphological/architectural adaptation (Bayuelo-Jimenez et al., 2011; Calderón-Vázquez et al., 2009; Chen et al., 2014; Zhu et al., 2005; Zhu and Lynch, 2004). Previous studies have shown that the maize cultivars with more lateral root branching, shallower root system and longer root hairs have greater capacity in the acquisition of soil P (Zhu et al., 2005, 2010; Zhu and Lynch, 2004). The genes, Rtcs, Bk2 and Rth3, associated with root morphology, exhibited higher expression in the P-efficient cultivar (L3) relative to the P-inefficient cultivar (L22). Thus, it can be expected that maize cultivars with larger root system and optimum root architecture are often able to accumulate more P in its aboveground when soil P is limited or sufficient. Unlike the expectation, XY335, with a similar (at low P supply) or larger (at high P supply) TRL and RSA, exhibited a lower shoot P content than ZD958. In addition, no significant difference was observed for SRL and ARD in the two cultivars under low P or high P supply, indicating that differences between the two cultivars in root morphological traits were mainly originated from TRL and RSA rather than root fineness. Root hairs play an important role in the acquisition of immobile P (Itoh and Barber, 1983). Several evidences have indicated that RHL of maize can largely be improved by low P availability, which contributes to P acquisition (Bayuelo-Jimenez et al., 2011). In accordance with these studies, our results showed that P shortage led to the significant increase of ARHL of the two maize cultivars (Figs. 2 and 3). Zhu et al. (2010) suggested that the maize cultivars with long root hairs under low P had significantly greater plant growth, P uptake and lower metabolic cost-benefit ratios than the short-haired cultivars. Similarly, Bayuelo-Jimenez et al. (2011) found that P-
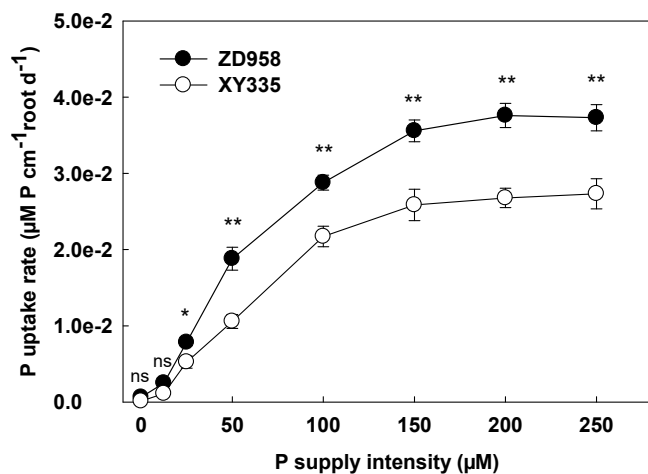
efficient accessions had greater RHL under P deficit than P-inefficient ones. In the present study, the ARHL remained the same in the two maize cultivars under low P or high P supply, indicating that ARHL was not associated with the improved P uptake by ZD958. Taken together, our results suggest that changes in root morphological traits do not explain the improved P uptake by ZD958 roots. It is possible that the changes in root physiological traits might explain the reason.

When subjected to low P stress, plant roots often exhibit a wide range of physiological adjustments to access soil P (Vance et al., 2003; Williamson et al., 2001). Such physiological changes for P acquisition is generally associated with rhizosphere acidification (Ligaba et al., 2004; Shen et al., 2006), exudation of organic acid (Ligaba et al., 2004) and APase activity (Kouas et al., 2009). In the present study, low P stress, did not alter the activity of APase, and caused a significant decrease of rhizosphere pH and an increase of organic acid exudation, suggesting that the dissolution of soil sparingly soluble P could be expected under low P supply to absorb P from the rhizosphere solutions more efficiently. Gaume et al. (2001) indicated that with P deficiency the exudation of malic and citric acids significantly increased in three of the four maize cultivars, which was in accordance with the present results. The rhizosphere acidification is known to be able to strengthen P intensity in the rhizosphere, and therefore to improve the uptake of soil P by plant roots (Gillespie and Pope, 1991; Pypers et al., 2006; Zeng et al., 2012). Studies with ^{32}P-labeled soil have shown unequivocally that species exuding organic acids can access fixed inorganic P that is unavailable to other plants (Hocking and Randall, 2001). At low P or high P supply, a similar rhizosphere pH and malic acid concentration in the two maize cultivars excluded their contribution to P uptake. In the present study, it was not expected previously that the concentration of citric acid was higher in ZD958 than in XY335. Gaume et al. (2001) claimed that low-P tolerant maize cultivar 'NST' could exudate higher amounts of citric acid than low-P susceptible cultivar 'SA3' under low P conditions. These results suggested that the higher P uptake in ZD958 than XY335 can be attributed to the ability to release more citric acids from roots. It is unlikely that the difference in P acquisition between the two maize cultivars is caused by the changes in APase activity, since the activity of the enzyme shows little difference in the two maize cultivars.

Apart from the root exudates, root physiological uptake capacity plays an essential role in the acquisition of soil P. Plant roots can improve P uptake capacity by adjusting morphological and physiological traits to meet changes in shoot P demand (Chen et al., 2013b; Clarkson, 1985; Clarkson and Hanson, 1980), which provides a key mechanistic explanation of why some species/cultivars are more effective in the acquisition of soil P than others. The results from the present study suggest that ZD958 exhibits a significantly higher P uptake rate under high P supply (not under low P) than XY335 (Table 2), indicating that P uptake rate is associated with the intensity of soil P supply. It can therefore be inferred that high P supply intensity may enlarge the difference of P uptake rate between two maize cultivars, while low P supply intensity may reduce the difference to not statistically obvious. This result was further validated in the separate hydroponic experiment demonstrating the correlation between P uptake rate and P supply intensity (Fig. 4). The results suggested that changes in P uptake rate were largely regulated by cultivars and P supply intensity, and a higher P uptake in ZD958 than XY335 can be largely attributed to the ability to improve P uptake rate.

In most of cases, P-efficient cultivars tend to respond to P stress in a less extreme way than P-inefficient ones (de Sousa et al., 2012; Vandamme et al., 2016), which implies that P-efficient cultivars are more tolerant to low P than P-inefficient cultivars. In the present study, ZD958 exhibited a lower responsiveness in shoots and roots growth than XY335, suggesting that ZD958 was more tolerant to P stress than XY335.

Nitrogen (N) and P are two essential macronutrients for plant growth and development. According to the ecological stoichiometry theory, the N:P ratio is relatively stable and maintain around the mean

value of 12.7 at a global scale (Elser et al., 2000). This is especially true for maize in an agricultural system with fertilization. When N and P are sufficient, the N:P ratio ranges from 18.75 to 22.63 (Wang et al., 2013), indicating that the growth of maize plants require more N than P. Therefore, it can be predicted that N-efficient maize cultivar have an advantage in plants growth over P-efficient cultivar. In a previous study, ZD958 has been identified as low-N efficient maize cultivar compared with the high N-efficient XY335. When simultaneously exposed to N and P deficiency, it is likely that XY335 as N-efficient cultivar has a greater superiority in growth than P-efficient ZD958, since there is a higher requirement for N than P in maize plants. When P is in limited supply, XY335 is inferior than ZD958. Further studies are required to examine interactive effects between N and P on root adaptation of the two maize cultivars.

In calcareous soil, where P is precipitated as calcium phosphates and soil P availability is low, both rhizosphere acidification and exudation of organic acids are obviously beneficial as they can dissolve P from Ca–P compounds. In neutral or near neutral soils, where soil P is less fixed and its availability relatively high, a better development of root system may be essential for the capture of soil available P. In acidic soils, where P is fixed as oxides and hydroxides of Al and Fe and soil P availability is low, exudation of organic acids is beneficial as it can dissolve P from Fe–P and Al–P compounds. Apart from the low available P, the crops in the acid soils also suffer from Al toxicity. The exudation of proton is not beneficial since concentration of Al^{3+} is higher in more acidic soil. Thus, different breeding strategies are required to improve P acquisition in three different soil types. Therefore, further studies involving different types of soils and more cultivars are required to determine root adaptation to low P stress.

Declaration of interest statement

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