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Enhanced adaptation to low-P stress by altering rhizosphere exudation and P-uptake rate other than root morphological traits in two maize genotypes

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Abstract: Alterations in root morphology and physiology are important strategies in plants to adapt to low-phosphorus (P) environments. Maize genotypes differed in nitrogen (N) efficiency may also respond differently to low P stress. This study aimed to investigate the responses of root morphological and physiological traits of these two maize cultivars to P deficit and how these traits were linked with the acquisition of soil P. Two maize cultivars, XY335 (N efficient) and ZD958 (N inefficient), were cultivated for 40 days in a calcareous loamy soil amended with (high P) or without (low P) P. Functional root traits were used to evaluate the morphological and physiological responses to low P supply. Two separate short-term experiments determined the correlation between P uptake rate and P supply intensity (hydroponic) or root hair length under two P treatments (rhizobox). Low P status significantly simulated biomass allocation to roots, specific root length and exudations of carboxylates, while decreased root diameter and rhizosphere pH in both maize cultivars. Two cultivars had different total root length and root surface area under low P stress: increased in ZD958 and decreased in XY335. Both genotypes developed longer root hair under P deficit. ZD958 (greater biomass and shoot P content) has a greater capability at accessing soil P than XY335. Rhizosphere exudation of citric acid was significantly higher in ZD958 than in XY335, while there was not significant genotypic difference in rhizosphere pH and exudation of malic acid and acid phosphatase activity. ZD958 had higher P uptake rate than XY335 when solution P was between 12.5 and 250 μM. This study identified ZD958 as a P-efficient genotype, which better adapted to low P stress by altering root physiological traits (exudation of citric acid and P uptake rate), rather than root morphological traits (total root length, root surface area, root hair length). Our results highlight the importance of analyzing root morphological and physiological traits to enhance our understanding of the physiological mechanisms of P acquisition.

Keywords: Carboxylate exudation; P efficiency; Root exudate; Root traits; Rhizosphere

1. Introduction

Phosphorus (P) is the second most important nutrient element after nitrogen (N) and it is a significant factor limiting agricultural production in many regions of the world [1]. In the soil, P often exists in unavailable forms [2]. In acidic soils, P is fixed to oxides and hydroxides of Al and Fe, and in calcareous soils, P is precipitated as calcium phosphates [3,4]. To overcome the constraint of low P availability, plants have evolved a number of adaptive strategies to acquire soil P and improve plant growth [5-8], including: (i) Root-foraging strategies that improve P acquisition by increasing

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root/shoot ratio, modifying root architecture and morphology, enhancing root hair length (RHL); (ii) P-mining strategies to enhance the desorption, solubilisation or mineralisation of P from sparingly-available sources in soil using root exudates (proton, organic anions, phosphatases); (iii) Modifying carbon metabolism and alternative respiratory pathways by balancing metabolic costs and metabolic benefits for improved P acquisition; (iv) Enhancing physiological P-uptake capacity through activating expression of high-affinity phosphate transporters, (v) Extending the soil exploration space beyond the roots by establishing a symbiotic associations with arbuscular mycorrhizal (AM) fungi. Additionally, plant growth promoting rhizobacteria (PGPR) around the roots also improve P acquisition by plants due to P solubilisation. The exploration of these strategies made crops more efficient in the acquisition of soil P and resulted in a reduction of requirements of P fertilizers [5,9,10].

Plant species or cultivars differ in the capacity of converting non-available forms of P into available forms and taking them up [11]. The capacity of P acquisition is largely related to the changes in root morphological traits (defined as the two-dimensional structure of root system, eg. root length, fineness) and architectural traits (defined as the spatial configuration of root system, eg. root branches, angle), allowing the plant to explore a larger volume of soil, and to 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), allowing the uptake of P from insoluble inorganic or organic forms [5,10,12,13]. Previous studies have suggested that higher tolerance of soybean and sunflower than maize to low-P stress may be associated to a more favorable root morphology and architecture [14,15]. At low P supply, P uptake of three herbaceous perennial legumes was closely correlated with total root length, rhizospheric carboxylate concentration and acid phosphatase activity [16]. Barley cultivars with long root hairs are better adapted to low-P soils than those cultivars with short root hairs [17]. Phosphorus 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 [18]. These adaptive changes in root morphological/architectural and physiological traits in response to low P availability play an important role for acquisition of sparingly soil P.

Maize is a cereal crop widely cultivated throughout the world in a range of agro-ecological environments. Maize cultivars made a historic contribution to the agricultural Green Revolution and still maintain a steady increase in grain yield due to continuously release of new cultivars with improved stress tolerance and nutrient efficiency [19,20]. P deficiency is a major constraint for maize production in many low-input agro-ecosystems. Maize display a variety of adaptations to cope with low P availability [21]. It has been shown that P deficiency affected the root morphology of maize mainly through its effect on the carbon budget [22]. Lateral rooting contributed to P acquisition when the metabolic costs from the production and maintenance of lateral roots were exceeded by the metabolic benefits of enhanced P acquisition [23]. Maize genotypes differ in the capacity at accessing P from soil. P-efficient maize genotypes had greater root to shoot ratio, root hair density and length of first-order laterals under P deficiency [24]. Maize genotypes with enhanced or sustained lateral rooting had greater P acquisition and biomass accumulation than genotypes with reduced lateral rooting at low P availability [25]. Modeling results indicated that maize genotypes with more branches were adapted to low-phosphorus environments while genotypes with fewer lateral root branching were adapted to low-nitrate environments [26]. Shallower maize genotypes had significantly greater growth and P accumulation compared with deeper genotypes at low P availability, suggesting that alterations in root architectural traits are important for improved P acquisition of maize [27]. RHL can largely be regulated by P availability, and genotypes with long root hairs under low P availability had significantly greater plant growth, P uptake and lower metabolic cost-benefit ratios than short-haired genotypes [24,28,29]. Low-P tolerant genotypes was characterized by higher organic acid exudation than low-P susceptible genotypes [30].

Two widely planted maize (*Zea mays* L.) cultivars in Northern China, ZD958 and XY335, have different genetic backgrounds. Genotype XY335 released in 2000 has been identified as N efficiency under low N environment in comparison with ZD958 released in 1996 [31]. Preliminary results from

a field experiment showed that ZD958 exhibited a higher tolerance to low P in shoot performance than XY335 when grown at a low-P soil, indicating possible contrasting response to P deficit. However, whether the N-efficient XY335 cultivar had a higher potential at accessing sparingly soil P than ZD958 remains unknown. We hypothesized that ZD958 have a superiority in shoot growth and accessing sparingly P from soil than XY335, and root physiological traits contribute better than 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 and their contributions to P acquisition between the two cultivars (Expt. 1 – soil columns), (2) whether P uptake rate and P supply intensity was correlated (Expt. 2 – hydroponics), and (3) variation in root hair length between the two cultivars under two 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 surface sterilized with 10% (v/v) H_2O_2 for 20 min, washed five times in deionized water, and then pre-germinated on wet filter paper at 25°C in the dark for 36 h before transferred as per respect experiments. All experiments were conducted in a controlled growth chamber with artificial light (day/night 16h/8h), 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 weekly to minimize any adverse effect generated by uneven environments.

2.1. Expt. 1 – soil columns

2.1.1. Experimental design and soil preparation

A complete randomized block design was used consisting of two cultivars and two P levels with four replicates per treatment (three plants per column). The experiment used a calcareous loamy soil collected from a farmland in Baoding city, Hebei Province, Northern China. Major soil properties were characterized as follows: organic carbon 8.42 g kg^{-1}, total N 0.85 g kg^{-1}, total P 0.58 g kg^{-1}, available P (Olsen-P) 9.7 mg kg^{-1}, NaOH-extractable N 65.74 mg kg^{-1}, ammonium acetate-exchangeable K 89.5 mg kg^{-1}, and pH (in $CaCl_2$) 8.02. The air-dried soil was sieved through a 2 mm mesh and thoroughly mixed and let aside for about two years before the use. Basal nutrients were added to the dry soil at the following rates (in mg kg^{-1} soil): $Ca(NO_3)_2{\cdot}4H_2O$ 1686.67, K_2SO_4 133.34, $MgSO_4{\cdot}7H_2O$ 43.34, $CaCl_2{\cdot}6H_2O$ 125.67, EDTA-FeNa 32.86, $MnSO_4{\cdot}4H_2O$ 6.67, $ZnSO_4{\cdot}7H_2O$ 10, $CuSO_4{\cdot}5H_2O$ 3.0, H_3BO_3 0.67, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.13. Phosphorus was added to the soil as KH_2PO_4 at a rate of 200 mg P kg^{-1} soil (High P). The soil without P addition was treated as low P treatment. Equivalent amount of K as KCl instead of KH_2PO_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 amended with basal nutrients and with (high P treatment) or without (low P treatment) P addition. Plants were thinned to three at emergence. The columns were supplied with deionized water and soil moisture was maintained at 75% of the field capacity prior to the planting and during the experiment by water to weight every two days.

Maize plants were harvested 7 and 40 days after shoot emergence for the first and second harvests, respectively. At harvest, 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), and 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. Whole-plant mass ratios were calculated as whole-plant mass in low P soil divided 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 baker containing 50 ml of 0.2 mM $CaCl_2$ solution and shaken for one minute to remove the tightly adhering soil (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 standing 20 minutes, 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 2ml 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 transfer to a −20°C freezer until analyzed (see below) [12,32]. The rest of rhizosphere extract was subsequently dried at 70℃ in an oven, and residual soil were used as a reference base for APase and carboxylates.

Root samples were scanned using 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 scanned root images in WinRHIZO (Regent Instructions, Quebec, Canada). Specific root length (SRL) was calculated as the root length per unit root dry weight (m g^{-1}).

2.1.4. Rhizosphere extract analysis

Rhizosphere extracts were used to determine variation in pH, activity of APase and carboxylates. The amount of rhizosphere soil differed between maize cultivars or P treatments, and the pH of the rhizosphere extracts might be strongly influenced by the amount of rhizosphere soil. Therefore, a modified pH method, derived from the pH of rhizosphere extracts, was used to denote the pH of the rhizosphere [33]. For the bulk soil, the pH was measured immediately after shaking in 50 ml 0.2 mM $CaCl_2$ solution for 30 minutes at a soil: water ratio of 1:5 using a pH meter (Sartorius PB-10, Germany).

The activity of APase in the rhizosphere was determined by the method of [34]. Details on the methods are as follows: 0.5 ml of rhizosphere extract was transferred to 2 ml Eppendorf reaction vials containing 0.4 mL Na-Ac buffer and 0.1 ml substrate (*p*-NPP) solution. After incubation for 1 hour at 25°C, 0.4 ml of 0.5 M NaOH was added to terminate the reaction. Soil was removed by centrifuging at 4,000 g for 10 min. Absorption of supernatants was measured at 405 nm on a spectrophotometer. Controls were performed with each rhizosphere soil suspension in order to eliminate the color not derived from the hydrolysis of *p*-NPP. Enzyme activity was expressed in katals where one katal respond to 1 mol of *p*-Nitrophenol converted by *p*-NPP per 1 second.

Malic and citric acids, two important carboxylates in the rhizosphere extracts, were analyzed by high-performance liquid chromatography (HPLC) in the ion suppression mode. 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. Identification of carboxylates was carried out by comparing the retention time and absorption spectra with those of known standards [12,32].

2.1.5. Tissue P concentration and P uptake calculation

To determine P concentration in shoots and roots, 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 concentrated H_2O_2-H_2SO_4. P concentrations were measured using the vanado-molybdate colorimetric reaction as described by Westerman [35].

Specific P uptake was calculated as the total amount (shoot and root) of P taken up from soil per unit root length. Phosphorus uptake rate was calculated as the net amount of P taken up per unit

root length per unit time. Assuming that plants have exponential root growth, the P uptake rate (P_u) was calculated from the formula of [36] 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 using 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), $(NH4)_6Mo_7O_{24}$ (0.05) and Fe-ethylene diamine tetraacetic acid (EDTA) (10). Phosphorus (P) was added to the nutrient as KH_2PO_4 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 as 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. K was balanced by supplying appropriate concentrations of KCl to the P treatments. The pH of nutrient solution was daily adjusted to 5.8 with 0.05 M NaOH. Maize plants were harvested 12 (before P treatment) and 15 days (3-day after P treatment) after 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 root hair length (RHL) according to the method of Zhu, Zhang and Lynch [29] 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 (no addition of P) and high P (200 mg P kg^{-1} soil as KH_2PO_4) 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 maximal RHL (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 quantitative analysis of the RHL.

2.4. Data analysis

Data collected from each experiment were subjected to a two-way analysis of variance (ANOVA) in SPSS 18.0 (IBM Inc., USA) to examine the impacts of P and cultivars, and their interactions on maize growth, root morphological and physiological traits. Data for shoot P content, rhizosphere pH and carboxylate concentration (Expt. 1) were log transformed to meet ANOVA assumptions. Comparisons between means were made using Tukey HSD test. Means were presented with standard error (SE), and significance was expressed at the 5% probability level ($P \leq 0.05$). For hydroponic experiment (Expt. 2), P uptake rates were presented as means with 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 between the two cultivars and between the two P treatments, respectively, on shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and root-shoot mass ratio (RMR), respectively, but there was no significant interaction between cultivar and P for these parameters except RDW (Table 1). Compared with high P, low P

supply markedly reduced TDW, SDW and RDW and increased RMR for both 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. By comparison, 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 difference between two maize cultivars in the acclimation to low P, 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 experiencing much higher degrees of P stress than XY335.

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
Root hair length (RHL)	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.

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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±0.07b	0.88±0.02d
Total dry weight (g plant^{-1})	2.35±0.11a	1.29±0.02c	1.99±0.07b	1.02±0.02d
Root dry weight (g plant^{-1})	0.19±0.02b	0.15±0.01c	0.25±0.01a	0.15±0.01c
Root mass ratio (%)	8.10±0.31c	11.68±0.80b	12.47±0.50b	14.40±0.53a
Whole-plant mass ratio	0.55±0.01a		0.51±0.01b	
Shoot P uptake				
Shoot P content (mg plant^{-1})	5.36±0.15a	1.50±0.05c	3.83±0.11b	1.16±0.11d
Root P content (mg plant^{-1})	0.64±0.06b	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±0.01b	0.08±0.005c
P uptake rate (μg cm^{-1}d^{-1})	0.70±0.02a	0.12±0.006c	0.32±0.02b	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.

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

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

Root morphological traits (Expt. 1)

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

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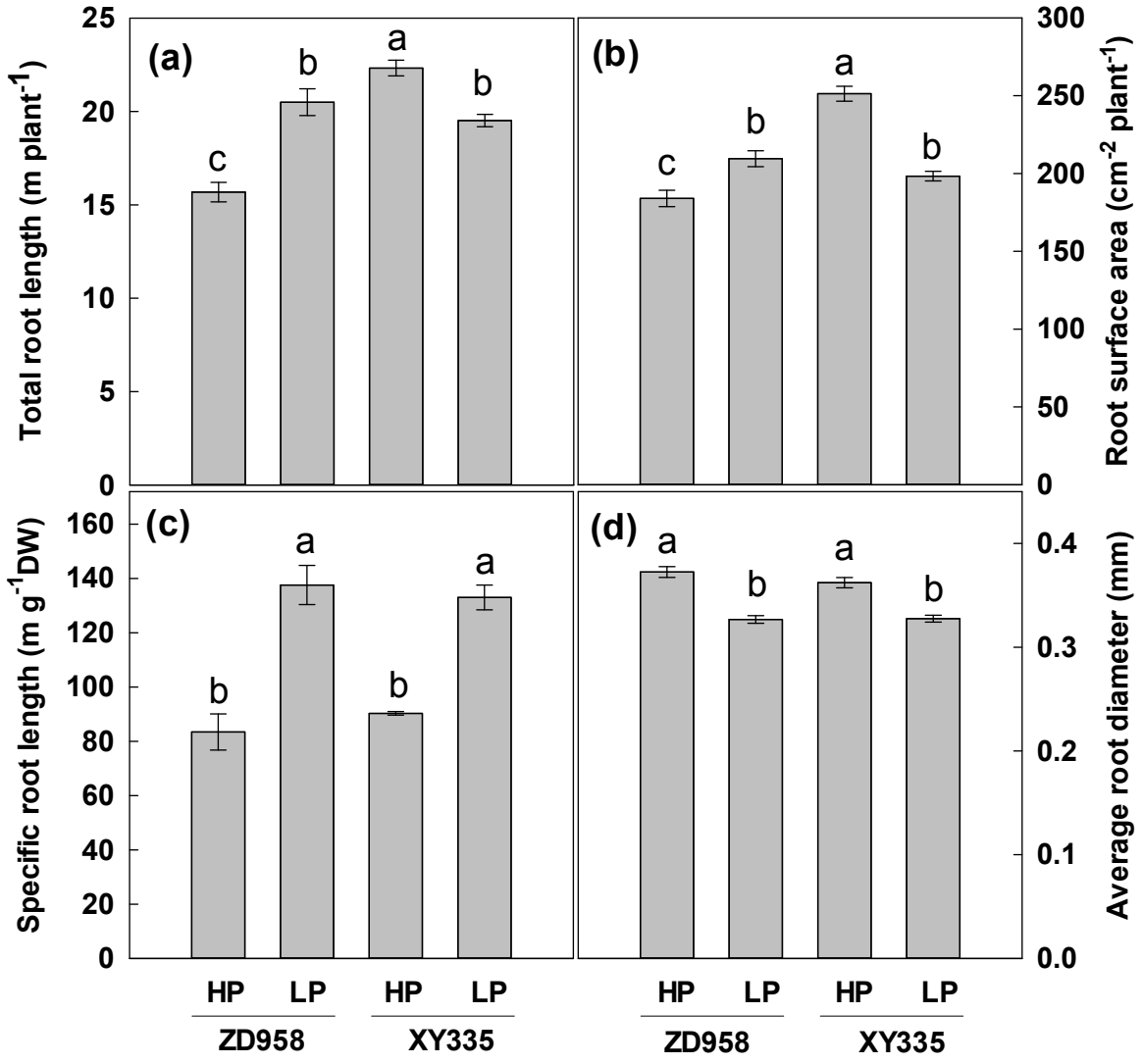

Figure 1. Total root length (a), specific root length (b), root surface area (c) and average root diameter (d) of two maize cultivars (ZD958 and XY335) grown at low P (LP) and high P (HP) soils. Data were the mean ±SE (n=4). For each trait, bars with the same letter are not significantly different.

3.3. Root hair length (Expt. 3)

Two-way ANOVA showed a significant effect of P on root hair length (RHL), no significant interaction between cultivar and P for RHL (Table 1). Low P supply significantly increased the RHL for both cultivars (Figure 2, Figure 3). At low P or high P supply, no significant difference was found for RHL between two cultivars.

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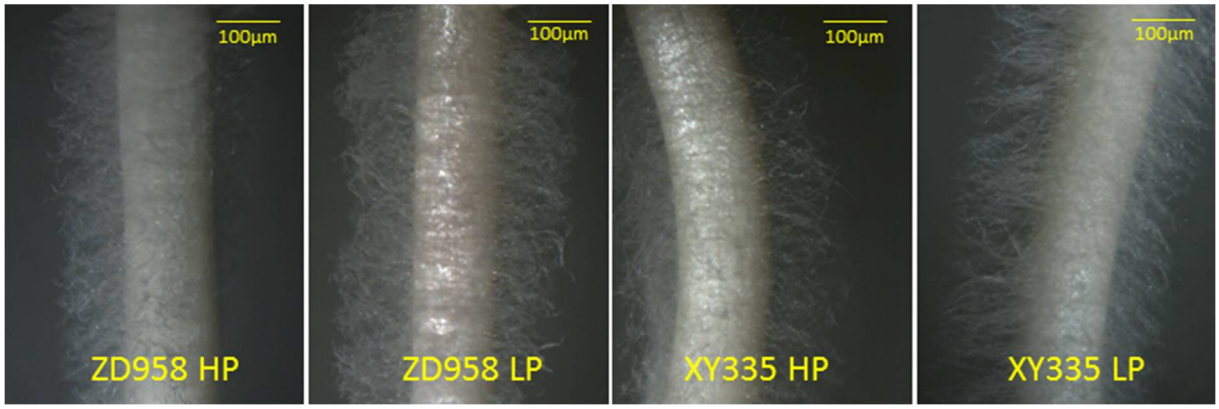

Figure 2. Root hair images of two maize cultivars, ZD958 and XY335, under low (LP) and high phosphorus (HP) soil. A section of lateral root with maximal root hair length (i.e. a zone with mature, fully elongated hairs) was selected for image capture under a stereo microscope (Leica Z16 APO, Germany)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

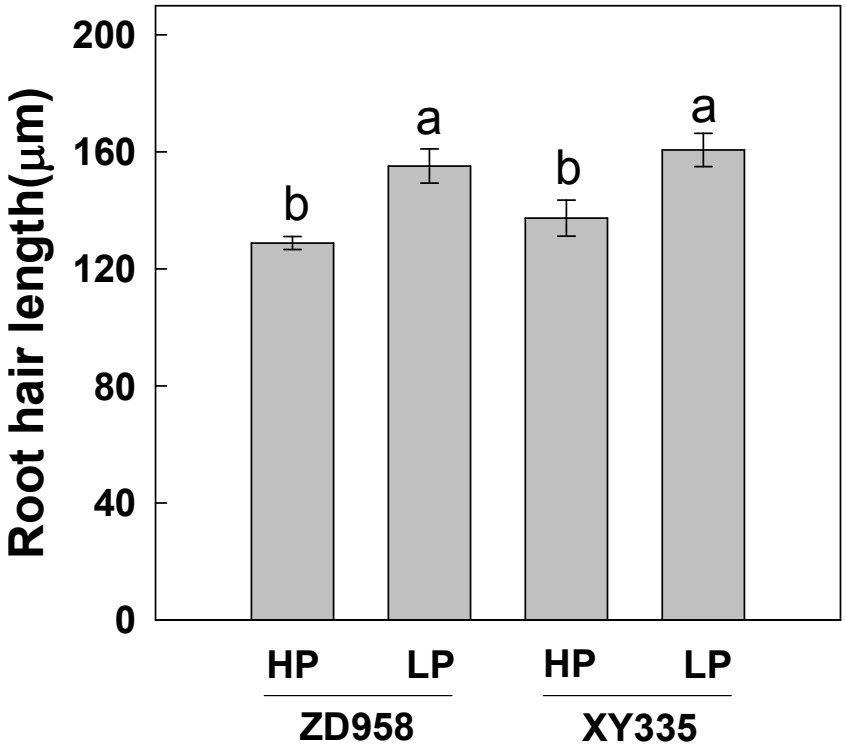

Figure 3. Mean root hair length for two maize cultivars, ZD958 and XY335, under low (LP) and high phosphorus (HP) soil. Data were the mean ±SE (n=4). Bars with the same letter are not significantly different.

Root physiological traits (Expt. 1)

Two-way ANOVA showed a significant effect of cultivar on citric acid concentration and of P on rhizosphere pH, citric and malic acid concentration, but no significant interaction between cultivar and P for 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. Low P supply significantly reduced rhizosphere pH in the both cultivars. 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 activity ranged from 367 to 389 μg *p*-nitrophenol h^{-1} g^{-1} dry soil. No significant difference was observed for APase activity between two P treatments and between the two cultivars. The

concentration of citric and malic acid was markedly enhanced under low P environment, but two maize cultivars presented different patterns (Table 3). The concentration of citric acid was significantly higher in ZD958 than in XY335 regardless P supplies. By comparison, the concentration of malic acid was not different between the two cultivars.

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 †	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±0.05b	1.57±0.10b
	LP	7.07±0.02b	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±0.02b
	LP	7.04±0.01b	389±34.8a	1.71±0.10b	2.74±0.18a

† Note: bulk soil pH: 7.97±0.03.

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 was progressively enlarged with the increase of P supply intensity (Figure 4). ZD958 had a significantly higher P uptake rate than XY335 when P supply intensity was greater than 12.5μM, with no obvious difference when supplied P less than 12.5μM.

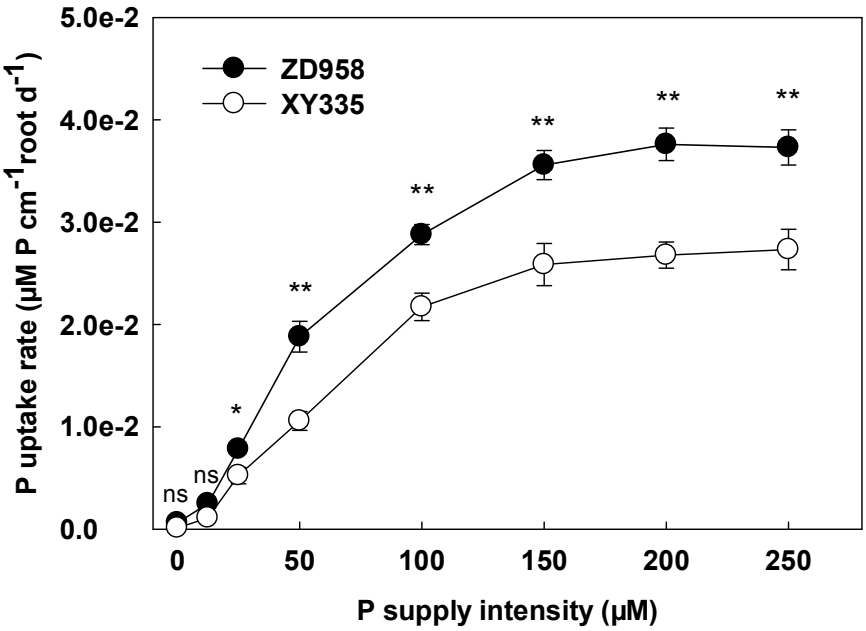

Figure 4. Effects of P supply intensity on P uptake rate in two maize cultivars (ZD958 and XY335). Data were the mean ±SE (n=4). Significant differences between the two cultivars for a given P supply: *, $P < 0.05$; **, $P < 0.01$.

4. Discussion

This study identified ZD958 as a P-efficient genotype and XY335 as a P-inefficient genotype, and explored the factors responsible for higher acquisition of soil P by ZD958 than XY335 by

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comparing root morphological and physiological adaptations to soil phosphorus deficit. The information generated by this study will be useful for establishing early selection strategies for P efficiency in maize.

In the present study, shoot P content of ZD958 was significantly higher than XY335 at both low- and high-P supplies, suggesting that ZD958 could access more P from soil than XY335. The difference in soil P acquisition was associated with the changes in biomass allocation, root morphology, root exudation, root physiological uptake capacity [5,10,13,37]. The patterns of biomass allocation are thought to assist in P acquisition by enhancing the capacity of root foraging and uptake [6,38]. When P is in short supply, plants tend to allocate a greater proportion of their biomass to their root system [38]. This study suggested that low P supply resulted in a greater biomass allocation to roots for the both cultivars, which confirms previous reports [16,22,32]. However, the two maize cultivars differed in biomass allocation as XY335 allocated a greater ratio of biomass to the roots (larger RMR) than ZD958 (lower RMR) regardless P supply (Table 2). Since P is relatively immobile in soil, increased biomass allocation to roots was thought to be beneficial for P acquisition of maize [22]. Interestingly, RMR does not closely correlate with shoot P content. XY335 with higher RMR produced a less shoot P (both P concentration and P content), whereas ZD958 with lower RMR produced higher shoot P. A detailed explanation about the relationship between root morphological and physiological traits with the acquisition of soil P is discussed below.

Root morphological traits play an essential role in the acquisition of soil P. The increases in TRL and RSA allow the plant to explore a larger volume of soil for the acquisition of soil P. When P is in a short supply, a positive response of TRL and RSA was observed for the acquisition of soil P. The results presented here revealed two different responses of maize roots to soil P shortage. Low P supply lead to a significant increase of TRL and RSA in ZD958 while an obvious decrease of these in XY335, suggesting that XY335 is more sensitive in response to soil P shortage and inferior in the acquisition of soil P as compared with ZD958. Maize has long been considered as a species with strong root morphological/architectural adaptations [21,24,25,27,39]. Previous studies have shown that maize genotypes with more lateral root branching, shallower root system and longer root hairs had greater the capacity in the acquisition of soil P [25,27,29]. The genes, Rtcs, Bk2 and Rth3, associated with root morphology, exhibited higher expression in the P efficient genotype (L3) relative to the P inefficient genotype (L22). Thus, it can be expected that maize genotypes with larger root system and optimum root architecture are often able to accumulate more P in its aboveground when soil P was in a limiting or sufficient supply. Different from the expectation, XY335, with a similar (at low P supply) or larger (at high P supply) TRL and RSA, exhibited the lower shoot P content than ZD958. In addition, no significant difference was observed for SRL and ARD between the two cultivars at 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 [41]. Several evidences indicated that RHL of maize can largely be improved by low P availability, which contributed to P acquisition [24,28]. In accordance with these studies, our results shown that P shortage significantly increased the RHL of two maize cultivars (Figure 2, Figure 3). Zhu et al. [29] suggested that maize genotypes with long root hairs under low P availability had significantly greater plant growth, P uptake and lower metabolic cost-benefit ratios than short-haired genotypes. Similarly, Bayuelo-Jimenez et al. [40] and Magdaleno-Armas et al. [24] found that P-efficient accessions had greater RHL under P deficit than P-inefficient ones. In the present study, the RHL was not different between two maize cultivars under low P or high P, indicating that RHL was not associated with the improved P uptake by ZD958. Taken together, our results suggest that changes in root morphological traits did not explain the improved P uptake by ZD958 roots. It is possible that the changes in root physiological traits might explain the improved P uptake by ZD958 roots.

When subjected to low P stress, plant roots often exhibit a wide range of physiological adjustments to access soil P [5,42]. Such physiological changes for P acquisition is generally associated with rhizosphere acidification [43,44], exudation of organic acid [43] and APase activity [45]. In the present study, low P stress, although not altered the activity of APase, caused a

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significant decrease of rhizosphere pH and an increase of organic acid exudation, suggesting that the dissolution of soil sparingly soluble P can be expected under low P supply, which enable maize plants efficiently to absorb P from the rhizosphere solutions. Gaume et al. [30] indicated that P deficiency significantly increased the exudation of malic and citric acids in three of the four maize genotypes, which was in accordance with the present results. The rhizosphere acidification has already been known to be able to increase P intensity in the rhizosphere, and therefore to improve the uptake of soil P by plant roots [46-48]. 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 [49]. At low P or high P supply, a similar rhizosphere pH and malic acid concentration in two maize cultivars excluded their contribution to P uptake. In the present study, it was not to be expected previously that the concentration of citric acid was higher in ZD958 than in XY335. Gaume et al. [30] claimed that low-P tolerant maize genotype 'NST' could exudate higher amounts of citric acid than low-P susceptible genotype 'SA3' under low P conditions. These results suggested that the higher P uptake by ZD958 than XY335 can be attributed to its ability to release more citric acids from its roots. It is unlikely that the difference in P acquisition between the two maize cultivars was caused by the changes in APase activity since the activity of the enzyme was not significantly different between two maize cultivars.

Apart from the root exudates, root physiological uptake capacity play an essential role in the acquisition of soil P. Plant roots can improve their P uptake capacity by adjusting their morphological and physiological traits to meet changes in shoot P demand [13,50,51], which provided 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 exhibited a significantly higher P uptake rate under high P supply but not under low P supply than XY335 (Table 2), indicating that P uptake rate was 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, making it not statistically obvious for P uptake rate. This result was further validated in the separate hydroponic experiment demonstrating the correlation between P uptake rate and P supply intensity (Figure 4). These results suggested that change in P uptake rate was largely regulated by cultivars and P supply intensity. Taken together, our results indicated that the higher P uptake by ZD958 than XY335 can be largely attributed to its ability to improve P uptake rate.

Although root physiological adaptations arise as a possible mechanism for better P acquisition, root architecture-based mechanisms are important for the acquisition of sparingly soil P as shown in maize by other studies [24,25,27]. The differences of two maize cultivars in root architectural traits have not yet been explored in this study. It is likely that ZD958 might have a greater lateral root branching and shallower root systems contributed to improved P uptake as compared with XY335. In P-limited soils, plants may depend largely upon the mycorrhizal association to acquire this nutrient [52]. It has been shown that mycorrhizal symbiosis provides up to 60% of the total requirement of P in maize [53]. Hyphal exudates of AM could release organic acids into soil and contribute to increased P uptake of colonized plants [54]. Extraradical hyphae of AM and ectomycorrhizal fungi also could release APase into the hyphosphere contributing to P uptake of plants [55,56]. It is suggested that a complex interaction exists between host plants and mycorrhizal fungi [57]. It is suggested that PGPRs associated with plant roots not only promote their growth and also improve P uptake and help in counteracting the detrimental effects of soil stresses [58]. Since the air-dried soil used in this study represented very low spore density of AM fungi and hyphal infection on maize roots at harvest (data not shown), the effects of AM fungi in P acquisition could be ignored. However, further studies are required for comparing the differences in root architectural traits, mycorrhizal associations and PGPRs between two maize cultivars.

In most of cases, P-efficient genotypes tend to respond to P stress in a less extreme way than inefficient ones [40,59]. This implies that P-efficient genotypes were more tolerant to low P than P-inefficient genotypes. In the present study, ZD958 exhibited a low responsiveness in shoots and roots growth than XY335, suggesting that ZD958 is 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, N:P ratios are relatively stable and maintain at the level of mean 12.7 ratio at a global scale [60]. This is especially true for maize in an agricultural system with fertilization. When N and P are in a sufficient supply, N:P ratios ranged from 18.75 to 22.63 [61], indicating that the growth of maize plants is required for more N than P. Therefore, it can be predicted that N-efficient maize genotype have an advantage in plants growth over P-efficient genotype. 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 genotype has a greater superiority in growth than P-efficient ZD958 since higher N requirement than P in maize plants. When P is in a limited supply, XY335 behave an inferior than ZD958. Further studies are required for examining interactive effect between N and P on root adaptations of two maize cultivars.

In this study, we added KCl to compensate KH_2PO_4 starvation in low P treatment. This compensate increased unavoidably the concentration of Cl^- in low P soil. It is suggested that critical toxic value of chloride for maize is 600 mg kg^{-1} soil [62]. The effect on maize growth and P uptake could be significant only when the Cl^- concentration is above the critical level i.e. 600 mgkg^{-1} soil. Concentration of initial soil chloride is 14.8 mg kg^{-1} soil. After adding compensated KCl to the low P soil, Cl concentration was 187.1 mg kg^{-1} soil. Therefore, it can be expected the compensated Cl could have less effect on maize growth and P uptake. In the future studies, we may consider the use of $NH_4H_2PO_4$ as P source and adjust N in low P soil with the addition of NH_4NO_3.

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 suffered from Al toxicity. The exudation of proton is not beneficial since concentration of Al^{3+} is higher in more acidic soil. Thus it can be seen that different breeding strategies are required for consideration to improve P acquisition in three different soil types. Therefore, further studies involving different types of soils and more genotypes are required to determine root adaptations to low P stress.

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