ORIGINAL ARTICLE

Divergent Molecular and Physiological Response of Two Maize Breeding Lines Under Phosphate Deficiency

Yanling Sun[1] · Hongxia Zheng[2]

Abstract
Inorganic phosphate (Pi) deficiency is a main limiting factor on crops growth and to select low-Pi tolerant breeding lines is very significant for crop breeding. Here, two contrasting maize (*Zea mays* L.) breeding lines showed different physiological response to Pi deficiency. The low-Pi tolerant QXN233 maintained normal growth, including high fresh weight, green leaves, strong shoots, and numerous roots relative to that of the sensitive MH05-4, mainly due to QXN233' high Pi content in shoots under Pi deficiency. Importantly, some Pi-responsive genes were detected, and among them, Pi transporters *ZmPHT1;1* and *ZmPHT1;9* as well as phytase gene *Zmphytase 2* were expressed increasingly in QXN233 compared to MH05-4 under Pi deprivation or Pi resupply. Moreover, QXN233 had higher proline content, soluble sugar content, and SOD activity than MH05-4, related with its tolerance. Taken together, this study enriches the understanding of the mechanism of maize responding to Pi deficiency.

Introduction

Phosphorus (P) is an essential plant macronutrient, involved in the protein and nucleic acid biosynthesis, and energy metabolism, photosynthesis, respiration, the regulation of enzymes, and signal transduction cascades (Yang and Finnegan 2010). P is mostly at its bound or organic forms in soil for plants hardly to acquire, and thus plants usually

Key Messages
- The tolerant QXN233 has better growth and high Pi content in shoots relative to that of the sensitive MH05-4
- Pi transporters ZmPHT1;1 and ZmPHT1;9 as well as phytase gene Zmphytase 2 could be important regulators for the tolerance to Pi deficiency

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exposed to Pi deficiency (Schachtman et al. 1998; Chiou and Lin 2011). Inorganic phosphate (Pi) has become one of the most limiting factors for plants growth and crop productivity. Plants have a variety of adaptive strategies to survive in Pi deficiency, including many physiological and biochemical responses to remobilize and optimize metabolic process to acquire more Pi into plant (Rausch and Bucher 2002; López-Arredondo et al. 2014; Sawers et al. 2017; Wang et al. 2017). However, the underlying mechanism of plants response to Pi deficiency is not clearly clarified (Chiou and Lin 2011; Liu et al. 2016; Ham et al. 2018).

Plant roots is directly sensing local Pi levels and influenced by Pi deficiency, including the inhibition of primary root (PR) growth and the promotion of lateral root formation (Vance et al. 2003). Recent studies showed that Pi deficiency induces plant to accumulate high level of iron (Fe) in the apoplast of roots, resulting in the production of reactive oxygen species (ROS) and the increase of callose deposition in plasmodesmata, which interferes with the intercellular movement of SHORT ROOT (SHR) protein and causes the impairment of PR growth (Müller et al. 2015; Wang et al. 2019). Besides root level modifications, aerial parts of plants also show key phenotypic modifications (Ruan et al. 2018; Ceasar et al. 2020). Responding to Pi deficiency, plants adapted themselves to optimize Pi acquisition and

redistribute Pi into the young or starved tissues under the control of the Pi uptake and regulatory pathway (Rausch and Bucher 2002; Chiou and Lin 2011; Liang et al. 2014; Mitra 2015; Ham et al. 2018). Among them, a MYB-like transcription factor phosphate starvation response 1 (PHR1) protein plays central role, which is a key molecular to regulate the expression of a large number of P starvation-responsive genes, such as many Pi transporters (PHTs), induced by phosphate starvation 1 (IPS1), RNS1 (a RNase), and SPX genes by binding to their promoters through the cis-element PHR1-binding sequence (P1BS; GNATATNC) (Rubio et al. 2001; Calderón-Vázquez et al. 2011; Liu et al. 2018; Xu et al. 2018). SPX (named after SYG1, PHO81, and Xpr1) families were classified into four families based on the SPX, SPX-EXS, SPX-MFS, and SPX-RING domain for each, and involved in Pi homeostasis via binding different inositol polyphosphate signalling molecules (InsPs) with several other proteins, including PHRs (Secco et al. 2012; Liu et al. 2018; Ried et al. 2021). IPS1 antagonizes miR-399 activity, and miR-399 can further guide the cleavage of PHO2 RNA, which encodes an E2 ubiquitin conjugase–related protein that negatively affects shoot Pi content (Franco-Zorrilla et al. 2007). Thus, PHO2 mutations masked the effects of IPS1 overexpression on Pi accumulation in shoots (Franco-Zorrilla et al. 2007). IPS1 was found to be induced by low-Pi (LP) stress and hormone treatment (Franco-Zorrilla et al. 2007; Zhang et al. 2019). The PHO1 family members, containing both the SPX and EXS domains, involved in Pi loading into the xylem in the long-distance Pi deficiency signalling network (Wang et al. 2004; Stefanovic et al. 2007; Secco et al. 2012). Importantly, PHT1 ~ 4 families were taken part in Pi uptake in plants (Poirier and Bucher 2002; Lin et al. 2009; Liu et al. 2016; Sawers et al. 2017; Wang et al. 2017). Previously, *AtPHT1;1* and *AtPHT1;4* were remarkably enhanced in *Arabidopsis* roots in response to Pi-starvation. Overexpression of *PHT1;1* improved Pi uptake to promote the cell growth in tobacco (Mitsukawa et al. 1997; Lin et al. 2009), and knocking out of *PHT1;1* or *PHT1;4* impeded Pi uptake (Misson et al. 2004; Shin et al. 2004). In maize (*Zea mays* L.), the *ZmPHT1* families had been identified to be contributed to maintain Pi uptake and regulate Pi homeostasis (Nagy et al. 2006; Gu et al. 2016; Liu et al. 2016; Wang et al. 2017). Moreover, phytate-P, which makes up 50% component of the total soil organic P, is poorly utilized by the roots of most plants, and phytases are a special class of phosphatase that hydrolyzes phytate to release inorganic Pi. Phytase-overexpressing transgenic maize could improve Pi availability (Chen et al. 2008).

Under different abiotic stresses, compatible osmoprotectants, such as proline, glycine betaine, trehalose, and polyols, are accumulated to protect plants from various abiotic stresses including Pi deficiency (Nuccio et al. 1999; Vinocur and Altman 2005). Under stresses, proline was highly increased and helpful to maintain osmosis balance, stabilize protein structure, and scavenge excess reactive oxygen species (ROS), associated with the increased/decreased expression of proline synthetases/dehydrogenase (Verslues and Sharma 2010; Spoljarević et al. 2011; Chen et al. 2013; Rai and Penna 2013; Sun et al. 2015). Recent research supported that proline content was positively correlated with maize crop yields (Spoljarević et al. 2011). Moreover, trehalose, as a non-reducing disaccharide, could be necessary for cellular membrane integrity to maintain osmotic balance in plants cell. It was all be accumulated among various plant species to resist with extreme conditions, and overexpression of *trehalose-6-phosphate synthase* (*TPS1*) gene could improve the tolerance of plants under stresses (Elbein et al. 2003; Avonce et al. 2005).

In recent years, plenty of Pi-responsive genes had been identified using high throughput sequencing technology and provided valuable information to understand the mechanism of plants adapt to Pi deficiency (Calderon-Vazquez et al. 2008; Lin et al. 2013; Oono et al. 2013; Pei et al. 2013; Sun et al. 2016a, b; Jiang et al. 2017). Here, two contrasting maize inbred lines, QXN233 and MH05-4, exhibited different physiological response under Pi deficiency, and this study tried to reveal the underlying mechanism of them during Pi deficiency.

Materials and Methods

Plant Growth and Treatments

QXN233 and MH05-4, as stable maize inbred lines, derived from Reid and Heterotic Pattern B (PB) inbred lines, respectively. Experiments were carried out in the artificial culture chamber at Shandong Academy of Agricultural Sciences/National Engineering Laboratory of Wheat and Maize. The light/dark time was 14/10 h and the temperature and relative humidity controlled at 26 ± 4 °C and 60–65%, respectively. The seeds were surface-sterilized by 10% NaClO for 10 min, germinated in a chamber at 28 °C, and then grown in the normal liquid Hoagland's nutrient solution for a week, its composition was as follows: 25 mM H_3BO_4, 2 mM $Ca(NO_3)_2$, 0.65 mM $MgSO_4$, 0.5 mM KH_2PO_4, 50 μM KCl, 25 μM Fe-EDTA, 5 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, and 0.005 μM $(NH_4)_6Mo_7O_{24}$. The fresh nutrient solution was replaced every day. Seven-day-old seedlings were treated with the low-Pi (LP) stress (0 mM Pi, K^+ was supplied by the equal concentration of KCl rather than KH_2PO_4) and optimum Pi (500 μM KH_2PO_4, control) solution for 0 day, 2 days, 14 days, or 45 days. In addition, after treated for 14 days under Pi deficiency, seedlings were then applied to the normal Hoagland's nutrient solution for 2 days as a Pi resupply. The 0.5 L solutions were applied newly every day.

For the quartz sand or soil analyses of pot, the seedlings at the three-leaf stage were treated with the LP solution for 20 days. The same 0.5 L fresh solutions as the above were applied every 2 days. The plant height, leaf width, leaf length, and fresh weight (FW) of leaves or roots were weighed by chemical balance and analyzed between each groups.

Measurements of MDA and Anthocyanin Contents

Seven-day-old seedlings were treated under Pi deficiency for 2 days, 14 days, or 45 days, respectively. MDA and anthocyanin contents were measured following the previous methods (Sunarpi et al. 2005; Sun et al. 2016a, b).

Measurements of Pi, Proline, Soluble Sugar, and Soluble Protein Content

The shoots were harvested from the different treated time points, and Pi content was determined following previous method (Sun et al. 2016a, b). Meanwhile, the leave samples were collected after treatment. The proline, soluble sugar, and soluble protein contents were analyzed, respectively, following as the previous protocols (Sun et al. 2015).

SOD Activity Measurement

Seven-day-old seedlings were treated for 2 days using a hydroponic assay under Pi deficiency and the leave samples were collected after treatment. Then, the leaves SOD activity from the Pi deficiency and control group was measured in accordance with the previously described protocols (Sun et al. 2015).

Quantitative Real-Time PCR (qRT-PCR) Analysis

Seven-day-old seedlings were treated for 14 days of Pi deficiency and 2 days of Pi resupply, respectively. Total RNA were extracted from the treated leave and root samples using the E-Z Nucleic Acid (E.Z.N.A) Plant RNA Kit (Cat.nos. R6827-01, OMEGA), and cDNA was synthesized by using 5×All-in-One RT MasterMix Kit (ABM, Canada). qRT-PCR was then performed using Ultra SYBR Mixture Kit on ABI 7500 Real-Time PCR System (ABI, USA) (low ROX, CW2601M, CWBIO), following the manufacturer's instructions: 95 °C for 2 min, 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. The primers for qRT-PCR were presented in Table 1. Using 18S rRNA as the endogenous control, each sample was analyzed thrice, and the $2^{-\Delta\Delta Ct}$ method was calculated the relative transcript abundance of the target gene (Schmittgen and Livak 2008).

Table 1 Primers used in qRT-PCR

| Gene ID	Gene name	Sequences (5′-3′)
AF168884	*18SrRNA*-F	CCATCCCTCCGTAGTTAGCTTCT
	18SrRNA-R	CCTGTCGGCCAAGGCTATATAC
GRMZM2G326707	*ZmPHT1;1*-F	TCGTCACGCTCGTCATCTCC
	ZmPHT1;1-R	GGCATCTTCATCCGCCAGTAG
GRMZM2G154090	*ZmPHT1;9*-F	CAACCAGGACAGGAGCAAGAC
	ZmPHT1;9-R	TGACGGGCTCCTCCTCTGA
GRMZM2G043336	*Zmphytase 2*-F	CGGGCAACCTGGCGTGG
	Zmphytase 2-R	CGCCTTGGTGACAGCCGC
GRMZM2G006477	*ZmPHR1*-F	TGGTGGGCAGATATAATGAATGATG
	ZmPHR1-R	CTATTGGGAGGACTAGCAACAGGAC
Zm00001d042935	*ZmIPS1*-F	GTCCTCATCCAACACCTTCC
	ZmIPS1-R	CACACGCACTCCATTACCAT
GRMZM2G171423	*ZmSPX1*-F	TTCGCATCACCAGCACCACC
	ZmSPX1-R	CGAACTTCATTGTTGCGATTGG
GRMZM2G466545	*ZmPHO1*-F	GCAGCAGCCCAGGAACACC
	ZmPHO1-R	GATGGACGCCGACAAGAGGA
DQ026301	*ZmP5CR*-F	CCAGCCTGTGCCAACCGC
	ZmP5CR-R	GTGCGGATGGCGGAGGC
DQ864376	*ZmP5CS*-F	GCGAGGAAGTGGGCAAGTGGT
	ZmP5CS-R	TTGGGGAGGTGGGGTGGC
AF529266	*ZmTPS1*-F	GGTTGCAGCGTTTCCTATTG
	ZmTPS1-R	AATCAAGAGATCGGTCCAGATG
GRMZM2G169890	*ZmSOD4*-F	TAAGCACCTGTGGCAACCGAT
	ZmSOD4-R	ACGAAACGGTCGGAATGCC
GRMZM2G427815	*ZmPOD3*-F	TGACTGCTTTGTCCAGGGGTG
	ZmPOD3-R	GACTGCCTCCACCTGTGCCTT

E
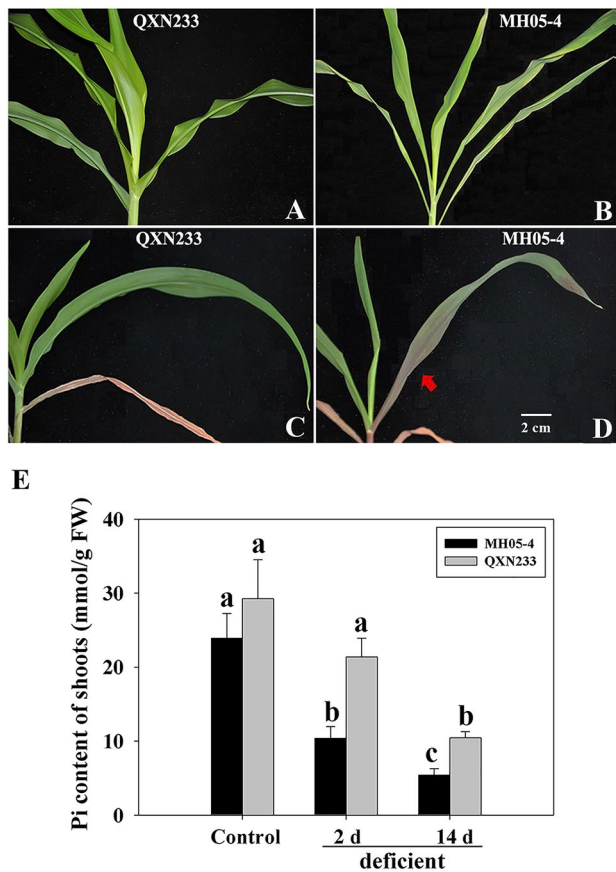
Fig. 1 The leaves phenotypic responses and Pi content of two contrasting maize breeding lines to Pi deficiency. The phenotype of QXN233 (**A**) and MH05-4 (**B**) under control condition and phenotype of QXN233 (**C**) and MH05-4 (**D**) under Pi deficiency for 45 days. The red arrow showed the red–purple leaves in MH05-4. Bar = 2 cm; Pi content of shoots in QXN233 and MH05-4 (**E**). Maize seedlings were grown under Pi deficiency for 2 days and 14 days. The lowercase indicates significantly different ($P < 0.05$, $n = 3$ replicates. 5–16 seedlings were tested for each genotype)

Statistical Analysis

The data were shown as average values ± standard deviations (SD) from three or five independent repeats. All data obtained were subjected to one-way analysis of ANOVA by Duncan's test with SPSS (17.0) software (IBM, USA), and the significant differences were defined at the P value (*$P < 0.05$).

Results

Characteristics of Two Contrasting Breeding Lines Under Pi Deficiency Stress

In the present study, several maize inbred lines were used to screen their tolerance under Pi deficiency based on the physiological LP-tolerance indexes. As a result, QXN233

kept the highest Pi content and superior performance, selected as the tolerant line, whereas MH05-4 showed the lowest Pi content and highest leaf anthocyanin content as the sensitive one, similarly to the sensitive QXH0121 in our previous study (Figs. 1 and 2; Table 2). In order to deplete endogenous Pi rapidly and exhibit a Pi deficiency symptom as soon as possible, 0 μM KH_2PO_4 condition was used as Pi deficiency for three-leaf-stage seedlings to suffer, while optimum Pi (500 μM KH_2PO_4) condition as a control. The results showed that QXN233 and MH05-4 both had weakly growth under Pi deficiency compared to normal conditions (Table 3). Obviously, MH05-4 seedlings exhibited an obvious red–purple in leaves, as a typical Pi-deficient symptom whereas the leaves of QXN233 seedlings remained green (Figs. 1, 2, S1 and S2). MH05-4 accumulated more MDA (Fig. 2A), and had smaller number of roots and lower fresh weight than QXN233 did under Pi deficiency (Fig. 2B–D; Table 3).

Significantly, Pi content of QXN233 has over twice than MH05-4 in shoots (Fig. 1E), indicating that an enhanced shoot Pi accumulation contributed to the tolerance of QXN233. Furthermore, the differences accumulation of compatible solutes between QXN233 and MH05-4 were investigated. The results showed that proline contents were both increased in two tested breeding lines under Pi deficiency, and QXN233 has higher proline content than that of MH05-4 (Fig. 3A); the same response of soluble sugar emerged between QXN233 and MH05-4 (Fig. 3B). Besides, QXN233 had slightly high soluble protein content than that of MH05-4 (Fig. 3C). In addition, SOD activity was higher in QXN233 than in MH05-4 under Pi deficiency (Fig. 3D).

Expression of Pi-Responsive Genes in Two Contrasting Breeding Lines

To test whether some important Pi-responsive genes were differently expressed between QXN233 and MH05-4 under Pi deficiency, the upstream regulators *ZmPHR1* (GRMZM2G006477), *ZmIPS1* (Zm00001d042935), *ZmSPX1* (GRMZM2G171423), and *ZmPHO1* (GRMZM2G466545), as well as Pi transporters *ZmPHT1;1* (GRMZM2G326707) and *ZmPHT1;9* (GRMZM2G154090), were examined by qRT-PCR. Under Pi deficiency, the tolerant QXN233 showed high expression levels of *ZmPHR1*, *ZmIPS1*, *ZmSPX1*, and *ZmPHO1* relative to that of MH05-4 (Fig. 4). For Pi transporters, *ZmPHT1;1* and *ZmPHT1;9* were both upregulated in QXN233 than MH05-4 under Pi deficiency (Fig. 5A–D), consistent with its high Pi content in shoots (Fig. 1E). Especially for *ZmPHT1;1* and *ZmPHT1;9*, were both increasingly in QXN233 leaves and roots after 14 days of Pi deficiency and subsequently 2 days of recovery, and enhanced by sevenfold and ninefold in QXN233 roots at 2 days of recovery,

Fig. 2 Changes in MDA and anthocyanin contents of leaves, the length of primary roots, and the number of total roots in QXN233 and MH05-4 under Pi deficiency. **A** MDA contents; **B** anthocyanin contents; **C** the length of primary roots; and **D** the number of total roots. Maize seedlings were treated for 14 days under Pi deficiency. The lowercase indicates significantly different ($P < 0.05$, $n = 3$ replicates. 10–12 seedlings were tested for each genotype)
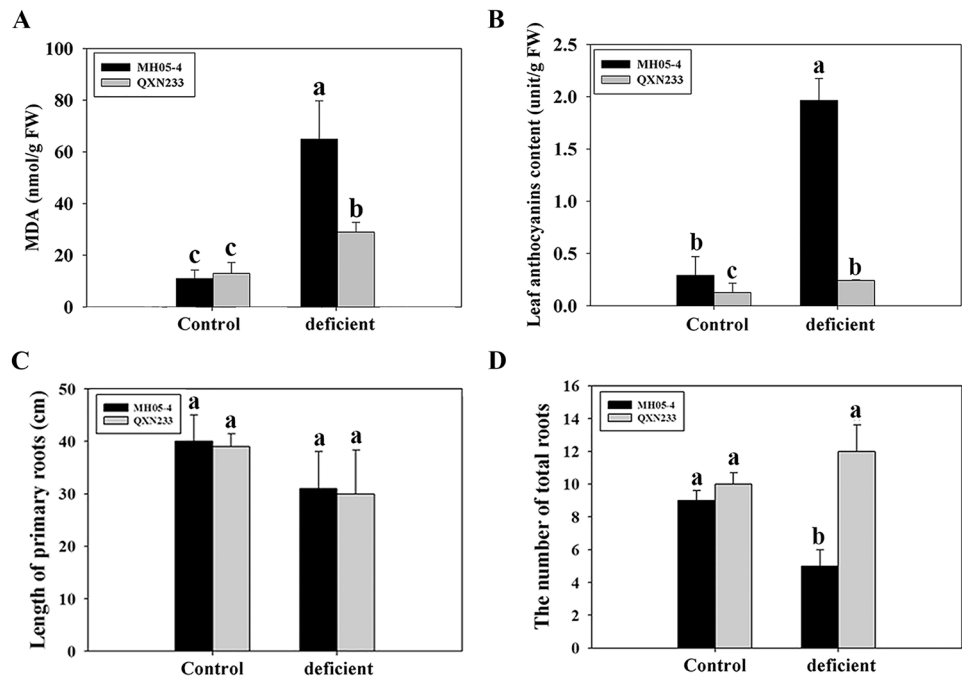
respectively (Fig. 5B and D). Also, MH05-4 has high expression levels of *ZmPHT1;1* and *ZmPHT1;9* in leaves or roots only at 2 days of recovery, and the degree of rise was low compared with that of QXN233 (Fig. 5A–D). Consistently, similar expressed pattern was also detected between QXN233 and the other sensitive QXH0121 (Fig. S4), previously reported in our study. These results suggested a stronger ability of Pi transport in tolerant QXN233 than the sensitive ones. Moreover, *Zmphytase 2* was elevated more in QXN233 than in MH05-4 under Pi deficiency or recovery (Fig. 5E and F). Especially in roots, the transcript level of *Zmphytase 2* was increased in QXN233, and had more than 2- and fourfold relative to that of MH05-4, suggesting that QXN233 has more phytase to catalyze phytate-P into available Pi, corresponding to its high Pi content (Fig. 1E). Also, a similar trend appeared between QXN233 and the sensitive QXH0121 (Fig. S4).

Expression of Stress-Responsive Genes in Two Contrasting Breeding Lines

Two proline-biosynthesis genes *ZmP5CR* and *ZmP5CS* were highly transcribed in QXN233 compared to MH05-4 (Fig. 6A and B), consistent with its higher proline content under Pi deficiency (Fig. 1E; Table 2). Moreover, QXN233 had increased expression of *ZmTPS1*, contributed to its high soluble sugar content under Pi deficiency (Figs. 3B and 6C). In addition, consistent with the high SOD activity in QXN233, the transcription levels of *ZmSOD4* and *ZmPOD3* were both enhanced 50- or 60-fold compared to that of MH05-4 (Figs. 3D and 6D–E), hinting that QXN233

possessed high SOD and POD activity to reduce oxidative damage and lipid peroxidation under LP stress.

Discussion

As one of common abiotic stresses, Pi deficiency severely impeded the plant growth and production in maize. To understand the mechanism of maize response to Pi deficiency, selecting tolerant materials are vital for breeding and crop yield. In our study, two maize breeding lines (QXN233 and MH05-4) were investigated to be exhibited contrasting responses under Pi deficiency. Obviously, QXN233 displayed a tolerant phenotype, including high fresh weight, strong shoots, and roots. Contrastingly,

Table 2 The measurements of anthocyanin and shoot Pi contents in eight tested maize genotypes. Maize seedlings were grown for 20 days under Pi deficiency using a quartz sand assay. Data were shown as means $\pm$ SD (*$P < 0.05$)

Maize inbred lines	Shoot Pi content (mmol/g FW)	Leaf anthocyanin content (unit/g FW)
MH05-4	18.9 $\pm$ 1.5	5.2 $\pm$ 0.3
Xianyu335	20.5 $\pm$ 5.8	0.7 $\pm$ 0.3
Qi319	21.4 $\pm$ 4.5	5.0 $\pm$ 1.0
Chang7-2	22.5 $\pm$ 5.3	4.4 $\pm$ 0.2
MH9311	24.0 $\pm$ 6.0	0.7 $\pm$ 0.4
Zheng58	28.3 $\pm$ 7.0	1.2 $\pm$ 0.6
MH03-2	28.7 $\pm$ 5.6	3.2 $\pm$ 0.1
QXN233	31.0 $\pm$ 6.7[*]	0.6 $\pm$ 0.1[*]

Table 3 Quantitative analyses of plant height, leaf width, and length of the longest leaf and fresh weights of leaves and roots of QXN233 or MH05-4 under Pi deficiency after 20 days, as assessed via a quartz sand pot experiment. The values represent the means $\pm$ SD (*$P < 0.05$)

Genotype	Treatment	Height (cm)	Leaf width (cm)	Leaf length (cm)	Fresh weight of leaves (g)	Fresh weight of roots (g)
QXN233	0 mM Pi	17.6 ± 4.3	2.4 ± 0.3	23.5 ± 3.0	2.8 ± 0.1	2.3 ± 0.4
	Control	33.2 ± 6.1	3.2 ± 0.1	33.3 ± 4.1	7.6 ± 0.5	5.4 ± 0.3
MH05-4	0 mM Pi	18.1 ± 2.3	1.8 ± 0.2*	21.6 ± 4.2	2.0 ± 0.2*	1.5 ± 0.3*
	Control	34.5 ± 1.2	3.1 ± 0.3	33.4 ± 3.2	7.2 ± 0.4	5.2 ± 0.2

MH05-4 exhibited weak performance and emerged purple leaves, as a typical symptom, and possessed high anthocyanin and MDA content, indicated that a high lipid peroxidation and ROS damage related with its inferior phenotype.

Previously reported, plants have adopted critical mechanisms to improve Pi acquisition and use efficiency under Pi deficiency (Poirier and Bucher 2002; Wang et al. 2017; Sawers et al. 2017). Many Pi-responsive genes, including Pi transporters and phytases, participated actively in Pi acquisition, transport, and homeostasis (Mudge et al. 2002; Nagy et al. 2006; Liu et al. 2016; Jiang et al. 2017; Wang et al. 2017; Ham et al. 2018). In the present study, the tolerant QXN233 showed twice high shoot Pi contents compared to MH05-4 under Pi deficiency, indicated that optimizing Pi allocation into shoot in maize probably as an important strategy to confront with Pi deficiency, which related with the roles of some Pi transporters. Among them, *ZmPHT1;1*, *ZmPHT1;9*, and *Zmphytase 2* were both enhanced obviously in QXN233 compared to that of the sensitive MH05-4 and QXH0121 under Pi deficiency or recovery, implying a vital role of them for Pi uptake and transport. Consistently, in our previous study, the RNA-Seq analysis showed that five maize inorganic Pi transporter genes, containing *ZmPHT1;1*, were highly expressed in the tolerant QXN233 (Sun et al. 2016a, b), associated with its tolerance. Thus, the Pi deficiency responsive genes are selected on the basis of their differential expression in tolerant and sensitive varieties based during Pi-deficient conditions. Nevertheless, they do not perfectly correlate with the Pi content, and possibly, these two genotypes have differential Pi requirements. Taken together, these results hinted us that *ZmPHT1;1*, *ZmPHT1;9*, and *Zmphytase 2* could be vital genes for the LP tolerance of maize, which could be checked in a multitude of breeding lines under Pi deficiency via GWAS (genome-wide association study). Moreover, PHT1;1 was reported to take part in xylem loading process of Pi in *Arabidopsis* (Fang et al. 2009; Lin et al. 2009). The biological functions of these Pi transporters could be confirmed via the single or combined overexpression or RNAi of them in plants. Significantly, the initial level of these transcripts is lower in the leaves of the tolerant QXN233 than the susceptible MH05-4, indicated


Fig. 3 Changes in compatible solutes contents and SOD activity in QXN233 and MH05-4 leaves. **A** Proline, **B** soluble sugar, **C** soluble protein contents, and **D** SOD activity in MH05-4 and QXN233 were measured after seedlings of them treated for 2 days under Pi deficiency. The lowercase indicates significantly different ($P < 0.05$, $n = 3$ replicates. 5–10 seedlings were tested for each genotype)
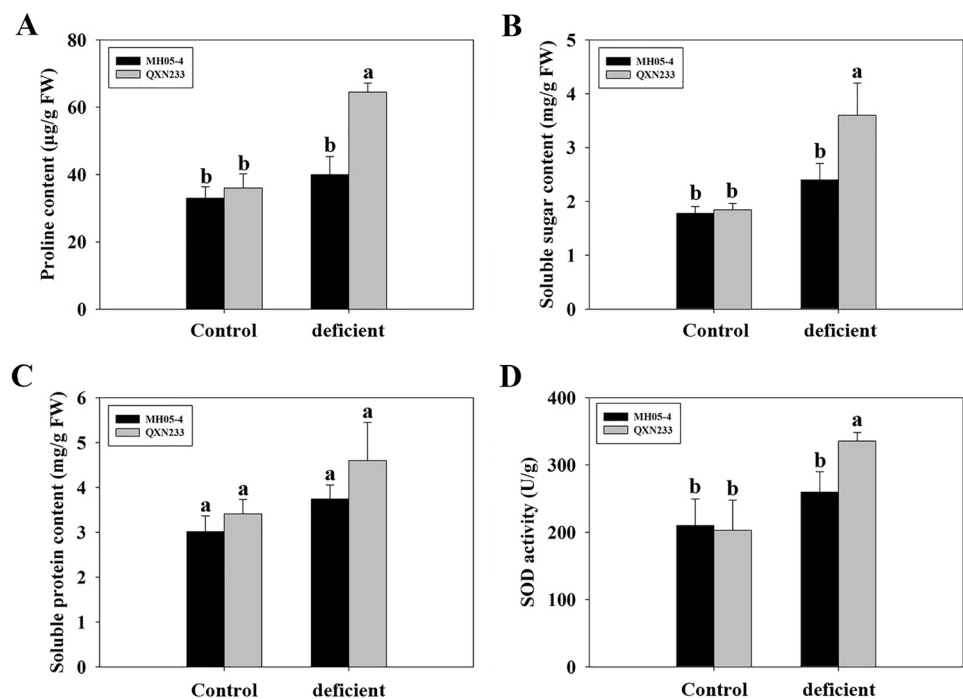
Fig. 4 Expression analysis of the upstream molecules of Pi signalling in QXN233 and MH05-4 by qRT-PCR. The transcript levels of *ZmPHR1* (**A**), *ZmIPS1* (**B**), *ZmSPX1* (**C**), and *ZmPHO1* (**D**) in leaves. Maize seedlings were treated for 2 days under Pi deficiency. Values given as mean ± SD ($P < 0.05$, $n = 5$ biological replicates)
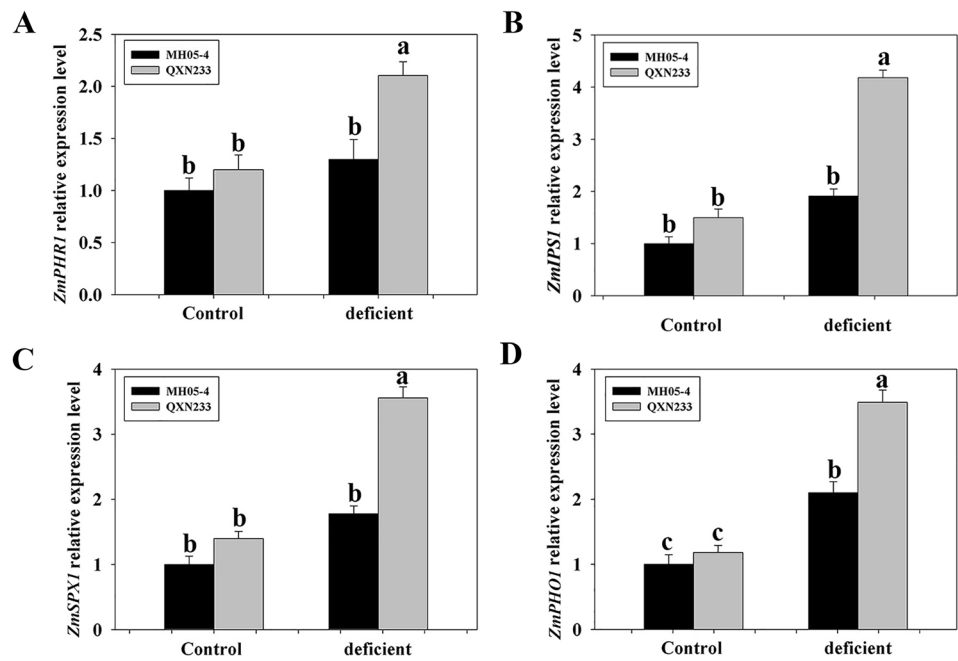
that not only the PHTs but also some upstream regulating factors of them are critical for the maintenance of plant LP tolerance. Correspondingly, the upstream regulators, such as *ZmPHR1*, *ZmIPS1*, *ZmSPX1*, and *ZmPHO1*, were detected to be upregulated in the tolerant QXN233 relative to that of the sensitive MH05-4. In addition, vacuolar phosphate efflux transporters were identified to be responsible for Pi release from the vacuoles to the cytoplasm in *Oryza sativa* (Xu et al. 2019), which should also be concerned on maize next.

Under various environmental stress, compatible solutes are accumulated to protect plant cell from osmotic pressure and oxidative damage (Matysik et al. 2002; Cuin and Shabala 2007; Szabados and Savouré 2010; Gupta and Huang 2014). In this study, proline and soluble sugar contents were induced in tolerant QXN233 under Pi deficiency, except for the soluble protein content, implying an importance of proline and soluble sugar in maize response to LP stress, which could be in function as osmoprotectants to reduce the membrane damage and maintain ion balance. In addition, proline content was found to be associated with grain productivity

in the maize (Spoljarević et al. 2011). Thus, it was inferred that the tolerant QXN233 had superior performance importantly related with its high proline content. Furthermore, proline accumulation is attributed to the enhanced transcript levels of proline–synthesis genes *P5CR* and *P5CS* in QXN233 plant. Sugars are required for the plant growth and development (Avonce et al. 2005; Elbein et al. 2003; Gupta and Kaur 2005), and also act as osmoprotectant or a signal molecule in signal transduction under different stresses (Yeo et al. 2000; Gupta and Kaur 2005). In this study, the tolerant QXN233 has higher soluble sugar content than MH05-4, consistent with its enhanced transcription of *ZmTPS1*, which could promote the strong growth under LP stress. Previously, SOD is considered as the most effective intracellular enzymatic protective system to scavenge excessive ROS under adverse stress (Gill and Tuteja 2010; Sun et al. 2016a, b). In this study, an increase of SOD activity was detected in QXN233 under Pi deficiency, in line with its remarkably high expression levels of *ZmSOD4* and *ZmPOD3*.
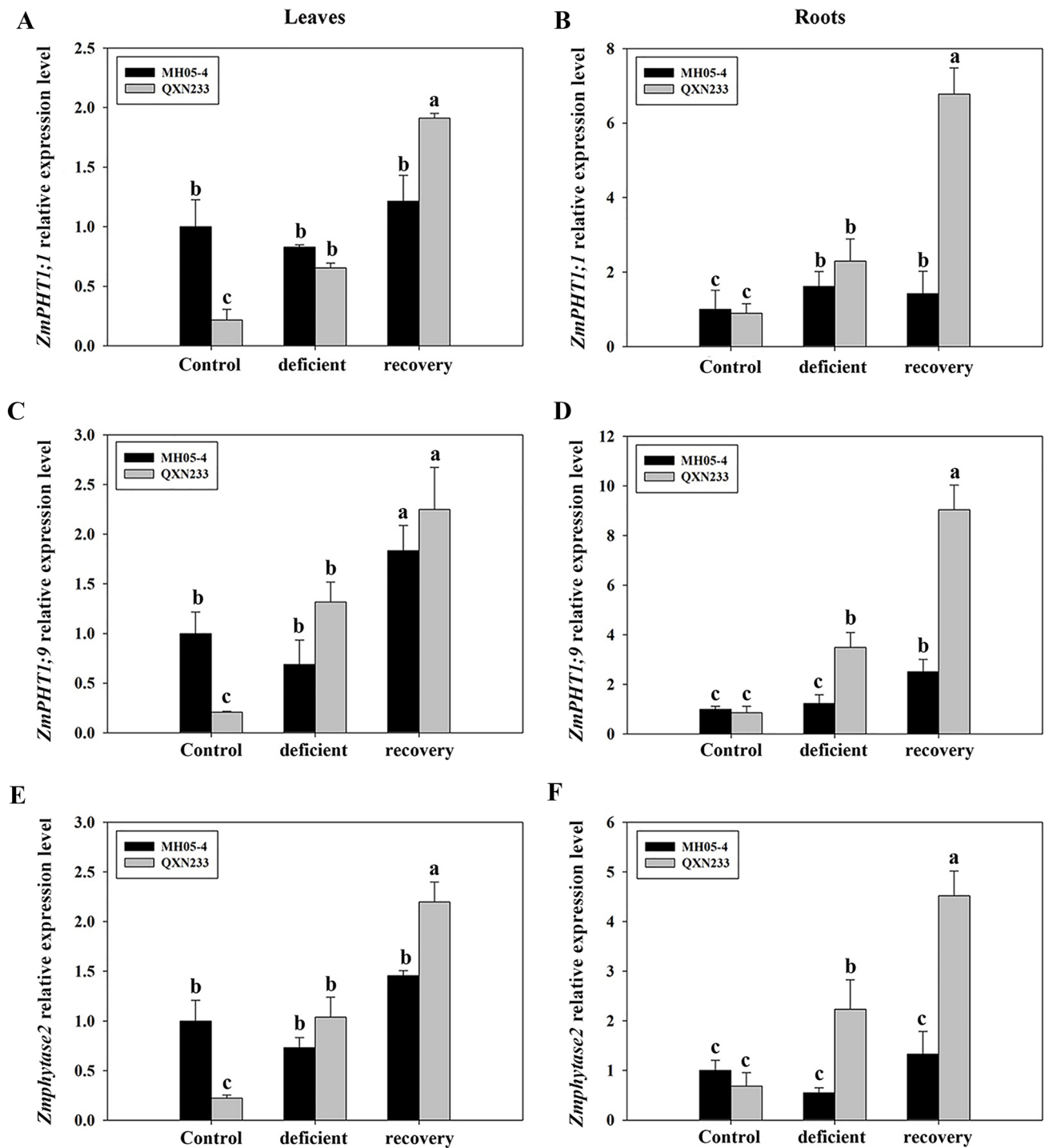
Fig. 5 Expression analysis of two Pi transporter genes and phytase in QXN233 and MH05-4 by qRT-PCR. The transcript levels of *ZmPHT1;1* (**A** or **B**), *ZmPHT1;9* (**C** or **D**), and *Zmphytase* (**E** or **F**) in leaves or roots. Maize seedlings were treated for 14 days under Pi deficiency and followed 2 days of Pi resupply. Values given as mean ± SD ($P < 0.05$, $n = 5$ biological replicates)

Fig. 6 Expression analysis of *ZmP5CR* (**A**), *ZmP5CS* (**B**), *ZmTPS1* (**C**), *ZmSOD4* (**D**), and *ZmPOD3* (**E**) genes in QXN233 and MH05-4. Maize seedlings were treated for 2 days under Pi deficiency. Values given as mean ± SD (*P* < 0.05, *n* = 5 biological replicates)
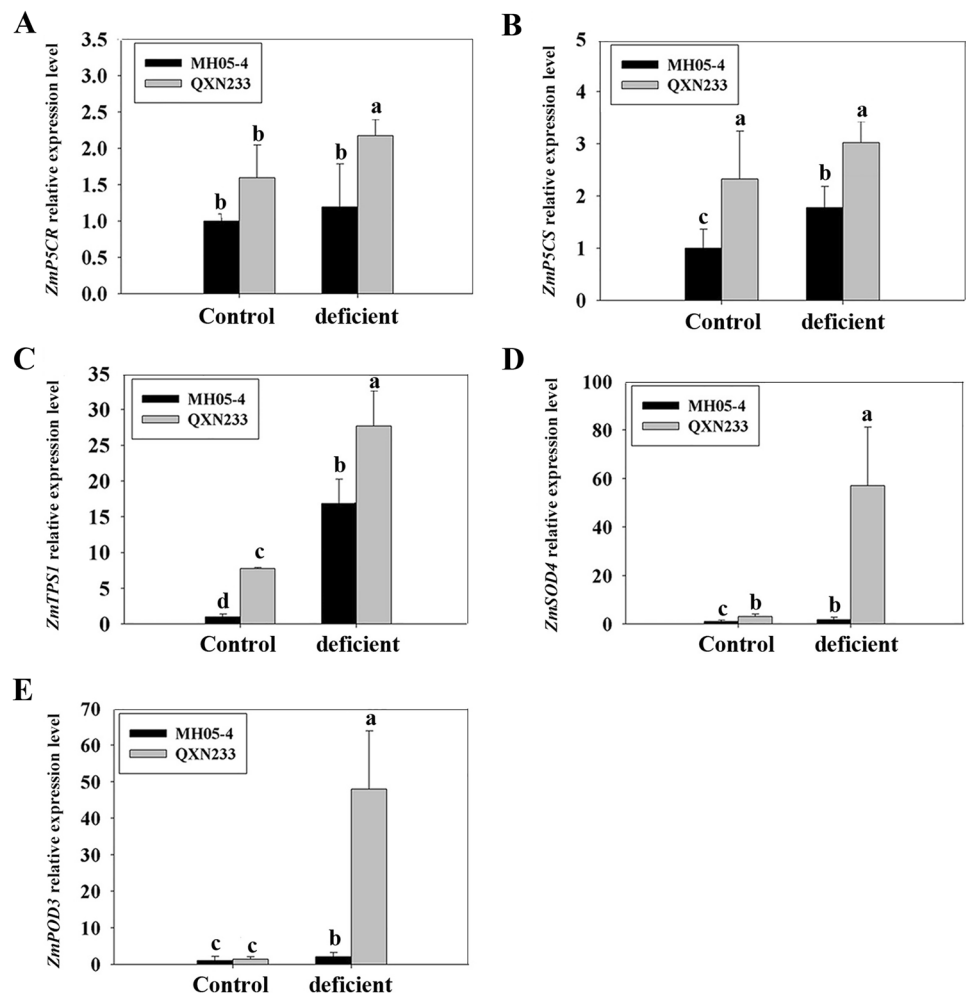
Conclusions

Two contrasting maize breeding lines, QXN233 and MH05-4, exhibit a tolerant and sensitive phenotype under Pi deficiency, respectively. QXN233 possessed high Pi content, compatible solutes, and SOD activity relative to MH05-4. Importantly, some Pi deficiency responsive genes, including *ZmPHT1;1*, *ZmPHT1;9*, and *Zmphytase 2*, play important roles for acquiring Pi in soil, especially for its roots under Pi recovery, indicated that these genes play vital roles in regulating Pi homeostasis under Pi deficiency.

Declarations

Conflict of Interest The authors declare no competing interests.

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