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Low straw phosphorus concentration is beneficial for high phosphorus use efficiency for grain production in rice recombinant inbred lines

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ABSTRACT

The objective of this study was to comprehensively investigate the relationship of phosphorus (P) concentration and accumulation with yield formation and P use efficiency for grain production (PUEg) using 127 rice recombinant inbred lines grown in a working field under low (LP) and high P (HP) conditions. Phosphorus concentration and accumulation, P translocation (PT) and translocation efficiency (PTE), PUEg, P harvest index (PHI), grain yield, and grain yield components were investigated. Wide ranges in grain yield, straw P concentration, total P accumulation, and PUEg were observed under LP and HP conditions. Coefficients of variance showed that the grain P concentration was considerably conserved, whereas the straw P concentration was relatively variable among inbred lines. In comparison with grain P, straw P made a larger contribution to total P accumulation (PUP) at maturity. Growth duration had no substantial effect on PUEg and positively affected P accumulation under both P conditions; however, it negatively affected the grain P concentration under the HP condition. The straw P concentration was negatively correlated with the grain filling percentage and harvest index. PUEg was negatively correlated with the grain and straw P concentrations, suggesting that low P concentrations, especially in straw, favored a high PUEg. There was no correlation between the P concentrations in grain and straw. The correlation analysis indicated that low straw P concentrations might be partly attributed to high PTE and PHI values. These results show that low straw P concentrations may simultaneously improve PUEg and grain yield by enhancing P translocation into grain, thus reducing the need for P fertilizer application.

1. Introduction

Phosphorus (P) is a critical element required for crop growth, development, and grain production. Rice is an extraordinarily important staple crop and a focus of significant effort aimed at improving its agricultural traits. A major focus of rice producers is increasing grain yield, which is often limited by the low availability of P in soil. P fertilizers are applied to alleviate P deficiency and have significantly contributed to increases in global food production since the green revolution (Fageria et al., 2011; Richardson et al., 2011). However, less than 20% of applied P is recovered by crops in the first year of growth, because most P applied to soil is converted into unavailable forms that cannot be easily absorbed and used by plants; therefore, farmers often apply additional P fertilizer to support high grain yield (Vance, 2001; Wang et al., 2010). However, excessive P application may exacerbate environmental degradation, so reducing P application by rice producers is desirable from an environmental point of view. Therefore, developing rice cultivars with high P uptake and use efficiency, and thus reduced P demand, is a significant research focus because of the potential for such a cultivar to offer increased grain yield and reduce the amount of P that must be applied during rice production.

Mechanisms for enhancing P efficiency include improvement of 1) P acquisition from the rooting environment; 2) P movement and redistribution within the plant; and 3) P utilization via metabolism and growth (Ahmad et al., 2001). Approaches for increasing P acquisition in a low-P environment include alteration of root morphology and architecture (Ramaekers et al., 2010; Richardson et al., 2011), activation of high-affinity transporters (Rausch and Bucher, 2002), secretion of phosphate scavenging and recycling enzymes into the

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rhizosphere (Hu et al., 2001), exudation of organic anions by roots (Hammond et al., 2004), and root symbiosis with mycorrhizae (Bucher, 2007). Further, P remobilization from senescing vegetative parts to young developing parts has been interpreted as a strategy to cope with P deficiency in some plants (Ahmad et al., 2001). P translocation from vegetative parts to grain also plays a key role in the development of wheat grain (Dordas, 2009; Horst et al., 1993). Additionally, low grain P concentration has been considered as a key trait for improving the P efficiency of a system via reduced P removal with grains (Vandamme et al., 2016a). Proper exploitation of these strategies can increase P uptake and enhance the utilization efficiency of P by cropping systems.

Phosphorus use efficiency is commonly defined as the total biomass or grain yield produced per unit of P absorbed by a crop. P use efficiency for biomass production is also equivalent to the reciprocal of the P concentration of the entire crop (Ahmad et al., 2001; Peng, 2011). Therefore, P efficient crops can maintain high biomass and grain yield under conditions of low tissue P concentration or low P demand (Richardson et al., 2011; Vandamme et al., 2016a). However, many reports have documented that P efficiency-related traits, such as P agronomic efficiency, physiological efficiency, and apparent recovery efficiency, are tightly associated with biomass accumulation and grain yield (Fageria et al., 2011; Zhu et al., 2012; Fageria, 2014). Several studies have revealed that P efficiency is positively correlated with grain yield in cereal crops. Su et al. (2009) found that there was a positive relationship between grain yield and PUE for grain yield in a doubled haploid population of wheat. Similarly, nitrogen use efficiency is positively correlated with grain yield in rice (Ju et al., 2006; Wei et al., 2011). These findings suggest that breeders can develop new rice cultivars with high grain yield and more efficient utilization of N/P (Peng, 2011). Additionally, PUE is significantly influenced by P application rate and plant genotype (Rose et al., 2016). These observations indicate that improving crop P use efficiency generally improves grain yield to some degree.

In addition to grain yield and total biomass accumulation, crop P demand and PUE are associated with P concentrations in grains, stems and leaves. Although reducing P application may reduce crop yield when crop P demand is unchanged (Vandamme et al., 2016a), lowering tissue P concentrations could reduce the P input required for crop production. Most studies related to P utilization reported the relationship among yield, yield components, the rate of P fertilizer application, and P accumulation in field and pot experiments (Fageria 2014; Vandamme et al., 2016a). Regarding tissue P concentrations, Batten (1992) and Calderini et al. (1995) reported that grain P concentration was significantly and negatively correlated with grain yield in wheat. However, Rose et al. (2010) did not observe a correlation of grain concentration with grain yield or grain weight, and grain P concentrations were also not associated with reduced grain yield in the 38 tested genotypes. Recently, Vandamme et al. (2016b) reported the genotypic variation in grain P concentration and estimated minimum grain P concentrations at various grain yields. However, there is little consistent information regarding the association among tissue P concentration and rice grain yield, which varies due to genotype, P application rate, and environmental conditions (Vandamme et al., 2016b).

Similarly, many studies reported that PUE was associated with grain yield, P fertilizer rate, and P uptake (Fageria 2014; Vandamme et al., 2016a). However, little information is available regarding the relationship between PUE and tissue P concentrations. High P acquisition efficiency and utilization efficiency are two key traits conferring high P efficiency in crops. Recently, low grain P concentration was identified as another key trait that may improve the P efficiency of cropping systems by minimizing P removal from fields (Rose et al., 2010; Vandamme et al., 2016a). Although a low grain P concentration may result in high-efficiency P utilization in a cropping system, the relationship of grain P concentration and PUE with grain/biomass production is not well understood. Few studies are available regarding the relationship between P efficiency-related traits and grain P concentration. Rose et al. (2010) observed no significant correlation between stem and leaf P concentrations and grain yield. Approximate 40–50% of P taken up by rice accumulates in the straw (stems and leaves). A high straw P concentration may result in low PUE for grain production because of enhanced P acquisition and P accumulation in straw biomass. However, little information is available regarding the relationship between straw P concentration and PUE-related traits.

Therefore, the objective of this study was to obtain a deeper insight into the relationships among P concentrations in grain and stems and P efficiency- related traits in rice using recombinant inbred lines. Such information should allow evaluation of the potential to improve P use efficiency and grain yield in rice by adjusting plant P concentrations in breeding programs.

2. Materials and methods

2.1. Plant materials and field experiments

A total of 127 lines were selected from recombinant inbred lines (RILs) derived by single-seed descent from a cross between Zhenshan 97 and Minghui 63 (*Oryza sativa* L. ssp. *indica*) (Xing et al., 2002), which are the parents of Shanyou 63, the elite hybrid most widely cultivated in the last two decades in China. The RILs show large genotypic variations in grain yield formation and nitrogen efficiency, and have been used in many studies with various intentions, such as identifying genes involved in grain formation (Xing et al., 2002), nitrogen use efficiency (Wei et al., 2011), and accumulation and translocation of stem nonstructural carbohydrates (Pan et al., 2011).

Field experiments were carried out in a farmer's paddy field in Dajin town, Wuxue City, Hubei Province, China (29°51′ N latitude, 115°33′E longitude), during the rice-growing seasons extending from May to October of 2008 and 2009. The experiments utilized a randomized complete block design with three replicates and two P conditions. For the low P condition (LP), no fertilizer P was applied. For the high P condition (HP), 40 and 60 kg P ha^{-1} was applied as basal fertilizer in the form of calcium superphosphate in 2008 and 2009, respectively. A total of 135 kg N ha^{-1} was applied as urea with three splits: 54 (40%) kg ha^{-1} as basal fertilizer, 40.5 (30%) kg ha^{-1} at 15 days after transplanting (DAT), and 40.5 (30%) kg ha^{-1} at 25 DAT. Potassium (100 kg K ha^{-1} as potassium chloride) was applied into two applications (50 kg ha^{-1} as basal fertilizer and 50 kg ha^{-1} at 25 DAT). Zinc (5 kg Zn ha^{-1}) was applied in the form of zinc sulfate heptahydrate as basal fertilizer. All fertilizers were applied during the early stages of growth because of the relatively short growth duration of several of the tested lines and the sampling schedules. Before the application of basal fertilizers, six soil samples were taken in the field and mixed thoroughly for measurement of soil characteristics. The soil was of the gleyed paddy soil type and had the following properties at 0–25 cm depth: pH 5.2, 25.9 g kg^{-1} organic C, 1.57 g kg^{-1} total N, 5.4 mg kg^{-1} available Olsen-P and 54.93 mg kg^{-1} exchangeable K

In both years, seeds were sown in a plastic plate in a nursery on May 17th, and seedlings were transplanted on June 15th at the 4th leaf stage. Transplanting was performed at a spacing of 0.20 m × 0.17 m with three seedlings per hill. The plot area was 8.2 m^2 with 16 hills per row and 14 hills per column. Appropriate irrigation was performed for high yield production. Pests, diseases, birds, and weeds were intensively controlled to ensure healthy crop growth and avoid yield losses.

Table 1
Mean ± SD, range and coefficient of variance (CV) for the investigated traits of the recombinant inbred lines (RILs) and their parents under low P (LP) and high P (HP) conditions.

Trait	LP						HP					
	Parents		RILs				Parents		RILs			
	Minghui63	Zhenshan97	Mean	Min.	Max.	CV (%)	Minghui63	Zhenshan97	Mean	Min	Max	CV (%)
PUEg (kg kg^{-1})	208 ± 41	225 ± 11	195	128	258	14.2	161 ± 13	188 ± 21	172**	92	227	15.5
Grain yield (g m^{-2})	700 ± 75	435 ± 38	570	316	890	16.5	655 ± 26	444 ± 47	578**	226	832	16.1
Dry weight of straw (g m^{-2})	886 ± 118	432 ± 41	728	434	1055	14.6	939 ± 25	418 ± 24	751**	436	1032	14.6
Grain P concentration (mg g^{-1})	3.43 ± 0.26	3.65 ± 0.35	3.51	2.79	4.08	7.0	3.69 ± 0.33	3.93 ± 0.52	3.58**	2.89	4.44	6.9
Straw P concentration (mg g^{-1})	1.51 ± 0.25	1.45 ± 0.08	1.71	1.14	2.53	16.2	2.09 ± 0.09	2.14 ± 0.16	2.15**	1.45	3.16	15.6
Plant P concentration (mg g^{-1})	2.29 ± 0.20	2.47 ± 0.12	2.44	1.96	2.88	7.0	2.70 ± 0.17	2.99 ± 0.23	2.73**	2.17	3.51	7.7
Grain P accumulation (g m^{-2})	2.06 ± 0.28	1.35 ± 0.08	1.72	0.97	2.56	16.7	2.08 ± 0.15	1.50 ± 0.12	1.77**	0.86	2.46	15.2
Straw P accumulation (g m^{-2})	1.36 ± 0.39	0.63 ± 0.09	1.26	0.60	2.13	23.4	1.96 ± 0.04	0.90 ± 0.12	1.62**	0.84	2.61	22.5
Total P accumulation (g m^{-2})	3.42 ± 0.56	1.98 ± 0.12	2.97	1.98	4.02	12.3	4.04 ± 0.17	2.39 ± 0.23	3.39**	2.32	4.22	11.1
PHI (%)	60.4 ± 7.5	67.8 ± 3.7	58.0	35.4	71.1	12.9	50.9 ± 1.1	62.3 ± 1.4	52.5**	31.6	67.7	14.5
PTE (%)	59.7 ± 10.8	76.3 ± 2.5	59.2	27.7	75.7	16.5	45.6 ± 6.2	71.3 ± 3.8	53.8**	24.4	73.3	20.1
PT (g m^{-2})	1.79 ± 0.28	1.07 ± 0.03	1.38	0.72	2.12	18.4	1.54 ± 0.33	1.41 ± 0.08	1.49**	0.68	2.12	19.5

PHI, P harvest index; PTE, P translocation efficiency; PT, P translocation; PUEg, P use efficiency for grain yield. *and ** represent significant differences for a trait at $p < 0.05$ and $p < 0.01$ between the low P and high P conditions among the RILs, respectively, as shown by two-way analysis of variance.

2.2. Sampling and measurement of traits

Heading date was defined as the time at which 50% of the hills in a plot had at least one panicle completely emerged and was quantified as the number of days from seeding to heading. The duration from heading to maturity showed very small variation across the lines (29–31 days), so the number of days from seeding to heading was considered as the growth duration in this study. Rice plants from each plot were diagonally sampled at ground level in the inner rows at the heading and maturity stages (8 and 12 hills, respectively). At heading, the samples were separated into leaves (including senesced and green leaves) and stems (including the young panicles and sheath). At maturity, the samples were separated into leaves, stems (including sheath) and panicles. The panicles were threshed by hand, after which filled and unfilled spikelets were divided by submerging them in tap water. Leaves, stems, rachis, and filled and unfilled spikelets were oven-dried at 80 °C to a constant weight for determination of dry weight. Grain yield (g m^2) and its components (panicle number per m^2, spikelets per panicle, grain filling percentage (%), 1000-grain weight (g), and harvest index (%)) were calculated.

Subsequently, oven-dried leaves, stems (including sheath and rachis) and grains were separately ground into powder using a grinder, mixed thoroughly, and passed through a 1-mm sieve. Approximately 0.2 g of sample powder was digested with 5 mL of concentrated sulfuric acid and hydrogen peroxide, after which the P concentration of the sample was determined via the molybdenum blue colorimetric method described by Murphy and Riley (1962) using a continuous-flow analyzer (Futura, Alliance, France). The P concentration (mg g^{-1}) was defined as the amount of P per g dry matter of each plant part. The straw P concentration (mg g^{-1}) was calculated as the amount of P per g dry weight of stems and leaves.

At maturity the whole rice plant was divided into fully filled grains and straw. The P accumulation (g m^{-2}) in each plant part was calculated by multiplying the P concentration by the dry weight. The P harvest index (PHI, %) was calculated as the ratio of the grain P accumulation to the total P accumulation of the aboveground parts at maturity (PUP). The P use efficiency for grain yield (PUEg, kg kg^{-1}) was calculated as the grain yield divided by the total aboveground P accumulation.

Phosphorus translocation (PT, g m^{-2}) and P translocation efficiency (PTE, %) were estimated according to the methods of Dordas (2009). PT (g m^{-2}) was defined as the plant P accumulation at heading minus the P accumulation in the leaves and stems at maturity. PTE (%) was calculated as the ratio of PT to the P accumulation at heading.

2.3. Data analysis

Mean values over the two years of the experiment were used for statistical analyses. The coefficient of variance (CV, %) was used to assess the degree of variation of a certain trait among RILs. The CV was calculated as the ratio of the standard deviation to the mean across 127 RILs. Two-way analysis of variance (ANOVA) was performed to determine significant trait differences among the RILs exposed to the low P and high P conditions. Pearson correlation analysis and Student's *t*-test (two-tailed) were performed using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). Direct (path coefficient) and indirect effects were calculated using a specific program performed with SAS software. Direct and indirect path coefficients (effects) were calculated as described by Dewey and Lu (1959) and Li (1975).

3. Results

3.1. Phenotypic variation

Under both P conditions, Minghui 63 showed higher performance in grain yield, dry weight of straw, grain and straw P accumulation, total P accumulation, and PT; however, Zhenshan 97 had higher PUEg, grain P concentration, plant P concentration, PHI, and PTE (Table 1). For the RILs, the mean values of PUEg, PHI and PTE were higher under LP conditions in comparison with those measured under HP conditions, while the mean grain yield, straw dry weight, and PT were lower under LP conditions. In general, the RILs showed wide variation in all investigated traits (Table 1).

Noticeably, under both P conditions, grain and whole plant P concentrations showed relatively little variation, with less than 8% coefficient of variance (CV), while the other ten traits showed more variation, with CVs from 11% to 23% (Table 1). However, the grain P concentrations and accumulations of the parent lines and the average values of these parameters across the RILs showed relatively small differences between the two P treatment groups. The straw P concentration and accumulation were higher under the HP condition in comparison with those measured under the LP condition. These findings indicate that the grain P concentration was relatively stable in comparison with the straw P concentration.

3.2. Relationships between P concentrations and grain yield-related traits

The whole plant P concentration at maturity was significantly negatively correlated with grain yield, especially under the HP con-

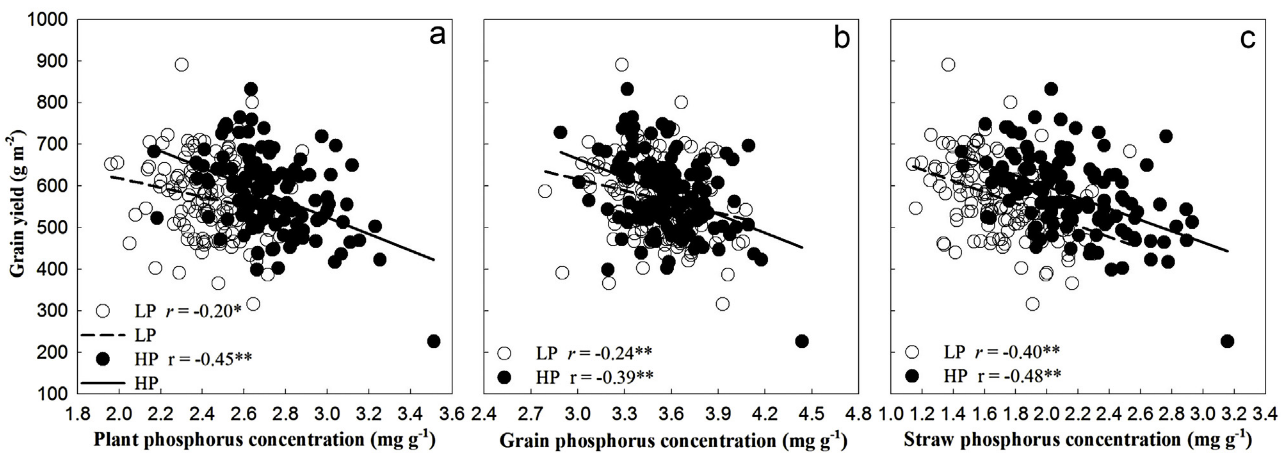

Fig. 1. Correlations of grain yield with plant P concentration (a), grain P concentration (b) and straw P concentration (c) under low P (LP) and high P (HP) conditions. * and ** indicate significance at $p < 0.05$ and $p < 0.01$ (n = 127), respectively.

Table 2
Relationship of P concentrations with yield components and harvest index under low P (LP) and high P (HP) conditions.

Trait	Panicles (No. m^{-2})	Spikelets $panicle^{-1}$	Spikelets (No. m^{-2})	Grain filling percentage (%)	1000-Grain weight (mg)	Harvest index (%)
LP						
Grain	0.04	−0.13	−0.12	−0.09	−0.09	−0.17
Straw	0.04	−0.07	−0.04	−0.38**	−0.01	−0.47**
Plant	0.20*	−0.15	0.02	−0.15	−0.11	−0.06
HP						
Grain	0.17	−0.21*	−0.11	−0.20*	−0.17	−0.16
Straw	0.03	−0.13	−0.15	−0.36**	−0.03	−0.49**
Plant	0.18*	−0.21*	−0.11	−0.27**	−0.16	−0.24**

* and ** indicate significance at $p < 0.05$ and $p < 0.01$ (n = 127), respectively.

Table 3
Relationships of P accumulations with yield components and harvest index under low P (LP) and high P (HP) conditions.

Trait	Panicles (No. m^{-2})	Spikelets $panicle^{-1}$	Spikelets (No. m^{-2})	Grain filling percentage (%)	1000-Grain weight (mg)	Harvest index (%)
LP						
Grain	−0.01	0.07	0.09	0.72**	0.17	0.62**
Straw	−0.20*	0.05	−0.12	−0.23**	0.18*	−0.69**
Plant	−0.17	0.09	−0.02	0.37**	0.28**	−0.06
HP						
Grain	0.06	0.01	0.09	0.72**	0.13	0.68**
Straw	−0.25**	−0.01	−0.23**	−0.24**	0.22*	−0.73**
Plant	−0.21*	0.01	−0.16	0.28**	0.30**	−0.22*

* and ** indicate significance at p < 0.05 and p < 0.01(n = 127), respectively.

dition (Fig. 1a); similar trends were also found between the grain P concentration and grain yield (Fig. 1b). In comparison with the whole plant and grain P concentrations, the straw P concentration showed a stronger negative correlation with grain yield under both P conditions (Fig. 1c). It is noteworthy that the correlation coefficients for the relationships between P concentrations and grain yield were generally low, regardless of significance. Moreover, straw P concentration was strongly and negatively correlated with grain filling percentage and harvest index under both P conditions (Table 2). These results show that exposure to a high straw P concentration adversely affected yield formation in the tested RILs.

3.3. Relationships between P accumulations and grain yield-related traits

Under both P conditions, the total P accumulation at maturity showed a significant positive correlation with grain yield (Fig. 2a). Strong positive correlations were observed between grain P accumulation and grain yield, with correlation coefficients of 0.91 under both P conditions (Fig. 2b). However, the straw P accumulation was negatively correlated with grain yield under both P conditions with low correlation coefficients (Fig. 2c). For the correlations between P accumulation and yield components, the total plant P accumulation was positively correlated with grain filling percentage and grain weight (Table 3). The grain P accumulation was significantly and positively correlated with the grain filling percentage and harvest index under both P conditions. It is noteworthy that the straw P accumulation was significantly and negatively correlated with the grain filling percentage and harvest index under both P conditions. These findings also demonstrate the adverse effects of high P accumulation in straw on yield formation by the tested RILs.

3.4. Effects of growth duration on P concentrations and accumulations

Under LP and HP conditions, P accumulations in grain, straw, and whole plants were significantly correlated with growth duration before heading; however, the PHI and PTE were negatively correlated with growth duration (Table 4). Growth duration had no effect on any P concentration under the LP condition; however,

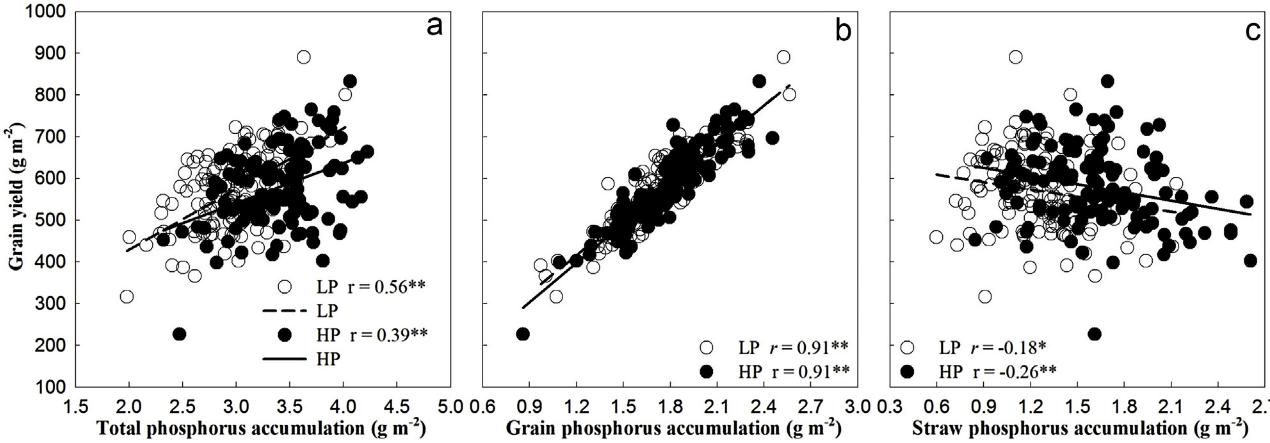

Fig. 2. Correlations of grain yield with total P accumulation (a), grain P accumulation (b) and straw P accumulation (c) under low P (LP) and high P (HP) conditions. * and ** indicate significance at $p < 0.05$ and $p < 0.01$ (n = 127), respectively.

Table 4
Relationships of growth duration before heading (days) with P concentration and accumulation and use efficiency under low P (LP) and high P (HP) conditions.

	P concentration			P accumulation			PHI	PUEg	PTE	PT
	Grain	Straw	Plant	Grain	Straw	Total				
LP	−0.13	0.09	−0.12	0.35**	0.54**	0.71**	−0.23**	−0.15	−0.40**	0.31**
HP	−0.36**	−0.02	−0.29**	0.25**	0.52**	0.68**	−0.26**	−0.10	−0.45**	0.02

PHI, P harvest index; PTE, P translocation efficiency; PT, P translocation; PUEg, P use efficiency for grain yield. * and ** indicate significance at $p < 0.05$ and $p < 0.01$ (n = 127), respectively.

growth duration was negatively associated with grain and whole plant P concentrations under the HP condition. Noticeably, growth duration had no effect on PUEg. Additionally, growth duration was positively correlated with PT under the LP condition.

3.5. Relationships of PHI with grain yield and PTE

Under the LP and HP conditions, the PHI showed a significant positive correlation with grain yield (Fig. 3a). The PHI was positively correlated with PTE under both P conditions (Fig. 3b). The straw P concentration was negatively correlated with PTE under both P conditions (Fig. 3c). Additionally, the straw P concentration was significantly and negatively correlated with PT ($r = -0.29$, $p < 0.01$, under the LP condition; −0.45, $p < 0.01$, under the HP condition) and the PHI ($r = -0.81$, $p < 0.01$, under the LP condition; −0.81, $p < 0.01$, under the HP condition). These results indicate that high P translocation to grain was associated with a high PHI and low straw P accumulation.

3.6. Contributions of grain and straw P concentrations to P accumulation

As shown in Fig. 4a, path analysis showed that the direct effects of pre-anthesis P accumulation on PUP (1.05 under the LP condition and 0.89 under the HP condition) were larger than those of post-anthesis P accumulation (0.65 under the LP condition and 0.77 under the HP condition, $p < 0.01$). However, the straw P concentration at maturity had larger direct effects (0.59 under the LP condition and 0.68 under the HP condition) on PUP, whereas the direct effects of grain P concentration on PUP were relatively small in comparison (0.32 under the LP condition and 0.30 under the HP condition, Fig. 4b). In comparison with the direct effects of straw

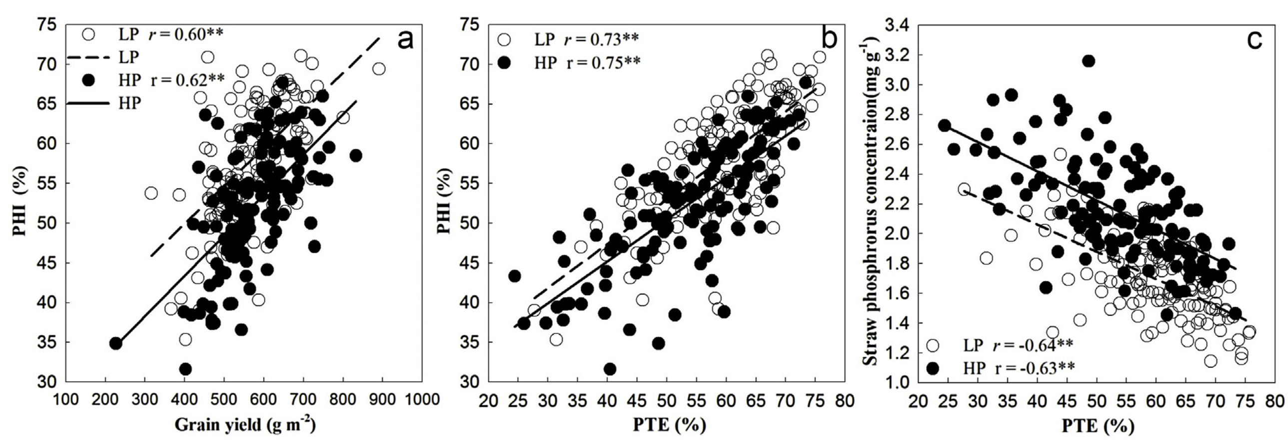

Fig. 3. Correlations between P harvest index (PHI) and grain yield (a), between PHI and P translocation efficiency (PTE, b), and between straw P concentration and PTE (c) under low P (LP) and high P (HP) conditions. * and ** indicate significance at $p < 0.05$ and $p < 0.01$ (n = 127), respectively.

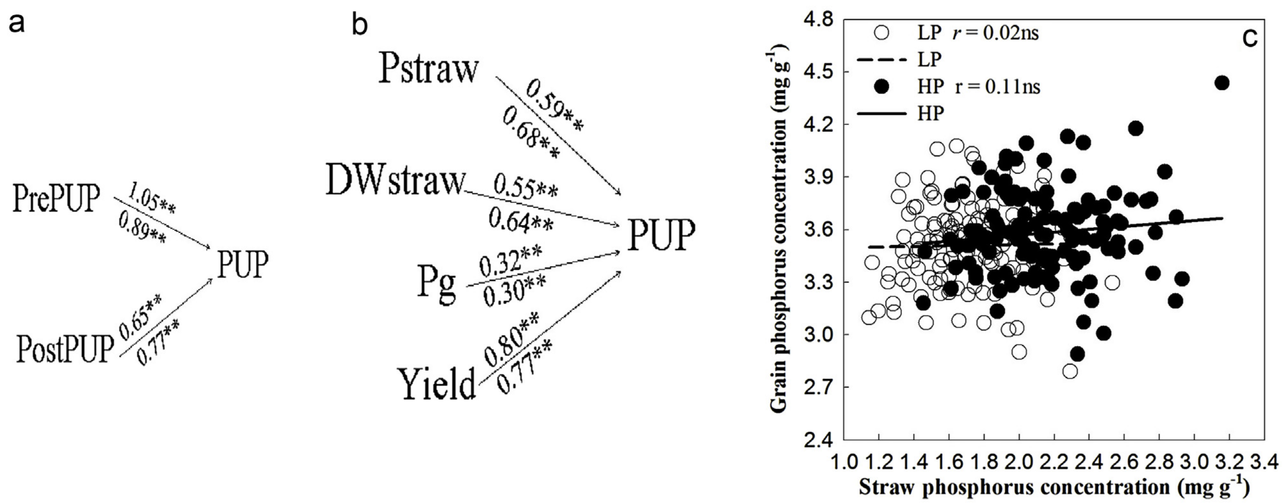

Fig. 4. Direct effects of pre-anthesis (PrePUP) and post-anthesis (PostPUP) P accumulations, straw and grain P concentrations (Pstraw, Pg), and straw biomass (DWstraw) and grain yield (Yield) on total P accumulation (PUP) under low P (above the arrow) and high P (below the arrow) conditions. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

biomass at maturity, grain yield had larger direct effects on PUP, especially under the LP condition.

As shown in Fig. 4c, there was no relationship between the straw and grain P concentrations under both P conditions ($r = 0.02$, $p > 0.05$, under the LP condition; $r = -0.11$, $p > 0.05$, under the HP condition)

3.7. Relationships of PUEg with grain and straw P concentrations

PUEg was significantly and positively correlated with grain yield (Fig. 5a). However, PUEg was significantly and negatively correlated with the grain and straw P concentrations under the LP and HP conditions (Fig. 5b and c). It is noteworthy that the correlation coefficients between the straw P concentration and PUEg were much stronger than those between grain P concentration and PUEg under both P conditions. However, PUEg showed a significant and negative correlation with straw biomass ($r = -0.41$, $p < 0.01$, under the LP condition; $r = -0.40$, $p < 0.01$, under the HP condition). The pathway analysis also showed that grain yield had positive direct effects on PUEg, whereas straw biomass had negative effects on PUEg (Fig. 5d). The straw P concentration showed a negative direct effect on PUEg; however, the grain P concentration showed a smaller negative direct on PUEg in comparison. These findings indicate that enhanced P distribution in straw adversely affected PUEg.

4. Discussion

4.1. Responses of plants to P fertilizer applications

Our study indicated that plant tissue P concentration and accumulation increased with the application of P fertilizer (Table 1). Similar results have been reported in wheat (Akhtar et al., 2011; Dordas, 2009) and rice (Fageria et al., 1988; Fageria et al., 2011). In contrast, Marschner (1995) reported that P content was not affected by application of P fertilizers because P is easily fixed to different soil constituents and therefore cannot be taken up by plants. In the present study, yield and P-related traits showed different responses to P fertilizer application. For example, total P accumulation increased by 14% on average; however, the straw and grain P accumulations increased by 29% and 3%, respectively. The average whole plant P concentration increased by 12%; however, the straw and grain P concentrations increased by 26% and 2%, respectively. Noticeably, these results suggest that the straw P concentration and accumulation responded well to P fertilizer application, while the grain P concentration and accumulation were relatively stable regardless of P application. These findings suggest that reducing P fertilizer application may reduce the straw P concentration and accumulation, but have only a small effect on grain. Therefore, crops may accumulate sufficient grain P for grain yield formation.

Fageria et al. (2011) reported that rice and wheat grain yields increased in a quadratic fashion as the P application rate was increased in the range of 0–88 kg P ha^{-1} in rice, while excessive P application reduced yield, depending on variety, and the optimal P application rate differed among varieties. Zhu et al. (2012) reported results in wheat similar to those of reported by Faggeria and colleagues in rice; however, in comparison with the LP treatment (no P fertilizer application), P application had little or no effect on grain yield regardless of large increases in P concentration and accumulation. It is possible that the environment provides sufficient P for yield formation, while further application of P results in accumulation of excess P, which is not always advantageous for grain formation. The detailed relationships between grain yield and P accumulation/concentration merit investigation in future studies.

4.2. Phosphorus concentrations/accumulations and yield formation

Rose et al. (2010) reported that the grain P concentration at maturity was not correlated with grain yield. However, in this study, the grain P concentration was found to be negatively and weakly correlated with grain yield under LP and HP conditions (Fig. 1b), despite the relatively small variation in grain P concentration among the genotypes. In wheat, Peterson et al. (1983), Calderini et al. (1995) and Manske et al. (2002) also found a negative relationship between the grain P concentration and grain yield. Therefore, to a certain extent, a low grain P concentration should ensure high P use efficiency without negatively affecting grain yield (Rose et al., 2010) and could improve the P efficiency of a cropping system by minimizing P removal with grain (Vandamme et al., 2016a). According to the theoretical framework for the relationship between grain yield and grain P concentration by Vandamme et al. (2016b), grain yield increases as the grain P concentration increases when the grain P concentration is low under P deficiency conditions. However, if the P supply is in excess of that required by the crop for optimal growth, excess grain P is unlikely to increase grain yield.

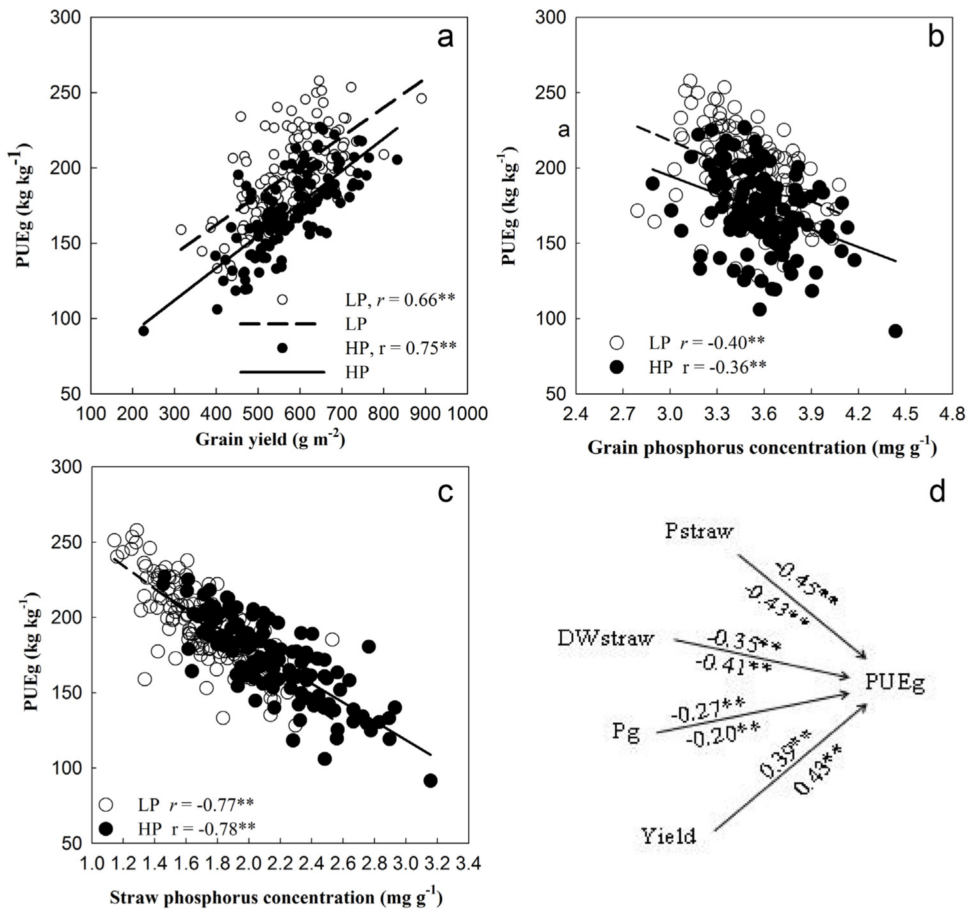

Fig. 5. Correlations of P use efficiency (PUEg) with straw and grain P concentrations (Pstraw, Pg) and grain yield, and direct effects of straw biomass (DW straw) and grain yield (Yield), Pstraw and Pg on PUEg under low P (above the arrow) and high P (below the arrow) conditions. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

In comparison with the relationship between the grain P concentration and grain yield, there was a much stronger and negative relationship between the straw P concentration and grain yield (Fig. 1c). A low straw concentration had positive impacts on the grain filling percentage and harvest index under both P conditions (Table 2). Therefore, our study and previous reports suggest that grain and straw P concentrations are closely associated with P efficiency, and it is possible for a genotype with relatively low P concentrations in particular parts of the plant, especially straw, to achieve high P efficiency. Although the P concentrations in grain and straw were found to be negatively correlated with grain yield, the negative correlation did not imply that low grain and straw P concentrations led to higher grain yield.

In contrast to the P concentration, the total plant P accumulation and grain P accumulation were positively correlated with grain yield (Fig. 2a and b). Similar results have been reported in rice and wheat (Calderini et al., 1995; Rose et al., 2010). However, straw P accumulation was negatively and weakly associated with grain yield (Fig. 2c). Generally, grain P accumulation showed significant positive correlations with the grain filling percentage and harvest index under LP and HP conditions, in contrast with the negative correlations between the grain P concentration and grain filling percentage under HP condition (Tables 2 and 3). Therefore, these findings suggest that the contributions of P accumulations in various plant parts to yield formation are different from those of P concentrations to some extent. Interestingly, high P distribution in the straw was associated with impaired yield formation. Taken together with the results described above, these findings suggest that a genotype yielding relatively more grain may show relatively high grain P accumulation and relatively low grain and straw P concentrations.

The straw P concentration showed large genotypic variation, as shown by analysis of the coefficients of variance of the RILs (Table 1). However, the analysis revealed a highly positive correlation between grain P accumulation and grain yield (Fig. 2b), highly negative correlations between grain yield and the straw P concentration (Fig. 1c)/straw P accumulation (Fig. 2c), no relationship between the straw and grain P concentrations (Fig. 4c), a small coefficient of variance for the grain P concentration (Table 1), and a negative correlation between the straw P concentration/accumulation and the two yield components (grain filling percentage and harvest index, Tables 2 and 3). These findings show that the grain P concentration was relatively stable across RILs, whereas the straw P concentration showed considerable genetic variation. Therefore, a low straw P concentration, which may be beneficial for yield formation, can be used as a criterion for estimation of high P use efficiency during the selection of genotypes for breeding programs.

4.3. Phosphorus concentrations and accumulations with PUEg

In this study, P concentrations in grain and straw were negatively correlated with PUEg (Fig. 5b and c) under both P conditions. Additionally, in comparison with the grain P concentration, the straw P concentration showed a stronger negative correlation and larger negative direct effects on PUEg (Fig. 5c and d). These results strongly suggest that the straw P concentration has a considerable influence on PUEg in comparison with that of the grain P concentration, implying that genotypes with low straw P concentrations are likely to have high PUEg. However, different relationships were observed between grain and straw P accumulations and PUEg in the present study. Grain P accumulation was positively correlated with PUEg ($r = 0.49$ under the LP condition and 0.64 under HP condition, $p < 0.01$), while straw P accumulation was negatively correlated with PUEg ($r = -0.78$ under the LP condition and −0.79 under the HP condition, $p < 0.01$). A low grain P concentration has been proposed as a trait that may improve P efficiency (Rose et al., 2010; Vandamme et al., 2016a). By considering the negative associations of P concentrations with grain yield (Fig. 1) and PUEg (Fig. 5), simultaneous improvement in P use efficiency and grain yield should be achievable in future breeding programs by reducing the straw P concentration. Additionally, a low straw P concentration may increase the P efficiency of a crop system by reducing the amount of P removed from fields at harvest, as has been reported for a low grain P concentration (Rose et al., 2010; Vandamme et al., 2016a). However, the critical and optimal grain and straw P concentrations with regard to increasing PUEg have not been identified and merit further study.

It is often argued that a low whole-plant P concentration can be achieved by reducing the concentration of inorganic P in vacuoles or by enhancing internal P reutilization (Richardson et al., 2011). Phosphorus-efficient plants with low P concentrations usually remobilize P from metabolically inactive sites to active sites in non-mature tissues, and this strategy is adopted by some plants to tolerate low P supply (Ahmad et al., 2001; Akhtar et al., 2008; Richardson et al., 2011). The studies of Dordas (2009) and Horst et al. (1993) also revealed that P re-translocation from vegetative tissues to reproductive tissues is important for grain development, as confirmed by the positive relationship between the PHI and grain yield in the present study (Fig. 3a). PUEg can also be calculated by dividing the PHI by the grain P concentration. In our study, significant positive correlations were observed between the PHI and PUEg ($r = 0.87$ under the LP condition and $r = 0.90$ under the HP condition, $p < 0.01$). Therefore, increased P translocation to grains was associated with an increased PHI, which in turn may have increased PUEg. Our results indicate that high PTE may increase the PHI and reduce the straw P concentration (Fig. 3b and c). The straw P concentration was significantly and negatively correlated with PT ($r = -0.29$ under the LP condition and −0.45 under the HP condition, $p < 0.01$) and the PHI ($r = -0.81$ under the LP condition and −0.80 under the HP condition, $p < 0.01$). Therefore, a lower straw P concentration may be partly attributed to more PT, higher PTE, and a higher PHI. These results suggest that, when P fertilizer application is slightly reduced, sufficient P may be accumulated in grains for yield formation by transferring straw P to grains, and high PUEg may be achieved.

As shown in Table 4, a long duration of growth may increase P accumulation and P translocation; however, growth duration had no effect, or a negative effect, on the P concentration, PHI, and PTE. Interestingly, we observed that growth duration had no effect on PUEg under either P application condition. These findings suggest that genotypes with growth periods of short duration may show reduced plant P concentrations and increased P re-distribution from straw to grain for yield formation. In several cropping systems aimed at achieving high annual efficiency of resource utilization, such as rice-rice-rapeseed systems, rice growth duration is a limiting factor on efficiency, and varieties with short growth duration are often utilized by breeders and farmers. Our results suggest that high-yield rice varieties with short periods of growth do not show low P efficiency, while the P demand for grain yield may be met by enhancing P movement from straw to grain.

Rose et al. (2011) showed that PUEg was always confounded by total plant P accumulation in rice. The most straightforward way to test whether PUEg represents true efficiency is to test for a positive correlation with grain yield (Rose and Wissuwa, 2012). In our study, PUEg was found to be tightly correlated with grain yield under both P conditions (Fig. 5a), while grain yield had a large positive direct effect on PUEg (Fig. 5d), suggesting that PUEg as investigated in our study reflected the true P use efficiency to a large extent. Therefore, considering the large genotypic variation in grain yield and PUEg, selection of a genotype with high yield and P use efficiency is possible.

Increasing grain yield without reducing grain P concentration can be achieved by improving translocation of P into grain (Manske et al., 2002). Selection of genotypes with high PTE and PHI might have the potential to prevent excessive reductions in grain P concentration and could alleviate the adverse effects associated with a low grain P concentration, including degraded seed quality, less vigorous seedlings, and reduced dry matter production (Bolland and Baker, 1988; Bolland and Paynter, 1990). Recently, Pariasca-Tanaka et al. (2015) reported that it may be possible to reduce seed P concentrations without negatively affecting seedling vigor or yield. In addition, the straw P concentration was found to have no association with the grain P concentration (Fig. 4c). Therefore, reducing the straw P concentration does not always reduce seedling vigor.

Vandamme et al. (2016a) proposed that a P-efficient genotype would thus have high P acquisition efficiency and P utilization efficiency traits in combination with a low grain P concentration trait. According to our data, high PUEg may be achieved by reducing straw P accumulation via lowering the straw P concentration and increasing P re-distribution to grains after flowering. Moreover, our results show that a low straw P concentration appears to be a suitable criterion for screening P-efficient rice genotypes, which generally show high PTE and a high PHI. Therefore, considering the large response of straw P concentration to P fertilizer application, it is significant that reducing the straw P concentration by enhancing P translocation to grains may simultaneously improve PUEg and grain yield, while reducing the required amount of P fertilizer application. Our data may provide a feasible approach to evaluate the PUEg of high yield rice by determination of the straw P concentration and P translocation rate during grain filling.

5. Conclusions

Wide genotypic variation was revealed for the straw P concentration, amount of straw P accumulation, PHI, PTE, PT, and PUEg in the RILs under the LP and HP conditions. The straw P concentration showed a large response to P application. However, the grain P concentration was relatively consistent among the genotypes and P conditions (Table 1). The contribution of the straw P concentration to PUP was greater than that of the grain P concentration at maturity; however, the contribution of grain yield to PUP was greater than that of straw dry weight (Fig. 4). Growth duration positively affected P accumulation, negatively affected the PHI and PTE, and did not affect PUEg under either P condition.

The grain P concentration was negatively correlated with grain yield, while grain P accumulation was positively correlated with grain yield (Figs. 1 and 2). The straw P concentration and accumulation were negatively correlated with grain yield (Figs. 1 and 2). Remarkably, the straw P concentration at maturity, which was independent of the grain P concentration and negatively correlated with PTE, showed significantly negative direct effects on PUEg (Fig. 5). This finding suggests that a low straw P concentration associated with enhanced P translocation from straw to grain allowed simultaneous improvement of PUEg and grain yield by reducing P fertilizer application. Therefore, adoption of a variety with low straw P concentrations in a cropping system may lead to increased P utilization by increasing PUEg and reducing P removal from the field.

Conflict of interest

The authors have no conflict of interest.

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