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Brief Report

Phosphorus Deficiency Alters Nutrient Accumulation Patterns and Grain Nutritional Quality in Rice

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Academic Editor: Gareth J. Norton
Received: 11 September 2016; Accepted: 24 October 2016; Published: 28 October 2016

Abstract: The accumulation of biomass and mineral nutrients during the post-anthesis period was investigated in field-grown rice plants cultivated in phosphorus (P)-sufficient vs. P-deficient soil. Phosphorus deficiency reduced biomass accumulation by around 30%, and reduced the accumulation of all nutrients in aboveground plant biomass except sulfur (S) and copper (Cu). Ultimately, grain zinc (Zn), Cu, and calcium (Ca) concentrations were significantly higher in P-deficient plants, while grain magnesium (Mg) concentrations were significantly lower. While P deficiency caused a 40% reduction in the concentration of the anti-nutrient phytate in the grain, this was offset by a 40% reduction in grain starch lysophospholipids, which have positive benefits for human health and grain quality.

Keywords: iron; lysophospholipids; magnesium; nitrogen; nutrient harvest index; *Oryza sativa*; potassium

1. Introduction

Rice (*Oryza sativa* L.) is grown on over 150 million ha worldwide and is the staple food for around three billion people. While rice provides a source of carbohydrate, in its polished form, it contains low concentrations of protein and key micronutrients needed in the human diet, including zinc (Zn) and iron (Fe) [1]. Unsurprisingly, a large proportion of the two-thirds of the world's population that lack one or more essential mineral elements in their diets rely on rice as their main staple [2].

Over 8 million ha of land used for rice production is classified as having a high P fixation capacity, and consequently, much of this land has inherently low bioavailable soil P levels [3]. Soil P deficiency leads to reduced tillering, bronzing of older leaves, stunting of rice plants during early growth, delayed flowering, and reduced grain yields [4]. However, little is known about the impact of P deficiency on the uptake and partitioning of mineral nutrients in the later stages of growth, and consequences for grain nutritional quality.

To our knowledge, only one study [5] has examined the impact of P deficiency on the accumulation of other nutrients in rice at maturity. That study found that grain nitrogen (N) concentrations increased with reductions in soil P status in four of the five cultivars investigated, despite all plots receiving N fertiliser [5]. However, the opposite trend was observed grain potassium (K), magnesium (Mg), and Zn, which all decreased in most rice cultivars with increasing P deficiency stress [5]. Since no biomass data were presented by Saleque et al. [5], it was not possible to ascertain whether total nutrient

accumulation (nutrient content of aboveground biomass) differed among P treatments or whether the lower or higher grain nutrient concentrations were the result of differences in nutrient partitioning between straw and grain.

The aim of this study was to investigate whether P deficiency impacts the accumulation and partitioning of key nutrients in rice, and whether any changes affect grain nutritional quality. While P deficiency often coincides with the presence of other stresses, such as aluminium toxicity in acid soils, this study focussed specifically on nutrient uptake and partitioning in rice under P deficiency in the absence of other nutritional stresses.

2. Results

2.1. Plant Growth, Biomass Accumulation, and Partitioning

Plants grown in the plots without P fertiliser showed typical P deficiency symptoms, including reduced tillering, reduced biomass production, and bronzing of older leaves during vegetative growth (data not shown). Flowering was delayed by around 10 days in the absence of P fertiliser, but the grain ripening period was the same (28 days) as for P-fertilised plots.

Phosphorus deficiency resulted in a biomass reduction of approximately 30% compared to P-fertilised plants (10.5 t·ha^{-1} in P-deficient plots compared to 16.9 t·ha^{-1} in P-fertilised plots; Table 1).

Despite the 10 days longer period of vegetative growth in P-deficient plants, their proportional biomass accumulation in the post-anthesis period was significantly higher than P-replete plants (69% vs. 51% of total biomass accumulated after anthesis, respectively; Table 1). Furthermore, P deficiency caused a significant reduction in harvest index (HI); the proportion of aboveground biomass located in the grains at maturity) ($p < 0.1$).

Table 1. Aboveground biomass and nutrient accumulation, harvest indices, and % post-anthesis accumulation of biomass and key nutrients in rice under Phosphorus (P)-deficient and P-fertilised growing conditions in the field. Significant differences are denoted by * ($p < 0.1$), ** ($p < 0.05$), or ns (not significantly different at $p = 0.1$).

Phosphorus Supply	Biomass	Macronutrients (kg·ha^{-1})						Micronutrients (g·ha^{-1})				
	(t·ha^{-1})	N	P	K	S	Ca	Mg	Cu	Fe	Zn	B	Mn
Whole shoot nutrient content												
+P	17	175	35	174	22	34	36	76	2986	343	523	1007
−P	11	115	11	123	18	23	18	67	1287	280	434	421
Significance	**	**	**	**	ns	*	**	ns	**	*	ns	**
% post-anthesis accumulation												
+P	51	20	68	22	37	32	58	40	10	49	46	53
−P	69	43	72	59	70	53	66	61	14	69	56	56
Significance	**	**	ns	**	*	**	ns	*	ns	**	ns	ns
Harvest index												
+P	0.62	0.69	0.90	0.42	0.61	0.15	0.56	0.71	0.28	0.64	0.48	0.36
−P	0.56	0.68	0.85	0.30	0.62	0.13	0.40	0.56	0.31	0.49	0.36	0.38
Significance	*	ns	**	*	ns	ns	**	ns	ns	**	ns	ns

2.2. Nutrient Accumulation

Total aboveground accumulation of Ca and Zn ($p < 0.1$), and N, P, K, Mg, Fe, and Mn ($p < 0.05$), was significantly lower in P-deficient plants (Table 1). The decreases in N and K uptake were concomitant with biomass reduction, since the concentration of these nutrients in whole shoots did not differ between P treatments (Table 2).

Table 2. Concentration of key nutrients in rice above-ground tissue, grain, and straw under P-deficient and P-replete growing conditions in the field. Significant differences are denoted by * ($p < 0.1$), ** ($p < 0.05$), or ns (not significantly different at $p = 0.1$).

Phosphorus Supply	Macronutrients (mg·g^{-1})						Micronutrients (mg·kg^{-1})				
	N	P	K	S	Ca	Mg	Cu	Fe	Zn	B	Mn
Whole shoot											
+P	10.4	2.05	10.4	1.32	2.00	2.15	4.49	178	20.3	31.1	59.7
−P	10.9	1.00	11.9	1.59	2.18	1.73	6.46	132	26.8	41.0	38.9
Significance	ns	**	ns	ns	ns	**	ns	*	**	*	**
Grain											
+P	11.7	3.01	6.98	1.30	0.431	1.96	5.16	77	21.1	23.4	34.9
−P	13.2	1.50	6.30	1.57	0.568	1.24	6.20	61	23.3	26.4	25.7
Significance	ns	**	ns	ns	*	**	**	ns	*	ns	ns
Straw											
+P	8.37	0.483	15.7	1.35	4.56	2.46	3.40	336	19.1	43.1	99.3
−P	7.89	0.347	19.0	1.56	4.32	2.38	6.80	228	31.3	59.8	56.6
Significance	ns	ns	ns	ns	ns	ns	ns	ns	**	*	**

However, the reduced accumulation of P, Mg, Fe, and Mn in P-deficient plants led to significantly lower shoot concentrations of these nutrients compared to P-fertilised plants ($p < 0.05$; Table 2). In contrast, Zn concentrations in whole shoots of P-deficient plants were higher than P-fertilised plants, despite a reduction in total Zn accumulation ($p < 0.1$; Table 2). Whole shoot concentrations of B were also significantly higher in P-deficient plants ($p < 0.1$).

Given biomass differences but a lack of difference in S and Cu accumulation in shoots in response to P treatments (Table 1), one would expect that concentrations of S and Cu would be higher in whole plant tissues of P-deficient plants; however, these differences were not significant due to high variability in concentrations.

Around 70% of P was taken up after anthesis regardless of whether P fertiliser was supplied, while only around 12% of Fe was taken up after anthesis, regardless of P treatment (Table 1). Phosphorus deficiency significantly increased the proportion of N, K, Ca, and Zn ($p < 0.05$) and Cu and S ($p < 0.1$) accumulated during the post-anthesis period (Table 1).

2.3. Nutrient Partitioning between Grain and Straw and Tissue Nutrient Concentrations

Nutrient harvest indices were significantly affected by P treatment, with P, Mg, and Zn ($p < 0.05$) and K ($p < 0.1$) harvest indices significantly lower in P-deficient plants (Table 1). The reduction in P and Mg harvest indices resulted in significantly lower concentrations of these nutrients in the grains of P-deficient plants ($p < 0.05$; Table 2). Grain Mn concentrations were also significantly lower in P-deficient plants (39 mg·kg^{-1}) than P-fertilised plants (60 mg·kg^{-1}). Despite the reduced Zn harvest index, Zn concentrations were significantly higher in P-deficient plants at 27 mg·kg^{-1}, compared to 20 mg·kg^{-1} in P-fertilised plants, and small but significant increases in grain Cu ($p < 0.05$) and Ca ($p < 0.1$) concentrations were also observed under P deficiency.

The only significant differences in straw nutrient concentration between P treatments were an increase in straw Zn concentrations and a decrease in straw Mn concentrations in P-deficient plants.

2.4. Grain Phytic Acid and Phospholipid Concentrations

Grain phytic acid levels were significantly reduced ($p < 0.05$) in the P-deficient plants (7.4 mg·g^{-1}) compared to plants in the P-fertilised plots (12.2 mg·g^{-1}). Similarly, grain starch lysophospholipids concentrations were significantly lower ($p < 0.05$) in P-deficient rice plants (2600 µg·g^{-1}) than plants in the P-fertilised plots (4306 µg·g^{-1}).

3. Discussion

3.1. Total Nutrient Uptake and Patterns of Accumulation

We hypothesised that due to biomass reductions in P-deficient crops, some nutrients would be accumulated in the shoots of P-deficient plants at luxury levels compared to P-sufficient plants, which may result in increased concentrations of these nutrients in grains under P deficiency. However, higher whole shoot concentration was only observed for Zn in P-deficient plants, likely due to the reported antagonistic effect of P on Zn uptake [6], resulting in proportionally less Zn per unit biomass in P-fertilised plants. The consequence of this was that Zn concentrations in both grain and straw were significantly higher in P-deficient plants—despite a reduction in the Zn harvest index compared to P-fertilised plants. This contradicts declines in grain Zn levels under P deficiency reported by Saleque et al. [5], but the absence of any Zn uptake data in the Saleque et al. [5] study makes it difficult to speculate on the cause of this discrepancy. Regardless, the higher grain Zn concentrations, and the significant increase in grain Cu and Ca concentrations in P-deficient plants may be beneficial, given that these nutrients are often lacking in the diets of people in under-developed countries [2].

Plants suffering from P-deficiency had significantly lower concentrations of P in grains compared to P-replete plants (Table 2), not unexpected since grain P levels in rice typically reflect soil P status [7]. This reduction in grain total P was reflected in the concentration of phytic acid in grains (7.4 $mg \cdot g^{-1}$ vs. 12.2 $mg \cdot g^{-1}$ in P-deficient and P fertilised plants, respectively), leading to an overall reduction in phytate: cation molar ratios in the P-deficient plants (data not shown). This ratio is frequently used to estimate mineral bioavailability [8], and the results therefore suggest that nutrients such as Zn, Cu, Mg, and Ca are more bioavailable in the grains of P-deficient plants; however, further detailed bioavailability studies would be needed before conclusions could be drawn. While a decrease in grain phytate likely has positive benefits for consumers of rice, this may be offset by the significant reduction in starch lysophospholipids (4306 $\mu g \cdot g^{-1}$ in P-fertilised plants vs. 2600 $\mu g \cdot g^{-1}$ in P-deficient plants), given that starch lysophospholipids not only have human health benefits but affect the physiochemical properties of starch (i.e., grain quality) [9].

Concentrations of Mg, Fe, and Mn were lower in whole shoots under P-deficiency, but the only consequence for grain nutritional quality was a significant reduction in grain Mg concentration. The decline in grain Mg concentrations under P deficiency is consistent with the results of Saleque et al. [5], and was a result of both decreased shoot accumulation of Mg and reduced Mg harvest index in P-deficient plants (Table 2). We have no logical hypothesis to explain this observation, and it remains unresolved.

3.2. Post-Anthesis Uptake of Key Nutrients and Potential Implications

Post-anthesis accumulation of N by rice plants is typically low [10], but is heavily influenced by the timing of N fertiliser applications, which tend to occur at establishment and early in vegetative growth [10,11]. However, under P deficiency, more than 40% of total aboveground N accumulation occurred after anthesis (Table 1). Given that the vast majority of fertiliser N is taken up by plants or lost in gaseous forms within weeks after application [12], the P-deficient plants may have acquired some of the 30 $kg \cdot ha^{-1}$ N applied around a week prior to anthesis during the post anthesis period, but most likely relied heavily on N mineralised from the soil during grain filling, which is not a favourable outcome.

The delay in the accumulation of key nutrients Ca, Zn, and K is less relevant in terms of nutrient losses, but may be problematic if climatic conditions during grain filling are not conducive to nutrient uptake. For example, in rainfed crops, drier periods during the grain filling period may limit nutrient uptake with consequences for grain nutrient levels [13]. Given that upland (non-flooded) rice crops that are often subject to water stress are also frequently cultivated in soils that are deficient in P [3], the reliance on post-anthesis accumulation of N, S, Ca, K, Zn, and Cu may have consequences for growth or grain nutritional quality in these regions.

We also acknowledge that the response of the IR64 rice plants to P deficiency (in terms of the uptake and partitioning of other nutrients to grains) was likely specific to the level of P deficiency stress suffered in the study. Had plants in our study only suffered a minor yield reduction (e.g., 10%–15%) in the absence of P fertiliser—which could have been achieved by growing plants in a field with higher soil P status or by growing a P-efficient rice cultivar that contains the *PSTOL1* gene [14]—the impacts on nutrient uptake and grain nutritional quality may have been different.

4. Experimental Section

4.1. Field Site

The experiment was undertaken at the P demonstration plots at the International Rice Research Institute (IRRI), Los Baños, The Philippines, from January–April 2013. The six 0.0135 ha plots (three +P, three −P) contained soil in bunkers transported from a P-deficient site at Pangil, Laguna, The Philippines. Briefly, soil in the −P plots had a pH (1:1 soil-water) 7.7, % C 2.34, % N 0.175, Bray P 1.6 mg·kg^{-1}, exchangeable K (meq·100 g^{-1}) 0.44, and available Zn 1.9 mg·kg^{-1}, while the +P soil had a pH (1:1 soil-water) 7.3, % C 2.33, % N 0.192, Bray P 8.5 mg·kg^{-1}, exchangeable K (meq·100 g^{-1}) 0.54, and available Zn 0.9 mg·kg^{-1}. On 4th January 2013, three days prior to transplanting, basal fertiliser was broadcast on the plots at rates (kg·ha^{-1}): 90 N, 26 P (+P plots only), 33 K, and 2 Zn.

4.2. Plant Cultivation

Rice (cv. IR64) seeds were sown into trays containing commercial nursery seedling mix in a glasshouse at IRRI. At 21 days after sowing (DAS), the 7th January 2013 seedlings were transplanted by hand into the field plots. Seedlings were transplanted in rows 0.2 m apart and a hill spacing of 0.2 m within rows (i.e., 25 hills·m^{-2}), with one plant per hill. A further 90 kg·ha^{-1} N fertiliser was applied (broadcast) in three splits of 30 kg·ha^{-1} N at 20, 40, and 60 days after transplanting. Plants were cultivated under standard, fully flooded practice, with weeds controlled by hand weeding.

4.3. Measurements

Anthesis occurred on the 18th March and 28th March for +P plots and −P plots, respectively, and plants reached physiological maturity 28 days after anthesis (DAA), regardless of P treatment. Three plants per plot were harvested at anthesis and at physiological maturity, by severing plant shoots 10 mm above the soil surface. Plant material was dried in an oven at 60 °C for 5 days, and then separated into grain (husk plus caryopsis), stem, flag leaf, second and third leaves, older leaves, and late tillers (tillers that had not yet reached anthesis). Any grains from late-emerging tillers that may not have been physiologically mature at the time of harvest, but were heavy enough to be separated from chaff during in the threshing operation, were recorded in the grain yield.

Samples were analysed for nutrient concentration at Environmental Analysis Laboratories, Lismore, NSW, Australia. A 0.2 g subsample of finely ground tissue was digested with nitric acid in a MARS microwave oven (CEM Corp., Matthews, NC, USA), and concentrations of P, K, Mg, Ca, Fe, Zn, Mn, and Cu in the digest solutions were quantified using inductively coupled plasma optical emission spectroscopy (ICP-OES 4300D, Perkin Elmer, Waltham, MA, USA). Tissue N and S concentrations were measured using a LECO TruMAC CNS analyser.

Grain lysophospholipids were measured as per Liu et al. [15]. In brief, the ground rice grain (15 mg) was extracted with 75% n-propanol (0.8 mL), and the extract was analysed using liquid chromatography mass spectrometry (LCMS) to quantify the ten major lysophospholipids in rice grain. The concentrations of these lysophospholipids were summed to obtain the total lysophospholipid concentration. Phytate was measured using the method of Shi et al. [16]. Briefly, ground rice grain was extracted using 0.4 M HCl, and the phytate in the extract was precipitated with an acidic iron-III-solution of known iron content. The phytate concentration was quantified by measuring the decrease of iron in the supernatant using colourimetry.

4.4. Statistical Analyses

Total nutrient content, % post anthesis accumulation, harvest index, grain lysophospholipid and phytic acid concentrations, and tissue concentration data for each nutrient were analysed using a one-way analysis of variance fitting P treatment (plus or minus P fertiliser) in Genstat. Because of the inherent high variability in field studies, a probability level of 0.1 was used: significance of differences between treatment mean values for each trait was tested using Duncan's multiple range test ($p \leq 0.1$).

Acknowledgments: The assistance of technical staff at the International Rice Research Institute and Southern Cross University is gratefully acknowledged.

Author Contributions: Terry Rose, Matthias Wissuwa and Tobias Kretzschmar designed the experiment and wrote the manuscript. Lei Liu and Graham Lancaster analysed the samples and contributed significantly to manuscript development.

Conflicts of Interest: The authors declare no conflict of interest.

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