Phosphorus toxicity disrupts Rubisco activation and reactive oxygen species defence systems by phytic acid accumulation in leaves

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Abstract

Phosphorus (P) is an essential mineral nutrient for plants. Nevertheless, excessive P accumulation in leaf mesophyll cells causes necrotic symptoms in land plants; this phenomenon is termed P toxicity. However, the detailed mechanisms underlying P toxicity in plants have not yet been elucidated. This study aimed to investigate the molecular mechanism of P toxicity in rice. We found that under excessive inorganic P (Pi) application, Rubisco activation decreased and photosynthesis was inhibited, leading to lipid peroxidation. Although the defence systems against reactive oxygen species (ROS) accumulation were activated under excessive Pi application conditions, the Cu/Zn-type superoxide dismutase activities were inhibited. A metabolic analysis revealed that excessive Pi application led to an increase in the cytosolic sugar phosphate concentration and the activation of phytic acid synthesis. These conditions induced mRNA expression of genes that are activated under metal-deficient conditions, although metals did accumulate. These results suggest that P toxicity is triggered by the attenuation of both photosynthesis and metal availability within cells mediated by phytic acid accumulation. Here, we discuss the whole phenomenon of P toxicity, beginning from the accumulation of Pi within cells to death in land plants.

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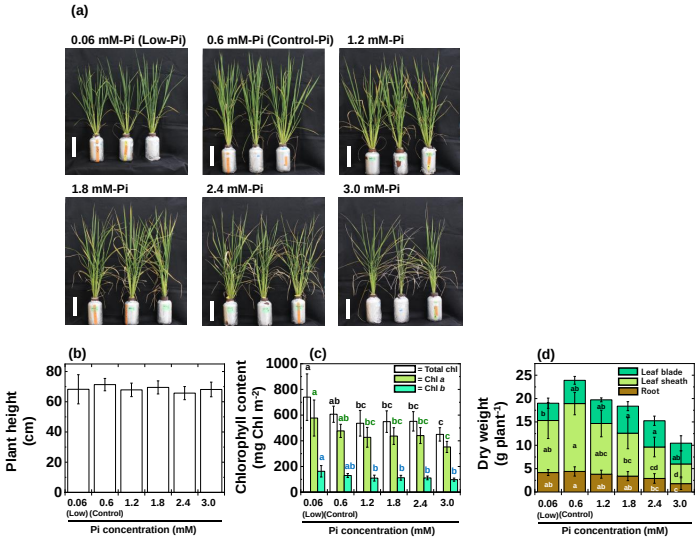
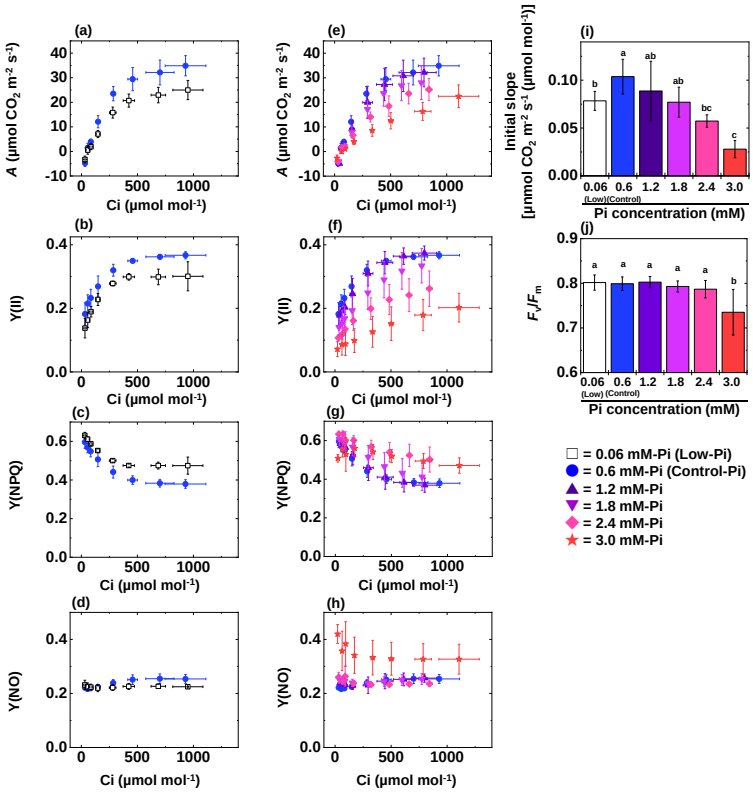


Figure 1

Growth phenotypes of rice plants grown under different inorganic phosphate (Pi) application rates. (a) Representative plants grown under different Pi application rates on the 70th day after germination. White bars indicate a scale of 10 cm. At this stage, the plant height (b) and chlorophyll content in the leaf blades (c) were examined. (d) Dry matter of the leaf blade, leaf sheath, and root in rice plants grown under different Pi application rates. Results are expressed as means ± SD (n = 6-8). Different letters indicate significant differences between different Pi application rates (Tukey's HSD test, $p < 0.05$).



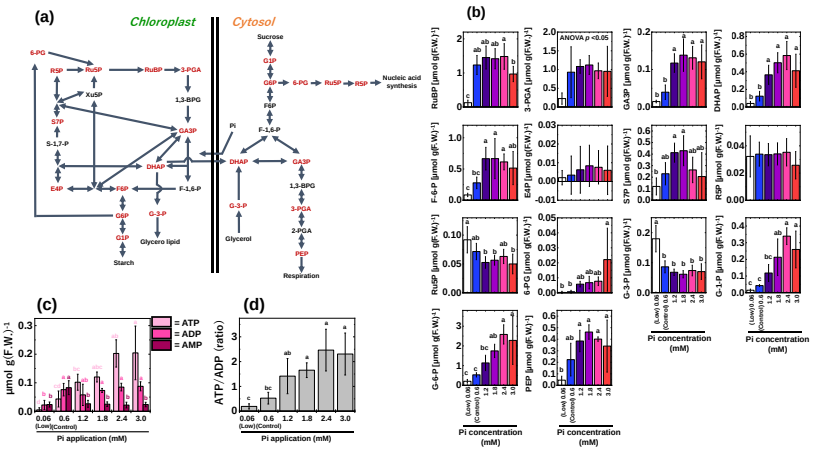


Figure 3

Effects of Pi application on the sugar phosphate and adenylate concentrations in rice leaf blades. (a) Scheme of sugar phosphate metabolism in chloroplasts and cytosol. The sugar phosphate concentrations represented by red characters indicates those quantified in this study. (b) Sugar phosphate concentration in rice leaf blades. White, blue, dark blue, purple, pink, and red bars indicate low (0.06 mM), control (0.6 mM), 1.2 mM, 1.8 mM, 2.4 mM, and 3.0 mM treatment conditions, respectively. (c) and (d) Adenylate concentration and ATP/ADP ratio in rice leaf blades. Results are expressed as means ± SD (n = 3-9). Different letters indicate significant differences among different Pi conditions (Tukey's HSD test, $p < 0.05$).

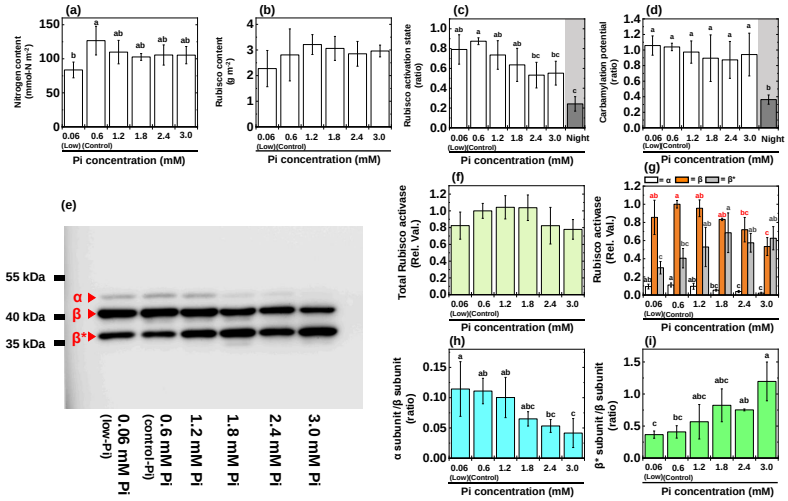


Figure 4

Effects of P toxicity on Rubisco concentration and activation by Rubisco activase (RCA). (a) Leaf nitrogen content in rice leaf blades and (b) Rubisco concentration in rice leaf blades (n = 3-4). The Rubisco activation was calculated from the ratio of the initial to maximum Rubisco activity (c), and the carbamylation potential was calculated from the ratio of the total to the maximum Rubisco activity (d) (n = 3-5). The white and dark grey bars indicate the results of the leaves sampled under illumination and at night, respectively, under control-Pi conditions. (e) Result of the western blot analysis, which targets RCA in rice leaves. Red arrows and Greek characters indicate the isoforms of RCA. Each sample was loaded on a leaf area basis (0.02 cm^2). (f) and (g) Relative concentrations of the total RCA and each isoform evaluated from the western blot analysis. The RCA concentration in the control-Pi plants is set as "1" and the relative content is shown. The black, red, and grey characters above the bars indicate the statistical results for the *α*, *β*, and *β** isoforms, respectively. (h) and (i) Isoform ratio between *α*/*β* and *β**/*β* (n = 4). Results are expressed as means ± SD. Different letters indicate significant differences among different Pi conditions (Tukey's HSD test, $p < 0.05$).

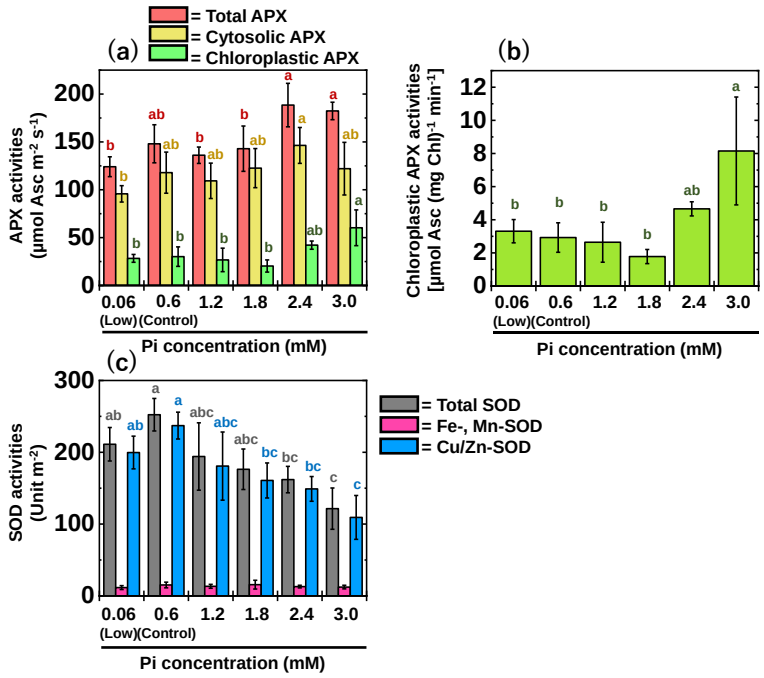


Figure 5

Ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities in rice leaf blades grown under different Pi conditions. (a) Results of the total, cytosolic, and chloroplastic APX activities evaluated on a leaf area basis. The red, yellow, and green characters above the bars indicate the statistical results of the total, cytosolic, and chloroplastic APX activities, respectively. (b) Results of the chloroplastic APX activities evaluated on a chlorophyll basis. (c) Results of the total, Fe-, Mn-, and Cu/Zn-SOD activities evaluated on a leaf area basis. The grey and blue characters above the bars indicate the statistical results of the total and Cu/Zn-SOD activities, respectively. Results are expressed as means ± SD (n = 3). Different letters indicate significant differences among different Pi conditions (Tukey's HSD test, $p < 0.05$).

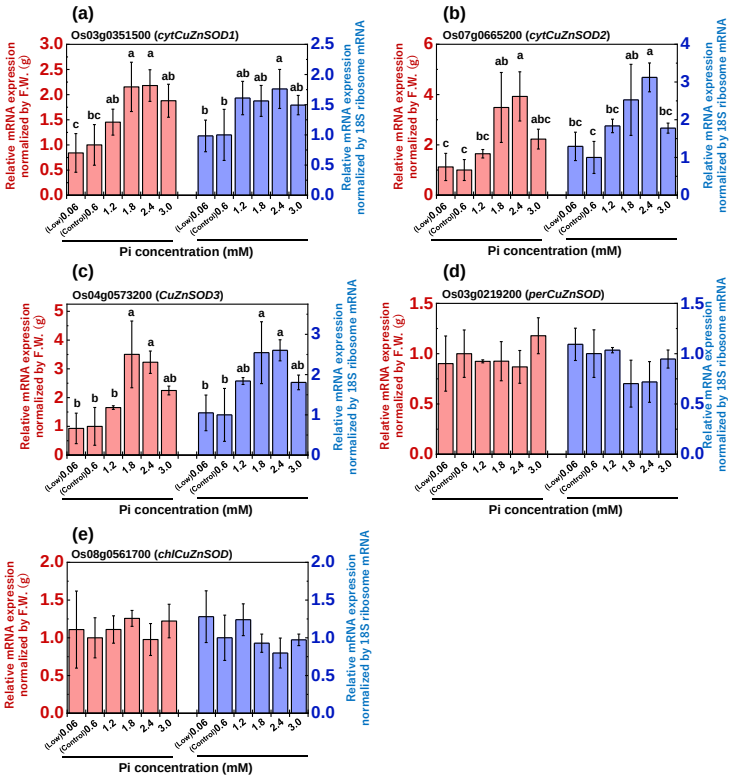


Figure 6

mRNA expressions of Cu/Zn-SOD genes in rice leaf blades. The mRNA expression is expressed on a fresh weight (F.W.) (red bars) and 18S ribosome expression (blue bars) basis. Results are expressed as means ± SD (n = 3-4). Different letters indicate significant differences among different Pi conditions (Tukey's HSD test, $p < 0.05$).

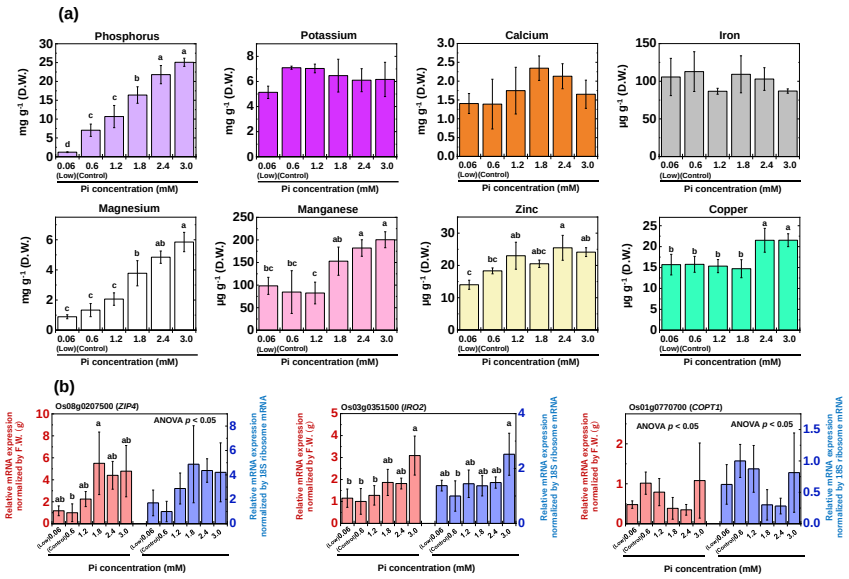
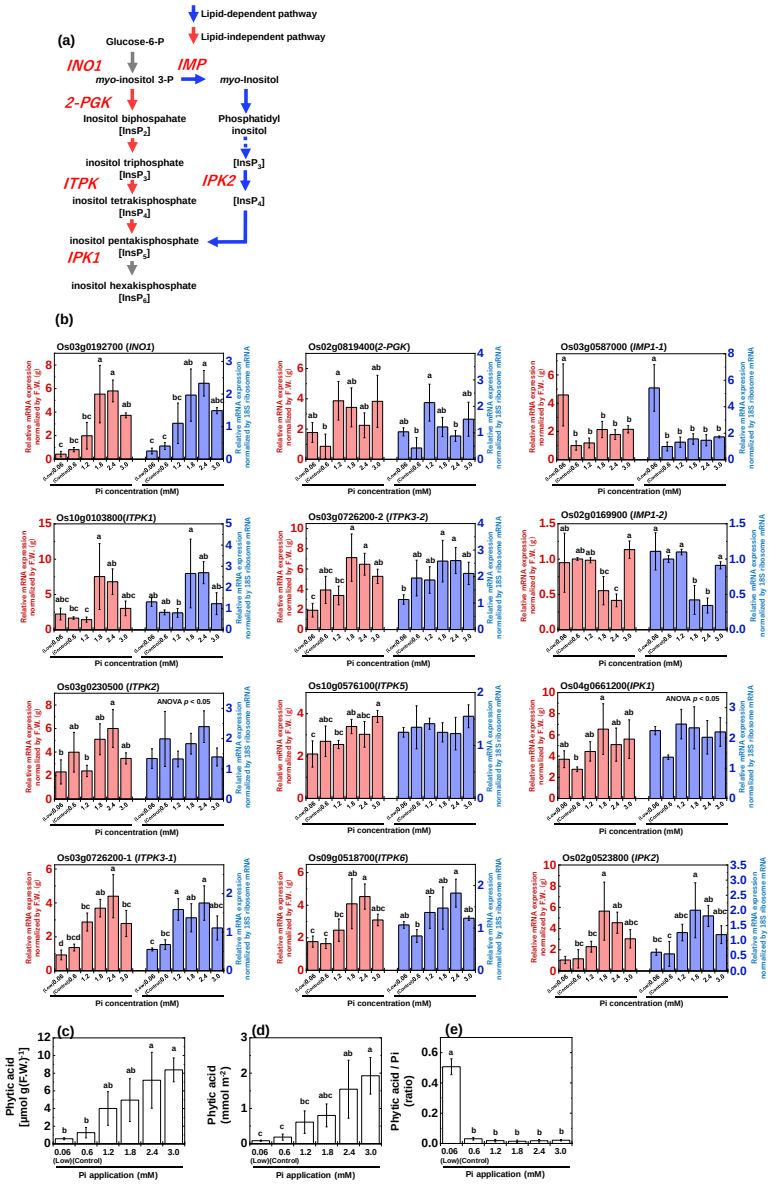
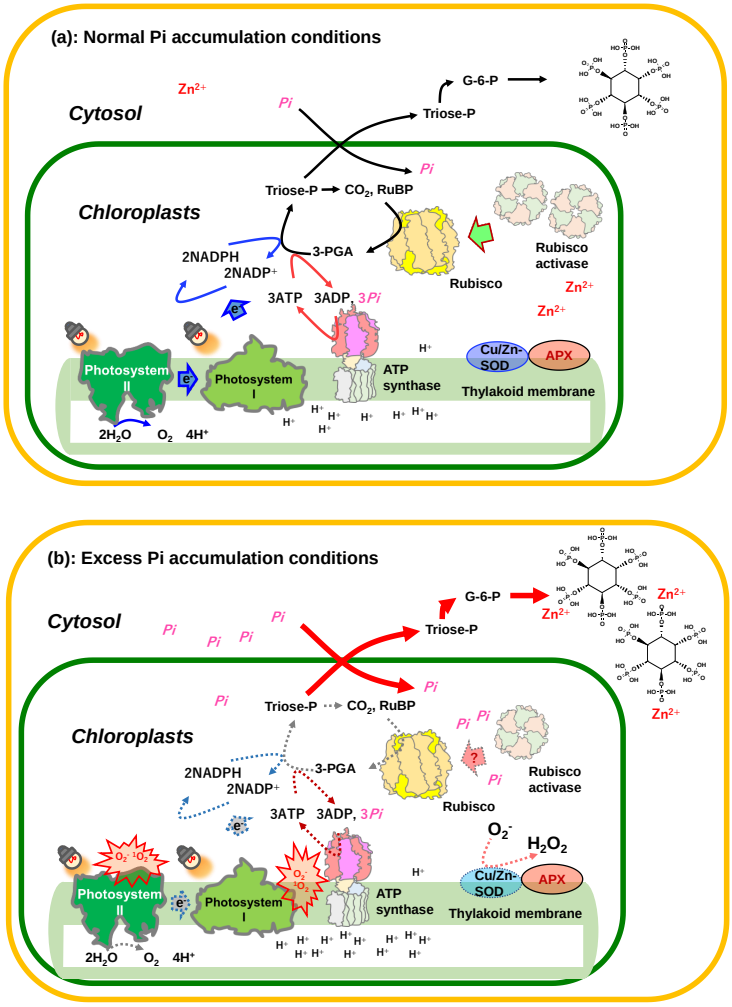


Figure 7

Leaf mineral concentration and metal-responsive gene expression in rice leaf blades grown under different Pi conditions. The concentration of each mineral nutrient was quantified on the leaf dry matter basis (a). Results are expressed as means ± SD (n = 3-4). (b) mRNA expressions of the metal-deficiency-responsive genes *ZIP4*, *IRO2*, and *COPT1* in the leaves. The mRNA expression is expressed on a fresh weight (F.W.) (red bars) and 18S ribosome expression (blue bars) basis. Results are expressed as means ± SD (n = 3-7). Different letters indicate significant differences among different Pi conditions (Tukey's HSD test, $p < 0.05$).





	Pi concentration					
	0.06 mM (n = 3)	0.6 mM (n = 3)	1.2 mM (n = 4)	1.8 mM (n = 3)	2.4 mM (n = 3)	3.0 mM (n = 3)
Pi (mmol m^{-2})	0.450 ± 0.018^{d}	9.16 ± 1.86^{cd}	26.0 ± 5.71^{c}	38.8 ± 1.89^{bc}	61.5 ± 13.0^{b}	79.2 ± 14.0^{a}
Pi (μmol g^{-1}[FW])	2.90 ± 0.48^{d}	57.0 ± 20.2^{cd}	114.3 ± 16.2^{c}	193.1 ± 18.7^{bc}	295.4 ± 66.8^{b}	365.4 ± 32.4^{a}
Po (mmol m^{-2})	0.180 ± 0.036^{c}	2.33 ± 1.19^{bc}	10.0 ± 2.95^{b}	7.40 ± 5.76^{bc}	14.87 ± 5.45^{a}	17.2 ± 3.9^{a}
Po (μmol g^{-1}[FW])	1.21 ± 0.40^{c}	14.9 ± 9.12^{bc}	44.1 ± 12.1^{b}	36.3 ± 27.1^{bc}	58.8 ± 20.5^{a}	75.0 ± 20.0^{a}

Table 1

Pi and Po concentrations in rice leaf blades. The different letters indicate significant differences between each Pi application (Tukey's HSD test, $p < 0.05$).