

Effect of Phosphorus Deficiency on Leaf Photosynthesis and Carbohydrates Partitioning in Two Rice Genotypes with Contrasting Low Phosphorus Susceptibility

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Abstract: To study the effect of phosphorus (P) deficiency on leaf photosynthesis and carbohydrates partitioning and to determine whether the characteristics of leaf photosynthesis and carbohydrates partitioning are related to low P tolerance in rice plants, a hydroponic culture experiment supplied with either sufficient P (10 mg/L) or deficient P (0.5 mg/L) was conducted by using two rice genotypes different in their responses to low P stress. Results showed that the plant growth of Zhenongda 454 (low P tolerant genotype) was less affected by P deficiency compared with Sanyang'ai (low P sensitive genotype). Under P-deficient conditions, photosynthetic rates of Zhenongda 454 and Sanyang'ai were decreased by 16% and 35%, respectively, and Zhenongda 454 showed higher photosynthetic rate than Sanyang'ai. Phosphorus deficiency decreased the stomatal conductance for both genotypes, but had no significant influence on leaf internal CO₂ concentration (C_i), suggesting that the decrease in leaf photosynthetic rate of rice plants induced by P deficiency was not due to stomatal limitation. Phosphorus deficiency increased the concentration of soluble carbohydrates and sucrose in shoots and roots for both genotypes, and also markedly increased the allocation of soluble carbohydrates and sucrose to roots. Under deficient P supply, Zhenongda 454 had higher root/shoot soluble carbohydrates content ratio and root/shoot sucrose content ratio than Sanyang'ai. In addition, phosphorus deficiency increased the concentration of starch in roots for both genotypes, whereas had no effect on the content of starch in shoots or roots. Compared to genotype Sanyang'ai, the better tolerance to low-P stress of Zhenongda 454 can be explained by the fact that Zhenongda 454 maintains a higher photosynthetic rate and a greater ability to allocate carbohydrates to the roots under P deficiency.

Key words: carbohydrate; phosphorus deficiency; photosynthesis; rice

Phosphorus (P) deficiency is one of the major limiting factors to the crop production in most of the soils throughout the world [1]. The traditional way to alleviate P deficiency is the application of P fertilizer. However, the scarcity of P mineral resource and extremely low utilization efficiency of P fertilizer indicate that applying P fertilizer annually is not the sustainable way to solve the problem of P deficiency in soil. Screening or breeding of plant species tolerant to P deficiency may be one of the effective alternatives to alleviate P deficiency and to increase the utilization efficiency of P fertilizer, since plants exhibit inter- and intra-specific variations in tolerance to low P stress [2-3].

Under P-deficient conditions, plants would develop numerous morphological, physiological, biochemical, and molecular adaptations to overcome P deficiency [2, 4]. Such adaptive mechanisms mainly include an increase in total root length and root hair growth [5-6], enhancement of organic acids, acid phosphatase and ribonuclease (RNase) secretion into the rhizosphere [7-9], increase in many proteins including phosphatase, inorganic phosphate (P_i) transporter, RNase, phosphoenolpyruvate carboxylase (PEPcase) in plant tissues [4], and so on.

It has been reported by many researchers that P deficiency has a significant influence on leaf photosynthesis and carbon metabolisms in plants [10]. Significant decrease in leaf photosynthesis induced by P deficiency has been reported in various plant species

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such as barley^[11], soybean^[12], and sugar beet^[13]. Inhibition of photosynthesis caused by P deficiency was mainly due to the decrease in ribulose-1,5-bisphosphate (RuBP) pool size^[14-15], or insufficient ATP production^[14]. P deficiency also leads to an increase in the allocation of carbohydrates to roots^[12, 16-17], which may account for the increased root/shoot dry weight ratio for most P-deficient plants. In addition, it is assumed that the decrease in cytosolic P_i level caused by P deficiency would result in the transport of less triose-P from the chloroplast to cytosol, which may further result in that more of the newly fixed carbon goes to starch than to sucrose^[10]. For example, the decrease in sucrose and increase in starch occurred in P-deficient leaves of soybean^[12, 18] and *Brachiaria* hybrid^[19]. However, Ciereszko and Barbachowska^[20] reported that P deficiency caused an increase in both sucrose content and starch content in plant tissues of bean. Therefore, the effect of P efficiency on sucrose and starch metabolisms varies among different plant species. Due to the fact that only a single genotype was involved in most of these earlier studies, it needs to be clarified whether the characteristics of photosynthesis and carbohydrates partitioning were related to low P tolerance of plants.

In the present study, a hydroponic culture experiment was conducted to study the effect of P deficiency on leaf photosynthesis and carbohydrates partitioning and to elucidate the relationship between low P tolerance and either leaf photosynthesis or carbohydrates distribution by using two rice genotypes identified to be different in low P susceptibility in a field trial^[21].

MATERIALS AND METHODS

Plant materials and plant culture

Two rice (*Oryza sativa* L.) genotypes (cv. Zhenongda 454 and Sanyang'ai) were used in the present experiment. Zhenongda 454 showed better tolerance to low P stress than Sanyang'ai according to the results from the field trial^[21]. Rice seeds were disinfected with 0.1% HgCl₂ solution for 1 min and then thoroughly washed with distilled water. Seeds were moistured in the distilled water for 36 h (30°C), and then sown on a nylon net supplied with half

strength of standard rice nutrient solution^[22]. The standard rice nutrient solution had the composition of the macronutrients NH₄NO₃ (1.4 mmol/L), NaH₂PO₄ (0.32 mmol/L), K₂SO₄ (0.5 mmol/L), CaCl₂ (1.0 mmol/L), and MgSO₄ (1.6 mmol/L), and the micronutrients MnCl₂ (9.5 μmol/L), (NH₄)₆Mo₇O₂₄ (0.01 μmol/L), H₃BO₃ (20 μmol/L), ZnSO₄ (0.15 μmol/L), CuSO₄ (0.15 μmol/L), and FeCl₃ (36 μmol/L). When the rice seedlings were at 2-leaf stage (about 10 days after germination), seedlings of each rice genotype uniform in size and vigor were transplanted into 2.5 L plastic pots (five holes per seedling holder, and two seedlings per hole) containing standard rice nutrient solution. After 2-day growth, the nutrient solution was renewed. Two P treatments were applied in the following period of the present experiment: (1) Control (sufficient P supply, 10 mg /L); (2) Low P (deficient P supply, 0.5 mg /L). Each treatment had three replicates. The pH of nutrient solution was adjusted to 5.0 by adding 1 mol/L HCl or NaOH daily and the nutrient solution was renewed every five days. The plants were grown in an environmentally controlled growth chamber, with a day (25°C, 14 h) / night (22°C, 10 h) cycling at a humidity of 70%. The light intensity in the day period was approximately 200 μmol photons / (m²·s).

Gas exchange measurements

The gas exchange of the youngest fully expanded leaves of rice plants was determined with a portable photosynthesis system (LiCor-6400; LiCor Inc. Lincoln, NE, USA) at 10:00 and 11:00 a.m. on the 19th day after treatments. The determination was conducted in the sample chamber, with a light intensity of 500 μmol photos / (m²·s), a leaf temperature of 28°C, and CO₂ concentration of 390±5 μmol /mol. Measurements were repeated at least six times on each.

Determination of total dry weight

After harvest on the 20th day after treatments, plant samples were divided into shoots and roots. Shoot and root samples were dried at 105°C for 30 min, and then oven-dried at 70°C to a constant weight. Dry weights (DW) of shoot and root samples were recorded. Shoot and root samples were ground with

a stainless steel mill and passed through 0.25 mm sieve for determination of carbohydrates.

Determination of carbohydrates

Carbohydrates were determined according to the method of Moya et al.^[23] with small modifications. Eight mL of ethanol (80%, V/V) was taken into a 10 mL plastic centrifuge tube containing 200 mg of powdered plant materials and the mixture was boiled for 30 min at 80°C. The procedure was repeated twice with the pellet obtained after centrifugation at 15 000 × *g* for 10 min. Supernatants were combined together and made up to 50 mL by the addition of ethanol (80% V/V). For the determination of soluble carbohydrates, an aliquot of the extract was transferred into a test-tube. The test-tube was immersed in a boiling water bath to evaporate ethanol and the aqueous residue was assayed with H₂SO₄-anthrone reagent as described by Fairbairn^[24] using glucose as standard. Another aliquot of the extract was used for the determination of sucrose according to the method of Roe^[25] after eliminating free fructose from the extracts^[26]. The pellets obtained after centrifugation were used for the determination of starch content by the method described as follows. The pellets were resuspended in 15 mL of 35% HClO₄ for 12 h at 25°C, and then the volume was made up to 25 mL by the addition of distilled water. After filtration through the filter paper, an aliquot was taken to measure the amount of glucose released using the method described above.

Statistical analysis

A one-way analysis of variation (ANOVA) was carried out on the data obtained from the present study, and means were compared using the least significant difference (LSD) test. The statistical analyses were performed according to the procedure of the SAS system.

RESULTS

Plant growth

The rice genotype Sanyang'ai had a greater shoot dry weight and root dry weight than Zhenongda 454 at sufficient P treatment, whereas there was no

significant difference at deficient P treatment (Fig. 1). Shoot dry weight of P-deficient plants of Zhenongda 454 and Sanyang'ai were 81% and 50% of their corresponding control plants, respectively (Fig. 1). Low P treatment decreased the root dry weight of Sanyang'ai, but had no influence on the root growth of Zhenongda 454 (Fig. 1). These two genotypes showed no difference in root/shoot dry weight ratio at sufficient P treatment, whereas Sanyang'ai showed

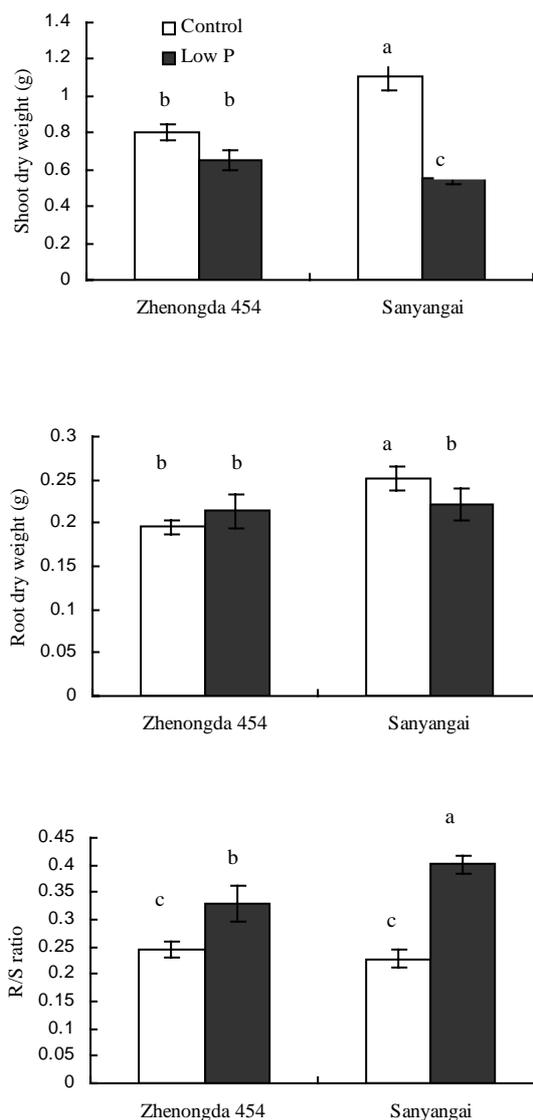


Fig. 1. Shoot dry weight and root dry weight of two rice genotypes, Zhenongda 454 and Sanyang'ai, grown with either sufficient P supply (Control) or deficient P supply (Low P).

Error bars show standard deviation (*n* = 3); columns labeled with different lowercase letters within each graph mean significantly different at *P* = 0.05 level according to the least significant difference (LSD) test.

higher root/shoot dry weight ratio than Zhenongda 454 at deficient P treatment (Fig. 1). Low P treatment markedly increased the root/shoot dry weight ratio for both genotypes (Fig. 1).

Leaf gas exchange

Zhenongda 454 showed higher photosynthetic rate than Sanyang'ai at deficient P treatment, whereas there was no difference at sufficient P treatment (Fig. 2). The photosynthetic rates of Zhenongda 454 and Sanyang'ai were decreased by 16% and 35%, respectively when grown with deficient P supply in comparison with P-sufficient plants (Fig. 2). There was no difference in stomatal conductance or in leaf internal CO₂ concentration between these two genotypes regardless of P levels (Fig. 2).

Low P treatment significantly decreased the stomatal conductance for both genotypes (Fig. 2), but had no significant effect on leaf internal CO₂ concentration (Fig. 2).

Carbohydrates

Under sufficient P conditions, these two genotypes showed no difference in the concentration of soluble carbohydrates in shoots or roots (Table 1). Under deficient P conditions, the concentration of soluble carbohydrates in roots of Zhenongda 454 was higher than that of Sanyang'ai, whereas the concentration of soluble carbohydrates was higher in shoots of Sanyang'ai than that of Zhenongda 454. For both rice genotypes, the concentration of carbohydrates in shoots and roots was higher at deficient P treatment than at sufficient P treatment. Content of total soluble carbohydrates was higher in shoots of Sanyang'ai than that of Zhenongda 454 at sufficient P treatment, whereas soluble carbohydrates content was higher that of Zhenongda 454 than in roots of Sanyang'ai at deficient P treatment. Low P treatment decreased the content of soluble carbohydrates in shoots of Sanyang'ai, but increased the content of soluble carbohydrates in roots of Zhenongda 454 (Table 1).

Under sufficient P conditions, concentration of sucrose in shoots was higher for Zhenongda 454 than Sanyang'ai, whereas there was no difference in sucrose concentration in roots (Table 2). Under

deficient P conditions, Sanyang'ai had higher concentration of sucrose in shoots than Zhenongda 454, while Zhenongda 454 had higher concentration of sucrose in roots than Sanyang'ai. For both genotypes, low P treatment increased the

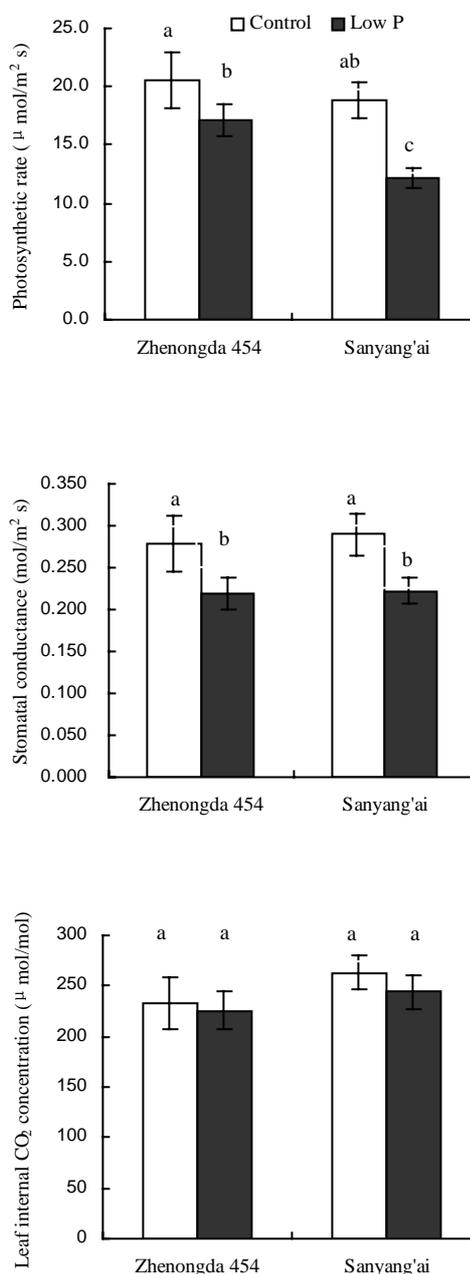


Fig. 2. Photosynthetic rate, stomatal conductance, leaf internal CO₂ concentration of two rice genotypes, Zhenongda 454 and Sanyang'ai, grown with either sufficient P supply (Control) or deficient P supply (Low P).

Error bars show standard deviation ($n = 3$); columns labeled with different lower case letters within each graph mean significantly different at $P = 0.05$ level according to the least significant difference (LSD) test.

Table 1. Concentration and content of soluble carbohydrates in shoots and roots of two rice genotypes grown with either sufficient P supply (Control) or deficient P supply (Low P).

Rice genotype	Concentration (mg/g)		Content (mg/plant)		
	Shoot	Root	Shoot	Root	Root/Shoot ratio
Control					
Zhenongda 454	56 c	32 c	45 b	6.3 c	0.14 b
Sanyang'ai	52 c	30 c	58 a	7.6 bc	0.13 b
Low P					
Zhenongda 454	68 b	55 a	44 b	11.8 a	0.27 a
Sanyang'ai	85 a	41 b	47 b	9.1 b	0.19 b
<i>LSD</i> _{0.05}	9.5	2.8	11	1.8	0.06

Data followed with different lowercase letters within each column mean significantly different at $P = 0.05$ level according to least significant different (LSD) test.

Table 2. Concentration and content of sucrose in shoots and roots of two rice genotypes grown with either sufficient P supply (Control) or deficient P supply (Low P).

Rice genotype	Concentration (mg/g)		Content (mg/plant)		
	Shoot	Root	Shoot	Root	Root/Shoot ratio
Control					
Zhenongda 454	23 c	16 c	18 b	3.1 b	0.17 c
Sanyang'ai	20 d	15 c	22 a	3.8 b	0.17 c
Low P					
Zhenongda 454	30 b	34 a	20 ab	7.2 a	0.37 a
Sanyang'ai	39 a	28 b	22 a	6.2 a	0.29 b
<i>LSD</i> _{0.05}	1.2	1.6	3.2	1.1	0.05

Data followed with different lowercase letters within each column mean significantly different at $P = 0.05$ level according to least significant difference (LSD) test.

Table 3. Concentration and content of starch in shoots and roots of two rice genotypes grown with either sufficient P supply (Control) or deficient P supply (Low P).

Rice genotype	Concentration (mg/g)		Content (mg/plant)		
	Shoot	Root	Shoot	Root	Root/Shoot ratio
Control					
Zhenongda 454	88 c	143 a	71 a	28 b	0.40 a
Sanyang'ai	69 d	141 a	76 a	36 a	0.48 a
Low P					
Zhenongda 454	121 b	155 a	79 a	33 ab	0.42 a
Sanyang'ai	138 a	159 a	76 a	35 a	0.46 a
<i>LSD</i> _{0.05}	12	NS	NS	5.3	NS

Data followed with different lowercase letters within each column mean significantly different at $P = 0.05$ level according to least significant difference (LSD) test. NS, Not significant.

concentration of sucrose in shoots and roots. Low P treatment increased content of sucrose in roots for both genotypes, but had no effect on content of sucrose in shoots. Zhenongda 454 had greater root/shoot sucrose content ratio than Sanyang'ai at deficient P treatment, while there was no difference at sufficient P treatment. Low P treatment increased

root/shoot sucrose content ratio for both rice genotypes (Table 2).

Zhenongda 454 had higher concentration of starch in shoots than Sanyang'ai at sufficient P treatment, whereas the concentration of starch in shoots was higher for Sanyang'ai than Zhenongda 454 at deficient P treatment (Table 3). There was no

difference in the concentration of starch in roots between Zhenongda 454 and Sanyang'ai regardless of P levels. For both genotypes, low P treatment increased the concentration of starch in shoots, but had no influence on the concentration of starch in roots. Shoot starch content, root starch content, root/shoot starch content ratio was not affected by low P treatment for both genotypes (Table 3).

DISCUSSION

The present study showed that the shoot dry weight of Zhenongda 454 and Sanyang'ai were reduced by 19% and 50%, respectively when grown with deficient P supply compared with P-sufficient plants (Fig. 1). Root dry weight of Sanyang'ai was decreased by 12% under deficient P supply, whereas P deficiency had no influence on root dry weight of Zhenongda 454 (Fig. 1). The above results indicated that the growth of Zhenongda 454 was less affected by P deficiency than Sanyang'ai, which confirmed the result that Zhenongda 454 was more tolerant to low P stress than Sanyang'ai in the field trial^[21].

Reduction in leaf photosynthetic rate of plants induced by P deficiency has been reported in both C₃^[11-12, 27] and C₄^[28-30] plant species. Our results also showed that photosynthetic rate of Zhenongda 454 and Sanyang'ai were decreased by 16% and 35%, respectively, when grown with deficient P supply (Fig. 2). There are two main factors that can cause a decrease in photosynthetic rate of plants, which are 1) a stomatal factor that depends on the number of stomatal pores, stomatal location and dimensions^[31] and 2) a non-stomatal factor which primarily depends on the activity of intrinsic enzymes, photosynthetic apparatus and their regulation mechanisms^[32]. It has been reported that leaf photosynthesis of plants was affected by low P treatment via stomatal and non-stomatal components^[12-13, 28, 33]. In the present study, low P treatment decreased stomatal conductance for both genotypes (Fig. 2), but had no significant influence on leaf internal CO₂ concentration, suggesting that the decrease in stomatal conductance did not restrict the diffusion rate of CO₂. Therefore, it could be concluded that photosynthetic rate of rice plants induced by P

deficiency was not due to stomatal limitation. This result was consistent with the result of Fredeen et al^[12] who suggested that non-stomatal limitation was much more important than stomatal limitation to account for the decrease in photosynthesis of soybean plants induced by P deficiency. In addition, we found that leaf photosynthetic rate of Zhenongda 454 was significantly higher than Sanyang'ai at deficient P treatment (Fig. 2). Considering the fact that maintaining higher photosynthetic rate is very important to sustain normal growth of plants when suffering from low P stress, it could be logical to deduce that higher photosynthetic rate of Zhenongda 454 than Sanyang'ai under P-deficient conditions would be one of the contributions to the fact that Zhenongda 454 is more tolerant to P deficiency than Sanyang'ai.

However, some of the earlier studies showed that P deficiency had no significant influence on leaf photosynthetic rate of plants^[34-35]. The discrepancy between different researches concerning the effect of P deficiency on photosynthetic rate may be resulted from the difference in plant materials used, duration time of P deficiency treatment or even growth environment of plants. Since the mechanisms involved in the photosynthesis inhibition induced by P deficiency were very complex^[36], more detailed biochemical characterization of these changes needed to be studied further.

Our results showed that the concentration of soluble carbohydrates in shoots and roots for both Zhenongda 454 and Sanyang'ai were significantly increased by low P treatment (Table 1). Increase of the soluble carbohydrate concentrations in plant tissues was not always caused by the increase of soluble carbohydrates production or export of soluble carbohydrates from shoots to roots because the inhibition of plant growth usually occurred when grown with low P supply. Low P treatment had no influence on soluble carbohydrates concentration in shoot of Zhenongda 454, whereas decreased soluble carbohydrates content in shoots of Sanyang'ai (Table 1). The possible explanation was that soluble carbohydrates production was inhibited for Sanyang'ai due to the fact that photosynthetic rate was decreased by 35% at deficient P treatment (Fig. 2),

whereas the photosynthetic rate was less affected by P deficiency for Zhenongda 454 (Fig. 2). Increase of soluble carbohydrates concentration in roots was observed for Zhenongda 454, but not observed for Sanyang'ai (Table 1), suggesting that Zhenongda 454 had higher ability to export soluble carbohydrates from shoots to roots compared with Sanyang'ai. A greater ability to allocate soluble carbohydrates to roots could be beneficial to maintain a relatively high growth rate of root, which could probably lead to the higher ability to acquire P from P-deficient environment. Therefore, compared to the genotype Sanyang'ai, the better tolerance to P deficiency of Zhenongda 454 may be related to its higher ability to export soluble carbohydrates from shoots to roots.

It is generally accepted that sucrose and starch metabolisms of plants are significantly affected by P deficiency^[10]. Some researchers suggested that enhancement of starch synthesis is induced when plants are starved of P because an increase in triose-P/P_i ratio in the chloroplast caused by P deficiency favors synthesis of starch by stimulating the ADP-glucose pyrophosphorylase, a key regulatory enzyme in starch synthesis pathway^[37-38] and diminishment in sucrose synthesis of plants is induced by P deficiency because triose-P exported from chloroplasts to the cytosol is likely to be restricted^[10]. However, such a mechanism does not appear to account for the results of the present study. Low P treatment did not increase the content of starch in shoots and roots for both genotypes (Table 3), but increased the concentration of sucrose in shoots and roots (Table 2). In addition, sucrose content in roots of both two rice genotypes was also increased by P deficiency (Table 2), suggesting that P deficiency treatment did not impair the sucrose synthesis, but enhanced the export of sucrose from shoots to roots for rice plants. The possible explanation to the results obtained here would be 1) restriction of triose-P export from chloroplasts to the cytosol via triose-phosphate is not always occurring for P-deficient plants^[19]; alternatively 2) low P treatment increased some key enzymes related to sucrose synthesis pathway, such as sucrose-phosphate synthase, cytosolic fructose-1,6-bisphosphatase (FBPase) and uridine-5-diphosphoglucose (UDPG)

pyrophosphorlase^[39]. In contrast, Zhenongda 454 had higher root/shoot sucrose content ratio than Sanyang'ai under P-deficient conditions, suggesting that Zhenongda 454 had greater ability to allocate sucrose to roots than Sanyang'ai, which might be one of the reasons for that Zhenongda 454 is more tolerant to low P stress than Sanyang'ai.

In conclusion, the present study demonstrated that P deficiency decreased photosynthetic rate, and increased partitioning of soluble carbohydrates and sucrose to roots for both rice genotypes. By comparison, Zhenongda 454 had a higher photosynthetic rate, and a greater ability to allocate carbohydrates to roots than Sanyang'ai under P-deficient conditions, which might account for the fact that Zhenongda 454 was more tolerant to low P stress than Sanyang'ai.

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