Response of root development and nutrient uptake of two chinese cultivars of hybrid rice to nitrogen and phosphorus fertilization in Sichuan Province, China

Guotao Yang1,2 · Farhan Nabi2 · Sumbal Sajid2 · Abdul Rasheed Kaleri2 · Ali Murad Jakhar2,3 · Liang Cheng2 · Martin Raspor4 · Noor Muhammad2 · Jun Ma1 · Yungao Hu2

Received: 18 August 2021 / Accepted: 12 October 2021 / Published online: 19 October 2021
© The Author(s) 2021

Abstract

Background Chemical fertilization helped modern agriculture in grain yield improvement to ensure food security. The response of chemical fertilization for higher hybrid rice production is highly dependent on optimal fertilization management in paddy fields. To assess such responses, in the current work we examine the yield, root growth, and expression of related genes responsible for stress metabolism of nitrogen (N) and phosphorus (P) in two hybrid-rice cultivars Deyou4727 (D47) and Yixiangyou2115 (Y21).

Methods and results The experiment followed four nitrogen (N) (N0, N60, N120, and N180 kg/ha) and phosphorus (P) (P0, P60, P90, and P120 kg/ha) fertilizer levels. The grain yield in D47 was more sensitive to nitrogen application, while Y21 was more sensitive to phosphorus application, which resulted in comparatively higher biomass and yield. Our findings were corroborated by gene expression studies of glutamine synthetase OsGS1;1 and OsGS1;2 and phosphate starvation-related genes PHR1 and SPX, confirming sensitivity to N and P application. The number of roots was less sensitive to nitrogen application in D47 between N0 and N60, but the overall nutrient response difference was significantly higher due to the deep rooting system as compared to Y21.

Conclusions The higher yield, high N and P use efficiency, and versatile root growth of D47 make it suitable to reduce unproductive usage of N and P from paddy fields, improving hybrid rice productivity, and environmental safety in the Sichuan basin area of China.

Keywords Deyou4727 · Yixiangyou2115 · Gene expression · Nutrient use efficiency · Root growth

Introduction

The extensive use of chemical fertilizers in current agricultural practice is detrimental to the environment and reduces soil quality and crop production in the long run. Consequently, finding sustainable solutions for the improvement of crop production has become a challenging global agricultural issue [1]. According to FAO [2], the annual world consumption of the three main fertilizer nutrients, nitrogen (N), phosphorus (P) applied as phosphate (P2O5), and potassium applied as potash (K2O), reached 185 million tons (total N, P2O5, and K2O) in 2016 and is expected to top 200 million tons annually by 2022. As the consumption increases, it will prompt the production of commercial fertilizers, intermediates, and raw materials, which will significantly contribute to global carbon emissions. China is among the top five markets of commercial fertilizer production and consumption;
according to FAO [3]. China consumed 27.3 million tons N and 15 million tons P fertilizer for rice and wheat fertilization in 2014.

It has been estimated that only about 1/3 of the applied nitrogen fertilizer is used by crops, whereas 2/3 remain in the environment, accounting for ~16 billion USD worth loss of N fertilizer worldwide on annual basis [4]. Unassimilated N fertilizer leaches from farming and creates environmental damage, including nitrate accumulation, water pollution and eutrophication [5], and soil deterioration [1]. In order to avoid excessive N fertilizer usage, current research in agronomy is focused on developing strategies for enhancing the nitrogen use efficiency of crop plants [1, 4, 6–8]. In cereal crops, enhancing grain yield without increasing fertilizer input remains one of the most important challenges [9].

Problems related to the use of phosphate fertilizer include both leaching of excess phosphate into the environment [10], and the prospective depletion of finite reserves of phosphate rock, which represents the raw material for the production of fertilizer [11]. Additionally, cereals and some other food crops convert a significant part of assimilated phosphate into phytic acid, which has no nutritive value and even acts as an antinutrient [12]. Agronomic measures for the prevention of phosphate leaks into the environment have proven relatively inefficient and transgenic approaches have been suggested for improving phosphate uptake and limiting the synthesis of phytic acid for improved phosphate use efficiency [13].

Recently, an array of various N and P management strategies have been launched for gradual or spatially targeted release of commercial fertilizers [1], which include combining organic manure [14], vermicompost [15], biochar [16] and usage of nano-fertilizers for delivery of nutrients to target plants [17]. However, the use of these strategies has so far faced limitations because they are either insufficient, costly, or labor-intensive [1].

According to FAO [2] data (http://www.fao.org/faostat/en/#data/QC), rice (Oryza sativa L.) is the world’s third most important crop after maize and wheat, in terms of both harvested area (162 million hectares worldwide) and annual production (755 million tonnes). Furthermore, for rice fertilization in China, a 75% higher (180 kg/ha) nitrogen input is used than the world average, whereas in the Guangdong province of Southern China fertilization rates as high as 250 kg/ha are applied [18]. Among the major food crops, rice accounts for the highest amounts of wasted nitrogen fertilizer [19]. Furthermore, optimizing the right measure of phosphate fertilizer to deliver sufficient phosphorus to rice crops without risking environmental damage has proven as a major challenge for rice cultivation, for instance in West African countries [20]. More than 3/4 of the applied phosphate fertilizer is wasted and remains in the environment, as only about 1/4 is taken up by rice crops [21]. Fertilization of rice paddy fields represents an important target for decreasing large-scale consumption of commercial fertilizers, reducing the environmental damage from fertilizer use, and at the same time cutting the costs for fertilizer purchase and transport. Thus, hybrid rice varieties, which show enhanced tolerance to abiotic stresses and produce high yields in low nitrogen and phosphorus fertilization, represent a promising perspective for reducing the use of chemical fertilizers for rice cultivation [22]. Like other cereal crops hybrid rice varieties have different fertilization patterns, highly depending on their genetic nature [8, 23]. For such hybrid rice types, effective fertilization management includes selecting the proportion of fertilizer, the source of fertilizer, the timing of fertilizer application, and the combination of fertilizers that match the needs of the crop to maximize fertilizer utilization efficiency, optimize crop production, and minimize the negative impact of fertilizer on the environment [24].

This study’s main objectives were to estimate the impact of low and high nitrogen and phosphorus input on the yield, nutrient uptake, use efficiency and root growth, of hybrid rice varieties Deyou4727 and Yixiangyou2115 under different N and P levels. Also, the expression levels of N and P metabolism-related genes OsGS1;1, OsGS1;2, PHR1, and SPX were quantified to assess the molecular response of the cultivars to different nutrient concentrations. The results provide a basis for planning measures to reduce the losses of N and P in paddy fields and for choosing a well-performing variety to improve rice yield in the Sichuan Basin area of China and other regions with similar environments.

Materials and methods

Study area

The experiments were carried out in the laboratory and field at the Southwest University of Science and Technology in Mianyang, Sichuan Province, China, in 2019. The soil of this region is clay-loamy soil, with a bulk density of 1.29 g/cm³ and organic matter content of 28.6 g/kg, while total nitrogen, phosphorus, and potassium content were 1.68, 0.37, and 1.86 g/kg respectively. The major food crops cultivated in this region are rice and oilseed rape, which form a paddy-dryland rotation system in this area, with oilseed rape cultivation occurring from late September to April, and rice cultivation from April to September.

Experimental design

Field experiment treatment sets were carried out for two rice varieties Deyou4727 (D47) and Yixiangyou2115 (Y21). Rice seeds were cultivated from March–September 2019. Field design was carried out as a randomized complete block
design, each plot was 3 m × 3.5 m with 0.5 m corridors. When the seedlings were around 7-8 cm long, field plots were treated. For the N application, four N application levels were used, i.e., N₀, N₁₂₀, N₁₈₀ [kg/ha]. To ensure that nutrients other than N would not limit rice growth, 90 kg P₂O₅/ha was applied to each plot. For the P application, four P application levels were used, i.e., P₀, P₆₀, P₉₀, P₁₂₀ [kg/ha]. To ensure that nutrients other than P would not limit rice growth, 120 kg N/ha was applied to each plot. Each treatment was replicated three times. The plant sampling was done at four time points after treatments, wrapped in aluminum foil, frozen in liquid nitrogen, and stored at -80 °C until further analysis.

**Sampling and measurement**

**Sampling**

Plant samples of all treatments were collected at four time points (10, 25, 40, 55 days after treatment) in 2019. Fresh samples were used for morphological measurements and gene expression studies. The soil samples for N and P uptake calculation were transported to the lab in ice boxes. Fresh samples were used to determine initial nutrient concentrations.

**Measurements**

At maturity, plant samples were separated into straw, leaves, and panicles for dry weight determination after oven-drying to constant weight at 70 °C. Plant roots were counted according to Gu et al., [25]. Grain yield was measured from a 5 m² area from the center of each plot at the maturity stage. Panicles were placed into bags and labeled to determine yield and yield components [26]. Grains and panicles were separated. Filled and empty panicles were separated and counted, grains were weighed. The seed setting rate was calculated according to Xiang et al., [27]. Yield component data (1000-grain weight, the number of panicles, and percentage of filled grains) from all treatments were determined according to Fageria [28].

Total nitrogen content in the soil was determined by the Kjeldahl method as described by Okalebo et al. [29]. Available phosphorus was determined by the Olsen method following the procedure described by Juo [30].

**Crop response to N and P inputs**

**N and P use efficiencies**

N and P use efficiency parameters were calculated based on the grain yield and N/P accumulation in plots treated at different N and P rates. The definitions are as follows [31]:

**Agronomic Efficiency (AE)**

$$AE = \frac{GY_{N/P} - GY_{N/P0}}{FN}$$ (kg grain kg⁻¹)

**Physiological Efficiency (PE)**

$$PE = \frac{GY_{N/P} - GY_{N/P0}}{TN/P_{N/P} - TN_{N/P0}}$$ (kg grain kg⁻¹)

**Recovery Efficiency (RE)**

$$RE = \frac{TN_{N/P} - TN_{N/P0}}{FN} \times 100\%$$ (%)

where \( GY_{N/P} \) is the grain yield of the plots that received N and P fertilizer, \( GY_{N/P0} \) is the grain yield in the N₀ and P₀ plots, \( FN \) is the amount of N and P fertilizer applied, \( TN_{N/P} \) is the total N and P accumulation in the plots that received N and P fertilizer at maturity, and \( TN_{N/P0} \) is the total N and P accumulation in the N₀ and P₀ plots at maturity.

**qPCR analysis**

Total RNA from flag leaves of mature rice plants was isolated using the RNeasy Plant Mini Kit (Qiagen, Venlo, The Netherlands) and reversely transcribed using Omniscript RT Kit (Qiagen). Quantitative real-time PCR (qPCR) was performed using the SYBR Premix Ex Taq (TaKaRa, Dalian, China) and the CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The PCR mix was composed of 5 µl SYBR Premix Ex Taq, cDNA corresponding to 30 ng RNA, 0.3 µl of each F and R primer (10 mM), and PCR grade water up to the final volume of 10 µl. The primer sequences are available in Supplementary Table S1. The incubation temperature of the reaction was as follows: (1) denaturation at a cycle of 95 °C for 3 min; (2) amplification: 40 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s; (3) final elongation 72 °C for 3 min, and (4) melting curve analysis (65–95 °C). Each sample was analyzed in triplicate, and the relative expression levels were calculated relative to control (N₀ or P₀ treatment) using the \(2^{-\Delta\Delta Ct} \) comparative CT method [32].

**Statistical analysis**

Origin 19.0 and Excel 2016 statistical software were used to analyze the experimental data. The correlation analysis between grain yield and N/P accumulation was done using ggpubr R package. Each treatment value is expressed as mean ± standard deviation (SD) of 3-6 biological replicates. The differences were determined by Student’s t-test at \( P < 0.05 \) (*), 0.01 (**), or 0.001 (***).
Results

Yield and physiological traits responses

The effect of nutrient and genotype on hybrid rice grain yield and effective panicles was significant \((P < 0.05)\). Both rice cultivars Deyou4727 (D47) and Yixiangyou2115 (Y21) showed a maximum yield of rice grains in high nitrogen (N) treatment \(N_{180}\) and the lowest yield observed in no-N treatment \(N_{0}\). The cultivar D47 showed a maximum yield of 9.17 t/ha, in the high N treatment \(N_{180}\), which was 5% higher than for Y21. The response of both cultivars to N application was significantly different. D47 was more sensitive to N application as compared to Y21, as it showed a significantly higher difference of yield response between \(N_{0}\)–\(N_{60}\), \(N_{60}\)–\(N_{120}\), \(N_{120}\)–\(N_{180}\) (Table 1).

Yield response was positively correlated with effective panicles in D47 and Y21, which resulted in yield difference. Y21 was less sensitive to N application.

The rice type D47 overall showed significantly higher grain yield in all treatments, more specifically under different nitrogen treatments it showed 3%, 3%, 4%, and 5% higher grain yield in \(N_{0}\), \(N_{60}\), \(N_{120}\), and \(N_{180}\) treatments respectively, as compared to Y21.

Among different phosphorus (P) treatments, both rice types favored the moderate phosphorus concentration \(P_{90}\). Likewise, in different phosphorus treatments, D47 showed, 6%, 10%, 4%, and 1% higher grain yield than Y21 in \(P_{0}\), \(P_{60}\), \(P_{90}\), and \(P_{120}\) treatments respectively. The response of P application for both cultivar yields were different. D47 showed higher sensitivity of yield response from \(P_{0}\)–\(P_{60}\), \(P_{60}\)–\(P_{90}\) and \(P_{90}\)–\(P_{120}\), as compared to Y21, which showed maximum sensitivity between \(P_{60}\)–\(P_{90}\). The P application response was equally observed for effective panicles that resulted in yield differences respectively.

Similarly, effective panicle significantly increased with increasing N and P treatments. Higher nutrient input caused higher effective panicles in both rice types, and they followed a \(N_{180} > N_{120} > N_{60} > N_{0}\) and \(P_{120} > P_{90} > P_{60} > P_{0}\) trend, for nitrogen and phosphorus respectively. Unlike EP, seed setting rate, the number of grains per panicle, and 1000-grain-weight were less affected by N and P supply (Table 1).

Correlation analyses of grain yield and N/P accumulation

A Pearson’s correlation was computed to assess the relationship between grain yield and nutrient accumulation for both rice types (Fig. S1). Mostly negative correlations between low or high nutrient accumulation and yield were
found in different nitrogen and phosphorus treatments. A high increase in nutrient uptake was negatively correlated with the amount of yield output. More specifically, in D47 in N_{180} treatment, was positively correlated (R = 0.69) while in Y21 N_{60} and N_{120} were more positively correlated with R values of (R = 0.76) and (R = 1) respectively.

Similarly in the case of phosphorus accumulation D47 showed a positive correlation in all treatments up to P_{120}. The correlation was highest up to P_{60} (R = 1), while it slightly decreased in P_{60} and P_{90} with R values near 0.94 and 0.97 respectively. Unlike D47, Y21 showed a negative correlation (R = -0.76), (R = -0.94) at P_{60} and P_{90} respectively, however, it had a good positive correlation at P_{120} with R-value near 0.99.

Plant biomass and nutrient accumulation in hybrid rice

Total biomass at maturity significantly (P < 0.05) increased with N application rate and differed in two hybrid rice cultivars (Table 2). The highest plant biomass, N, and P accumulation were found in high N and P treatments for both hybrid rice varieties. Overall D47 showed 4%, 9%, 3%, and 6% higher biomass production than Y21 in N_{0}, N_{60}, N_{120}, and N_{180} treatments respectively. A similar trend was found for different P treatments, in which D47 showed 10%, 7%, 3%, and 7% higher biomass production than Y21 in P_{0}, P_{60}, P_{90}, and P_{120} treatments respectively. Like biomass, N and P also followed higher accumulation in high treatments and low accumulation in low treatments. N accumulation followed the N_{180} > N_{120} > N_{60} > N_{0} trend, and phosphorus accumulation followed the P_{120} > P_{90} > P_{60} > P_{0} trend in both rice types, at constant environmental factors. D47 accumulated 5–10% greater total biomass compared to Y21 at all fertilizer treatments (Table 2). Accumulation of nitrogen biomass was similar for both cultivars at all treatments, however, D47 accumulated 10–15% more phosphorus biomass on most treatments (Table 2).

N/P accumulation, biomass and N/P concentration in both cultivars were sensitive to N and P application. Like yield and yield parameters, N/P accumulation response and biomass were higher in D47. It showed a more sensitive response between N_{0}–N_{60}, N_{60}–N_{120}, N_{120}–N_{180}. Unlike N/P accumulation and biomass, D47 showed less sensitivity in N/P concentration. That makes it more nitrogen efficient, as compared to Y21. Conversely, Y21 showed less response sensitivity in biomass and N/P concentration, while N/P accumulation was significantly more sensitive between N_{0}–N_{60}, N_{60}–N_{120}, N_{120}–N_{180}.

Nutrient use efficiency

Nutrient uptake and nutrient use efficiency varied among treatments and hybrid rice varieties. The AE, PE, and RE significantly (P < 0.05) increased with nutrient application, thus the highest values were found in N_{120} and P_{90} treatments for both hybrid rice types (Table 3). The AE for N_{120} treatment in D47 showed 6%, 65%, and 2% higher AE, PE, and RE respectively as compared to Y21. Unlike N_{120}, in the case of P_{90}, Y21 showed 6%, 15%, and 5% higher AE, PE, and RE in P_{90} treatment as compared to D47. The response of nutrient use efficiency of both cultivars was highly sensitive to N application and showed a significant difference of AE and PE. A more sensitive response was found between N_{60}–N_{120}, while Y21 showed less sensitivity for these values as compared to D47. In case of NRE most sensitive difference was found between N_{120}–N_{180} for both cultivars, with the less significant difference (P <0.05). Similarly, the P application resulted in a high response difference of AE and PE between P_{60}–P_{90} for both cultivars, however, D47 was more sensitive for P application as compared to Y21. When it comes to RE, D47 showed a high response difference.

Table 2 Biomass, N, P content, and accumulation for two rice varieties Deyou4727 (D47) and Yixiangyou2115 (Y21)

| Treatments | D47 N/P accumulation (g m⁻²) | Biomass (g m⁻²) | N/P concentration (%) | Y21 N/P accumulation (g m⁻²) | Biomass (g m⁻²) | N/P concentration (%) |
|------------|-------------------------------|----------------|------------------------|-------------------------------|----------------|------------------------|
| N_{0}      | 7.7d                          | 1325d          | 0.58b                  | 7.5d                          | 1277c          | 0.59b                  |
| N_{60}     | 9.9c                          | 1572c          | 0.63b                  | 9.8c                          | 1424b          | 0.69a                  |
| N_{120}    | 12.4b                         | 1713b          | 0.73a                  | 12.2b                         | 1663a          | 0.73a                  |
| N_{180}    | 14.1a                         | 1848a          | 0.76a                  | 13.8a                         | 1738a          | 0.79a                  |
| P_{0}      | 2.68c                         | 1249d          | 0.21a                  | 2.21b                         | 1119c          | 0.19b                  |
| P_{60}     | 3.15b                         | 1488c          | 0.21a                  | 2.84b                         | 1378b          | 0.20b                  |
| P_{90}     | 3.66b                         | 1713b          | 0.21a                  | 3.24a                         | 1663a          | 0.19b                  |
| P_{120}    | 4.19a                         | 1817a          | 0.23a                  | 3.82a                         | 1698a          | 0.22a                  |

Values obtained at different N, or different P treatments, at the same time point for the same cultivar, and labeled with different letters are significantly different from each other at P < 0.05.
between P60–P90 while Y21 showed more response difference between P90–P120 and no significant difference found between P60–P90. These findings suggest that D47 had a more sensitive response difference for N and P applications in each treatment, while Y21 was less sensitive to nutrient application. The above results indicate higher nitrogen use efficiency in D47.

Impact of nutrient input on root number

The number of roots increased progressively from day 10 to day 55 in all treatments, but the number of roots and dynamics of root appearance differed between treatments (Fig. 1), which indicates nutrient (N, P) application had a low response difference in initial days. Among the nitrogen treatments, the highest number of roots on day 55 was recorded in N120 (Fig. 1a, b). Similarly, in the case of different phosphorus concentrations, D47 and Y21 favored the P90 phosphorus concentration (Fig. 1a, b). As compared with Y21, D47 collectively showed 12.4% higher root numbers in N120 P90 (478 for D47, as compared to 425 for Y21). D47 showed better development of root architecture and higher root numbers in all treatments of N and P, as compared to Y21 (Fig. S2). For N application higher sensitive difference was found between N60–N120 for both cultivars, while in the case of P application higher sensitive difference was found between P60–P120 in both cultivars. However, D47 was less sensitive in low nitrogen application N0–N60, but the overall nutrient response difference was significantly higher due to the deep rooting system as compared to Y21.

Expression of glutamine synthetase genes OsGS1;1 and OsGS1;2 with nitrogen fertilization

The expression levels of two genes of the glutamine synthetase family, OsGS1;1 and OsGS1;2, which are considered important markers of nitrogen metabolism, have been analyzed in response to nitrogen availability in hybrid rice cultivars D47 and Y21.

Interestingly, OsGS1;1 was upregulated in both low (N0, N60) and high (N180) nitrogen compared to moderate nitrogen treatment (N120), where its expression in both cultivars was relatively low (Fig. 2a). The expression of OsGS1;2 followed the opposite pattern in D47, being strongly upregulated on moderate nitrogen treatment compared to low and high nitrogen treatments. However, in Y21, OsGS1;2 was upregulated at low nitrogen and downregulated on moderate and high nitrogen (Fig. 2b).

Expression of PHR1 and SPX genes with phosphorus fertilization

The expression levels of two genes that are considered important markers of phosphorus availability, PHR1, and SPX, have been analyzed in response to phosphorus availability in hybrid rice cultivars D47 and Y21.

The gene PHR1 was strongly upregulated on the no-phosphorus (P0) treatment compared to the other three treatments for the cultivar D47, while in Y21 its expression was also higher on moderate (P90) than on high (P120) phosphorus treatment (Fig. 2c). On the other hand, the expression of SPX was remarkably upregulated at P90 compared to other treatments, while in Y21 it was downregulated on higher P concentrations compared to low P treatments (Fig. 2d).

Discussion

Optimization of fertilizer dosage has become one of the most important challenges in the current agronomy of cereal crops [33, 34]. This work represents a comparative analysis of the effects of high, moderate, low-dosage application and non-application of nitrogen and phosphorus fertilizer on the growth, yield, yield-related traits and expression of selected genes.
genes in two Chinese hybrid rice cultivars. The cultivar Deyou4727 (herein shortened to D47) has been previously described as drought-tolerant, with a particularly well-developed root system [35], while Yixiangyou2115 (referred to as Y21) was reported as relatively nitrogen-inefficient compared to other hybrid rice cultivars and restorer lines [36].

**Yield and yield-related parameters and correlation analysis**

Our results showed that grain yield progressively increased with nitrogen fertilization in both cultivars, although in Y21 the yield difference between N$_{120}$ and N$_{180}$ was not statistically significant (Table 1). The underperformance of Y21 with high nitrogen fertilization is in concordance with its low nitrogen efficiency as compared to other rice cultivars, as has been previously reported by Li et al. [36], and demonstrated further by comparing the nitrogen use efficiency of the two cultivars in this study.

Both cultivars performed better at moderate (P$_{90}$) than at high (P$_{120}$) phosphorus fertilization (Table 1). It was reported before that high phosphorus fertilization is inefficient in promoting the agronomic performance of rice, as no significant differences in grain yield were found between phosphorus applied at 90 and 135 kg/ha in both upland and paddy rice [37].

Effective panicle was positively affected by both high N and P fertilization in both cultivars, but neither high N nor high P fertilization could significantly enhance the seed-setting rate, the number of grains per panicle, or 1000-grain weight in cultivars (Table 1). Overall, it can be concluded that while high N fertilizer dosage contributed...
to higher yield in D47 (but not Y21), a high dosage of P fertilizer does not confer any significant benefit to the most important agronomic parameters of either D47 or Y21 output.

Hybrid rice varieties possess different nutrient uptake abilities, the low and high N and P application rate determines plant physiological needs. In our study, we assessed 2 rice types for yield and nutrient uptake correlation. We found D47 had a positive correlation with N application only in N_{180} treatment (R = 0.69), while Y21 on the other hand had a positive correlation at N_{60} and N_{120} with R-values of 0.76 and 1 in respective treatments. In our study D47 findings are positively consistent with [38], in which they found increasing N application positively correlated yield uptakes up to N_{180}, but in the case of Y21, the results were different. It showed a positive correlation of R=1 in the N_{120} treatment. Similarly, many studies have shown that rice yield increases with the increase of nitrogen application within a certain range, but the yield decreases when the nitrogen application is too high [39, 40].

In the case of P accumulation and yield correlation, the hybrid variety D47 showed a positive correlation up to P_{120}. The correlation was highest up to P_{60} (R = 1), while it slightly decreased in P_{90} and P_{120} with R-values near 0.94 and 0.97 respectively. Unlike D47, Y21 showed a negative correlation at P_{60} and P_{90}, however, it had a positive correlation at P_{120} with R-value near 0.99. The above findings suggest that D47 is a more phosphorus efficient type and its yield highly depends on phosphorus input, while in the case of Y21 the low P input resulted in a negative correlation, which makes it less phosphorus efficient type. Similar results were stated by Zhang et al. [37], in which they reported that P accumulation highly depends on rice variety used and P input. High input resulted in high yield (positive correlation), while between varieties the P accumulation and yield output differed greatly.
Nitrogen and phosphorus uptake and use efficiency

In our study, high N and P fertilization positively affected both total plant biomass, and accumulation of N or P, respectively, in the plant biomass (Table 2). It is well known that N is the limiting nutrient for plant growth because it is at the same time both largely required for the synthesis of proteins and nucleic acids, and limitedly available to the plants [19]. High nitrogen fertilization is known to positively affect total biomass in rice, as well as height, grain harvest index, and components related to grain yield [38, 41–43]. Phosphorus fertilization up to 90 kg/ha positively affected plant growth, yield, formation of panicles, tillers and leaves in hybrid rice [44], whereas increasing P fertilization over that threshold was not found agronomically useful in previous reports [37].

The genotypes D47 and Y21 differed in N and P uptake (Table 2) and use efficiency (Table 3) at different fertilizer dosages. The genotype D47 was superior (~5–10 %) at total biomass compared to Y21 at all fertilizer treatments (Table 2). It showed similar N uptake rates as Y21 (Table 2) but 1.5-3-fold higher nitrogen use efficiency on all fertilizer treatments (Table 3). It is already known that nitrogen use efficiency can considerably vary between lowland rice genotypes [41]. Superior nitrogen use efficiency of D47 compared to Y21 is likely accounting for its more successful total biomass accumulation, as these two parameters are known to greatly coincide [45].

When it comes to phosphate assimilation, D47 accumulated slightly higher phosphate biomass than Y21, but this was probably only because of its greater total biomass, as its percentage of phosphorus biomass was similar to Y21 (Table 2). However, Y21 showed considerably higher values for phosphorus use efficiency compared to D47 on all P fertilizer treatments, especially for physiological P use efficiency (Table 3). Similar to nitrogen, phosphate use efficiency has also been proven to considerably vary between lowland rice genotypes, and it also importantly correlates with rice grain yield [46].

Both nitrogen and phosphate use efficiency are reported to have relatively low values in rice – NUE typically ranges from 30 to 50% [4] while PUE is typically less than 30% [47]. Therefore, breeding the rice varieties with improved nutrient use efficiency is the most promising strategy to raise both grain yield and nutritional quality with limited fertilizer input [48]. The nutrient use efficiency parameters such as agronomic use efficiency (AE), physiological use efficiency (PE), and recovery efficiency (RE) were studied in this experiment. AE, PE, and RE significantly (P < 0.05) increased with the nutrient application, but both AE and PE dropped at the highest fertilizer dosage of both nitrogen and phosphate. An important exception is the PE value for nitrogen fertilization of the Y21 cultivar, which was extremely nitrogen-inefficient at low and moderate doses and continued to grow even at N180 (Table 3). We conclude that the values of nitrogen and phosphate use efficiency of rice cultivars D47 and Y21 argue in favor of moderate fertilizer application to achieve better nutrient use efficiency.

Root growth of D47 and Y21 genotypes at different fertilizer treatments

In our findings, root growth depended on both genotype, and fertilizer dosage (Fig. 1). In most treatments, root growth was more pronounced in D47, which is a cultivar known for drought tolerance and a well-developed root system [35]. It has been suggested before, that root growth is strongly related to nitrogen use efficiency in cereals [49, 50] so it is unsurprising that these two traits are co-occurring in the same genotype, D47.

Both nitrogen and phosphorus fertilization had a positive impact on root growth up to moderate doses (N120, or P90), whereas high fertilizer doses of either N or P (N180, or P120, respectively) had an inhibitory effect on root growth, resulting in significantly lesser number of roots compared to moderate doses starting from day 40 (Fig. 1). Ammonium toxicity from excess N fertilization can negatively affect root growth [51, 52]. Furthermore, on day 10, both D47 and Y21 had developed a greater number of roots when grown at N60 compared to N120 and N180 treatments, whereas since day 25 this relationship was reversed to favor higher doses of N fertilizer application (Fig. 1). It has been recently reported that urea from the N fertilizers inhibits the early stages of root growth, but does not affect shoot growth [8]. When it comes to the effect of phosphorus fertilizer on root growth, it has been shown that the number of roots in upland rice is positively affected by growing amounts of P fertilizer up to a certain limit [53], which was confirmed by our results.

Expression of nitrogen and phosphate homeostasis-related genes

The need for improving the nutrient use efficiency of large-scale cultivated crops has pushed the research towards the identification of target genes for genetic modifications [54].

The key step in nitrogen assimilation in higher plants is the incorporation of the inorganic ammonium ion (NH4+) into organic compounds. The initial step in this pathway is the synthesis of the amino acid glutamine through the activity of the enzymes glutamine synthetases (GS) [55]. Thus, the genes encoding glutamine synthetases are important genetic markers for nitrogen assimilation and were proven related to the nitrogen use efficiency in crop species such as maize [56] and tobacco [57]. The glutamine synthetase gene family in plants consists of several genes coding for different enzyme isoforms that are differentially regulated [58]. In this study, we investigated the expression of two
cytosolic GS1 (OsGS1;1 and OsGS1;2) genes in rice cultivars D47 and Y21 to assess their role in response to different N fertilization treatments. The two GS1 genes have different roles in rice, with OsGS1;1 being responsible for the initiation of synthesis of a broader range of metabolites [59, 60], whereas OsGS1;2 has diverse functions, like the primary assimilation of ammonium ions in roots [61] and cross-talk with other signaling pathways, like cytokinins which affect the outgrowth of axillary buds in the rice shoot [62]. Previous research has shown that expression profiles of OsGS1;1 and OsGS1;2 at different N fertilizer treatments can vary between rice cultivars, with expression peaks coinciding with optimal levels of N fertilization for each cultivar [63]. Our results for OsGS1;2 expressions suggest that moderate N levels (N120) are optimal for the cultivar D47, whereas in Y21 which is a less nitrogen-efficient cultivar, OsGS1;2 has relatively lower expression and is more active at lower doses of nitrogen fertilization (Fig. 2).

Members of the PHOSPHATE STARVATION RESPONSE (PHR) gene family have been identified as MYB transcription factors with a regulatory role in response to phosphorus nutrient deficiency [64, 65]. The PHR1 family has 12 members in rice, designated OsPHR1-OsPHR12 [66]. The role in phosphate homeostasis was confirmed for OsPHR2 [67, 68] and OsPHR4 [69]. Our results show that in both D47 and Y21 OsPHR1 has high transcriptional activity when P fertilizer is not applied, whereas their activity is downregulated with the growing application of P fertilization (Fig. 2), confirming its role in regulating plant metabolism at low P nutrition.

Members of the PHR family are subject to negative regulation by members of another family of transcription factors, SPX [45, 70]. The SPX genes might have a role in a broader array of physiological processes, but just like PHR, they might serve as genetic targets for improving phosphorus use efficiency [45]. The expression profiles of the OsSPX gene studied in our work revealed a similar pattern as for OsPHR, with high transcript levels when P fertilizer is not applied, but being downregulated at higher P treatments. Expression levels for both OsPHR and OsSPX were similar for both cultivars D47 and Y21 (Fig. 2).

Conclusions

In this research, we investigated an array of agronomic traits related to yield and nutrient use efficiency of the rice cultivars Deyou4727 (D47) and Yixiangyou2115 (Y21) at low, moderate and high levels of nitrogen and phosphorus fertilization. D47 was more sensitive to nitrogen application, the response of total biomass and yield was greater than Y21, but Y21 was more sensitive to phosphorus application. We confirmed our outcomes by gene expression studies of glutamine synthetase OsGS1;1 and OsGS1;2 and phosphate starvation-related genes PHR1 and SPX, which confirmed the D47 response sensitivity to N application and Y21 response sensitivity to P application.

Similarly, root numbers of D47 were less sensitive in low nitrogen application N5 and N60, but the overall nutrient response difference was significantly higher due to the deep rooting system as compared to Y21. Our results suggest that large amounts of N fertilizer have limited benefits for D47, whereas P fertilization with more than 90 kg/ha brings no significant agronomic benefit for the cultivation of D47 or Y21 rice. We further recommend research should be undertaken in the area of optimal fertilization management based on hybrid rice type, soil properties, and regional conditions to solve agronomic needs and environmental concerns.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06835-7.

Acknowledgements Special thanks are extended to the staff of the agricultural station of SWUST for their irreplaceable work to manage the experiment field and to collect the samples. We would also like to thank our friends Awais Ahmed, Abdul Hakeem and also the reviewers and editor who provided valuable suggestions to improve this paper.

Author contributions JM, YH designed the study and supervision. NM, LC collected the samples. GY, FN conducted the laboratory experiments, writing—original draft preparation, and formal analysis. MR, AMJ writing—review and editing. SS, ARK conducted bioinformatics and statistical analyses. All authors have read and agreed to the published version of the manuscript.

Funding This study was supported by Sichuan Science and Technology program (2021NZZJ0024, 2021YFN0053, 2021YFN0049, 2021 ZHFP0126).

Declarations

Conflict of interest The authors declare no conflict of interest.

Consent for participate All authors have materially participated in the research and manuscript preparation.

Consent for publication Consent for publication submission is approved by all authors.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Springer
1. Iqbal A, He L, Khan A, Wei S, Akhtar K, Ali I, Ullah S, Munsif F, Zhao Q, Jiang L (2019) Organic manure coupled with inorganic fertilizer: an approach for the sustainable production of rice by improving soil properties and nitrogen use efficiency. Agron 9:651. https://doi.org/10.3390/agronomy9100651
2. FAO (2015) World food and agriculture. Food and Agriculture Organization, Rome
3. FAO (2015) World food and agriculture. Food and Agriculture Organization, Rome
4. Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357-63. https://doi.org/10.2134/agronj1999.00021962009100030001x
5. Tahir MA, Rasheed H (2008) Distribution of nitrate in the water resources of Pakistan. Afr J Environ Sci 2.397-403. https://doi.org/10.5897/AJEST.9000034
6. Fageria N, Baligar V (2005) Enhancing nitrogen use efficiency in crop plants. Adv Agron 88:97-185. https://doi.org/10.1016/S0065-2113(05)80004-6
7. Li S, Wang Z, Hu T, Gao Y, Stewart B (2009) Nitrogen in dryland soils of China and its management. Adv Agron 101.123-81. https://doi.org/10.1016/S0065-2113(08)00803-1
8. Sharma N, Sinha VB, Gupta N, Rajpal S, Kuchi S, Sitaramam V, Parsad R, Raghunam R (2018) Phenotyping for nitrogen use efficiency: rice genotypes differ in N-responsive germination, oxygen consumption, seed urease activities, root growth, crop duration, and yield at low N. Front Plant Sci 9:1452. https://doi.org/10.3389/fpls.2018.01452
9. Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, Foley JA (2012) Closing yield gaps through nutrient and water management. Nature 490.254-7. https://doi.org/10.1038/nature11420
10. Chen M, Chen J, Sun F (2008) Agricultural phosphorus flow and its environmental impacts in China. Sci Total Environ 405:140-52. https://doi.org/10.1016/j.scitotenv.2008.06.031
11. Baker A, Ceasar SA, Palmer AJ, Paterson JB, Qi W, Muench SP, Baldwin SA (2015) Replace, reuse, recycle: improving the sustainable use of phosporous by plants. J Exp Bot 66:3523-3540. https://doi.org/10.1093/jxberv210
12. Gupta RK, Gangoliya SS, Singh NK (2015) Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J Food Sci Technol 52:676-84. https://doi.org/10.1007/s13197-013-0978-y
13. Kopriwa S, Chu C (2018) Are we ready to improve phosphorus homeostasis in rice? J Exp Bot 69:3515-22. https://doi.org/10.1093/jxberv163
14. Ye L, Zhao X, Bao E, Li J, Zou Z, Cao K (2020) Bio-organic fertilizer with reduced rates of chemical fertilization improves soil fertility and enhances tomato yield and quality. Sci Rep 10:1-11. https://doi.org/10.1038/s41598-019-56954-2
15. Mondal T, Datta JK, Mondal NK (2017) Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop. J Saud Soc Agric Sci 16:135–144. https://doi.org/10.1016/j.jssas.2015.05.001
16. Ayaz M, Feizienê D, Tiilikienê V, Akhtar K, Stulpinaite U, Iqbal R (2021) Biochar role in the sustainability of agriculture and environment. Sustainability 13:1330. https://doi.org/10.3390/su13031330
17. Kah M, Tufenkji N, White JC (2019) Nano-enabled strategies to enhance crop nutrition and protection. Nat Nanotechnol 14:532-40. https://doi.org/10.1038/s41565-019-0439-9
18. Welmeyer H, de Guia AH, Connor M (2020) Reduction of fertilizer use in South China—Impacts and implications on smallholder rice farmers. Sustainability 12:2240. https://doi.org/10.3390/su12062240
19. Anas M, Liao F, Verma KK, Sarwar MA, Mahmood A, Chen Z-L, Li Q, Zeng X-P, Liu Y, Li Y-R (2020) Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biol Res 53:1-20. https://doi.org/10.1186/s40659-020-00312-4
20. Dogbe W, Sogbedji J, Buah S (2015) Site-specific nutrient management for lowland rice in the northern Savannah zones of Ghana. Curr Agric Res J 3.109. https://doi.org/10.12944/CARJ.3.2.04
21. Qin Q, Wang F, Wang Y, Lu H, Yang Z, Lv Q, Mao C (2019) Molecular control and genetic improvement of phosphorus use efficiency in rice. Mol Breed 39:1–15. https://doi.org/10.1007/s11032-019-1059-3
22. Chaturvedi I (2005) Effect of nitrogen fertilizers on growth, yield and quality of hybrid rice (Oryza sativa). J Cent Eur Agric. https://doi.org/10.5513/cej.v6i4.343
23. Xie X, Shan S, Wang Y, Cao F, Chen J, Huang M, Zou Y (2019) Dense planting with reducing nitrogen rate increased grain yield and nitrogen use efficiency in two hybrid rice varieties across two light conditions. Field Crops Res 236.24-32. https://doi.org/10.1016/j.fcr.2019.03.010
24. Malhi S, Grant C, Johnston A, Gill K (2001) Nitrogen fertilization management for no-till cereal production in the Canadian Great Plains: a review. Soil Tillage Res 60:101–122
25. Gu D, Zhen F, Hannaway DB, Zhu Y, Liu L, Cao W, Tang L (2017) Quantitative classification of rice (Oryza sativa L.) root length and diameter using image analysis. PLoS One 12:e0169968. https://doi.org/10.1371/journal.pone.0169968
26. Qi D, Wu Q, Zhu J (2020) Nitrogen and phosphorus losses from paddy fields and the yield of rice with different water and nitrogen management practices. Sci Rep 10:1–12. https://doi.org/10.1038/s41590-020-66757-5
27. Xiang X, Zhang P, Yu P, Zhang Y, Yang Z, Sun L, Wu W, Khan RM, Abbas A, Cheng S (2019) LSSR1 facilitates seed setting rate by promoting fertilization in rice. Rice 12:1–14. https://doi.org/10.1007/s12284-019-0280-3
28. Fageria N (2009) The use of nutrients in crop plants. CRC Press, Florida
29. Okalebo JR, Gathua KW, Woomer PL (2002) Laboratory methods of soil and plant analysis: a working manual, 2nd edn. Sacred Heart Africa, Nairobi, p 21
30. Juo A (1978) Selected methods for soil and plant analysis. IITA, Ibadan
31. Chen S, Liu S, Zheng X, Yin M, Chu G, Xu C, Yan J, Chen L, Wang D, Zhang X (2018) Effect of various crop rotations on rice yield and nitrogen use efficiency in paddy–upland systems in southeastern China. Crop J 6.576-88. https://doi.org/10.1016/j.cj.2018.07.007
32. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C T method. Nat Protoc 3.1101. https://doi.org/10.1038/nprot.2008.73
33. Dicasono M, Rubino P, Montemurro F (2013) Precision nitrogen management of wheat: a review. Agron Sustain Dev. https://doi.org/10.1007/s11865-013-0111-z
34. Fageria N, Baligar V, Heinemann A, Carvalho M (2015) Nitrogen uptake and use efficiency in rice. Nutrient use efficiency: from basics to advances. Springer, Berlin, pp 285–296
35. Wang X, Samo N, Li L, Wang M, Qadir M, Jiang K, Qin J, Rasul F, Yang G, Hu Y (2019) Root distribution and its impacts on the drought tolerance capacity of hybrid rice in the sachuan basin area of China. Agronomy 9:79
36. Li J, Feng Y, Wang X, Peng J, Yang D, Xu G, Luo Q, Wang L, Ou D, Su W (2020) Stability and applicability of the leaf value model for variable nitrogen application based on SPAD value in...
37. Zhang Y, Hua J, Li Y, Chen Y, Yang J (2012) Effects of phosphorus on grain quality of upland and paddy rice under different cultivation. Rice Sci 19.135-42. https://doi.org/10.3936/jxb.12014

38. Cazetta DA, Arf O, Buzetti S, Sá MEd, Rodrigues RAF (2008) Desempenho do arroz de terras altas com a aplicação de doses de nitrogenéio e em sucessão às culturas de cobertura do solo em sistema de plantio direto. Bragantia 67.471–479. https://doi.org/10.1590/S0006-87052008000300023

39. Pan J, Liu Y, Zhong X, Lampayan RM, Singleton GR, Huang N, Liang K, Peng B, Tian K (2017) Grain yield, water productivity and nitrogen use efficiency of rice under different water management and fertilizer-N inputs in South China. Agric Water Manag 184.191-200. https://doi.org/10.1016/j.agwat.2017.01.013

40. Zhang J, Tong T, Potcho PM, Huang S, Ma L, Tang X (2020) Nitrogen effects on yield, quality and physiological characteristics of giant rice. Agronomy 10.1816. https://doi.org/10.3390/agronomy10111816

41. Fageria N (2007) Yield physiology of rice. J Plant Nutr 30:843–79. https://doi.org/10.1080/01026550701734831

42. Fageria N, De Morais O, Dos Santos A (2010) Nitrogen use efficiency in upland rice genotypes. J Plant Nutr 33:1696–1711

43. Moro E, Crusciol CAC, Cantarella H, Nascente AS (2013) Upland rice under no-tillage preceded by crops for soil cover and nitrogen fertilization. Rev Bras Cienc Solo 37.1669–77. https://doi.org/10.1590/S0103-00312013000600023

44. Khan S-u-T, Iqbal A, Fahad S (2016) Growth and productivity response of hybrid rice to application of animal manures, plant residues and phosphorus. Front Plant Sci 7:1440. https://doi.org/10.3389/fpls.2016.01440

45. Li S, Tian Y, Wu K, Ye Y, Yu J, Zhang J, Liu Q, Hu M, Li H, Tong Y (2018) Modulating plant growth–metabolism coordination for sustainable agriculture. Nature 560.595-600. https://doi.org/10.1038/s41586-018-0415-5

46. Fageria N (2014) Yield and yield components and phosphorus use efficiency of lowland rice genotypes. J Plant Nutr 37:979–89. https://doi.org/10.1080/010265514.2014.888735

47. López-Arredondo DL, Leyva-González MA, González-Morales SI, López-Bucio J, Herrera-Estrella L (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. Annu Rev Plant Biol 65.95–123. https://doi.org/10.1146/annurev-arplplant-050213-035949

48. Zhang Z, Gao S, Chu C (2020) Improvement of nutrient efficiency in rice: current toolbox and future perspectives. Theor Appl Genet 133.1365-84. https://doi.org/10.1007/s00122-019-0527-6

49. Li P, Chen F, Cai H, Liu J, Pan Q, Liu Z, Gu R, Mi G, Zhang F, Yuan L (2015) A genetic relationship between nitrogen use efficiency and seedling root traits in maize as revealed by QTL analysis. J Exp Bot 66:3175–3188. https://doi.org/10.1093/jxberv127

50. Xie Q, Fernando KM, Mayes S, Sparkes DL (2017) Identifying seedling root architectural traits associated with yield and yield components in wheat. Ann Bot 119.1115-29. https://doi.org/10.1093/aob/mcx001

51. Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 159.567-84. https://doi.org/10.1016/S0176-1617-0774

52. Lips S, Leidi E, Silberbusch M, Soares M, Lewis O (1990) Physiological aspects of ammonium and nitrate fertilization. J Plant Nutr 13:1271–1289. https://doi.org/10.1080/01904169009364151

53. De Bauw P, Mai TH, Schnepp A, Merckx R, Smolders E, Vanderborght J (2020) A functional–structural model of upland rice root systems reveals the importance of laterals and growing root tips for phosphate uptake from wet and dry soils. Ann Bot 126.789–806. https://doi.org/10.1093/aob/mca2120

54. Vinod K, Heuer S (2012) Approaches towards nitrogen- and phosphorus-efficient rice. AoB Plants. https://doi.org/10.1093/aobpla/pls028

55. James D, Borphukan B, Fartyal D, Ram B, Singh J, Manna M, Sheri V, Panditi V, Yadav R, Achary VMM (2018) Concurrent overexpression of OsG51; 1 and OsG52 genes in transgenic rice (Oryza sativa L.): impact on tolerance to abiotic stresses. Front Plant Sci 9.786. https://doi.org/10.3389/fpls.2018.00786

56. Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falque M (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiol 125:1258–1270. https://doi.org/10.1104/pp.125.3.1258

57. Oliveira IC, Brears T, Knight TJ, Clark A, Coruzzi GM (2002) Overexpression of cytosolic glutamine synthetase. Relation to nitrogen, light, and photosynthesis. Plant Physiol 129:1170–1180. https://doi.org/10.1104/pp.2002013

58. Dragićević M, Simonović A, Bogdanović M, Subotić A, Ghalawenji N, Dragićević I, Todorić S (2016) Differential regulation of GS-GOGAT gene expression by plant growth regulators in arabidopsis seedlings. Arch Biol Sci 68:399–404. https://doi.org/10.2298/ABSI150608087D

59. Kusano M, Fukushima A, Tabuchi-Kobayashi M, Funayama K, Kojima S, Maruyama K, Yamamoto YY, Nishizawa T, Kobayashi M, Wakazaki M (2020) Cytosolic glutamine synthetase 1; 1 modulates metabolism and chloroplast development in roots. Plant Physiol 182:1894–1909. https://doi.org/10.1104/pp.19.01118

60. Kusano M, Tabuchi M, Fukushima A, Funayama K, Diaz C, Kobayashi M, Hayashi N, Tsuichiya YN, Takahashi H, Kamata A (2011) Metabolomics data reveal a crucial role of cytosolic glutamine synthetase 1; 1 in coordinating metabolic balance in rice. Plant J 66.456-66. https://doi.org/10.1111/j.1365-313X.2011.04506.x

61. Funayama K, Kojima S, Tabuchi-Kobayashi M, Sawa Y, Nakayama Y, Hayakawa T, Yamaya T (2013) Cytosolic glutamine synthetase 1; 2 is responsible for the primary assimilation of ammonium in rice roots. Plant Cell Physiol 54.934–943. https://doi.org/10.1093/pcp/pct046

62. Ohashi M, Ishiyama K, Kojima S, Kojima M, Sakakibara H, Yamaya T, Hayakawa T (2017) Lack of cytosolic glutamine synthetase 1; 2 activity reduces nitrogen-dependent biosynthesis of cytokinin required for axillary bud outgrowth in rice seedlings. Plant Cell Physiol 58.679–690. https://doi.org/10.1093/pcp/pcx022

63. Gaur VS, Singh U, Gupta AK, Kumar A (2012) Influence of different nitrogen inputs on the makers of ammonium transporter and glutamine synthetase genes in two rice genotypes having differential responsiveness to nitrogen. Mol Biol Rep 39:8035–8844. https://doi.org/10.1007/s11033-012-1650-8

64. Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva A, Paz-Ares J (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev 15.2122–2133

65. Sega P, Pacak A (2019) Plant PHR transcription factors: put on a map. Genes 10.1018. https://doi.org/10.3390/genes10120188

66. Xu Y, Liu F, Han G, Cheng B (2018) Genome-wide identification and comparative analysis of phosphate starvation-responsive transcription factors in maize and three other graminaceous plants. Plant Cell Rep 37:711–26. https://doi.org/10.1007/s00207-018-2262-0
67. Wu P, Wang X (2008) Role of OsPHR2 on phosphorus homoeostasis and root hairs development in rice (Oryza sativa L.). Plant Signal Behav. https://doi.org/10.4161/psb.3.9.5781

68. Zhou J, Jiao F, Wu Z, Li Y, Wang X, He X, Zhong W, Wu P (2008) OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. Plant Physiol 146:1673-86. https://doi.org/10.1104/pp.107.111443

69. Ruan W, Guo M, Wu P, Yi K (2017) Phosphate starvation induced OsPHR4 mediates Pi-signaling and homeostasis in rice. Plant Mol Biol 93:327-40. https://doi.org/10.1007/s11103-016-0564-6

70. Jung J-Y, Ried MK, Hothorn M, Poirier Y (2018) Control of plant phosphate homeostasis by inositol pyrophosphates and the SPX domain. Curr Opin Biotechnol 49:156-62. https://doi.org/10.1016/j.copbio.2017.08.012

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.