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Phosphorus application rates affect the grain yields of different phosphorus-tolerant rice cultivars by regulating grain filling and leaf senescence characteristics

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Abstract: The grain filling and physiological traits of different phosphorus-tolerant rice cultivars and phosphorus fertiliser rates have not been fully studied. A pot-growth experiment with cv. Lianjing 7 (weak phosphorus tolerance) and cv. Yongyou 2640 (strong phosphorus tolerance) was conducted using four phosphorus rates, namely, 0 (P0), 0.44 (P1), 0.88 (P2), and 1.32 g/pot (P3). Results indicated that grain yield, net photosynthetic rate, soil and plant analyser development (SPAD) value, superoxide dismutase (SOD) and catalase (CAT) activity in leaves, and adenosine diphosphate glucose pyrophosphorylase (AGPase) and sucrose synthase (SuSase) activity in grains increased and then decreased with increasing phosphorus fertiliser rate, whereas malondialdehyde (MDA) content in leaves decreased first and then increased. The above indexes of cv. Lianjing 7 and cv. Yongyou 2640 were optimal at P2 and P1 treatments, respectively. The grain yield, net photosynthetic rate, SPAD value, AGPase content, SuSase content in grains, and SOD and CAT activity in the leaves of cv. Yongyou 2640 were higher, whereas the MDA content was lower than those of cv. Lianjing 7. Correlation analysis showed that AGPase and SuSase activity in superior and inferior grains, photosynthetic rate, and SOD and CAT activity in the leaves were significant or highly significantly positively correlated with grain-filling rate and rice yield. Therefore, the adoption of appropriate phosphorus fertiliser rates can increase the activity of enzymes related to starch synthesis in different phosphorus-tolerant rice, enhance antioxidant systems in leaves at the filling stage, reduce leaf MDA content, and delay leaf senescence. These effects are beneficial to grain filling and increase grain yield.

Keywords: *Oryza sativa* L.; nutrition; adaptability; grain filling characteristics; starch metabolic enzyme; antioxidant enzyme

Rice (*Oryza sativa* L.) is one of the world's primary food crops and an important staple food for most of the world's population. Approximately 90% of the world's rice is grown and consumed in Asia (Anand et al. 2017, Kumar et al. 2024). The world population is growing rapidly, and the addition of fertilisers has become necessary to improve rice growth and productivity according to the global demand (Singh 2017, Albahri et al. 2023).

Phosphorus, as an essential nutrient for plant growth and development, not only is a component of many important organic compounds, including nucleic acids, phospholipids, and ATP but also participates in various metabolic processes, such as

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energy transfer and protein activation (Shamsuddin et al. 2015, Qaswar et al. 2020, Prathap et al. 2023, Sun et al. 2024). Phosphate fertiliser plays an extremely important role in agricultural production (Khalf et al. 2021, Wendimu et al. 2023, Yang et al. 2024). Phosphorus deficiency restricts plant growth and development and reduces leaf area. Conversely, plant respiration is enhanced under conditions of excessive phosphorus application, leading to substantial consumption of sugars and energy, which ultimately inhibits plant growth (Veronica et al. 2017, Deng et al. 2021, Sun et al. 2024). The excessive application of phosphorus can harm the environment by promoting surface runoff loss, groundwater leaching, and eutrophication in water bodies (Shamsuddin et al. 2015, Nawaz et al. 2022, Yahaya et al. 2023, Hu et al. 2024). Furthermore, it restricts the increase in rice yield (Shamsuddin et al. 2015, Rinasoa et al. 2023). Therefore, improving the utilisation efficiency of phosphorus fertiliser resources is essential to sustainable rice development.

Starch biosynthesis and accumulation in endosperm cells occur during grain filling and enrichment (Yang et al. 2004, Li et al. 2018, Iqbal et al. 2021, Fei et al. 2024). Photosynthetic assimilates produced by source organs are mainly transported to the grains in the form of sucrose, which is then transformed into starch, adenosine diphosphate glucose pyrophosphorylase (ADGPase), starch synthase (SS), and starch-branching enzymes (SBEs) in the grains, playing an important role in starch synthesis (Yang et al. 2004, Li et al. 2018, Iqbal et al. 2021, Chen et al. 2023, Yu et al. 2024). Improper phosphorus application results in abnormal grain filling, reducing grain-filling rate and grain yield (Li et al. 2018, Iqbal et al. 2021, Sun et al. 2023). How to maintain a high rice yield and even further improve yield when phosphorus resources are limited has been explored. The application of appropriate amounts of phosphorus fertiliser is beneficial for starch synthesis in grains, resulting in plumper grains and increasing grain weight and yield (Qaswar et al. 2020, Deng et al. 2021, Wendimu et al. 2023, Yang et al. 2024). An increase in the activity of key enzymes in the sucrose-starch metabolism pathway considerably increases starch content in rice grains (Yang et al. 2004, Iqbal et al. 2021, Sun et al. 2023, Zang et al. 2024). In addition, suitable phosphorus application promotes the decomposition of sucrose in grains, providing precursors for starch synthesis, and the activity of sucrose synthase (SuSase) in grains of different cultivars is affected differently by phosphorus fertiliser (Dissanayaka et al. 2018, Hayes et al. 2022, Sun et al. 2024). Deng et al. (2021) observed that the superiority of YJ2 in low phosphorus was attributed to high tillers, increased root dry weight, enhanced root-shoot ratio, remobilisation of NSC from stem to grain, and enhanced ATPase activity in the roots and POD activity in the grains (Deng et al. 2021). Previous studies focused on the low phosphorus tolerance of rice cultivars under different phosphorus levels and the selection of phosphorus-efficient cultivars (Veronica et al. 2017, Dissanayaka et al. 2018, Zhang et al. 2021, Verbeeck et al. 2023, Kumar et al. 2024, Sun et al. 2024). However, little is known about changes in rice's grain filling and physiological traits combined with phosphorus-tolerant cultivars during the whole growth period at different phosphorus rates.

Leaf senescence is an adaptive mechanism formed by plants during long-term evolution, closely related to the formation of harvesting organs and nutrient transport, regulated by internal factors of the plant, and is an irreversible physiological and biochemical process (Farooq et al. 2019, Zhou et al. 2023, Averill-Bates 2024). In rice, grains compete with leaves for phosphorus during the grain-filling stage, and this competition accelerates leaf senescence, which is inconducive to photosynthesis and affects grain weight under phosphorus-deficient conditions (Kwanho et al. 2017, Cao et al. 2023, Zhou et al. 2023). The effects of phosphate fertiliser rate on the senescence characteristics of crops have been extensively studied, but conclusions are inconsistent (Kwanho et al. 2017, Wu et al. 2022, Zhou et al. 2023). The activity of antioxidant enzymes increases under low-phosphorus conditions, which can effectively scavenge reactive oxygen species (ROS; Sewelam et al. 2016, Cao et al. 2023). POD activity in leaves first increases and then decreases with increasing phosphorus application rate, malondialdehyde (MDA) content is inhibited, and leaf senescence is delayed at an appropriate phosphorus application rate (Dang et al. 2023). Notably, excessive phosphorus application increases MDA content, accelerates leaf senescence, and is thus inconducive to photosynthesis (Song et al. 2015). Compared with varieties with weak low-phosphorus tolerance, cultivars with strong low-phosphorus tolerance have stronger adaptability to low-phosphorus environments and higher ROS-scavenging ability (Deng et al. 2021, Zhou et al. 2023, Kumar et al. 2024, Sun et al. 2024). However, evidence of leaf senescence traits is limited, especially

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evidence regarding increased yield and enhanced grain-filling rate at different phosphorus fertiliser rates and cultivar types.

High chlorophyll content and photosynthetic rate at the reproductive growth period can promote grain filling, enhance sucrose synthesis and transport in leaves, and is conducive to assimilation accumulation and yield formation (Xu et al. 2019, 2020, Vishwakarma et al. 2023, Fei et al. 2024). However, the mechanism of key enzymes that are involved in grain filling and leaf senescence and affect chlorophyll content and photosynthetic rate in leaves and their relationship to grain filling and rice yield at different phosphorus fertiliser rates and in different cultivar types remain unclear.

This study aimed to investigate grain filling and the physiological performance of different phosphorus-tolerant cultivars at different phosphorus fertiliser rates. We examined changes in grain filling and physiological characteristics throughout the rice growth season, including changes in maximum grain-filling rate, average grain-filling rate, active filling period, photosynthetic rate, soil and plant analyser development (SPAD) value, SOD activity, catalase (CAT) and MDA content in leaves, and adenosine diphosphate glucose pyrophosphorylase (AGPase) and SuSase activity in grains. Furthermore, we analysed their correlations with grain yield and grain filling. The results can provide novel information for the selection of phosphorus-tolerant cultivars and guidance regarding the reasonable use of phosphate fertilisers.

MATERIAL AND METHODS

Plant materials. Experiments were conducted at Henan University of Science and Technology, Luoyang, China (34°39'N, 112°26'E) from May to October in 2022 and repeated in 2023. Cv. Lianjing 7 (weak phosphorus tolerance) and cv. Yongyou 2640 (strong phosphorus tolerance) were grown in pots. Each pot was 25 cm in diameter and 30 cm in height, and 13 kg of soil was placed in each pot. The soil from the field was clay loam (Typic Fluvaquents, Entisols, US Taxonomy) containing 11.3 g/kg organic carbon, soil pH value of 7.4 and 105.1, 5.2, and 118.6 g/kg available nitrogen, phosphorus, and potassium. The air temperatures for each month were 29, 26.5, 26.5, 22.5, and 14.5 °C in 2022 and 25.5, 28.5, 27, 22.5, and 17.5 °C in 2023 from transplantation (June) to harvesting (October).

Treatments. A completely randomised experiment was performed. Four levels of phosphorus fertiliser application were designed, that is, 0, 0.44, 0.88 and 1.32 g P/pot, and were labelled as P0, P1, P2, and P3, respectively. The same amounts of nitrogen and potassium were applied to all treatments, which were 2.0 and 0.83 g/pot, respectively. Submerged irrigation with a water depth of 2–3 cm was adopted during the regreening stage according to farming practices, and wetting and moderate-drying irrigation were alternated between stages, except in the late tillering stage, when light-field drainage was conducted, and 1 week before the final harvest. The plots were not re-watered until the soil water potential reached –20 kPa in the alternate wetting-and-moderate-drying irrigation. Soil water potential was monitored with a vacuum-suction gauge (produced by the Institute of Soil Science, Chinese Academy of Sciences). The gauges were installed in the pools by placing each pottery head 15 cm below the soil. 30% of nitrogen fertiliser (urea) was applied 1 day before transplantation, 30% was applied 7 days after transplantation, and 40% was applied in the panicle-initiation period. Before transplantation, partial nitrogen fertiliser, total superphosphate (containing 5.89% phosphorus) and potassium chloride (43% potassium) were applied in each treatment. During field cultivation, sowing was performed on May 10th, transplantation was performed on June 10th to pots with three hills, two seedlings were sown per hill, and 25 pots were performed for each treatment. Diseases, pests, and weeds were strictly controlled throughout the entire growth period in both years.

Sampling and measurement

Sampling. A total of 150 spikes that headed on the same day were selected and labelled for each plot. Fifteen labelled spikes from each pot were sampled at 6-day intervals from anthesis to maturity. All grains except the second grain in the primary branch at the top of the spikelet and the first grain in the secondary branch were regarded as superior grains. The second grains in the first and secondary branches were regarded as inferior grains (Wei et al. 2017). Half of the sampled grains were frozen in liquid nitrogen for 30 s and then stored in a –70 °C refrigerator for the determination of enzyme activity. The other sampled grains were dried at 70 °C to constant weight and weighed. The processes of grain filling were fitted with the growth equation of Richards (1959), as described by Zhu et al. (1988). The flag leaves of rice were sampled at 6 (labelled

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as early grain filling stage), 18 (labelled as middle grain filling stage), and 30 days (labelled as late grain filling stage) after anthesis for determination of senescence-related indexes.

Adenosine phosphate glucose pyrophosphorylase (AGPase) and SuSase enzyme activity. Extraction methods for enzyme activity were described by Yang et al. (2004). Grains (40–50) were ground using a mortar and extracted in 9 mL of 50 mmol/L HEPES-NaOH extract (pH 7.5) containing 5 mmol/L $MgCl_2$, 5 mmol/L DTT, and 2% (*w/v*) PVP 40. A filter solution was centrifuged for 10 min at 10 000 rpm and 4 °C, and the supernatant was used for enzyme determination.

Adenosine phosphate glucose pyrophosphorylase (AGPase) activity was measured using the procedure of Nakamura et al. (1989). The reaction mixture consisted of 100 μL of 5 mmol ADPG, 50 μL of 50 mmol $MgCl_2$, 100 μL of 50 mmol Hepes-NaOH, and 50 μL enzyme extract. The reaction was started by adding 100 μL of 20 mmol pyrophosphoric acid. The reaction was terminated by adding 100 μL of 6 mmol oxidised coenzyme II, 50 μL of 1.5 IU phosphoglucose mutase, 50 μL of 5 IU glucose-6-phosphate dehydrogenase, and 1.4 mL of 50 mmol Hepes-NaOH (pH = 7.5) after cooling, and change in absorbance was measured at 340 nm.

SuSase activity was determined using the methods described by Ranwala and Kato (1995). The reaction mixture consisted of 50 μL of HEPES-NaOH buffer (pH = 7.5), 20 μL of 50 mmol/L $MgCl_2$, 20 mL of 100 mmol/L fructose solution, 20 μL of 100 mmol/L UDPG, and 90 μL of an enzyme extract. The reaction was started by adding 1.5 mL of 300 g/L HCl and 0.5 mL of 1 g/L resorcinol, and the change in absorbance was measured at 480 nm—the amount of sucrose after the enzymatic reaction was calculated according to the standard curve.

Malondialdehyde content and superoxide dismutase and catalase enzyme activity. Malondialdehyde content in leaves was determined as described by Velikova et al. (2000). Leaf samples (0.1 g) were homogenised in 5 mL of 5% trichloroacetic acid. The homogenate was centrifuged at 12 000 rpm for 15 min and 4 °C, and the supernatant was used in determining MDA content at 532, 600, and 450 wavelengths.

Leaf samples (0.1 g) were homogenised in 10 mL of 50 mmol/L phosphate buffer (pH = 7.0). The homogenate was centrifuged at 12 000 rpm for 15 min and 4 °C. The supernatant was used to determine the levels of superoxide dismutase and catalase activity. SOD activity was measured according to the method described by Dhindsa et al. (1981). The 3 mL of reaction mixture consisted of 13 mmol/L methionine, 75 mmol/L nitroblue tetrazolium chloride (NBT), 2 μmol/L riboflavin, 10 μmol/L EDTA, and 0.05 mol/L phosphate buffer (pH = 7.5). One unit of SOD activity was deemed as the amount of enzyme that was required to inhibit 50% photochemical reduction of nitro blue tetrazolium.

CAT activity was measured using Aebi's method (1984). An assay mixture (3 mL) composed of 1 mL of 0.3% hydrogen peroxide, 1.9 mL of distilled water, and 0.1 mL of enzyme extract was used. The reaction was started by adding an enzyme extract, and a decrease in absorbance was recorded at 240 nm. CAT activity was expressed as U/g/min FW.

Photosynthesis rate and SPAD value. Plants from 50 hills in each treatment were sampled to measure photosynthesis rate and SPAD value at mid-tillering, panicle initiation, heading, and maturity stages. Chlorophyll content in the leaves was measured using SPAD-502 and the method of Li et al. (2012).

The photosynthesis rate of the upmost fully expanded leaves in each treatment was measured using a Li 6800 photosynthesis analyser (LI-COR, Co., Ltd., Lincoln, USA) from 09:00 to 11:00, when photosynthesis-activity radiation above the canopy was 1 500 $\mu mol/m^2/s$.

pH and chemical properties of soil. Pot soil samples were collected before transplantation at a 0–20 cm depth with 3 replications. The soil pH and chemical indicators were determined following the method described by Gajda et al. (2017).

Grain yield. Rice plants were harvested on October 15, 2022, and October 16, 2023—five pots for each treatment obtained grain yield. Yield components, namely panicles per pot, spikelets per panicle, filled-grain rate, and 1 000-grain weight, were determined in the five pots of each treatment. The filled-grain rate was expressed as the ratio of ripened grains (specific gravity ≥ 1.06) to the total spikelet.

Data analysis

Data was analysed using the SAS/STAT statistical analysis package for variance (version 9.2, SAS Institute, Cary, USA). Mean values were tested using the least significant difference at $P_{0.05}$ ($LSD_{0.05}$). The Spearman model was used to evaluate the relationships between enzyme activity and photosynthesis rate with grain yield and grain-filling characteristics.

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RESULTS

Grain yield and its component. Cultivar, phosphorus application rate, and their interaction considerably affected grain yield and its components ($P < 0.05$, Table 1). Specifically, the grain yield of cv. Lianjing 7 reached the highest value at the P2 treatment and increased by 55.0% (2022) and 50.1% (2023) compared with the grain yield obtained when no phosphorus was applied. The grain yield of cv. Yongyou 2640 was the highest at the P1 treatment, and the yield increased by 73.1% (2022) and 68.4% (2023) compared with the grain yield obtained when no phosphorus was applied. P3 treatment significantly reduced the grain yield when compared with the optimal phosphorus application rate of the two cultivars. Compared with cv. Lianjing 7, cv. Yongyou 2640 increased grain yield by 15.2% (2022) and 18.7% (2023).

Panicle, spikelets, and filled grain rate significantly increased in cv. Lianjing 7 after P2 treatment and cv. Yongyou 2640 after P1 treatment compared with those obtained without phosphorus application. This result indicated that suitable phosphorus application improved total spikelets and grain filling, thus enhancing yield. Although panicle and grain weight decreased, increases in spikelets and grain-filling rate can compensate for the reduction. Consequently, the yield increased significantly compared with the yield of cv. Lianjing 7.

Table 1. Grain yield and its components of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice

Year	Cultivar	Treatment	Panicles (pot)	Spikelet per panicle	Filled grain rate (%)	1 000-grain weight (g)	Yield (g/pot)
2022	Lianjing 7	P0	33.5[c]	154.3[e]	55.4[de]	24.3[c]	69.6[d]
		P1	34.0[c]	158.4[e]	59.0[cd]	25.5[b]	81.0[cd]
		P2	40.3[a]	161.8[de]	61.5[c]	26.8[a]	107.9[b]
		P3	37.0[b]	176.7[cd]	52.3[e]	23.8[c]	81.2[cd]
	Yongyou 2640	P0	26.7[d]	185.1[c]	61.5[c]	23.1[d]	69.8[d]
		P1	26.0[de]	208.9[b]	87.8[a]	25.3[b]	120.8[a]
		P2	24.3[de]	229.5[a]	83.8[ab]	23.9[c]	111.8[ab]
		P3	23.7[e]	203.3[b]	80.5[b]	22.7[d]	87.6[c]
2023	Lianjing 7	P0	30.0[b]	135.3[e]	63.8[d]	27.4[ab]	71.0[e]
		P1	31.0[ab]	138.4[e]	67.9[d]	27.8[a]	80.8[d]
		P2	32.0[a]	151.8[d]	79.9[bc]	27.7[a]	107.2[b]
		P3	32.0[a]	144.7[de]	66.9[d]	26.4[b]	81.8[cd]
	Yongyou 2640	P0	23.0[d]	184.3[c]	75.5[c]	23.8[c]	76.0[de]
		P1	25.7[c]	234.0[a]	91.1[a]	23.4[c]	128.0[a]
		P2	24.7[c]	229.2[a]	83.5[b]	23.4[c]	110.2[b]
		P3	23.0[d]	206.4[b]	79.3[bc]	23.2[c]	87.1[c]
ANOVA							
Cultivar (C)			748.9**	503.6**	412.2**	308.9**	72.0**
Phosphorus fertiliser rate (P)			6.6**	21.8**	54.2**	22.1**	110.5**
Year (Y)			82.5**	7.0*	97.5**	40.9**	ns
C × P			16.2**	12.1**	20.6**	4.8**	36.7**
C × Y			33.9**	27.7**	26.3**	72.7**	ns
P × Y			ns	ns	ns	9.6**	ns
C × P × Y			7.7**	ns	10.8**	4.0*	ns

P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Different letters indicate statistical significance at $P < 0.05$ within a column in the same year. * and ** indicate *F*-values significance at $P < 0.05$ and $P < 0.01$; ns – not significant at $P = 0.05$

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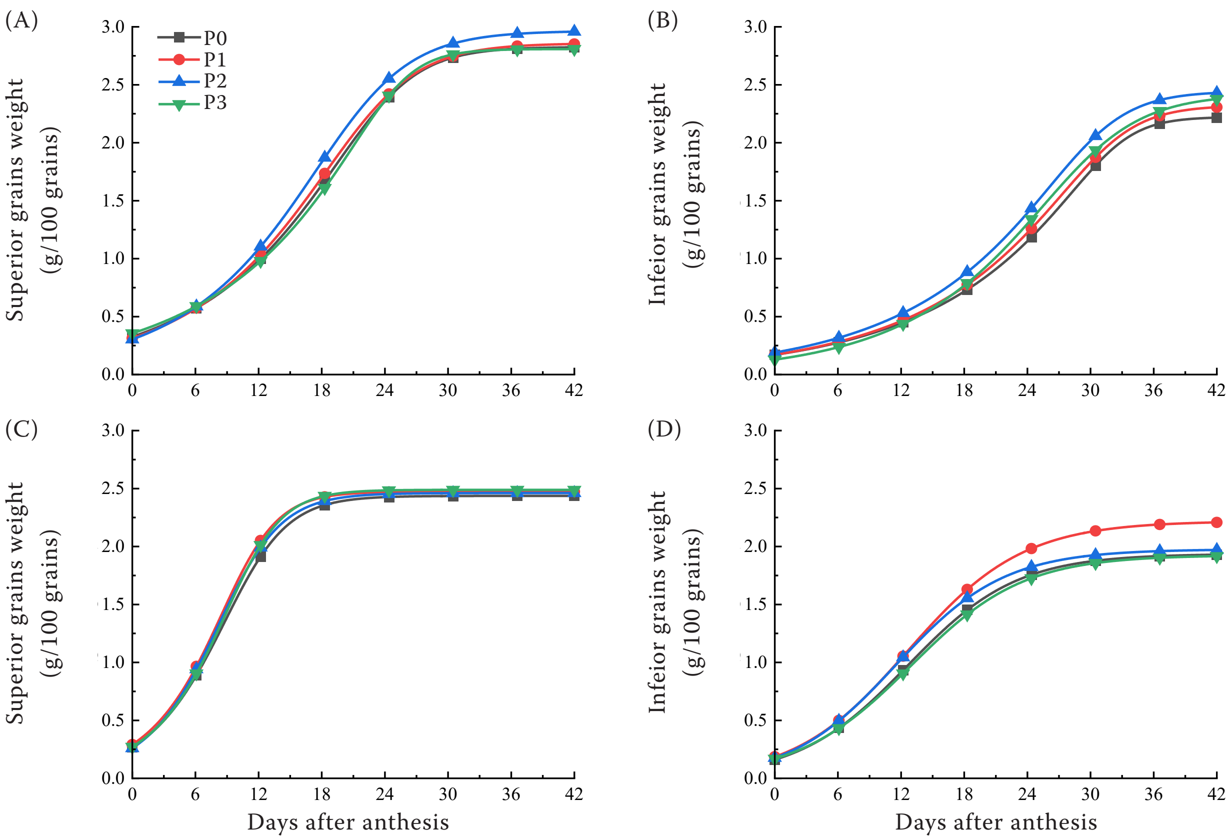

Figure 1. Superior and inferior grains weight of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was a low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar; A – superior grains of cv. Lianjing 7; B – inferior grains of cv. Lianjing 7; C – superior grains of cv. Yongyou 2640; D – inferior grains of cv. Yongyou 2640

Grain-filling characteristics of superior and inferior grains. Grain weight rapidly increases and then slowly increases (Figure 1). The inferior grains in both cultivars were lower in weight than the superior grains. Phosphorus application exerted a considerable effect on the weight of superior grains. Cv. Lianjing 7 had the highest grain weight under P2 treatment, while cv. Yongyou 2640 had the highest weight of superior grains under the P1 treatment. The weight of an inferior cv. Lianjing 7 and cv. Yongyou 2640 grains showed 12.6% and 9.2% increases 30 days after anthesis, compared with that obtained without phosphorus application. The results suggested that the grain weight of different phosphorus-tolerant rice was affected by phosphorus fertiliser rates, and excessive phosphorus application would reduce the grain weight.

The grain-filling rate of rice initially increased and then decreased during growth (Figure 2). Considerable differences in grain-filling rate were found between the superior and inferior grains of different phosphorus-tolerant rice cultivars under phosphorus application conditions. Specifically, the grain-filling rate of superior grains was obviously higher than those of inferior grains. Similar to grain yield, the maximum filling rate of cv. Lianjing 7 reached its highest value after P2 treatment, increasing by 8.0% (superior grain) and 7.0% (inferior grain) compared with the maximum filling rate obtained without phosphorus application. In addition, cv. Yongyou 2640 had the maximum filling rate after the P1 treatment, showing an increase of 15.3% (superior grains) and 9.7% (inferior grains). P3 treatment significantly reduced the grain-filling rate compared with the optimal phosphorus application rate of the two cultivars. Compared with cv. Lianjing 7, cv. Yongyou 2640 showed an increase in the maximum grain-filling rate of its superior and a decrease in inferior grains. The result indicated that the application of phosphorus fertiliser can improve the grain-filling rate,

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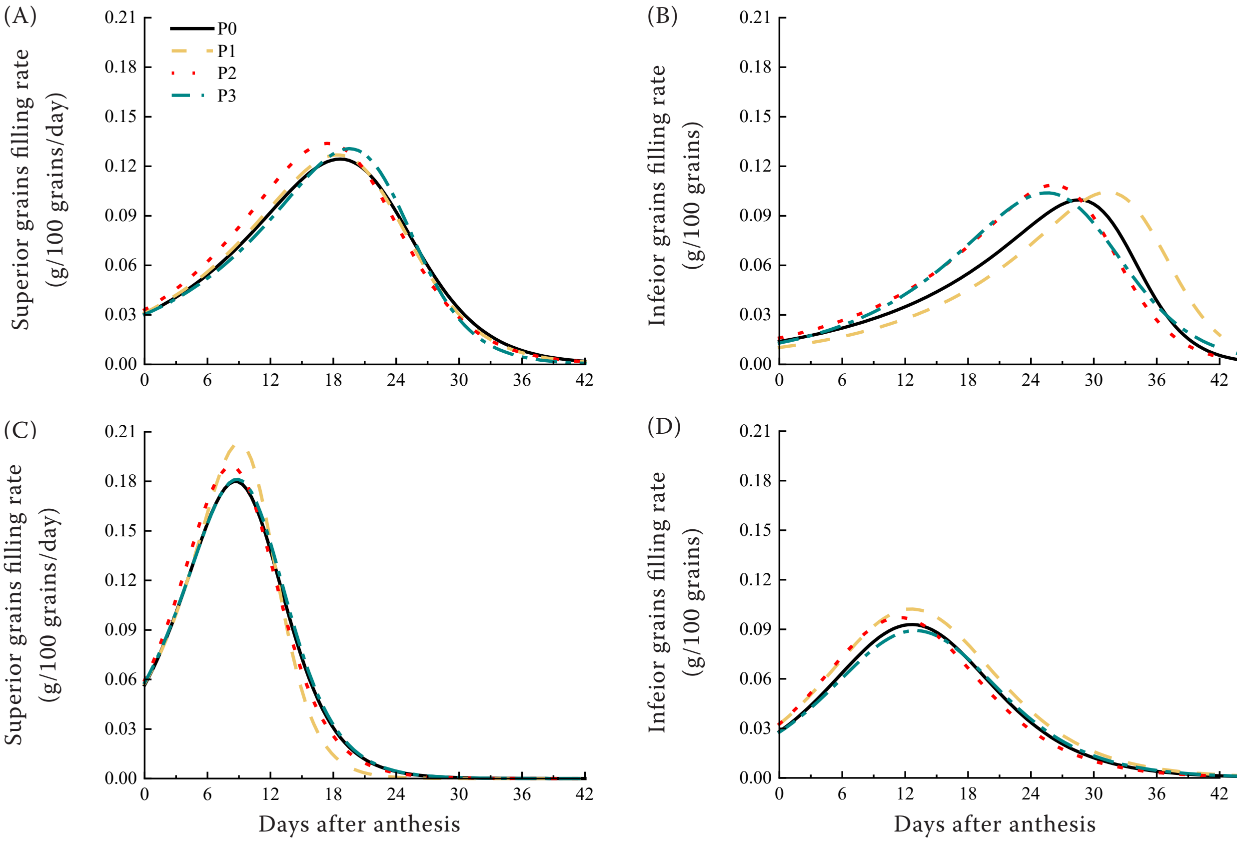

Figure 2. Superior and inferior grains filling of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar; A – superior grains of cv. Lianjing 7; B – inferior grains of cv. Lianjing 7; C – superior grains of cv. Yongyou 2640; D – inferior grains of cv. Yongyou 2640

but excessive phosphorus application inhibited the grain-filing rate of rice.

Photosynthetic rate and SPAD value. Analysis of variance indicated a significant effect of cultivar and phosphorus fertiliser rates, as well as their interaction, on leaf photosynthetic rate at different stages ($P < 0.05$; Table 2). Similar to the grain-filling rate, the photosynthetic rate of cv. Lianjing 7 reached the highest at P2 treatment, which increased by 10.4–56.7% (2022) and 17.1–45.6% (2023) compared with the photosynthetic rate without phosphorus application. Meanwhile, the leaf photosynthetic rate was the highest under the P1 treatment, followed by the P2 and P3 treatment, and the lowest under the P0 treatment of cv. Yongyou 2640. Under the same phosphorus fertiliser rates, compared with cv. Lianjing 7, cv. Yongyou 2640 increased leaf photosynthetic rate by 17.6% (panicle initiation) and 22.4% (maturity) in 2022 and 21.7% (panicle initiation) and 35.3% (maturity) in 2023.

The SPAD value of the leaves varied by cultivar, phosphorus fertiliser rate, and growth stage (Figure 3). It presented an up-down trend during growth, peaked at the heading stage, and subsequently declined. The highest SPAD value of cv. Lianjing 7 was obtained with the P2 treatment, increasing by 13.7–19.6% (2022) and 10.9–45.9% (2023) compared with that without phosphorus application. Meanwhile, the SPAD value of cv. Yongyou 2640 peaked in the P1 treatment, increasing by 10.3–16.0% (2022) and 7.3–28.8% (2023). Compared with cv. Lianjing 7, cv. Yongyou 2640 showed significantly increased SPAD values in its leaves, especially at both years' mid-tillering and heading stages. This result indicated that the SPAD values of rice leaves with different phosphorus-tolerant cultivars were affected differently by phosphorus application rate.

AGPase and SuSase activity of the superior and inferior grains. Different cultivars and phosphorus application rates significantly affected AGPase activity

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Table 2. Photosynthetic rate of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice

Year	Cultivar	Treatment	Mid-tillering	Panicle initiation	Heading	Maturity
2022	Lianjing 7	P0	16.20^{f}	15.21^{e}	13.87^{f}	7.89^{c}
		P1	17.36^{e}	17.24^{d}	14.52^{ef}	8.42^{c}
		P2	24.14^{b}	23.83^{ab}	15.31^{e}	10.68^{ab}
		P3	22.78^{c}	20.14^{c}	14.03^{f}	9.46^{bc}
	Yongyou 2640	P0	18.74^{d}	17.19^{d}	16.71^{d}	10.11^{b}
		P1	26.97^{a}	24.60^{a}	21.14^{a}	12.12^{a}
		P2	25.20^{b}	23.86^{ab}	19.95^{b}	11.14^{ab}
		P3	24.61^{b}	23.08^{b}	17.60^{c}	10.72^{ab}
2023	Lianjing 7	P0	15.85^{f}	12.32^{e}	15.87^{e}	4.45^{e}
		P1	17.81^{e}	14.58^{d}	18.67^{b}	5.11^{cd}
		P2	21.39^{b}	17.94^{b}	20.60^{a}	5.21^{cd}
		P3	20.30^{cd}	15.32^{d}	18.43^{b}	5.00^{d}
	Yongyou 2640	P0	17.32^{e}	16.80^{c}	16.71^{d}	5.35^{c}
		P1	23.14^{a}	19.17^{a}	20.10^{a}	7.60^{a}
		P2	20.43^{c}	18.83^{a}	18.57^{b}	6.97^{b}
		P3	19.43^{d}	17.47^{bc}	17.67^{c}	6.93^{b}
ANOVA						
Cultivar (C)			240.38**	312.11**	250.96**	101.02**
Phosphorus fertiliser rate (P)			248.58**	189.13**	103.72**	14.37**
Year (Y)			247.80**	560.75**	155.40**	537.51**
C × P			112.00**	43.41**	22.27**	5.61**
C × Y			60.74**	ns	282.54**	ns
P × Y			21.10**	25.46**	3.92*	ns
C × P × Y			4.36*	10.65**	13.05**	3.30*

P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Different letters indicate statistical significance at $P < 0.05$ within a column in the same year. * and ** indicate F-values significance at $P < 0.05$ and $P < 0.01$; ns – not significant at $P = 0.05$

in the superior and inferior grains at different grain-filling stages each year (Figure 4). AGPase activity in the grains was higher in metaphase than in prophase and anaphase of the filling stage. AGPase activity in superior grains was higher than that of the inferior grains under different phosphorus rates at the growth stage. Specifically, cv. Lianjing 7 showed an 11.7–33.5% increase in phosphorus rate compared with that of the inferior grains and cv. Yongyou 2640 showed a 12.4–44.4% increase. AGPase activity in the grains significantly increased with phosphorus content, but excessive phosphorus application inhibited AGPase activity. Similar to the leaf photosynthesis rate, the AGPase activity of cv. Lianjing 7 reached its highest value after the P2 treatment, increasing by 18.3% (superior grain) and 20.3% (inferior grain) compared with AGPase activity without phosphorus application. In addition, cv. Yongyou 2640 had the maximum enzyme activity under the P1 treatment, which was enhanced by 19.9% (superior grains) and 27.8% (inferior grains). Compared with cv. Lianjing 7, cv. Yongyou 2640 elevated AGPase activity at each stage for both years.

SuSase activity in rice grains varied with cultivar, phosphorus fertiliser rates, and growth stages (Figure 5). It presented an up-down trend with the grain process, peaked at the metaphase stage, and subsequently declined. SuSase activity in superior grains was obviously higher than those of inferior grains. Specifically, the enzyme activity during the prophase stage of grain filling in superior grains of cv. Lianjing 7 increased by 9.9–22.0%, and

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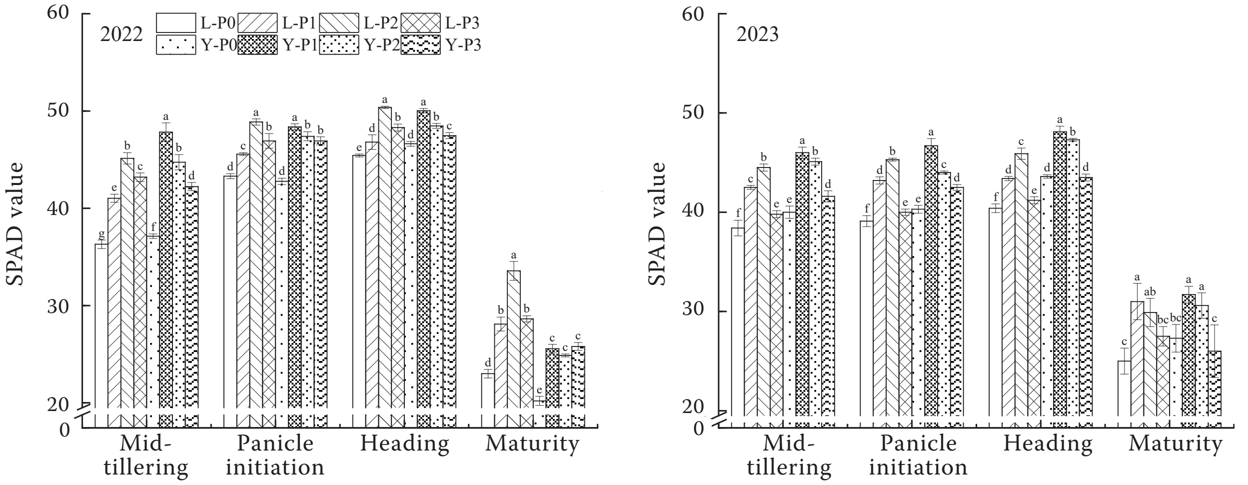

Figure 3. Soil and plant analyser development (SPAD) value in leaves of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar; Vertical bars represent ± standard error (SE) of the mean. The SE was calculated across three replications for each year. Different letters indicate statistical significance at $P < 0.05$ within the same stage

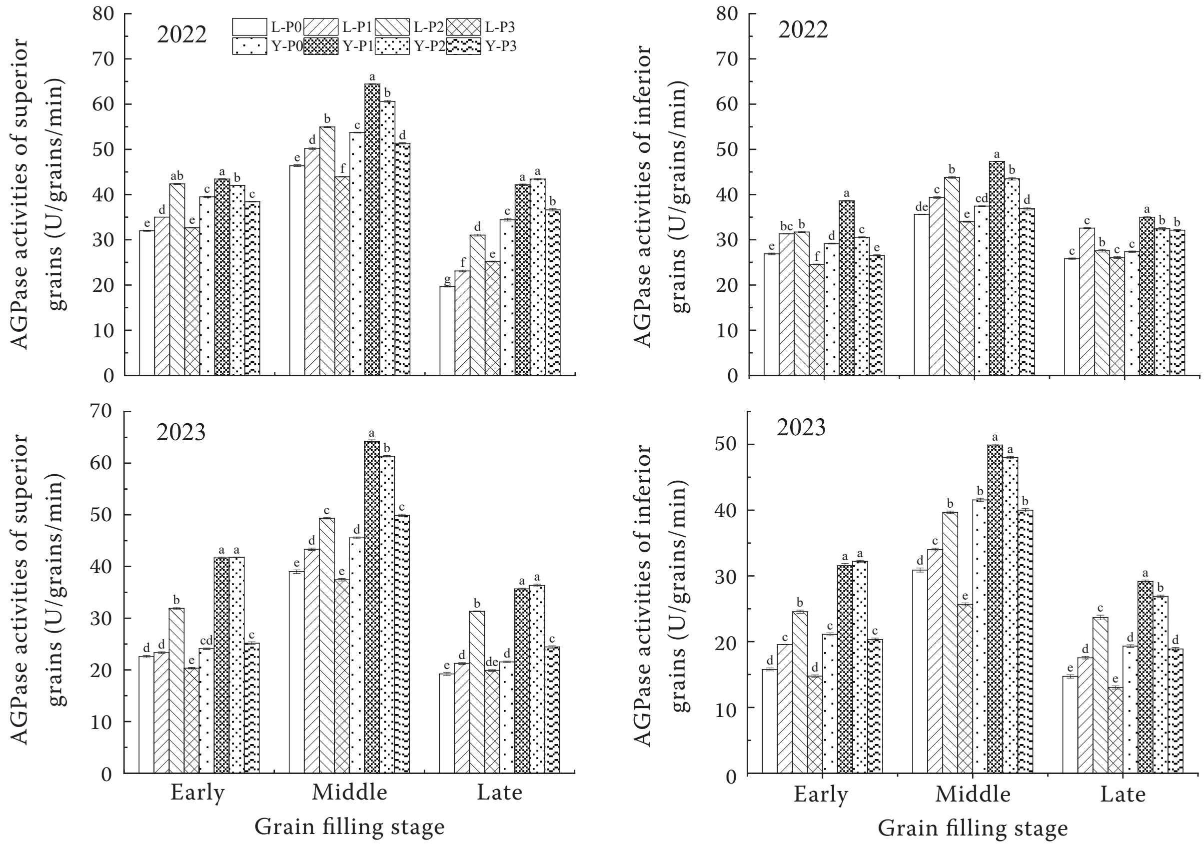

Figure 4. Adenosine diphosphate glucose pyrophosphorylase (AGPase) activities of superior and inferior grains of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Early, middle, late represented 6^{th}, 18^{th}, 30^{th} after anthesis. Vertical bars represent ± standard error (SE) of the mean. The SE was calculated across three replications for each year. Different letters indicate statistical significance at $P < 0.05$ within the same stage

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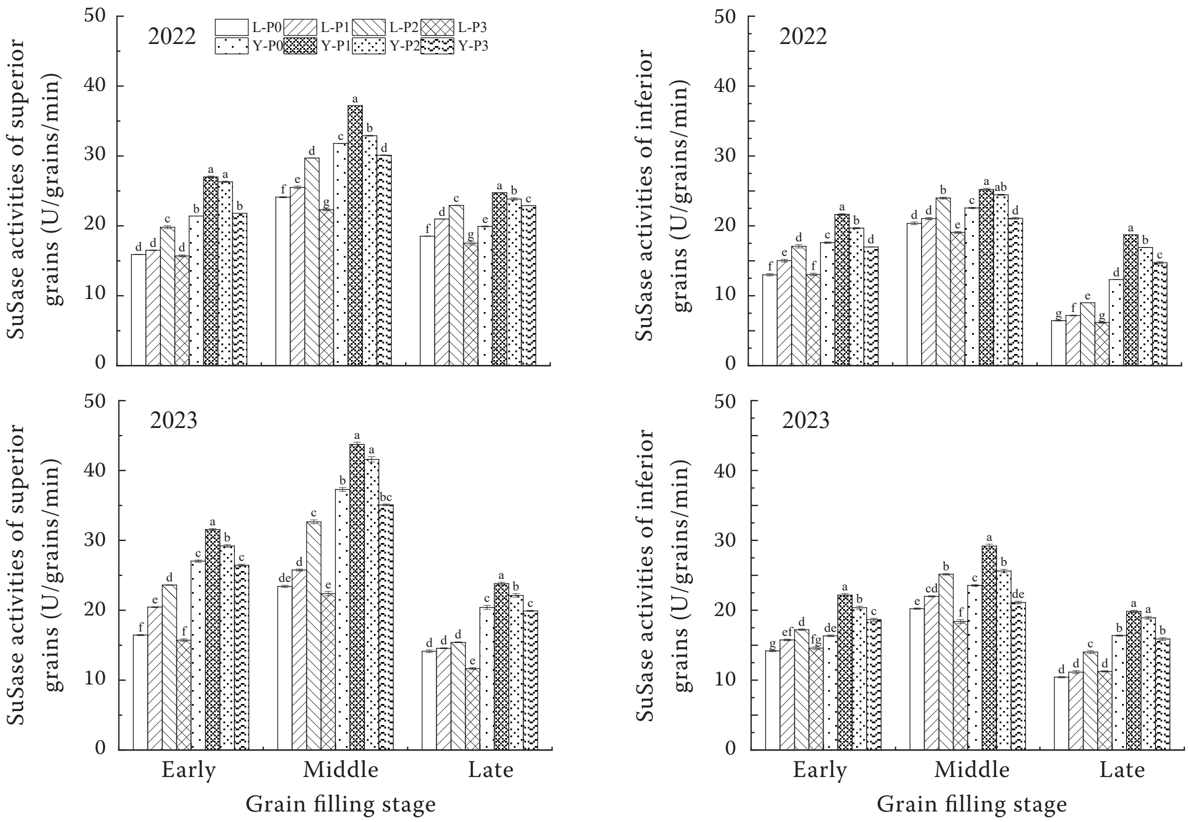

Figure 5. Ssucrose synthase (SuSase) activities of superior and inferior grains of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot;P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Early, middle, late represented 6^{th}, 18^{th}, 30^{th} after anthesis. Vertical bars represent ± standard error (SE) of the mean. The SE was calculated across three replications for each year. Different letters indicate statistical significance at $P < 0.05$ within the same stage

cv. Yongyou 2640 increased by 21.3–33.9% compared to inferior grains. Among the four phosphorus fertiliser treatments, the SuSase activity of cv. Lianjing 7 was the highest under P2 treatment, which increased by 24.8% (superior grain) and 31.4% (inferior grain) in the prophase of the filling stage relative to the SuSase activity obtained without phosphorus application. Meanwhile, cv. Yongyou 2640 had its maximum SPAD value after the P1 treatment, showing 26.3% (superior grain) and 22.8% (inferior grain) increases. Excessive phosphorus application (P3 treatment) inhibited SuSase activity and thus was inconducive to grain growth. At the same phosphorus fertiliser rates, SuSase activity in cv. Yongyou 2640 increased by approximately 52.6% (early grain filling stage), 53.3% (mid grain filling stage), and 55.5% (late grain filling stage) in 2022 and 25.5% (early grain filling stage), 16.4% (mid grain filling stage), and 52.8% (late grain filling stage) in 2023, compared with that in cv. Lianjing 7.

Leaf senescence characteristics. Different cultivars and phosphorus application rates significantly affected MDA content in the leaves at different grain-filling stages and each year (Figure 6). In contrast to the SPAD value in the leaves, MDA content in the leaves decreased first and then increased with increasing phosphorus content. Specifically, the MDA content of cv. Lianjing 7 reached its lowest value after the P2 treatment, decreasing by 23.8% on average relative to that obtained without phosphorus application. Meanwhile, the MDA content of cv. Yongyou 2640 reached its lowest value after the P1 treatment, decreasing by 24.5% on average. Under the same phosphorus fertiliser rates, the MDA content of cv. Yongyou 2640 decreased by 19.4% (early grain filling stage), 6.5% (mid grain filling stage), and 17.0% (late grain filling stage) in 2022 and 18.0% (early grain filling stage), 5.5% (mid grain filling stage), and 14.5% (late grain filling stage) in 2023, compared with that of cv. Lianjing 7. Therefore, an

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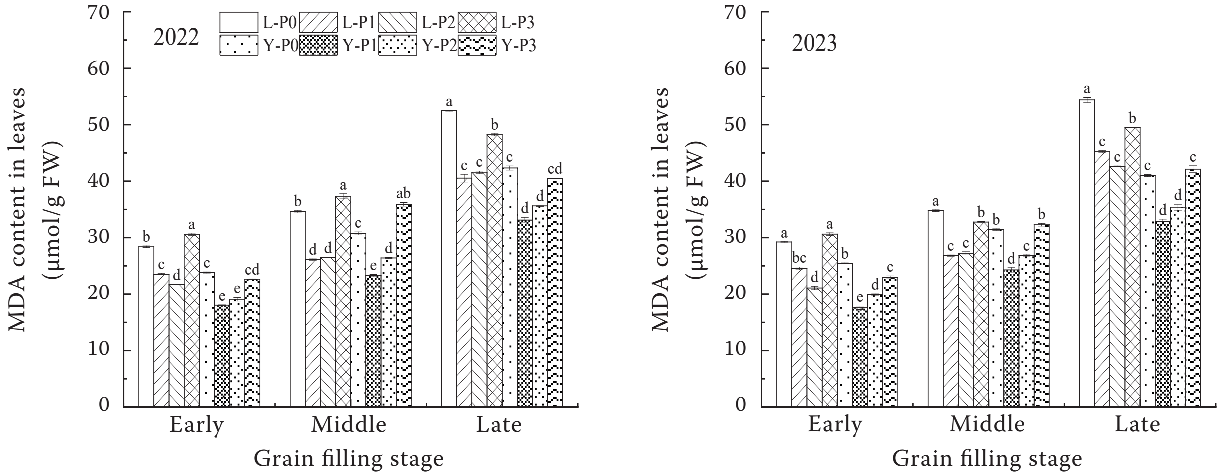

Figure 6. Mmalondialdehyde (MDA) content in leaves of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Early, middle, late represented 6th, 18th, 30th after anthesis. Vertical bars represent ± standard error (SE) of the mean. The SE was calculated across three replications for each year. Different letters indicate statistical significance at $P < 0.05$ within the same stage. FW – fresh weight

appropriate amount of phosphate fertiliser can effectively regulate MDA content in leaves and improve their physiological activity.

SOD activity in the leaves varied with cultivar and phosphorus fertiliser rate and decreased gradually at the growth stage (Figure 7). The SOD activity of cv. Lianjing 7 peaked after the P2 treatment, increasing by 31.2–61.4% (2022) and 35.8–97.3% (2023) compared with that obtained without phosphorus application. Meanwhile, the SOD activity of cv. Yongyou 2640 showed its maximum value after the P1 treatment, increasing by 66.5–153.4% (2022) and 63.9–78.5% (2023). Compared with cv. Lianjing 7, cv. Yongyou 2640 showed elevated SOD activity at each stage for both years. The result indicated that an appropriate amount of phosphorus fertiliser

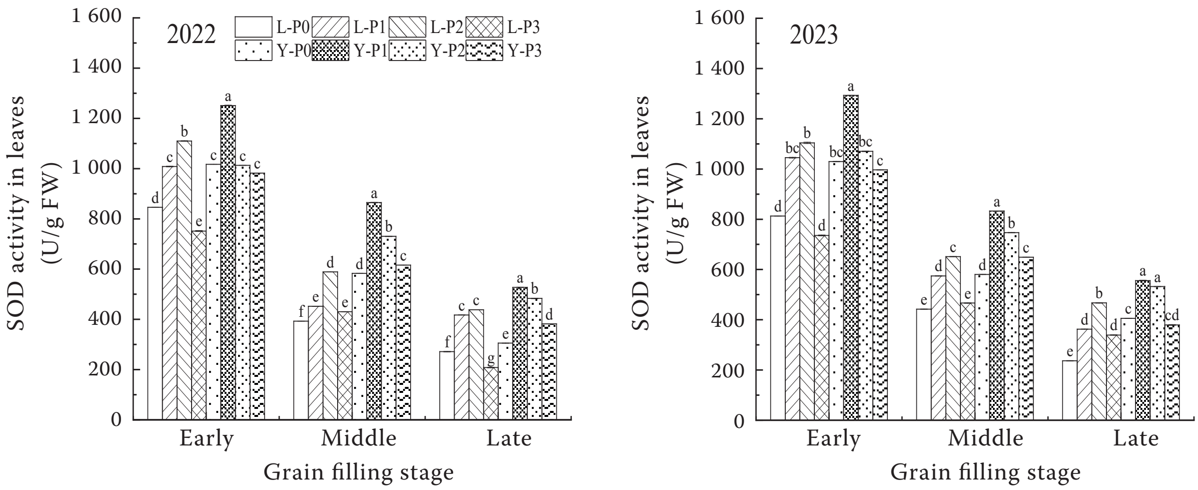

Figure 7. Ssuperoxide dismutase (SOD) activity in leaves of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Early, middle, late represented 6th, 18th, 30th after anthesis. Vertical bars represent ± standard error (SE) of the mean. The SE was calculated across three replications for each year. Different letters indicate statistical significance at $P < 0.05$ within the same stage. FW – fresh weight

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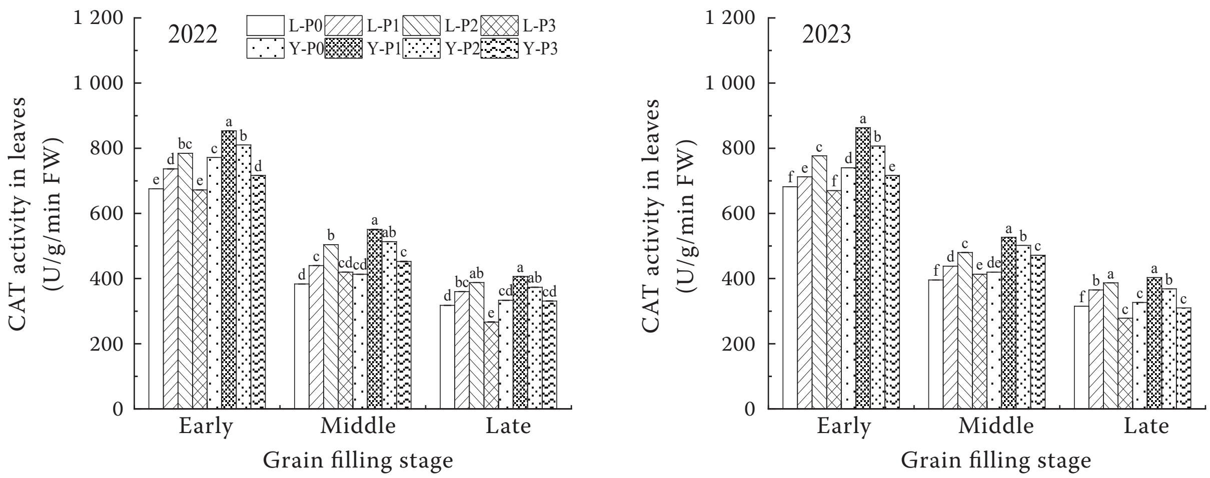

Figure 8. Ccatalase (CAT) activity in leaves of cvs. Lianjing 7 and Yongyou 2640 under phosphorus fertiliser rates in different phosphorus-tolerance cultivars of rice. P0 – no phosphorus application; P1 – 0.44 g/pot; P2 – 0.88 g/pot; P3 – 1.32 g/pot; cv. Lianjing 7 was low-phosphorus-sensitive cultivar and cv. Yongyou 2640 was low-phosphorus-tolerant cultivar. Early, middle, late represented 6^{th}, 18^{th}, 30^{th} after anthesis. Vertical bars represent ± standard error (SE) of the mean. The SE was calculated across three replications for each year. Different letters indicate statistical significance at $P < 0.05$ within the same stage

is conducive to SOD activity in leaves. In contrast, excessive phosphorus fertiliser application not only reduces SOD activity but also causes the waste of phosphorus resources.

Analysis of variance indicated the significant effects of cultivar, phosphorus fertiliser rate, and their interaction on CAT activity at different stages (Figure 8). Like SOD activity in the leaves, CAT activity in the leaves decreased gradually at the growth stage. Specifically, CAT activity in the leaves of cv. Lianjing 7 showed its highest value after the P2 treatment, increasing by 19.3% on average, compared with no phosphorus application. Meanwhile, after the P1 treatment, cv. Yongyou 2640 showed maximum CAT activity, which increased by 21.8%. Under the same phosphorus fertiliser rates, CAT activity in cv. Yongyou 2640 increased by 10.0% (early grain filling stage), 10.6% (mid grain filling stage), and 9.6% (late grain filling stage) in 2022 and 10.1% (early grain filling stage), 11.3% (mid grain filling stage), and 5.2% (late grain filling stage) in 2023, compared with that in cv. Lianjing 7. Therefore, appropriate phosphate fertiliser rates can effectively regulate CAT content in leaves, delay leaf senescence, and improve the physiological function of leaves.

Correlation of rice's photosynthetic rate, SPAD value, leaf senescence, and starch synthase activity with grain yield and grain filling. The photosynthetic rate, SPAD value, leaf senescence, and SuSase activity of rice were related to yield and grain-filling characteristics (Figure 9). Correlation analysis showed a significant or extremely significant positive correlation between AGPase and SuSase activity in superior and inferior grains, photosynthetic rate, SPAD value, SOD and CAT activity in the leaves, and grain yield and a negative correlation between MDA content and grain yield. Furthermore, a remarkable or considerably remarkable positive correlation was observed among AGPase and SuSase activity in the superior and inferior grains, SOD and CAT activity in the leaves, grain weight, and grain-filling rate and negative correlations among MDA content, grain weight, and grain-filling rate.

DISCUSSION

Effects of phosphorus application rates on yield and the component of different phosphorus-tolerant rice cultivars. The grain yield and quality of rice are affected by genetic and environmental factors, such as genotype, temperature, light, moisture, fertiliser, geographical site, and soil health (Iqbal et al. 2021, Kesh et al. 2022, Aqib et al. 2022, Radha et al. 2023, Yu et al. 2024, Yuan et al. 2024). The effects of phosphorus application rates on the phosphorus-use efficiency, growth and development, physiological characteristics, morphology trait, and yield performance of rice have been extensively studied (Deng et al. 2021, Patrick et al. 2022, An et al. 2023, Wendimu et al. 2023, Sun et al. 2024, Kaur

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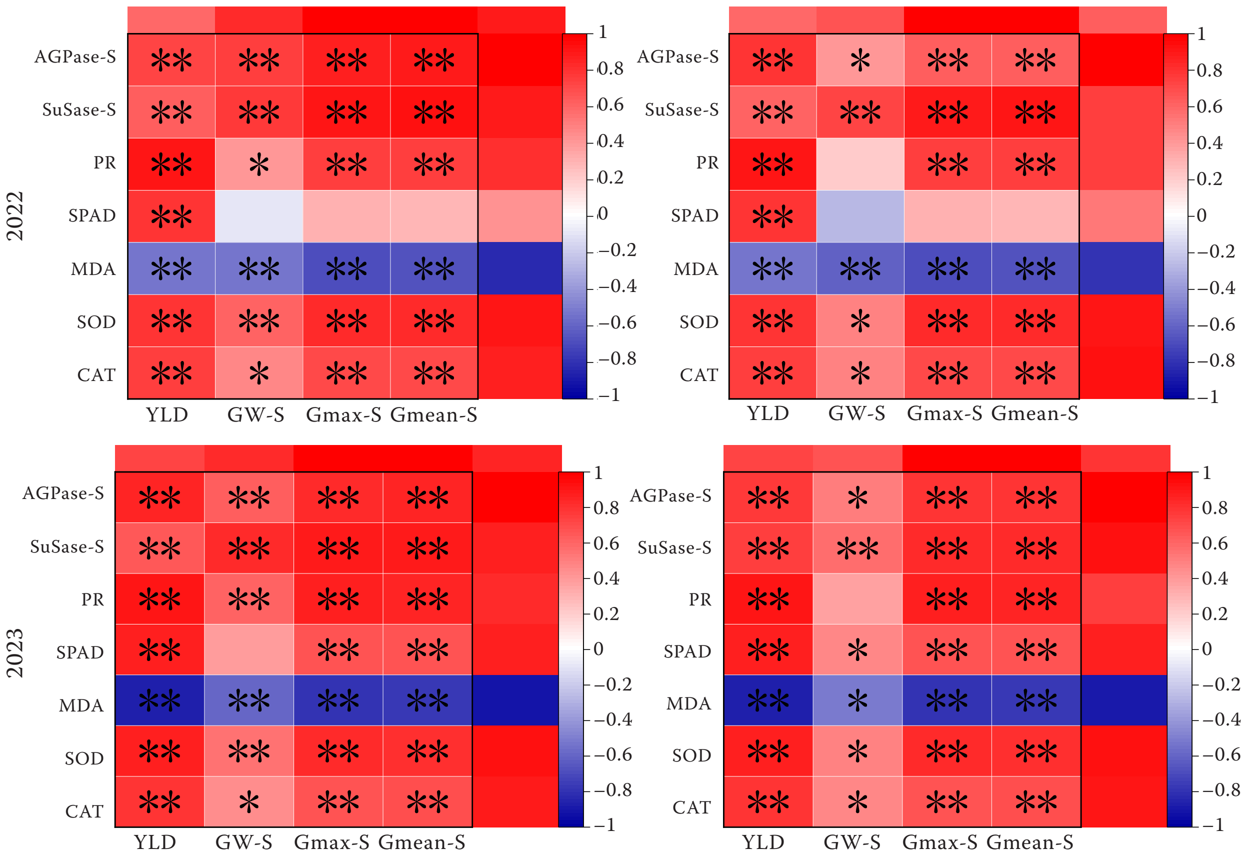

Figure 9. Relationship between adenosine diphosphate glucose pyrophosphorylase (AGPase) activity, sucrose synthase (SuSase)activity in superior and inferior grains, photosynthetic rate, soil and plant analyser development (SPAD) value, malondialdehyde (MDA) content, superoxide dismutase (SOD), catalase (CAT) enzyme activities in rice leaves and grain yield, grain weight, grain filling rate of different phosphorus-tolerance rice. * and ** indicate *F*-values significance at $P < 0.05$ and $P < 0.01$; ns – not significant at $P = 0.05$; YLD – yield; GW-S – grain weight of superior grains; Gmax-S – maximum grain filling rate of superior grains; Gmean-S – mean grain filling rate of superior grains; GW-I – grain weight of inferior grains; Gmax-I – maximum grain filling rate of inferior grains; Gmean-I – mean grain filling rate of inferior grains

et al. 2024). However, few studies have investigated the effects of combined phosphorus application rates and different phosphorus-tolerant cultivars on the grain yield of rice, the activity of starch-metabolising enzyme at the grain-filling stage, leaf senescence characteristics, and their relationship, especially in the phosphorus-deficient soil. The present results showed that the grain yield of rice initially increased and then decreased with increasing phosphorus application rate (Table 1). The suitable phosphorus application rates for cv. Lianjing 7 and cv. Yongyou 2640 were 0.88 and 0.44 g/pot, respectively. Total spikelets and filled-grain rate improved after suitable phosphorus application, resulting in a considerable yield increase. This finding is similar to that of Massawe et al. (2017), who observed that increased grain yield was partly due to the large spikelet number and high grain-filling rate. The possible reasons were improved soil environment of the rice roots, root activity, and nutrient absorption and utilisation by root. In addition, the relationship between source and sink becomes increasingly coordinated after the application of phosphorus fertiliser, which ultimately increases grain yield (Alkahtani et al. 2020, Zhang et al. 2021, Prodhan et al. 2022, Sun et al. 2023, Zhang et al. 2023, Yuan et al. 2024, Sun et al. 2024). Meanwhile, low phosphorus application considerably reduces rice yield by inhibiting each yield component. The grain yields of different phosphorus-tolerant cultivars considerably increased after the P1 treatment relative to those obtained without phosphorus application. The increase in the yield of the cultivar

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with strong low-phosphorus tolerance was higher than that of the variety with weak low-phosphorus tolerance, indicating that low phosphorus application had a relatively small effect on the former. Consequently, the rational application of phosphorus fertiliser can increase yield and ensure the efficient utilisation of phosphorus fertiliser by different rice cultivars in different planting environments. Notably, this method can conserve phosphorus fertilisers.

Effects of phosphorus application rates on the grain-filling characteristics of different phosphorus-tolerant rice cultivars. Grain filling is an important physiological process that determines grain weight and is closely related to grain yield (Parida et al. 2022, Farooq et al. 2022, Ma et al. 2023, Zeng et al. 2024). The process is actually starch accumulation. AGPase and SuSase are the two key enzymes in starch synthesis, and their activity is closely related to the filling rate in developing rice grains (Yang et al. 2004, Wenting et al. 2020, Wang et al. 2021, Tong et al. 2022, Chen et al. 2023, Yu et al. 2024). Our results indicated that increasing the application of phosphorus fertiliser enhanced the filling rate of the superior and inferior grains, promoting grain weight and ultimately enhancing grain yield. However, the excessive application of phosphorus (1.32 g/pot) reduced the maximum filling rate, average filling rate, and grain weight (Figures 1 and 2). This result indicated that the maximum filling rate and grain weight of cv. Lianjing 7 was optimal after the P2 treatment and cv. Yongyou 2640 after the P1 treatment. Appropriate-phosphorus application increased the activity of ribulose diphosphate carboxylase in leaves, sucrose phosphate synthase in stems, and related enzymes in grains and thus increased the leaf area index of plants; thus, it is conducive to photosynthesis and grain yield during the grain-filling stage of rice (Yu et al. 2024). Meanwhile, AGPase and SS activity in the grains were enhanced after suitable phosphorus application in cv. Lianjing 7 and cv. Yongyou 2640, as indicated by the increased grain-filling rate and grain weight (Figures 4 and 5). Correlation analysis confirmed these results (Figure 9), which were similar to those of Zang (2024), who observed that AGPase, StSase, and SBE activity promoted grain filling, increased grain weight by 7.5%, and increased yield (Zang et al. 2024).

Generally, the earlier flowering glumes in the upper part of rice panicles are considered superior grains, whereas the latter are considered inferior grains. Superior grains have higher grain-filling rates and grain weight (Zhao et al. 2020, Dou et al. 2021, Lu et al. 2022, Chen et al. 2023, Panigrahi et al. 2023). Our results indicated that the maximum and average grain filling rates of cv. Yongyou 2640 superior grains were higher than those of cv. Lianjing 7. The underlying reasons can be elucidated as follows: cv. Lianjing 7 had lower physiological activity, lower SPAD value, and faster leaf senescence characteristics in the early stage of grain filling in grains (Figures 3, 6 and 8). These features limited the grain-filling rate of the grains (Zhang et al. 2021, Panigrahi et al. 2023, Zhang et al. 2024). Meanwhile, insufficient photosynthetic assimilation was considered the main reason for the limited filling rate of the inferior grains (Yin et al. 2022, Zhang et al. 2024). In addition, the expression levels of AGPS2b, SSS1, GBSS1, and GBSE11b genes were inhibited, and thus, the activity of key enzymes in the sucrose-starch metabolic pathway decreased at the grain-filling stage (Iqbal et al. 2021). Therefore, selecting suitable varieties in production, coordinating the growth between superior and inferior grains through the regulation of starch-related enzyme activity, and improving the maximum and average grain filling rates will increase grain weight and rice yield.

Effect of phosphorus fertiliser rates on the leaf senescence characteristics of different phosphorus-tolerant rice cultivars. Excessive ROS generated by abiotic stress can be oxidised into lipids, proteins, and nucleic acids, including the excessive oxidation of chloroplast thylakoid membranes, leading to severe structural and functional damage in crops (Raza et al. 2022, Liang et al. 2023). To protect themselves against the harmful effects of ROS, plants typically boost the antioxidant defence systems, including enzymatic and nonenzymatic systems, and thereby eliminate harmful substances, such as CAT, POD, SOD, glutathione peroxidase, and ascorbate peroxidase (Cao et al. 2017, Liang et al. 2023, Averill-Bates 2024). The present results indicated that the SOD and CAT activity in the leaves remarkably increased, whereas the MDA content decreased with increasing phosphorus fertiliser rate (Figures 5–7). Our results also demonstrated that the highest levels of antioxidant enzymes were obtained at suitable phosphorus application rates of 0.88 g/pot for cv. Lianjing 7 and 0.44 g/pot for cv. Yongyou 2640, which delayed the senescence of plants, prolonged the photosynthesis period and facilitated grain filling and yield formation. Correlation analysis supported these results (Figure 9). The present results were consistent with those of Dang et al. (2023), who found that an appropriate amount of phosphate fertiliser can alleviate damage to plants caused by stress conditions and reduce the content of MDA (Dang et al. 2023). However, the excessive use of phosphorus fertiliser can inhibit the activity of antioxidant enzymes and increase the content of MDA. The possible mechanism was that an appropriate amount of phosphorus fertiliser promoted the antioxidant defence systems and accelerated the clearance of free radicals, but high concentrations of phosphorus can actually lead to lipid membrane peroxidation in crop cells and exacerbate damage to plants (Barbhuiya et al. 2024, Huang et al. 2024). In our study, SOD and CAT activity in the leaves of cv. Yongyou 2640 were remarkably higher than those of cv. Lianjing 7, and MDA content was lower than that of cv. Lianjing 7 (Figures 5–7). The results indicated that cultivars with strong low-phosphorus tolerance can improve the antioxidant systems of crops and maintain vigorous physiological functions when subjected to abiotic stress. Therefore, selecting cultivars with strong low-phosphorus tolerance and the suitable application of phosphorus fertiliser can delay leaf senescence, improve leaf photosynthetic rate, optimise grain-filling rate characteristics, and thus promote grain weight and yield in production.

Correlation analysis demonstrated that increases in photosynthetic rate, SPAD value, SOD and CAT activity in leaves, and sink activity, including starch synthesis, are essential to the enhancement of grain yield and grain-filling rate (Figure 9). The activity of starch-synthesising enzymes, including AGPase, SS, SBE, and antioxidant enzymes, are significantly positively correlated with grain filling and the formation of rice yield (Yang et al. 2018, Iqbal et al. 2021, Zhu et al. 2022, Liu et al. 2023, Zang et al. 2024). Therefore, parents with highly active starch-metabolising enzymes in grains, antioxidant enzymes, and high photosynthetic rates in leaves are expected to generate high-yield rice cultivars. Furthermore, regulating and improving the activity of these enzymes and photosynthetic assimilation at the grain-filling stage with suitable phosphorus fertiliser rates and cultivars with strong low-phosphorus tolerance cultivar can improve grain filing rate and rice yield.

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