https://doi.org/10.17221/530/2024-PSE

Irrigation methods and nitrogen-form interactions regulate starch-metabolising enzyme activity to improve rice yield and quality

Haojing Li, Hairun Li, Danke Zhang, Mengmeng Jiang, Jing Cao, Guowei Xu**

College of Agriculture, Henan University of Science and Technology, Luoyang, Henan, P.R. China
**Corresponding authors: caojing0111@163.com; gwxu2007@163.com*

Citation: Li H.J., Li H.R., Zhang D.K., Jiang M.M., Cao J., Xu G.W. (2025): Irrigation methods and nitrogen-form interactions regulate starch-metabolising enzyme activity to improve rice yield and quality. Plant Soil Environ., 71: 185–201.

Abstract: Nitrogen management and irrigation methods play crucial roles in determining rice's grain yield and quality (*Oryza sativa* L.). However, limited knowledge exists on how interactions between nitrogen forms and irrigation regimes regulate starch-metabolising enzyme activity to influence rice yield and quality. A soil-growth experiment was conducted using a high-lodging-resistance rice cultivar under three irrigation methods, namely, submerged irrigation (0 kPa), alternate wetting and moderate drying (–20 kPa), and alternate wetting and severe drying (–40 kPa), as well as three nitrogen forms, namely, ammonium nitrogen (NH_4^+-N), mixed ammonium + nitrate (50:50), hereafter denoted as 50:50, and nitrate nitrogen (NO_3^--N). Results indicated that compared with the other treatments, alternate wetting and moderate drying interacted with 50:50 treatment, resulting in the following: improved grain yield by 11.7–21.0%, milling, appearance, eating and cooking, and nutritional qualities including milled-rice and gel consistency; and decreased chalky rice, chalky size, chalky degree, amylose content, and protein content by 20.0–23.1, 29.6–33.3, 44.1–48.5, 6.2–9.6 and 10.1–13.9%, respectively. The activities of adenosine phosphate glucose pyrophosphorylase (AGPase), starch synthase (SS), starch-branching enzyme (SBE), and adenosine triphosphate (ATP) enzyme in the grains also improved, with an increase of 20.0–35.0, 11.8–20.0, 13.6–26.3 and 21.2–39.6%, respectively. Conversely, severe drying and NO_3^--N treatment negatively impacted grain yield and quality due primarily to decreased SS activity in grains under each irrigation method. Correlation analysis showed that starch-metabolising enzyme (AGPase, SS and SBE) activity at 14 days after anthesis (DAA) and 28 DAA exhibited a positive correlation with grain yield, milling quality and gel consistency, whereas negatively correlated with appearance and nutritional qualities. In summary, the adoption of alternate wetting and moderate drying and 50:50 interaction treatment can synergistically boost grain yield by increasing the filled-grain rate and 1 000-grain weight and enhance grain quality of rice by upregulating the activities of starch-metabolising enzyme activity.

Keywords: rice (*Oryza sativa* L.); production; water management; water stress; nutrition; quality formation; starch synthesis

Rice (*Oryza sativa* L.) is one of the world's most crucial food crops, playing a prominent role in safeguarding China's food security (Li et al. 2018, Hou et al. 2024). In China, rice is the largest consumer of agricultural water, accounting for over 70% of the nation's total agricultural water usage. Nevertheless, the water resources earmarked for rice irrigation are declining, seriously threatening rice production

Supported by the National Natural Science Foundation of China grants, Project No. U1304316; by the Joint Fund for Science and Technology Research and Development of Henan Province, Project No. 242103810031; by the Scientific and Technological Research Projects in Henan Province, Project No. 242102110169; by the Open Foundation for Key Laboratory of Cultivation and Physiology of Jiangsu Province, Project No. 027388003K11009, and by the Doctoral Research Initiation Fund Project of Henan University of Science and Technology, Project No. 13480108.

https://doi.org/10.17221/530/2024-PSE

(Ishfaq et al. 2021). Maintaining high rice yields and further boosting yields under limited water resources has become an issue of concern. Scientists have invented many efficient water-utilisation technologies. Among them, alternate wetting-and-dry irrigation technology is an outstanding and effective water-saving technology. It is widely recommended and implemented in numerous rice-producing countries across Asia, such as Bangladesh, India, Vietnam, the Philippines, and China (Carrijo et al. 2017, Maneepitak et al. 2019, Ishfaq et al. 2020, Zhang et al. 2021, Jiang et al. 2024). This technology has brought about significant economic and ecological benefits, including a reduction in irrigation quota, methane emissions (Abu-Ali et al. 2023), arsenic concentration in grain and yield in milled rice (Xu et al. 2019a, Vicente et al. 2025), and methylmercury accumulation in soil (Ishfaq et al. 2021). However, alternate wetting-and-drying irrigation reportedly causes nitrogen loss, decreased aboveground dry-matter weight and shortened grain-filling period, ultimately resulting in lower yield (Wang et al. 2016, Vuciterna et al. 2024). Therefore, the impacts of this technology on rice yield and quality vary significantly due to factors like variety adaptability, soil moisture-monitoring methods, different cultivation methodology, soil properties, and rainfall during the rice-growth period (Wang et al. 2016, Prasad et al. 2017, Wand et al. 2019, Xu et al. 2018, 2020, 2021, Jiang et al. 2024, Vicente et al. 2025).

In traditional continuous-flooding irrigation systems, nitrogen predominantly exists as ammonium nitrogen (NH_4^+-N). In contrast, nitrate nitrogen (NO_3^--N) is mainly prevalent in arid and semiarid irrigation regions. In the soil, NH_4^+-N is assimilated into amino acids or amides. A portion of this assimilated nitrogen is utilised to meet the plant's metabolic requirements. In contrast, the remaining portion is translocated to the plant's above-ground parts to carry out its physiological functions. NO_3^--N needs to be transported in plants by nitrate transporters and finally absorbed and assimilated (Wang et al. 2017, González et al. 2024). Under alternate wetting-and-drying irrigation, the rhizosphere environment of rice undergoes significant alterations compared with flooded irrigation. In this case, the water management in farmland shifts from the traditional flooded condition to a regime of controlled irrigation or a state of relatively moderate water stress. Consequently, the forms of nitrogen in the soil start to transform, leading to the coexistence of NH_4^+-N and NO_3^--N (Xu et al. 2021).

Changes in the forms of available nitrogen in the soil have a certain degree of impact on the nutrient absorption and utilisation patterns of rice, in close relation to the growth and development of crops and ultimately affecting yield and quality (Zheng et al. 2018, Yi et al. 2019, Hamoud et al. 2019, Xu et al. 2020, Fu et al. 2021, Pereira et al. 2024). Previous research has shown that rice seedlings treated with NH_4^+-N exhibit a stronger water-absorption capacity. Specifically, they can maintain higher aquaporin activity when subjected to water stress conditions. In contrast, water stress significantly reduces the root surface area and root length of rice under NO_3^--N treatment but increases morphological parameters such as root length, root tips, and root volumes of NH_4^+-N nutritional treatment, as well as significantly increases the biomass and photosynthesis rate of rice at tillering stage (Yang et al. 2018, Pereira et al. 2024). Zheng et al. (2018) reported that the grain yield of rice can be maximised under NO_3^--N application in the black soil region of the Songnen Plain; the underlying mechanism is associated with enhancing the photosynthetic electron-transfer rate, the light-capturing ability, and the photosynthetic activity of the rice plants. These enhancements are conducive to the rice leaves' more efficient absorption and utilisation of light energy (Zheng et al. 2018). Other studies have pointed out that grain yield and nutrient absorption and utilisation can be maximised through treatment with an ammonium nitrate mixture by optimising root morphology, increasing the synthesis, distribution and transportation capacity of hormones (such as Z + ZR and IAA) and physiological activity in the root system; regulating small-miRNA expression; and enhancing carbon and nitrogen metabolism and leaf photosynthesis rate (Xu et al. 2020, 2021, Chen et al. 2024a). Most previous studies have focused on dryland crops, a single growth period, or a hydroponic experiment (Zaghdoud et al. 2016, Guo et al. 2019, Fu et al. 2021, Mi et al. 2024). However, the grain yield and quality of rice under irrigation methods and nitrogen form and their relationship to the key enzymes involved in the grain-filling stage are not well understood.

The process of grain filling and enriching is actually the process of starch biosynthesis and accumulation in endosperm cells (Yang et al. 2004, Li et al. 2018, Iqbal et al. 2021, Yu et al. 2024). Photoassimilates generated by the source organs, such as leaves, are transported into the grain sinks as sucrose. Subsequently, this sucrose undergoes a series of biochemical trans-

https://doi.org/10.17221/530/2024-PSE

formations to be converted into starch. Adenosine diphosphate glucose pyrophosphorylase (ADGPase), starch synthase (SS), and starch-branching enzymes (SBE) in the grains are considered to play important roles in starch synthesis (Yang et al. 2003, 2004, Li et al. 2018, Ali et al. 2021, Yu et al. 2024). Studies have shown that unreasonable nitrogen application or irrigation management can disrupt the normal grain-filling process. This disruption often leads to a decrease in rice yield and a deterioration in grain quality (Li et al. 2018, Iqbal et al. 2021, Dou et al. 2024). However, the evidence of the difference in starch-metabolising enzyme activity involved in the grain-filling stage and their relationship to grain yield and quality of rice under different irrigation methods and nitrogen forms is considerably scarce.

The present study aimed to determine whether alternate wetting-and-moderate-drying irrigation and mixed ammonium + nitrate treatment could promote rice's grain yield and quality. Three irrigation methods and three nitrogen forms were subjected to soil-growth experiments. The characteristics closely associated with rice quality and physiological traits, including milling and appearance quality, eating-and-cooking quality, nutritional quality and the key enzymes involved in the grain-filling stage, were explored. The results can provide new information on improving rice's grain yield and quality.

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 2019 and repeated in 2020. A high-lodging-resistance cultivar of Liangeng 7 was grown in a pool field. The soil of the field was clay loam (Typic Fluvaquents, Entisols, US Taxonomy) containing soil pH value of 7.4, organic carbon at 11.26 mg/kg and available N-phosphorus-potassium at 105.1, 5.2, and 118.6 mg/kg, respectively. The average air temperatures and rainfall for each month from transplanting (June) to harvest (October) are shown in Table 1 and Figure 1.

Treatments. A wholly randomised experiment comprising two factors, namely, irrigation method and nitrogen form, was adopted with three replications. Each plot, measuring 9 m × 2 m, was separated by a 40-cm-wide alley. Waterproof material was installed in the alley to a depth of 40 cm to form a barrier. Three irrigation methods were set, i.e., submerged irrigation (SI), alternate wetting-and-moderate-drying irrigation, and alternate wetting-and-severe-drying irrigation. Submerged irrigation refers to a water depth kept 2–3 cm throughout the entire growth stage as farming practices recommend, except for light field drainage at the late tillering stage and one week before the final harvest. Under the alternate wetting-and-moderate-drying irrigation, the plots were not rewatered until soil water potential reached –20 kPa. Severe drying irrigation refers to a field not irrigated until Ψ_{soil} decreased to –40 kPa at 15 cm soil depth (Xu et al. 2021). 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 its pottery head 15 cm below the soil; the pool was sheltered from the rain with a plastic canopy during the growing season.

Three different nitrogen forms were set, namely, NH_3-N, ammonium + nitrate mixed 50:50 (hereafter denoted as 50:50), and NO_3-N according to the ratio of NH_3-N to NO_3-N with the same nitrogen rates of 240 kg/ha. NH_4^+-N and NO_3^--N were provided by analytical pure ammonium sulfate [$(NH_4)_2SO_4$], ammonium nitrate (NH_4NO_3), and calcium nitrate [$Ca(NO_3)_2$]. Dicyandiamide (DCD), a nitrification inhibitor by controlling the activities of nitrifying bacteria to prevent the change in nitrogen form, was added with 5% of total nitrogen (Xu et al. 2020, 2021). Nitrogen fertiliser (30%) was applied 1 day before transplanting (BBCH 22), 30% was applied 7 days after transplanting, and 40% was applied in the panicle-initiation period (BBCH 30). Before transplanting, 300 kg/ha superphosphate (5.89% P) and 195 kg/ha potassium chloride (43% K) were applied in each treatment. The field-cultivation procedure was sowing on May 10th and transplanting on June 8th to pools at 20 cm × 20 cm spacing, two seedlings per hill. Each treatment had three replications. Diseases, pests, and weeds were strictly controlled throughout the entire growth period in both years.

Table 1. Monthly mean temperature (T_{ave}), and rainfall during the growing stage of 2019 and 2020 in Luoyang, China

Month	T_{ave} (°C)		Rainfall (mm)	
	2019	2020	2019	2020
June	27.0	26.3	138.7	116.4
July	28.6	25.9	93.2	104.6
August	26.0	26.3	134.8	143.0
September	21.8	23.1	94.1	32.9
October	15.3	14.9	90.1	59.0

https://doi.org/10.17221/530/2024-PSE

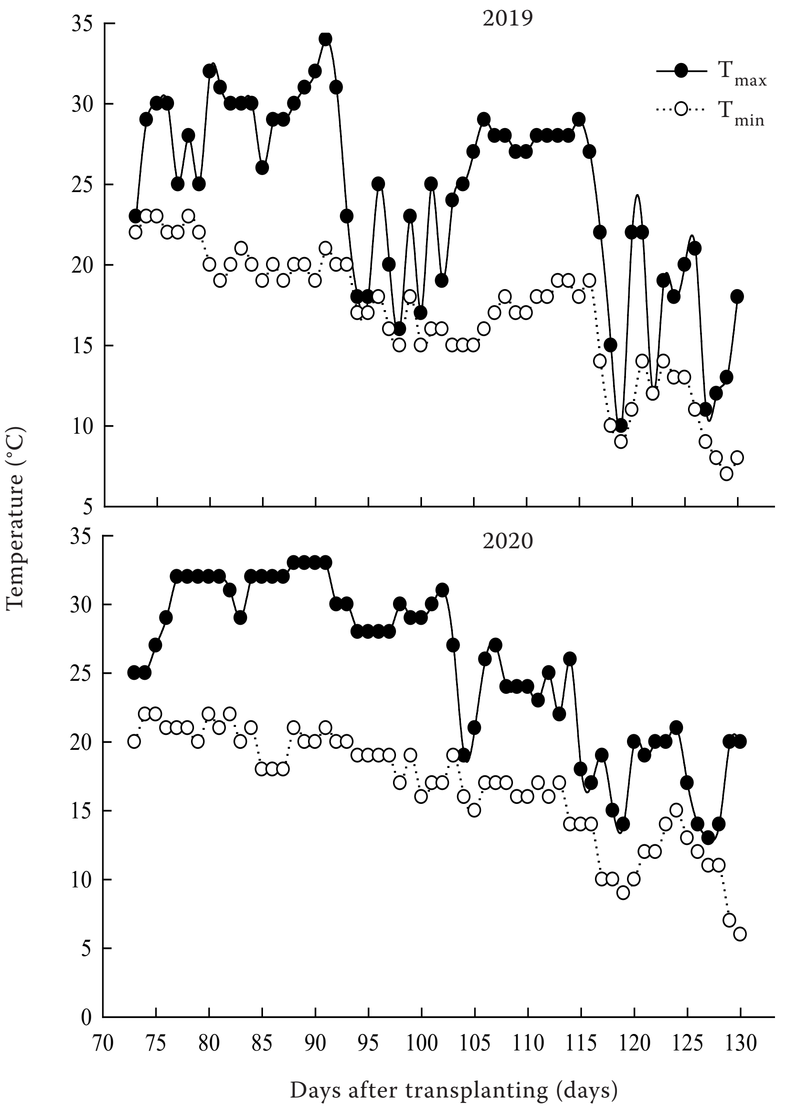

Figure 1. The maximum and minimum air temperatures during grain filling stage

Sampling and measurement

Harvesting (BBCH 99). Plants were harvested on 17 October 2019 and 18 October 2020. Grain yield was obtained by 5 m^2 for each treatment (excluding border ones). Yield components, i.e., panicles per unit area, spikelets per panicle, filled-grain rate and 1 000-grain weight, were determined by 50 plants (excluding the border ones) samples of each plot. The filled-grain rate was defined as a percentage of filled grains (specific gravity ≥ 1.06) to the total spikelets.

Measurements of milling, appearance, cooking, and nutritional quality. The method of determining rice's milling and appearance quality was performed as described by Xu et al. (2019). After the selected rice grains were dried and stored for 3 months, 130 g grains of each sample were shelled using a rice huller (Sy88-138 HT; Seoul, Korea) twice, and then the brown rice was pressurised and finely milled with a rice miller (NSART100, Seoul, Korea). The milled rice was divided into head rice (unbroken grain) and broken grain. Head rice was defined as the kernels that retained more than three-quarters of their original length. After the sorting, the grains were weighed to calculate the brown rice rate, the milled rice rate, and the head rice rate.

The chalkiness rate was calculated as the proportion of chalky kernels among 100 randomly selected intact milled rice grains, expressed as a percentage. The chalky size was measured by randomly taking 10 chalky rice grains, placing them horizontally in the spotlight, measuring the percentage of each chalky area in the area of the head rice, and calculating the average value. The chalkiness degree was the percentage of the total chalky area in the sample, that is, the product of chalkiness rate and size.

https://doi.org/10.17221/530/2024-PSE

A near-infrared grain rapid quality analyser (manufactured by FOSS Tecator, Sweden) determined amylose content, gel consistency, and protein content.

Sampling. 80–150 spikes headed on the same day were selected and labelled for each plot in the pool fields. Fifteen labelled spikes from each plot were sampled 14 and 28 days after anthesis (BBCH 55, hereafter denoted as 14 DAA and 28 DAA), and all grains from each spike were removed. All sampled grains were frozen in liquid nitrogen for 30 s to arrest metabolic activity and stored in a –70 °C refrigerator for enzyme-activity determination.

Starch-synthase and ATP enzyme activity. The procedures for extracting enzyme activity were by the methods described by Yang et al. (2004). 40–50 g were cryogenically homogenised in liquid nitrogen using a pre-chilled porcelain mortar and pestle and then extracted in 3–5 mL of mmol/L Tricine-NaOH (pH 8.0) containing 10 mmol/L $MgCl_2$, 2 mmol/L EDTA, 50 mmol/L 2-mercaptoethanol, 12% (*v*/*v*) glycerol, and 5% (*w*/*v*) PVP 40. The mixture was filtered through four layers of gauze, and the filtered solution was centrifuged for 10 min at 15 000 rpm and 4 °C. The supernatant was used for enzyme determination. SS activity was determined as described by Ranwala and Mille (1998). Adenosine phosphate glucose pyrophosphorylase (AGPase) and SBE were measured according to the procedure of Nakamura et al. (1989). ATP enzyme activity in grains was measured by the method of Xu et al. (2021).

Physical and chemical properties of soil. Soil samples were collected from the fields before transplanting with 3 replications at 0–20 cm depths. The physical and chemical indicators of the soil were analysed according to Gajda et al. (2017): pH (electrometric), soil organic carbon (Walkley and Black), available nitrogen (alkali-hydrolyzable diffusion method), available phosphorus (modified Olsen), exchangeable potassium (ammonium acetate).

Data analysis

The dataset was meticulously analysed using the SAS/STAT statistical analysis software package for variance analysis (version 9.2; SAS Institute Inc., Cary, USA). The statistical model employed comprehensively accounted for multiple sources of variation. These sources included replication, year, irrigation method, nitrogen forms and the interaction of year × irrigation method, year × nitrogen forms and irrigation method × nitrogen forms (model y = year, irrigation method, nitrogen forms, year × irrigation method, year × nitrogen forms, irrigation method × nitrogen forms). Means were tested using the least significant difference at $P_{0.05}$ ($LSD_{0.05}$).

RESULTS

Grain yield and its component. Nitrogen form, irrigation method, and their interaction significantly affected grain yield and its components (P < 0.05, Table 2). Specifically, grain yield showed no significant differences between NH_4^+-N and 50:50 treatment under the SI method. However, in both years, it was substantially higher in the 50:50 than in the NH_4^+-N treatment under alternate wetting-and-drying irrigation. NO_3^--N reduced rice yield under various irrigation methods for both years. Alternate wetting-and-moderate-drying irrigation increased grain yield by an average of 15.0% (2019) and 10.2% (2020) under the same nitrogen form. In contrast, severe drying significantly reduced grain yield by 32.4% (2019) and 36.4% (2020) compared with SI. The 50:50 treatment interacted with moderate-drying irrigation, the best combination in this experiment; it promoted the filled-grain rate and 1 000-grain weight and consequently increased the rice yield. The decrease in grain yield under NO_3^--N, severe-drying irrigation, and its interaction treatments were primarily attributed to the decrease in panicle numbers, spikelets per panicle, filled-grain rate, and 1 000-grain weight.

Grain quality. Analysis of variance revealed significant impacts of nitrogen form, irrigation method, and their interactions on milling and appearance quality, cooking, and nutritional quality (Tables 3–5). Brown rice, milled rice, and head rice showed no remarkable differences between NH_4^+-N and 50:50 treatments under SI (Table 3). In contrast, 50:50 treatment significantly increased brown rice, milled rice, and head rice by 4.8, 5.6, and 3.1% in 2019 and 3.4, 4.9, and 5.6% in 2020, respectively, under alternate wetting-and-drying irrigation compared with NH_4^+-N treatment. Milling quality dramatically declined in the NO_3^--N treatment compared with those in the NH_4^+-N treatment. Compared with SI, alternate wetting and moderate drying increased brown rice, milled rice, and head rice under the same irrigation method. However, severe-drying irrigation significantly decreased brown rice, milled rice, and head rice by 4.4, 6.3, and 1.7% in 2019 and 4.1, 4.0, and 4.6% in 2020, respectively.

https://doi.org/10.17221/530/2024-PSE

Table 2. Grain yield and its components of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation methods

Treatment		Panicle numbers ($1/m^2$)	Spikelet per panicle	Filled grain rate (%)	10^3-grain weight (g)	Yield (t/ha)
2019						
AN	0 kPa	339.3 ± 5.1^a	131.1 ± 2.4^b	79.0 ± 1.0^c	27.1 ± 0.4^b	9.5 ± 0.2^c
50:50		337.0 ± 6.6^a	129.5 ± 2.1^b	79.3 ± 2.1^c	26.9 ± 0.3^b	9.3 ± 0.3^c
NN		301.7 ± 6.5^c	126.1 ± 1.8^c	76.0 ± 1.0^d	26.0 ± 0.4^c	7.5 ± 0.1^e
AN	–20 kPa	333.3 ± 4.5^a	134.5 ± 1.8^a	84.0 ± 1.7^b	27.4 ± 0.3^b	10.3 ± 0.1^b
50:50		332.7 ± 4.0^a	136.9 ± 1.7^a	89.7 ± 1.5^a	28.3 ± 0.4^a	11.5 ± 0.3^a
NN		294.3 ± 6.7^c	129.1 ± 1.2^{bc}	80.7 ± 1.2^c	26.9 ± 0.2^b	8.3 ± 0.2^d
AN	–40 kPa	321.3 ± 6.1^b	111.4 ± 2.6^e	66.3 ± 2.1^f	25.9 ± 0.2^c	6.2 ± 0.1^g
50:50		320.1 ± 4.7^b	116.6 ± 2.1^d	72.0 ± 1.7^e	26.1 ± 0.2^c	7.0 ± 0.3^f
NN		272.3 ± 6.5^d	109.1 ± 1.8^e	63.0 ± 0.6^g	25.2 ± 0.3^d	4.7 ± 0.1^h
2020						
AN	0 kPa	349.3 ± 3.1^a	137.7 ± 1.7^a	81.3 ± 0.6^c	27.3 ± 0.5^c	10.7 ± 0.3^b
50:50		348.0 ± 2.7^{ab}	133.0 ± 2.1^b	80.7 ± 0.6^c	27.1 ± 0.3^{cd}	10.1 ± 0.1^c
NN		304.2 ± 3.2^e	122.8 ± 2.3^c	78.0 ± 1.0^d	26.2 ± 0.5^e	7.8 ± 0.1^e
AN	–20 kPa	345.8 ± 2.7^{ab}	135.5 ± 0.8^{ab}	84.7 ± 0.6^b	27.6 ± 0.1^b	11.0 ± 0.2^b
50:50		342.5 ± 2.2^b	132.0 ± 2.7^b	92.7 ± 1.2^a	28.4 ± 0.2^a	11.9 ± 0.4^a
NN		311.7 ± 6.7^d	123.6 ± 2.2^c	85.3 ± 0.6^b	26.8 ± 0.2^d	8.6 ± 0.4^d
AN	–40 kPa	321.7 ± 4.9^c	112.2 ± 1.6^e	65.7 ± 1.5^f	26.0 ± 0.2^e	6.3 ± 0.1^g
50:50		327.7 ± 3.1^c	116.9 ± 2.6^d	71.0 ± 1.7^e	26.2 ± 0.5^e	7.0 ± 0.4^f
NN		272.5 ± 8.6^f	110.6 ± 3.2^e	64.7 ± 0.6^f	25.3 ± 0.3^f	4.9 ± 0.1^h
ANOVA						
Year (Y)		ns	ns	ns	ns	ns
Nitrogen form (N)		157.1**	164.2**	834.4**	186.6**	641.2**
Irrigation (I)		420.7**	42.4**	63.7**	82.8**	226.1**
Y × N		ns	ns	ns	ns	ns
Y × I		ns	ns	ns	ns	ns
N × I		8.8**	6.9**	12.9**	5.2**	10.8**

AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; –20 kPa – alternate wetting and moderate drying; –40 kPa – alternate wetting and severe drying. Values followed by different letters indicate statistical significance at $P < 0.05$ within the same column and year. *$P < 0.05$ and **$P < 0.01$ indicate significance difference of F-values; ns – not significant difference

In contrast to milling quality, chalky rice, chalk size, and chalk degree were the highest in NO_3^--N followed by NH_4^+-N, and the lowest was in the 50:50 treatment with the same irrigation method, especially in alternate wetting-and-drying irrigation (Table 4). Compared with SI, alternate wetting and moderate drying decreased chalky rice, chalk size, and chalk degree under the same nitrogen form. Conversely, severe-drying irrigation significantly increased chalky rice, chalk size, and chalk degree by averages of 13.6, 6.5, and 22.4% in 2019 and 17.6, 3.7, and 22.5% in 2020, respectively.

Similar to appearance quality, differences in amylose content and protein content between NH_4^+-N and 50:50 treatment were not significant but were lower than those of NO_3^--N treatment in SI (Table 5). However, amylose and protein content were the lowest in the 50:50 treatment, followed by NH_4^+-N, and the highest in NO_3^--N with alternate wetting-and-drying irrigation. Compared with SI, alternate wetting and moderate drying remarkably decreased amylose and protein content under the same nitrogen form by averages of 4.1% and 11.0% in 2019 and 6.1% and 9.9% in 2020, respectively. However, severe drying

https://doi.org/10.17221/530/2024-PSE

Table 3. Milling quality of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation methods interactions

Treatment		Brown rice (%)	Milled rice (%)	Head rice (%)
2019				
AN	0 kPa	76.0 ± 1.0^{d}	71.0 ± 1.0^{cd}	60.3 ± 0.6^{bc}
50:50	0 kPa	78.7 ± 1.5^{ab}	73.7 ± 1.5^{bc}	59.3 ± 1.5^{cd}
NN	0 kPa	75.7 ± 0.6^{d}	70.3 ± 0.6^{d}	55.7 ± 0.6^{e}
AN	−20 kPa	78.0 ± 0.6^{bc}	73.3 ± 0.6^{b}	63.0 ± 1.7^{a}
50:50	−20 kPa	80.0 ± 0.6^{a}	75.7 ± 0.6^{a}	63.7 ± 2.1^{a}
NN	−20 kPa	78.7 ± 0.6^{bc}	72.3 ± 0.6^{bc}	62.0 ± 0.0^{ab}
AN	−40 kPa	71.3 ± 1.2^{e}	66.3 ± 1.2^{e}	57.7 ± 0.6^{d}
50:50	−40 kPa	76.7 ± 0.6^{cd}	71.7 ± 0.6^{bc}	60.7 ± 0.6^{bc}
NN	−40 kPa	71.0 ± 1.0^{e}	63.7 ± 0.6^{f}	54.0 ± 1.0^{e}
2020				
AN	0 kPa	75.7 ± 0.6^{b}	70.7 ± 0.6^{d}	61.7 ± 0.6^{c}
50:50	0 kPa	76.0 ± 1.0^{b}	71.0 ± 1.0^{d}	62.0 ± 1.0^{c}
NN	0 kPa	71.3 ± 1.2^{c}	66.3 ± 1.2^{e}	57.3 ± 1.2^{d}
AN	−20 kPa	78.7 ± 1.5^{a}	73.7 ± 0.6^{b}	64.7 ± 0.6^{b}
50:50	−20 kPa	80.0 ± 0.0^{a}	76.0 ± 1.0^{a}	67.0 ± 1.0^{a}
NN	−20 kPa	78.7 ± 0.6^{a}	72.3 ± 0.6^{c}	62.7 ± 0.6^{c}
AN	−40 kPa	71.0 ± 1.0^{c}	66.0 ± 1.0^{e}	57.0 ± 1.0^{d}
50:50	−40 kPa	75.3 ± 0.6^{b}	70.3 ± 0.6^{d}	61.3 ± 0.6^{c}
NN	−40 kPa	68.3 ± 0.6^{d}	63.3 ± 0.6^{f}	54.3 ± 0.6^{e}
ANOVA				
Year (Y)		ns	ns	ns
Nitrogen form (N)		215.1**	243.4**	209.6**
Irrigation (I)		69.3**	112.6**	112.9**
Y × N		ns	ns	ns
Y × I		ns	ns	ns
N × I		14.1**	8.8**	6.6**

AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; −20 kPa – alternate wetting and moderate drying; −40 kPa – alternate wetting and severe drying. Values followed by different letters indicate statistical significance at $P < 0.05$ within the same column and year. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significance difference of F-values; ns – not significant difference

irrigation significantly increased amylose and protein content for both years.

In contrast to amylose content and protein content, gel consistency was the highest at 50:50, followed by NH_4^+-N, and the lowest was in NO_3^--N with the same irrigation method, especially in alternate wetting-and-drying irrigation (Table 5). Compared with SI, alternate wetting and moderate drying remarkably promoted gel consistency under the same nitrogen form by averages of 6.2% in 2019 and 5.3% in 2020, respectively. However, severe drying irrigation significantly decreased gel consistency by 6.4% in 2019 and 6.9% in 2020.

Starch synthase activity. Different irrigation regimes, nitrogen forms, and their interaction significantly affected the activity of the SS enzyme. The difference in AGPase activity between NH_4^+-N and 50:50 treatment exhibited no significance under SI (Table 6), whereas 50:50 treatment significantly increased AGPase activity by 6.5–11.3% (14 DAA) and 8.3–13.6% (28 DAA) in 2019 and 7.8–18.1% (14 DAA) and 13.5–17.8% (28 DAA) in 2020 under alternate wetting-and-drying irrigation method compared with NH_4^+-N treatment. Compared with SI, moderate-drying irrigation considerably elevated

https://doi.org/10.17221/530/2024-PSE

Table 4. Appearance quality of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation methods interactions

Treatment		Chalky rice	Chalk size	Chalk degree
		(%)		
2019				
AN	0 kPa	25.7 ± 0.6^{bcd}	27.0 ± 2.6^{de}	6.8 ± 0.5^{de}
50:50		22.3 ± 0.6^{e}	22.0 ± 1.5^{f}	4.8 ± 0.3^{f}
NN		27.0 ± 1.0^{b}	32.0 ± 1.3^{ab}	8.6 ± 0.4^{b}
AN	–20 kPa	24.0 ± 1.0^{d}	24.8 ± 1.0^{ef}	6.0 ± 0.2^{ef}
50:50		20.3 ± 1.2^{f}	18.5 ± 2.2^{g}	3.8 ± 0.3^{g}
NN		24.7 ± 0.6^{cd}	30.2 ± 1.0^{bc}	7.5 ± 0.4^{cd}
AN	–40 kPa	28.7 ± 1.2^{a}	28.7 ± 1.8^{cd}	8.2 ± 0.5^{bc}
50:50		26.3 ± 0.6^{bc}	24.3 ± 0.6^{f}	6.4 ± 0.1^{de}
NN		30.0 ± 1.0^{a}	32.8 ± 2.8^{a}	9.8 ± 1.1^{a}
2020				
AN	0 kPa	25.0 ± 0.6^{d}	27.0 ± 1.6^{d}	6.8 ± 0.7^{cd}
50:50		24.7 ± 1.2^{d}	26.4 ± 2.5^{de}	6.5 ± 0.8^{cd}
NN		27.0 ± 1.0^{c}	32.7 ± 1.0^{b}	8.8 ± 0.6^{b}
AN	–20 kPa	23.3 ± 0.6^{e}	23.8 ± 0.8^{e}	5.6 ± 0.1^{e}
50:50		19.7 ± 0.6^{f}	17.8 ± 2.0^{f}	3.5 ± 0.4^{f}
NN		24.7 ± 0.6^{d}	29.8 ± 0.8^{c}	7.4 ± 0.1^{c}
AN	–40 kPa	30.0 ± 1.0^{b}	29.7 ± 1.0^{c}	8.9 ± 0.5^{b}
50:50		26.7 ± 0.6^{c}	24.3 ± 0.6^{e}	6.5 ± 0.3^{d}
NN		33.7 ± 1.2^{a}	35.7 ± 0.6^{a}	12.0 ± 0.5^{a}
ANOVA				
Year (Y)		ns	ns	ns
Nitrogen form (N)		210.0**	46.3**	143.2**
Irrigation (I)		82.8**	112.4**	161.1**
Y × N		ns	ns	ns
Y × I		ns	ns	ns
N × I		8.3**	5.0**	10.5**

AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; –20 kPa – alternate wetting and moderate drying; –40 kPa – alternate wetting and severe drying. Values followed by different letters indicate statistical significance at $P < 0.05$ within the same column and year. *$P < 0.05$ and **$P < 0.01$ indicate significance difference of F-values; ns – not significant difference

AGPase activity at 14 DAA and 28 DAA for both years. Conversely, severe-drying irrigation significantly decreased AGPase activity by averages of 35.8% (14 DAA) and 23.6% (28 DAA) in 2019 and, 17.0% (14 DAA) and 29.6% (28 DAA) in 2020. The activity changes of SS and SBE were similar to those of AGPase activity (Table 6).

ATP activity in grains. ATP activity in grains exhibited no remarkable differences between NH_4^+-N and 50:50 under SI (Figure 2), whereas 50:50 significantly increased ATP activity in grains by 14.0–14.6% (14 DAA) and 16.6–28.8% (28 DAA) in 2019 and 9.0–12.6% (14 DAA) and 10.5–12.8% (28 DAA) in 2020 under alternate wetting-and-drying irrigation compared with NH_4^+-N treatment. ATP activity in grains dramatically declined in NO_3^--N compared with those in NH_4^+-N when the irrigation method was the same. Compared with SI, moderate drying remarkably improved ATP activity in grains under the same irrigation method. Conversely, severe drying significantly decreased ATP activity in grains by averages of 28.8% (14 DAA) and 32.7% (28 DAA) in 2019, as well as 40.3% (14 DAA) and 18.6% (28 DAA) in 2020.

Correlation analysis of starch synthase activity with grain yield and grain quality. As shown in

https://doi.org/10.17221/530/2024-PSE

Table 5. Cooking and nutritional quality of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation methods interactions

Treatment		Amylose content (%)	Gel consistency (mm)	Protein content (%)
2019				
AN	0 kPa	24.1 ± 0.5^{de}	86.1 ± 1.4^{c}	7.9 ± 0.3^{de}
50:50		24.6 ± 0.8^{d}	86.3 ± 0.9^{c}	8.0 ± 0.2^{d}
NN		26.4 ± 0.5^{b}	80.6 ± 1.0^{e}	8.7 ± 0.2^{b}
AN	–20 kPa	23.6 ± 0.5^{e}	88.4 ± 0.3^{b}	7.1 ± 0.1^{f}
50:50		22.6 ± 0.1^{f}	93.3 ± 1.1^{a}	6.8 ± 0.1^{g}
NN		25.6 ± 0.1^{c}	86.7 ± 2.7^{c}	7.8 ± 0.1^{e}
AN	–40 kPa	25.6 ± 0.6^{c}	78.9 ± 1.1^{e}	8.3 ± 0.1^{c}
50:50		24.8 ± 0.5^{d}	83.4 ± 2.4^{d}	8.0 ± 0.1^{d}
NN		27.2 ± 0.3^{a}	74.5 ± 4.6^{f}	9.2 ± 0.1^{a}
2020				
AN	0 kPa	24.9 ± 0.3^{d}	87.0 ± 1.7^{c}	7.9 ± 0.1^{e}
50:50		25.1 ± 0.1^{d}	87.3 ± 0.6^{c}	8.0 ± 0.1^{de}
NN		26.7 ± 0.1^{b}	80.8 ± 0.6^{f}	8.7 ± 0.1^{b}
AN	–20 kPa	23.8 ± 0.2^{e}	89.1 ± 0.1^{b}	7.4 ± 0.1^{g}
50:50		22.5 ± 0.1^{f}	93.1 ± 0.4^{a}	7.1 ± 0.1^{h}
NN		25.7 ± 0.1^{c}	85.3 ± 0.8^{d}	7.6 ± 0.1^{f}
AN	–40 kPa	25.8 ± 0.4^{c}	79.0 ± 1.5^{g}	8.5 ± 0.2^{c}
50:50		24.9 ± 0.1^{d}	82.8 ± 0.5^{e}	8.1 ± 0.1^{d}
NN		27.5 ± 0.5^{a}	74.9 ± 0.3^{h}	9.2 ± 0.2^{a}
ANOVA				
Year (Y)		ns	ns	ns
Nitrogen form (N)		167.7**	482.3**	1 450.9**
Irrigation (I)		223.5**	247.5**	678.4**
Y × N		ns	ns	ns
Y × I		ns	ns	ns
N × I		8.8**	3.4*	67.6**

AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; –20 kPa – alternate wetting and moderate drying; –40 kPa – alternate wetting and severe drying. Values followed by different letters indicate statistical significance at P < 0.05 within the same column and year. *P < 0.05 and **P < 0.01 indicate significance difference of F-values; ns – not significant difference

Figure 3, starch-metabolising enzyme activity showed a significant positive correlation with grain yield, brown rice, milled rice, head rice and gel consistency. Conversely, a negative correlation of starch-metabolising enzyme activity was observed with chalky rice, size, degree, amylose content, and protein content.

DISCUSSION

Effect of different irrigation methods and nitrogen-form interaction on grain yield and quality. Grain yield and quality of rice are influenced by complex interactions between genetic determinants and environmental variables, including genotype selection, thermal conditions, solar radiation patterns, water availability, fertilisation regimes, geographic location, and edaphic characteristics (Chen et al. 2024b). Most recent studies have extensively investigated the effect of alternate wetting-and-drying irrigation on water- and nitrogen-use efficiency, methane emission, heavy-metal residue, pest and disease infestation, and yield performance of rice (Carrijo et al. 2018, Abu-Ali et al. 2023, Vuciterna et al. 2024, Vicente et al. 2025). However, few studies have investigated the

https://doi.org/10.17221/530/2024-PSE

Table 6. Starch synthase activity of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation methods interactions

Treatment		AGPase activity (unit/grain/h)		SS activity (unit/grain/h)		SBE activity (unit/grain/min)	
		14 DAA	28 DAA	14 DAA	28 DAA	14 DAA	28 DAA
2019							
AN	0 kPa	5.5 ± 0.3^{c}	4.2 ± 0.3^{c}	8.3 ± 0.3^{bc}	5.1 ± 0.2^{bc}	6.6 ± 0.1^{b}	3.8 ± 0.2^{c}
50:50	0 kPa	5.4 ± 0.4^{c}	3.7 ± 0.2^{d}	7.9 ± 0.4^{cd}	5.0 ± 0.2^{cd}	6.5 ± 0.1^{bc}	3.7 ± 0.1^{cd}
NN	0 kPa	4.4 ± 0.1^{e}	3.1 ± 0.1^{e}	7.1 ± 0.1^{e}	4.4 ± 0.2^{e}	5.3 ± 0.4^{e}	2.8 ± 0.1^{e}
AN	−20 kPa	6.2 ± 0.2^{b}	4.8 ± 0.1^{b}	8.8 ± 0.1^{b}	5.3 ± 0.2^{b}	7.2 ± 0.1^{a}	4.3 ± 0.1^{b}
50:50	−20 kPa	6.6 ± 0.2^{a}	5.2 ± 0.2^{a}	9.5 ± 0.3^{a}	5.7 ± 0.1^{a}	7.5 ± 0.1^{a}	4.7 ± 0.1^{a}
NN	−20 kPa	4.8 ± 0.1^{d}	4.0 ± 0.3^{c}	7.4 ± 0.1^{de}	4.8 ± 0.1^{d}	6.3 ± 0.1^{c}	3.4 ± 0.1^{d}
AN	−40 kPa	3.3 ± 0.1^{g}	2.8 ± 0.1^{f}	6.5 ± 0.3^{f}	4.1 ± 0.1^{f}	5.2 ± 0.1^{e}	2.8 ± 0.1^{e}
50:50	−40 kPa	3.7 ± 0.2^{f}	3.3 ± 0.2^{e}	7.2 ± 0.1^{e}	4.8 ± 0.1^{d}	5.8 ± 0.2^{d}	3.5 ± 0.2^{d}
NN	−40 kPa	2.9 ± 0.3^{h}	2.3 ± 0.3^{g}	5.8 ± 0.5^{g}	3.7 ± 0.4^{g}	4.4 ± 0.2^{f}	2.4 ± 0.2^{f}
2020							
AN	0 kPa	5.3 ± 0.3^{cd}	4.0 ± 0.2^{cd}	8.3 ± 0.3^{c}	4.5 ± 0.2^{c}	6.4 ± 0.2^{bc}	3.8 ± 0.1^{c}
50:50	0 kPa	5.2 ± 0.4^{d}	3.7 ± 0.2^{d}	8.2 ± 0.4^{c}	4.6 ± 0.2^{bc}	6.2 ± 0.1^{c}	3.8 ± 0.2^{c}
NN	0 kPa	4.5 ± 0.2^{e}	3.1 ± 0.2^{e}	7.4 ± 0.2^{d}	3.9 ± 0.2^{d}	5.2 ± 0.1^{e}	2.4 ± 0.1^{g}
AN	−20 kPa	6.4 ± 0.2^{b}	4.8 ± 0.1^{b}	9.0 ± 0.2^{b}	4.9 ± 0.2^{b}	7.1 ± 0.1^{a}	4.3 ± 0.1^{b}
50:50	−20 kPa	6.9 ± 0.2^{a}	5.4 ± 0.3^{a}	9.9 ± 0.2^{a}	5.4 ± 0.2^{a}	7.4 ± 0.1^{a}	4.8 ± 0.2^{a}
NN	−20 kPa	5.6 ± 0.3^{c}	4.2 ± 0.2^{c}	7.9 ± 0.1^{c}	4.3 ± 0.1^{c}	6.6 ± 0.3^{b}	2.8 ± 0.1^{f}
AN	−40 kPa	4.0 ± 0.1^{f}	2.6 ± 0.2^{f}	6.6 ± 0.3^{e}	3.8 ± 0.1^{d}	4.8 ± 0.1^{f}	3.1 ± 0.1^{e}
50:50	−40 kPa	4.6 ± 0.2^{e}	3.1 ± 0.1^{e}	7.3 ± 0.2^{d}	4.4 ± 0.2^{c}	5.7 ± 0.2^{d}	3.5 ± 0.1^{d}
NN	−40 kPa	3.6 ± 0.4^{g}	2.0 ± 0.2^{g}	6.0 ± 0.4^{f}	3.2 ± 0.5^{e}	4.3 ± 0.1^{g}	2.4 ± 0.2^{g}
ANOVA							
Year (Y)		ns	ns	ns	ns	ns	ns
Nitrogen form (N)		324.5**	279.5**	102.8**	86.9**	257.6**	182.9**
Irrigation (I)		75.9**	74.1**	51.1**	58.2**	136.7**	154.2**
Y × N		ns	ns	ns	ns	ns	ns
Y × I		ns	ns	ns	ns	ns	ns
N × I		6.7**	7.3**	4.5*	4.5*	3.5*	10.6**

AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; −20 kPa – alternate wetting and moderate drying; −40 kPa – alternate wetting and severe drying. Values followed by different letters indicate statistical significance at $P < 0.05$ within the same column and year. *$P < 0.05$ and **$P < 0.01$ indicate significance difference of F-values; ns – not significant difference; AGPase – adenosine phosphate glucose pyrophosphorylase; SS – starch synthase; SBE – starch-branching enzyme; DAA – days after anthesis

effects of combined alternate wetting-and-drying irrigation and nitrogen-form application on rice grain yield and quality, the activity of starch-metabolising enzymes in the grain-filling stage, and their relationship. Zheng et al. (2018) reported that the grain yield of rice can be maximised under NO_3^--N application and water-saving irrigation in the black soil region of the Songnen Plain (Zheng et al. 2018). Our results showed that alternate wetting-and-moderate-drying irrigation interacted with 50:50 treatment, which was the best combination of this experiment, which significantly promoted the filled-grain rate and 1 000-grain weight and consequently increased rice yield. The underlying reasons can be elucidated as improvements in root morphology and root oxidation activity, enhancements in carbon and nitrogen metabolism, and elevations in the leaf photosynthesis rate have been demonstrated to contribute to an

https://doi.org/10.17221/530/2024-PSE

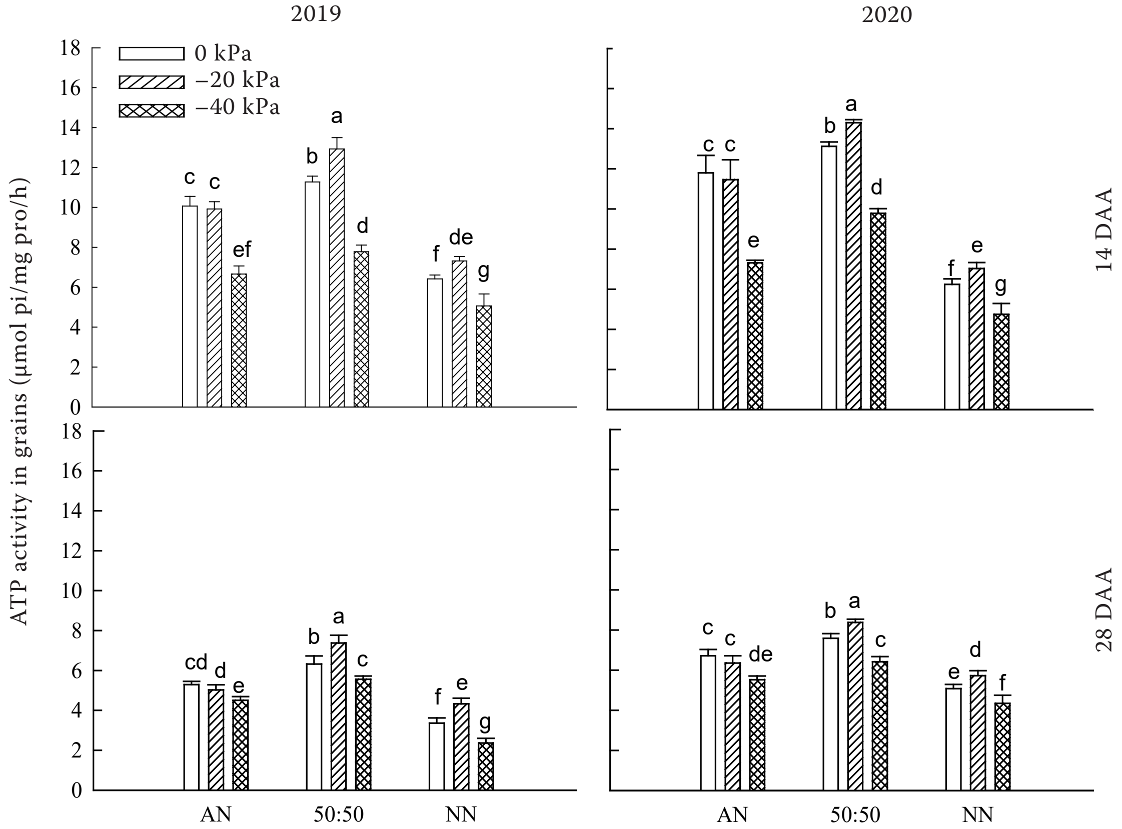

Figure 2. Adenosine triphosphate (ATP) content of a japonica cultivar Liangeng 7 at 14 DAA (days after anthesis) and 28 DAA under nitrogen form and irrigation method. AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; −20 kPa – alternate wetting and moderate drying; −40 kPa – alternate wetting and severe drying. Vertical bars represent ± standard error of the mean. Different letters of the column indicate statistical significance at $P < 0.05$ within the same stage and the same year

increase in grain yield (Xu et al. 2020). In addition, promoting leaf-source and grain-sink activity in rice also plays a crucial role in achieving the highest grain yield (Fei et al. 2024). Conversely, severe-drying irrigation interacted with NO_3^--N treatments significantly decreased rice yield due to each yield component's inhibition (Table 2). The present results were inconsistent with the conclusion of Zheng et al. (2018). The test soil of Zheng was acidic owing to the nitrogen-based urea as the solo nitrogen fertiliser over a long period of time. Therefore, they suggested that some alkaline fertilisers were more suitable for pH adjustment in that area. Our experiment was carried out in neutral soil; the ammonium-nitrate mixture can provide different nitrogen forms, better supporting the selective nitrogen absorption by roots and creating a more favourable rhizosphere environment (Figure 4) (Xu et al. 2020, Pereira et al. 2024). In addition, the ventilation environment and oxygen content in the soil were improved under alternate wetting-and-moderate-drying irrigation, and the relationship between aboveground and underground was more coordinated (Xu et al. 2020, Jiang et al. 2024). Consequently, rice's growth potential was maximised, contributing to increased yield (Figure 5).

Previous studies have shown that rice quality is predominantly governed by the genetic characteristics of different cultivars and by many environmental and cultivation factors, particularly nitrogen fertilisation regimes, planting density, and irrigation methods (Xu et al. 2020, Dou et al. 2024). Several studies have demonstrated that appropriate nitrogen applications can effectively reduce the chalkiness rate and chalkiness degree in rice grain and improve the appearance quality, milling quality, and taste quality of rice (Zhou et al. 2019, Dou et al. 2024). Most recent investigations have indicated that alternate wetting-and-moderate-soil-drying irrigation promotes the proportion of head rice and decreases chalkiness, protein, and prolamin content (Xu et al.

https://doi.org/10.17221/530/2024-PSE

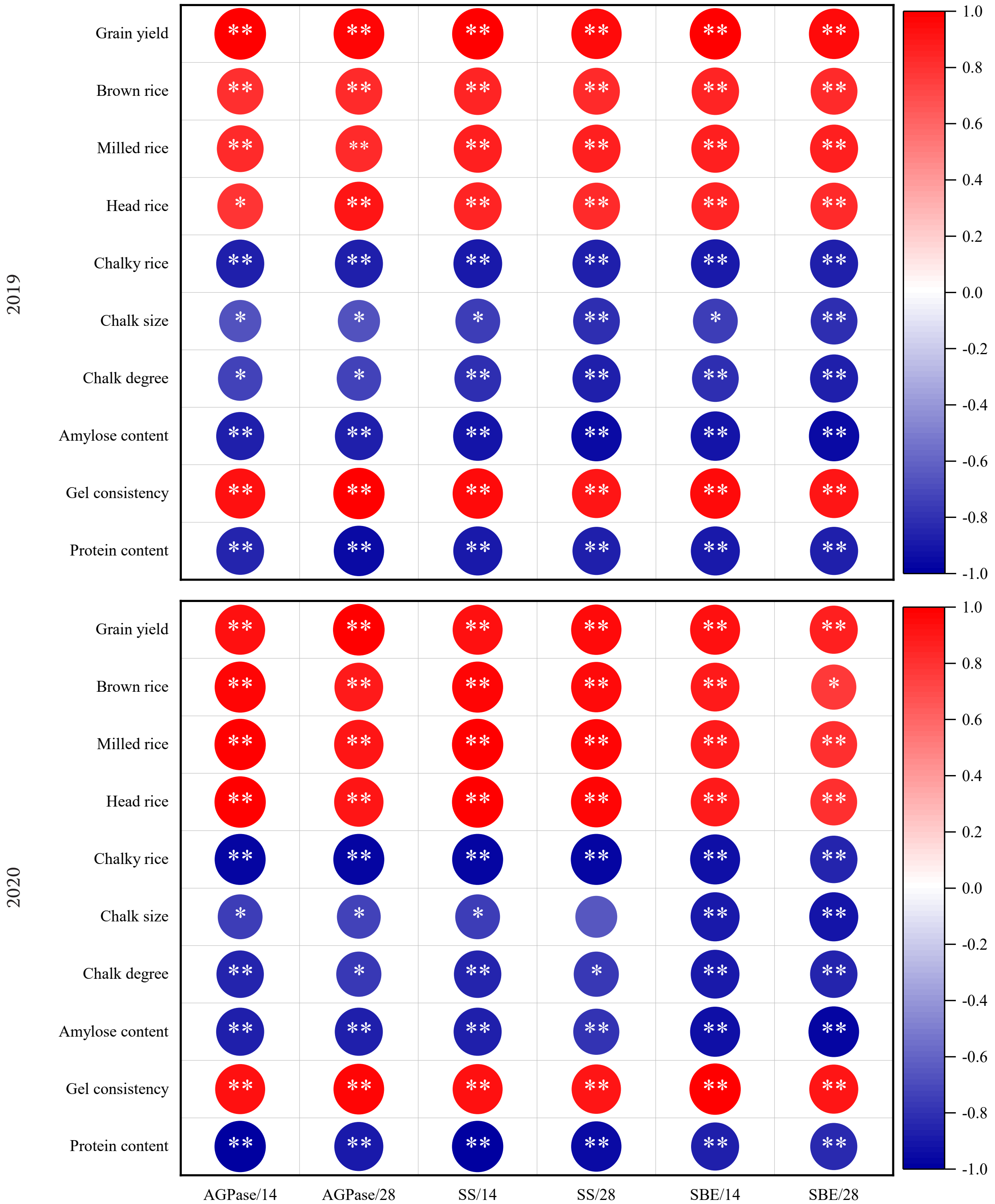

Figure 3. Correlation coefficients of grain yield and quality with starch metabolising enzyme activity at 14 DAA (days after anthesis) and 28 DAA of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation method interactions. AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; –20 kPa – alternate wetting and moderate drying; –40 kPa – alternate wetting and severe drying. *P < 0.05 and **P < 0.01 indicate significance difference of F-values. Red and blue means positive and negative correlation respectively. AGPase – adenosine phosphate glucose pyrophosphorylase; SS – starch synthase; SBE – starch-branching enzyme

https://doi.org/10.17221/530/2024-PSE

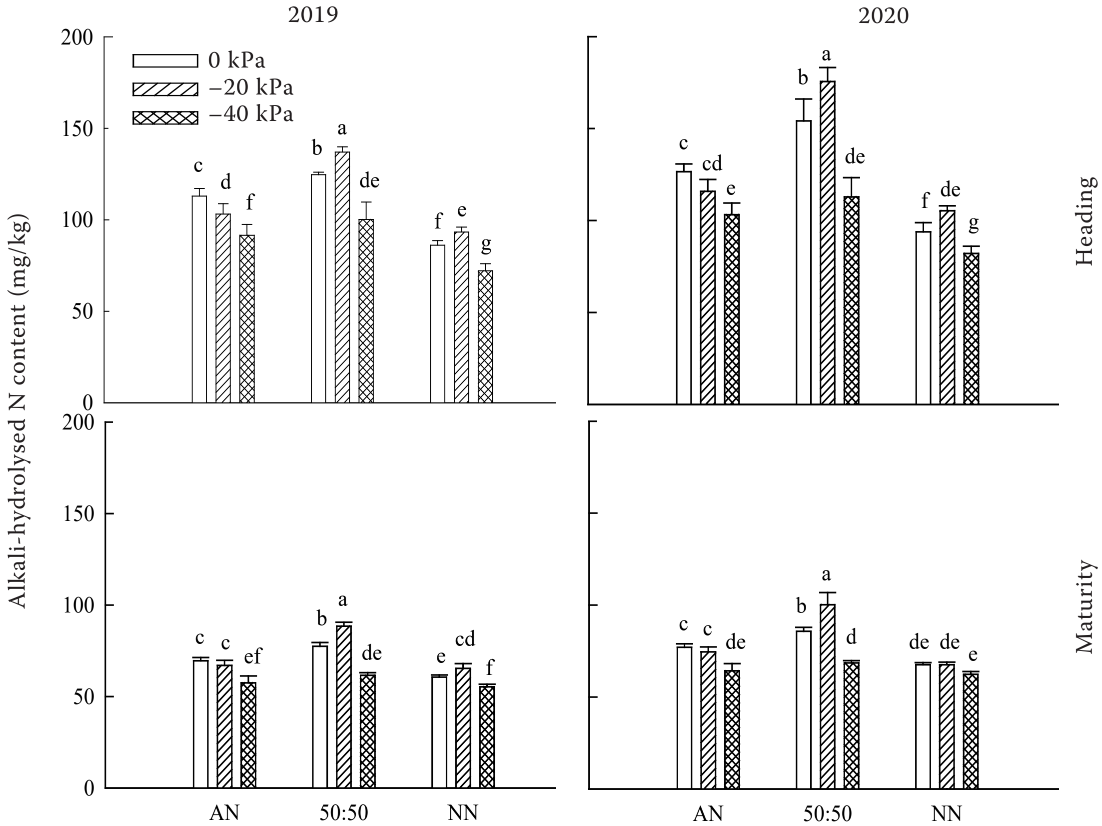

Figure 4. Alkali-hydrolysed nitrogen content in soil of a japonica cultivar Liangeng 7 at heading stage and maturity stage under nitrogen form and irrigation method. AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; −20 kPa – alternate wetting and moderate drying; −40 kPa – alternate wetting and severe drying. Vertical bars represent ± standard error of the mean. Different letters of the column indicate statistical significance at $P < 0.05$ within the same stage and the same year

2020, Zou et al. 2024). However, before the present study, little evidence exists on the grain quality of rice under alternate wetting-and-drying irrigation and nitrogen-form interaction. Our results indicated that alternate wetting and moderate drying interacted with 50:50 treatment and significantly improved rice quality. Specifically, it increased the proportion of head rice and gel consistency while simultaneously decreasing the chalkiness, amylose content, and protein content (Tables 3–5). The possible mechanism was that the enhancement of abscisic acid levels in the grains, the elevation of photosynthesis rate, and the inhibition of protein synthesis, achieved by improving the remobilisation of nutrients from vegetative tissues to the grains, all contribute to the improvement of rice grain quality (Ju et al. 2021). Additionally, a reduction in protein synthesis, particularly the content of glutelin and prolamin, and an increase in the long-chain amylopectin in starch can enhance the eating quality of japonica rice by improving the water absorption, expansion, and gelatinisation of starch particles (Ma et al. 2025). Notably, results demonstrated that NO_3^--N treatment decreased the proportion of head rice and gel consistency but increased chalkiness, amylose content, and protein content, regardless of irrigation method (Tables 3–5). This finding revealed that NO_3^--N application did not improve rice quality. The present results suggested that alternate wetting and moderate drying, that is, plants were reirrigated when soil water potential dropped to −20 kPa, interacted with 50:50 treatment represented a superior water- and fertiliser-management practice for enhancing both the grain yield and quality of rice.

Effect of different irrigation regimes and nitrogen-form interaction on starch-metabolising enzyme activity. SuS in the grains serves as a pivotal enzyme in catalysing the degradation of sucrose, and

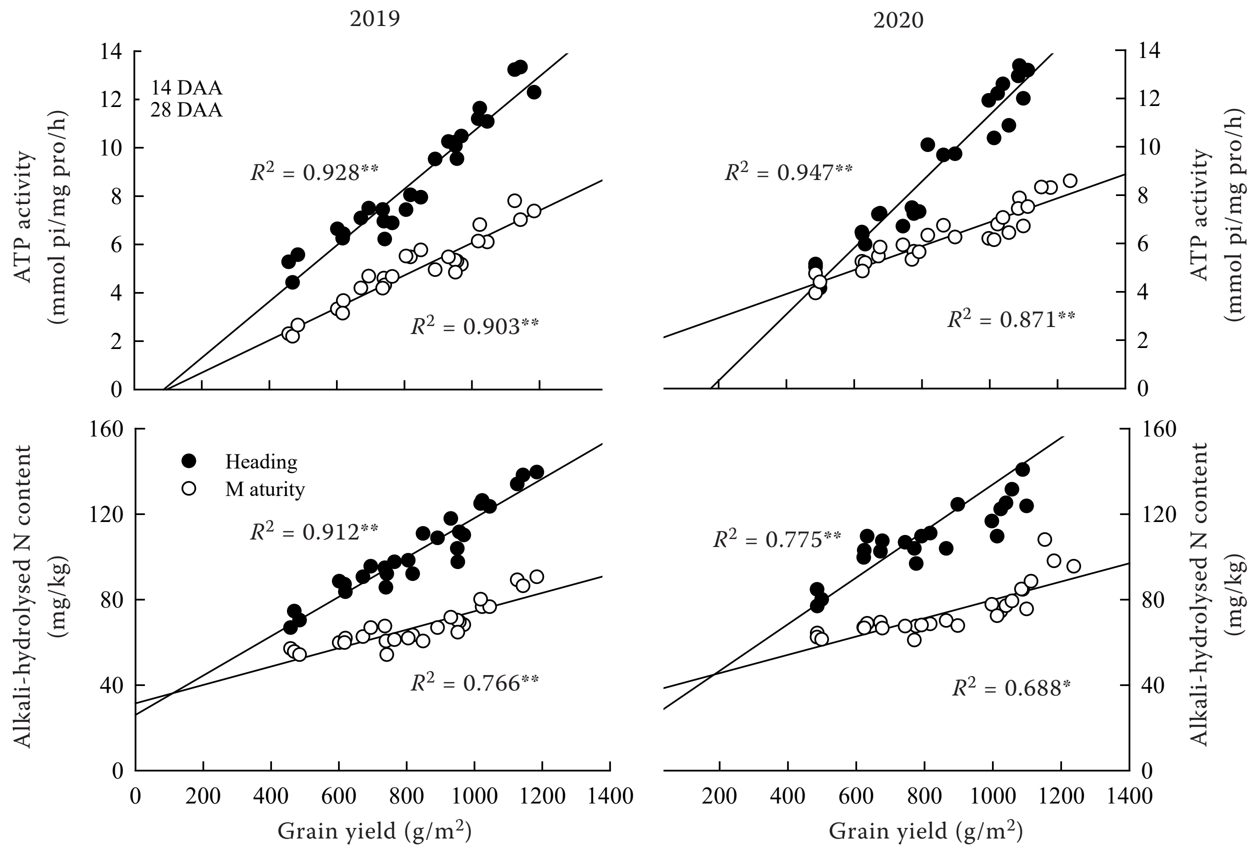

Figure 5. Correlation coefficients of grain yield with alkali-hydrolysed nitrogen and adenosine triphosphate (ATP) content of Liangeng 7 (japonica cultivar) under nitrogen form and irrigation method interactions. AN – ammonium nitrogen; 50:50 – ammonium nitrate mixed 50:50; NN – nitrate nitrogen; 0 kPa – submerged irrigation; –20 kPa – alternate wetting and moderate drying; –40 kPa – alternate wetting and severe drying. $^*P < 0.05$ and $^{**}P < 0.01$ indicate significance difference of *F*-values; DAA – days after anthesis

its activity is regarded as a crucial indicator of sink strength during the grain-filling stage. AGPase and SS are two key enzymes involved in the starch synthesis pathway, and their activities are intimately associated with the filling rate in developing rice grains (Iqbal et al. 2021, Yu et al. 2024). Previous research has shown that reducing nitrogen application or implementing mild water-deficit management can improve the activities of AGPase, SS, and SBE during the grain-filling stage. This enhancement contributes to an increase in grain weight by promoting the accumulation and remobilisation of starch (Iqbal et al. 2021, Wang et al. 2022). However, few studies have investigated the effects of combined alternate wetting-and-drying irrigation and nitrogen-form application on the activity of starch-metabolising enzymes in the grain-filling stage. In the present study, AGPase, SS, SBE and ATP enzyme activities in grains remarkably increased under alternate wetting-and-moderate-drying irrigation interacted with 50:50 treatment (Tables 6, Figure 2). These results were consistent with those reported by Yang et al. (2004), who also observed that the mild water deficits of rice and wheat cultivars improve starch accumulation and grain-filling rates by accelerating the remobilisation of stored reserves from stems to grains. The underlying mechanisms may be as follows: the activities of SuS and ATP were regarded as sink strength during the grain development of rice (Wei et al. 2023). Higher activities of these enzymes facilitate the transportation of assimilates from the stems to the grain sinks and enhance starch accumulation, which is beneficial for promoting grain filling and increasing grain weight. Previous studies have shown that ABA increased the activity of phloem cells of sink organs by stimulating ATP enzyme activity. This stimulation regulated the metabolism of assimilates in the cells, promoting the activities of key enzymes involved in the starch metabolism pathway (Wei et al. 2023). Moreover, the expres-

https://doi.org/10.17221/530/2024-PSE

sion levels of genes such as AGPS2b, SSS1, GBSS1, and GBSE11b were upregulated, thereby promoting the activities of key enzymes in a sucrose-starch metabolic pathway during the grain-filling period (Warwate et al. 2024). Notably, we observed that the activities of AGPase, SS, SBE, and ATP enzyme in grains decreased under severe-drying irrigation and NO_3^--N treatment (Table 6, Figure 2), which led to a reduction in starch synthesis and grain weight, ultimately resulting in a decrease in yield. This result indicated that the inability of the source to supply sufficient carbohydrates to the grain sink, which decreased the grain-filling rate and grain weight by regulating translocation efficiency, consequently contributed to lower grain yields (Aye et al. 2020, Wei et al. 2023). Therefore, increasing the application of ammonium nitrate mixed fertiliser during alternate wetting-and-drying irrigation can improve the activity of starch-metabolic enzymes, which is conducive to improving grain filling and increasing the yield level of the rice.

Relationship between grain quality and starch-metabolising enzyme activity. The activities of AGPase, SS, and SBE significantly influenced the rate and quantity of starch accumulation and played important roles in determining the quality of rice (Wei et al. 2023). Correlation analysis showed that the activities of starch-metabolic enzymes exhibited a significantly positive correlation with the milling quality of rice and gel consistency but remarkably negatively correlated with rice's appearance quality and amylose content (Figure 3). Our outcomes were also in agreement with Iqbal et al. (2021), who stated that starch-metabolising enzyme activity was significantly positively correlated with grain weight, milling quality, appearance quality, and the texture of rice. A previous study has shown that the genes associated with starch synthesis govern the quality of appearance and eating through the formation of an intricate regulatory network in combination with the activity of starch enzymes (Warwate et al. 2024). The present result showed that wetting-and-moderate-drying irrigation interacted with 50:50 treatment, which can improve the taste quality of rice by reducing the amylose content and increasing the gel consistency of rice (Figure 3). Generally, as the amylose content drops and the gel consistency extends, rice's breakdown value and peak viscosity tend to rise. In contrast, the setback value and peak time decline, leading to a remarkable improvement in the cooking quality of rice (Wei et al. 2024). Notably, the protein content in grains was significantly negative with the activities of starch-metabolic enzymes, and the overall quality of rice decreased remarkably (Figure 3). A possible mechanism could be that a high protein content was generally believed to inhibit the water absorption, expansion, and gelatinisation of starch particles, resulting in inferior eating quality of rice. Another important reason was that a decrease in starch biosynthesis rate, which affected cell metabolism and function, resulted in the formation of a chalk ring (Ma et al. 2025). Consequently, the overall quality of rice deteriorated. The results reminded us that selecting parents with high activity of starch-metabolising enzymes in grains holds promise for cultivating high-quality rice cultivars in production practice. Furthermore, regulating and improving the activities of the above enzymes in grains at the grain-filling stage through an appropriate irrigation method and nitrogen management can help improve rice grain and quality.

Acknowledgements. The authors gratefully acknowledge the contributions of the rice team lead in managing the experiment's field and collecting data. We also acknowledge the significant contribution of the reviewers through their constructive feedback.

REFERENCES

Ali L., Ullah S., Iqbal A., Quan Z., Liang H., Ahmad S., Muhammad I., Amanullah I., Guo Z.X., Wei S.Q. (2021): Combined application of biochar and nitrogen fertilizer promotes the activity of starch metabolism enzymes and the expression of related genes in rice in a dual cropping system. BMC Plant Biology, 21: 1–15.

Abu-Ali L., Maguffin S.C., Rohila J.S., McLung A.M., Reid M.C. (2023): Effects of alternate wetting and drying on oxyanion-forming and cationic trace elements in rice paddy soils: impacts on arsenic, cadmium, and micronutrients in rice. Environmental Geochemistry and Health, 45: 8135–8151.

Aye M.A., Thu C.N., Htwe N.M. (2020): Source-sink relationship and their influence on yield performance of YAU promising rice lines. Journal of Experimental Agriculture International, 42: 124–135.

Carrijo D.R., Akbar N., Reis A.F.B., Li C.Y., Gaudin A.C.M., Parikh S.J., Green P.G., Linquist B.A. (2018): Impacts of variable soil drying in alternate wetting and drying rice systems on yields, grain arsenic concentration and soil moisture dynamics. Field Crops Research, 222: 101–110.

Carrijo D.R., Lundy M.E., Linquist B.A. (2017): Rice yields and water use under alternate wetting and drying irrigation: a meta-analysis. Field Crops Research, 203: 173–180.

Chen B.P., Guo L.J., Tang J.C., Li Y.S., Li C.F. (2024a): Comprehensive impacts of different integrated rice-animal co-culture systems on rice yield, nitrogen fertilizer partial factor productivity

https://doi.org/10.17221/530/2024-PSE

and nitrogen losses: a global meta-analysis. Science of the Total Environment, 915: 169994.

Chen R.R., Li D.X., Fu J., Qin P., Zhang X.W., Sun Z.B., He K., Li L., Zhou W., Wang Y.J., Wang K., Liu X.M., Yang Y.Z. (2024b): Exploration of quality variation and stability of hybrid rice under multi-environments. Molecular Breeding, 44: 4.

Dou Z., Zhou Y.C., Zhang Y.Y., Guo W., Xu Q., Gao H. (2024): Influence of nitrogen applications during grain-filling stage on rice (*Oryza sativa* L.) yield and grain quality under high temperature. Agronomy, 14: 216.

Fei L.W., Pan Y.H., Ma H.L., Guo R.J., Wang M., Ling N., Shen Q.R., Guo S.W. (2024): Optimal organic-inorganic fertilization increases rice yield through source-sink balance during grain filling. Field Crops Research, 308: 109285.

Fu H.Y., Cui D.D., Shen H. (2021): Effects of nitrogen forms and application rates on nitrogen uptake, photosynthetic characteristics and yield of double-cropping rice in south China. Agronomy, 11: 158.

Gajda A.M., Czyz E.A., Stanek-Tarkowska J., Dexter A.R., Furtak K.M., Grzadziel J. (2017): Effects of long-term tillage practices on the quality of soil under winter wheat. Plant, Soil and Environment, 63: 236–242.

González A.F., Fang Z.T., Sen D., Henrich B., Nagashima Y., Sokolov A.V., Okumoto S., Verhoef A.J. (2024): *In-vivo* Raman microspectroscopy reveals differential nitrate concentration in different developmental zones in *Arabidopsis* roots. Plant Methods, 20: 185.

Guo J.X., Jia Y.M., Chen H.H., Zhang L.J., Yang J.C., Zhang J., Hu X.Y., Ye X., Li Y., Zhou Y. (2019): Growth, photosynthesis, and nutrient uptake in wheat are affected by differences in nitrogen levels and forms and potassium supply. Scientific Report, 9: 1248.

Hamoud Y.A., Shaghaleh H., Sheteiwy M., Guo X.P., Elshaikha N.A., Khan N.U., Oumarou A., Rahim S.F. (2019): Impact of alternative wetting and soil drying and soil clay content on the morphological and physiological traits of rice roots and their relationships to yield and nutrient use-efficiency. Agricultural Water Management, 223: 105706.

Hou D.P., Liu K., Liu S.K., Li J.C., Tan J.S., Bi Q.Y., Zhang A.N., Yu X.Q., Bi J.G., Luo L.J. (2024): Enhancing root physiology for increased yield in water-saving and drought-resistance rice with optimal irrigation and nitrogen. Frontiers in Plant Science, 15: 1370297.

Iqbal A., Xie H.M., He L., Ahmad S., Hussain I., Raza H., Khan A., Wei S.Q., Zhao Q., Wu K., Ali I., Jiang L.G. (2021): Partial substitution of organic nitrogen with synthetic nitrogen improves rice yield, grain starch metabolism and related gene expression under the dual cropping system. Saudi Journal of Biological Sciences, 28: 1283–1296.

Ishfaq M., Akbar N., Anjum S.A., Anwar-Ul-haq M. (2020): Growth, yield and water productivity of dry direct seeded and transplanted aromatic rice under different irrigation management regimes. Journal of Integrative. Agriculture, 19: 2656–2673.

Ishfaq M., Akbar N., Zulgiqar U., Ali N., Ahmad M., Anjum S.A., Farooq M. (2021): Influence of water management techniques on milling recovery, grain quality and mercury uptake in different rice production systems. Agricultural Water Management, 243: 106500.

Jiang Y., Tao W.L., Zhang W.Y., Wang Z.Q., Yang J.C. (2024): Wetting alternating with partial drying during grain filling increases lysine biosynthesis in inferior rice grain. The Crop Journal, 12: 262–270.

Ju C.X., Liu T., Sun C.M. (2021): Effect of controlled soil drying on the grain yield and nutritional quality of rice. Crop Science, 61: 1426–1435.

Li G.H., Hu Q.Q., Shi Y.G., Cui K.H., Nie L.X., Huang J.L., Peng S.B. (2018): Low nitrogen application enhances starch-metabolizing enzyme activity and improves accumulation and translocation of non-structural carbohydrates in rice stems. Frontiers in Plant Science, 9: 1128.

Ma Z.T., Chen X., Cao J.L., Yu J.H., Zhu Y., Liu G.D., Xu F.F., Hu Q., Zhang H.C., Li G.Y., Wei H.Y. (2025): Carbon and nitrogen metabolism effects on eating quality in grains of diverse *Japonica* rice cultivars from the middle and lower Yangtze river. Food and Energy Security, 14: e70051.

Maneepitak S., Ullah H., Paothong K., Kachenchart B., Datta A., Shrestha R.P. (2019): Effect of water and rice straw management practices on yield and water productivity of irrigated lowland rice in the central plain of Thailand. Agricultural Water Management, 211: 89–97.

Mi W.H., Hong Y., Gao F., Ma Y.Y., Sun T., Wu L.H., Wang G.Z., Chen S.T. (2024): Effect of different form of N fertilization on yield sustainability and soil quality in double cropped rice system in a long-term experiment. Journal of Soil Science and Plant Nutrition, 2: 2815–2824.

Nakamura Y., Yuki K., Park S.Y. (1989): Carbohydrate metabolism in the developing endosperm of rice grains. Plant Cell Physiology, 30: 833–839.

Pereira E.G., Santos L.A., Chapeta A.C., Ribeiro Y.D., Santa-Catarina C., Bucher C.P., Bucher C.A., Garcia A.C., Fernandes M.S. (2024): Disruption of amino acid transporter *OsAAP1* impairs rice seedling establishment and nitrate uptake and assimilation. Journal of Plant Growth Regulation, 43: 2841–2854.

Prasad R., Shivay Y.S., Kumar D. (2017): Current Status, Challenges, and Opportunities in Rice Production. Rice Production Worldwide. New York, Springer.

Ranwala A.P., Miller W.B. (1998): Sucrose-cleaving enzymes and carbohydrate pools in *Lilium longiflorum* floral organs. Physiologia Plantarum, 103: 541–550.

Vicente L., Alvarenga P., Pena D., Fernandez D., Albarran A., Rato-Nunes J.M., Lopez-Pineiro A. (2025): Controlling As, Cd, and Pb bioaccumulation in rice under different levels of alternate wetting and drying irrigation with biochar amendment: a 3-year field study. Chemosphere, 372: 144114.

Vuciterna R., Ruggeri G., Corsi S., Facchi A., Gharsallah D. (2024): A bibliometric analysis of scientific literature on alternate wetting and drying (AWD). Paddy and Water Management, 22: 415–430.

https://doi.org/10.17221/530/2024-PSE

Wand P., Wang Z.K., Sun X.C., Mu X.H., Chen H., Chen F.J., Ye L.X., Mi G.H. (2019): Interaction effect of nitrogen form and planting density on plant growth and nutrient uptake in maize seedlings. Journal of Integrative Agriculture, 18: 1120–1129.

Wang Z.Q., Zhang W.Y., Beebout S.S., Zhang H., Liu L.J., Yang J.C., Zhang J.H. (2016): Grain yield, water and nitrogen use efficiencies of rice as influenced by irrigation regimes and their interaction with nitrogen rates. Field Crops Research, 193: 54–69.

Wang J.Z., Zhang J.B., Zhao W., Müller C., Cai Z.C. (2017): Nitrification is the key process determining N use efficiency in paddy soils. Journal of Plant Nutrition and Soil Science, 180: 648–658.

Wang X.P., Fu J.X., Min Z.S., Zou D.T., Liu H.L., Wang J.G., Zheng H.L., Jia Y., Yang L.M., Xin W., Sun B., Zhao H.W. (2022): Response of rice with overlapping growth stages to water stress by assimilates accumulation and transport and starch synthesis of superior and inferior grains. International Journal of Molecular Science, 23: 11157.

Warwate S.I., Awana M., Thakare S.S., Krishnan V., Kumar S., Bollinedi H., Arora A., Sevanthi A.M., Ray M., Praveen S., Sinth A. (2024): Exploring the synergy of enzymes, nutrients, and gene networks in rice starch granule biogenesis. Frontiers in Nutrition, 11: 1448450.

Wei C.H., Jiang J.J., Liu C., Fang X.C., Zhou T.Y., Xue Z.Y., Wang W.L., Zhang W.Y., Zhang H., Liu L.J., Wang Z.Q., Gu J.F., Yang J.C. (2023): Effects of source strength and sink size on starch metabolism, starch properties and grain quality of rice (*Oryza sativa* L.). Agronomy, 13: 1288.

Wei X.D., Zhang Y.D., Song X.M., Zhao L., Zhao Q.Y., Chen T., Lu K., Zhu Z., Huang S.D., Wang C.L. (2024): Silicon and zinc fertilizer application improves grain quality and aroma in the *japonica* rice variety Nanjing 46. Foods, 13: 152.

Xu G.W., Lu D.K., Wang H.Z., Li Y.J. (2018): Morphological and physiological traits of rice roots and their relationships to yield and nitrogen utilization as influenced by irrigation regime and nitrogen rate. Agricultural Water Management, 203: 385–394.

Xu Y.J., Gu D.J., Li K., Zhang W.Y. (2019): Response of grain quality to alternate wetting and moderate soil drying irrigation in rice. Crop Science, 59: 1261.

Xu G.W., Jiang M.M., Lu D.K., Wang H.Z., Chen M.C. (2020): Nitrogen forms affect the root characteristic, photosynthesis, grain yield, and nitrogen use efficiency of rice under different irrigation regimes. Crop Science, 60: 2594–2610.

Xu G.W., Zhao X.H., Jiang M.M., Lu D.K., Chen M.C. (2021): Nitrogen forms and irrigation regimes interact to affect rice yield by regulating the source and sink characteristics. Agronomy Journal, 113: 4022–4036.

Yang J.C., Zhang J.H., Wang Z.Q., Zhu Q.S., Liu L.J. (2003): Activities of enzymes involved in sucrose-to-starch metabolism in rice grains subjected to water stress during filling. Field Crops Research, 81: 69–81.

Yang J.C., Zhang J.H., Wang Z.Q., Xu G.W., Zhu Q.S. (2004): Activities of key enzymes in sucrose-to-starch conversion in wheat grains subjected to water deficit during grain filling. Plant Physiology, 135: 1621–1629.

Yang J.C., Zhou Q., Zhang J.H. (2017): Moderate wetting and drying increases rice yield and reduces water use, grain arsenic level, and methane emission. The Crop Journal, 5: 151–153.

Yang X.X., Rasheed A., Yan H., Zhou C.H., Shang Q.Y. (2018): Effects of nitrogen forms and osmotic stress on root aerenchyma formation of japonica rice and its relationship with water absorption. International Journal of Agricultural Biology, 20: 2287–2292.

Yi J., Gao J.P., Zhang W.Z., Zhao C., Wang Y., Zhen X.X. (2019): Differential uptake and utilization of two forms of nitrogen in japonica rice cultivars from north-eastern China. Frontier in Plant Science, 4: 1061.

Yu T., Xin Y.N., Liu P. (2024): Exogenous abscisic acid (ABA) improves the filling process of maize grains at different ear positions by promoting starch accumulation and regulating hormone levels under high planting density. BMC Plant Biology, 24: 80.

Zaghdoud C., Carvajal M., Ferchichi A., Martínez-Ballesta M. (2016): Water balance and N-metabolism in broccoli (*Brassica oleracea* L. var. *Italica*) plants depending on nitrogen source under salt stress and elevated CO_2. Science of the Total Environment, 571: 763–771.

Zhang W.Y., Yu J.X., Xu Y.J., Wang Z.Q., Liu L.J., Zhang H., Gu J.F., Zhang H., Zhang J.H., Yang J.C. (2021): Alternate wetting and drying irrigation combined with the proportion of polymer-coated urea and conventional urea rates increases grain yield, water and nitrogen use efficiencies in rice. Field Crops Research, 268: 108165.

Zheng E.N., Yang H., Zhang Z.X. (2018): Influence of different nitrogen forms application on rice photosynthesis: fluorescence with water-saving irrigation in black soil region of Songneng Plain, Northeast China. Paddy and Water Environment, 16: 795–804.

Zhou C.C., Huang Y.C., Jia B.Y., Wang Y., Li R.F., Wang S., Feng Y., Dou F. (2019): Effect of interaction between nitrogen rate and irrigation regime on grain quality of japonica rice in northeast China. Chinese Journal of Rice Science, 33: 357–367.

Zou J.N., Pang Z.Q., Li Z., Guo C.L., Lin H.M., Li Z., Chen H.F., Huang J.W., Chen T., Xu H.L., Qin B., Letuma P., Lin W.W., Lin W.X. (2024): The underlying mechanism of variety-water-nitrogen-stubble damage interactions on yield formation in ratoon rice with low stubble height under mechanized harvesting. Journal of Integrative Agriculture, 23: 806–823.

Received: October 3, 2024
Accepted: March 10, 2025
Published online: March 14, 2025