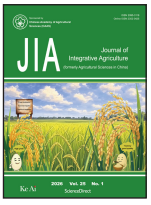
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**Journal of Integrative Agriculture**



RESEARCH ARTICLE

# Alternate wetting and moderate drying irrigation improves rice cooking and eating quality by optimizing lipid and fatty acid synthesis in grains

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**Highlights**

- Alternate wetting and moderate drying irrigation (AWMD) regime significantly increased contents of lipid, TFFAs, FUFAs, linoleic acid and oleic acid in milled rice.
- Elevated levels of lipid, TFFAs, FUFAs, linoleic acid, and oleic acid contributed more favorably to enhanced rice cooking and eating quality.
- The AWMD improved rice cooking and eating quality through optimization of lipid and free fatty acid biosynthesis in rice grains.

**Abstract**

Alternate wetting and drying irrigation (AWD) significantly influences the cooking and eating quality of rice (*Oryza sativa* L.). However, the mechanisms by which AWD affects rice cooking and eating quality remain unclear. Lipid and free fatty acid contents in grains correlate positively with cooking and eating quality of rice. This study examined Yangdao 6 (YD6, a conventional taste *indica* inbred) and Nanjing 9108 (NJ9108, a superior taste *japonica* inbred) cultivated under conventional irrigation (CI), alternate wetting and moderate drying irrigation (AWMD), and alternate wetting and severe drying irrigation (AWSD) from 10 days after transplanting to maturity. The research investigated the relationship between lipid and free fatty acid biosynthesis in grains and the cooking and eating quality of rice. Compared to CI treatment, AWMD significantly enhanced the contents of lipid, total free fatty acids (TFFAs), free unsaturated fatty acids (FUFAs), linoleic acid, and oleic acid in milled rice by increasing activities of enzymes associated with lipid synthesis, while AWSD produced opposite effects. Correlation analysis revealed that elevated levels of lipid, TFFAs, FUFAs, linoleic acid, and oleic acid contribute to improved rice cooking and eating quality. The findings demonstrate that AWMD enhances cooking and eating quality of milled rice through optimization of lipid and fatty acid synthesis in rice grains.

**Keywords:** rice (*Oryza sativa* L.), cooking and eating quality, lipid content, fatty acid content, alternate wetting and drying irrigation

## 1. Introduction

Rice (*Oryza sativa* L.) ranks among the three major food crops globally, providing sustenance for more than half of the world's population (Bhullar and Gruissem 2013; Rong *et al.* 2021). As rice production advances and living standards improve, rice quality has become increasingly significant (Zhou *et al.* 2020). Plant breeders focus on developing rice varieties with superior appearance, nutritional value, and taste characteristics (Lau *et al.* 2016; Gong *et al.* 2023; Sailimuhan *et al.* 2023). Among quality traits, cooking and eating quality significantly influences consumer preferences and market value (Lau *et al.* 2016; Sultana *et al.* 2022). Currently, rice eating and cooking quality assessment

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relies on starch physicochemical properties and cooked rice sensory characteristics (Gu *et al.* 2015; Xu *et al.* 2018; Ding *et al.* 2023). Beyond varietal breeding, cultivation practices, including irrigation and fertilizer management, play essential roles in enhancing rice cooking and eating quality (Ashraf *et al.* 2022; Peng *et al.* 2022; Chen *et al.* 2023).

In irrigation management, alternate wetting and drying irrigation (AWD), a water-saving technique, significantly affects rice eating and cooking quality formation (Yang *et al.* 2005; Graham-Acquaah *et al.* 2019; Zhao *et al.* 2021). Research indicates that alternate wetting and moderate drying irrigation (AWMD) enhances rice eating and cooking quality by improving starch physicochemical properties and cooked rice sensory characteristics, while alternate wetting and severe drying irrigation (AWSD) demonstrates contrary effects (Yang *et al.* 2005; Xu *et al.* 2019; Zhao *et al.* 2021). Studies have established that starch, protein, and lipid constitute the three primary components in rice grains and serve as key determinants of rice quality (Zhou *et al.* 2002; Guo *et al.* 2023). Previous research demonstrates that AWMD increases amylopectin and total starch levels, as well as the quantity and volume percentage of medium starch granules in milled rice, without significantly affecting amylose content (Xu *et al.* 2019; Zhang *et al.* 2020). By comparison, research regarding the effects of AWD regimes on milled rice protein remains limited. While a research found no significant difference in total protein content between AWD irrigation and continuous flooding (Song *et al.* 2021), our previous study observed that AWMD reduced total protein and amino acid contents in milled rice (Xu *et al.* 2019), potentially due to variations in soil dryness, soil type, and rice variety. These findings suggest that different AWD regimes can influence starch and protein synthesis in rice grains. Nevertheless, studies examining the effects of different AWD regimes on lipid dynamics in rice grains remain scarce.

In rice grains, starch (80–85%) and protein (7–12%) are major components, lipid accounts for a relatively minor proportion with approximately 0.3–3% (Zhou *et al.* 2002; Kim *et al.* 2015). The distribution of rice lipids occurs primarily in the embryo, followed by the aleurone layer and endosperm (Resurreccion *et al.* 1979). These lipids can be categorized into six groups: glycerophospholipids, sphingolipids, glycerolipids, fatty acyls, sterol lipids, and prenol lipids (Concepcion *et al.* 2020). Research indicates that lipid content correlates strongly with rice luster and eating quality, with superior cooking and eating quality varieties typically containing higher lipid level in milled rice (Yoon *et al.* 2012a; Wu *et al.* 2020). Additionally, free fatty acid levels during rice storage positively correlate with fragrance and overall rice quality (Concepcion *et al.* 2020; Wang *et al.* 2020). These findings suggest that elevated levels of lipid and free fatty acids enhance rice taste quality. However, the influence of AWMD regime on lipid and fatty acid biosynthesis in rice grains remains largely unexplored, as does its potential role in improving rice cooking and eating quality through modifications in lipid and fatty acid content.

In higher plants, lipid biosynthesis occurs through the catalytic action of several key enzymes, mainly including acetyl-CoA carboxylase (ACCase), fatty acid synthase (FAS), stearate dehydrogenase (SAD), fatty acid dehydrogenase (FAD), glycerol 3-phosphate dehydrogenase (G3PD), phosphatidate phosphatase (PPase), and diacylglycerol acyltransferase (DGAT) (Sidorov and Tsydendambaev 2014; Chen *et al.* 2017). These enzymes play crucial roles in the fat or triglyceride (TAG) synthesis (Chen *et al.* 2017), with the related biosynthetic pathway illustrated in Appendix A. Research demonstrates that increased phosphorus or potassium application rates can enhance fatty acid compositions and elevate lipid content and eating quality of rice through improved enzymatic activities related to lipid synthesis in rice grains (Peng *et al.* 2022; Chen *et al.* 2023). Similarly, AWMD regime may potentially enhance these key enzyme activities to improve fatty acid compositions and lipid content in rice grains.

The aim of this study was to examine the hypothesis that an AWMD regime can improve rice cooking and eating quality by optimizing lipid and fatty acid synthesis in grains. In our study, the enzymatic activities related to lipid synthesis in filling grains, contents of lipid and diverse free fatty acids in milled rice, and rice cooking and eating quality were monitored.

## 2. Materials and methods

### 2.1. Experimental materials and planting conditions

Two varieties differing in taste quality, Yangdao 6 (YD6, a conventional taste *indica* inbred) and Nanjing 9108 (NJ9108, a superior taste *japonica* inbred), were chosen as experimental materials. This study was conducted in soil pond on the farm of Yangzhou University, located in Jiangsu Province, China (32°30′N, 119°25′E).

The experiment was conducted during two rice planting seasons of 2021 and 2022. Rice seeds were sown in a seedling nursery on 17 May, 2021 and 15 May, 2022, respectively, and young seedlings were transplanted into the soil pond on 16 June. The soil pond contained sandy loam soil with 106.2 and 105.1 mg $kg^{-1}$ alkali-hydrolyzable nitrogen, 22.5 and 24.0 mg $kg^{-1}$ available phosphate, 77.2 and 78.8 mg $kg^{-1}$ exchangeable potassium, and 22.9 and 19.8 g $kg^{-1}$ organic matter, in 2021 and 2022, respectively. The cultivation density was 13.3 cm×30 cm with two rice seedlings per hill. Nitrogen (240 kg N $ha^{-1}$) in urea form was applied in three split applications, with 50% as basal fertilizer, 20% as tillering fertilizer, and 30% as panicle fertilizer. Additionally, both of calcium superphosphate ($P_2O_5$, 13.5%) and potassium chloride ($K_2O$, 62%) were used as the basal fertilizer, at the rate of 400 and 160 kg $ha^{-1}$, respectively. Weeds were removed mainly by hands, and pests and diseases were prevented by spraying pesticides.

### 2.2. Irrigation regime

Three irrigation regimes were implemented in the study: conventional irrigation (CI), AWMD, and AWSD. The CI treatment maintained a 2–3 cm water level above ground until 1 wk before harvest, except for drainage at the late tillering stage. The AWMD and AWSD regimes were regulated according to soil water potential (SWP). In the AWMD regime, rice plants received re-flooding when SWP reached

–15 kPa (soil water content 0.170 g $g^{-1}$) at 15–20 cm soil depth, beginning 10 days after transplanting. For the AWSD regime, re-flooding occurred when SWP reached –35 kPa (0.153 g $g^{-1}$) at 15–20 cm soil depth, starting 10 days after transplanting. A removable rain shelter was installed above the soil pond for rain protection. Field irrigation utilized manually controlled tap water. SWP monitoring in each plot employed soil moisture tension meters (Nanjing Institute of Soil Science, Chinese Academy of Sciences, China). The field experiment followed a complete randomized block design, with three replicate trial plots per experimental treatment and plot dimensions of 3.2 m×4.5 m. Cement ridges separated experimental plots to prevent water and fertilizer runoff. The specific SWP changes for both rice varieties under different irrigation regimes are presented in Appendix B.

### 2.3. Sampling

For each experimental treatment, 600 panicles that flowered simultaneously were tagged (200 panicles per plot). Forty labeled rice panicles from each treatment were collected at 6-day intervals from 12 days post-anthesis to maturity. Grains were sampled from middle branches of rice panicles as previously described (Xu *et al.* 2021), then fixed in liquid nitrogen and stored at –75°C for enzymatic activity analysis. At maturity, 120 tagged rice panicles were selected, and their middle grains were naturally dried to 14% water content in a cool, ventilated environment to measure lipid and free fatty acid contents in milled rice.

At harvest, rice plants from a 1 $m^2$ area (excluding border plants) were collected to assess theoretical grain yield and its components, with four replications. After 3 months of storage, the mature grains underwent evaluation for cooking and eating quality.

### 2.4. Analysis of the enzymatic activity in lipid synthesis in filling grains

Rice grains sampled during the filling stage were hulled in an ice bath, and 1.0 g rice kernels underwent thorough grinding in a high-throughput tissue grinder. Subsequently, 10 mL phosphate buffer (pH 7.4) was added and swirled for 10 min at low temperature. After 5 min on ice, the extraction solution underwent centrifugation at 10, 000 r $min^{-1}$ for 20 min, and the supernatant was collected for enzyme activity determination. The activities of lipid synthesis enzymes including ACCase, FAS, SAD, FAD, G3PD, PPase, and DGAT in rice grains were analyzed using corresponding enzyme-linked immunoassay kits from Shanghai Tongwei Biotechnology Co., Ltd. (China).

### 2.5. Measurement of lipid and free fatty acid contents in milled rice

Lipid extraction from milled rice utilized the ultrasound-assisted extraction (UAE) method, with lipid content analysis performed according to previously described methods (Tu *et al.* 2020; Peng *et al.* 2022; Chen *et al.* 2023). BioNovoGene Co., Ltd. (Suzhou, China) conducted free fatty acid detection. Individual free fatty acid composition analysis employed gas chromatography-mass spectrometry, utilizing working standard solutions of 51 fatty acid compositions. Total free fatty acids (TFFAs) were categorized into free saturated fatty acids (FSFAs) and free unsaturated fatty acids (FUFAs) based on the presence of double bonds.

### 2.6. Evaluation of rice cooking and eating quality

Milled rice was pulverized and sieved through a 100-mesh screen for cooking quality analysis (starch viscosity properties). The primary cooking quality parameters included peak viscosity (PV), hot viscosity (HV), final viscosity (FV), setback (SB), breakdown (BD), and gelatinization temperature (GT) of rice starch. These parameters were measured using a super rapid viscosity analyzer as described in our previous study (Xu *et al.* 2019). Cooked rice eating quality assessment (including taste value and values of appearance, hardness, viscosity, and balance) utilized a rice sensory analyzer following earlier reports (Zhang *et al.* 2019).

### 2.7. Statistical analysis

Statistical analyses, including variance, regression, and correlation, were conducted using SPSS 16.0 statistical software (SPSS Institute Inc., Chicago, USA). The means were evaluated using the least significant difference at $P$=0.05 ($LSD_{0.05}$). Regression analysis examined the relationships between enzymatic activities and levels of lipid and free fatty acids. Correlation analysis evaluated the relationship between lipid and free fatty acid contents in milled rice and its cooking and eating properties.

## 3. Results

### 3.1. Rice yield and yield components

Compared to CI treatment, the AWMD regime significantly increased grain yield of both test varieties across both years, while the AWSD regime substantially decreased grain yield (Appendix C). Relative to CI, both AWMD and AWSD regimes reduced panicle number in both rice varieties, though AWMD regime notably enhanced spikelets per panicle and filled grain percentage. The reduction in grain yield under AWSD regime primarily resulted from decreases in spikelets per panicle, grain weight, and filled grain percentage (Appendix C). Grain yield and its components demonstrated similar patterns of change among irrigation regimes between study years (Appendix C). Additionally, yields of both rice varieties were notably lower in 2022 compared to 2021 (Appendix C). This study observed exceptionally high daily temperatures (particularly daily maximum temperatures exceeding 40°C) and extended heat duration during flowering and early grain-filling stages (August), coupled with low temperature and reduced sunshine hours during late grain filling (September to October) in 2022 (Appendices D–F), potentially explaining the substantial decreases in grain yield and filled grain percentage for both varieties.

### 3.2. Lipid content in milled rice

In comparison with CI regime, AWMD significantly enhanced lipid content in milled rice of both varieties, while AWSD regime notably reduced lipid content (Fig. 1). Calculations revealed lipid content increases of 43.05–52.76% in YD6 (Fig. 1-A

and C) and 30.63–38.64% in NJ9108 (Fig. 1-B and D) under AWMD treatment compared to CI. Lipid content decreased by 57.04–57.47% in YD6 (Fig. 1-A and C) and 36.26–45.45% in NJ9108 (Fig. 1-B and D) under AWSD treatment relative to CI. These findings indicate that YD6's lipid content exhibits greater sensitivity to irrigation treatment than NJ9108. Furthermore, between the two varieties, NJ9108 demonstrated higher milled rice lipid levels than YD6 (Fig. 1).

### 3.3. Free fatty acid content in milled rice

The study identified 51 distinct free fatty acids in milled rice of both varieties (Table 1). The contents of various fatty acid compositions differed substantially, with marked variations between rice varieties (Table 1). YD6 exhibited the highest content of free linoleic acid (985.4–1,248 μg $g^{-1}$ DW), followed by oleic acid (790.2–915.9 μg $g^{-1}$ DW), while NJ9108 showed the highest content of oleic acid (2,100–2,506 μg $g^{-1}$ DW), followed by linoleic acid (1,105–1,378 μg $g^{-1}$ DW) (Table 1). The next four most abundant fatty acid compositions were palmitic acid, stearic acid, petroselic acid, and vaccenic acid, respectively (Table 1). These main free fatty acids (comprising the above six fatty acids) constituted approximately 90% of total free fatty acids. Different irrigation regimes influenced the contents of various fatty acid compositions (Table 1). Analysis of main fatty acid composition changes revealed that compared to CI regime, AWMD increased linoleic and oleic acid contents while reducing palmitic and stearic acid contents in milled rice, whereas AWSD produced opposite effects (Table 1).

AWMD regime significantly elevated TFFAs and FUFAs levels in milled rice compared to CI, while AWSD increased FSFAs content (Fig. 2). The levels of TFFAs, FSFAs and FUFAs in NJ9108 milled rice were significantly higher than those in YD6 (Fig. 2).

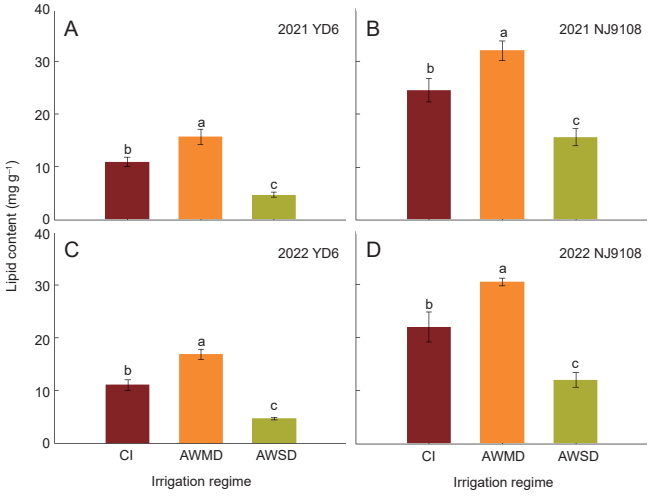



**Fig. 1 Effects of irrigation regimes on lipid content in milled rice across two rice varieties.** CI, conventional irrigation; AWMD, alternate wetting and moderate drying irrigation; AWSD, alternate wetting and severe drying irrigation. YD6, Yangdao 6; NJ9108, Nanjing 9108). Data are represented as the mean±SE ($n$=6). Different letters above the bars denote statistical significance at $P$=0.05 among irrigation treatments within the same year.

### 3.4. Rice cooking and eating quality

Relative to CI regime, AWMD increased PV and BD values of starch in milled rice while reducing HV, FV and SB values, whereas AWSD produced contrary effects (Table 2). These parameter trends remained consistent across both rice varieties and years (Table 2). However, AWMD regime significantly reduced GT in NJ9108 milled rice but showed no significant effect on YD6. AWSD significantly increased GT in both rice varieties during the study years (Table 2).

Compared to the CI regime, the AWMD treatment resulted in improved taste values of cooked rice for both varieties, characterized by enhanced appearance, reduced hardness, and better viscosity and balance, while the AWSD regime produced opposite effects (Table 2). Furthermore, NJ9108 demonstrated substantially superior taste quality compared to YD6 (Table 2).

### 3.5. Enzymatic activity in lipid synthesis and their relationships with lipid and free fatty acid levels

The activities of seven key enzymes (ACCase, FAS, SAD, FAD, G3PD, PPase, and DGAT) involved in lipid biosynthesis in 2021 are presented in Figs. 3 and 4, generally exhibiting an initial increase followed by a decrease in both varieties, with peak enzymatic activity occurring 18–30 days post-anthesis. Across different irrigation treatments, activities of ACCase (Fig. 3-A and B), FAS (Fig. 3-C and D), FAD (Fig. 3-G and H), and PPase (Fig. 4-C and D) demonstrated a pattern of AWMD>CI>AWSD throughout the grain-filling period, while activities of SAD (Fig. 3-E and F), G3PD (Fig. 4-A and B), and DGAT (Fig. 4-E and F) showed varying patterns, with a distinct AWMD>CI>AWSD trend during early grain filling. Similar patterns were observed for both rice varieties in 2022 (Appendices G–H). Additionally, enzymatic activities in lipid synthesis were generally lower in 2022 compared to 2021 (Figs. 3 and 4; Appendices G–H).

Regression analysis revealed that activities of ACCase (Fig. 5-A) and PPase (Fig. 6-B) in filling grains throughout the grain filling period showed highly significant positive correlations with lipid content in milled rice ($r$=0.72$^{**}$–0.89$^{**}$, $P$=0.01, $n$=12). The activities of FAS (Fig. 5-B) and FAD (Fig. 5-D) during the middle and late grain filling stages demonstrated highly significant positive correlations with lipid content ($r$=0.68$^{*}$ ($P$=0.05)–0.96$^{**}$ ($P$=0.01), $n$=12). SAD activity during early and middle grain filling stages exhibited strong positive correlations with lipid content ($r$=0.71$^{*}$ ($P$=0.05)–0.81$^{**}$ ($P$=0.01), $n$=12) (Fig. 5-C), while G3PD activity during the middle grain-filling stage showed a highly significant positive correlation with lipid content ($r$=0.74$^{**}$, $P$=0.01, $n$=12) (Fig. 6-A).

Among these seven key enzymes, FAS activity during middle and late grain filling stages showed high or very high positive correlations with contents of TFFAs, FUFAs, linoleic acid, oleic acid, petroselic acid, and vaccenic acid in milled rice ($r$=0.87$^{*}$ ($P$=0.05)–0.97$^{**}$ ($P$=0.01), $n$=6), while early grain filling stage activity showed significant negative correlations with contents of FSFAs, palmitic acid, and stearic acid ($r$=–0.83$^{*}$–(–0.86)$^{*}$, $P$=0.05, $n$=6) (Table 3). FAD activity during middle grain filling stage demonstrated high or very

**Table 1 Impact of different irrigation regimes on contents (μg g$^{-1}$ DW) of varied free fatty acid compositions in milled rice of two rice varieties in 2022**

| Fatty acid composition | YD6[1)] | | | NJ9108[1)] | | |
|---|---|---|---|---|---|---|
| | CI | AWMD | AWSD | CI | AWMD | AWSD |
| Linoleic acid (C18:2) | 1,099.0 b | 1,248.0 a | 985.4 c | 1,286.0 b | 1,378.0 a | 1,105.0 c |
| Oleic acid (C18:1) | 824.0 b | 915.9 a | 790.2 c | 2,338.0 b | 2,506.0 a | 2,100.0 c |
| Palmitic acid (C16:0) | 743.8 b | 722.2 c | 773.9 a | 1,052.0 b | 1,028.0 b | 1,194.0 a |
| Stearic acid (C18:0) | 192.6 b | 170.1 c | 217.6 a | 358.3 b | 321.4 c | 375.0 a |
| Petroselic acid (C18:1N12) | 115.3 a | 114.6 a | 69.2 b | 155.5 a | 158.6 a | 105.8 b |
| Vaccenic acid (C18:1N7) | 50.63 a | 53.80 a | 44.58 b | 118.60 a | 115.60 a | 106.40 b |
| Myristic acid (C14:0) | 37.37 ab | 43.87 a | 33.25 b | 68.04 a | 65.07 a | 45.20 b |
| *trans*-9-Palmitoleic acid (C16:1T) | 36.17 a | 35.30 a | 24.34 b | 50.95 a | 52.17 a | 38.22 b |
| Arachidic acid (C20:0) | 34.57 b | 33.06 b | 41.08 a | 78.92 b | 80.80 b | 93.81 a |
| Alpha linolenic acid (C18:3N3) | 34.58 b | 42.69 a | 46.77 a | 34.58 a | 33.29 a | 26.74 b |
| *cis*-11-Eicosenoic acid (C20:1) | 34.27 b | 41.60 a | 34.16 b | 30.43 b | 38.16 a | 31.12 b |
| Lignoceric acid (C24:0) | 25.70 b | 32.41 a | 24.70 b | 54.07 a | 54.12 a | 40.97 b |
| *cis*-9-Palmitoleic acid (C16:1) | 10.94 a | 11.39 a | 11.25 a | 16.95 b | 18.20 a | 15.22 b |
| *cis*-9-Myristylic acid (C14:1) | 10.47 a | 9.549 a | 10.10 a | 10.25 ab | 10.55 a | 9.874 b |
| *trans*-9-Myristic acid (C14:1T) | 10.39 a | 8.683 b | 10.61 a | 13.34 b | 18.28 a | 13.96 b |
| Behenic acid (C22:0) | 9.987 b | 11.92 a | 9.503 b | 39.81 a | 41.95 a | 40.74 a |
| Nervonic acid (C24:1) | 9.397 c | 11.17 b | 17.34 a | 7.657 c | 12.07 b | 20.24 a |
| Lauric acid (C12:0) | 9.203 a | 8.799 a | 8.721 a | 29.23 a | 31.33 a | 26.25 b |
| *trans*-Petroselic acid (C18:1N12T) | 9.202 a | 9.004 a | 8.674 a | 6.112 a | 6.098 a | 6.095 a |
| Elaidic acid (C18:1N9T) | 8.595 a | 8.395 ab | 8.144 b | 6.442 ab | 5.872 b | 6.875 a |
| *cis*-10-Pentadecenoic acid (C15:1) | 6.837 a | 6.537 a | 6.443 a | 4.804 a | 4.758 a | 4.866 a |
| *trans*-11-Eicosenoic acid (C20:1T) | 6.479 a | 6.477 a | 6.468 a | 4.102 a | 4.086 a | 4.105 a |
| Heptadecanoic acid (C17:0) | 6.421 a | 6.945 a | 6.501 a | 6.321 a | 6.061 a | 6.006 a |
| *trans*-10-Pentadecenoic acid (C15:1T) | 6.386 a | 6.098 a | 6.089 a | 9.202 a | 9.004 a | 9.674 a |
| *cis*-10-Heptadecenoic acid (C17:1) | 6.125 a | 5.807 a | 5.885 a | 8.595 a | 8.395 a | 8.124 a |
| *trans*-10-Heptadecenoic acid (C17:1T) | 4.894 a | 4.657 a | 4.865 a | 6.837 a | 6.537 a | 6.443 a |
| *trans*-Vaccenic acid (C18:1N7T) | 4.753 a | 4.686 a | 4.805 a | 7.479 a | 7.427 a | 7.828 a |
| Brassidic acid (C22:1N9T) | 4.381 a | 4.061 a | 4.006 a | 6.421 a | 6.945 a | 6.501 a |
| Erucic acid (C22:1N9) | 4.038 a | 3.703 a | 3.610 a | 4.234 a | 3.662 b | 3.862 ab |
| Pentadecanoic acid (C15:0) | 3.954 b | 4.386 a | 3.882 b | 3.866 b | 4.481 a | 3.290 c |
| *cis*-11,14-Eicosadienoic acid (C20:2) | 2.569 a | 2.745 a | 2.519 a | 2.062 a | 2.048 a | 2.212 a |
| Linoelaidic acid (C18:2N6T) | 2.541 a | 2.748 a | 2.354 a | 2.523 a | 2.706 a | 2.453 a |
| Tricosanic acid (C23:0) | 1.747 a | 1.569 a | 1.013 b | 1.446 a | 1.360 a | 1.480 a |
| *cis*-13,16-Docosadienoic acid (C22:2) | 1.602 a | 1.449 ab | 1.060 b | 1.622 a | 1.338 b | 0.980 c |
| Gamma linolenic acid (C18:3N6) | 1.372 a | 1.458 a | 1.458 a | 19.680 a | 18.400 a | 18.330 a |
| *trans*-10-Decanoenoic acid (C19:1N9T) | 1.181 a | 0.9990 b | 0.8850 b | 0.6833 c | 1.244 b | 1.772 a |
| Undecanoic acid (C11:0) | 0.8845 a | 0.8425 a | 0.8595 a | 0.4345 c | 0.6535 a | 0.5273 b |
| Heneicosan oic acid (C21:0) | 0.8080 a | 0.6780 ab | 0.5640 b | 0.4675 c | 0.6850 a | 0.5540 b |
| Arachidonic acid (C20:4N6) | 0.8695 a | 0.8870 a | 0.8745 a | 0.4565 a | 0.4695 a | 0.4420 a |
| Homogamma linolenic acid (C20:3N6) | 0.7270 a | 0.7810 a | 0.7615 a | 0.4125 ab | 0.4430 a | 0.4070 b |
| *cis*-7,10,13,16-Docosatetraenoic acid (C22:4) | 0.6695 c | 0.9800 b | 1.605 a | 0.4802 c | 0.8144 a | 0.6402 b |
| *cis*-11,14,17-Eicosatrienoic acid (C20:3N3) | 0.6385 a | 0.6435 a | 0.5740 a | 1.720 a | 1.885 a | 0.1005 b |
| EPA (C20:5N3) | 0.4675 b | 0.6850 a | 0.5545 a | 0.6802 b | 0.6715 b | 0.8032 a |
| Tridecylic acid (C13:0) | 0.4565 a | 0.4695 a | 0.4420 a | 0.7242 a | 0.6398 b | 0.6072 b |
| Caproic acid (C6:0) | 0.4125 a | 0.4430 a | 0.4075 a | 0.8611 a | 0.8737 a | 0.8615 a |
| Capric acid (C10:0) | 0.3295 a | 0.3240 a | 0.3145 a | 0.7270 a | 0.7810 a | 0.7615 a |
| Caprylic acid (C8:0) | 0.3115 a | 0.3090 a | 0.3120 a | 0.3650 a | 0.3156 b | 0.3162 b |
| *cis*-7,10,13,16,19-Docosapentaenoic acid (C22:5N3) | 0.3320 b | 0.8975 a | 0.7955 a | 0.2394 b | 0.2284 b | 0.3340 a |
| *cis*-4,7,10,13,16-Docosapentaenoic acid (C22:5N6) | 0.2440 b | 0.8200 a | 0.8565 a | 0.4324 b | 0.7882 a | 0.8000 a |
| *trans*-7-Nonadecenoic acid (C19:1N12T) | 0.1825 b | 0.2775 a | 0.1510 c | 0.1024 c | 0.1843 a | 0.1430 b |
| DHA (C22:6N3) | 0.0410 b | 0.4865 b | 0.5325 a | 0.0635 b | 0.3565 a | 0.3320 a |

[1)] YD6, Yangdao 6; NJ9108, Nanjing 9108. CI, conventional irrigation; AWMD, alternate wetting and moderate drying irrigation; AWSD, alternate wetting and severe drying irrigation.
Different letters denote statistically significant differences at the $P$=0.05 level among irrigation treatments.

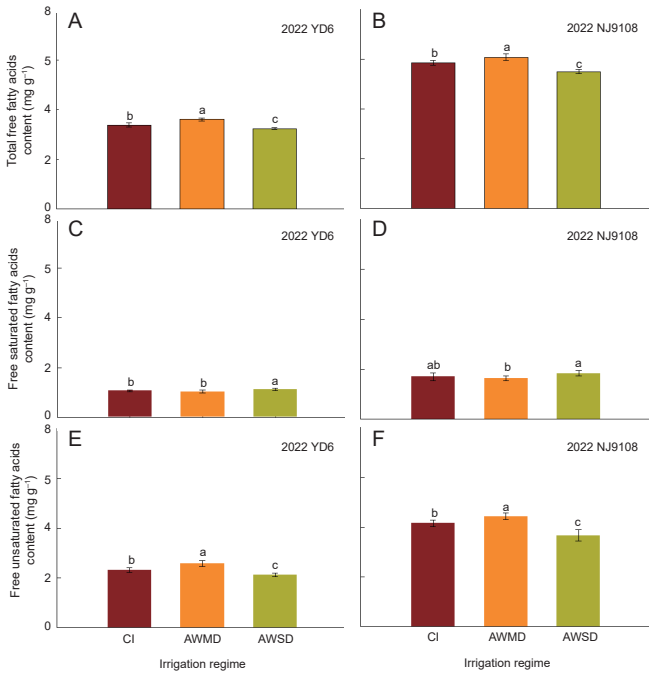



**Fig. 2 Effects of irrigation regimes on the contents of total free fatty acids (TFFAs, A and B), free saturated fatty acids (FSFAs, C and D), and free unsaturated fatty acids (FUFAs, E and F) in milled rice from two rice varieties.** CI, conventional irrigation; AWMD, alternate wetting and moderate drying irrigation; AWSD, alternate wetting and severe drying irrigation. YD6, Yangdao 6; NJ9108, Nanjing 9108. Data are represented as the mean±SE ($n$=3). Different letters above the bars indicate statistical significance at $P$=0.05 among irrigation treatments.

high positive correlations with contents of TFFAs, FUFAs, linoleic acid, oleic acid, petroselic acid, and vaccenic acid in milled rice ($r$=0.88$^{*}$ ($P$=0.05)–0.96$^{**}$ ($P$=0.01), $n$=6) (Table 3). Furthermore, ACCase and PPase activities in filling grains showed high or very high positive correlations with linoleic acid content ($r$=0.88$^{*}$ ($P$=0.05)–0.98$^{**}$ ($P$=0.01), $n$=6), while PPase activity during early grain-filling stage also showed significant positive correlations with contents of FUFAs, petroselic acid, and vaccenic acid in milled rice ($r$=0.81$^{*}$ ($P$=0.05)–0.93$^{**}$ ($P$=0.01), $n$=6) (Table 3).

### 3.6. Correlations of lipid and free fatty acid levels and cooking and eating quality in milled rice

Correlation analysis revealed that lipid level in milled rice exhibited strong negative correlations with HV, FV, SB, and GT of milled rice starch ($r$=–0.74$^{**}$–(–0.90)$^{**}$, $P$=0.01, $n$=12) (Table 4), suggesting that higher lipid content enhances cooking quality. The lipid content in milled rice demonstrated strong positive correlations with taste, appearance, viscosity, and balance value of cooked rice ($r$=0.92$^{**}$–0.98$^{**}$, $P$=0.01, $n$=12), while showing significant negative correlation with hardness of cooked rice ($r$=–0.61$^{*}$, $P$=0.05, $n$=12) (Table 4), indicating that increased lipid content enhances eating quality.

Regarding free fatty acids in milled rice, FSFAs, palmitic acid, and stearic acid contents showed significant negative correlations with PV, FV, or GT of milled rice starch ($r$=–0.84$^{*}$ ($P$=0.05)–(–0.97)$^{**}$ ($P$=0.01), $n$=6) (Table 4), suggesting that higher levels of these compounds improve cooking quality. Contents of TFFAs, FUFAs, linoleic acid,

**Table 2 Impact of different irrigation regimes on cooking and eating quality of two rice varieties**

| Year | Variety[1)] | Irrigation regime[2)] | Cooking quality[3)] | | | | | | Eating quality | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PV (cP) | HV (cP) | FV (cP) | BD (cP) | SB (cP) | GT (°C) | Taste value | Appearance | Hardness | Stickiness | Balance |
| 2021 | YD6 | CI | 2,824 d | 1,677 d | 2,361 c | 1,147 d | –463 f | 80.2 c | 63 fg | 6.4 ef | 6.8 bc | 7.2 ef | 7.0 cd |
| | | AWMD | 2,946 b | 1,591 f | 2,225 e | 1,355 b | –721 i | 79.5 c | 70 e | 6.8 c | 6.4 d | 7.6 cd | 7.5 b |
| | | AWSD | 2,706 f | 1,833 b | 2,492 b | 873 h | –214 c | 81.6 b | 61 g | 6.0 g | 6.9 ab | 6.7 h | 6.5 f |
| | NJ9108 | CI | 2,640 h | 1,615 e | 2,054 g | 1,025 f | –586 g | 73.9 e | 76 bc | 7.6 b | 6.5 d | 7.9 b | 7.5 b |
| | | AWMD | 2,758 e | 1,502 h | 1,899 h | 1,256 c | –859 k | 72.0 g | 81 a | 8.2 a | 6.0 e | 8.5 a | 8.1 a |
| | | AWSD | 2,518 i | 1,622 e | 2,195 f | 896 g | –323 d | 76.1 d | 71 de | 7.0 c | 6.4 d | 7.4 de | 6.8 de |
| 2022 | YD6 | CI | 2,886 c | 1,762 c | 2,480 b | 1,124 e | –406 e | 81.2 b | 65 f | 6.5 de | 7.1 a | 7.3 ef | 6.7 ef |
| | | AWMD | 3,105 a | 1,607 e | 2,310 d | 1,498 a | –795 j | 81.1 b | 70 e | 6.7 cd | 6.6 cd | 7.8 bc | 7.2 c |
| | | AWSD | 2,690 g | 1,946 a | 2,548 a | 744 j | –142 b | 82.8 a | 64 fg | 6.2 fg | 6.9 ab | 6.9 gh | 6.5 f |
| | NJ9108 | CI | 2,302 k | 1,516 h | 1,900 h | 786 j | –402 e | 74.4 e | 74 cd | 7.4 b | 6.4 d | 7.8 bc | 7.1 c |
| | | AWMD | 2,474 j | 1,462 i | 1,785 i | 1,012 f | –689 h | 73.0 f | 79 ab | 8.0 a | 6.1 e | 8.3 a | 8.0 a |
| | | AWSD | 2,185 l | 1,533 g | 2,064 g | 652 k | –121 a | 75.8 d | 72 de | 6.8 c | 6.6 cd | 7.1 fg | 6.7 ef |
| Analysis of variance | | | | | | | | | | | | | |
| Year (Y) | | | ** | NS | ** | ** | ** | ** | NS | NS | * | NS | ** |
| Variety (V) | | | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Irrigation (I) | | | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Y×V | | | ** | ** | ** | ** | ** | ** | * | * | NS | ** | NS |
| Y×I | | | ** | ** | ** | ** | ** | NS | NS | NS | NS | NS | * |
| V×I | | | ** | ** | ** | ** | ** | ** | NS | ** | NS | NS | ** |
| Y×V×I | | | ** | ** | ** | ** | ** | NS | NS | NS | * | NS | NS |

[1)] YD6, Yangdao 6; NJ9108, Nanjing 9108.
[2)] CI, conventional irrigation; AWMD, alternate wetting and moderate drying irrigation; AWSD, alternate wetting and severe drying irrigation.
[3)] PV, peak viscosity; HV, hot viscosity; FV, final viscosity; BD, breakdown; SB, setback; GT, gelatinization temperature.
Different letters denote significant differences at the $P$=0.05 level within the same column. * and **, $F$-values indicate significance at the $P$=0.05 and $P$=0.01 levels, respectively; ns, not significant at the $P$=0.05 level.

oleic acid, petroselic acid, and vaccenic acid exhibited significant negative correlations with PV, HV, FV, or GT of milled rice starch ($r$=–0.82$^{*}$ ($P$=0.05)–(–0.99)$^{**}$ ($P$=0.01), $n$=6), while showing significant positive correlations with taste, appearance, viscosity, and balance value of cooked rice ($r$=0.84$^{*}$ ($P$=0.05)–0.98$^{**}$ ($P$=0.01), $n$=6) (Table 4), demonstrating that higher levels of these compounds enhance cooked rice eating quality.

## 4. Discussion

### 4.1. Impact of different AWD regimes on contents of lipid and free fatty acid in milled rice and their relations with cooking and eating quality of rice

AWD, a water-saving and ecofriendly rice production system, has gained significant attention due to its impact on rice growth, grain filling and yield formation, grain quality and heavy metal accumulation, water and fertilizer use

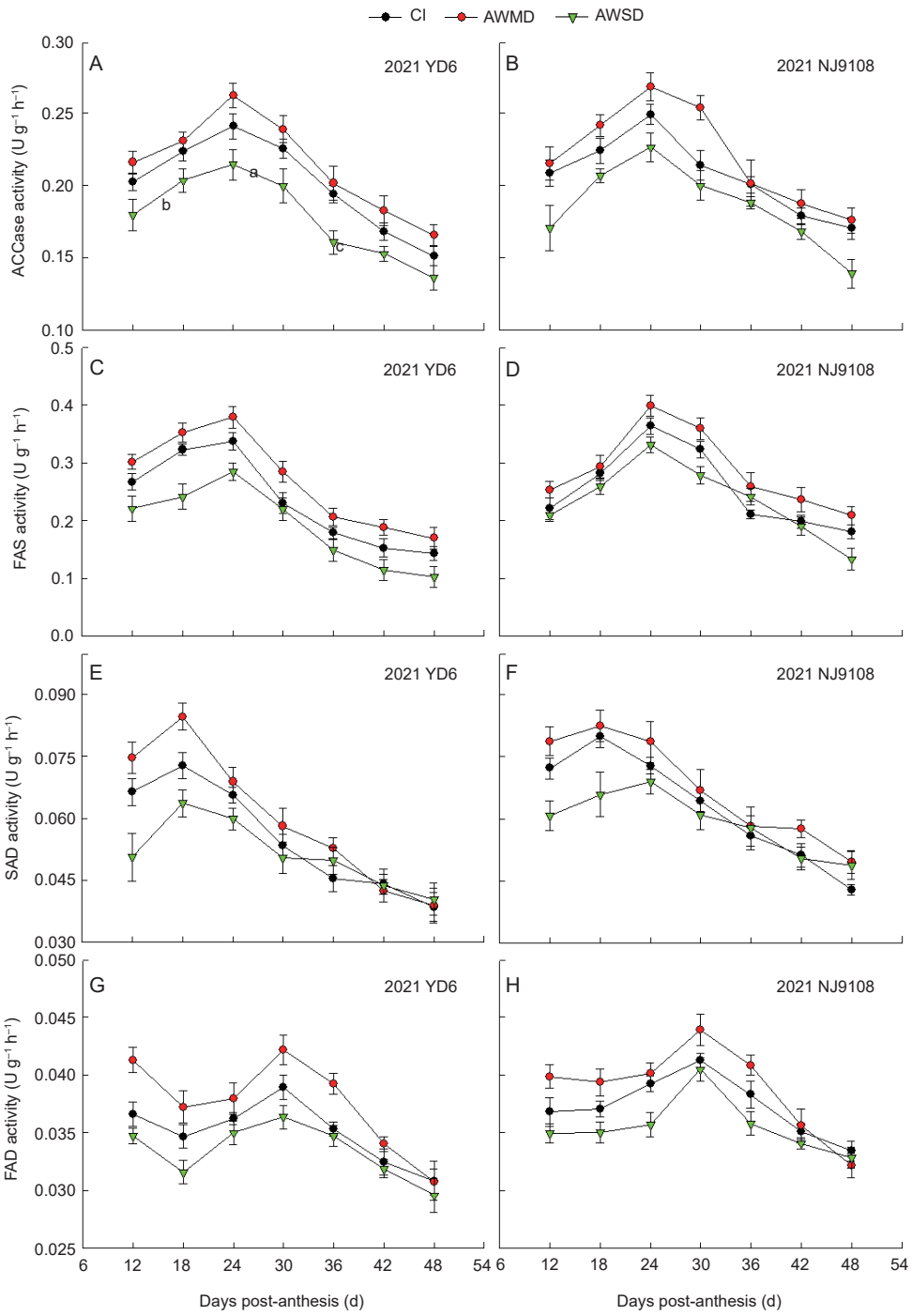



**Fig. 3 Effects of irrigation regimes on the activities of acetyl-CoA carboxylase (ACCase) (A and B), fatty acid synthase (FAS) (C and D), stearate dehydrogenase (SAD) (E and F), and fatty acid dehydrogenase (FAD) (G and H) in filling grains of two rice varieties in 2021.** CI, conventional irrigation; AWMD, alternate wetting and moderate drying irrigation; AWSD, alternate wetting and severe drying irrigation. YD6, Yangdao 6; NJ9108, Nanjing 9108. Vertical bars indicate ±SE of the mean ($n$=6) when exceeding symbol size.

efficiencies, and greenhouse gas emissions (Ishfaq *et al.* 2020). The effectiveness of AWD varies considerably depending on rice varieties, degree of soil dryness, soil type, and crop growth stage (Graham-Acquaah *et al.* 2019; Ashraf *et al.* 2022). Current literature indicates that AWMD positively affects rice yield and cooking and eating quality compared to CI treatment, while AWSD negatively impacts these parameters (Yang *et al.* 2009; Zhou *et al.* 2017; Xu *et al.* 2019; Zhang Q *et al.* 2022). Our findings confirm that AWMD regime significantly increased yield in both test varieties (Appendix C) and improved their cooking and eating quality (Table 2), while AWSD substantially reduced rice yield (Appendix C) and diminished cooking and eating quality (Table 2). These results confirm that AWMD regime enhances rice cooking and eating quality while maintaining yield.

Research indicates that while rice has relatively low lipid content, these lipids significantly influence rice cooking and eating quality (Concepcion *et al.* 2020; Shen *et al.* 2021). High-quality rice varieties typically exhibit elevated lipid content in their grains, with fatty acids contributing substantially to taste quality (Yoon *et al.* 2012b; Jianggu *et al.* 2016). Research demonstrates that elevated levels of unsaturated fatty acids (UFAs) significantly enhance rice eating quality (Yoon *et al.* 2009, 2012a). The present study revealed that the superior taste variety (NJ9108) contained higher levels of lipids and free fatty acids in milled rice compared to the conventional taste variety (YD6) (Figs. 1 and 2). Although genetic factors primarily control rice lipid content, external cultivation practices also influence these levels (Baud and Lepiniec 2010). Research shows that increased phosphorus or potassium fertilization can modify fatty acid compositions and enhance lipid levels, thereby improving rice eating quality (Peng *et al.* 2022; Chen *et al.* 2023). Previous research confirmed that AWMD regime enhances rice eating and cooking quality (Xu *et al.* 2019), however, the effects of AWMD regime on lipid profiles in milled rice remain unstudied. This investigation demonstrated that both AWMD and AWSD regimes significantly influenced lipid and free fatty acid contents in milled rice of both varieties

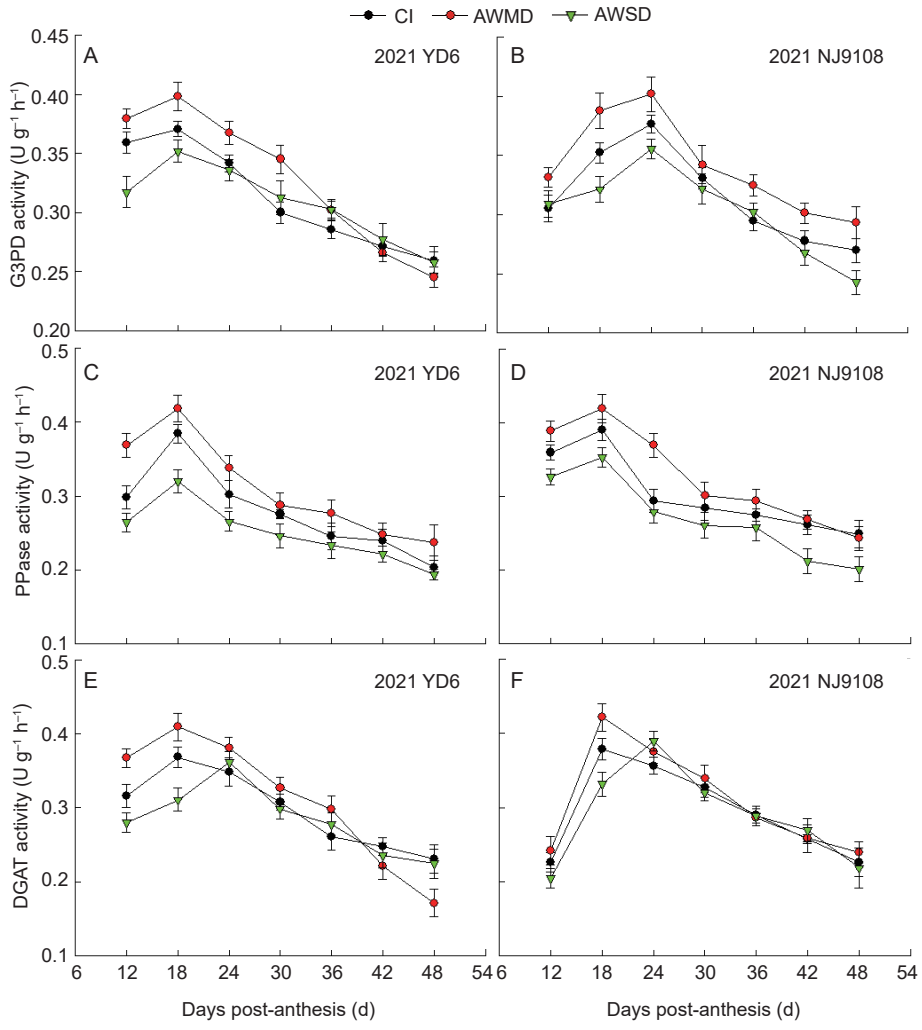



**Fig. 4 Effects of irrigation regimes on activities of glycerol 3-phosphate dehydrogenase (G3PD) (A and B), phosphatidate phosphatase (PPase) (C and D) and diacylglycerol acyltransferase (DGAT) (E and F) in filling grains of two rice varieties in 2021.** CI, conventional irrigation; AWMD, alternate wetting and moderate drying irrigation; AWSD, alternate wetting and severe drying irrigation. YD6, Yangdao 6; NJ9108, Nanjing 9108. Vertical bars indicate ±SE of the mean ($n$=6) when exceeding symbol size.

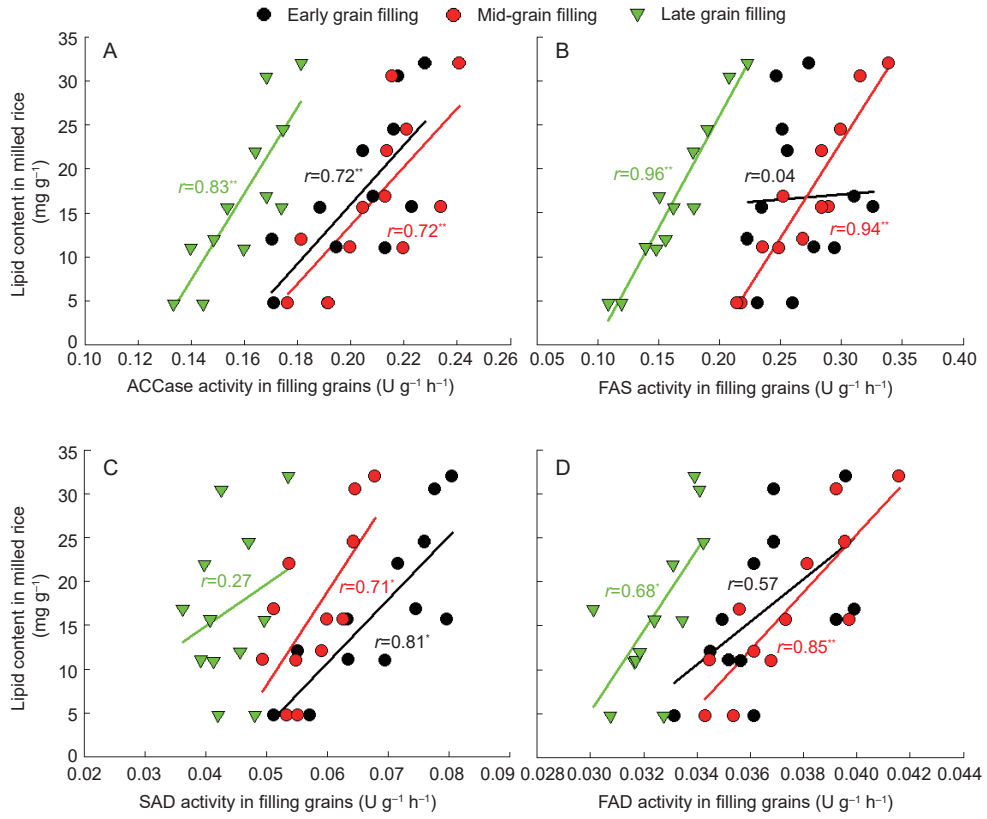



**Fig. 5 Correlation between acetyl-CoA carboxylase (ACCase), fatty acid synthase (FAS), stearate dehydrogenase (SAD), or fatty acid dehydrogenase (FAD) activity in filling grains and lipid content in milled rice of two rice varieties.** Data for calculations were derived from Fig. 3 and Appendix G. Determination coefficient (*r*) is indicated, with * and ** denoting significance at the *P*=0.05 and *P*=0.01 levels, respectively (*n*=12).

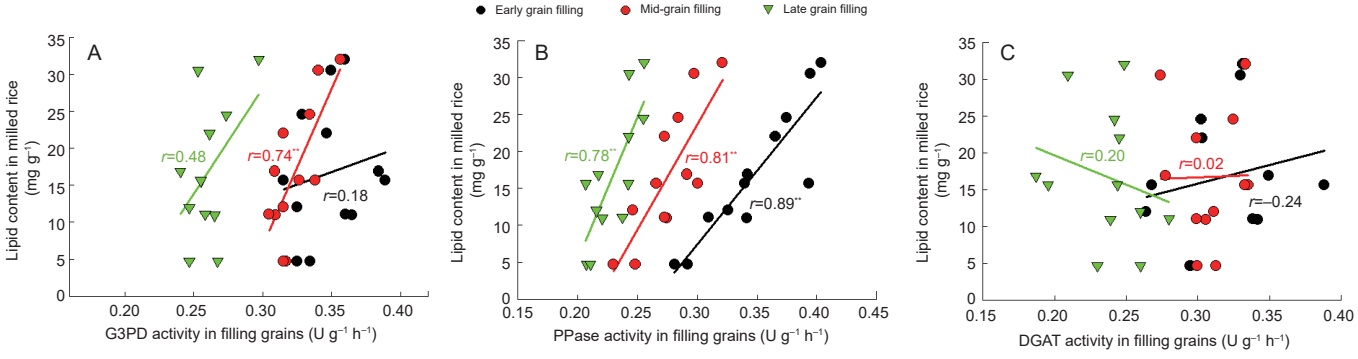



**Fig. 6 Correlation between glycerol 3-phosphate dehydrogenase (G3PD), phosphatidate phosphatase (PPase) or (DGAT) activity in filling grains and lipid content in milled rice of two rice varieties.** Data for calculations were derived from Fig. 4 and Appendix H. Determination coefficient (*r*) is indicated, and * and ** represent significance at the *P*=0.05 and *P*=0.01 levels, respectively (*n*=12).

(Figs. 1 and 2). Compared to CI, AWMD regime notably increased lipid content, TFFAs, and FUFAs in milled rice of YD6 and NJ9108, while showing minimal impact on FSFAs content. Conversely, AWSD substantially reduced TFFAs and FUFAs contents in both varieties (Fig. 1). Correlation analysis revealed significant positive relationships between lipid content and rice cooking and eating quality (Table 4), further indicating that higher TFFAs and FUFAs contents enhance cooking and eating quality of milled rice (Table 4). These findings suggest that increased lipid and FUFAs contents under AWMD treatment may substantially contribute to improved rice cooking and eating quality. Additionally, while previous studies detected relatively few fatty acid compositions (12–16 types) in rice grains (Kitta *et al.* 2005; Tsuzuki *et al.* 2014; Peng *et al.* 2022), this study identified 51 distinct free fatty acid compositions (Table 1). Consistent with previous research (Kitta *et al.* 2005; Chen *et al.* 2023), linoleic acid, oleic acid, palmitic acid, and stearic acid emerged as the predominant

**Table 3 Correlations between the activities of key enzymes involved in lipid synthesis during grain filling and free fatty acid content in milled rice[1)]**

| Correlations with[2)] | Grain-filling period | TFFAs content | FSFAs content | FUFAs content | Linoleic acid content | Oleic acid content | Palmitic acid content | Stearic acid content | Petroselic acid content | Vaccenic acid content |
|---|---|---|---|---|---|---|---|---|---|---|
| ACCase activity | Early grain filling | 0.33 | –0.01 | 0.44 | 0.90* | 0.32 | –0.11 | –0.07 | 0.64 | 0.36 |
| | Middle grain filling | 0.37 | 0.06 | 0.48 | 0.91* | 0.36 | –0.04 | 0.00 | 0.68 | 0.42 |
| | Late grain filling | 0.59 | 0.34 | 0.66 | 0.94** | 0.57 | 0.27 | 0.26 | 0.75 | 0.60 |
| FAS activity | Early grain filling | –0.66 | –0.83* | –0.57 | 0.08 | –0.67 | –0.86* | –0.85* | –0.35 | –0.62 |
| | Middle grain filling | 0.91* | 0.73 | 0.95** | 0.89* | 0.91* | 0.67 | 0.67 | 0.97** | 0.90* |
| | Late grain filling | 0.87* | 0.65 | 0.92** | 0.92** | 0.87* | 0.58 | 0.60 | 0.95** | 0.87* |
| SAD activity | Early grain filling | 0.41 | 0.08 | 0.51 | 0.94** | 0.39 | –0.00 | 0.02 | 0.70 | 0.43 |
| | Middle grain filling | 0.72 | 0.69 | 0.71 | 0.40 | 0.74 | 0.68 | 0.65 | 0.54 | 0.66 |
| | Late grain filling | 0.07 | 0.30 | –0.00 | –0.55 | 0.10 | 0.35 | 0.35 | –0.32 | 0.02 |
| FAD activity | Early grain filling | –0.24 | –0.46 | –0.15 | 0.44 | –0.25 | –0.51 | –0.52 | –0.05 | –0.23 |
| | Middle grain filling | 0.91* | 0.72 | 0.96** | 0.88* | 0.92** | 0.64 | 0.69 | 0.91* | 0.92* |
| | Late grain filling | 0.61 | 0.54 | 0.61 | 0.29 | 0.64 | 0.48 | 0.58 | 0.44 | 0.62 |
| G3PD activity | Early grain filling | –0.26 | –0.51 | –0.17 | 0.48 | –0.29 | –0.55 | –0.58 | 0.14 | –0.24 |
| | Middle grain filling | 0.68 | 0.53 | 0.71 | 0.59 | 0.70 | 0.47 | 0.49 | 0.60 | 0.64 |
| | Late grain filling | 0.38 | 0.31 | 0.39 | 0.24 | 0.39 | 0.24 | 0.37 | 0.45 | 0.45 |
| DGAT activity | Early grain filling | –0.35 | –0.64 | –0.23 | 0.43 | –0.35 | –0.69 | –0.69 | 0.06 | –0.32 |
| | Middle grain filling | –0.05 | 0.27 | –0.16 | –0.70 | –0.05 | 0.34 | 0.34 | –0.30 | –0.04 |
| | Late grain filling | –0.19 | 0.05 | –0.27 | –0.70 | –0.17 | 0.10 | 0.14 | –0.32 | –0.17 |
| PPase activity | Early grain filling | 0.80 | 0.55 | 0.86* | 0.98** | 0.79 | 0.47 | 0.49 | 0.93** | 0.81* |
| | Middle grain filling | 0.30 | –0.01 | 0.40 | 0.88* | 0.28 | –0.09 | –0.10 | 0.66 | 0.32 |
| | Late grain filling | 0.53 | 0.31 | 0.59 | 0.68 | 0.53 | 0.22 | 0.31 | 0.75 | 0.59 |

[1)] Activities of key enzymes during early grain, middle, and late grain-filling stages represent mean values measured at 12 and 18 days post-anthesis, 24, 30, and 36 days post-anthesis, and 42 and 48 days post-anthesis, respectively. Data for calculations were derived from Figs. 3 and 4 and Appendices G–H. TFFAs, total free fatty acids; FSFAs, free saturated fatty acids; FUFAs, free unsaturated fatty acids.
[2)] ACCase, acetyl-CoA carboxylase; FAS, fatty acid synthase; SAD, stearate dehydrogenase; FAD, fatty acid dehydrogenase; G3PD, glycerol 3-phosphate dehydrogenase; DGAT, diacylglycerol acyltransferase; PPase, phosphatidate phosphatase.
* and **, significant correlations at the $P$=0.05 and $P$=0.01 levels, respectively ($n$=12).

fatty acids in milled rice of both varieties (Table 1). The study demonstrated that AWMD significantly elevated linoleic acid and oleic acid levels compared to CI regime (Table 1), with these fatty acids showing strong positive correlations with rice cooking and eating quality (Table 4). These findings suggest that increased linoleic acid and oleic acid contents under AWMD treatment may significantly contribute to enhanced rice cooking and eating quality.

### 4.2. Impact of different AWD regimes on the activities of key enzymes related to lipid biosynthesis in filling grains of rice

The biosynthesis of lipid in rice grains is catalyzed by multiple key enzymes including ACCase, FAS, SAD, FAD, G3PD, PPase, and DGAT (Chen *et al.* 2017). Beyond genetic factors, the activities of these enzymes are susceptible to external influences such as cultivation practices. Research indicates that nitrogen fertilizer application enhances the activities of lipid synthesis-related enzymes in rice grains, including ACCase, FAS, G3PD, PPase, and glycerol 3-phosphate acyhransferase (GPAT) (Tu *et al*. 2020). Peng *et al.* (2022) demonstrated that phosphorus fertilizer supplementation optimizes fatty acid compositions and increases lipid content by enhancing lipid synthesis enzyme activities in rice grains, thereby improving rice eating quality. Furthermore, studies show that potassium fertilizer application can optimize fatty acid compositions and enhance both lipid content and eating quality by increasing lipid synthesis enzyme activities (Chen *et al.* 2023). Consistent with these findings, our research revealed that the AWMD regime positively regulated lipid synthesis enzyme activities in filling grains of two rice varieties, while AWSD significantly inhibited these enzyme activities (Figs. 3 and 4; Appendices G–H). Consequently, the AWMD regime increased lipid and FUFAs contents (primarily linoleic acid and oleic acid) in milled rice, while the AWSD regime decreased these contents (Figs. 1 and 2; Table 1). Therefore, the increased lipid and FUFAs contents in milled rice under AWMD treatment can be attributed to enhanced enzymatic activities related to lipid biosynthesis in rice filling grains.

### 4.3. Future research for the effect and mechanism of different AWD regimes on cooking and eating quality of rice

Lipid substances are predominantly stored in grain embryo (51%) and aleurone layer (32%), with a smaller proportion in endosperm (17%) (Resurreccion *et al.* 1979). This distribution raises an important question regarding the effects of different AWD regimes on lipid content in brown rice (containing embryo and aleurone layer) of two varieties. Currently, limited research exists on the relationship between irrigation regimes and brown rice lipid content. However, our additional trial revealed

**Table 4 Correlations of lipid and free fatty acid levels and cooking and eating quality in milled rice[1)]**

| Correlations with[2)] | Cooking quality | | | | | | Eating quality | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PV | HV | FV | BD | SB | GT | Taste value | Appearance | Hardness | Viscosity | Balance value |
| Lipid content | –0.16 | –0.82** | –0.90** | 0.30 | –0.74** | –0.88** | 0.94** | 0.97** | –0.61* | 0.98** | 0.92** |
| TFFAs content | –0.81 | –0.86* | –0.97** | –0.41 | –0.07 | –0.99** | 0.91* | 0.87* | –0.88* | 0.56 | 0.67 |
| FSFAs content | –0.94** | –0.72 | –0.84* | –0.64 | 0.25 | –0.90* | 0.74 | –0.57 | –0.79 | 0.25 | 0.41 |
| FUFAs content | –0.74 | –0.88* | –0.99** | –0.32 | –0.18 | –0.99** | 0.95** | 0.92** | –0.89* | 0.66 | 0.75 |
| Linoleic acid content | –0.12 | –0.84* | –0.82* | 0.35 | –0.77 | –0.73 | 0.88* | 0.85* | –0.57 | 0.98** | 0.92** |
| Oleic acid content | –0.82* | –0.84* | –0.97** | –0.43 | –0.05 | –0.99** | 0.91* | 0.88* | –0.90* | 0.55 | 0.67 |
| Palmitic acid content | –0.95** | –0.69 | –0.79 | –0.66 | 0.32 | –0.85* | 0.68 | 0.58 | –0.73 | 0.15 | 0.35 |
| Stearic acid content | –0.97** | –0.65 | –0.80 | –0.71 | 0.33 | –0.87* | 0.68 | 0.63 | –0.79 | 0.18 | 0.33 |
| Petroselic acid content | –0.31 | –0.84* | –0.85* | 0.14 | –0.57 | –0.82* | 0.84* | 0.87* | –0.67 | 0.88* | 0.79 |
| Vaccenic acid content | –0.83* | –0.85* | –0.96** | –0.44 | –0.03 | –0.99** | 0.88* | 0.84* | –0.86* | 0.52 | 0.62 |

[1)] PV, peak viscosity; HV, hot viscosity; FV, final viscosity; BD, breakdown; SB, setback; GT, gelatinization temperature.
[2)] TFFAs, total free fatty acids; FSFAs, free saturated fatty acids; FUFAs, free unsaturated fatty acids.
* and **, significant correlations at the $P$=0.05 and $P$=0.01 levels, respectively ($n$=12 for lipid content and $n$=6 for free fatty acid content).

significant differences in lipid metabolic expression patterns in YD6 milled rice under different AWD regimes using untargeted lipidomics technology (Appendix I). These findings suggest that different AWD irrigations likely influence lipid metabolism in both embryo and aleurone layer. Without specific experimental data, predicting the effects of different AWD regimes on brown rice lipid content remains challenging. Additional research is needed to examine lipid profiles and alterations in brown rice under various irrigation conditions, and their subsequent impact on rice cooking and eating quality.

Rice cooking and eating quality primarily depends on its fundamental components: starch, protein, and lipid (Zhang *et al.* 2021; Xia *et al.* 2022; Guo *et al.* 2023). These three major constituents in rice grains interact to determine the cooking and eating quality. In rice grain endosperm, starch comprises two types of glucose polymers: amylose and amylopectin. Research demonstrates that rice with higher amylose content (AC) produces a firmer, less-sticky texture after cooking compared to rice with lower AC (Lu *et al.* 2009; Jang *et al.* 2016; Zhang W *et al.* 2022). Studies also indicate that amylopectin structure significantly influences rice texture, where reduced degree of polymerization and chain length of amylopectin results in softer cooked rice (Nakamura *et al.* 2006; Chung *et al.* 2011). Previous research suggested that while starch determines overall rice hardness, surface hardness correlates strongly with protein content (PC) (Okadome *et al.* 1999). Recently, rice varieties with low AC and PC have been identified as optimal for superior eating quality in the middle and lower reaches of Yangtze River (Liu *et al.* 2020). Additionally, lipid is increasingly recognized as a crucial determinant of rice taste quality (Yoon *et al.* 2012a; Concepcion *et al.* 2020). While these components clearly play vital roles in rice quality, limited research exists on their interactions. Previous studies primarily examined the effects of different AWD regimes on starch and protein metabolism in rice grains (Liu *et al.* 2012; Chen *et al.* 2016; Li *et al.* 2018). This study innovatively examines how different AWD regimes affect enzymatic activities in lipid synthesis and levels of lipid and free fatty acid. Further research is recommended to investigate the interaction between starch, protein, and lipid metabolism in rice grains and their relationship to rice cooking and eating quality.

## 5. Conclusion

The AWMD significantly increased contents of lipid, TFFAs, FUFAs, linoleic acid and oleic acid in milled rice by enhancing the activities of key enzymes involved in lipid biosynthesis, while AWSD regime demonstrated opposite effects. Higher concentrations of lipid, TFFAs, FUFAs, linoleic acid, and oleic acid contributed more favorably to enhanced rice cooking and eating quality. The AWMD regime improved cooking and eating quality of milled rice through optimization of lipid and free fatty acid biosynthesis in rice grains.

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## Declaration of competing interest

The authors declare that they have no conflict of interest.

## Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

**Appendices** associated with this paper are available at https://doi.org/10.1016/j.jia.2024.05.015

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