

ORIGINAL RESEARCH

Physiological and Agronomic Insights Into Water Use Efficiency Differences Among Mid-Season *Indica* Rice Varieties

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ABSTRACT

A major challenge in rice (*Oryza sativa* L.) production is addressing freshwater scarcity without compromising grain yield. Identifying rice varieties with high yield and water use efficiency (WUE) is critical for sustainable agriculture. However, limited information exists regarding the agronomic and physiological traits associated with varying WUE. In this 2-year field study, six mid-season *indica* rice varieties with different WUE levels were categorized as low (LWVs), medium (MWVs), and high WUE varieties (HWVs). Agronomic and physiological traits were systematically evaluated. The HWVs exhibited significantly higher grain yield and WUE, accompanied by a greater productive tiller percentage, harvest index, and leaf area index (LAI) at key stages. These varieties also showed an increased grain-leaf ratio, flag leaf length and width, specific leaf weight and nitrogen content at heading, and an enhanced net photosynthetic rate during panicle initiation, heading, and mid-grain filling. Additionally, HWVs had higher non-structural carbohydrate (NSC) levels in stems and sheaths, elevated antioxidant enzyme activities (catalase, peroxidase, superoxide dismutase), and superior root biomass, root oxidation activity, and zeatin + zeatin riboside (Z + ZR) contents. Principal component analysis revealed that productive tiller percentage, effective LAI, leaf photosynthesis, root dry weight, and Z + ZR levels in leaves were strongly associated with yield and WUE. These results suggest that enhanced shoot and root traits contribute to high WUE and yield performance. This study provides clear guidance and data support for screening rice varieties suitable for water-limited conditions, contributing to sustainable rice production and food security.

1 | Introduction

Rice (*Oryza sativa* L.), a pivotal food crop globally, holds particular significance in Asia, nourishing more than half of its population (Wu et al. 2021; Song et al. 2022). Over the past five decades, significant advancements in agricultural management and breeding technologies have played a crucial role in the substantial increase of rice grain yields. Notably, the implementation of the semi-dwarf characteristic and the exploitation of heterosis have been instrumental in these developments (Yu et al. 2022; Cheng et al. 2021; Yang et al. 2022; Shen et al. 2021). As the primary consumer of the world's irrigated freshwater resources, rice stands at the forefront of agricultural water usage among various crops. This critical dependence on water poses a significant challenge to rice cultivation, particularly in the face of declining water availability. To address this, sustainable strategies are essential to balance water conservation with the imperative of maintaining rice grain yield (Zhang, Mo, et al. 2021; Yu et al. 2021). The concept of water use efficiency (WUE), defined in terms of grain yield (kg) per cubic meter of irrigation water, and correlating with biomass produced for each unit of water transpired, is a vital measure for understanding the nexus between water consumption and agricultural productivity. Consequently, enhancing WUE in rice cultivation and mitigating the mismatch between water resource availability and demand emerges as a pivotal challenge to safeguarding global food security. Achieving both high grain yield and elevated WUE concurrently remains a challenging task within the rice ecosystem. A variety of water-saving methodologies have been formulated to augment WUE. These include the development and selection of rice varieties with both high yield and enhanced WUE, coupled with the implementation of appropriate management techniques (Qin et al. 2013; Sui et al. 2013; Wang et al. 2016, 2020; Abdelrahman et al. 2021; Zhang et al. 2023). Notably, the cultivation of biologically water-efficient rice varieties stands out as an effective tactic to counteract the adverse effects of water scarcity and to fortify food security.

Currently, the physiological underpinnings that contribute to elevated WUE levels remain largely unexplored. The variance in achieving high yield and high WUE across different rice varieties is yet another aspect that lacks clarity. Research has indicated that superior agronomic and physiological traits above ground play a pivotal role in enhancing both grain yield and WUE (Peng et al. 2010; Zhang 2011). WUE was defined as the grain production per unit of water used in irrigation, which can potentially be optimized through strategic management of biomass and harvest index (HI), thereby improving WUE without adversely affecting grain yield (Zhang and Yang 2004). The storage and subsequent mobilization of carbon reserves, particularly the non-structural carbohydrates (NSC) in the stems and sheaths, play a pivotal role in enhancing the grain sink strength. This process is crucial for influencing endosperm development and grain filling, thereby boosting grain yield and its efficiency (Xu et al. 2021). Leaves, being the primary sites of photosynthesis and major contributors to transpiration and water loss, reach their peak leaf area index (LAI) at the culmination of vegetative growth in rice (Arp 1991). Water stress conditions impede leaf cell

expansion and differentiation, leading to a deceleration in leaf growth (Lu and Neumann 1998; Latif et al. 2023). Yang et al. (Yang and Zhang 2010) reported that a reduction in both the maximum tiller count and overall LAI can positively impact WUE. This improvement is attributed to the minimization of unnecessary vegetative growth and optimization of canopy structure. Stomatal dynamics are pivotal in mediating gas exchange between vegetation and the surrounding atmosphere, playing a crucial role in optimizing photosynthesis and enhancing plant water use efficiency (Lawson and Vialet-Chabrand 2019; Eyland et al. 2021). Within a specified range of stomatal conductance, an elevated rate of leaf photosynthesis contributes significantly to improved WUE (Drake et al. 2013; Han et al. 2020). Concurrently, rice cultivation strategies aimed at maximizing grain yield with minimal water usage necessitate the development of a sophisticated antioxidative enzyme system. This system encompasses enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), which are vital in water-limited environments (Liu et al. 2011).

The root system plays a pivotal role in securing plants in soil and facilitating the uptake of water and nutrients. In response to water scarcity, rice plants adapt by minimizing water loss through stomatal closure, maintaining cellular turgor, and enhancing both the efficiency of water extraction (ability of rice roots to effectively absorb water from the soil under water scarcity) and overall WUE (ratio of biomass produced to the amount of water taken up by the roots) within the root system (Gao et al. 2023). Root oxidation activity (ROA) is a key indicator of root physiological functionality. Elevated ROA levels are crucial for sustaining root biomass, promoting root growth, and optimizing water and nutrient absorption (Meng et al. 2018; Chen et al. 2019; Henry et al. 2012; Yang et al. 2008). Enhanced root biomass and ROA are instrumental in increasing rice grain yield and water productivity (Chu et al. 2014). In terms of plant physiology, cytokinins have diverse roles, including the regulation of leaf aging, chloroplast development, and cellular division (Li, Zheng, et al. 2021; Cortleven and Schmölling 2015; Rani Debi et al. 2005). Predominantly synthesized in the root system, cytokinins are conveyed through the transpiration stream to aboveground parts, where they exert significant influence on plant growth and development (Zhao et al. 2021; Gu et al. 2018). Numerous studies have highlighted the substantial and positive correlation between the concentrations of zeatin (Z) and zeatin riboside (ZR) in rice roots and key parameters such as grain yield and WUE. It has been observed that rice varieties exhibiting high yields and efficiency tend to demonstrate elevated photosynthetic rates, accompanied by increased Z + ZR levels in both roots and leaves (Zhang, Jing, et al. 2021). Despite these findings, there remains a notable gap in the research concerning the comparative analysis of root morphology and physiological traits across rice varieties with varying levels of WUE.

In this study, we hypothesized that mid-season *indica* rice varieties with higher WUE achieve superior grain yield and WUE through a coordinated improvement in shoot and root traits. To test this hypothesis, we conducted a 2-year field comparison of six varieties differing in WUE and quantified agronomic and

physiological traits of shoots and roots (canopy architecture, leaf photosynthetic capacity, antioxidant enzyme activities, and root growth and activity), and their relationships with yield and WUE to inform variety screening for sustainable, water-saving rice production.

2 | Materials and Methods

2.1 | Cultivation

Our field experiments were carried out at the research farm of Yangzhou University, located in Jiangsu Province, China (32°30' N, 119°25' E), during the rice cultivation seasons of 2020 and 2021, spanning May to October each year. The soil had a sandy loam texture. Key physicochemical characteristics included a pH of 6.1 (measured in a 1:2.5 soil-water suspension), 22.5 g kg⁻¹ of organic matter, 105.1 mg kg⁻¹ of alkali hydrolyzable nitrogen (N), 30.7 mg kg⁻¹ of Olsen phosphorus (P), and 87.2 mg kg⁻¹ of exchangeable potassium (K). The cation exchange capacity (CEC) was 11.2 cmol kg⁻¹, the moisture soil content at field capacity was 0.2 g g⁻¹, and the soil bulk density was 1.4 g cm⁻³ (Table S1). Meteorological data, including average air temperature, precipitation, and sunshine hours for these periods, were recorded at a nearby weather station (Figure S1).

The Yangtze River's middle and lower reaches, a premier rice-producing region in China, traditionally cultivate both *indica* and *japonica* rice varieties. Prior to the 1990s, mid-season *indica* rice (in the rice-growing regions of China, mid-season rice was defined as varieties with a growth period between 120 and 150 days) was the dominant variety. In this study, six representative *indica* varieties (Taizhongxian, Zhenzhu'ai, Yangdao 2, Yangdao 6, Yangliangyou 6, and Liangyoupeijiu) were grown in the field. These varieties were widely cultivated over areas exceeding 6.67 × 10⁴ ha and were known to head normally in Yangzhou. In 2020 and 2021, the seedlings were initially grown in a seedbed, sown on 15 May, followed by transplanting on 10 June of both years. After leveling the seedbed, seedling trays were neatly arranged in the field with appropriate spacing between varieties (about 20 cm). Bird netting was installed to protect the seedlings. For the first 7 days after sowing, no water was applied to the seedbed. After this period, the field was irrigated with a water layer until transplanting. One week before transplanting, urea was applied as a supplementary fertilizer. Transplanting was conducted with a hill spacing of 30.0 cm × 11.0 cm, with two seedlings placed per hill. Each experimental plot measured 5.0 m × 3.0 m. A total of 240 kg N ha⁻¹ was applied at a ratio of 4:2:2 during the pre-transplanting, mid-tillering, panicle initiation, and spikelet differentiation stages. One day prior to transplanting, phosphorous (P₂O₅ 13.5%) as calcium superphosphate (CaH₆O₉P₂) and potassium chloride (K₂O 52.0%) were applied at rates of 300 and 195 kg ha⁻¹, respectively. These application rates were determined based on local agronomic recommendations for high-yielding *indica* rice production in the Yangtze River basin, taking into account the baseline soil fertility status at the experimental site (Table S1).

Each rice variety had two plots as repetitions with a complete randomized block design. An alternate wetting and

drying irrigation regime was implemented from 10 days post-transplanting until maturity. In this regime, irrigation was withheld until soil water potential at 15–20 cm depth reached -15 ± 5 kPa. Tensiometers in each plot recorded soil water potential at 1200 h, and plots were irrigated with 1.0–2.0 cm depth of water upon reaching this threshold. Strict management of water, weeds, insects, and diseases was maintained to mitigate yield loss risks.

2.2 | Sampling and Measurements

In each experimental plot, we meticulously selected and labeled 20 plants to monitor the number of tillers at key growth stages: mid-tillering, panicle initiation, heading, and maturity, across both study years. The percentage of productive tillers was calculated as the proportion of tillers that developed panicles to the total tiller count at maturity. Additionally, for comprehensive analysis, 10 representative plants (excluding the previously selected 20) from each plot were examined. These plants, selected for their average stem number, were used to measure root and shoot biomass, leaf area, leaf nitrogen content, leaf antioxidant enzyme activity, root oxidation activity (ROA), and levels of zeatin (Z) and zeatin riboside (ZR) in roots and leaves during mid-tillering, panicle initiation, heading, middle grain filling, and maturity stages.

Furthermore, in each plot, five hills of plants were systematically sampled and dissected into roots, leaves, stems, and sheaths to ascertain the dry weights of roots and shoots at mid-tillering, panicle initiation, heading, and maturity. Each plant component's dry weight was determined post a 72-h drying period at 70°C. Non-structural carbohydrate (NSC) contents in stems and sheaths during the heading and maturity stages were quantified as per the methodology outlined by Li et al. (2017). After removing the leaves from the stem, the leaf area was immediately measured using an LI-3000 leaf area meter (LiCor Corp.).

The procedure for measuring catalase (CAT) activity was adapted from Chandless and Scandalios (1984), with slight modifications. The 3 mL CAT assay mixture comprised 50 mM phosphate buffer (pH 7.8), 5.9 mM hydrogen peroxide, and 0.1 mL of enzyme extract. The reaction was initiated by adding H₂O₂, and CAT activity was monitored over 3 min, recording absorbance decreases at 240 nm every 20 s using a spectrophotometer.

For estimating peroxidase (POD) activity, we employed the guaiacol oxidation technique as outlined by Doerge et al. (1997). The 3 mL reaction mixture for POD contained 0.1 mL of enzyme extract, 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, and 40 mM H₂O₂. Absorbance changes in the mixture were noted at 470 nm every 20 s for a total duration of 180 s.

Superoxide dismutase (SOD) activity was ascertained by assessing the inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT), as per the method described by Giannopolitis and Ries (1977). For SOD, 50 μL of the enzyme extract was added to a 1 mL reaction solution containing 50 μM NBT (dissolved in ethanol), 1.3 μM riboflavin, 13 mM methionine, 75 nM EDTA, and 50 mM phosphate buffer (pH 7.8). The solutions were placed in an aluminum-coated chamber and exposed to 30 W

fluorescent lamps. The reaction, initiated and terminated by switching the lamps on and off respectively, lasted 5 min. The resultant blue formazan from NBT photoreduction was measured by observing the increase in absorbance at 560 nm. The control, devoid of leaf extract, was similarly irradiated. Absorbance for the solutions was measured using a UV–visible spectrophotometer (IRMECO U2020). SOD activity was defined as the enzyme amount that inhibits 50% of the NBT reduction rate at 560 nm, compared to samples without plant extract.

The net photosynthetic rate of the flag leaf was quantified at key stages: panicle initiation, heading, and filling. This metric was assessed using the LI-6400 portable photosynthesis system (LiCor Corp.), with measurements conducted from 0900 to 1200 h to coincide with peak photosynthetic active radiation (1300–1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$) above the canopy. For each assessment, six leaves per treatment were analyzed.

Root samples were collected via a systematic approach, extracting a soil block of dimensions 20 cm $\times$ 20 cm $\times$ 20 cm around each hill with a sampling core. The roots were then isolated from the core segments using a hydropneumatic elutriation system (Gillison's Variety Fabrications). Approximately 10 g of roots from each sample were instantly frozen in liquid nitrogen for 1 min before being preserved at -80°C for analysis of Z + ZR contents. Root oxidation activity (ROA) was determined by measuring alpha-naphthylamine (α -NA) oxidation, following the methods outlined by Ramasamy et al. (1997), and expressed as $\mu\text{g } \alpha\text{-NA}$ per gram of dry weight per hour.

The cytokinins (Z + ZR) extraction method followed the protocol established by Pan et al. (2010), using $[^2\text{H}_5]\text{-Z}$, $[^2\text{H}_5]\text{-ZR}$, $[^2\text{H}_6]\text{-iP}$, and $[^2\text{H}_6]\text{-iPR}$ as internal standards. Detection of cytokinins was performed using a multiple reaction monitoring (MRM) liquid chromatography–tandem mass spectrometry (LC–MS/MS) system (TSQ Vantage, Thermo Fisher Scientific), as described by Müller and Munné-Bosch (2011). Quantification was based on a calibration curve, correlating known amounts of standards to the summed area ratios of the MRM transitions for cytokinins and their internal standards, analyzed using the Xcalibur Data System (Thermo Fisher Scientific).

For the calculation of indices, we employed the following formulas:

$$\begin{aligned} \text{Yield – level water use efficiency (WUE}_Y, \text{ kg m}^{-3}) \\ = \text{grain yield/the amount of irrigation water} \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Leaf – level water use efficiency (WUE}_L, \mu\text{mol mmol}^{-1}) \\ = \text{leaf net photosynthesis rate/leaf transpiration rate} \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Harvest index (HI)} = \frac{\text{total grain weight (dry weight)}}{\text{total aboveground biomass (dry weight)}} \end{aligned} \quad (3)$$

$$\begin{aligned} \text{NSC translocation amount (g m}^{-2}) \\ = \text{NSC content in stems and sheaths at heading} \\ - \text{NSC content in stems and sheaths at maturity} \end{aligned} \quad (4)$$

NSC remobilization (%)

$$\begin{aligned} = & (\text{NSC content in stems and sheaths at heading} \\ & - \text{NSC content in stems and sheaths at maturity}) / \\ & \text{NSC content in stems and sheaths at heading} \times 100 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Sugar – spikelet ratio (mg spikelet}^{-1}) \\ = \text{NSC content in stems and sheaths at heading} / \\ \text{total number of spikelets} \end{aligned} \quad (6)$$

$$\text{Specific leaf weight (SLW, g m}^{-2}) = \text{leaf dry weight/leaf area} \quad (7)$$

$$\begin{aligned} \text{Specific leaf nitrogen content (SLNC, g m}^{-2}) \\ = \text{leaf nitrogen content/leaf area} \end{aligned} \quad (8)$$

2.3 | Harvest

For our harvest assessment, we meticulously recorded the panicle density (panicles per square meter), the rate of grain filling (percentage of filled grains), and individual grain weight. This data was meticulously collected from a representative sample of 50 plants, deliberately excluding border plants, from each experimental plot (the plants used for yield measurement were distinct from those used to assess other traits). We calculated the grain yield based on the harvest from a designated 5 m² area in each plot, again excluding border plants. All grains were harvested and threshed manually to ensure accurate yield measurement. This yield was then standardized to a uniform moisture content of 14% (0.14 g H₂O g⁻¹ fresh weight). The protocols for assessing the proportion of filled grains and counting the spikelets per panicle adhered strictly to the methodology established by Zhang et al. (2018).

2.4 | Data Analysis

Analysis of variance (ANOVA) was conducted using IBM SPSS Statistics 27 (IBM Corp.). The means were compared using the least significant difference (LSD) method at a threshold of $p \leq 0.05$. Before statistical analysis, data were examined for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. A two-way ANOVA was utilized to examine the effects and interactions of the year and variety on the observed parameters. Figures were plotted using Origin 2021 (OriginLab Corp.). Principal component analysis (PCA) and path analysis were conducted using the “ggbiplot” package and “plspm” package in RStudio software (version 4.2.1), respectively.

3 | Results

3.1 | Classification of Different Rice Varieties, Grain Yield, and the Components of Yield

According to Tables 1 and 2, the yield of Taizhongxian and Zhenzhu'ai was less than 8 t ha⁻¹, the yield of Yangdao 2 and Yangdao 6 was between 8 and 10 t ha⁻¹, and the yield of Yangliangyou 6 and Liangyoupeijiu was more than 10 t ha⁻¹.

TABLE 1 | Grain yield and its components of *indica* rice varieties.

Year	Variety	Number of panicles ($\times 10^4 \text{ ha}^{-1}$)	Spikelets per panicle	Total spikelets ($\times 10^6 \text{ ha}^{-1}$)	Filled grain rate (%)	1000-grain weight (g)	Grain yield (t ha^{-1})
2020	Taizhongxian	239c ^a	138e	332f	80.25c	25.16c	6.68e
	Zhenzhu'ai	253a	148d	376e	78.30d	26.18b	7.72d
	Yangdao 2	239c	166b	397c	85.02a	25.95b	8.78c
	Yangdao 6	242b	157c	379d	84.87a	29.16a	9.38b
	Yangliangyou 6	221d	196a	432b	81.11bc	28.87a	10.11a
	Liangyoupeijiu	242b	196a	476a	82.28b	26.03b	10.16a
2021	Taizhongxian	241b	140d	334e	81.35d	26.46cd	7.27d
	Zhenzhu'ai	247a	149c	379c	79.60e	27.18b	7.96c
	Yangdao 2	241b	168b	383c	87.12a	26.25d	9.27b
	Yangdao 6	242b	167b	340d	86.07b	26.76bcd	9.32b
	Yangliangyou 6	224c	198a	436b	82.91c	30.07a	11.05a
	Liangyoupeijiu	245a	198a	480a	83.78c	26.83bc	10.91a
Analysis of variance ^b							
Year		NS	NS	**	**	NS	*
Variety		**	**	**	**	**	**
Year $\times$ variety		NS	NS	**	NS	**	NS

^aDifferent letters indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

^b*, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicate not significant at $p \leq 0.05$.

The yield-level water use efficiency (WUE_Y) of Taizhongxian and Zhenzhu'ai was less than 1.6 kg m^{-3} , the WUE_Y of Yangdao 2 and Yangdao 6 was between 1.6 and 1.8 kg m^{-3} , and the WUE_Y of Yangliangyou 6 and Liangyoupeijiu was more than 1.8 kg m^{-3} . Similar to WUE_Y , the leaf-level WUE (WUE_L) of Taizhongxian and Zhenzhu'ai was between 1.8 and $2.0 \mu\text{mol mmol}^{-1}$, the WUE_L of Yangdao 2 and Yangdao 6 was between 2.0 and $2.2 \mu\text{mol mmol}^{-1}$, and the WUE_L of Yangliangyou 6 and Liangyoupeijiu was between 2.2 and $2.4 \mu\text{mol mmol}^{-1}$. Based on these results, we defined Taizhongxian and Zhenzhu'ai as low-water use efficiency varieties (LWVs), Yangdao 2 and Yangdao 6 as medium-water use efficiency varieties (MWVs), and Yangliangyou 6 and Liangyoupeijiu as high-water use efficiency varieties (HWVs; Table 3).

The yields of HWVs were significantly higher than those of LWVs and MWVs in the 2 years. In 2 years, compared with the LWVs, the yields in MWVs significantly increased by an average of 24.13%. Compared with the MWVs, the yields in HWVs significantly increased by an average of 14.85% (Table 1). For the yield components, there was a significant difference in total spikelets between the LWVs, MWVs, and HWVs. Compared with the LWVs, the total spikelets in MWVs significantly increased by an average of 5.50%. Compared with the MWVs, the total spikelets in HWVs significantly increased by an average of 21.85%. The filled grain rate and 1000-grain weight were comparatively steady in this study. This indicated that a high grain yield was mainly contributed to by a large sink size as a result of the total number of spikelets. The trend in grain yield and its components was similar during the 2 years (Table 1).

3.2 | WUE, Percentage of Productive Tillers, and Harvest Index (HI)

A significant difference in WUE, percentage of productive tillers, and HI was observed between the three types (LWVs, MWVs, HWVs; Table 2). In 2 years, compared with the LWVs, MWVs significantly increased the WUE_Y , percentage of productive tillers, and HI by an average of 24.13%, 16.47%, and 9.47%, respectively. Compared with the MWVs, HWVs also significantly increased the three parameters by an average of 14.85%, 2.98%, and 17.98%, respectively. The trend of WUE_L was similar to that of WUE_Y (Table 2).

3.3 | Leaf Area Index (LAI), Grain-Leaf Ratio, Flag Leaf Net Photosynthetic Rate, Transpiration Rate, and Stomatal Conductance

The LAI at mid-tillering, panicle initiation, and heading, grain-leaf ratio at heading, and flag leaf net photosynthetic rate, transpiration rate, and stomatal conductance at mid-tillering, heading, and mid-grain filling stage were significantly greater in HWVs than in LWVs and MWVs, and the trend of the above-mentioned parameters was similar in 2020 and 2021 (Tables 4 and 5, Figure 1). In 2 years, compared with the LWVs, the total LAI, effective LAI, and high-effective LAI in MWVs at heading significantly increased by an average of 14.38%, 10.46%, and 15.26%, respectively. Compared with the MWVs, HWVs significantly increased the above three LAI parameters by an average of 2.61%, 1.75%, and 4.75%, respectively.

TABLE 2 | Water use efficiency (WUE), percentage of productive tillers, and harvest index (HI) of *indica* rice varieties.

Year	Variety	Yield-level WUE (kg m ⁻³)	Leaf-level WUE (μmol mmol ⁻¹)			Percentage of productive tillers (%)	HI
			PI	HD	MF		
2020	Taizhongxian	1.26e ^a	1.99a	2.03c	1.70d	69.36d	0.30c
	Zhenzhu'ai	1.46d	1.94a	2.23b	1.82c	74.85c	0.49a
	Yangdao 2	1.66c	1.97a	2.28b	1.94b	82.76b	0.38bc
	Yangdao 6	1.77b	2.02a	2.21b	1.99b	82.94b	0.44ab
	Yangliangyou 6	1.91a	2.11a	2.52a	1.96b	84.67a	0.48ab
	Liangyoupeijiu	1.92a	2.03a	2.55a	2.32a	85.56a	0.52a
2021	Taizhongxian	1.37d	1.79a	2.15c	1.66d	66.85e	0.27b
	Zhenzhu'ai	1.50c	1.82a	2.09c	1.77cd	71.32d	0.48a
	Yangdao 2	1.75b	1.84a	2.63b	1.81cd	80.66c	0.42a
	Yangdao 6	1.76b	1.86a	2.48b	2.05bc	82.45b	0.44a
	Yangliangyou 6	2.08a	1.86a	2.69b	2.22b	83.93a	0.46a
	Liangyoupeijiu	2.06a	1.97a	2.91a	2.52a	84.41a	0.51a
Analysis of variance ^b							
Year		NS	**	**	NS	*	NS
Variety		**	NS	**	**	**	*
Year × variety		NS	NS	**	NS	NS	NS

^aDifferent letters indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

^b*, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicate not significant at $p \leq 0.05$.

TABLE 3 | Classification of *indica* rice varieties differing in WUE.

Type ^a	Variety	Grain yield (t ha ⁻¹)	Yield-level WUE (kg m ⁻³)	Leaf-level WUE (μmol mmol ⁻¹)
LWVs	Taizhongxian	< 8	< 1.6	1.8–2.0
	Zhenzhu'ai	< 8	< 1.6	1.8–2.0
MWVs	Yangdao 2	8.0–10.0	1.6–1.8	2.0–2.2
	Yangdao 6	8.0–10.0	1.6–1.8	2.0–2.2
HWVs	Yangliangyou 6	> 10.0	> 1.8	2.2–2.4
	Liangyoupeijiu	> 10.0	> 1.8	2.2–2.4

^aLWVs denotes low-water use efficiency varieties, MWVs denotes medium-water use efficiency varieties, and HWVs denotes high-water use efficiency varieties.

The trend of LAI at mid-tillering and panicle initiation was similar to that at heading (Table 4). For the grain-leaf ratio, compared with the LWVs, the No. of spikelets/leaf, No. of grains/leaf, and grain weight/leaf in MWVs significantly increased by an average of 2.80%, 1.35%, and 7.51%, respectively. Compared with the MWVs, HWVs significantly increased the above three indexes by an average of 10.58%, 6.72%, and 10.87%, respectively (Table 5). Compared with the LWVs, the flag leaf net photosynthetic rate and stomatal conductance in MWVs significantly increased by an average of 23.11% and 20.15% at panicle initiation, 19.25% and 23.41% at heading, and 48.61% and 31.10% at MF, respectively. Compared with the MWVs, the flag leaf net photosynthetic rate in HWVs

significantly increased by an average of 23.28% and 35.08% at panicle initiation, 22.84% and 30.04% at heading, and 12.20% and 40.92% at mid-grain filling stage, respectively (Figure 1).

3.4 | Leaf Length and Width, Specific Leaf Weight (SLW), and Specific Leaf Nitrogen Content (SLNC)

The leaf length, leaf width, SLW, and SLNC at heading were shown in Table 5. In 2 years, compared with the LWVs, the above four parameters in MWVs significantly increased by an average of 8.23%, 5.62%, 15.03%, and 11.41%, respectively. Compared with the MWVs, the above four parameters in HWVs

TABLE 4 | Leaf area index (LAI) of *indica* rice varieties differing in WUE.

					Heading		
Year	Type ^a	Variety	Mid-tillering	Panicle initiation	Total LAI	Effective LAI	High-effective LAI
2020	LWVs	Taizhongxian	0.91d ^b	2.93c	5.84c	5.29c	4.89c
		Zhenzhu'ai	1.02cd	3.14c	6.68b	6.32ab	5.44b
	MWVs	Yangdao 2	1.24c	4.07a	7.18ab	6.14b	5.98a
		Yangdao 6	1.23c	3.44bc	7.31a	6.66a	6.26a
	HWVs	Yangliangyou 6	1.63b	3.95ab	7.18ab	6.18b	6.16a
		Liangyoupeijiu	1.92a	3.76ab	7.46a	6.72a	6.46a
2021	LWVs	Taizhongxian	0.91d	2.66c	5.80d	5.51c	4.99c
		Zhenzhu'ai	0.93d	3.19bc	7.00c	6.29b	5.49bc
	MWVs	Yangdao 2	1.29c	4.00ab	7.19bc	6.49ab	5.95ab
		Yangdao 6	1.27c	3.91ab	7.27bc	6.57ab	5.79ab
	HWVs	Yangliangyou 6	1.60b	4.47a	7.38ab	6.54ab	6.12a
		Liangyoupeijiu	1.90a	4.02ab	7.69a	6.88a	6.37a
Analysis of variance ^c							
Year			NS	NS	NS	NS	NS
Variety			**	**	**	**	**
Year × variety			NS	NS	NS	NS	NS

^aLWVs denotes low-water use efficiency varieties, MWVs denotes medium-water use efficiency varieties, and HWVs denotes high-water use efficiency varieties.

^bDifferent letters indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

^c*, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicate not significant at $p \leq 0.05$.

significantly increased by an average of 8.30%, 2.75%, 8.03%, and 10.55%, respectively. The differences between 2 years were not significant (Table 6).

3.5 | The Activities of Catalase (CAT), Peroxidase (POD), and Superoxide Dismutase (SOD) in Leaves

The maximum activities of CAT and POD in leaves occurred both at the heading stage (Figure 2A–F), and the maximum SOD activity in leaves occurred at panicle initiation (Figure 2G–I). In 2 years, compared with the LWVs, the CAT activity at heading, POD activity at heading, and SOD activity at panicle initiation in MWVs significantly increased by an average of 19.19%, 17.50%, and 21.52%, respectively. Compared with the MWVs, the above three parameters in HWVs significantly increased by an average of 11.20%, 8.04%, and 26.97%, respectively. The trend of the above-mentioned parameters was similar in 2020 and 2021 (Figure 2).

3.6 | Non-Structural Carbohydrate (NSC) Translocation Amounts, NSC Remobilization, and Sugar-Spikelet Ratio

In 2 years, compared with the LWVs, the NSC translocation amount and remobilization in MWVs significantly increased by an average of 53.25% and 15.48%, respectively (Table 7).

Compared with the MWVs, the above two parameters in HWVs significantly increased by an average of 59.68% and 40.47%, respectively. Compared to LWVs and MWVs, the HWVs had a higher sugar-spikelet ratio by an average of 33.78% and 10.26%, respectively. There were no significant differences in the NSC translocation amount and remobilization across the 2 years (Table 7).

3.7 | Root Biomass, Root-Shoot Ratio, and Root Oxidation Activity (ROA)

Compared with the LWVs in 2 years, the root dry weight at mid-tillering, panicle initiation, heading, and maturity in MWVs significantly increased by an average of 19.36%, 8.98%, 37.67%, and 16.14%, respectively. Compared with the MWVs, the above four parameters in HWVs significantly increased by an average of 9.13%, 18.12%, 7.46%, and 25.36%, respectively (Figure 3A–D). The root-shoot ratio was decreased significantly from mid-tillering to maturity. At mid-tillering, compared with the LWVs, the root-shoot ratio in MWVs and HWVs was increased. At maturity, the root-shoot ratio showed no significant differences among LWVs, MWVs, and HWVs (Figure 3E–H). The ROA reached a maximum at panicle initiation. Compared with the LWVs, the ROA at panicle initiation in MWVs significantly increased by an average of 10.60%. Compared with the MWVs, the ROA at panicle initiation in HWVs significantly increased by an average of 9.07% (Figure 4A). The trend of the above three

TABLE 5 | Grain-leaf ratio at heading stage of *indica* rice varieties differing in WUE.

Year	Type ^a	Variety	No. of spikelets/ leaf (cm ⁻²)	No. of grains/ leaf (cm ⁻²)	Grain weight/ leaf (mg cm ⁻²)
2020	LWVs	Taizhongxian	0.57bc ^b	0.46c	11.71d
		Zhenzhu'ai	0.56c	0.46c	11.95cd
	MWVs	Yangdao 2	0.59bc	0.45c	12.30cd
		Yangdao 6	0.60b	0.47bc	12.94bc
	HWVs	Yangliangyou 6	0.63a	0.49ab	14.04ab
		Liangyoupeijiu	0.65a	0.50a	14.58a
2021	LWVs	Taizhongxian	0.53b	0.42c	10.17c
		Zhenzhu'ai	0.57b	0.43bc	10.32bc
	MWVs	Yangdao 2	0.55b	0.42bc	10.83bc
		Yangdao 6	0.56b	0.44abc	11.37ab
	HWVs	Yangliangyou 6	0.59ab	0.45ab	12.08a
		Liangyoupeijiu	0.66a	0.47a	11.97a
Analysis of variance ^c					
Year			NS	**	**
Variety			**	**	**
Year × variety			NS	NS	NS

^aLWVs denotes low-water use efficiency varieties, MWVs denotes medium-water use efficiency varieties, and HWVs denotes high-water use efficiency varieties.

^bDifferent letters indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

^c*, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicate not significant at $p \leq 0.05$.

parameters (root dry weight, root-shoot ratio, and ROA) was similar during the 2 years.

3.8 | Zeatin (Z) + Zeatin Riboside (ZR) Contents in Leaves and Roots

The trend of the Z + ZR contents in leaves and roots was consistent (Figure 5). With the process of growth, the Z + ZR contents in leaves and roots decreased gradually. The Z + ZR contents in roots were higher than those in leaves (Figure 5D–F). In 2 years, compared with the LWVs, the Z + ZR contents in leaves and roots at panicle initiation in MWVs significantly increased by an average of 16.72% and 36.04%, respectively. Compared with the MWVs, the Z + ZR contents in leaves and roots at panicle initiation in HWVs significantly increased by an average of 11.09% and 32.93%, respectively. The trend of the Z + ZR contents in leaves and roots at heading and mid-grain filling stage in three types (LWVs, MWVs, and HWVs) was similar to that at panicle initiation (Figure 5).

3.9 | Relationships Between Main Agronomic and Physiological Traits

The relationships between different agronomic and physiological traits with grain quality and WUE were presented in Figure 6. The PCA showed that PC1 explained 72.2% of the variance, and PC2 accounted for 6.9%. The activities of CAT,

POD, and SOD, leaf net photosynthesis rate, root dry weight, and Z + ZR contents in leaves were closely associated with grain yield and WUE (Figure 6).

4 | Discussion

Water scarcity has emerged as a critical bottleneck in ensuring food security in the world, so it is important to make full use of water resources while increasing crop production, which will be a huge challenge (Bonetti et al. 2022; Sun et al. 2023). In rice cultivation, varieties characterized by high yield and enhanced WUE are pivotal for sustaining productivity and stability (Yu et al. 2023). Our research highlights the possibility of simultaneous improvement in grain yield and WUE through strategic selection of rice varieties (Nawaz et al. 2022). This study demonstrates a progressive increase in grain yield correlating with the rise in WUE of *indica* rice. This yield enhancement is primarily linked to an augmented total spikelet count, particularly in HWVs with total spikelet numbers exceeding $430 \times 10^6 \text{ ha}^{-1}$. Furthermore, we observed that varieties yielding higher grain production typically exhibit superior WUE, exceeding 1.8 kg m^{-3} (Tables 1 and 2). These findings suggest that HWVs can concurrently achieve heightened grain yield and WUE.

The percentage of productive tillers is a crucial indicator of rice population growth. A higher ratio of productive tillers suggests a reduction in unproductive tiller growth, thus conserving water and nutrient resources. Our observations indicate that HWVs

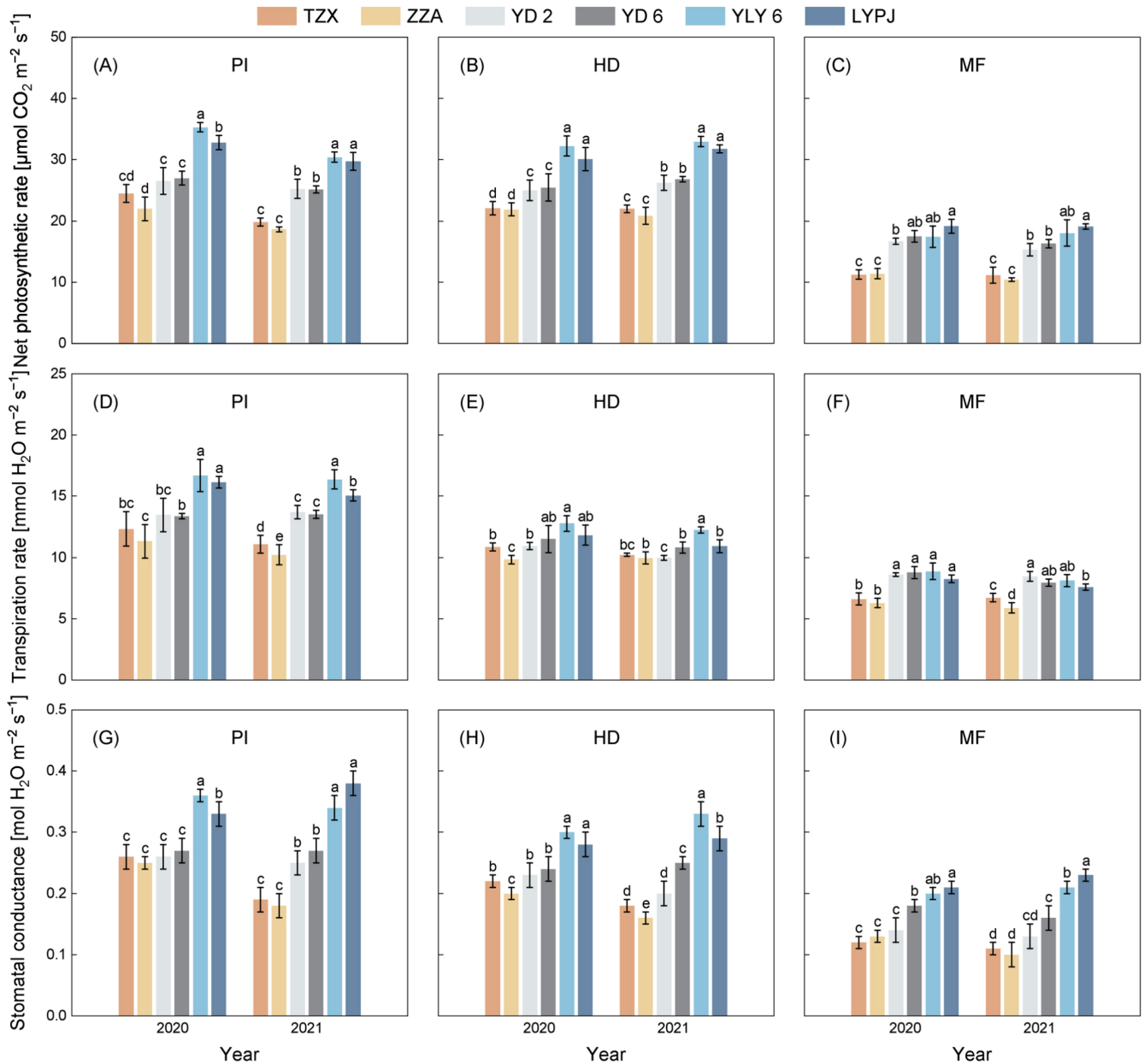


FIGURE 1 | Flag leaf net photosynthetic rate, transpiration rate, and stomatal conductance of *indica* rice varieties differing in WUE. (A) Flag leaf net photosynthetic rate at PI. (B) Flag leaf net photosynthetic rate at HD. (C) Flag leaf net photosynthetic rate at MF. (D) Transpiration rate at PI. (E) Transpiration rate at HD. (F) Transpiration rate at MF. (G) Stomatal conductance at PI. (H) Stomatal conductance at HD. (I) Stomatal conductance at MF. TZX denotes Taizhongxian, ZZA denotes Zhenzhu'ai, YD 2 denotes Yangdao 2, YD 6 denotes Yangdao 6, YLY 6 denotes Yangliangyou 6, and LYPJ denotes Liangyoupeijiu. PI, HD, and MF represent panicle initiation, heading, and mid-grain filling stage, respectively. Vertical bars represent $\pm$ standard error of the mean where these exceed the size of the symbol. Different letters above the columns indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

possess a higher percentage of productive tillers compared to LWVs and MWVs (Table 2). Studies have shown that a high percentage of productive tillers contributes to an improved canopy structure, enhanced air circulation, and light penetration, thereby boosting dry matter production from heading to maturity and optimizing nitrogen extinction coefficient in rice leaves for augmented photosynthesis and nitrogen distribution in the canopy (Huang et al. 2022; Wei et al. 2020; Li, Tang, et al. 2021; Wang et al. 2016; Gu et al. 2017). Hence, it is plausible that the increased productive tiller percentage in HWVs may confer benefits to the grain yield and WUE of mid-season *indica* rice.

Previous research has established a linear relationship between plant biomass production and transpired water, where a reduction in transpiration often compromises biomass production. Enhancing the HI has been identified as an effective strategy to increase both grain yield and water productivity (grain yield/water transpired) in rice (Yang 2015; Yan et al. 2022; Zhang, Uwimpaye, et al. 2021; Deng et al. 2022). Our findings corroborate these observations (Figure 6), indicating a trend of increasing HI with enhanced WUE. Moreover, the sink capacity in rice and its enrichment are closely associated with matter accumulation pre-anthesis and post-anthesis (Zhu et al. 2022).

TABLE 6 | Leaf length, leaf width, specific leaf weight, and specific leaf nitrogen content at heading stage of *indica* rice varieties differing in WUE.

Year	Type ^a	Variety	Leaf length (cm)	Leaf width (cm)	Specific leaf weight (g m ⁻²)	Specific leaf nitrogen content (g m ⁻²)
2020	LWVs	Taizhongxian	38.04b ^b	16.57a	51.26d	1.48c
		Zhenzhu'ai	33.47c	17.53a	57.73c	1.51c
	MWVs	Yangdao 2	38.22b	17.54a	59.65c	1.71b
		Yangdao 6	39.77ab	17.97a	67.16b	1.78ab
	HWVs	Yangliangyou 6	40.77ab	18.87a	65.76b	1.87ab
		Liangyoupeijiu	43.18a	17.65a	75.51a	1.89a
2021	LWVs	Taizhongxian	37.45bc	16.25b	51.76c	1.44c
		Zhenzhu'ai	36.30c	16.90ab	59.81b	1.66b
	MWVs	Yangdao 2	40.51abc	17.50ab	60.11b	1.66b
		Yangdao 6	38.68abc	18.00a	66.77a	1.63b
	HWVs	Yangliangyou 6	42.98ab	18.45a	66.27a	1.85a
		Liangyoupeijiu	43.30a	18.00a	66.52a	1.88a
Analysis of variance ^c						
Year			NS	NS	NS	NS
Variety			**	**	**	**
Year × variety			NS	NS	*	NS

^aLWVs denotes low-water use efficiency varieties, MWVs denotes medium-water use efficiency varieties, and HWVs denotes high-water use efficiency varieties.

^bDifferent letters indicate statistical significance at $p \leq 0.05$ ($n=4$) within the same year.

^c*, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicate not significant at $p \leq 0.05$.

We observed that HWVs had higher NSC content in stems and sheaths at the heading and maturity stages, providing a material foundation for a greater number of spikelets and filled grain rate (Table 7). The grain yield is determined by the transport of dry matter accumulated before anthesis and the post-anthesis accumulation of photosynthates (Huang et al. 2019; Fang et al. 2021). Our findings reveal that HWVs not only exhibit higher post-anthesis assimilate accumulation but also more efficient assimilate translocation (Figure 3).

Photosynthesis predominantly governs leaf metabolism. Enhanced photosynthetic activity, particularly post-anthesis, is crucial for effective biofabrication and is significantly influenced by leaf longevity and optimal light exposure (Wang et al. 2023). The effectiveness of photosynthetic matter production is intricately linked to the quality of the leaves, with the photosynthetic functionality of the leaves playing a pivotal role in determining the capacity for leaf assimilation, subsequent photosynthate accumulation, and grain filling post-heading (Gu et al. 2012). Research has highlighted the integral relationship between leaf functionality and quantitative parameters such as LAI, photosynthesis rate, and SLNC (Flack-Prain et al. 2021). Our findings demonstrate that both total and effective LAI in HWVs at the heading stage significantly surpass those in LWVs and MWVs, as shown in Table 4. This is coupled with enhanced leaf photosynthetic rate and SLNC in HWVs at the heading stage (Figure 1, Table 6), suggesting a notable improvement in leaf

quantity and quality correlating with increased WUE, which fosters greater photosynthate accumulation after heading.

The availability of carbohydrates plays a critical role in the development and establishment of sink organs, thereby influencing carbohydrate utilization and impacting the overall activity of the source (Zang et al. 2024; Krahmer et al. 2018). The grain-leaf ratio is a key indicator of the source-sink balance in rice populations (Zhou et al. 2023). Our study reveals that increases in total spikelet number, LAI (total, effective, and high-effective), and grain-leaf ratio (number of spikelets per leaf, number of grains per leaf, and grain weight per leaf) correlate with increased grain yield (Table 1, Tables 4 and 5). This indicates that higher grain yields are achieved through a well-coordinated leaf source-sink relationship, underpinned by an appropriate LAI. Additionally, our observations show that HWVs exhibit a higher sugar-spikelet ratio, which aids in the mobilization of assimilates pre-stored in stems and sheaths pre-anthesis (Table 7). A higher sugar-spikelet ratio at the heading stage indicates a larger reserve of NSC in vegetative tissues, primarily in stems and sheaths. These reserves are crucial in initiating grain filling by modulating sink activity (including hormonal contents, energy levels, and enzyme activities involved in sucrose-to-starch conversion in rice grain; Fu et al. 2011; Yang et al. 2002). The sugar-spikelet ratio shows a significant positive correlation with NSC remobilization from vegetative tissues to grains during the heading

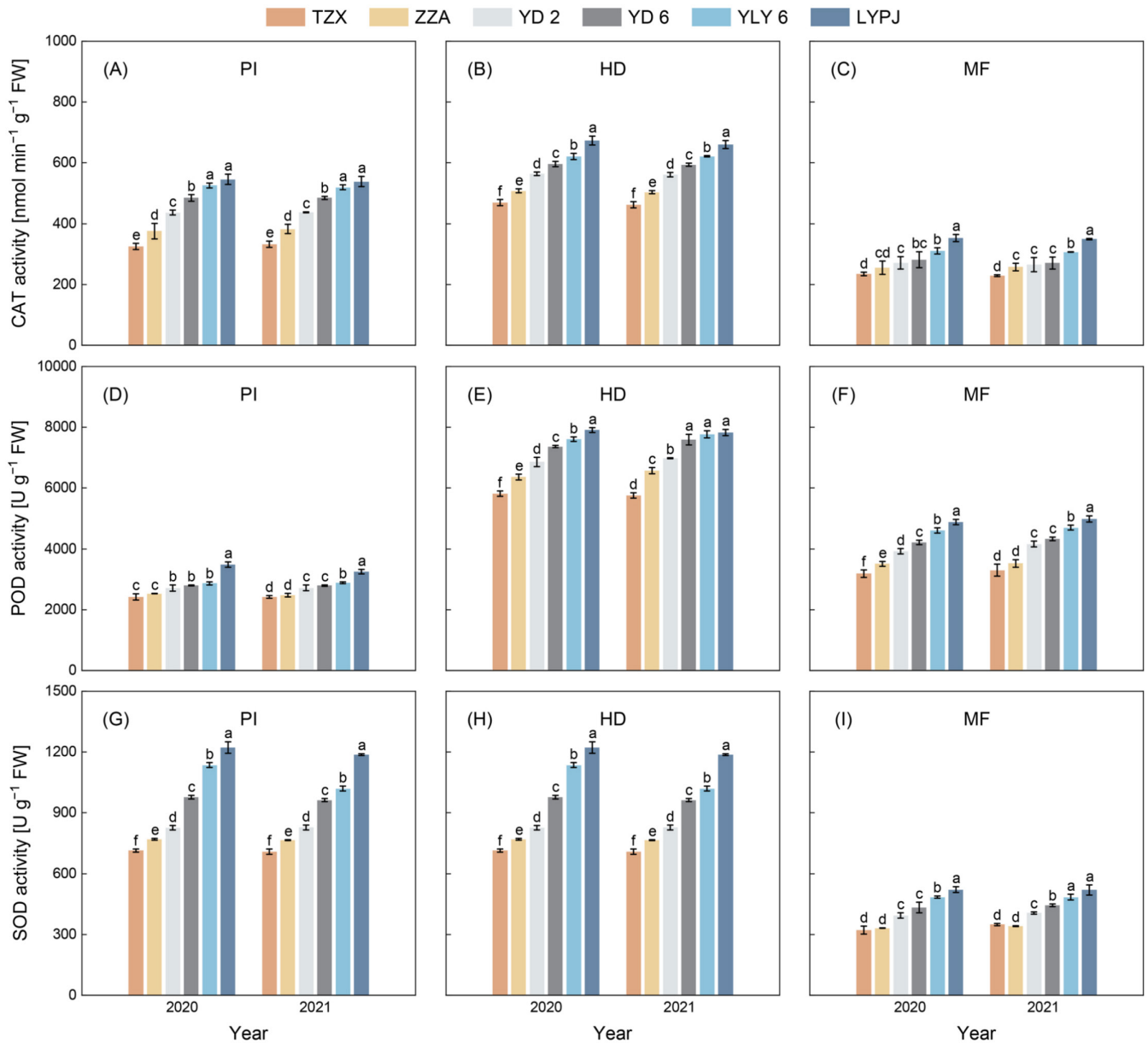


FIGURE 2 | The activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in leaves of *indica* rice varieties differing in WUE. (A) CAT activity at PI. (B) CAT activity at HD. (C) CAT activity at MF. (D) POD activity at PI. (E) POD activity at HD. (F) POD activity at MF. (G) SOD activity at PI. (H) SOD activity at HD. (I) SOD activity at MF. TZX denotes Taizhongxian, ZZA denotes Zhenzhu'ai, YD 2 denotes Yangdao 2, YD 6 denotes Yangdao 6, YLY 6 denotes Yangliangyou 6, and LYPJ denotes Liangyoupeijiu. PI, HD, and MF represent panicle initiation, heading, and mid-grain filling stage, respectively. Vertical bars represent $\pm$ standard error of the mean where these exceed the size of the symbol. Different letters above the columns indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

and filling stage (Figure 6), underscoring the importance of enhancing the sugar-spikelet ratio as a strategy to boost sink activity and grain filling in HWVs.

In plants, the generation of reactive oxygen species (ROS) typically coincides with normal metabolic functions. However, elevated ROS levels under adverse conditions can compromise cellular membrane permeability and disrupt the cellular oxidative equilibrium, resulting in pronounced toxicity. The antioxidant enzyme system in crops plays a pivotal role in scavenging ROS, thereby mitigating cellular damage. SOD is instrumental in managing oxidation, acting as the primary remover of O_2^- . It facilitates the conversion of surplus O_2^- into H_2O_2 and O_2 ,

subsequently neutralized to H_2O by POD and CAT, thereby mitigating crop stress responses (Kanazawa et al. 2000; Nie et al. 2019). Our study revealed that HWVs exhibited superior CAT, SOD, and POD activities compared to LWVs and MWVs, as shown in Figure 2. We hypothesize that the enhanced functioning of these enzymes might be advantageous for rice growth in water-scarce environments.

Understanding rice root water uptake capabilities and associated root characteristics is also essential. Crops with robust root systems typically show increased yield and enhanced tolerance to stress (Prince et al. 2017; Topp 2016; Liu 2021). Root attributes are known to be intimately linked with

TABLE 7 | Non-structural carbohydrate (NSC) content in stems and sheaths, NSC translocation amount, NSC remobilization, and sugar-spikelet ratio of *indica* rice varieties differing in WUE.

Year	Type ^a	Variety	NSC contents in stems and sheaths (g m ⁻²)		NSC translocation amount (g m ⁻²)	NSC remobilization (%)	Sugar-spikelet ratio (mg spikelet ⁻¹)
			Heading	Maturity			
2020	LWVs	Taizhongxian	175e ^b	121c	54e	30.81d	5.27d
		Zhenzhu'ai	207d	138b	69d	33.30d	5.51d
	MWVs	Yangdao 2	241c	162a	79d	32.72d	6.08c
		Yangdao 6	265b	155a	109c	41.32c	6.99a
	HWVs	Yangliangyou 6	285a	154a	131b	46.00b	6.6ab
		Liangyoupeijiu	293a	155a	170a	58.01a	6.16bc
2021	LWVs	Taizhongxian	204d	128b	75c	37.05b	6.12b
		Zhenzhu'ai	203d	130b	73c	36.20b	5.38c
	MWVs	Yangdao 2	239c	153a	85c	35.86b	6.25b
		Yangdao 6	263b	149a	114b	43.45a	7.76a
	HWVs	Yangliangyou 6	279a	153a	125ab	44.98a	6.40b
		Liangyoupeijiu	291a	149a	141a	48.64a	6.07b
Analysis of variance ^c							
Year			NS	NS	NS	NS	*
Variety			**	**	**	**	**
Year × variety			*	NS	**	*	**

^aLWVs denotes low-water use efficiency varieties, MWVs denotes medium-water use efficiency varieties, and HWVs denotes high-water use efficiency varieties.
^bDifferent letters indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.
^c*, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicate not significant at $p \leq 0.05$.

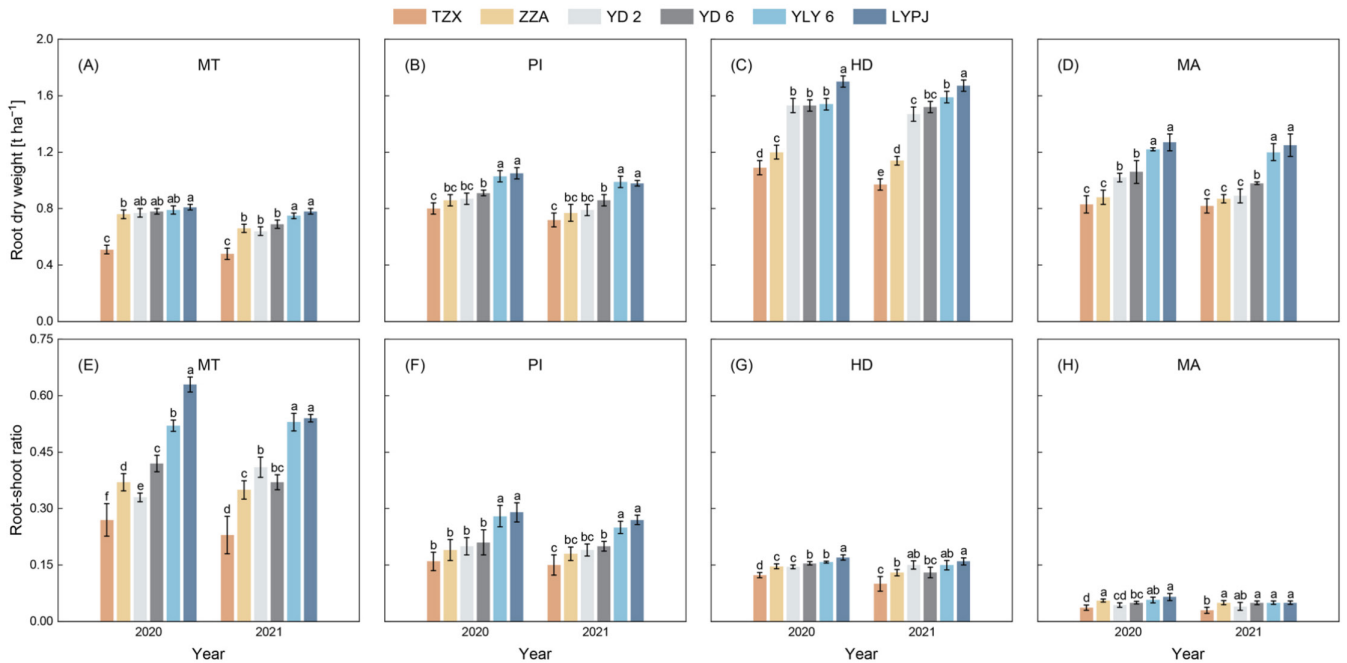


FIGURE 3 | Root dry weight and root-shoot ratio of *indica* rice varieties differing in WUE. (A) Root dry weight at MT. (B) Root dry weight at PI. (C) Root dry weight at HD. (D) Root dry weight at MA. (E) Root-shoot ratio at MT. (F) Root-shoot ratio at PI. (G) Root-shoot ratio at HD. (H) Root-shoot ratio at MA. TZX denotes Taizhongxian, ZZA denotes Zhenzhu'ai, YD 2 denotes Yangdao 2, YD 6 denotes Yangdao 6, YLY 6 denotes Yangliangyou 6, and LYPJ denotes Liangyoupeiuiu. MT, PI, HD, and MA represent mid-tillering, panicle initiation, heading, and maturity stage, respectively. Vertical bars represent $\pm$ standard error of the mean where these exceed the size of the symbol. Different letters above the columns indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

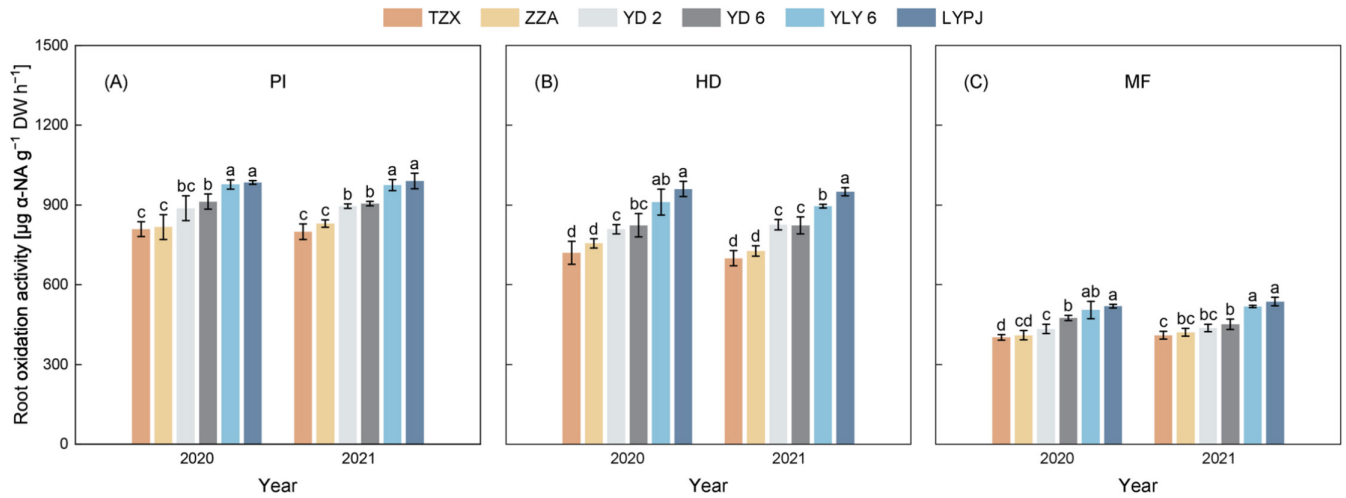


FIGURE 4 | Root oxidation activity (ROA) of *indica* rice varieties differing in WUE. (A) ROA at PI. (B) ROA at HD. (C) ROA at MF. TZX denotes Taizhongxian, ZZA denotes Zhenzhu'ai, YD 2 denotes Yangdao 2, YD 6 denotes Yangdao 6, YLY 6 denotes Yangliangyou 6, and LYPJ denotes Liangyoupeijiu. PI, HD, and MF represent panicle initiation, heading, and mid-grain filling stage, respectively. Vertical bars represent $\pm$ standard error of the mean where these exceed the size of the symbol. Different letters above the columns indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

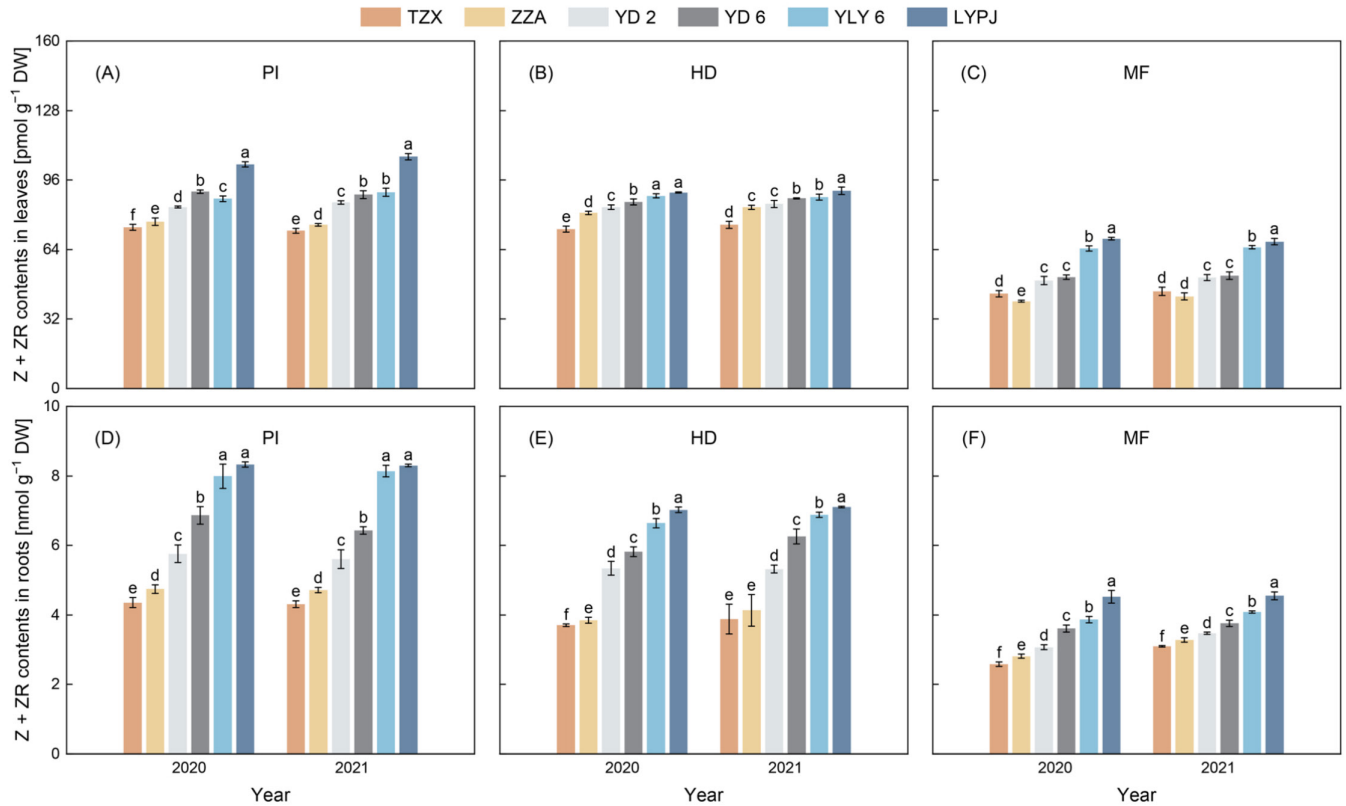


FIGURE 5 | Zeatin (Z)+zeatin riboside (ZR) contents in leaves and roots of *indica* rice varieties differing in WUE. (A) Z+ZR contents in leaves at PI. (B) Z+ZR contents in leaves at HD. (C) Z+ZR contents in leaves at MF. (D) Z+ZR contents in roots at PI. (E) Z+ZR contents in roots at HD. (F) Z+ZR contents in roots at MF. TZX denotes Taizhongxian, ZZA denotes Zhenzhu'ai, YD 2 denotes Yangdao 2, YD 6 denotes Yangdao 6, YLY 6 denotes Yangliangyou 6, and LYPJ denotes Liangyoupeijiu. PI, HD, and MF represent panicle initiation, heading, and mid-grain filling stage, respectively. Vertical bars represent $\pm$ standard error of the mean where these exceed the size of the symbol. Different letters above the columns indicate statistical significance at $p \leq 0.05$ ($n = 4$) within the same year.

shoot growth, yield development, and water utilization (Fan et al. 2016; Chen et al. 2022). In our study, HWVs demonstrated higher root biomass at main growth stages compared to LWVs and MWVs (Figure 3A–D). Furthermore, the

root-shoot ratio in HWVs showed an increasing trend during these stages (Figure 3E–H). These findings suggest that improvements in root growth contribute to enhanced grain yield and water uptake in HWVs.

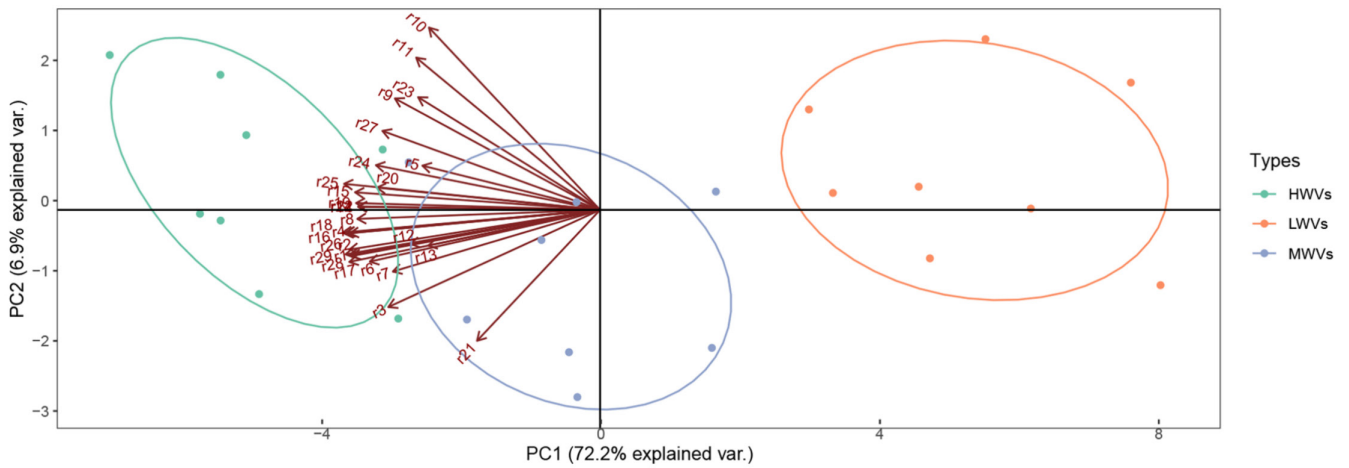


FIGURE 6 | Principal component analysis (PCA) of main agronomic and physiological traits of *indica* rice varieties differing in WUE. r1, grain yield; r2, yield-level water use efficiency; r3, leaf-level water use efficiency; r4, percentage of productive tillers; r5, harvest index; r6, total leaf area index; r7, effective leaf area index; r8, high-effective leaf area index; r9, No. of spikelets/leaf; r10, No. of grains/leaf; r11, No. of grain weight/leaf; r12, leaf length; r13, leaf width; r14, specific leaf weight; r15, specific leaf nitrogen content; r16, catalase activity; r17, peroxidase activity; r18, superoxide dismutase activity; r19, non-structural carbohydrate translocation amount; r20, non-structural carbohydrate remobilization; r21, sugar-spikelet ratio; r22, leaf net photosynthesis rate; r23, leaf transpiration rate; r24, leaf stomatal conductance; r25, root oxidation activity; r26, root dry weight; r27, root-shoot ratio; r28, zeatin + zeatin riboside contents in leaves; r29 zeatin + zeatin riboside contents in roots; LWVs, low-water use efficiency varieties; MWVs, medium-water use efficiency varieties; HWVs, high-water use efficiency varieties.

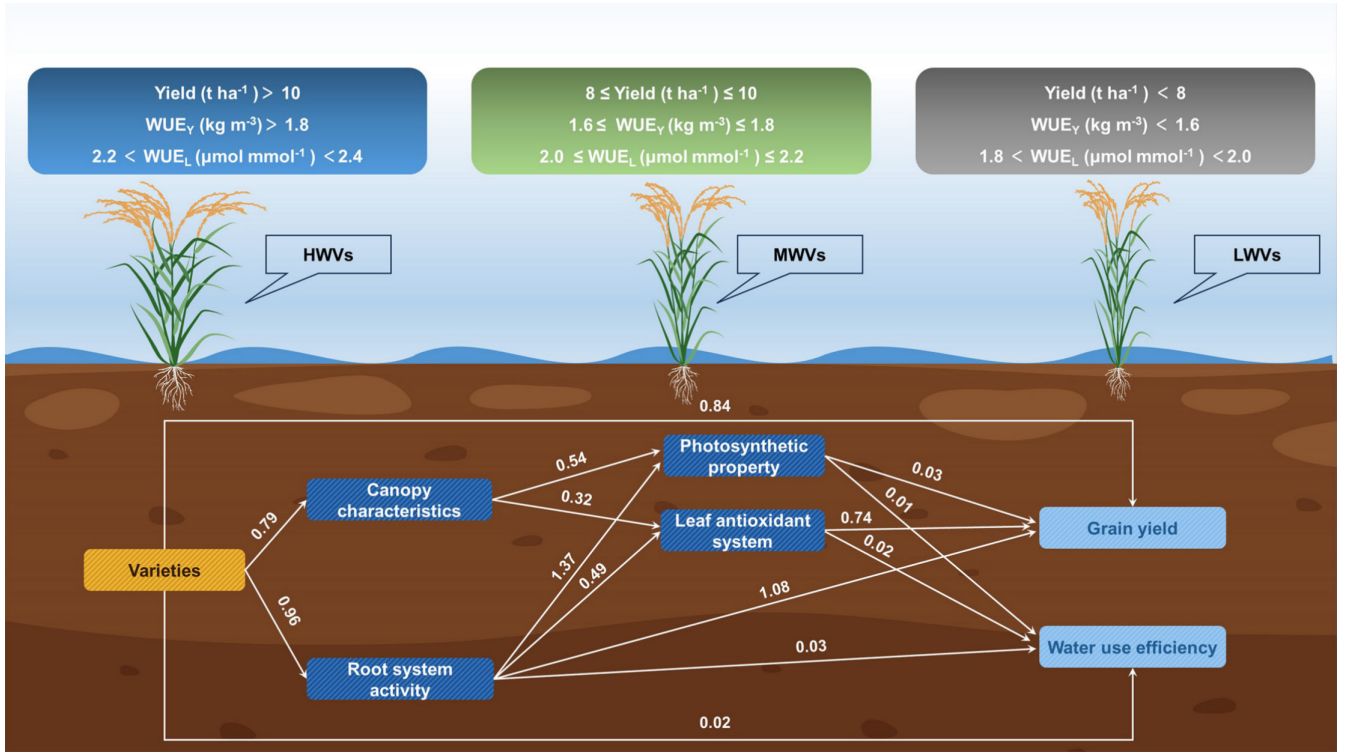


FIGURE 7 | Path analysis of main agronomic and physiological traits of *indica* rice varieties differing in WUE. HWVs, high-water use efficiency varieties; MWVs, medium-water use efficiency varieties; LWVs, low-water use efficiency varieties.

Roots, as crucial organs for resource transposition, metabolic activities, and water and nutrient absorption, play a significant role in the overall growth, yield, and stress resilience of rice. Efficient water utilization is closely related to root activity and the concentration of root-promoting hormones in rice. Cytokinins, Z + ZR, synthesized in roots, act as vital signals for

root-to-shoot communication, influencing cytokinin-regulated processes in the shoot (Yang 2002; Kudoyarova et al. 2014). In this study, HWVs displayed higher ROA and Z + ZR contents in leaves and roots compared to LWVs and MWVs (Figures 4 and 5). These results imply that HWVs possess superior root physiological traits, thereby enhancing shoot growth and grain yield

formation, a key physiological factor in achieving high yield and efficient water utilization. In summary, a robust root system provides a foundation for high yield by coordinating the relationship between shoots and roots, which is critical for attaining high yield and WUE (Figure 7).

It is worth noting that, in addition to the six varieties examined in this study, two water-efficient types now widely promoted in China, namely water-saving and drought-resistant rice (WDR) and intersubspecific hybrid rice, represent a new benchmark for integrating high yield with improved WUE. Studies on WDR varieties such as Hanyou 73 have demonstrated stable yields and water-saving advantages under water-conserving regimes, driven by root- and canopy-level adaptations relevant to our trait framework (Xia et al. 2022). At the irrigation-regime scale, alternate wetting and drying irrigation (AWD) typically reduces irrigation inputs by 30%–35% while enhancing WUE with minimal yield penalties, and soil-water-potential-based AWD has the potential to increase water productivity across large irrigated rice areas, including central China (Gao et al. 2024; Bo et al. 2024). Modern intersubspecific hybrids also exhibit strong physiological and management responsiveness, including improved canopy light–nitrogen coordination and enhanced resource use efficiency, which support high productivity under water-saving management (Liu et al. 2024; Chen et al. 2023). Our recent research demonstrates that AWD enhances the yield and WUE of the modern *indica-japonica* hybrid rice Yongyou 2640, while simultaneously reducing methane emissions (Xiao et al. 2025). These findings highlight the need for future research to benchmark WDR and intersubspecific hybrids against the variety series used here under AWD conditions, with integrated evaluations of agronomic and physiological traits in both shoots and roots, thereby advancing the selection of water-efficient varieties and contributing to the resilience and sustainability of future rice production systems.

5 | Conclusion

Screening of high-yield and water use efficiency (WUE) varieties could provide more opportunities for sustainable rice production and food security. The high-water use efficiency varieties (HWVs) performed at high grain yield and WUE. The increased yield and WUE in HWVs were attributed mainly to the improvement in the canopy characteristics, photosynthetic property, leaf antioxidant enzyme activities, and root system. The superior yield and WUE in HWVs primarily benefit from the simultaneous improvement of the agronomic and physiological traits in shoots and roots.

Author Contributions

Wenjiang Jing: investigation, data curation, writing – original draft, writing – review and editing. Xiping Lv, Yu Yan, Jia Yin, Rumeng Sun, and Ying Zhang: investigation and data curation. Danping Hou: investigation, conceptualization, and methodology. Jianhua Zhang: resources, conceptualization, methodology, validation, project administration. Hao Zhang: resources, conceptualization, methodology, writing – original draft, writing – review and editing, supervision, validation, and project administration. All the authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Soil classification and physical–chemical properties of the experimental field (0–20cm layer). **Figure S1:** Mean air temperature, precipitation, and sunshine hours during the rice growing seasons. (A) Mean air temperature in 2020. (B) Mean air temperature in 2021. (C) Precipitation in 2020. (D) Precipitation in 2021. (E) Sunshine hours in 2020. (F) Sunshine hours in 2021.