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Comparison of Main Agronomic Traits and Identification of Important Genes in Japonica Rice Cultivars Grown in the Jianghuai Region

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Abstract: A exploration and understanding of cultivar adaptability to specific environmental conditions are critical in rice breeding. This study aimed to compare the agro-morphological data of 36 japonica rice cultivars (*Oryza sativa* L.) from Chinese rice accessions grown under two different environments (Fengyang and Hexian) and to identify important genes associated with key traits in the cultivars. Higher significant differences were observed between Fengyang and Hexian in traits like, grain width, grain length, yield per plot, plant height, and tiller number with cultivars grown in Hexian having the greatest values. This revealed that the environment in Hexian favored these traits, and most cultivars performed better in Hexian than in Fengyang. Correlation analysis also showed strong positive correlations between tiller number and yield per plot in both environments, indicating the influence of tiller number on rice yield potential. The PCR analysis showed the amplification of *DEP1*, *Ghd7*, *Wx*, *Chalk5*, *COLD1*, *DST*, *Xa13*, and *Bph6* in most japonica cultivars, indicating presence of these genes in the cultivars. This study suggests that differences in agronomic performance between the cultivars grown in Fengyang and Hexian might be caused by differences in environmental conditions. This finding could be valuable for future breeding of high-yielding and climate-resilient cultivars.

Keywords: agronomic traits; molecular marker analysis; japonica rice; Jianghuai region; comparative analysis

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1. Introduction

Rice (*Oryza sativa* L.) is a pivotal crop that serves as a staple food for more than half of the world's population [1]. During 2015-2030, the global population is expected to increase by 12.8%. To meet the global rice demand by 2030, the total rice production output must reach 771 million tons, reflecting an increase of at least 11.8% from 2015 output levels [2]. Therefore, cultivation of high-yielding rice varieties is crucial for the increasing population demand for rice. China is the world's leading producer, importer, and consumer of rice. Rice serves as a major food for more than 80% of the population [3]. China plays a vital role in the cultivation of Asian rice, specifically *O. sativa*, due to its abundant genetic resources [4]. *Oryza. sativa* spp. *japonica* is one of the two main ecogeographic species of rice cultivated in northern and eastern China [5] and is mainly grown in more temperate regions at higher latitudes. Japonica rice is distinct from indica rice in terms of plant architecture, and agronomic and physiological traits such as stress resistance, cold tolerance, and grain quality [6]. Its production area has increased over time in China, with yield accounting for 45% of national production [7].

The Jianghuai region, located between the Yangtze River and the Huai River in Anhui Province, is known for rice cultivation due to its favourable environmental conditions and fertile soil [8,9]. However, climate change and climatic factors such as flood and drought pose challenges for rice cultivation, highlighting the need for cultivars with high yield and strong adaptability. Different rice cultivars thrive in specific ecological conditions. However, it is vital to select cultivars adaptable to specific conditions to ensure optimum yield. Photoperiod is the main climatic factor influencing rice's ability to adapt to various ecological circumstances [10]. There are many reports that photoperiod sensitivity is crucial for grain filling and affects flowering time regulation [11,12]. Rice cultivars can only be grown in a limited range of latitude due to photoperiod sensitivity and this is an important factor in cultivar adaptation to different ecological zones. Rice grows in a wide range of latitudes across the world (50°N-35°S) [13]. Therefore, it is important to identify traits that help define the performance of the most stable cultivars at different latitudes [14]. Although Jianghuai provides unique prospects for japonica rice cultivation, the development of superior cultivars faces significant challenges [15].

Despite decades of enormous breeding efforts, progress in developing high-yielding and adaptable rice cultivars has not met expectations, because most agronomic traits such as plant height, tiller number, and panicle length, and other morphological traits are quantitative in nature [16]. These traits are typically controlled by several genes rather than single genes, and their expression is influenced by various environmental factors [17]. The genes controlling these quantitative trait loci may reside on different chromosomes, but their products work together as part of complex metabolic pathways and physiological processes [18]. Previous studies investigated individual genes that influence rice productivity [19,20]. However, the influence of genetic factors on agronomic traits in japonica cultivars under different environments remains unknown. This is important for developing high-yielding cultivars that can thrive in different ecological zones.

The objective of this study was to collect, analyze, and compare the morphological and agronomic traits of 36 japonica cultivars from the Chinese rice accessions grown in two planting areas: Fengyang and Hexian. To achieve this objective, PCR analysis was performed using molecular markers associated with eight key genes - DENSE AND ERECT PANICLE 1 (*DEP1*) and GRAIN NUMBER, PLANT HEIGHT, AND HEADING DATE 7 (*Ghd7*) for grain yield and heading date; CHALKINESS 5 (*Chalk5*) and WAXY (*Wx*) for grain quality; COLD-TOLERANCE 1 (*COLD1*) and DROUGHT AND SALT TOLERANCE (*DST*) for stress tolerance; and XANTHOMONAS RESISTANCE 13 (*Xa13*) and BROWN PLANTHOPPER RESISTANCE 6 (*Bph6*) for disease resistance - across the 36 japonica cultivars to understand the influence of these genes on cultivar performance. The comparison of agronomic traits and PCR analysis of japonica cultivars will facilitate development of rice varieties with high adaptability and superior qualities.

2. Materials and Methods

2.1. Plant Materials

In this study, 36 rice accessions were collected from academic and research institutes located in different provinces in China. Among the 36 rice cultivars, one was from Shanxi Province, five were from Zhejiang Province, one was from Chongqing Province, 16 were from Jiangsu Province, 11 were from Anhui Province, and two were from Shandong Province. 10 indica rice cultivars were used as controls to understand the genetic differences between the two subspecies. The detailed background information for each cultivar is presented in Table S1.

2.2. Field Trials

All rice germplasms were cultivated in two distinct locations of Anhui Province, Fengyang County, Chuzhou (32°52′30″ N, 117°33′15″ E), and Hexian County Maashan (118°17′12.02″ N, 31°56′25.45″ E) in the summer of 2023. Both locations are situated within the Jianghuai region. Each rice cultivar's seeds were soaked and treated before being planted in a nursery field. 30 days after nursery, the seedlings were transplanted onto the main paddy field in a Randomized Complete Block Design with two replications per cultivar, with a row spacing of 20 cm and an intra-row spacing of 15 cm, accommodating 30 plants per cultivar. Throughout the growth period, crop management practices were applied. The rice fields were irrigated at 5-7 cm water depth during vegetative growth, and reduced to intermittent flooding during the post-tillering stage; fertilization included 150 kg/ha of nitrogen (urea), 60 kg/ha of phosphorus (P_2O_5) and 80 kg/ha of potassium (K_2O); and weeding was performed manually every 15 days after transplanting, and it was supplemented by herbicide application to protect and manage the crops until they reached maturity stage.

2.3. Data Collection

10 agronomic traits were recorded on 36 japonica cultivars following the Standard Evaluation System for Rice [21]. Data was recorded on randomly selected cultivars for traits such as plant height (average of 15 plants per cultivar) abbreviated as PH, tiller number (average of 15 plants per cultivar) abbreviated as TN, panicle length (average of 15 plants per cultivar) abbreviated as PL, number of primary branches (average of 15 plants per cultivar) abbreviated as PB, number of secondary branches (average of 15 plants per cultivar) abbreviated as SB, grain length (average of 15 plants per cultivar) abbreviated as GL, grain width (average of 15 plants per cultivar) abbreviated as GW, thousand-grain weight (average of 5 plants per cultivar) abbreviated as TGW, seed-setting rate (average of 5 plants per cultivar) abbreviated as SR, and yield per plot (estimated average yield per cultivar from a 66.67 m^2 plot) abbreviated as YD.

2.4. Analysis of Molecular Markers

DNA extraction for PCR amplification was carried out by the CTAB method [22]. Eight genes including *DEP1* [23], *Ghd7* [24], *Wx* [25], *Chalk5* [26], *COLD1* [27], *DST* [28], *Xa13* [29], and *Bph6* [30], were selected as target genes in this study. Based on the above research, primers were designed to amplify molecular markers associated with these genes (Table S3), and they were confirmed in the Gramene database (http://archive.gramene.org/markers/) and RiceData (https://www.ricedata.cn/gene/).

10 µL volume of PCR reaction mixture containing 50 ng genomic DNA, 5 µL 2 × Santaq PCR mix (Vazyme Biotech Co., Ltd., Nanjing, China), 30 ng of each forward and reverse primer was performed using LongGene (T20, LongGene Scientific Instrument Co., Ltd., Hangzhou, China). The PCR conditions were: 95℃ for 3 min; 34 cycles of 95℃ for 30 s; 56℃ for 1 min; 72℃ for 30 s and 72℃ for 5 min. The amplified PCR products were separated on 2% agarose gels stained with ethidium bromide and visualized using GelDocXR (Bio-Rad, USA).

2.5. Statistical Analysis

Several statistical methods were used in the data analysis. Descriptive statistics were used to analyze the means (±) and standard deviation (SD) for all the agronomic traits among the cultivars grown in Fengyang and Hexian. Student's *t*-test was used to determine the significant difference between agronomic traits in a pairwise comparison ($P < 0.05$) and significant difference percentages were calculated by dividing the number of cultivars that showed significant differences in each environment by the total number of significant differences observed across both environments. Pearson correlation coefficient was used to assess the linear relationship between agronomic traits and GraphPad Prism (GraphPad Software Inc., San Diego, USA) was used to visualize the data.

3. Results

3.1. Variation in Agronomic Traits Under Different Environmental Conditions

3.1.1. Variation in Agronomic Traits in Fengyang

Under the environmental conditions in Fengyang, the 36 japonica accessions showed variation in agro-morphological traits including plant height, tiller number, panicle length, number of primary branches, number of secondary branches, grain length, grain width, thousand-grain weight, seed-setting rate, and yield per plot (Figures 1–3). Cultivars NJ46, YJG6, and ZD28 showed the greatest plant heights with means and standard deviations of 103.13 ± 2.59, 96.90 ± 3.60, and 96.73 ± 2.43 cm, respectively. Cultivars WKG3, HLPR1, and FG629 had the lowest plant height, with 69.63 ± 4.23, 74.83 ± 2.62, and 79.83 ± 2.83 cm, respectively (Figure 2a). The number of tillers varied greatly among cultivars (Table 1), highest for ZD28 (10.3 ± 3.1) and SXN1 (9.9 ± 3.1) while NG5718 (3.9 ± 1.5) and HLPR1 (4.1 ± 1.3) were the lowest (Figure 2b). Similarly, ZJN1 (19.86 ± 2.10 cm), WKN3 (19.75 ± 1.30 cm), WYG31 (19.36 ± 1.20 cm), ZD11 (19.20 ± 1.20 cm), ZD18 (19.20 ± 1.65 cm), and HZN1 (19.08 ± 1.10 cm) had the longest panicles whereas YNN418 (15.33 ± 0.77 cm), WKN3 (15.40 ± 0.87 cm), HLPR1 (15.55 ± 1.00 cm), and NG5718 (15.58 ± 1.10 cm) had the shortest (Figures 1a and 2c). The greatest number of primary branches was observed in HN168 (18.67 ± 2.41) while SXN1 (13.61 ± 1.45) and NG5718 (13.00 ± 1.30) had the least (Figures 1b and 2d). In contrast, WKN3 (54.20 ± 10.67) had the highest number of secondary branches whereas NG5718 (20.00 ± 6.74) had the lowest (Figure 2e). Cultivar FN6 (1715 ± 106 g/m^2) had the highest yield per plot and NG5718 (472 ± 167 g/m^2) had the lowest among the cultivars (Figure 2f).

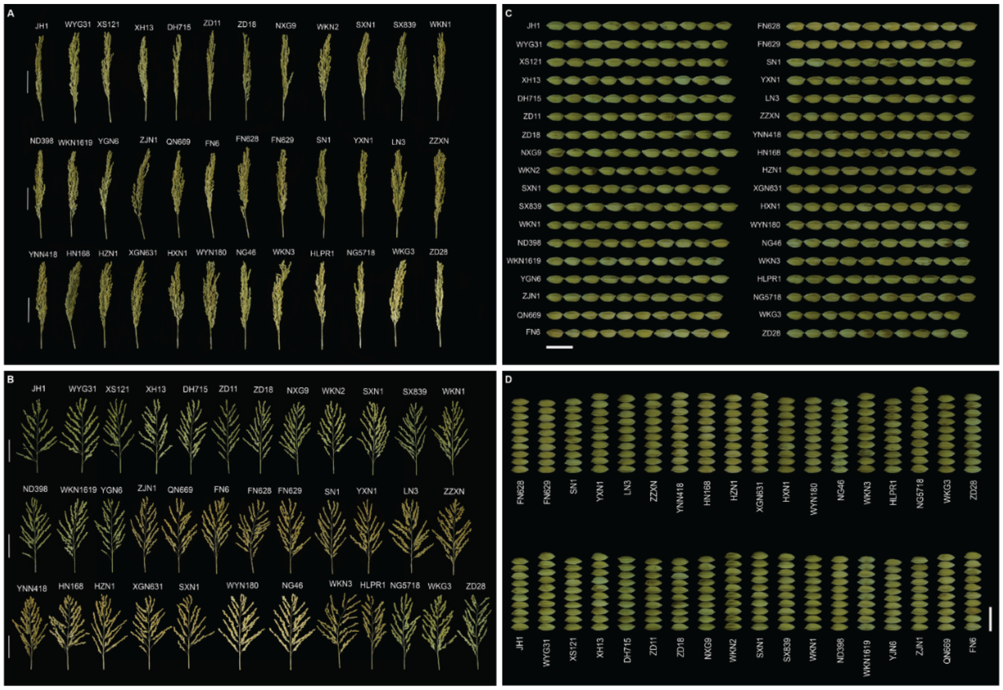

Figure 1. Variation in morphological traits among the 36 japonica cultivars grown in Fengyang. (a) panicle length, (b) number of primary branches; Scale bar, 5 cm. (c) grain length, and (d) grain width; Scale bar, 1 cm.

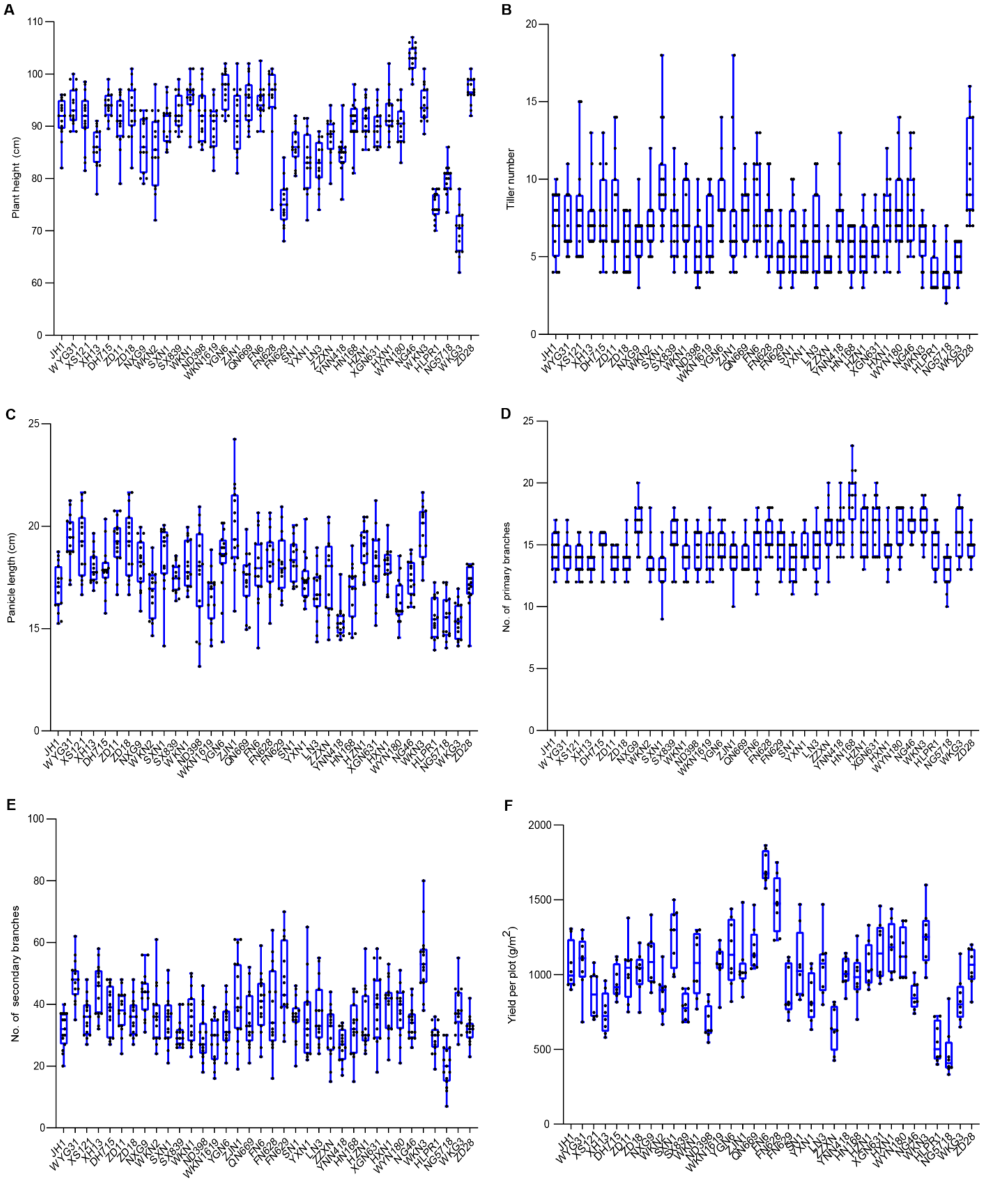

Figure 2. Box plot showing variation in agronomic traits among the 36 japonica cultivars grown in Fengyang. (a) plant height, (b) tiller number, (c) panicle length, (d) number of primary branches, (d) number of secondary branches, and (f) yield per plot. The box plot represents the median (line), 25-75 quartiles (boxes), and individual data points (dots).

Table 1. Descriptive statistics of agronomic traits of japonica rice cultivars grown in Fengyang and Hexian.

Traits	Env.	Mean ± SD	Range	CV (%)	Skewness	Kurtosis
PH/ cm	FY	89.44 ± 4.12	69.63 - 103.13	4.60	-1.07	1.63
	HX	92.32 ± 3.47	73.53 - 100.68	3.76	-0.10	1.90
TN	FY	6.8 ± 2.1	3.9 - 10.3	30.70	0.16	-0.02
	HX	8.5 ± 2.4	5.3 - 16.9	28.69	2.03	4.22
PL/ cm	FY	17.71 ± 1.29	15.33 - 19.86	7.28	-0.28	-0.42
	HX	17.63 ± 1.34	15.94 - 19.23	7.60	0.03	-1.20
PB	FY	14.95 ± 1.54	12.93 - 18.67	10.30	0.76	0.74
	HX	14.60 ± 1.79	12.33 - 17. 67	12.26	0.47	-0.31
SB	FY	36.20 ± 8.16	20.00 - 54.20	22.53	0.34	1.46
	HX	37.94 ± 7.18	24.93 - 50.53	21.61	0.31	-0.14

GL/ mm	FY	7.50 ± 0.13	7.16 - 7.85	1.73	0.18	-0.9
	HX	7.64 ± 0.14	7.12 - 8.04	1.83	-0.25	0.58
GW/ mm	FY	3.67 ± 0.10	3.43 - 4.21	2.72	0.46	0.03
	HX	3.74 ± 0.09	3.29 - 4.14	2.41	0.24	0.53
TGW/ g	FY	25.89 ± 1.15	22.60 - 30.10	4.44	0.15	-0.50
	HX	25.36 ± 1.15	20.40 - 32.10	4.53	0.54	1.75
SR/ %	FY	89.69 ± 3.46	61.77 - 96.08	3.86	-2.83	11.18
	HX	87.69 ± 4.06	69.09 - 95.88	4.63	-1.31	0.95
YD/ g/m^2	FY	1005 ± 160	472 - 1715	15.95	0.30	1.72
	HX	1362 ± 175	787 - 2716	12.89	1.84	3.60

Note: Data represents plant height (PH), tiller number (TN), panicle length (PL), number of primary branches (PB), number of secondary branches (SB), grain length (GL), grain width (GW), thousand-grain weight (TGW), seed-setting rate (SR), yield per plot (YD), Environment (Env), Fengyang (FY), Hexian (HX), standard deviation (SD), and coefficient of variation (CV).

Among grain characteristics, SX839 (7.85 ± 0.12 mm), NG5718 (7.85 ± 0.12 mm), NXG9 (7.85 ± 0.10 mm), and FN628 (7.83 ± 0.12 mm) had the greatest grain lengths while WKN2 (7.16 ± 0.11 mm) had the lowest (Figures 1c and 3a). For grain width, NG5718 (4.02 ± 0.15 mm) had the highest and YGN6 (3.42 ± 0.12 mm) the lowest (Figures 1d and 3b). The highest thousand-grain weight was for NG5718 (30.10 ± 2.10 g) while XH13 (22.60 ± 0.42 g) had the lowest (Figure 3c). For seed-setting rate, ND398 (96.07 ± 1.08%) and ZJN1 (96.08 ± 1.08%) had the highest while XH13 (61.77 ± 4.36%) had the lowest (Figure 3d).

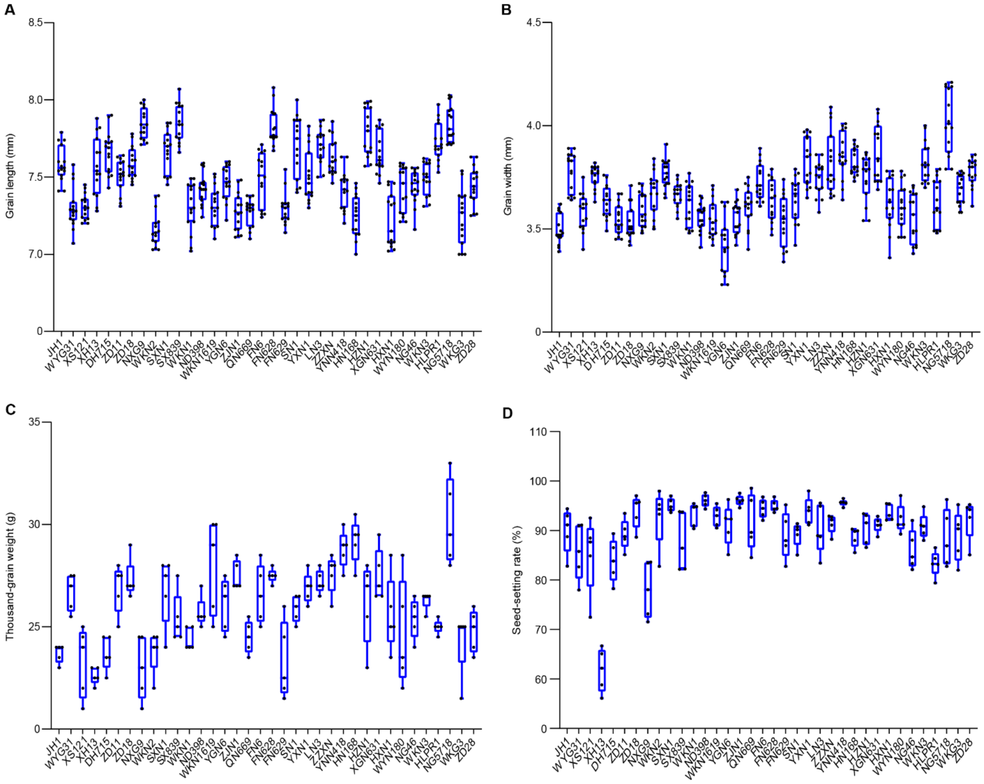

Figure 3. Box plot showing variation in agronomic traits among the 36 japonica cultivars grown in Fengyang. (a) grain length, (b) grain width, (c) thousand-grain weight, and (d) seed-setting rate. The box plot represents the median (line), 25-75 quartiles (boxes), and individual data points (dots).

3.1.2. Variation in Agronomic Traits in Hexian Rice Fields

In Hexian, the 36 japonica cultivars exhibited distinct variations in agronomic traits (Figures 4–6). Cultivars WKN2, HN168, QN669, NG46, and YXN1 had the greatest plant heights, with means of 100.67 ± 4.28, 100.07 ± 3.75, 99.73 ± 3.35, 99.40 ± 3.66, and 99.17 ± 3.03 cm, respectively. The shortest plants were for FN629 with 73.53 ± 2.86 cm (Figure 5a). Tiller numbers varied significantly among cultivars (Table 1), with the greatest for HLPR1 (16.9 ± 2.3), WKG3 (15.6 ± 2.3), and ZD28 (15.2 ± 3.0) while WYG31 (5.3 ± 1.4) and XGN631 (5.7 ± 2.6) had the least (Figure 5b). Panicle length was greatest for, NXG9 (19.22 ± 1.52 cm), ZD11 (19.15 ± 1.16 cm), SXN1 (19.14 ± 1.53 cm), WKN3 (19.14 ± 1.36 cm), and SN1(19.07 ± 1.25 cm) while JH1 (15.94 ± 1.15 cm) and HLPR1 (15.96 ± 1.23 cm) had the least (Figures 4a and 5c). The greatest number of primary branches was for HLPR1 (17.67 ± 2.00), NXG9 (16.67 ± 1.88), HN168 (16.53 ± 2.33), XGN631 (16.53 ± 2.61), WKG3 (65.20 ± 1.33), and SX839 (16.07 ± 1.44), while SXN1 (12.33 ± 1.65) had the least (Figures 4b and 5d). Cultivar WKN3 (50.53 ± 9.59) and SN1 (50.07 ± 7.35) had the highest number of secondary branches while HN168 (24.93 ± 7.75) and JH1 (27.93 ± 5.30) had the least (Figure 5e). Yield per plot was greatest for WKG3 (2716 ± 189 g/m^2) and HLPR1 (2701 ± 144 g/m^2) but least for ZD18 (787 ± 198 g/m^2) (Figure 5f).

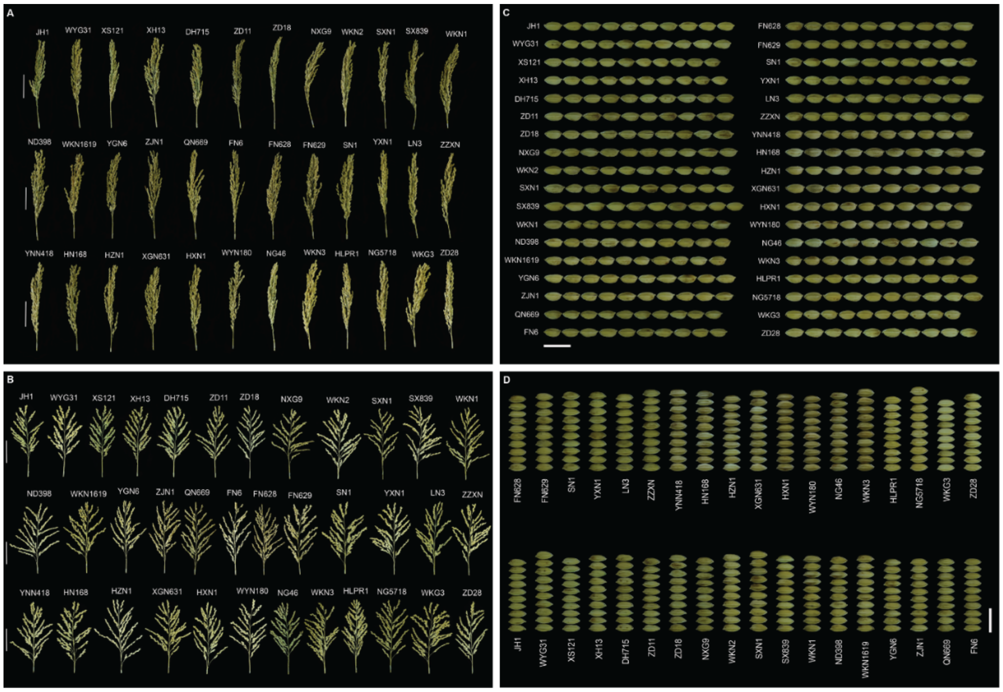

Figure 4. Variation in morphological traits among the 36 japonica cultivars grown in Hexian. (a) panicle length, (b) number of primary branches; Scale bar, 5 cm. (c) grain length, and (d) grain width; Scale bar, 1 cm.

Grain length was greatest for SX839 (8.04 ± 0.19 mm) and HN168 (8.01 ± 0.07 mm) whereas WKG3 (7.12 ± 0.22 mm) and XS121 (7.19 ± 0.11 mm) had the least (Figures 4c and 6a). However, grain width was greatest for NG5718 (4.14 ± 0.08 mm), XGN631 (4.07 ± 0.06 mm), and YNN418 (4.01 ± 0.07 mm) while WKG3 (3.34 ± 0.11 mm) had the least (Figures 4d and 6b). Thousand-grain weight was greatest for NG5718 (32.00 ± 1.46 g) but least for XH13 (20.40 ± 0.65 g) (Figure 6c). Seed-setting rate was highest in NG5718 (95.88 ± 2.87%) and SXN1 (95.80 ± 2.71%), and lowest in WKN2 (69.09 ± 5.70%) among the cultivars (Figure 6d).

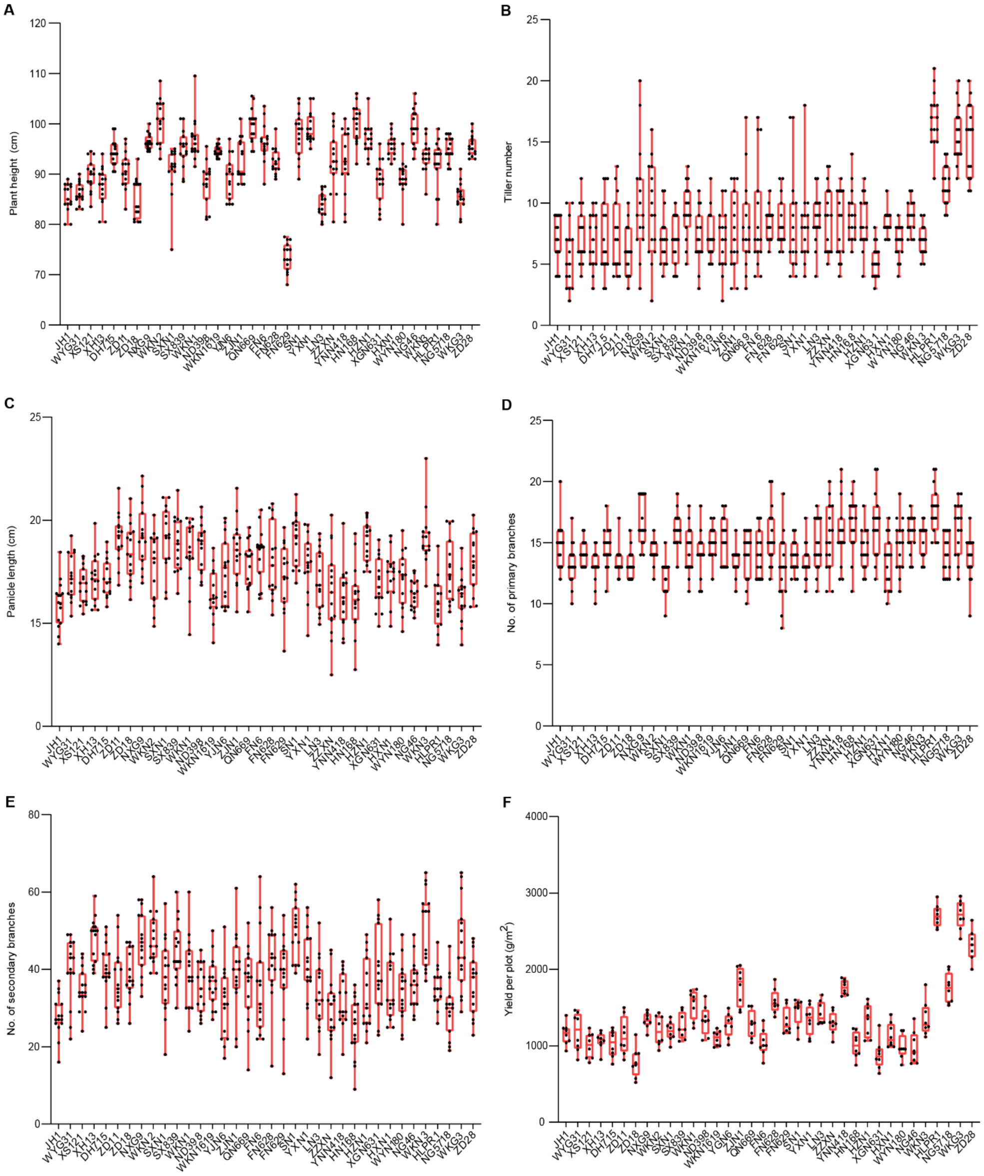

Figure 5. Box plot showing variation in agronomic traits among the 36 japonica cultivars grown in Hexian. (a) plant height, (b) tiller number, (c) panicle length, (d) number of primary branches, (d) number of secondary branches, and (f) yield per plot. The box plot represents the median (line), 25-75 quartiles (boxes), and individual data points (dots).

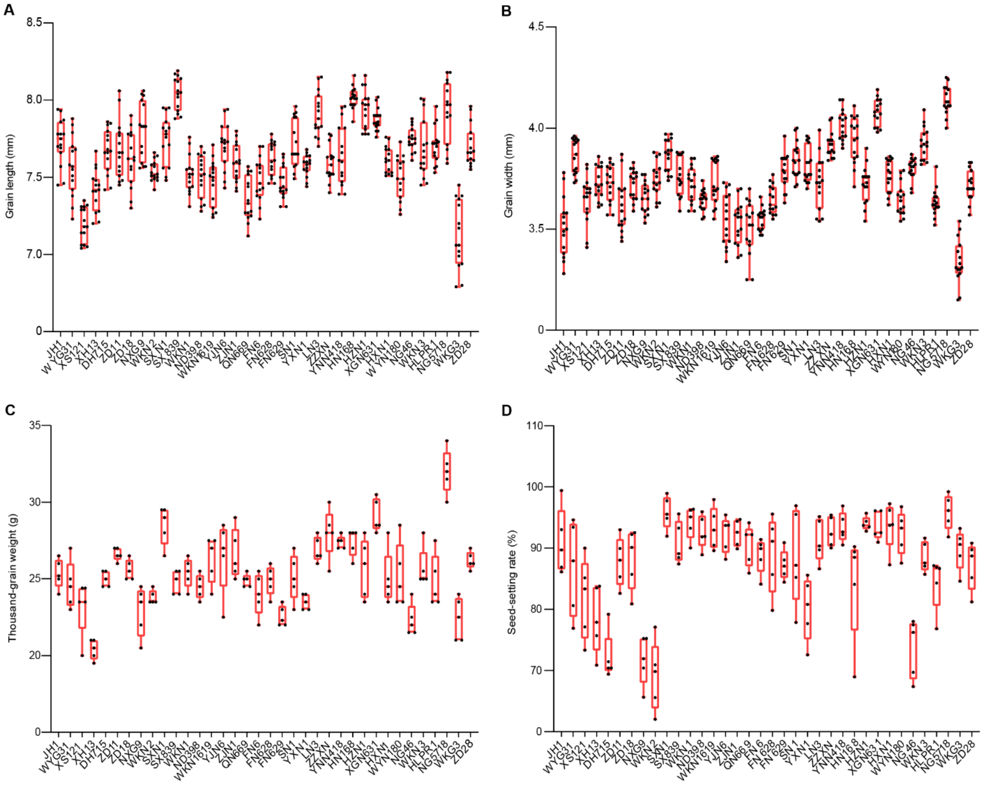

Figure 6. Box plot showing variation in agronomic traits among the 36 japonica cultivars grown in Hexian. (a) grain length, (b) grain width, (c) thousand-grain weight, and (d) seed-setting rate. The box plot represents the median (line), 25-75 quartiles (boxes), and individual data points (dots).

3.2. Trait Variation and Distribution Patterns in Fengyang and Hexian

A statistical summary of agronomic traits across the 36 japonica cultivars grown in Fengyang and Hexian is presented in Table 1. The average mean values indicated that GL and GW showed high uniformity in both sites, shown by their low coefficient of variations (CVs) (1.73%-2.72%). In contrast, TN (28.69%-30.70%) and SB (21.61%-22.53%) exhibited the highest CVs, indicating substantial variation among cultivars. Skewness for most traits was between -0.5 and 0.5 (Table 1) in both environments, showing an approximate symmetry except for PL (-1.07) and SR (-2.83) in Fengyang, which showed obvious left skewness, suggesting a concentration of higher mean values in these traits. In Hexian, TN (2.03) and YD (1.84) showed right skewness, indicating more cultivars with lower mean values in these traits. The kurtosis coefficient for most traits exhibited moderate deviation from zero. However, SR in Fengyang (11.18) and TN in Hexian (4.22) showed a heavily tailed distribution, indicating the presence of outliers.

3.3. Comparative Analysis of Agronomic Traits Between Fengyang and Hexian Rice Fields

The agronomic traits exhibited variation between Fengyang and Hexian across the 36 japonica cultivars (Tables 2 and S2). Overall, **52.8%** of the cultivars exhibited significant differences in PH, with a higher proportion of cultivars showing higher significant differences in Hexian (**36.11%**) than in Fengyang (**16.66%**). Similarly, TN showed significant differences in **44.44%** of the cultivars, with **38.89%** of significant differences observed in Hexian and **5.55%** in Fengyang. Trait PL exhibited significant differences in **30.77%** of cultivars, with **19.44%** and **11.11%** of significant differences in Fengyang and Hexian, respectively. Traits PB and SB showed lower levels of significant differences, with **12.12%** and **25%** of the cultivars affected, respectively. Notably, more significant differences in SB were observed in cultivars in Hexian (**19.44%**) compared to Fengyang (**5.55%**). Traits GL and GW displayed significant differences of **61.11%** and **72.22%**, respectively. The highest significant differences in GL were in Hexian (**52.78%**) compared to Fengyang (**8.33%**) and GW also showed higher significant differences in Hexian (**44.44%**) than Fengyang (**27.78%**). For TGW, significant differences were observed in 36.11% of cultivars, with 25.00% of significant differences in Fengyang and 11.11% in Hexian. Trait SR displayed significant differences in 22.22% of cultivars, with Fengyang (16.67%) having slightly higher significant differences than Hexian (5.55%). Trait YD showed significant differences in 61.11% of cultivars, with higher significant differences in Hexian (50.00%). The *P*-values for all traits ranged within **0.0001-0.0492**, confirming the environmental influence on trait performance between cultivars in Fengyang and Hexian.

Table 2. Proportional analysis of significant differences in agronomic traits in Fengyang and Hexian.

Traits	No. of Cultivars	Sig. Diff. (%)	FY (%)	HX (%)	*P* - Value Range
PH	36	52.77	16.66	36.11	0.0001 < 0.0460
TN	36	44.44	5.55	38.89	0.0001 < 0.0455
PL	36	30.77	19.44	11.11	0.0001 - 0.0443
PB	36	12.12	8.33	2.78	0.0004 - 0.0473
SB	36	25.00	5.55	19.44	0.0001 < 0.0362
GL	36	61.11	8.33	52.78	0.0001 < 0.0126
GW	36	72.22	27.78	44.44	0.0001 < 0.0448
TGW	36	36.11	25.00	11.11	0.0004 - 0.0393
SR	36	22.22	16.67	5.55	0.0009 - 0.0492
YD	36	61.11	11.11	50.00	0.0001 < 0.0165

Note: Data shows plant height (PH), tiller number (TN), panicle length (PL), number of primary branches (PB), number of secondary branches (SB), grain length (GL), grain width (GW), thousand-grain weight (TGW), seed-setting rate (SR), yield per plant (YD), significant difference (Sig. Diff.), Fengyang (FY), Hexian (HX) and *P* – value ($P < 0.05$).

3.4. Trait Relationships Between Fengyang and Hexian

We next analyzed the relationships between agronomic traits across the 36 japonica cultivars grown in Fengyang and Hexian (Table 3). The results from Fengyang showed strong positive correlations of PH with TN (0.65), PL (0.50), and YD (0.47), indicating taller cultivars with higher TN, PL, and YD. Trait TN was also positively correlated with YD (0.57), indicating that higher TN contributed to higher YD. Traits PH (0.47), TN (0.57), PL (0.40), SB (0.44), and SR (0.35) were positively correlated with YD, suggesting their influence on YD in this environment. Trait TGW was positively correlated with GW (0.48) and SR (0.48) but not significantly correlated with YD (0.07). In Fengyang, agronomic traits such as PB (0.07), GL (-0.07), and GW (-0.14) showed no significant correlations with YD and other key traits, indicating their limited contribution to YD.

In Hexian, there was a strong positive correlation between TN and YD (0.87), indicating the critical role of TN in YD. Trait PL was positively correlated with SB (0.46), indicating that higher PL contributed to higher SB. In contrast, PL was negatively correlated with PB (-0.40), indicating a trade-off between these traits in Hexian. Trait TGW was positively correlated with GL (0.53) and GW (0.53), indicating interdependence of grain size traits. Trait SR was positively correlated with TGW (0.59) but negatively with SB (-0.40), suggesting that higher SR contributes to higher TGW but lower SB. There were no significant correlations between YD and PH (-0.02), PL (-0.13), PB (0.29), SB (0.08) GL (-0.12), GW (-0.23), TGW (0.03), and SR (0.14), indicating that these traits did not contribute to YD in Hexian.

Table 3. Relationship between agronomic traits in Fengyang and Hexian.

Traits	Env.	PH	TN	PL	PB	SB	GL	GW	TGW	SR	YD
PH	FY	-									
	HX	-									
TN	FY	0.65****	-								

	HX	0.15	-								
PL	FY	0.50***	0.27	-							
	HX	0.20	-0.23	-							
PB	FY	0.06	-0.21	-0.18*	-						
	HX	0.19	0.35*	-0.40*	-						
SB	FY	0.08	0.14	0.62****	0.11	-					
	HX	0.07	-0.01	0.46**	-0.06	-					
GL	FY	-0.05	-0.20	0.01	0.02	-0.20	-				
	HX	0.14	-0.09	0.19	0.27	-0.22	-				
GW	FY	-0.24	-0.25	-0.26	0.17	-0.11	0.23	-			
	HX	0.14	-0.21	-0.03	0.01	-0.09	0.51***	-			
TGW	FY	0.04	-0.22	-0.09	0.14	-0.38*	0.15	0.48**	-		
	HX	0.10	-0.06	0.03	0.05	-0.49***	0.53***	0.53***	-		
SR	FY	0.18	0.08	-0.01	0.02	-0.25	-0.22	-0.04	0.48**	-	
	HX	-0.25	-0.11	0.08	-0.12	-0.40*	0.09	0.09	0.59***	-	
YD	FY	0.47**	0.57***	0.40*	0.07	0.44**	-0.07	-0.14	0.07	0.35*	-
	HX	-0.02	0.87****	-0.13	0.29	0.08	-0.12	-0.23	0.03	0.14	-

Note: Data represents plant height (PH), tiller number (TN), panicle length (PL), number of primary branches (PB), number of secondary branches (SB), grain length (GL), grain width (GW), thousand-grain weight (TGW), seed-setting rate (SR), yield per plot (YD), Environment (Env), Fengyang (FY), Hexian (HX), no significance (ns), significant at 5% level (*), significant at 1% level (**), significant at 0.1% level (***), and significant at 0.01% level (****).

3.5. PCR Analysis of Japonica Cultivars

To study the genes linked with these agronomic traits, seven SSR markers were used in this study: *Ghd7* for heading date; *Wx*, and *Chalk5* for grain quality; *COLD1*, and *DST* for stress tolerance; *Xa13*, and *Bph6* for disease resistance; and an EST marker *DEP1* for grain yield. The PCR amplification and gel electrophoresis revealed distinct band patterns across the 36 japonica cultivars, while the indica cultivars exhibited variation in some genes (Figure 7a–h). There were consistent band locations among most japonica cultivars at the expected fragment sizes for each gene: *DEP1* (694 bp) (Figure 7a), *Ghd7* (161 bp) (Figure 7b), *Wx* (124 bp) (Figure 7c), *Chalk5* (217 bp) (Figure 7d), *COLD1* (200 bp) (Figure 7e), *DST* (113 bp) (Figure 7f), *Xa13* (111 bp) (Figure 7g), and *Bph6* (238 bp) (Figure 7h and Supplementary Figures S3–S10). However, gene *Wx* was absent from japonica rice cultivar LN3, and two indica cultivars (HHZ and YD6) showed band variation (Figures 7c and S5), suggesting a null allele and an allelic difference in *Wx* between japonica and indica rice cultivars. Similarly, *DST* was absent for japonica cultivars JH1 and XH13 (Figures 7f and S8), indicating that they may lack *DST* alleles or carry a non-functional variant. Japonica cultivar WKN2 showed no amplification of *Xa13* (Figures 7g and S9), suggesting absence of a *Xa13* allele, which is associated with bacterial blight resistance. Gene *Bph6* was also absent from japonica cultivar ZD28 (Figures 7h and S10), indicating lack of a *Bph6* allele, which confers resistance to brown planthopper. Interestingly, japonica cultivar HZN1 showed band variation in *COLD1* similar to indica cultivars (Figures 7e and S7), suggesting that the function of the *COLD1* allele in HZN1 may be similar to that in indica cultivars. The indica cultivars displayed variation in band pattern for *DEP1* (Figure 7a), *Ghd7* (Figure 7b), *Wx* (Figure 7c), *COLD1* (Figure 1e), and *Xa13* (Figure 7g and Supplementary Figures S3–S5, S7 and S9).

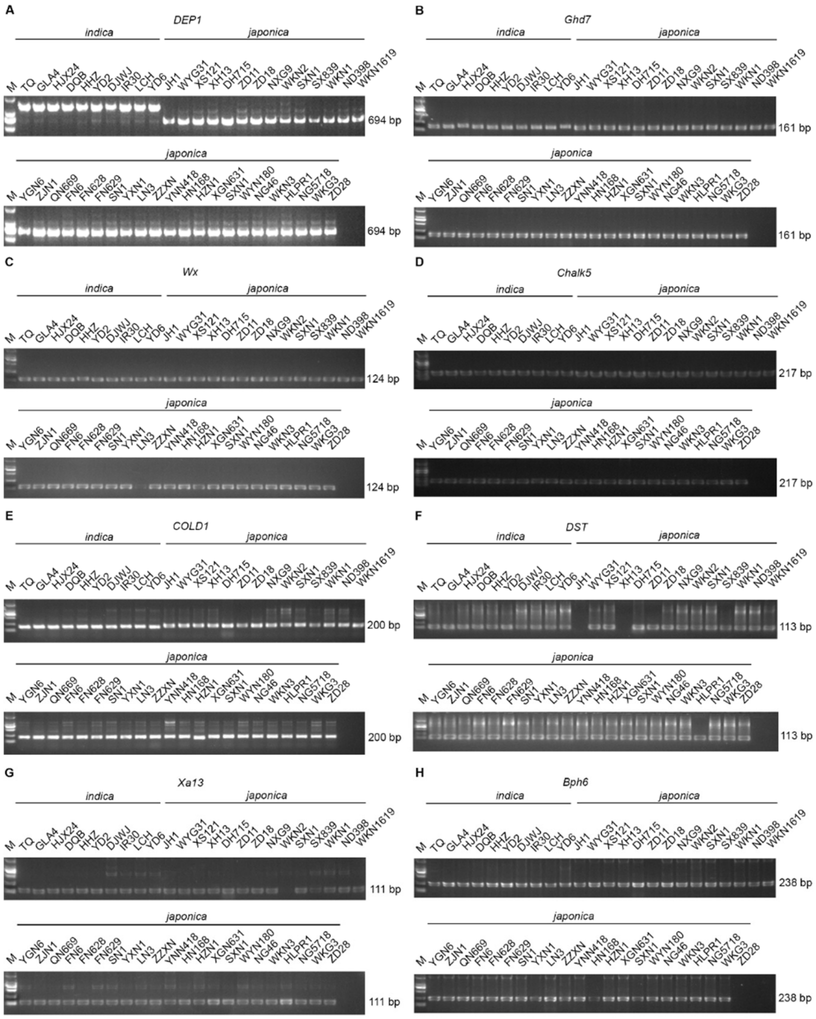

Figure 7. A representative gel picture depicting PCR amplification of key rice genes in 36 japonica cultivars and 10 indica cultivars. PCR product of (**a**) *DEP1* gene, (**b**) *Ghd7* gene, (**c**) *Wx* gene, (**d**) *Chalk5* gene, (**e**) *COLD1* gene, (**f**) *DST* gene, (**g**) *Xa13* gene, and (**h**) *Bph6* gene in the 36 japonica cultivars and 10 indica cultivars: TQ, GLA4, HJX24, DQB, HHZ, YD2, DJWJ, IR30, LCH, and YD6. M represents 1000 bp DNA marker.

4. Discussion

The Jianghuai region is the main japonica rice cultivation zone in Anhui Province, with Fengyang tending to have slightly more sunshine hours and Hexian to receive more rainfall [31]. These climatic differences influence the type of rice cultivars suitable for each environment. Our findings of agronomic performance and gene detection in the japonica cultivars grown in Fengyang and Hexian confirmed the influence of environmental conditions on trait expression, consistent with previous findings [32–35]. Our results clearly showed significant differences among cultivars, with traits like grain width, grain length, yield per plot, and plant height having the highest significant differences (Tables 2 and S2). There were strong positive correlations between tiller number and yield per plot in both Fengyang and Hexian (Table 3), while gene identification showed minimal band variation among the 36 japonica cultivars (Figures 7 and S3–S10).

4.1. Analysis of Trait Variability and Distribution

In this study, cultivars like WKG3 and HLPR1 recorded the highest yield per plot (2716. ± 189 and 2701 ± 144 g/m^2, respectively) and tiller number (15.6 ± 2.3 and 16.9 ± 2.3, respectively) in Hexian (Figure 5f), compared to Fengyang where WKG3 and HLPR1 displayed low yield per plot (832 ± 155 and 545 ± 133 g/m^2, respectively) and tiller number (4.9 ± 1.0 and 4.1 ± 1.3, respectively) (Figure 2f). This suggests that the higher rainfall and warmer climate in Hexian [31] enhanced vegetative growth and promoted higher tillering and yield. In contrast, FN6 recorded the highest yield per plot in Fengyang (1715 ± 106 g/m^2) (Figure 2f) but only moderate yield in Hexian (1036 ± 171 g/m^2) (Figure 5f). This suggests that FN6 may be suited for the slightly longer sunshine hours and reduced rainfall conditions in Fengyang [31]. Cultivar FN628 showed balanced performance in Fengyang and Hexian for traits like plant height (95.27 ± 2.68 and 92.47 ± 2.60 cm, respectively) (Figures 2a and 5a), tiller number (7.3 ± 2.1 and 8.4 ± 1.3, respectively) (Figures 2b and 5b), panicle length (18.31 ± 1.50 and 18.01 ± 1.80 cm, respectively) (Figures 1a and 2c) (Figures 4a and 5c), number of primary branches (15.87 ± 1.46 and 15.60 ± 2.41, respectively) (Figures 1b and 2d) (Figures 4b and 5d) and yield per plot (1478 ± 186 and 1579 ± 159 g/m^2, respectively) (Figures 2f and 5f). This indicates a balanced and adaptable cultivar suited for diverse growing conditions.

The CVs showed differences in trait stability among cultivars grown at both sites (Table 1). Compared to other traits, TN, SB, and YD displayed higher variability in both environments, suggesting that these traits are more environmentally sensitive and genetically influenced [13,36]. The higher CVs for these traits could result from influence of environmental factors such as soil type, temperature fluctuation, and water availability [37]. However, GL and GW showed the lowest CVs (Table 1), suggesting that these traits were relatively stable in both environments. The lower variability in most agronomic traits in Fengyang and Hexian suggests more consistent growing conditions, resulting in more uniform plant growth and development [35]. The skewness and kurtosis values further showed that most traits exhibited approximate symmetry (Table 1), indicating a normal distribution with no extreme deviations. However, there were exceptions for cultivars in Fengyang, where PH and SR exhibited negative skewness. This suggests that majority of the cultivars had higher values for these traits, with fewer cultivars exhibiting lower values. These trends suggest potential limitations of the adaptability of certain cultivars to the environmental conditions in Fengyang [38,39]. In Hexian, tiller number and yield per plot exhibited positive skewness, indicating that fewer high-performing cultivars contributed to the overall distribution, while the majority had lower values. Kurtosis values for most traits showed moderate deviation from zero. However, SR in Fengyang showed high kurtosis, suggesting the presence of extreme values and potential outliers within the cultivars. These findings align with a previous study [40] that suggested the need for evaluating cultivars for location-specific breeding strategies to identify stable and high-yielding cultivars.

4.2. Influence of Environment on Trait Expression

We also observed significant differences in agronomic traits between cultivars grown in each environment, highlighting the influence of environmental conditions on cultivar performance [41,42]. Traits PH and TN were significantly greater in Hexian compared to Fengyang (Table 2), suggesting that environmental factors such as soil composition and climatic conditions have a stronger influence on these traits in this environment. This is consistent with studies [43,44] suggesting that plant height and tiller number are highly responsive to environmental variations including temperature and soil compositions. Grain-related traits like GL and GW also showed high sensitivity to environmental conditions in Hexian, likely due to temperature and nutrient availability during grain-filling stages. Similar reported findings emphasize the impact of environmental factors on grain development [33,45]. Trait YD was significantly higher in Hexian than in Fengyang (Table 2), indicating that yield-related traits are more influenced by location-specific factors such as soil fertility and management practice [46]. Other traits, such as PL and TGW, showed lower values but notable significant differences, in Fengyang. Several studies have shown that environmental conditions such as temperature, soil type, and water availability significantly affect rice agronomic traits, leading to variation in cultivar performance across different growing conditions [13,47]. The observed significant differences across multiple traits emphasize the importance of genotype-environment interactions in determining cultivar performance [32,41].

4.3. Correlation Analysis

Correlation analysis is important in the indirect selection for high yield in genotypes [48]. The relationship between cultivars grown in Fengyang and Hexian will provide insight into how agronomic traits perform across these two environments. In Fengyang, PH showed strong positive correlations with TN, PL, and YD (Table 3). This suggests that taller cultivars with greater number of tillers and longer panicles contribute to higher yield per plot, similar to previous studies that linked plant height with tiller number to increase biomass and grain production in an environment with favorable growing conditions [49]. The positive correlation between TN and YD highlights the importance of tiller formation in yield improvement as observed in other japonica cultivars [44,50]. Traits PL, SB, and SR were also positively correlated with YD, indicating their roles in enhancing yield in japonica cultivars. These results align with studies on the influence of longer panicles and seed-setting rates on increased grain filling and yield stability in an environment with adequate water and nutrient availability [51,52]. In contrast, GL, GW, and TGW showed no significant correlations with YD, suggesting that grain size may not directly contribute to yield, as reported in similar environments where yield is mainly influenced by panicle size and tiller number [16,53].

Trait TN exhibited a strong positive correlation with YD in Hexian, highlighting its critical role in rice yield (Table 3). This finding is similar to studies that have linked tiller number as the key determinant of yield in high-yielding rice cultivars, particularly in environments with moderate stress levels [54]. Trait PL was positively correlated with SB, suggesting that longer panicles are associated with increased numbers of secondary branches, which may enhance grain production. However, the negative correlation between SB and TGW indicates a trade-off between branch formation and grain weight, similar to other rice-growing regions with similar environmental constraints [55]. This trade-off suggests that while increased numbers of secondary branches may contribute to yield potential, this may also reduce grain weight, highlighting the need for balanced trait selection in rice breeding. Trait TGW was positively correlated with GL and GW, indicating interdependence of these traits [55]. Additionally, SR was positively correlated with TGW but negatively with SB, suggesting that a higher seed-setting rate is associated with higher grain weight but reduced numbers of secondary branches. These findings help us to understand the complexity of trait interactions in rice yield formation, particularly in environments where environmental factors play a critical role in rice growth and development [13].

4.4. Molecular Characterization and Gene Detection

The PCR results highlighted the influence of rice genes among key traits including grain yield, heading date, grain quality, stress tolerance, and disease resistance. Genes *DEP1* and *Ghd7* play important roles in regulating grain yield and heading date in rice. Gene *DEP1* is associated with dense and erect panicles and influences rice yield. Gene *DEP1* was amplified at a 694-bp band in all 36 japonica cultivars (Figures 7a and S3) which aligns with previous findings [23], indicating the presence of the *DEP1* mutant allele which is widely utilized in japonica breeding programs. This suggests its contribution to higher tiller number and grain yield [23]. However, the indica cultivars displayed a slightly higher band size, suggesting a different allele, leading to less dense panicles and lower grain yield [56]. Similarly, *Ghd7*, which regulates heading date, was amplified at 161 bp in japonica cultivars (Figures 7b and S4), suggesting a similar maturity rate among these cultivars [24]. This is consistent with their adaptation to temperate regions with predictable growing seasons [57]. However, *Ghd7* displayed higher bands in some indica cultivars (HJX24 and YD6), indicating alleles linked to delayed flowering, which is advantageous in long-day environmental conditions [58,59]. Grain quality traits in rice including appearance, size, and milling quality are important traits in the marketing and consumption of rice [60]. Genes *Wx* and *Chalk5* are two key genes regulating grain quality. Gene *Wx* regulates amylose content at 124 bp in both japonica and indica cultivars (Figures 7c and S5), suggesting a low amylose content (≤ 20) consistent with the *Wxb* allele resulting in soft and sticky texture [61]. However, slightly higher bands in two indica cultivars (HHZ and YD6) suggest allelic variations that may elevate amylose levels while the absence of amplification in japonica cultivar LN3 suggests a null allele or structural deletion [25]. Gene *Chalk5*, known to affect grain chalkiness, was amplified at 217 bp in both japonica and indica cultivars (Figures 7d and S6), indicating no observable variation across all rice accessions. Unlike previous reports which link *Chalk5* polymorphism to increased chalkiness in indica cultivars [62], this uniformity suggests that both subspecies share a common allele at this locus, which is associated with high gene expression and reduced chalkiness, as observed in japonica cultivars with improved grain quality [63]. Chinese breeders selected this gene to eliminate chalkiness in rice for grain quality improvement [60]. Genes *COLD1* and *DST* are regulatory and can be used to develop rice cultivars more resistant to cold, drought, and salinity [64]. Gene *COLD1* confers chilling tolerance in rice through a critical single nucleotide polymorphism (SNP2), which differentiates phenotype between japonica and indica cultivars [27]. In our study, *COLD1* was amplified at 200 bp in all japonica cultivars (Figures 7e and S7), consistent with its role in enhancing cold adaptation by increasing RGA1 GTPase activity and triggering Ca^{2+} influx under cold stress, a trait selected during japonica rice domestication of Chinese *Oryza rufipogon* for colder environments [27]. However, japonica cultivar HZN1 showed a slightly lower band pattern similar to indica cultivars. This suggests the presence of a *COLD1* allele in these cultivars, encoding methionine or threonine, which is linked to reduced chilling tolerance due to weaker RGA1 activation and reduced Ca^{2+} signaling [65]. Similarly, *DST*, which regulates drought and salt tolerance in rice, was amplified at 113 bp in both indica and japonica cultivars (Figures 7f and S8), suggesting that stress tolerance differences arise from sequential level differences rather than structural changes. Notably, the absence of amplification in JH1 and XH13 indicates a potential deletion, which could increase drought and salt tolerance by reducing *DST* function. This can lead to stomatal closure, thereby improving tolerance to drought and salt as observed in dst mutants [28]. Also, genes like *Xa13* and *Bph6* confer resistance to bacterial blight and planthopper disease. Gene *Xa13* was amplified at 113 bp in both japonica and indica cultivars (Figures 7g and S9). This confirms the presence of promoter mutation leading to expression of *Xa13* and confers resistance to *Xanthomanas oryzae* pv. *oryzae*. However, the absence of amplification in WKN2 indicates complete deletion or a null allele, which suggests susceptibility due to lack of *Xa13*-mediated resistance [29]. Similarly, *Bph6* was amplified at 238 bp in both subspecies (Figures 7h and S10), indicating the presence of a functional *Bph6* encoding an exocyst-localized protein [30][66], suggesting resistance to brown planthopper. In contrast, its absence in ZD28 suggests a non-functional *Bph6* due to a null allele or deletion.

5. Conclusions

Gene detection and agronomic performance of 36 japonica rice cultivars in Fengyang and Hexian rice fields showed that cultivar FN628 exhibited exceptional balance and adaptability, and achieved a consistent moderate to high plant height, tiller number, panicle length, number of primary branches, and yield per plot in both environments. We also observed the presence of all eight key genes in FN628, making this cultivar suitable for breeding programs targeting stable performance in the Jianghuai region.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, Figure S1: Boxplot showing variation in yield per plant among the 36 japonica cultivars grown in Fengyang; Figure S2: Boxplot showing variation in yield per plant among the 36 japonica cultivars grown in Hexian; Figure S3: PCR amplification of *DEP1* gene in the 36 japonica cultivars and 10 indica cultivars; Figure S4: PCR amplification of *Ghd7* gene in the 36 japonica cultivars and 10 indica cultivars; Figure S5: PCR amplification of *Wx* gene in 36 japonica cultivars and 10 indica cultivars; Figure S6: PCR amplification of *Chalk5* gene in the 36 japonica cultivars and 10 indica cultivars; Figure S7: PCR amplification of *COLD1* gene in the 36 japonica cultivars and 10 indica cultivars; Figure S8: PCR amplification of *DST* gene in the 36 japonica cultivars and 10 indica cultivars; Figure S9: PCR amplification of *Xa13* gene in the 36 japonica cultivars and 10 indica cultivars; Figure S10: PCR amplification of *Bph6* gene in the 36 japonica cultivars and 10 indica cultivars; Table S1: Plant materials used in this study; Table S2: Comparison of agronomic traits between Fengyang and Hexian for each japonica cultivar; Table S3: Eight key genes and their corresponding primer sequence.

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