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ARTICLE

Preponderant alleles at *Hd1* and *Ehd1* lead to photoperiod insensitivity in *japonica* rice varieties

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Abstract: *Adaptation of photoperiod-sensitive japonica rice varieties from long-day (LD) to short-day (SD) conditions is impeded by their extremely early flowering in response to photoperiod change, but the genetic factor underlying this is still elusive. We detected mutations in Hd1 in Zhenjing2400 through gene mapping and sequencing analysis. Genome resequencing of the varieties Zhenjing2400 and Jiahe218 identified single nucleotide polymorphisms (SNPs) in the other flowering-related genes Ehd1, SDG725, OsCOL15, DTH2, and DTH7. We constructed recombinant inbred lines (RILs) derived from a cross between Zhenjing2400 and Jiahe218, and selected homozygous lines with these six genes. We established that photoperiod insensitivity is caused by a defective Hd1 gene. In addition, the effect of Hd1 and Ehd1 on heading date was stronger than that of the other four genes. Measurements of agronomic traits and quality traits in homozygous lines demonstrated the superiority of the hd1 ehd1 genotype for eating quality and photoperiod-insensitive high yield.*

Keywords: *Molecular breeding, Heading date, Yield- and quality-related indexes, Recombinant inbred lines, Photoperiod insensitivity*

INTRODUCTION

Heading date in rice and other cereals is affected by exogenous factors such as photoperiod, temperature and nutrient availability, among which photoperiod is a key factor. The molecular mechanisms of photoperiod-controlled flowering in rice have been intensively studied and largely deciphered. Two distinct pathways have been reported to regulate the expression of *Hd3a/RFT1* (*Heading date 3a/RICE FLOWERING LOCUS T 1*), two genes encoding florigen in rice (Turck et al. 2008). The first is the *GI-Hd1-Hd3a* (*GIGANTEA-Heading date1-Hd3a*) pathway, in which Hd1 receives signals from GI to affect the expression of *Hd3a*, to regulate flowering time (Dong et al. 2013, Lee et al. 2016). Recent studies have shown that the conversion of Hd1 function between LD and SD conditions depends on the activity of its interaction partners, Ghd7 (Grain number, plant height and heading date 7), DTH8 (Days To Heading on chromosome 8, also named Ghd8), and DTH7 (Zhang et al. 2019, Zong et al. 2021). The other photoperiodic flowering pathway is *Ehd1-Hd3a/RFT1* (*Early heading date1-Hd3a/RFT1*), which is unique to rice (Doi et al. 2004, Komiya et al. 2008). *Ehd1* is a type B response regulator that promotes flowering by inducing the expression of

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RFT1 and *Hd3a* under both LD and SD conditions (Doi et al. 2004). *Ehd1* expression can be up-regulated by *DTH3* (also named *OsSOC1* and *OsMADS50*) (Lee et al. 2004, Bian et al. 2011), *Ehd3* (Matsubara et al. 2011) and *Ehd4* (Gao et al. 2013), and down-regulated by *DTH8* (*DTH8*, also known as *Ghd8*) (Yan et al. 2011), *OsCOL4* (OsCONSTANS-like 4) (Lee et al. 2010), *OsCOL10* (Tan et al. 2016), *OsCOL15* (Wu et al. 2018), *Heme Activator Protein like 1* (*OsHAPL1*) (Zhu et al. 2017), and *SDG723/OsTrx1/OsSET33*.

China has a long history of rice cultivation and a broad range of rice cultivation regions (from 18° N to 53° N). Most *japonica* rice varieties suitable for planting in such locations are sensitive to photoperiod or temperature. Indeed, their heading date will be seriously shortened if grown at low latitudes under SD and high-temperature conditions, resulting in reduced yield. To solve this problem, breeders need to select photoperiod-insensitive varieties, so as to delay the heading date and improve the yield of *japonica* rice varieties to adapt to low-latitude environment. However, changing the heading date in rice may affect grain qualities (Cho et al. 2013). Amylose content (AC), Gel consistency (GC) and Gelatinization temperature (GT) are the key indexes of rice flour that affect eating and cooking quality (ECQ). GT is mainly controlled by the *Alkali degeneration* (*ALK*) gene encoding soluble starch synthase □a (SS□a) (Shimbata et al. 2012). AC and GC are mainly regulated by *Waxy* (*Wx*), which encodes granule-bound starch synthase. Few studies have explored the effect of heading date change on ECQ of rice varieties harboring the same alleles at the *ALK* and *Wx* loci.

In this study, the phenotypes of Zhenjing2400 were examined under LD and SD conditions and the key gene for photoperiod insensitivity was elucidated through gene mapping and genome resequencing of the varieties Zhenjing2400 and Jiahe218. Furthermore, allele typing of the major heading date genes and observation of agronomic traits and quality traits were examined in homozygous lines and the use of allele types of the major heading date genes for adaptation of *japonica* rice varieties from LD to SD conditions was discussed. The result of this study will provide valuable data and a new strategy for generating varieties more suited to different ecological areas.

MATERIAL AND METHODS

Plant materials and plant growth conditions

Jiahe218, Huaidao5, Zhendao18 and Zhendao21 are all *japonica* conventional cultivated rice varieties. Zhenjing2400 was selected from an M_2 population derived from treating rice breeding materials (Zhen68990) with methylnitrosourea (MNU). Zhenjing2400 was stabilized through selfing for at least 10 generations. All experiments were performed at the experimental farm of Zhenjiang in Jiangsu (ZJ, 31.9□N) and Lingshui in Hainan (LS, 18.5□N). The planting density was 16.5 cm×19.8 cm. Each *japonica* variety or line was planted with 5 rows with 10 holes in one row. Agronomic traits and quality traits were determined for 12 strains from each line to obtain the average of the line. The *Hd1* GenBank of 'Nipponbare' (*Oryza sativa* L) was Os06g0275000 and 'Shuhui498' (R498) (*Oryza sativa* L) was OsR498G0612090700.01. The *Hd1* gene sequencing was entrusted to Nanjing GenScript Biotechnology Co., Ltd.

Gene mapping and whole genome re-sequencing (WGS)

For genetic analysis, F_2 and $F_{2:3}$ populations were generated from the reciprocal crosses of Zhenjing2400 × Zhen68990 and Zhen68990 × Zhenjing2400. Photoperiod insensitivity was scored when the difference between heading date under LD and SD conditions was less than or equal to 0; a positive difference was considered an indication of photoperiod sensitivity. The leaves of Zhenjing2400 and Jiahe218 were sent to Novogene Co., LTD (www.novogene.com) to perform library construction and genome resequencing. Genetic variation was analyzed related to heading date between Zhenjing2400 and Jiahe218 using the software Notepad++. A total of 371 InDel markers discriminating between Zhenjing2400 and Jiahe218 were developed and designed to map the photoperiod-insensitive candidate gene (Supplemental Table 1). The coding regions of candidate genes within the mapping interval were amplified from Zhenjing2400, Zhen68990 and Jiahe218, sequenced for verification, and compared to the reference genomes from Nipponbare (http://rapdb.dna.affrc.go.jp/download/irgsp1.html) and R498 (http://www.mbkbase.org/R498/).

DNA extraction, PCR and molecular marker screening of RILs

Genomic DNA was extracted from fresh leaves of each line using the cetyltrimethylammonium bromide (CTAB) method. Genotyping tests were carried out with PARMS (Penta-primer amplification refractory mutation system) primer

sets commercially synthesized by Gentides Biotech Co., Ltd. (Wuhan, China). The composition of each PCR and PCR conditions followed the recommendations of PARMS manual for SNP detection.

RNA extraction and reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted from flag leaves in rice at heading date with an RNA Prep Pure Plant kit (Tiangen) following the manufacturer's instructions. Each RNA sample (2-µg) was reverse transcribed into first-strand cDNA using a QuantiTect reverse transcription kit (Qiagen). Quantitative PCR analysis was performed using an ABI7300HT fast real-time PCR system with SYBR Premix Ex Taq (TaKaRa; catalog no. RR041A). Rice *ACTIN* was used as an internal reference transcript. Primer pairs for *Hd1* were designed using Primer Express (Applied Biosystems) and are listed in Supplemental Table 2.

Determination of quality-related indexes

The harvested mature seeds were air-dried and then dehusked on a vertical hulling machine (FC2K, Otake, Japan) to obtain brown rice samples, which were subsequently polished using a grain polisher (VP-32, Yamamoto, Japan) to obtain milled whole rice kernels for quality-related index analysis. Milled rice samples (80g in weight) were sent to Huazhi Biotechnology Co., Ltd to determine amylose content (AC), gel consistency (GC), and gelatinization temperature (GT). The overall eating quality of the cooked rice was evaluated as previously described (Zhang et al. 2016).

Statistical analysis

Significant differences were determined by Student's *t* test analysis using GraphPad Prism 7.0 software. Duncan's multiple comparison test was performed by IBM SPSS Statistics 20 software. Sequences of *Hd1*, *Ehd1*, *SDG725*, *OsCOL15*, *DTH2* and *DTH7* were aligned between Zhenjing2400 and Jiahe218 by DANMAN 8.0 software. The structural elements of *Hd1*, *Ehd1*, *SDG725* and *OsCOL15* genes were identified using the online tool exon intron graphic maker (http://www.wormweb.org/exonintron). The daily average temperature records under SD and LD conditions are from the website https://lishi.tianqi.com/. Jurong City, Jiangsu Province corresponds to LD condition, while Lingshui Li Autonomous County, Hainan Province corresponds to SD condition.

RESULTS AND DISCUSSION

Performance of Zhenjing2400 under LD and SD conditions

We grew Zhenjing2400 and some *japonica* varieties at two latitudes: in Jiangsu with a subtropical climate and an LD photoperiod and in Sanya with a tropical climate and a SD photoperiod. The heading date of Zhenjing2400 was significantly different from that of other *japonica* varieties (Supplemental Table 3). The heading date of Jiahe218 was 105.20 days under LD and 90.60 days under SD conditions, indicating early flowering under SD condition (Supplemental Table 3). Similar situations also occurred in varieties Zhen68990, Huaidao5, Zhendao18 and Zhendao21, indicative of photoperiod sensitivity, while Zhenjing2400 flowered after 96.60 days under LD and 101.60 days under SD conditions, suggestive of photoperiod insensitivity (Supplemental Table 3).

We investigated the agronomic traits (Figure 1A and Supplemental Table 4) and quality-related traits (Figure 1B and Supplemental Table 5) of the two varieties, Zhenjing2400 and Jiahe218, under LD and SD conditions. The number of both primary and secondary rachis branches (PBN and SBN, respectively) in Jiahe218 was significantly lower under SD than under LD conditions (Supplemental Table 4). In addition, the number of spikelets per panicle (SPP), thousand-grain weight (TGW), and yield per plant of Jiahe218 were significantly lower under SD than under LD conditions; by contrast, we observed no significant differences in Zhenjing2400 between the two photoperiods (Figure 1A and Supplemental Table 4). Moreover, the overall

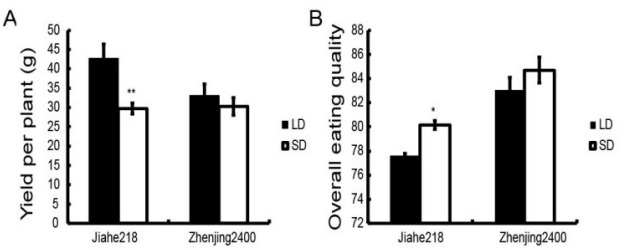

Figure 1. Yield per plant (A), and overall eating quality (B) for the cultivars Jiahe218 and Zhenjing2400 grown under LD and SD conditions. Data are means ± standard deviation, n represents 5 strains; **P* < 0.05, ***P* < 0.01, (Student's *t* test).

eating quality, and GC and AC values of Jiahe218 showed significant differences between SD and LD conditions, while we again detected no differences for Zhenjing2400 under the same conditions (Figure 1B and Supplemental Table 5). These results indicate that the agronomic and quality-related traits of Zhenjing2400, which was less sensitive to photoperiod than Jiahe218, are not affected by daylength.

Verification of photoperiod insensitivity gene in Zhenjing2400

Genetic analysis of reciprocal crosses between Zhenjing2400 and Zhen68990 showed that the photoperiod-insensitive phenotype of Zhenjing2400 is controlled by a single recessive nuclear locus (Supplemental Table 6). Molecular analysis of a segregating F_2 population and its derived $F_{2:3}$ population from the cross Zhenjing2400 × Jiahe218 allowed us to map the causal locus between markers C6-4 and C6-8 (Supplemental Tables 1 and 2) on chromosome 6. Notably, this interval contains the major flowering gene *Hd1*. We detected two single nucleotide polymorphisms (SNPs) and a 123-bp insertion in *Hd1* in Zhenjing2400 (Figure 2A). Only 123-bp insertion in the first exon of *Hd1* was observed in Zhenjing2400 compared with *Hd1* in Zhen68990 (Supplemental Figure 1). We also sequenced *Hd1* of some *japonica* varieties (Supplemental Figure 1). The results showed that the *Hd1* sequence of Zhenjing2400, Zhen68990 and Jiahe218 was more closely related to that of R498 and only the first exon of *Hd1* in Zhenjing2400 had 123-bp insertion among these varieties (Supplemental Figure 1). We designed two pairs of primers, QHd12 and QHd11 (Supplemental Table 7), to amplify the first or second exon of *Hd1*, respectively. Analysis by reverse transcription quantitative PCR (RT-qPCR) indicated that *Hd1* transcript levels are dramatically lower in Zhenjing2400 compared to Jiahe218 at the heading stage (Supplemental Figure 2). These results showed that the insertion of 123-bp in first exon of *Hd1* in Zhenjing2400 caused its photoperiod insensitivity.

By means of forward and reverse genetics, as well as population genetic analyses, 71 genes involved in photoperiodic flowering have been cloned in rice (Zhou et al. 2021). We sequenced the genomes of Zhenjing2400 and Jiahe218, which allowed us to assess sequence variation for all 71 cloned heading date genes in Zhenjing2400, followed by confirmation by Sanger sequencing of targeted PCR products. We detected polymorphisms in five genes in Zhenjing2400 when comparing their sequences to those in Jiahe218: *Ehd1*, *SDG725*, *OsCOL15*, *DTH2*, and *DTH7* (Figure 2). In Zhenjing2400, we observed a G-to-A SNP 655-bp in the coding sequence of *Ehd1* (Figure 2B), which was identical to that in Taichung 65. The non-synonymous SNPs in the coding sequences of *SDG725*, *OsCOL15*, *DTH2*, and *DTH7* were all from natural polymorphic variants between *indica* and *japonica* (Figure 2). For example, nine SNPs in *SDG725* and two SNPs in *OsCOL15* were shared with Nipponbare (Nip) in Zhenjing2400 and from R498 in Jiahe218 (Figure 2C and 2D). However, SNPs in the coding sequences of *DTH2* and *DTH7* originated from R498 in Zhenjing2400 and from Nip in Jiahe218 (Figure 2E and 2F).

Ehd1, a rice-specific flowering regulator and core signal integrator, is regulated by diverse activators and repressors. The expression levels of *Ehd1* in *SDG725*-RNA interference (RNAi) lines were significantly down-regulated under either SD or LD conditions (Sui et al. 2013). The SNP S7 of *SDG725* between Zhenjing2400 and Jiahe218 affects the region encoding the CW-type zinc-finger domain (Figure 2C). OsCOL15 was shown to have transcriptional activation activity, with the central region of the protein between the B-box and CCT domain being required for this activity (Wu et al. 2018). Notably, two SNPs between Zhenjing2400 and Jiahe218 in *OsCOL15* both located to the sequence encoding the region in between the B-box and CCT domains (Figure 2D). In addition, two FNPs (functional nucleotide polymorphisms) were described in *DTH2* that correlate with early heading (Wu et al. 2013). In our study, the SNP 1896-bp in the *DTH2* coding region was the same as the second FNP reported by Wu et al. (2013) and had the same allele as Nip, leading to early flowering. Ten SNPs were detected in *DTH7* between the varieties Kita-ake and PA64S and were shown to affect DTH7 function (Gao et al. 2014). The SNP 140-bp in the *DTH7* coding region between Zhenjing2400 and Jiahe218 was different from any of the 10 SNPs mentioned above.

Development of SNP markers and screening of homozygous RILs

We developed six SNP markers to genotype the six genes with polymorphisms in this study (Supplemental Table 7). Specifically, we based these markers on polymorphisms S2 of *Hd1*, S7 of *SDG725*, and S1 of *OsCOL15*. We performed genotyping using 502 lines from a RIL population constructed from a cross between Zhenjing2400 and Jiahe218 to identify homozygous lines harboring the Zhenjing2400 or Jiahe218 allele at each locus, in all possible combinations. The assay successfully discriminated between plants homozygous for either parental allele and heterozygous plants (Supplemental

Figure 3). In our study, we detected polymorphisms in six genes between Zhenjing2400 and Jiahe218, each gene thus being represented by two alleles, resulting in 64 different genotype combinations. The number of RILs carrying each of the 64 combinations of alleles varied, with several genotypes being represented by fewer RILs than other genotypes, possibly leading to errors in the role of single genes in the control of heading date. To remedy this issue, we grouped RILs into 16 combinations based on their genotypes to investigate their heading dates under LD conditions.

Among the four minor genes regulating heading stage, *SDG725* and *DTH2* promote flowering, while *OsCOL15* and *DTH7* delay flowering, and their associated phenotypes are not all affected by daylength (Sui et al. 2013, Wu et al. 2013, Gao et al. 2014, Wu et al. 2018). We therefore identified RILs for each of the 16 possible gene combinations and investigated their heading dates under LD conditions (Supplemental Table 8). We detected no significant difference in heading date between lines harboring the *Hd1* and *Ehd1* wild-type functional alleles (*Hd1 Ehd1*). In lines with the *Hd1 ehd1* (*Ehd1* being non-functional) genotype, we observed the earliest flowering time for the *SDG725 DTH2* (Nip) *oscol15 dth7* (R498) lines at 112.3 days, while the latest flowering times were observed for the *sdg725 dth2* (R498) *OsCOL15 DTH7* (Nip) lines with 119.8 days. When the *Hd1* allele was non-functional (*hd1 Ehd1*), RILs with the genotype *sdg725 dth2* (R498) *OsCOL15 DTH7* (Nip) flowered significantly later than the other three possible genotype combinations. When

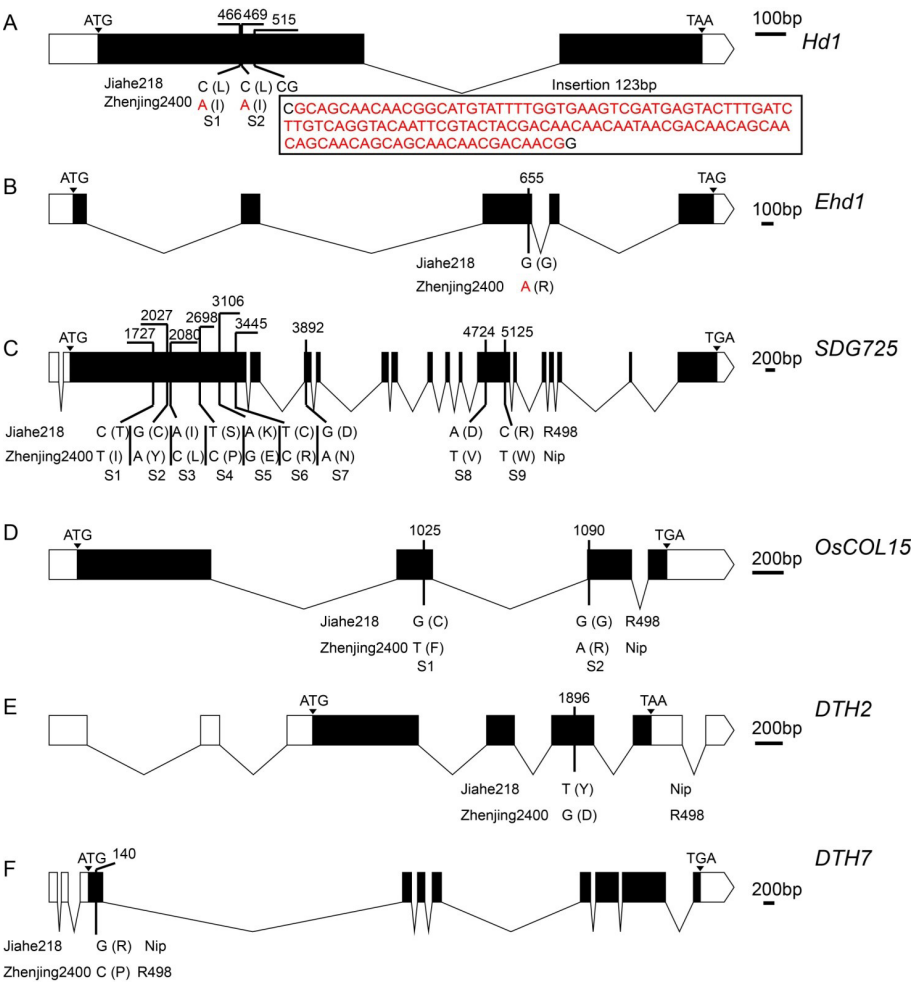

Figure 2. Schematic diagrams of the *Hd1* (A), *Ehd1* (B), *SDG725* (C), *OsCOL15* (D), *DTH2* (E), and *DTH7* (F) genes. White boxes represent the 5′ untranslated regions (5′ UTRs, left) and 3′ UTRs (right). The numbers represent the positions of variants in the coding regions. S1, S2, and S3 represent the first, second, and third SNPs. The letters in parentheses are amino acids; the letters in front of them are the polymorphic nucleotides.

both genes were non-functional (*hd1 ehd1*), RILs with the genotype *SDG725 DTH2* (Nip) *oscol15 dth7* (R498) flowered earlier than the other genotypes (Supplemental Table 8).

This result indicated that the lines with the allele combination *SDG725 DTH2* (Nip) *oscol15 dth7* (R498) flower earlier when they also carry the *ehd1* allele, regardless of the genotype at *Hd1*. Photoperiod-mediated control of heading date is dictated by two pathways in rice, involving either Hd1 or Ehd1. When either *Hd1* or *Ehd1* was non-functional, the RILs harboring the *sdg725 dth2* (R498) *OsCOL15 DTH7* (Nip) genotypes flowered later compared to the other three lines. Conversely, when RILs carried the *Hd1* and *Ehd1* alleles, the influence of the genotype at the minor genes (*SDG725*, *OsCOL15*, *DTH2*, and *DTH7*) on heading date ranged from none (*Hd1 Ehd1*) to 7 days (*Hd1 ehd1*) (Supplemental Table 8). However, the heading date of *Hd1 Ehd1* lines was 103.0–104.4 days, that of *Hd1 ehd1* lines was 112.3–119.8 days,

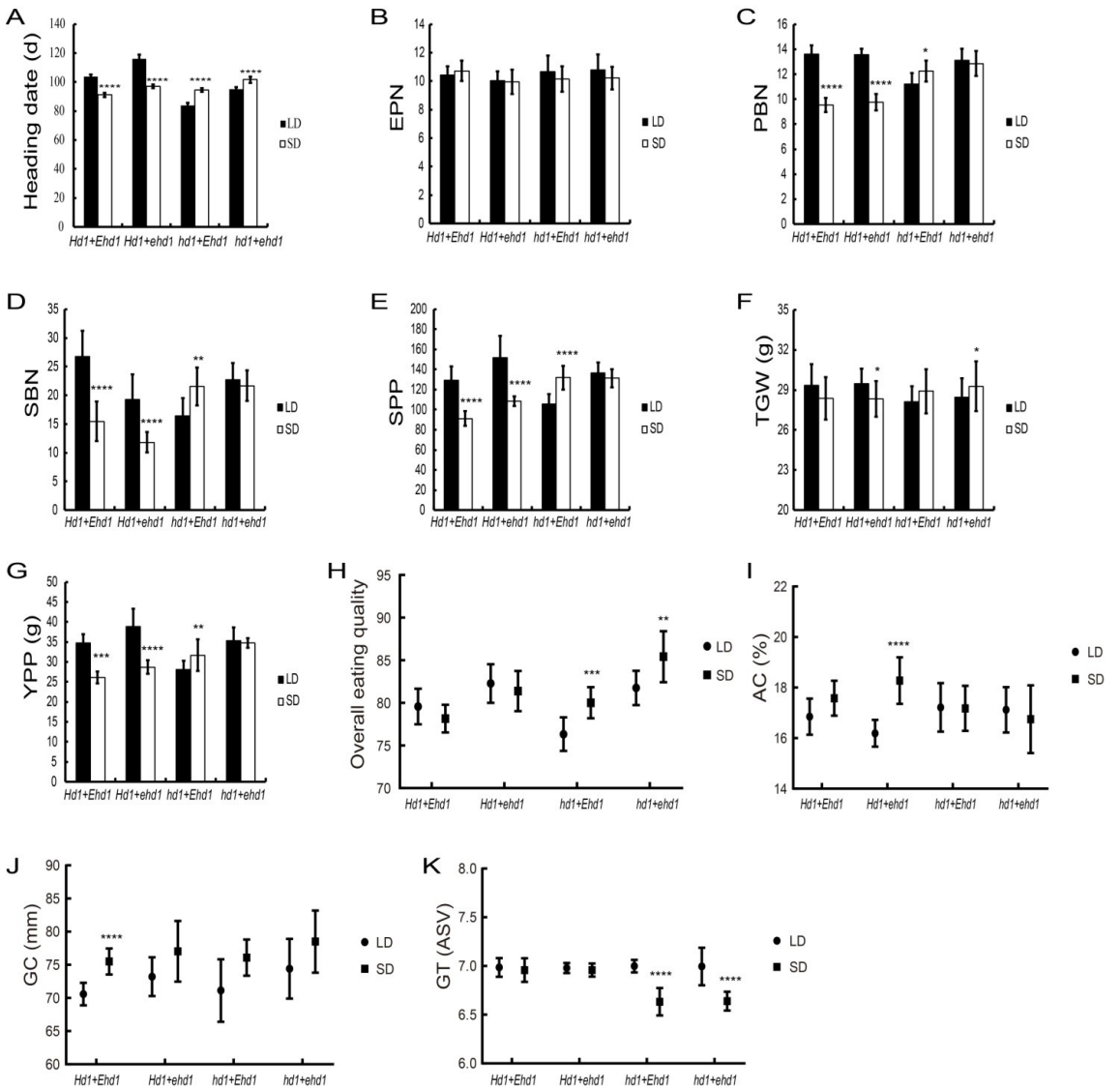

Figure 3. Analysis of agronomic traits and quality-related indexes of RILs grown under LD and SD conditions. A Heading date; B Effective panicle number (EPN); C Primary branch number (PBN); D Secondary branch number (SBN); E Spikelets per panicle (SPP); F Thousand-grain weight (TGW); G Yield per plant (YPP); H Overall eating quality of RIL lines; I Amylose content (AC) of RIL lines; J Gel consistency (GC) of RIL lines; K Alkali spreading value (ASV) of RIL lines. Data are means ± standard deviation, n=12; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 (Student's *t* test).

and that of *hd1 Ehd1* lines was 82.6–85.5 days. These flowering times demonstrated that the effect of *Hd1* and *Ehd1* on heading date is greater than that of the four minor genes combined.

Analysis of agronomic and quality related traits in RILs

As the genotype at *Hd1* and *Ehd1* appeared to exert a stronger influence on heading date than the other four loci identified in this study, we focused on *Hd1* and *Ehd1* in our follow-up research. We determined the heading date of these four sets of lines at different stages under LD conditions (Supplemental Figure 4). A comparison of the changes in heading date of each of the four sets of lines under LD and SD conditions revealed that the difference in heading date for each line is extremely significant (Figure 3A). The *Hd1 Ehd1* lines and the *Hd1 ehd1* lines flowered earlier under SD compared to LD conditions, while the *hd1 Ehd1* lines and the *hd1 ehd1* lines showed delayed flowering under SD compared to LD conditions (Figure 3A). This result suggested that variation at *Hd1* leads to the photoperiod-insensitive phenotype of Zhenjing2400. We also investigated the sum of effective temperature under LD and SD conditions (Supplemental Table 9). The result showed that the sum of effective temperature under SD (1296) was lower than under LD (1597.5). The heading date of Zhenjing2400, the *hd1 Ehd1* lines and the *hd1 ehd1* lines showed extremely significant difference between SD and LD conditions (Supplemental Table 3, Figure 3A). We speculate that the extremely significant difference is caused by temperature.

There was no significant difference between plants grown under LD and SD conditions for EPN of the four sets of lines (Figure 3B). We detected a significant drop between LD and SD conditions for PBN, SBN, and SPP in the sets of lines harboring *Hd1 Ehd1* and *Hd1 ehd1*, while the set of lines with a significant increase in these traits carried the allele combination *hd1 Ehd1*; the lines with the *hd1 ehd1* genotypes returned comparable values under SD and LD conditions (Figure 3C, D and E). The thousand-grain weight (TGW) of *Hd1 ehd1* lines decreased significantly under SD conditions compared to LD conditions, while the *hd1 ehd1* lines showed the opposite trend (Figure 3F). Looking at YPP values, the sets of RILs with the genotype *Hd1 Ehd1* and *Hd1 ehd1* had lower YPP under SD conditions compared to LD conditions, while YPP increased in *hd1 Ehd1* lines; the *hd1 ehd1* lines showed no significant difference in YPP values between SD and LD conditions (Figure 3G). We conclude that the yield in *hd1 ehd1* lines is not affected by daylength, while yield has the potential to increase under SD conditions in *hd1 Ehd1* lines.

A change in heading date in rice may affect grain qualities (Cho et al. 2013). We therefore sequenced the *Wx* and *ALK* genes in Zhenjing2400 and Jiahe218. We determined that the allele of *Wx* is Wx^b, while the allele at *ALK* was ALK^b in both Zhenjing2400 and Jiahe218 (Supplemental Figure 5), indicating that all RILs have the same genetic background for *Wx* and *ALK*. We thus measured quality-related traits in RILs under LD and SD conditions (Figure 3H, I, J and K). The overall eating quality of rice flour obtained from *hd1 Ehd1* and *hd1 ehd1* lines grown under SD conditions was higher than that under LD conditions; notably, the overall eating quality of rice flour from *hd1 Ehd1* lines was the lowest compared to the other three lines when grown under LD conditions (Figure 3H). Compared to LD conditions, the AC of flour from *Hd1 ehd1* lines increased significantly (Figure 3I). The GC of flour from *Hd1 Ehd1* lines also increased significantly (Figure 3J), while the GT of flour from *hd1 Ehd1* and *hd1 ehd1* lines was significantly lower (Figure 3K) under SD conditions. This analysis revealed that the overall eating quality of flour from *hd1 Ehd1* and *hd1 ehd1* lines is higher when they are grown under SD conditions compared to LD conditions, while we measured the best overall eating quality for flour from *hd1 ehd1* lines. Combining agronomic traits and quality traits, the optimal line for planting under LD and SD conditions among the four sets of lines is any line harboring *hd1* and *ehd1*.

Grain quality is controlled by genetic factors and affected by heading date. ECQ is one of the most important evaluation indexes to assess rice quality and is mainly measured by three physicochemical properties: AC, GC, and GT (Lanceras et al. 2000). *Wx* and *SSIIa* (*ALK*) are central genes in determining grain ECQ (Tian et al. 2009). The G-to-T SNP at *Wx* at the +1 position of the consensus cleavage site in intron 1 is responsible for the characteristics of the Wx^b allele with low AC (Chen et al. 2008). The ALK^a (A733-G864C865) or ALK^b (G733-T864T865) alleles resulted in low GT (Luo et al. 2015, Shimbata et al. 2012), with ALK^b being associated with a lower GT than ALK^a (Chen et al. 2020). The Zhenjing2400 and Jiahe218 parental varieties carry the alleles Wx^b and ALK^b, such that both parents and all derived RILs have excellent alleles for ECQ. The changes in ECQ-related indexes in RILs were closely related to heading date. Indeed, the overall eating quality of flour from *Hd1 Ehd1* lines or *Hd1 ehd1* lines was the same for plants grown under LD and SD conditions. This result showed that photoperiod-sensitive lines may be less affected by the environment when they harbor excellent

quality alleles at the major genes *Wx* and *ALK*. However, the overall eating quality trait of *hd1 Ehd1* lines and *hd1 ehd1* lines was higher under SD conditions than under LD conditions. This difference may be caused by the delayed heading date of *hd1 Ehd1* lines and *hd1 ehd1* lines under SD conditions compared to LD conditions. We speculate that planting photoperiod-insensitive lines from LD to SD conditions has the potential to improve ECQ.

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