Genetic Interactions Among Ghd7, Ghd8, OsPRR37 and Hd1 Contribute to Large Variation in Heading Date in Rice

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Abstract

Background: Heading date is crucial for rice reproduction and geographic expansion. Many heading date genes are sensitive to photoperiod and jointly regulate flowering time in rice. However, it is not clear how these genes coordinate rice heading.

Results: Here, we performed a genetic interaction analysis among four major rice heading date genes Ghd7, Ghd8, OsPRR37/Ghd7.1 (hereafter PRR37) and Hd1 in the near-isogenic background under both natural long-day (NLD) and natural short-day (NSD) conditions. The 4-gene segregating population exhibited a large heading date variation with more than 95 days under NLD and 42 days under NSD conditions. Tetragenic, trigenic and digenic interactions among these four genes were observed under both conditions but more significant under NLD conditions. In the functional Hd1 backgrounds, the strongest digenic interaction was Ghd7 by Ghd8 under NLD but was Ghd7 by PRR37 under NSD conditions. Interestingly, PRR37 acted as a flowering suppressor under NLD conditions, while it functioned alternatively as an activator or a suppressor under NSD conditions depending on the status of the other three genes. Based on the performances of 16 homozygous four-gene combinations, a positive correlation between heading date and spikelets per panicle (SPP) was found under NSD conditions, but changed to a negative correlation when heading date was over 90 days under NLD conditions.

Conclusions: These results demonstrate the importance of genetic interactions in the rice flowering regulatory network and will help breeders to select favorable combinations to maximize rice yield potential for different ecological areas.

Keywords: Rice, Heading date, Genetic interaction, Alternative function, Genotype combination, Correlation, Spikelets per panicle

Background

Heading date, a crucial trait for rice expansion to high latitudes, is determined by both genetic factors and environmental cues (Andres and Coupland 2012). Cultivars with an appropriate heading date will be conductive to high grain yield by fully utilizing the light and temperature resources in their growing regions (Zhang et al. 2015a).

In the last two decades, dozens of quantitative trait loci (QTLs) for rice heading date have been cloned by using biparental populations, germplasm resources and mutants with forward- or reverse-genetics approaches (Yamamoto et al. 2012; Hori et al. 2016; Yano et al. 2016). Among these genes, several major QTLs, especially those cloned from natural variations, have pleiotropic effects on heading date, plant height and grain yield, which have been widely subjected to artificial selection in the process of rice genetic improvement. For example, Heading date1 (Hd1), the homolog of Arabidopsis CONSTANS (CO), encodes a zinc finger CCT (CO, CO-LIKE and TIMING OF CAB1) domain and acts as a major flowering activator in rice (Yano et al. 2000; Zhang et al. 2017). Hd1 delays heading date in some varieties under long-day (LD) conditions by interacting with other flowering genes such as Ghd7, resulting in a taller plant and more grain yield (Nemoto et al. 2016; Zhang et al. 2017). Ghd7 is
a rice-specific gene encoding a CCT domain protein and is important for heading date, grain yield, rice adaptation and drought resistance (Xue et al. 2008; Weng et al. 2014). Another major QTL, Ghd8 (allelic to Hd5 and DTH8), encodes a HAP3 subunit of heterotrimeric heme activator protein (HAP) and simultaneously controls heading date, plant height and grain number (Wei et al. 2010; Yan et al. 2011; Fujino et al. 2013). OsPRR37, allelic to Gh7.1, DTH7 and Hd2 and encoding a PSEUDO-RESPONSE REGULATOR 7-like protein harboring the CCT domain, greatly represses heading and increases grain yield under LD conditions (Koo et al. 2013; Liu et al. 2013; Gao et al. 2014). Natural variations in OsPRR37/Ghd7.1 also contribute to rice cultivation at a wide range of latitudes (Koo et al. 2013; Yan et al. 2013). It was initially demonstrated that these genes are in separate branches in the flowering regulatory network and have partially unrelated effects on transcription level (Brambilla and Fornara 2013; Song et al. 2015).

Photoperiod sensitivity largely determines heading date in rice. There are two independent genetic pathways involved in photoperiod sensitivity. One is the OsGI-Hd1-Hd3a pathway, which is conserved with the GI-CO-FT pathway in Arabidopsis (Shrestha et al. 2014). Hd1 is upregulated by OsGI and activates the expression of Hd3a to promote rice heading under both short-day (SD) and LD conditions (Hayama et al. 2003; Zhang et al. 2017). Another is the Ehd1-Hd3a pathway, a unique pathway in rice regulated by many genes (Doi et al. 2004; Tsuji et al. 2011). Among these genes, Ehd2, Ehd3, Ehd4 and OsMADS51 always promote rice heading by directly or indirectly upregulating the expression of Ehd1 under both SD and LD conditions (Kim et al. 2007; Matsubara et al. 2008; Matsubara et al. 2011; Gao et al. 2013). In contrast, other genes including Gh7, Gh8, OsPRR37, Hd16, OsCOL4 and OsCOL10 repress the expression of Ehd1, resulting in late flowering under LD conditions (Xue et al. 2008; Lee et al. 2010; Yan et al. 2011; Hori et al. 2013; Yan et al. 2013; Tan et al. 2016). The recent finding that the Gh7-Hd1 complex represses Ehd1 by binding to a cis-regulatory region in the Ehd1 5′-UTR suggested that Hd1 was integrated into the rice-specific genetic pathway (Nemoto et al. 2016).

Our previous studies indicated that Gh7 and Gh8 in the ZS97 background greatly delayed heading date (non-heading) under NLD conditions because of the presence of Hd1, indicating a strong genetic interaction among Gh7, Gh8 and Hd1 (Zhang et al. 2015a). PRR37 shared the conserved CCT domain with Hd1 and Gh7, and formed a heterotrimer with Gh8 and NF-YCs similar to Hd1 (Zhang et al. 2015b; Goret et al. 2017). Thus, we hypothesized that PRR37 is involved in genetic interactions with the three other genes. To test this hypothesis, we further conducted genetic interaction analysis among Gh7, Gh8, PRR37 and Hd1 in the ZS97 background under NLD and NSD conditions in this study. Tetragenic, trigeneric and digeneric interactions among these four genes were observed under both conditions. PRR37 always acts as a flowering suppressor under NLD conditions but exhibits an alternative function (either suppression or activation) in heading date under NSD conditions.

Materials and methods
Construction of NILs and segregating populations
We previously developed a near-isogenic line (NIL1) pyramiding functional Gh7MH63 and Gh89311 in the ZS97 background (Zhang et al. 2015a). Another near-isogenic line (NIL2) in the ZS97 background, which harbored functional PRR37TQ and nonfunctional hd1TQ derived from Teqing (TQ), was crossed with NIL1. Therefore, NIL-F1 plants carried heterozygous Gh7, Gh8, PRR37 and Hd1 (Additional file 1: Figure S1a; Table S1). The NIL-F2 population was developed by self-crossing a NIL-F1 plant that was genotyped by using the RICE6K SNP array (Yu et al. 2014) (Additional file 1: Figure S1b). To avoid genetic background noise, a NIL-F2 individual harboring heterozygous alleles at all four of these genes was used to produce a NIL-F3 population by self-pollination. All individuals of the NIL-F2 and NIL-F3 populations were genotyped at these four gene loci. According to the genotypes of the NIL-F3 population, 8 NIL-F3 plants, each carrying heterozygous PRR37 but with different homozygous combinations of the other three genes, were used to generate 8 NIL-F4 populations for estimating the genetic effects of PRR37. Sixteen NIL-F3 plants with different homozygous four-gene combinations were selected to generate 16 four-gene homozygous lines for evaluating yield performance.

Field experiments and growth conditions
Rice seeds were sown in a seedling bed in the middle of May at the experimental station of Huazhong Agricultural University, Wuhan, China (30.5°N). The 25-day-old seedlings were transplanted into the field with a distance of 16.5 cm between plants within a row and 26.5 cm between rows. The plants were subsequently grown in the field under NLD conditions (a day length of more than 13.5 h) until the beginning of August (Additional file 1: Table S2). For the field experiments under NSD conditions, the plant materials were sown in Lingshi, Hainan (18.5°N), at the beginning of December and were transplanted into the field after 1 month, at the same planting density as that used in Wuhan, and grown under an average day length of less than 12.5 h from December to April (Additional file 1: Table S2).

The NIL-F2 population consisting of 680 individuals was grown in Wuhan in 2016. Excluding the marginal...
plants and abnormally growing individuals, 509 individuals were used for analysis of genetic interactions among Ghd7, Ghd8, PRR37 and Hd1 under NLD conditions. A total of 900 NIL-F3 plants derived from an F2 individual segregating for these four genes were grown in Lingshi from Dec 2016 to Apr 2017, and a total of 679 non-marginal individuals were used for analysis of genetic interactions among these four genes under NSD conditions. Eight NIL-F3 populations were grown in Wuhan (~ 60 plants per population) in summer 2017 (from May to October) and in Lingshi (~ 40 plants per population) in winter (from Dec 2017 to Apr 2018). Meanwhile, 16 four-gene homozygous lines were also grown in Wuhan and Lingshi in summer and winter of 2017, respectively. Three additional PRR37-segregating population (~ 80 plants per population) with the backgrounds Ghd7Ghd8Hd1, Ghd7Ghd8hd1 and Ghd7ghd8Hd1 were also grown in Lingshi in winter 2017. In addition, four plants of each four-gene homozygous combination were grown in the field to implement a short-day treatment with a day length of 11 h and darkness of 13 h in the summer of 2018. A set of plants from these genotypes were planted in the same field at the same density under NLD conditions and served as the control group.

DNA extraction, polymerase chain reaction and genotyping
At the tillering stage, leaf blades were collected for DNA extraction using a modified cetyl-trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). Genomic DNA was amplified using rTaq polymerase from Takara in Buffer I according to the manufacturer’s indications. For each PCR reaction, DNA was initially incubated for 5 minutes at 95 °C, followed by 35 cycles of amplification (95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s). The simple sequence repeat (SSR) marker MRG4436, which is tightly linked to Ghd7, and the functional markers Z9M, InDel37 and S56 designed from Ghd8, PRR37 and Hd1 (Additional file 1: Table S1), respectively, were used to genotype the individuals of all populations and NILs. All markers used for genotyping are listed in (Additional file 1: Table S7).

RNA extraction and qRT-PCR analysis
Seedlings were grown in a seedbed under NLD conditions for 30 days and were subsequently transplanted to a plot in the field for the short-day treatment (started on the 11th of June, light treatment from 7:00 am to 6:00 pm every day). After treatment for 15 days (from the 11th to 26th of June), the young leaves in the short-day treatment and control group (treated with LD condition, i.e., more than 14 h day length per day from the 11th to 26th of June) were collected at 9:00 am for RNA extraction. For each genotype, leaves from three different individuals were collected as biological replicates. Total RNA was extracted using TRIzol reagent (TransGen Biotech, Beijing) and treated with DNase I (Invitrogen, USA). cDNA was synthesized from 3 µg of RNA using SuperScript III Reverse Transcriptase (Invitrogen, USA). The quantitative analysis of gene expression was performed with SYBR Premix EX Taq reagent (Takara, Dalian) on the ABI ViiA7 Real-time PCR System (Applied Biosystems, USA). The data were analyzed using the relative quantification method. The primers used for real-time PCR are listed in (Additional file 1: Table S7).

Trait measurement and data analysis
Heading date was individually scored as the number of days from sowing to the emergence of the first panicle on the plant. The total number of spikelets per plant was measured by the Yield Traits Scorer (Yang et al. 2014). The number of spikelets per panicle (SPP) of each homozygous combination line was recorded as the total number of spikelets divided by the number of panicles. The comparison between genotypes was performed by Student’s t-test. To verify the existence of high order genetic interactions, the three-way ANOVA or factorial ANOVA were performed under the condition of fixation of the allele at the fourth gene. The statistical significance of three-way interactions was evaluated by a general linear model (GLM) using the program STATISTICA 8.0 (Statsoft 1995).

Results
Composition of major heading date genes in ZS97
Our previous studies confirmed that ZS97 carried a functional allele of Hd1 and nonfunctional alleles of Ghd7, Ghd8 and PRR37/Ghd7.1 (Xue et al. 2008; Yan et al. 2011; Yan et al. 2013; Zhang et al. 2017). To clarify the genetic background on heading date, the coding sequences and functional nucleotide polymorphisms of other 10 major flowering genes were downloaded from the reference genome of ZS97 and Rice SNP-Seek Database, respectively (Alexandrov et al. 2015; Song et al. 2018; Wang et al. 2018). Alignment of coding sequence were used to compare allele identity between ZS97 and varieties used in previous studies (Additional file 1: Table S3). Alleles of DTH3/OsMADS50 and Hd6 were the same as the one carried by Dianjingyou 1 and Kasalath, respectively, which were the functional alleles (Takahashi et al. 2001; Lee et al. 2004; Bian et al. 2011). The haplotypes of Hd16/EL1, Hd3a and Ehd1 were identified as Type 4, Type 3 and Type 6, respectively, which were also confirmed as the functional types (Takahashi et al. 2009; Hori et al. 2013; Kwon et al. 2014). Allele of Ehd4 in ZS97 was the same as the 93–11 haplotype, Hap_2, which was a weak functional allele demonstrated by transgenic verification (Gao et al. 2013). The haplotype
of Hd17 in ZS97 was consistent with that in Koshihikari, which was a weak allele compared with Nipponbare (Matsubara et al. 2012). Allele of Hd18 in ZS97 was the same as that in Hayamasari, acted as a weak allele (Shibaya et al. 2016). The haplotype of DTH2 in ZS97 was consistent with Group A1, which was a nonfunctional allele (Wu et al. 2013). RFT1 in ZS97 belonged to Type IIb with E105K variation and also exhibited a loss of function (Zhao et al. 2015).

The genetic interactions among Ghd7, Ghd8, PRR37 and Hd1 under NLD conditions

The NIL-F1 plant carrying heterozygous alleles at these four genes (Additional file 1: Figure S1a) was genotyped by the RICE6K SNP array. More than 90% of the NIL-F1 plant background was consistent with ZS97, but the segments harboring Ghd7, Ghd8, PRR37 and Hd1 were heterozygous. The segments harboring other 10 flowering gene regions were fixed with ZS97 genotype in the NIL-F1 plant (Additional file 1: Figure S1b). In the NIL-F2 population, large variation in heading date was observed, ranging from 65 days to no heading after 160 days under NLD conditions (Fig. 1a). For convenience, 160 days was recorded as the heading date of these non-heading plants. Two-way and three-way ANOVA separately showed that all 6 pairs of digenic interactions and 4 trigenic interactions were highly significant (Additional file 1: Table S4). Four-way ANOVA revealed that the tetragenic interaction among these four genes was also highly significant (Additional file 1: Table S4). To better understand the four-way interaction, we classified the populations into three subpopulations based on Hd1 genotypes: homozygous Hd1, heterozygous Hd1 (Hd1H) and homozygous hd1. A significant three-way interaction was detected among Ghd7, Ghd8 and PRR37 at $P < 1.0 \times 10^{-10}$ in both the Hd1 and Hd1H backgrounds and at $P = 6.9 \times 10^{-4}$ in the hd1 background (Additional file 1: Figure S2a-c; Table 1). Additionally, all digenic interactions were detected among Ghd7, Ghd8 and PRR37. The Ghd7 by Ghd8 interaction contributed more to heading date variation than the other digenic interactions. The square of this interaction accounted for 5.9% and 5.8% of the total sum-of-squares in the Hd1 and Hd1H backgrounds, respectively, and 5.8% of that in the nonfunctional hd1 background (Table 1). The main effects of Ghd7, Ghd8 and their digenic interaction effects explained more than 70% of the variation in heading date in both the Hd1 and Hd1H backgrounds. The genetic square of PRR37 accounted for 17.0% of the total sum-of-squares in the hd1 background, which was much larger than that observed in the Hd1 and Hd1H backgrounds (Table 1). Taken together, these results revealed that a strong trigenic interaction existed among Ghd7, Ghd8 and PRR37 regardless of the genotype of Hd1, and the interaction between Ghd7 and Ghd8 showed the strongest digenic interaction among these three genes under NLD conditions.

The genetic interactions among Ghd7, Ghd8, PRR37 and Hd1 under NSD conditions

The heading date variation of NIL-F3 population exhibited a continuous distribution ranging from 82 days to 124 days (Fig. 1b). Accordingly, all digenic and trigenic interactions (except the Ghd8 by PRR37 by Hd1 interaction) among these four genes were significant under NSD conditions (Additional file 1: Table S4). A significant tetragenic interaction was also observed in the NIL-F3 (Additional file 1: Table S4). Following the analysis performed for NLD conditions, this population were also classified into 3 classes according to Hd1 genotypes. Significant interactions were identified among Ghd7, Ghd8 and PRR37 in the hd1, Hd1H and Hd1 backgrounds (Additional file 1: Figure S2d-f; Table 2). However, the
digenic interactions among these three genes were different from those detected under NLD conditions. The Ghd7 by PRR37 interaction contributed much more to heading date variation than the other two digenic interactions in the functional Hd1 backgrounds, in which the genetic square accounted for 20.3% and 20.4% of the total sum-of-squares in the Hd1 and HdlH backgrounds, respectively (Table 2). Notably, the effect of Ghd7 on heading date was the strongest under NLD conditions, explaining 58%, 21.7% and 29.1% of the variation in the hd1, HdlH and Hd1 backgrounds, respectively (Table 2). These results indicated that Ghd7, Ghd8 and PRR37 interacted under NSD conditions and the Ghd7 by PRR37 interaction showed the strongest epistatic effect among the digenic interactions in the functional Hd1 backgrounds.

**PRR37 acts as a heading date suppressor under NLD conditions**

To estimate the additive and dominance effects of PRR37 in different genetic backgrounds under NLD conditions, we developed 8 PRR37-segregating populations (NIL-F4) with different homozygous combinations of the other three genes. The NIL-F4 population with the Ghd7Ghd8Hd1 background did not head even after October 24th, when the low temperature is unfavorable to rice growing in Wuhan (Fig. 2a). Therefore, no data were used to evaluate the genetic effect of PRR37 in this population (Table 3). We merged the 8 NIL-F4 populations for interaction analysis because these populations shared similar genetic background and were grown in the same condition. All digenic and trigenic interactions or even tetragenic interaction among these four genes were also highly significant (Additional file 1: Figure S3a-c; Table S5). To confirm whether PRR37 also delayed rice heading in the Ghd7Ghd8Hd1 background, we took the young panicles of the main stems of the two homozygous combinations, namely, Ghd7Ghd8Hd1PRR37 and Ghd7Ghd8Hd1ppr37, on September 30th and compared their lengths (Fig. 2b). The young panicle length of Ghd7Ghd8Hd1PPR37 (0.87 cm) was significantly shorter than that of Ghd7Ghd8Hd1ppr37 (1.55 cm), which suggested that PRR37 suppressed heading in the Ghd7Ghd8Hd1 background (Fig. 2c). The additive effect of PRR37 in the other 7 populations ranged from 5.6–19.4 days, indicating that PRR37 always plays as a suppressor of heading date in these backgrounds under NLD conditions (Table 3). The dominance effects and degrees of dominance of PRR37 ranged from 2.4–10.7 days and from 0.28–0.93, respectively (Table 3). Accordingly, we observed large heading date variations in the ghd7ghd8hd1 and Ghd7Ghd8Hd1 backgrounds, ranging from 69 to 115 days and from 94 to 127 days, respectively (Table 3). The effects

### Table 1 Three-way ANOVA analysis of Ghd7, Ghd8 and PRR37 in NIL-F2 population under NLD conditions

| Effect          | df | F    | P       | G.T (%) | df | F    | P       | G.T (%) | df | F    | P       | G.T (%) |
|-----------------|----|------|---------|---------|----|------|---------|---------|----|------|---------|---------|
| Ghd7            | 2  | 787.7| < 1.0E-10| 28.6    | 2  | 12145.5| < 1.0E-10| 29.6    | 2  | 9627.4| < 1.0E-10| 31.9    |
| Ghd8            | 2  | 800.5| < 1.0E-10| 29.1    | 2  | 14400.5| < 1.0E-10| 35.1    | 2  | 11278.0| < 1.0E-10| 37.4    |
| PRR37           | 2  | 468.3| < 1.0E-10| 17.0    | 2  | 747.6 | < 1.0E-10| 1.8     | 2  | 799.1 | < 1.0E-10| 2.6     |
| Ghd7 by Ghd8    | 4  | 80.1 | < 1.0E-10| 5.8     | 4  | 11834.4| < 1.0E-10| 5.8     | 4  | 893.3 | < 1.0E-10| 5.9     |
| Ghd7 by PRR37   | 4  | 9.6  | 1.1E-06  | 0.7     | 4  | 116.5 | < 1.0E-10| 0.6     | 4  | 239.9 | < 1.0E-10| 1.6     |
| Ghd8 by PRR37   | 4  | 21.1 | < 1.0E-10| 1.5     | 4  | 34.8  | < 1.0E-10| 0.2     | 4  | 31.1  | < 1.0E-10| 0.2     |
| Ghd7 by Ghd8 by PRR37 | 8 | 3.7  | 6.9E-04  | 0.5     | 8  | 88.5  | < 1.0E-10| 0.9     | 8  | 63.8  | < 1.0E-10| 0.8     |

*Range of heading date variation; HdlH heterozygous allele of Hdl; DF Degree of freedom, G.T Ratio of the genetic to the total of sum-of-squares

### Table 2 Three-way ANOVA analysis of Ghd7, Ghd8 and PRR37 in NIL-F2 population under NSD conditions

| Effect          | df | F    | P       | G.T (%) | df | F    | P       | G.T (%) | df | F    | P       | G.T (%) |
|-----------------|----|------|---------|---------|----|------|---------|---------|----|------|---------|---------|
| Ghd7            | 2  | 371.1| < 1.0E-10| 58.0    | 2  | 144.55| < 1.0E-10| 21.7    | 2  | 107.0 | < 1.0E-10| 29.1    |
| Ghd8            | 2  | 11.2 | 3.2E-05 | 1.7     | 2  | 8.4  | 2.9E-04 | 1.3     | 2  | 36.2 | < 1.0E-10| 9.8     |
| PRR37           | 2  | 16.9 | 2.6E-07 | 2.6     | 2  | 55.7 | < 1.0E-10| 8.4     | 2  | 10.9 | 4.0E-05 | 2.9     |
| Ghd7 by Ghd8    | 4  | 23.1 | < 1.0E-10| 7.2     | 4  | 9.6  | 2.4E-07 | 2.9     | 4  | 14.5 | 5.4E-10 | 7.9     |
| Ghd7 by PRR37   | 4  | 13.0 | 5.0E-09 | 4.1     | 4  | 67.9 | < 1.0E-10| 20.4    | 4  | 37.4 | < 1.0E-10| 20.3    |
| Ghd8 by PRR37   | 4  | 15.0 | 3.1E-10 | 4.7     | 4  | 19.5 | < 1.0E-10| 5.9     | 4  | 6.5  | 7.6E-05 | 3.5     |
| Ghd7 by Ghd8 by PRR37 | 8 | 3.0  | 4.1E-03 | 1.9     | 8  | 8.3  | 3.1E-10 | 5.0     | 8  | 4.4  | 9.3E-05 | 4.8     |

*Range of heading date variation; HdlH heterozygous allele of Hdl; DF Degree of freedom, G.T Ratio of the genetic to the total of sum-of-squares
Fig. 2 PRR37 delays heading date under NLD conditions. Non-heading plants of prr37 and PRR37 in the Ghd7Ghd8Hd1 background, b their young panicles of main stems and c the comparison of panicle length. d The plants of ghd7Ghd8Hd1PPR37 and ghd7Ghd8Hd1prr37. e The large effect of PRR37 on heading date in the ghd7Ghd8Hd1 background. f The plants of Ghd7Ghd8Hd1PPR37 and Ghd7Ghd8Hd1prr37. g The strong effect of PRR37 on heading date in the ghd7Ghd8Hd1 background. **, P < 0.01 based on Student's t-test; n = 15 for each combination in c, and n ≥ 10 for each genotype in e and g. PRR37H, the heterozygous allele of PRR37. Scale bars: 20 cm for a, d and f, and 1 cm for b.

Table 3 The genetic effects of PRR37 on heading date in 8 NIL-F4 populations under NLD conditions

| Background   | Size | Heading date (d) | Range         | prr37 | PRR37H | PRR37 | A   | D   | [D/A] |
|--------------|------|------------------|---------------|-------|--------|-------|-----|-----|-------|
| ghd7Ghd8hd1  | 47   | 71.0 ± 1.7       | 70–88         | 70.0 ± 1.7 | 81.3 ± 2.3 | 84.7 ± 1.8 | 6.8 | 3.4 | 0.50  |
| ghd7Ghd8hd1  | 51   | 75.7 ± 1.2       | 74–97         | 75.7 ± 1.2 | 84.9 ± 1.8 | 92.7 ± 2.3 | 8.5 | NS  |       |
| Ghd7Ghd8hd1  | 52   | 79.1 ± 1.8       | 77–93         | 79.1 ± 1.8 | 87.0 ± 1.6 | 90.2 ± 1.8 | 5.6 | 2.4 | 0.43  |
| Ghd7Ghd8hd1  | 59   | 96.4 ± 1.4       | 94–127        | 96.4 ± 1.4 | 119.1 ± 2.4 | 124.5 ± 1.2 | 14.0 | 8.7 | 0.62  |
| ghd7Ghd8Hd1  | 47   | 61.5 ± 1.5       | 59–82         | 61.5 ± 1.5 | 73.2 ± 2.7 | 79.8 ± 1.7 | 9.1 | 2.5 | 0.28  |
| ghd7Ghd8Hd1  | 47   | 71.9 ± 1.5       | 69–115        | 71.9 ± 1.5 | 101.5 ± 1.7 | 110.7 ± 2.5 | 19.4 | 10.2 | 0.53  |
| Ghd7Ghd8Hd1  | 58   | 81.6 ± 1.3       | 80–108        | 81.6 ± 1.3 | 103.7 ± 1.3 | 104.5 ± 1.6 | 11.5 | 10.7 | 0.93  |
| Ghd7Ghd8Hd1  | 60   | NH               | NH            | NH    | NH     | NH    |     |     |       |

Size, the number of plants of segregating population; PRR37H, heterozygous allele of PRR37; A, additive effect; D, dominance effect; [D/A], the degree of dominance; NS, no significance; NH, no heading.
of PRR37 on heading date were 38.8 and 28.1 days in these two backgrounds, respectively, which were much larger than that in the ghd7ghd8hd1 background (Fig. 2d-g; Table 3). These results revealed that the large genetic effects of PRR37 on heading date were dependent on the combinations of Ghd7, Ghd8 and Hd1.

**Alternative functions of PRR37 in repressing or promoting heading under NSD conditions**

Heading dates of these 8 PRR37-segregating populations (NIL-F4) also exhibited a continuous distribution ranging from 95 to 135 days under NSD conditions (Additional file 1: Figure S3d). We merged these 8 populations together for interaction analysis. Accordingly, most of digenic and trigenic interactions and tetragenic interaction among these four genes were significant (Additional file 1: Figure S3e-f; Table S5). The additive effects of PRR37 were 1.8 days, 5.0 days and 2.2 days in the ghd7ghd8hd1, ghd7ghd8Hd1 and ghd7Ghd8Hd1 backgrounds, respectively (Table 4), indicating that PRR37 acted as a flowering suppressor in these three backgrounds. However, the effect on delaying heading date was much smaller than that observed under NLD conditions. The genetic effect of PRR37 disappeared in the ghd7Ghd8hd1 and Ghd7ghd8hd1 backgrounds (Additional file 1: Figure S3d), indicating that PRR37 acted as a flowering activator in these backgrounds. Interestingly, a converse effect of PRR37 was observed in the Ghd7ghd8Hd1 background (Fig. 2d-g; Table S5). These results revealed that the large genetic effects of PRR37 were largely influenced by the genetic background.

**Transcriptional analysis of Ehd1 and Hd3a in the Ghd7ghd8Hd1, Ghd7Ghd8Hd1 and Ghd7Ghd8hd1 backgrounds**

Considering that PRR37 has an alternative function in these three backgrounds under different day-length conditions, the expression of PRR37 downstream genes, Ehd1 and Hd3a, was compared between prr37 and PRR37 in these three backgrounds under LD and SD conditions, respectively. The relative expression levels of Ehd1 and Hd3a in Ghd7ghd8Hd1prr37 and Ghd7Ghd8hd1prr37 genotypes decreased under LD conditions but increased under SD conditions compared with those in Ghd7ghd8Hd1PRR37 and Ghd7Ghd8hd1PRR37, respectively (Fig. 4). The expression of Ehd1 and Hd3a showed no significant difference between prr37 and PRR37 in the Ghd7Ghd8Hd1 background under LD conditions but increased with the presence of PRR37 under SD conditions (Fig. 4). These results indicated that PRR37 promoted the expression of Ehd1 and Hd3a in these three backgrounds under SD conditions, resulting in an early heading date. In contrast, PRR37 delayed rice heading in the ghd7ghd8Hd1 background by repressing the expression of Ehd1 and Hd3a under both LD and SD conditions (Additional file 1: Figure S4).

**Correlation between heading date and SPP under NLD and NSD conditions**

We identified the relationship between heading date and SPP on the basis of performance of 16 homozygous 4-

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**Table 4** The genetic effects of PRR37 on heading date in 8 NIL-F4 populations under NSD conditions

| Background               | Size | Heading date (d) | prr37 | PRR37° | A | D | [D/A] |
|--------------------------|------|------------------|-------|--------|---|---|------|
| ghd7ghd8hd1              | 40   | 112–122          | 114.9 ± 1.5 | 118.2 ± 1.5 | 118.4 ± 2.0 | 1.8 | 1.6 | 0.88 |
| ghd7Ghd8hd1              | 39   | 110–117          | 114.9 ± 1.6 | 114.7 ± 1.0 | 113.5 ± 1.6 | NS | NS |
| Ghd7Ghd8hd1              | 38   | 120–126          | 123.2 ± 0.8 | 123.3 ± 1.9 | 123.7 ± 1.0 | NS | NS |
| Ghd7Ghd8Hd1              | 40   | 123–135          | 133.4 ± 1.3 | 127.1 ± 1.5 | 126.0 ± 1.2 | −3.7 | −2.6 | 0.71 |
| ghd7ghd8Hd1              | 39   | 97–112           | 100.0 ± 2.1 | 106.9 ± 3.0 | 110.0 ± 1.4 | 0.0 | 1.9 | 0.38 |
| ghd7Ghd8Hd1              | 40   | 102–112          | 104.5 ± 1.9 | 108.1 ± 1.7 | 108.9 ± 1.6 | 2.2 | 1.4 | 0.64 |
| Ghd7Ghd8Hd1              | 38   | 108–116          | 113.3 ± 1.6 | 111.9 ± 1.5 | 109.3 ± 1.3 | −2.0 | NS |
| Ghd7Ghd8Hd1              | 40   | 113–136          | 133.4 ± 1.7 | 116.2 ± 2.3 | 113.6 ± 1.1 | −9.9 | −7.3 | 0.74 |

Size, the number of plants of segregating population; PRR37°, heterozygous allele of PRR37; Negative value indicates the functional allele of PRR37 promotes rice heading. A, additive effect; D, dominance effect; [D/A], the degree of dominance; NS, no significance.
Under NLD conditions, the heading date of these 16 combinations exhibited a continuous distribution ranging from 60 days to 130 days except for the two non-heading combinations Ghd7Ghd8PRR37Hd1 and Ghd7Ghd8prr37Hd1. The earliest heading combination was ghd7ghd8prr37Hd1 with 60.8 days, which was the ZS97 genotype (Fig. 5a; Additional file 1: Table S6).

Unexpectedly, SPP of these 14 combinations showed an inverse correlation with heading date. The SPP increased with later heading dates when the heading date was earlier than 90 days, while the SPP decreased with later heading dates when heading date was after 90 days (Fig. 5b). The curve-fitting plots of heading date with SPP under NLD conditions also revealed the inverse correlation with an inflection point at 90.0 days (Fig. 5c). The

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**Fig. 3** PRR37 promotes rice heading in the specific backgrounds under NSD and SD conditions. Comparisons of heading date among different alleles of PRR37 in the backgrounds Ghd7ghd8Hd1 a, Ghd7Ghd8Hd1 b and Ghd7Ghd8hd1 c under NSD conditions (n ≥ 10 for each combination). d-f, Pictures (top) and heading dates (bottom) of prr37 and PRR37 in each corresponding background under SD conditions (n = 4 for each combination). **, P < 0.01 based on Student’s t-test. PRR37H, the heterozygous allele of PRR37. Scale bars: 20 cm for d, e and f.
combination *ghd7ghd8prr37Hd1* had the most SPP, with 199.9 ± 7.3 under NLD conditions, and the second most was *Ghd7ghd8prr37Hd1*, with 184.1 ± 9.8 (Additional file 1: Table S6). Under NSD conditions, the heading date of the 16 combinations also showed a continuous distribution with a range from 98 days to 132 days. The combination with the earliest heading date was also the ZS97 genotype, *ghd7ghd8prr37Hd1*, at 98.7 days, while the combination with the latest heading date was *Ghd7Ghd8prr37Hd1*, at 131.8 days (Fig. 5d; Additional file 1: Table S6). The SPP of these 16 combinations increased with the later heading dates, indicating that SPP was positively correlated with heading date under NSD conditions (Fig. 5e-f).

**Discussion**

*Ghd7, Ghd8, PRR37/Ghd7.1 and Hd1* are all photo-period sensitive genes that respond to day-length changes and play important roles in rice adaptation to high latitude regions (Yano et al. 2000; Xue et al. 2008; Yan et al. 2011; Liu et al. 2013; Koo et al. 2013). Their combinations also largely determine the adaptation and yield potential of rice cultivars. Loss-of-function allele combination (NNN) and pre-existing strong allele combination
(SSF) of *Ghd7, Ghd8* and *Hd1* allow rice cultivars to adapt to temperate and tropical regions, respectively (Zhang et al. 2015a). Loss-of-function alleles of *Ghd7, PRR37/DTH7* and *Hd1* contributed to early rice heading dates in the northern regions of northeast China, while functional alleles delayed heading in the southern regions of northeast China, indicating that divergent alleles of these three genes largely determined rice adaptation in northeast China (Ye et al. 2018). In this study, the combinations of *Ghd7, Ghd8, PRR37* and *Hd1* in ZS97 background exhibited stronger photoperiod sensitivity under NLD conditions than under NSD conditions. Significant digenic, trigenic or even tetragenic interactions of these four genes were detected under both conditions (Additional file 1: Table S4), but the significance detected under NLD conditions was much greater than that detected under NSD conditions, where the effects of *Ghd7, Ghd8* and *PRR37* were decreased. The OsHAPL1-DTH8-Hd1 complex acts as a transcriptional regulator of heading date by interacting with the HAP complex and GTFs (Zhu et al. 2017).

![Fig. 5 Performances of 16 4-gene homozygous combinations on heading date, SPP under NLD and NSD conditions. Heading date (a, d), SPP (b, e) and curve-fitting plots of heading date with SPP (c, f) under NLD and NSD conditions, respectively. The combinations in a, b, d and e are ordered by the increasing heading date. Curves fitting the trait change in c and f are calculated by the quadratic and liner equation with $r^2$ values, respectively. G7, G8, P37 indicate functional alleles of Ghd7, Ghd8 and PRR37, respectively. g7, g8, p37 indicate nonfunctional alleles of Ghd7, Ghd8 and PRR37, respectively. "160<", non-heading after 160 days from sowing. $20 \leq n \leq 24$ for each combination under NLD conditions and $10 \leq n \leq 16$ for each combination under NSD conditions.](image-url)
subunit, which can form a multicomplex with HAP2 and HAP5 (Thirumurugan et al. 2008). Gh7, PRR37 and Hd1 encode transcription factors containing CCT domains, which are similar to HAP2 and responsible for DNA binding and protein-protein interaction (Wenkel et al. 2006; Thirumurugan et al. 2008). Thus, interactions among these genes probably indicate physical interactions among their encoding proteins or between proteins (transcriptional factors) and DNA elements (gene promoters). In addition, only strong functional and non-functional alleles were taken into consideration in this study. The heading date of these 16 four-gene combinations showed a continuous distribution with a range of 60–130 days and no heading under NLD conditions in Wuhan and a range of 98–132 days under NSD conditions in Hainan (Fig. 5). In nature, there are more diverse alleles for each gene (Koo et al. 2013; Zhang et al. 2015a). It is expected that different gene combinations will have similar heading dates due to the comprehensive effect of single gene and interaction effects. A better understanding of these four major flowering genes will aid in breeding design for developing cultivars for local rice production. It is noticed that these findings are derived from typical Xian (indica) cultivar, ZS97. It is not clear whether similar results would be obtained in Geng (japonica), which is worth testing in the future.

Grain yield is positively correlated with heading date, especially in low latitude areas where the temperature is warm year-round (Gao et al. 2014; Li et al. 2018). In this study, due to continuously high temperature stress during the rice flowering stage in Wuhan, the seed setting rates were significantly decreased; therefore, we analyzed the relationship between heading date and SPP instead of that between heading date and grain yield. The SPP is consistently and positively correlated with heading date under NSD conditions. Nevertheless, the SPP exhibited an inverse correlation with heading date under NLD conditions. The SPP increased with increasing days from sowing to heading when the heading date was earlier than 90 days, while it decreased with increasing days when the heading date was later than 90 days. Based on this finding, optimized combinations can be suggested for local regions to maximize rice production in indica varieties. For example, varieties with the Gh7Ghd8pr37Hd1 and Gh7Ghd8pr37Hd1 combinations will produce more grains in low latitude regions (tropical regions) with short-day and warm conditions such as Hainan. In subtropical regions like Wuhan, the gh7Ghd8pr37Hd1 and Gh7Ghd8pr37Hd1 combinations will have the highest yield potential. In this study, the set of materials was grown at only two locations. If they were tested in multiple diverse ecological areas, the favorable gene combinations could be defined for each area.

Previous studies showed that PRR37 inhibited heading date under LD conditions but seemed to have no effect under SD conditions (Koo et al. 2013; Liu et al. 2013; Gao et al. 2014). However, in this study, PRR37 delayed rice heading in the ghd7ghd8hd1, ghd7ghd8hd1 and ghd7ghd8hd1 backgrounds but significantly promoted heading in the Gh7Ghd8hd1, Gh7Ghd8hd1 and Gh7ghd8hd1 backgrounds under NSD conditions (Fig. 3; Table 4), which clearly demonstrated that PRR37 had alternative functions under SD conditions. PRR37 suppressed heading date by inhibiting the expression of its downstream genes Ehd1 and Hd3a under LD conditions. In contrast, PRR37 acted as an activator of rice heading by promoting Ehd1 and Hd3a expression in the Gh7Ghd8hd1, Gh7Ghd8hd1 and Gh7ghd8hd1 backgrounds under SD conditions (Fig. 4). All these three backgrounds had functional allele of Gh7, indicating that Gh7 played an essential role in the function inversion of PRR37. However, Gh7 and PRR37 are both transcriptional suppressors (Weng et al. 2014; Liu et al. 2018). The effect of Gh7 on heading date was the largest in the 3-gene segregating populations with fixed Hd1 genotypes, and the Gh7 by PRR37 interaction was the strongest digenic interaction in these populations under NSD conditions (Table 2). Consequently, the enhanced genetic interaction between Gh7 and PRR37 under SD conditions most likely attenuated the interaction of Gh7 with other genes, and ultimately weakened the ability of Gh7 and PRR37 or their complexes to inhibit the expression of downstream genes, Ehd1 and Hd3a, resulting in an early heading date. This hypothesis deserved to be further validated and improved by more genetic and molecular biology evidences.

Conclusions

Multi-order genetic interactions among Gh7, Gh8, PRR37 and Hd1 were observed in the 4-gene segregating population under both NLD and NSD conditions. These four genes jointly determined a large heading date variation and their homozygous combinations exhibited a continuous distribution under both conditions except two non-heading combinations under NLD conditions. Coupled with the correlation between heading date and SPP, the favorable combinations were suggested for local regions to maximize rice production. Furthermore, we revealed that PRR37 acted as a heading date suppressor under NLD conditions but it functioned alternatively under NSD conditions depending on the status of the other three genes, indicating different interactions among these four genes under different conditions. These findings revealed the importance of genetic interactions of these four genes in the photoperiod flowering pathways and contributed to a comprehensive insight into how these genes coordinate rice heading date under different day-length conditions.
Additional file

**Additional file 1**: Figure S1. Development and genome composition of the rice populations. Figure S2. Genetic interaction analysis among Ghd7, Ghd8, PRR37 and Hd1 in the 4-gene segregating populations under NLD and NSD conditions. Figure S3. Genetic interaction analysis of Ghd7, Ghd8, PRR37 and Hd1 in the merged PRR37-segregating populations (NLD–F2) under NLD and NSD conditions. Figure S4. PRR37 delays the heading date in the ghd7ghd8Hd1 background under both LD and SD conditions. Table S1. Characteristics of four heading date genes and linked markers. Table S2. The monthly average day length of growing seasons at Wuhan and Lingshui. Table S3. Haplotypes of 10 heading date genes in ZS97. Table S4. The genetic interactions in the 4-gene segregating populations under NLD and NSD conditions. Table S5. The genetic interactions among four genes on the basis of the merged PRR37-segregating populations (NLD–F2) under NLD and NSD conditions. Table S6. The heading date and spikelets per panicle of 16 homozygous 4-gene combinations under NLD and NSD conditions. Table S7. Primers used in this study. (DOCX 1337 kb)

**Abbreviations**

CCT: CO, CO-LIKE and TIMING OF CAB1; HAP: Heterotrimeric heme activator protein; LD: Long-day; NLD: Natural long-day; NSD: Natural short-day; QTLs: Quantitative trait loci; SD: Short-day; SPP: Spikelets per panicle; SSR: Simple sequence repeat

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**Authors’ contributions**

YX, HL and BZ planned and designed the research. BZ, HL and ZZ prepared the materials. BZ performed the experiments and data analysis, FQ and QL contributed to QTL genotyping. ZH contributed to data analysis. BZ and YX wrote and revised the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets supporting the conclusions of this article are included within the article and its additional files.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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