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Dominance complementation of *Hd1* and *Ghd8* contributes to extremely late flowering in two rice hybrids

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Abstract A rice hybrid is the product of a cross between two genetically distinct inbred lines. Owing to yield heterosis in rice hybrids, hybrid rice breeding has been developed and widely used in agriculture. In some cases, the hybrid F_1 lines show extremely late flowering. However, the genetic basis of the phenomenon is not very clear. In this study, two hybrids were found to have heading date of ~ 130 days, which was much later than their inbred parents. To elucidate the underlying mechanism, mapping populations consisting ~ 2000 F_2 individuals were developed, and eight pools were created based on the flowering time of the F_2 individuals. Whole genome deep sequencing of the parental lines and the eight pools enabled the identification of two major loci underlying heading date, on chromosome 6 and 8, respectively. Follow-up PCR amplification and Sanger sequencing showed *Hd1* (*Heading date1*) and *Ghd8*/*DTH8* (*Grain number*, *plant height and heading date 8*) were probably the causal genes on the two loci. The female parent had the genotype $Hd1^{WT}$ $ghd8^{1bp\text{-}del}$ while the male parents had the genotype $hd1^{4bp\text{-}del}$ $Ghd8^{WT}$. Furthermore, genotypes of F_2 individuals at *Hd1* and *Ghd8* were investigated to estimate the genetic effects of different genotype combinations. The result showed that dominance complementation effects of the two genes leaded to extremely late flowering in the F_1 hybrid crosses with the genotype $Hd1^{WT/4bp\text{-}del}$ $Ghd8^{WT/1bp\text{-}del}$. Taken together, this study lays a foundation for further functional validation and breeding utilization of important flowering genes in genetic improvements of hybrid rice.

Keywords Hybrid rice · Next-generation sequencing · Flowering time · *Hd1* · *Ghd8* · Heterosis · Breeding

Introduction

Hybrid rice is the first filial generation (F_1) of the cross between two diverse inbred lines. The heterozygous hybrids (that is, F_1 lines) often display superior performance over their inbred parents, particularly in grain yield and disease resistance, which is called as heterosis or hybrid vigor (Liu et al. 2020). For the utilization of heterosis, the systems of male sterile lines (e.g., cytoplasmic male sterility (CMS) for three-line hybrid system and photo-thermo genic male sterility (P-TGMS)

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for two-line hybrid system) were developed several decades ago (Balaji Suresh et al. 2012; Ding et al. 2012; Li et al. 2007; Luo et al. 2013; Tang et al. 2014; Zhou et al. 2014), since rice is self-pollinated. The three-line hybrid rice system using CMS line, maintainer line, and restorer line, and the two-line hybrid rice system using P-TGMS line and restorer line are widely used, which contribute a large portion of rice production in China (Cheng et al. 2007). Owing to the development of male sterile systems and the understanding of heterosis in rice, hybrid rice breeding was technically available and became very popular in China and many other countries (Cheng et al. 2007). Up to now, thousands of hybrid rice varieties have been created, including the famous varieties Shanyou63, LiangYouPeiJiu, and YongYou series (Huang et al. 2015). These hybrid rice varieties contributed greatly to the increase of grain yield per unit area—it was estimated that hybrid rice lines may have a 10–30% rise in grain yield when compared with inbred lines (Huang et al. 2016).

In hybrid rice crosses, the F_1 lines generally displayed similar heading date with its inbred parents. However, in a few cases, the hybrids showed extremely late flowering, which was unfavorable in agricultural production. During the past two decades, dozens of flowering-related genes have been identified and functionally confirmed in *Arabidopsis* and rice through mutant mapping, quantitative trait locus (QTL) mapping, and reverse genetics approach. Considering the importance of flowering in plant growth and grain yield, the accumulation of knowledge on gene functions of these flowering genes greatly help both the advances of basic studies and molecular breeding designs. However, the causal genes leading to extremely late flowering in hybrid crosses were not very clear. Besides, the occurrence of the extremely late flowering may result from overdominance effects of a single gene (Krieger et al. 2010), dominance complementation effects of two or several genes (Li et al. 2015), or complicated epistatic effects (Zhou et al. 2012), which is also unclear and under investigations. Therefore, the identification of the causal genes and the understanding of the underlying genetic mechanisms of extremely late flowering in these rice hybrids could help to guide a better design of hybrid combinations.

Cutting-edge approaches including whole genome sequencing make segregating F_2 population from the hybrids with extremely late flowering a good choice for rapid genetic mapping of the causal genes. Recently, a method "GradedPool-Seq" was developed for high-resolution QTL mapping with F_2 progeny (Wang et al. 2019). This method combines both next-generation sequencing technology and bulked-segregant analysis (BSA) for complex trait mapping. When the method was applied in plant height, heading date, tiller angle, flag leaf angle, and grain weight, major QTLs were detected at relatively high-resolution (~ 400 kb) using F_2 populations. Hence, it is hopeful to map the causal loci for extremely late flowering by using "GradedPool-Seq."

In this study, we firstly identified two hybrid crosses with extremely late flowering. Using "GradedPool-Seq" for high-resolution genetic mapping in resulting F_2 populations, two previously reported genes, *Hd1* and *Ghd8*/*DTH8*, were detected as the major genes resulting in flowering time segregation. Further genetic analysis showed dominance complementation effects of the two genes leaded to extremely late flowering in the F_1 hybrid crosses. These results provide a genetic clue for molecular breeding in hybrid rice.

Materials and methods

Population construction and phenotype evaluation

Plant materials were developed from a cross between SE21S and HR1128, and a cross between SE21S and R317, all of which belong to *indica* subspecies. Heading date (50% head emergence) of the parental lines and the F_1 plants was evaluated in the experimental fields of China National Rice Research Institute in Hangzhou, China (N 30.32, E 120.12). The F_1 plants were used to produce progeny by self-pollination, and the two F_2 populations (SE21S × HR1128 and SE21S × R317) were created for genetic mapping. All the individuals in the two F_2 populations were sown, transplanted and watered, fertilized, and managed following local standard practices. Flowering time of each individual in the two populations was recorded when the first inflorescences emerged above the flag leaf sheath. In each population, F_2 individuals were divided into four groups according to flowering time trait (group1: ≤ 90 days; group2: 90–130 days; group3: 130–150 days; group4: > 150 days).

Pooling sequencing, read alignment, and genetic mapping

The genomic DNA of the parental lines was extracted from the fresh leaf tissue using Tiangen gDNA Kit. In the two populations, equal masses of fresh leaves of ~ 200 randomly chosen individuals within the same group were combined together for genomic DNA extraction. The genomic DNA was fragmented by ultrasonic treatment, and the sequencing library was constructed using TruSeq Nano Kit, with an insert size of 300–500 bp. The DNA libraries of each parental line and each F_2 pool were loaded into Illumina sequencing system HiSeqX. The resulting 150-bp paired-end reads of three parental lines and eight F_2 pools (Table 1) were aligned against the reference genome sequence (IRGSP releases build 4.0 pseudomolecules of rice) using the software BWA (version 0.7.1) with default parameters (Li et al., 2009). After the BAM files were generated, PCR duplicates were then removed by the "MarkDuplicates" module (Picard package, version 1.119). Furthermore, "IndelRealigner" module (GenomeAnalysisTK, version 3.4.0) was used for realignment of the reads at the complicated regions (DePristo et al., 2011). SNP calling was performed by using "UnifiedGenotyper" module (GenomeAnalysisTK, version 3.4.0). Using the two VCF files as inputs, the software "GradedPool-Seq" was applied for genetic mapping of heading date (including Ridit analysis and background noise reduction) as previously reported (Wang et al. 2019). Candidate genetic intervals responsible for heading date were identified by plotting the ratio of the number of statistically significant variants beyond the set threshold to the total number of variants in each window.

PCR validation and genotyping

The genomic regions of the two candidate genes, *Hd1* and *Ghd8*/*DTH8*, from the three parental lines were amplified and sequenced by Sanger sequencing system. For each gene, the sequences of the parental lines were compared with each other using BLAST2 to detect causative mutations. According to the variation sites of *Hd1* and *Ghd8*/*DTH8*, four SNP/Indel-based molecular markers were designed. Local sequences of *Hd1* and *Ghd8*/*DTH8* were assessed using specific primers (Table 2). Population-scale genotyping was performed using ligation detection reaction (LDR) system (Table 2), generating the genotypes of *Hd1* and *Ghd8*/*DTH8* in each F_2 individual.

Genetic analysis of two major loci

After the genotypes of *Hd1* and *Ghd8*/*DTH8* were identified for the two populations, allele effects were assessed for both homozygous genotypes and heterozygous genotypes (that is, the genotypes of AA, Aa, and aa). For each genotype combination of *Hd1* and *Ghd8*/*DTH8*, the means and the standard errors were calculated using all the individuals with the same genotypes of *Hd1* and *Ghd8*/*DTH8*.

Results

Phenotypes of the parents and hybrids

In our hybrid breeding studies, we found that a few F_1 lines displayed extremely late flowering. Among those hybrid combinations, SE21S × HR1128 and SE21S × R317 are two typical ones. The accession SE21S is a thermo-sensitive genic male sterile line of *indica* type for two-line hybrid rice (Hu et al. 2001). HR1128 is an *indica* variety with strong stem and large panicle (Liu et al. 2012), and R317 is an *indica* variety which is developed from Gui 99 (Qin et al. 1994) and IR30 (IR2153-159-1-4). HR1128 and R317 are used as restorer lines in hybrid breeding. The heading date of the three accessions (50% head emergence) was ~ 90 days in Hangzhou, China (long-day conditions) (Table 1). The hybrid between SE21S and HR1128 and that between SE21S and R317, however, had heading date of ~ 130 days. The growth duration of the hybrids is too long and not suitable in most agricultural productions.

Construction of the mapping population

It was not clear why the two hybrids showed much later flowering than their parents. In order to identify the causal loci, two mapping populations were constructed by self-pollination of the two hybrids (Fig. 1). A total of 1452 and 1722 F_2 individuals were generated from the cross of SE21S × HR1128 and that of SE21S × R317. The F_2 populations were then planted and phenotyped in Hangzhou, China, and flowering time showed to be diverse among the F_2 individuals. According to the phenotypic distribution of flowering time, the F_2

individuals were divided into four groups in each mapping population—no more than 90 days, between 90 and 130 days, between 130 and 150 days, and more than 150 days.

GradedPool-Seq for genetic mapping

Within each group, equal masses of fresh leaves from randomly chosen individuals were combined together for DNA extraction. The genomic DNA of three parental lines (SE21S, HR1128, and R317) and eight pools (four pools for each two populations) were all deeply sequenced using Illumina platform (> 75× coverage for parental lines and > 100× coverage for F_2 pools) (Table 1). After read alignment and variant calling, the pipeline in GradedPool-Seq was then used for genetic mapping on heading date. In both populations, the same two major QTLs were identified on chromosome 6 and chromosome 8, respectively (Fig. 2). The mapping signals of the two QTLs were very high, which may explain a large proportion of heading date variation between parents and hybrids.

Detection of the candidate genes

Owing to high-resolution mapping from high-throughput sequencing, it is possible to identify the causal genes at the two loci. Based on knowledge on function studies of flowering-related genes, two strong candidate genes, *Hd1* and *Ghd8*/*DTH8*, were found to be located within the QTL intervals. Previous studies showed that *Hd1* is the rice orthologous gene to the famous *Arabidopsis* flowering time gene *CONSTANS* and is a major photoperiod sensitivity quantitative trait gene in rice (Yano et al. 2000). *Ghd8* is another major quantitative trait gene underlying grain yield, plant height, and heading date in rice (Yan et al. 2011). The two genes could form a module to mediate day-length-dependent regulation of heading date in rice (Du et al. 2017). Both genes were PCR-amplified from genomic DNA of three parents, and then sequenced and aligned to Nipponbare reference (Fig. 3). We found that a 4-bp deletion was located in the second exon, which encodes the CCT domain, of *Hd1* gene in HR1128 and R317, thus resulting in a frameshift of *Hd1* coding (Fig. 3a). The sequence of *Hd1* gene in SE21S was the same with that in Nipponbare reference and should be of the wild type. For *Ghd8*/*DTH8* gene, a 1-bp deletion was detected within the coding region in SE21S, leading to a premature termination of *Ghd8*/*DTH8* coding (Fig. 3b). The sequence of *Ghd8*/*DTH8* gene in HR1128 and R317 was generally the same with that in Nipponbare reference except for 10 SNPs (weak effect) and a 3-bp indel (non-frameshift), and should be of the wild type as well. Therefore, based on Sanger sequencing-based validations, the female parent SE21S has the genotype $Hd1^{WT}$ $ghd8^{1bp\text{-}del}$ while the male parent HR1128 or R317 has the genotype $hd1^{4bp\text{-}del}$ $Ghd8^{WT}$. Thus, the genotypes of both F_1 hybrids should be $Hd1^{WT/4bp\text{-}del}$ $Ghd8^{WT/1bp\text{-}del}$.

Since *Ghd7* and *Ghd7.1/OsPRR37* were reported to genetically interact with *Hd1* and *Ghd8* contributing to

Table 1 Summary of the whole genome sequencing data DNA sample

	Heading date (days)	Pool size	Data yield (Gb)	Reads (M)
SE21S	88	1	33.362	222.41
HR1128	90	1	43.639	290.92
R317	77	1	29.281	195.21
F2-21-P1	P1 < =90	R198*	45.048	300.32
F2-21-P2	90 < P2 < =130	R199	47.907	319.38
F2-21-P3	130 < P3 < =150	R197	50.839	338.93
F2-21-P4	P4 > 150	A97**	49.503	330.02
F2-22-P1	P1 < =90	R196	44.081	293.88
F2-22-P2	90 < P2 < =130	A115	53.144	354.29
F2-22-P3	130 < P3 < =150	R199	49.924	332.83
F2-22-P4	P4 > 150	R199	53.659	357.73

*R for Random Chosen; **A for All

Table 2 Primers used in this study

Primer name	Primer sequence(5′-3′)
Hd1-F3	CACACACGACACATAGAGAGAG
Hd1-R6	TAGAACTACTCCCACTGGATCG
Ghd8-F7	AGCATCACCACTCCATCCTC
Ghd8-R8	TGGATCACAACCGAACTCCTAC
H1-L1_F	CGAGGAGGTGGACTCTTG
H1-L1_R	TCACCAAAATACATGCCG
H1-S1_F	GGTGCTCAGGTACAGGGA
H1-S1_R	GGTCCACTTCGATCTGCA
G8-L2_F	GAGTGCGTGTCGGAGTTC
G8-L2_R	GTTGATGGTCTTCCGCTT
G8-S2_F	GAGGGTGATGGAGTTCGA
G8-S2_R	GCGTGTGCCTAGCTAGCT
G8-L2_M	P-GTCGGAGGCCTCGCCTGTAACGAAGTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
G8-L2_DEL	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTGGTCTTCCGCTTCTCGCGCTGGCACT
G8-L2_IN	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTGGTCTTCCGCTTCTCGCGCTGGCACTT
H1-S1_M	P-TTGTTTCATAACGTATTGTCTTCTCTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
H1-S1_DEL	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTCCGGGGTCGTGCCTCTGCATACGCC
H1-S1_IN	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTCCGGGGTCGTGCCTCTGCATACGCCTTTC
G8-S2_M	P-GCCGCCGCCTCCGCTCTCCTCCTCGTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
G8-S2_DEL	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTGGAGGTGGCCGGCG AACCCCCTCTC
G8-S2_IN	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTGGAGGTGGCCGGCGA ACCCCCTCTCGCCGCCGCC
H1-L1-R_M	P-GACAACAACAACAGCAACAGCAGCATTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
H1-L1-R_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTACAACAACAAC GACAACGACAATAAG
H1-L1-R_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACAACAACA ACGACAACGACAATAAC

flowering time regulation via rice florigen Hd3a (Zhang et al. 2019a; Zhang et al. 2015; Zhang et al. 2019b), the alleles of *Ghd7*, *Ghd7.1*, and *Hd3a* in three parental lines were also checked using aforementioned whole genome sequencing data. The results showed that they were all wild type as them in Nipponbare.

Genotyping of F_2 individuals

Although pooling sequencing and statistical analysis using GradedPool-Seq enabled the identification of the causal genes, the exact genotypes of *Hd1* and *Ghd8*/*DTH8* in each F_2 individual were still unknown. LDR-

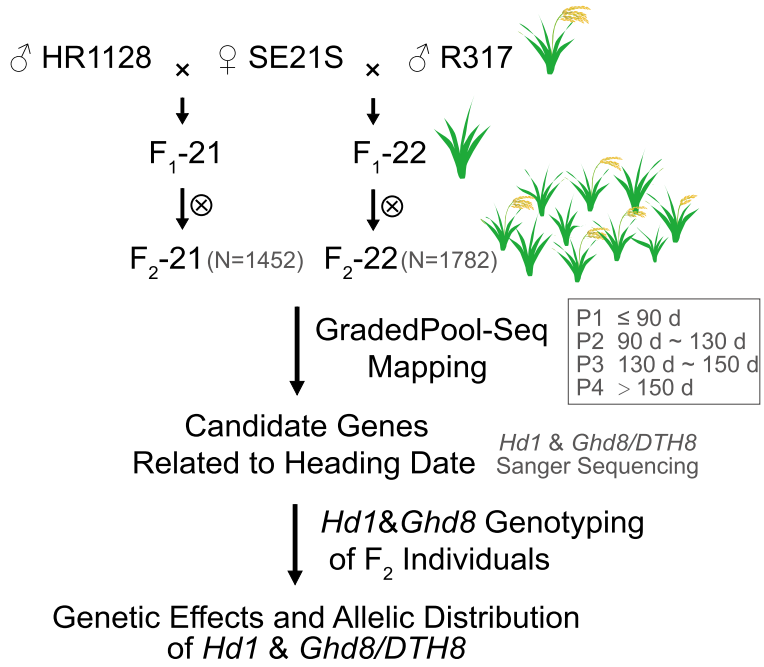

Fig. 1 Identification of *Hd1-Ghd8* module contributing to extremely late flowering in two rice hybrids. Two F_2 populations were constructed from self-pollination of ♀SE21S × ♂HR1128 and ♀SE21S × ♂HR1128 F_1 hybrids, which have an extremely late flowering. Two candidate genes, *Hd1* and *Ghd8*, were identified with next-generation sequencing and GradedPool-Seq mapping and further confirmed by Sanger sequencing. After genotyping of each F_2 individuals, genetic effects and allelic distribution of *Hd1* and *Ghd8* were estimated in the F_2 populations and 17 representative hybrid rice varieties, respectively

PCR method was used to validate the exact genotypes of *Hd1* and *Ghd8*/*DTH8* in each F_2 individual. In F_2 populations, there were three genotypes $Hd1^{WT}$, $Hd1^{WT/4bp\text{-}del}$, and $hd1^{4bp\text{-}del}$ for *Hd1* gene, and three genotypes $Ghd8^{WT}$, $Ghd8^{WT/1bp\text{-}del}$, and $ghd8^{1bp\text{-}del}$ for *Ghd8*/*DTH8* gene. Because the two genes were located in different chromosomes (that is, without the problem of highly genetic linkage), there were nine (that is, 3*3)

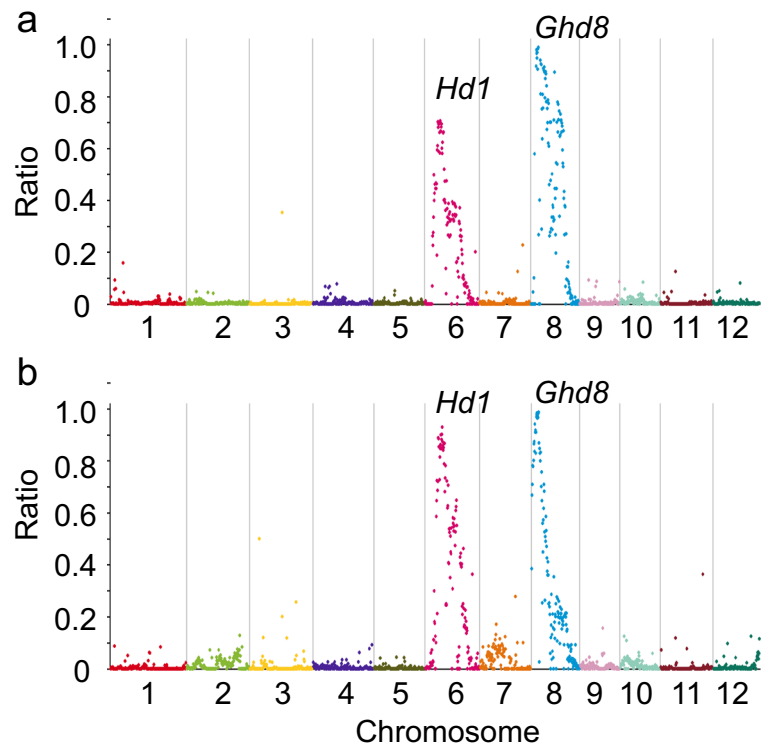

Fig. 2 Genetic mapping in two F_2 populations. Two major QTLs for heading date were identified by using GradedPool-Seq. Ratio plots show two loci corresponding to *Hd1* and *Ghd8* were both identified in SE21S × HR1128 (**a**) and SE21S × R317 (**b**) F_2 populations. The y-axis refers to the ratio of the number of statistically significant variants beyond the set threshold to the total number of variants in each window

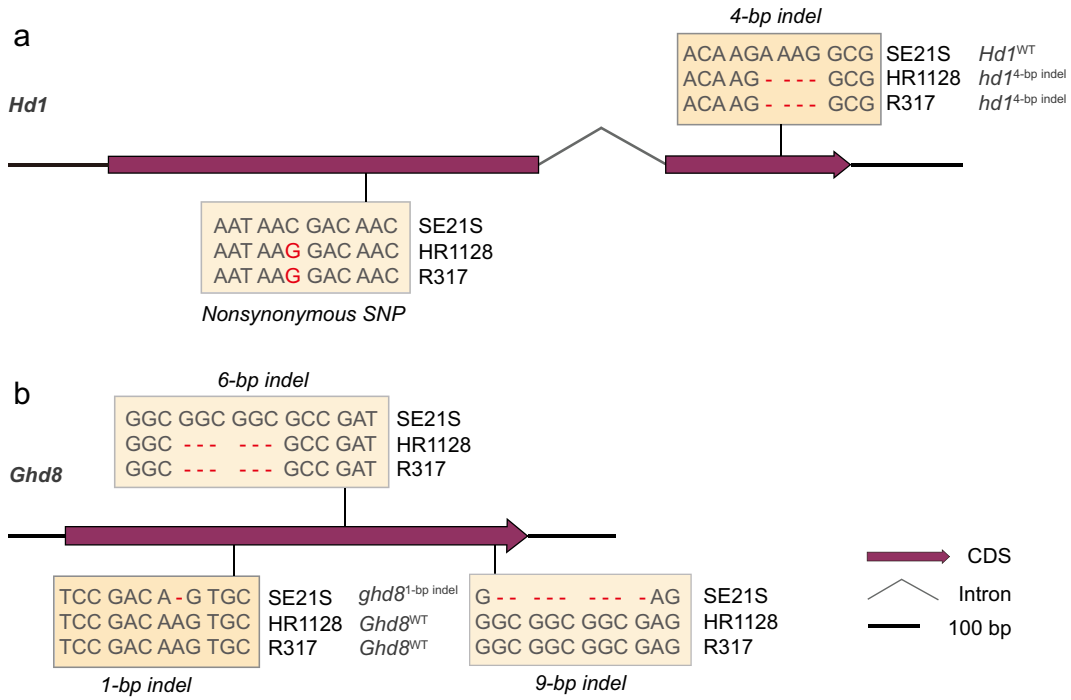

Fig. 3 *Hd1* and *Ghd8* alleles in three parents. A 4-bp deletion in second exon, which encodes CCT domain, of *Hd1* (**a**) and 1-bp deletion in coding region of *Ghd8* (**b**) lead to loss function of *Hd1* or *Ghd8* in male parents (HR1128 and R317) and female parent (SE21S), respectively

genotype combinations between the two loci. By LDR-PCR at the two genes, the genotype combination was assigned to each individual.

Estimates of genetic effects

Considering that *Hd1* and *Ghd8*/*DTH8* are the only two major genes underlying heading date in the two populations, we further estimated the genetic effects for each of nine genotype combinations (Fig. 4). The parental genotypes $Hd1^{WT}ghd8^{1bp\text{-}del}$ and $hd1^{4bp\text{-}del}Ghd8^{WT}$ were equivalent to single mutants of *Ghd8* and *Hd1*, respectively. For the genotype $hd1^{4bp\text{-}del}ghd8^{1bp\text{-}del}$ that was equivalent to double mutant, heading date was earlier than the parental genotype (~ 10 days ahead). For the genotype $Hd1^{WT}Ghd8^{WT}$ that was equivalent to "wild type," heading date was strongly delayed—~ 50 days later than the single mutants. Because of dominance effects for the heterozygous genotypes, the hybrid $Hd1^{WT/4bp\text{-}del}$ $Ghd8^{WT/1bp\text{-}del}$ was close to the

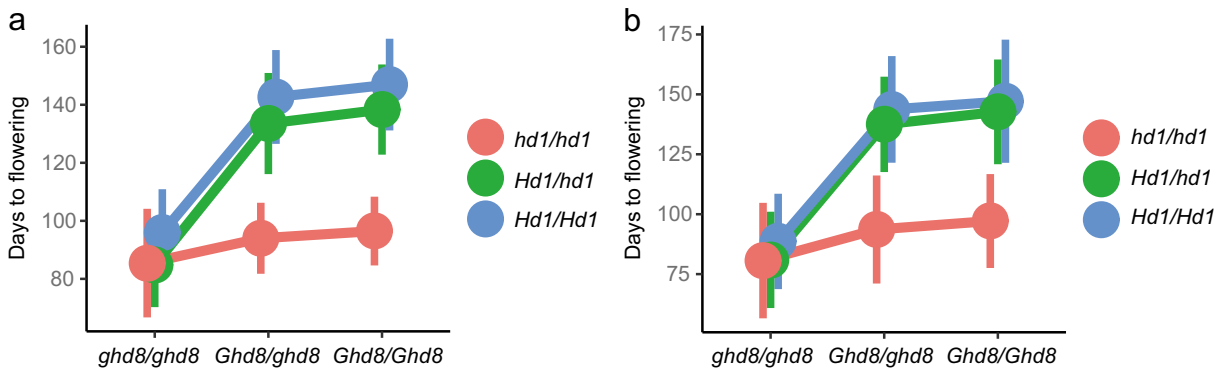

Fig. 4 Incomplete dominance effects of *Hd1* and *Ghd8* in flowering time regulation. Flowering time of 9 genotype combinations of *Hd1* and *Ghd8* in SE21S × HR1128 (**a**) and SE21S × R317 (**b**) F_2 populations were shown as dots in different colors. Error bars show the standard variations

performance of "wild type"—much later than the parental genotypes. Therefore, dominance complementation effect was the major cause of extremely late flowering in the F_1 hybrids. However, it should be noticed that the dominance effects were incomplete for both genes. Hence, the heading date of heterozygous genotypes *Hd1*$^{WT/4bp\text{-}del}$ *Ghd8*$^{WT/1bp\text{-}del}$ was still earlier than that of "wild type" (~ 10 days ahead).

Allelic distribution of *Hd1* and *Ghd8*/*DTH8* in hybrid rice parents

Since the hybrid genotype with *Hd1*$^{WT/4bp\text{-}del}$ *Ghd8*$^{WT/1bp\text{-}del}$ would lead to strongly delayed flowering, why did most hybrid varieties show suitable heading date? To address the question, genetic data of 17 representative hybrid rice combinations (Huang et al. 2016) at the two major loci was investigated. In two-line hybrid rice from *indica-indica* crosses, most combinations contained the genotype *Hd1*$^{Mut/Mut}$*Ghd8*$^{WT/Mut}$, which was nearly equivalent to single mutant. In three-line hybrid rice from *indica-indica* crosses, most combinations contained the genotype *Hd1*$^{WT/Mut}$*Ghd8*$^{Mut/Mut}$ or *Hd1*$^{Mut/Mut}$*Ghd8*$^{Mut/Mut}$, which was nearly equivalent to single mutant or double mutant. Hence, the problem of extremely late flowering was avoided by the reasonable genotypic combinations.

Discussion

Importance of flowering-related genes in hybrid rice breeding

In a previous study, 17 populations containing totally 10,074 F_2 individuals were sequenced and phenotyped to search for the key heterosis-related genes in hybrid rice (Huang et al. 2016). It was found that several major heterosis-related genes are the key nodes in the flowering time pathway, including *Hd3a*, *Ghd8*, *Ghd7*, and *Hd1*. It was also reported that the tomato ortholog of *Hd3a* showed a strong overdominance effect for yield per plant (Krieger et al. 2010). Hence, flowering-related genes play a very important role in hybrid rice breeding. The knowledge derived from genetic mapping and flowering gene functions is hopeful to be applied in agricultural breeding. Particularly, the genotypes of *Hd3a*, *Ghd8*, *Ghd7*, *Ghd7.1*, and *Hd1* could be screened and checked to facilitate genetic improvements of parental lines and the designs of hybrid crosses, which may help hybrid rice breeding via a more efficient way. For instance, it should be avoided to develop F_1 hybrids that contained all the four wild-type alleles (*Hd1*, *Ghd7*, *Ghd7.1*, and *Ghd8*).

Rapid identification of important QTLs using high-throughput sequencing

Next generation sequencing technology provides a powerful tool for genetic mapping of complex traits. The methods that have been used in rice to accelerate genetic mapping include high-density genotyping in linkage populations (Huang et al. 2009), genome-wide association studies (Huang et al. 2010; Huang et al. 2011), and bulked-segregant analysis (Wang et al. 2019). In this study, we adopted the following strategies—(i) generation of F_2 populations, (ii) pooled sequencing and bulked-segregant analysis, (iii) PCR-based validation of the strong candidates. Considering a high level of genetic diversity in cultivated and wild rice accessions (Zhao et al. 2018), there are a number of novel QTLs to be mapped and the causal genes to be identified. In future, using high-throughput sequencing, generation of permanent populations, and next-generation analysis methods, it will be possible to identify important QTLs more rapidly and more precisely (Zhou and Huang 2019).

Construction of *Hd1* and *Ghd8*/*DTH8* mutants through genome editing

The development of genome editing approaches, especially CRISPR-Cas9 technology, has greatly accelerated genetic research in rice. To better understand the function of *Hd1* and *Ghd8*/*DTH8* in different genetic backgrounds, we are planning to construct CRISPR-Cas9 lines for *Hd1* and *Ghd8*/*DTH8* in *japonica*, *indica*, and *aus*. The CRISPR-Cas9 technology could provide enormous opportunities to precisely evaluate the effects of diverse allelic combinations of flowering genes and to de novo create ideal alleles of the functionally characterized genes. All of these efforts, including genetic mapping of important genes in hybrid rice and functional investigation of the causal genes, will provide a new avenue for yield improvement in hybrid rice.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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