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Identification and fine mapping of a major QTL, *qHD19*, that plays pleiotropic roles in regulating the heading date in rice

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Abstract As a food consumed by more than half of the world's population, rice (*Oryza sativa*) has always been a hot spot in plant science research. The three most important agronomic traits of rice, the yield, plant height, and flowering time, are controlled by many quantitative trait locus (QTLs). In this study, a newly identified QTL, *qHD19*, was found to be controlled by a single recessive gene. Using two populations with chromosome segment substitutions, *qHD19* was narrowed to a 22.5-kb region containing three putative genes, one of which encoded a ZOS5-02-C2H2 zinc finger protein. This gene was regarded as the *qHD19* candidate. Further analysis showed that the amino acid sequence encoded by the *qHD19* gene included two amino acid mutations, (84): lysine (L) replaced by phenylalanine (F), and (169): alanine (A) replaced valine (V), and these mutations are expected to significantly alter the functions of the protein. Additionally, the CSSL87 and CSSL88 lines containing the *qHD19* gene not only exhibited a shorter plant height but also exhibited a higher yield, which showed that the *qHD19* gene presents good application prospects in rice breeding. Taken together, these data indicate that *qHD19* probably plays an important role in the signaling network of photoperiodic flowering as well as the regulation of plant height and yield potential.

Keywords Rice (*Oryza sativa* L. subsp. *indica*) · The ZhangPu wild rice (*Oryza rufipogon* Griff.) · Chromosome segment substitution lines · Heading date · Fine mapping

Abbreviations

CSSLs	Chromosome segment substitution lines
QTLs	Quantitative trait locus
SSR	Simple sequence repeat
INDEL	Insertion/deletion
ORF	Open reading frame
BAC	Bacterial artificial chromosome
PAC	P1-derived artificial chromosome

Introduction

Flowering time in plants is a critical determinant of the distribution and regional adaptability of plants (Andrés and Coupland 2012). As food for more than half of the world's population, rice has always been a hot spot in plant science research. Rice yield is not only determined by spike number, grain weight, plant height, and the number of grains per panicle but is also affected by flowering time (Li et al. 2003; Wang et al. 2008). The adaptation of flowering plants is largely determined by their flowering time, which is mainly controlled by photoperiod and temperature (Izawa 2007).

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Molecular genetic studies conducted in the past few decades have identified a number of flowering time locus. To date, 734 quantitative trait locus (QTLs) related to the heading stage have been identified, which are distributed among all of the chromosomes of rice (http://www.gramene.org/qtl/). A number of QTLs have been recently revealed in rice (Yano et al. 2000; Kojima et al. 2002; Xue et al. 2008; Wei et al. 2010; Gao et al. 2013; Wu et al. 2013; Gao et al. 2014; Liu et al. 2016; Kim et al. 2018; Zhang et al. 2019). Among these QTLs, dozens of early heading date genes have been identified. *Ehd1*, encoding a B-type response regulator, upregulates florigen gene expression under both short-day conditions and long-day conditions. *Grain number*, *plant height*, and *heading date 7* (*Ghd7*), encoding a CO, CO-like, TOC1 (CCT) domain protein, and *Days to heading 8* (*DTH8*), encoding a putative HAP3 subunit of a CCAAT box-binding transcription factor, act as LD-specific repressors of *Ehd1* (Xue et al. 2008; Wei et al. 2010). On the other hand, several positive *Ehd1* regulators have also been cloned. For example, it was shown that *Early heading date 2* (*Ehd2*)/*RID1*/*OsId1*, encoding a Cs2/His2-type zinc finger protein; *Early heading date 3* (*Ehd3*), encoding a putative plant homeo domain finger-containing protein; and *Early heading date 4* (*Ehd4*), encoding a CCCH-type zinc finger protein, independently promote *Ehd1* expression under both short-day conditions and long-day conditions (Matsubara et al. 2008; Matsubara et al. 2011; Gao et al. 2013).

The development of chromosome segment substitution lines (CSSLs), as suggested by Doi et al. (1997) and Kubo et al. (2002), allows the detection of QTLs for complex agronomical traits in plants and may well resolve issues related to the precise mapping of QTLs (Li et al. 2015). Specifically, CSSLs can be used for the detection and fine mapping QTLs as single Mendelian factors by blocking background genetic noise. Several CSSLs have been developed in rice, and many QTLs for traits of biological and economic interest have been detected (Kubo et al. 2002; Ebitani et al. 2005; Mei et al. 2006; Takai et al. 2007; Zhu et al. 2009; Chen et al. 2014; Subudhi et al. 2015; Qi et al. 2017; Liu et al. 2018; Balakrishnan et al. 2019; Sui et al. 2019). These achievements have undoubtedly enhanced the understanding of complex traits and promoted plant genomic studies.

In this study, using a bin map converted from an ultrahigh-quality physical map associated with the heading dates of 146 CSSLs, we identified a heading date gene, *qHD19*, by map-based cloning. The molecular cloning of *qHD19* and phenotypic analysis of the *qHD19* gene were also reported. Additionally, the CSSL87 and CSSL88 lines containing the *qHD19* gene not only exhibited a shorter plant height but also exhibited a higher yield, which showed that the *qHD19* gene presents good application prospects in rice breeding. Taken together, these data indicate that *qHD19* probably plays an important role in the signaling network of photoperiodic flowering as well as the regulation of plant height and yield potential.

Materials and methods

Plant materials

A total of 146 chromosome segment substitution lines (CSSLs) derived from DongNanHui 810/ZhangPu wild rice with DongNanHui 810 as the recurrent parent were used to analyze QTLs controlling the heading date. Among the 146 CSSLs, only one CSSL carried two substituted segments, and the remaining 145 carried only one substituted segment. The physical map indicated that the average length of the substituted segments per chromosome was 95.47 Mb in the CSSLs. The total length of the substituted segments in the CSSLs was 1145.65 Mb, which was 3.04 times the total length of the rice genome, and all of the chromosomes exhibited 100% coverage in both cases (Yang et al. 2016).

Identification and substitution mapping of QTLs for heading date

DongNanHui 810, ZhangPu wild rice, and 146 CSSLs were grown in a paddy field under natural conditions at the experimental farm of the Fujian Academy of Agricultural Sciences (Fuzhou, China) at the end of 2017. The field experiment was designed in randomized plots with three plots per genotype. For the parents and each CSSL, 64 plants were planted in eight rows, and three plots were selected to investigate the characteristics of the heading date. The heading date was reported as the mean value from three plots, and QTLs were identified on the basis of significant differences between the parents and each CSSL, as determined by *t* tests. All plants were grown according to standard commercial practices, with spacing of 13.3 cm between plants within each row and 26.4 cm between rows. Field management essentially followed normal agricultural practices, and the amounts of N,

P_2O_5, and K_2O applied were 127.5 kg/hm^2, 45.0 kg/hm^2, and 30.0 kg/hm^2, respectively.

Construction of the mapping population

The mapping population was constructed by crossing CSSL87 and CSSL88 with DongNanHui 810, and a total of 3769 recessive plants in the F_2 population were selected for fine mapping.

PCR amplification and marker detection

Plant DNA was extracted from the frozen leaves of rice plants using the CTAB method (Murray and Thompson 1980) with minor modifications. The extracted DNA was dissolved in ddH_2O. DNA amplification was performed by PCR with the following parameters: 5 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 60 °C (for Indels) or 55 °C (for SSRs), and 50 s at 72 °C, with a final extension of 10 min at 72 °C. For the PCR amplification of markers, each 20 μL reaction mixture contained 50 ng of DNA, 5 μmol of each primer, 10× PCR buffer [100 mM Tris (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 2 μg of gelatin], each dNTP at 250 μM and 0.5 U of *Taq* polymerase. The amplified PCR products were resolved by electrophoresis in 8% polyacrylamide denaturing gels with silver staining for SSR markers (Panaud et al. 1996).

Molecular mapping of the *HD19* gene

A physical map of the target gene was constructed through bioinformatic analysis using (bacterial artificial chromosome) BAC and P1-derived artificial chromosome (PAC) clones of cvar Nipponbare released by the International Rice Genome Sequencing project (IRGSP, http://rgp.dna.affrc.go.jp/IRGSP/index.html). The clones were anchored with the target gene-linked markers, and the alignment of sequences was then carried out using the pair wise Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast/bl2seq/b12.html).

Bioinformatic analysis

Candidate genes were predicted according to the available sequence annotation databases (http://rice.plantbiology.msu.edu/; http://www.tigr.org/). DNA and amino acid sequences were employed for complete alignment using Clustal X version 1.81.

Results

Substitution mapping of QTLs for heading date in the CSSLs

To evaluate the potential advantages of the CSSLs for QTL detection, phenotypic variations in the heading date were observed in the 146 CSSLs. There were two lines, CSSL87 and CSSL88, that showed earlier maturation (Fig. 1). Using three CSSLs, one QTL, *qHD19*, was identified and mapped within the marker interval between RM153 and RM17919, which spanned a genetic distance of 24.7 cM on rice chromosome 5 (Fig. 2).

Main agronomic characteristics of CSSL87 and CSSL88

To elucidate the genes that control the development of the rice heading date, we performed screening for the comparison of phenotypes between CSSL87, CSSL88, and DongNanHui 810. In addition to showing earlier

Fig. 1 Phenotypes of DongNanHui 810 (left) and CSSL87 (right)

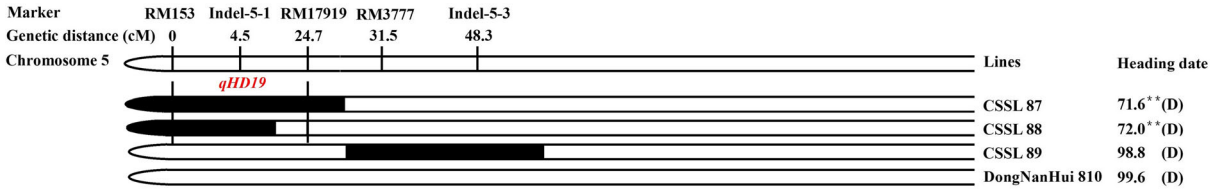

Fig. 2 Substitution mapping of the *qHD19* gene. The substituted segments from ZhangPu wild rice are indicated by black bars. The substituted segments from ZhangPu wild rice are indicated by black bars with the assumption that a segment flanked by one marker of the donor type and one marker of the recipient type constitutes a 50% donor genotype. For the purposes of mapping, however, the full region between one marker of the donor type and one marker of the recipient type was used as the boundary on each end. The vertical bars through the CSSLs indicate the region to which the gene was mapped. Note: **Shows significance at the 0.01 level

maturation, CSSL87 and CSSL88 showed a number of special traits (Table 1). For example, CSSL87 and CSSL88 presented a shorter plant height and more effective panicles than DongNanHui 810, showing a difference at the 0.05 probability level or a significant difference at the 0.01 probability level. Additionally, CSSL87 and CSSL88 displayed shorter panicle lengths and fewer spikelets per panicle than DongNanHui 810, differing at the 0.05 probability level. However, we observed that the seed setting rate and the 1000-grain weight of CSSL87 and CSSL88 showed no difference compared with those of DongNanHui 810. It is worth mentioning that the yields of CSSL87 and CSSL88 were 44.06 g and 43.61 g, respectively, which were higher compared with that of DongNanHui 810 (Table 1).

Genetic analysis of the gene for the *qHD19* trait

To determine whether *qHD19* was controlled by a single gene or multiple genes, CSSL87 and CSSL88 were crossed with DongNanHui 810. All F_1 hybrids showed normal phenotypes, and all F_2 populations showed normal Mendelian segregation (Table 2). The segregation of the DongNanHui 810 and CSSL87 or CSSL88 plants fit a 3:1 segregation ratio in the two F_2 populations ($\chi 2 = 0.430$~0.688, $P > 0.05$) (Table 2). Therefore, these results indicated that the early maturation phenotype was controlled by a single recessive gene.

Fine mapping of the *qHD19* gene

To map the *qHD19* gene to a smaller region, 3769 recessive individuals were identified from the two F_2 populations (Table 2). Another map was constructed using published markers (http://archive.gramene.org/markers/) in the region between RM153 and RM17919, the *qHD19* gene was mapped between molecular markers RM17777 and RM17790, and the physical distance between the two markers was 162 kb (Fig. 3b). To further map *qHD19*, two polymorphic markers were selected between molecular markers RM17777 and RM17790. The results showed that the *qHD19* gene was mapped between molecular markers RM17777 and RM17783 on chromosome 5, and the physical distance between the two markers was 69 kb (Fig. 3c and Table 3).

To fine map the *qHD19* gene, seven polymorphic InDels were selected from 24 new InDels (Table 3). The InDel markers were designed from publicly

Table 1 Comparison of the main agronomic traits between CSSL87, CSSL88, and the DongNanHui 810

Name	Heading date (D)	Plant height (cm)	Panicle length (cm)	Number of effective panicles	Spikelets per panicle	Seed setting rate (%)	The 1000-grain weight (g)	Yield per plant (g)
DongNanHui 810	99.6	118.2	24.8	8.0	137.2	92.79	30.25	30.81
CSSL87	71.6**	107.6**	22.1*	13.2**	121.4*	93.47	29.42	44.06**
CSSL88	72.0**	106.8**	21.7*	12.8**	122.2*	92.99	29.98	43.61**

* Difference between CSSL and DongNanHui 810 at $P < 0.05$; ** difference between CSSL and DongNanHui 810 at $P < 0.01$. Data are derived from the trial performed at Fuzhou experimental station in October 2017

Table 2 Segregations of F_2 populations crossed by CSSL87 and CSSL88

Crosses	F_1 phenotype	F_2 population			$\chi^2(3:1)$	P
		Earliness plants	Normal type	Total plants		
CSSL87/DongNanHui 810	Normal type	48	160	208	0.430*	0.5–0.75
CSSL88/DongNanHui 810	Normal type	52	137	189	0.648*	0.25–0.5

*Denote the segregation ratio of normal plants to mutant plants complied with 3:1 at 0.05 significant probability level

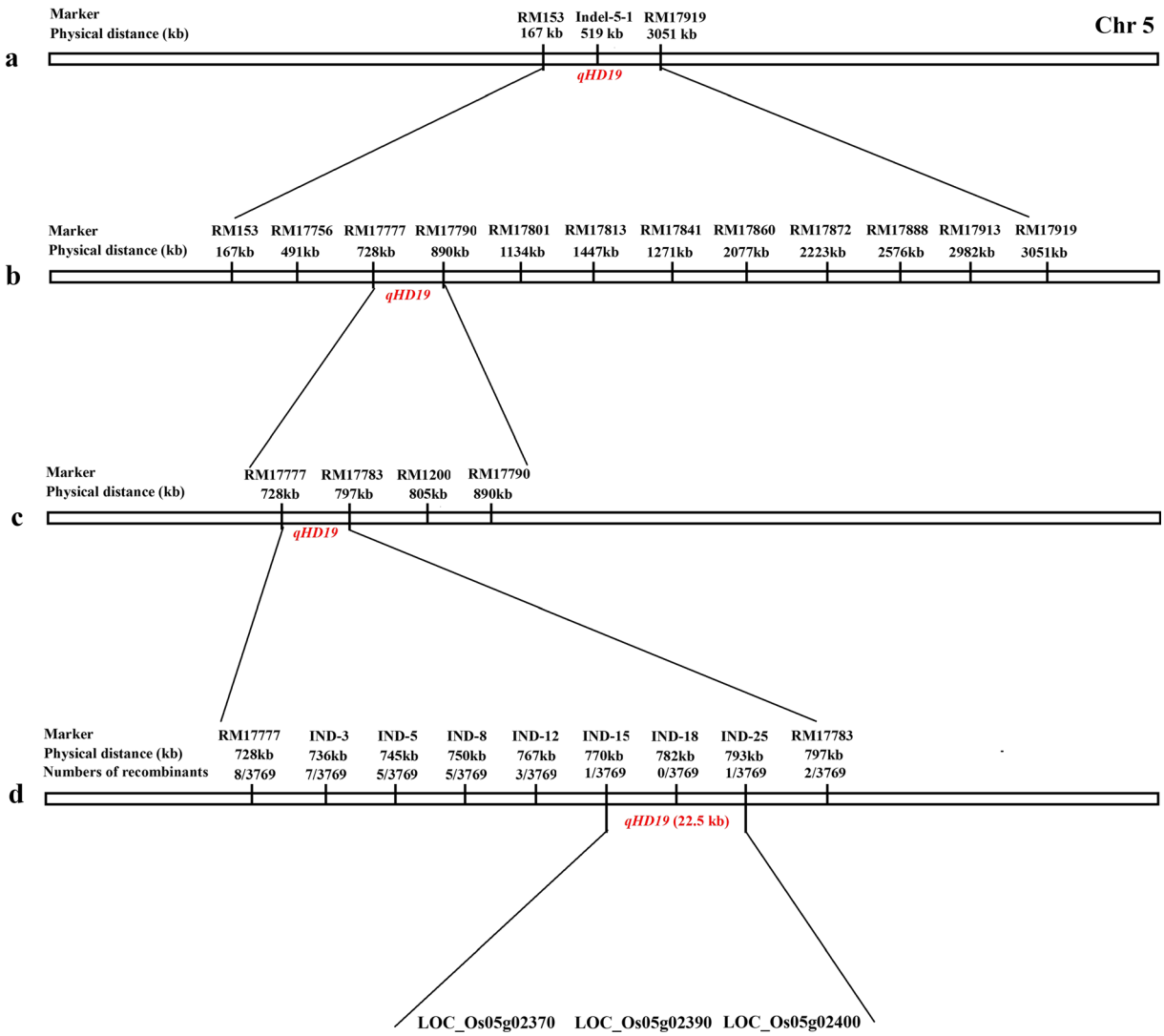

Fig. 3 Genetic and physical maps of the *qHD19* gene. **a** Primary mapping of the *qHD19* gene. The introgressed segment of *qHD19* on the short arm of chromosome 5, and the gene was mapped to the region between markers RM153 and RM17919. **b** Further mapping of the *qHD19* gene. The gene was mapped to the region between markers RM17777 and RM17790. **c** Fine mapping of the *qHD19* gene. The gene was mapped to the region between markers RM17777 and RM17783. **d** High-resolution mapping of *qHD19*. The *qHD19* gene was finally localized to a 22.5-kb region between markers IND-15 and IND-25, and the number of recombinants between the markers and the target genes was indicated under the linkage map

Table 3 Indel and SSR molecular marker used for fine mapping of the *qHD19* gene

Marker	Sequence of forward primer	Sequence of reverse primer
RM17813	TGTACAGGAAGTCCCTCCGATCC	CCGTGGATTTCTGGTGGTTGG
RM17841	TTGTCCGTCTACCTAGCAAAGC	AATTAATCCGCCCTCTACATCC
RM17860	GTTCCAACGAGATCCCGTACAATAGC	CGCCGGTTTCTACACTATTTCTGACG
RM17872	ATTCAACTGGTCGGAGACAAGTAACC	CTGACGACGACGATGATGAGG
RM17888	CAGTAGGCGTGCAGCGAAGG	CCCGACGAGGATGAAATCGTAGC
RM17913	CATGTCGGAGGAGGAGGAAGACG	ACGACGCGGAGGAAGTTGAGG
RM17919	CCCTGCAACTATACTTGATCGATGG	GTTGTTGGAGCTTCTCGTCATCC
RM17883	TGCACTTAACGAAGGAAGAAGAGG	GCGGATTTAATCTCCCACAGC
RM1200	AGTTTGCTGTTCTTGGAGTCGTAGC	GAGAAGAATTCCAGCACGTCAGC
IND-3	TAAAATTTTCCTACACACAC	CGTCGTGAAGACTGAAGAGT
IND-5	ATCACCGTTGTCTCAGTCTC	TGAAATATCAAAAATGCCCT
IND-8	ACCGGTACTTTGGACTTTTC	ATTGGATTGAGGGGATATTT
IND-12	GGTAGTACTGGGCAGAAAAA	CCAGCTGGTGTAATTCACTT
IND-15	GAGAAGAGCTCGAGACGG	ACCAATTGATAGAAGGGGAG
IND-18	ATGCATCGAGCAAGTAGC	CAACAAGGGTGATGTGAAC
IND-25	ATGACTAATTTGCGAGATGA	AACTAATAACAGAACCCGTC

available rice genome sequences, and the likelihood of detecting polymorphisms between ZhangPu wild rice and DongNanHui 810 was predicted by comparing sequences from Nipponbare (http://rgp.dna.affrc.go.jp/) and *indica* cultivar 93-11 (http://rice.genomics.org.cn/). First, the BAC clone sequences of *japonica* and *indica* were aligned; primers were then designed using Primer Premier 5.0 based on the polymorphic region between the two rice subspecies; and polymorphic markers were finally used for gene mapping. Recombinant screening with seven markers (IND-3, IND-5, IND-8, IND-12, IND-15, IND-18 and IND-25) located within the *qHD19* locus detected seven, five, five, three, one, zero, and one recombinant, respectively. Thus, the *qHD19* gene was precisely mapped within a 22.5-kb region between IND-15 and IND-25 (Fig. 3d).

Candidate genes in the 22.5-kb region

There were three candidate genes (LOC_Os05g02370, LOC_Os05g02390, and LOC_Os05g02400) in the 22.5-kb region (Fig. 3d), according to the available sequence annotation databases (http://rice.plantbiology.msu.edu/; http://www.tigr.org/). LOC_Os05g02370 encodes an expressed protein, LOC_Os05g02390 encodes a ZOS5-02-C2H2 zinc finger protein, and LOC_Os05g02400 encodes an RNA recognition motif-containing protein.

Sequence analyses of the *qHD19* gene

To investigate which gene was responsible for the observed phenotype, the sequencing of three genes in DongNanHui 810, ZhangPu wild rice, CSSL87, and CSSL88 revealed that two base substitutions (A to G and C to T) occurred in LOC_Os05g02390 (Fig. 4), whereas no differences in LOC_Os05g02370 and LOC_Os05g02400 were observed in DongNanHui 810, ZhangPu wild rice, CSSL87, and CSSL88. Thus, it was concluded that the LOC_Os05g02390 locus corresponded to *qHD19*.

The analysis of the open reading frame (ORF) region showed that the *qHD19* gene (LOC_Os05g02390) had one exon and no introns. *qHD19* was a two-point mutant, exhibiting the exchange of A for G (positions 269) and C for T (positions 506) in its cDNA (Fig. 5).

Further analysis showed that the amino acid sequence encoded by the *qHD19* gene presented two amino acid mutations, (84): lysine (L) replaced by phenylalanine (F), and (169): alanine (A) replaced by valine

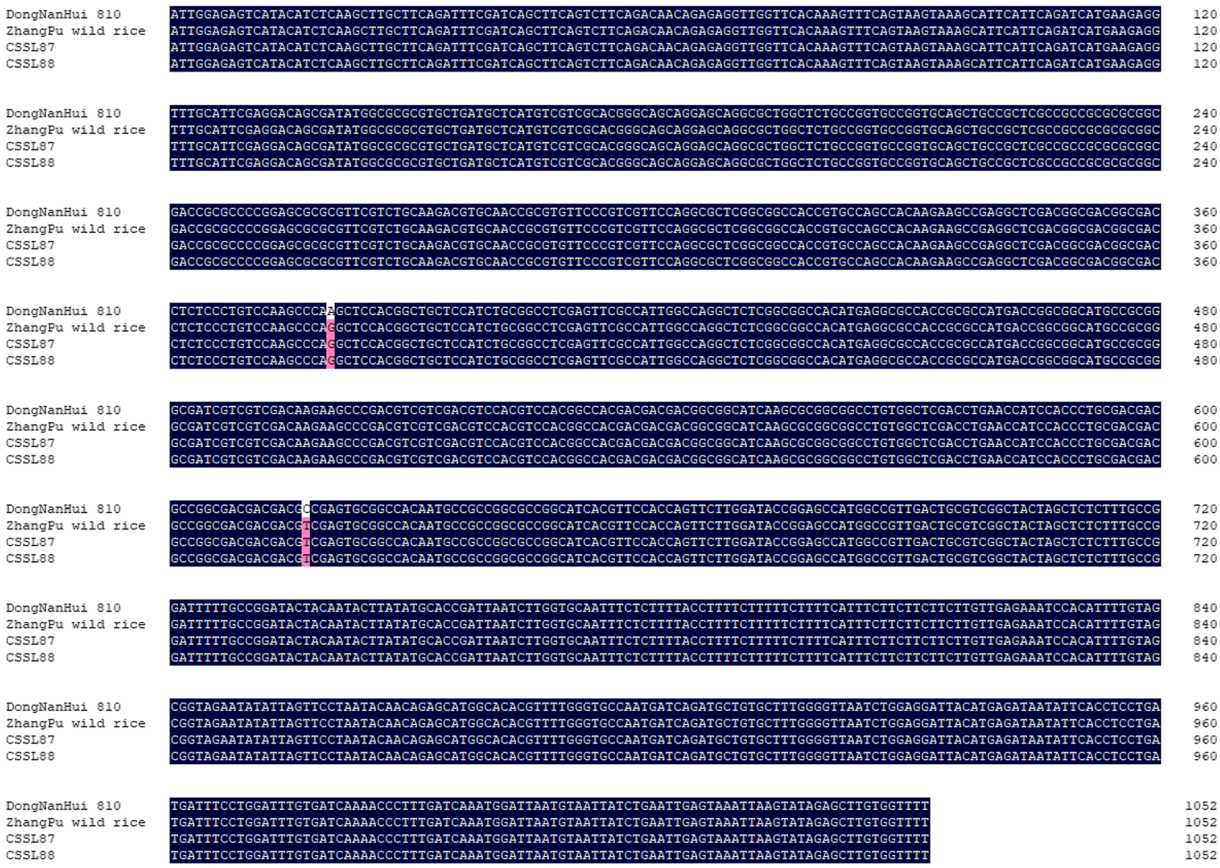

Fig. 4 Sequence comparison in DongNanHui 810, ZhangPu wild rice, CSSL87, and CSSL88 in LOC_Os05g02390

(V) (Fig. 6). Lysine is a positively charged, basic amino acid, while phenylalanine is a nonpolar, hydrophobic amino acid. As such, these mutations would be expected to alter the function of the protein significantly.

Phylogenetic tree for the *qHD19* gene

To gain insight into the function of *qHD19*, a phylogenetic tree was generated using the zinc finger protein

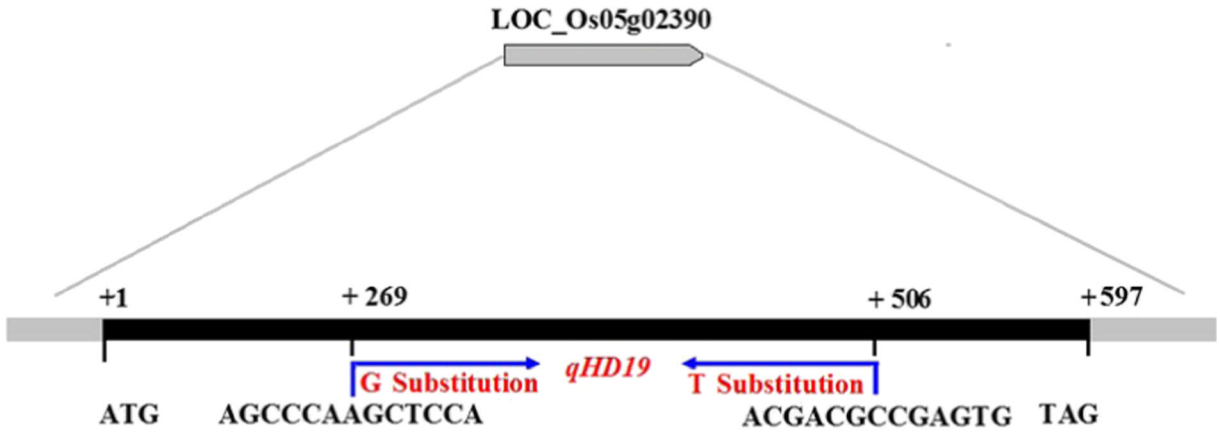

Fig. 5 The structure of the *qHD19* gene and LOC_Os05g02390. The start (ATG) and stop codons (TAG) are indicated. Black and gray boxes indicate the ORF region and the untranslated region (UTR) of the *qHD19* gene, respectively. There were two substitutions in the ORF region of *qHD19*

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                                  10        20        30        40        50        60
                         ....|....|....|....|....|....|....|....|....|....|....|....|
DongNanHui810       1    MKRFAFEDSDMARVLMLMSSHGQQEQALALPVPVQLPLAAARGDRAPERAFVCKTCNRVF  60
ZhangPu             1    MKRFAFEDSDMARVLMLMSSHGQQEQALALPVPVQLPLAAARGDRAPERAFVCKTCNRVF  60
CSSL87              1    MKRFAFEDSDMARVLMLMSSHGQQEQALALPVPVQLPLAAARGDRAPERAFVCKTCNRVF  60
CSSL88              1    MKRFAFEDSDMARVLMLMSSHGQQEQALALPVPVQLPLAAARGDRAPERAFVCKTCNRVF  60
Clustal Consensus   1    ************************************************************  60

                                  70        80        90       100       110       120
                         ....|....|....|....|....|....|....|....|....|....|....|....|
DongNanHui810       61   PSFQALGGHRASHKKPRLDGDGDLSLSKPKLHGCSICGLEFAIGQALGGHMRRHRAMTGG  120
ZhangPu             61   PSFQALGGHRASHKKPRLDGDGDFSLSKPRLHGCSICGLEFAIGQALGGHMRRHRAMTGG  120
CSSL87              61   PSFQALGGHRASHKKPRLDGDGDFSLSKPRLHGCSICGLEFAIGQALGGHMRRHRAMTGG  120
CSSL88              61   PSFQALGGHRASHKKPRLDGDGDFSLSKPRLHGCSICGLEFAIGQALGGHMRRHRAMTGG  120
Clustal Consensus   61   **********************:*****:******************************  120

                                 130       140       150       160       170       180
                         ....|....|....|....|....|....|....|....|....|....|....|....|
DongNanHui810       121  MPRAIVVDKKPDVVDVHVHGHDDDGGIKRGGLWLDLNHPPCDDAGDDDAECGHNAAGAGI  180
ZhangPu             121  MPRAIVVDKKPDVVDVHVHGHDDDGGIKRGGLWLDLNHPPCDDAGDDDVECGHNAAGAGI  180
CSSL87              121  MPRAIVVDKKPDVVDVHVHGHDDDGGIKRGGLWLDLNHPPCDDAGDDDVECGHNAAGAGI  180
CSSL88              121  MPRAIVVDKKPDVVDVHVHGHDDDGGIKRGGLWLDLNHPPCDDAGDDDVECGHNAAGAGI  180
Clustal Consensus   121  ************************************************.***********  180

                                 190
                         ....|....|....|...
DongNanHui810       181  TFHQFLDTGAMAVDCVGY  198
ZhangPu             181  TFHQFLDTGAMAVDCVGY  198
CSSL87              181  TFHQFLDTGAMAVDCVGY  198
CSSL88              181  TFHQFLDTGAMAVDCVGY  198
Clustal Consensus   181  ******************  198
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Fig. 6 Sequence comparison of amino acids in DongNanHui 810, ZhangPu wild rice, CSSL87, and CSSL88 at LOC_Os05g02390

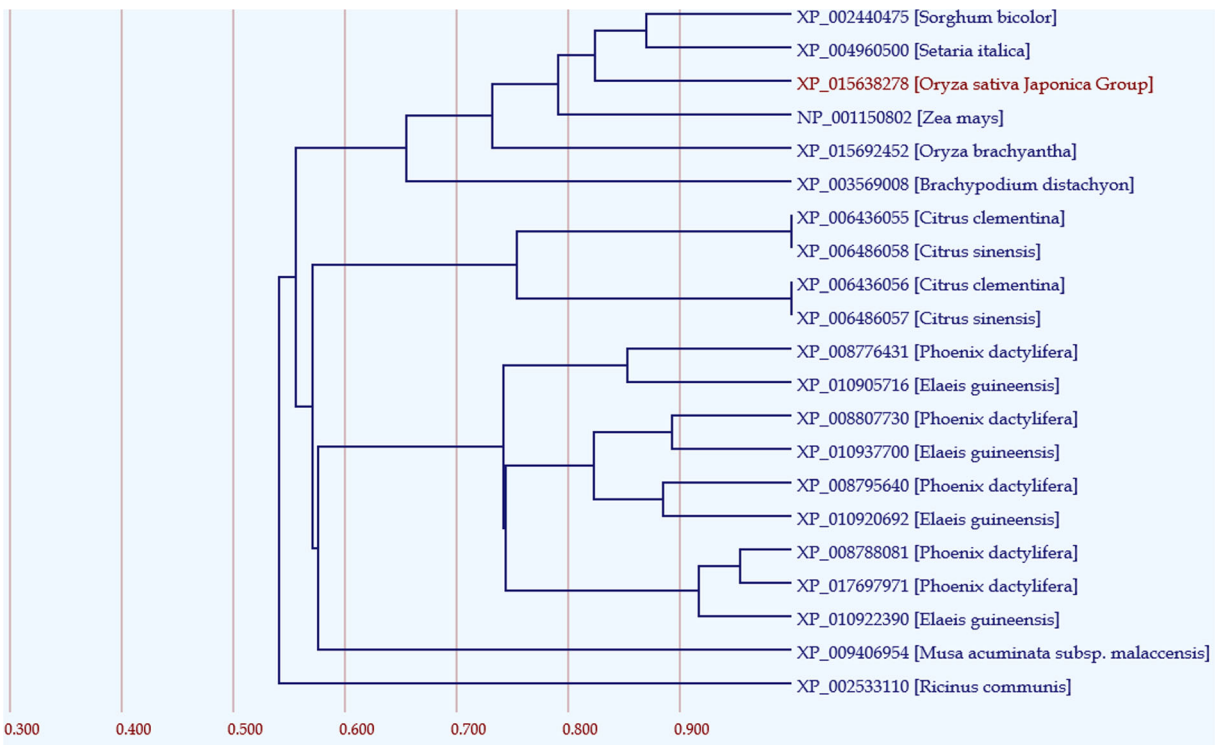

Fig. 7 Phylogenetic tree of the *qHD19* gene

Table 4 Homolog gene of the *qHD19* gene by species

Species	Repr. RefSeq ID	Annotation
Sorghum bicolor	XP_002440475	Hypothetical protein; C2H2-type zinc finger
Setaria italica	XP_004960500	Zinc finger protein ZAT12-like; C2H2-type zinc finger; pfam13912; C2H2 Zn finger
Oryza sativa Japonica Group	XP_015638278	Zinc finger protein ZAT12; C2H2-type zinc finger; pfam13912; C2H2 Zn finger
Zea mays	NP_001150802	ZFP16-1; C2H2-type zinc finger
Oryza brachyantha	XP_015692452	Uncharacterized protein LOC102713636; C2H2-type zinc finger
Brachypodium distachyon	XP_003569008	Zinc finger protein ZAT12-like; C2H2-type zinc finger; pfam13912; C2H2 Zn finger
Citrus clementina	XP_006436055	Hypothetical protein; C2H2-type zinc finger
Citrus sinensis	XP_006486058	Zinc finger protein ZAT12-like; C2H2-type zinc finger
Citrus clementina	XP_006436056	Hypothetical protein; C2H2-type zinc finger
Citrus sinensis	XP_006486057	Zinc finger protein ZAT12-like; C2H2-type zinc finger
Phoenix dactylifera	XP_008776431	Zinc finger protein ZAT11-like; C2H2-type zinc finger
Elaeis guineensis	XP_010905716	Zinc finger protein ZAT12-like; C2H2-type zinc finger
Phoenix dactylifera	XP_008807730	Zinc finger protein ZAT12; C2H2-type zinc finger
Elaeis guineensis	XP_010937700	Zinc finger protein ZAT12-like; C2H2-type zinc finger; pfam13912
Phoenix dactylifera	XP_008795640	Zinc finger protein ZAT12-like; C2H2-type zinc finger
Elaeis guineensis	XP_010920692	Zinc finger protein ZAT12-like; C2H2-type zinc finger
Phoenix dactylifera	XP_008788081	Zinc finger protein ZAT11; C2H2-type zinc finger
Phoenix dactylifera	XP_017697971	Zinc finger protein ZAT11-like
Elaeis guineensis	XP_010922390	Zinc finger protein ZAT11; C2H2-type zinc finger; pfam13912; C2H2 Zn finger
Musa acuminata subsp. *malaccensis*	XP_009406954	Zinc finger protein ZAT12-like; C2H2-type zinc finger
Ricinus communis	XP_002533110	Zinc finger protein ZAT12; C2H2-type zinc finger; pfam13912; C2H2 Zn finger

sequences from rice and other plants (Fig. 7) according to the available sequence annotation databases (http://www.plant.osakafu-u.ac.jp/~kagiana/gcorn/p/). The phylogenetic tree analysis showed highly homologous genes of *qHD19* in more than 20 different species (Fig. 7), and these genes all encoded zinc finger proteins (Table 4). These results indicated that the *qHD19* gene presented high homology and conservation among different plants.

Discussion

qHD19 may regulate grain productivity, plant height, and heading date

In rice, the three most important agronomic traits are yield, plant height, and flowering time. The cloned QTLs/genes that control important agronomic traits can be classified according to their functions into the three following groups: genes (*Gn1a* and *GIF1*) that underlie grain productivity (Ashikari et al. 2005; Wang et al. 2008), genes (*d2* and *d11*) that regulate plant height (Hong et al. 2003; Tanabe et al. 2005), and genes (*Ehd1* and *RID1*) that regulate heading date (Doi et al. 2004; Wu et al. 2008). Although there are close correlations between yield, plant height, and flowering time, most of these genes have been reported to regulate only one of the three traits. Recently, several QTLs/genes, which contained *ghd7*, *DTH8,DTH7*, *Ghd2*, and *OsNF-Y*, have been proven to have large pleiotropic effects on an array of traits, including grain number, flowering time, and plant height (Xue et al. 2008; Wei et al. 2010; Gao et al. 2014; Liu et al. 2016; Yang et al. 2017). In this study, a rice early maturation gene, *qHD19*, was isolated by map-based cloning. Additionally, CSSL87 and CSSL88 containing *qHD19* showed a shorter plant height and higher yields compared with DongNanHui 810 (Table 1). These results indicate that the *qHD19* gene presents good application prospects for rice breeding in the future.

Utilization of *qHD19* in rice breeding

Plant breeding aimed at improving the genetic basis of new varieties of crops with increased productivity and quality, combines art with science (Xi et al. 2006). In general, traditional breeding is predominantly based on phenotypic assays (Xu et al. 2010). However, this approach has targeted QTLs for very few traits for genetic improvement. On the other hand, CSSLs, which are selected at the level of the whole genome with multi-trait breeding objectives, have expanded the available targets and thus become important in improving the properties of plants (Xu et al. 2010). In this study, the *qHD19* gene was associated with not only a shorter plant height but also a higher yield (Table 1). Therefore, the *qHD19* gene presents good application prospects for rice breeding. First, breeders can develop excellent conventional rice varieties using *qHD19*. For example, CSSL87 and CSSL88 containing the *qHD19* gene showed a shorter plant height and higher yield compared with DongNanHui 810. These two lines can be used as conventional rice lines that can be directly included in regional rice tests and may be certified as new rice varieties. Second, the *qHD19* gene was found to be controlled by a single recessive gene (Table 2). Therefore, to breed a new hybrid rice variety, breeders can transfer this gene into both restorer and sterile lines with the assistance of molecular markers.

Why does *qHD19* result in early maturation in DongNanHui 810?

Although the *qHD19* gene came from the ZhangPu wild rice, ZhangPu wild rice did not show early maturation in the same culture environment. We transferred the *qHD19* gene into DongNanHui 810 and obtained two stable lines (CSSL87 and CSSL88) via molecular marker-assisted selection. However, the two lines both showed early maturation (Fig. 2), giving rise to the question of why this occurs? *Ehd1* is a pivotal convergence point that integrates multiple signaling pathways to regulate the flowering time of rice under diverse environmental conditions (Tsuji et al. 2011). Studies have shown that the *Ehd4* gene encodes a new CCH zinc finger protein and that *Ehd4* is upregulated by *Ehd1*, leading to the expression of the anthocyanin genes *Hd3a* and *RFT1* to promote flowering, independent of other known *Ehd1* regulators (Gao et al. 2013). We hypothesized that *qHD19* may also promote flowering by upregulating the expression of genes related to anthocyanin through *Ehd1*. In cultivated rice, the *qHD19* gene can normally regulate the expression of related genes and promote flowering, while in ZhangPu wild rice, due to the absence of related genes, flowering cannot be normally regulated, thus delaying heading.

Author's contribution statement DY drafted the manuscript. DY, XY, XZ, CC, and NY contributed to the data analysis. DY participated in the design of the study and the interpretation of the results and wrote and edited the manuscript.

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Compliance with ethical standards

Ethics approval and consent to participate The authors declare that the experiments comply with the current laws of Italy and the P. R. of China.

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

References

Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. Science 309:741–745

Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet 13(9):627–639

Balakrishnan D, Surapaneni M, Mesapogu S, Neelamraju S (2019) Development and use of chromosome segment substitution lines as a genetic resource for crop improvement. Theor Appl Genet 132(1):1–25

Chen JB, Li XY, Cheng C, Wang YH, Qin M, Zhu HT, Zeng RZ, Fu XL, Liu ZQ, Zhang GQ (2014) Characterization of epistatic interaction of QTLs *LH8* and *EH3* controlling heading date in rice. Sci Rep 4:4263

Doi K, Iwata N, Yoshimura A (1997) The construction of chromosome substitution lines of African rice (*Oryza* glaberrima Steud.) in the background of japonica rice (*O. sativa* L.). Rice Genet News 14:39–41

Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of *Hd1*. Genes Dev 18:926–936

Ebitani T, Takeuchi Y, Nonoue Y, Yamamoto T, Takeuchi K, Yano M (2005) Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of indica rice cultivar 'Kasalath' in a genetic background of japonica elite cultivar 'Koshihikari'. Breed Sci 55: 65–73

Gao H, Zheng XM, Yuan DY, Wang MQ, Zhang Z, Sheng P, Ma WW, Jiang L, Wang HY, Yuan LP (2014) *Days to heading 7*, a major quantitative locus determining photoperiod sensitivity and regional adaptation in rice. Proc Natl Acad Sci U S A 111(51):16337–16342

Gao H, Zheng XM, Fei GL, Chen J, Jin MN, Ren YL, Wu WX, Zhou KN, Sheng PK, Zhou F, Jinag L, Wang J, Zhang X, Guo XP, Wang LJ, Cheng ZJ, Wu CY, Wang HY, Wan JM (2013) *Ehd4* encodes a novel and Oryza-genus-specific regulator of photoperiodic flowering in rice. PLoS Genet 9: e1003281

Hong Z, Ueguchi-Tanaka M, Umemura K, Uozu S, Fujioka S, Takatsuto S, Yoshida S, Ashikari M, Kitano H, Matsuoka M (2003) A rice brassinosteroid-deficient mutant, ebisu dwarf (*d2*), is caused by a loss of function of a new member of cytochrome P450. Plant Cell 15:2900–2910

Izawa T (2007) Adaptation of flowering-time by natural and artificial selection in Arabidopsis and rice. J Exp Bot 58(12):3091–3097

Kim SR, Torollo G, Yoon MR, Kwak J, Lee CK, Prahalada GD, Choi IR, Yeo US, Jeong OY, Jena KK, Lee JS (2018) Loss-of-function alleles of *heading date 1* (*Hd1*) are associated with adaptation of temperate *Japonica* rice plants to the tropical region. Front Plant Sci 9:1827

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of hd1 under short-day conditions. Plant Cell Physiol 43(10):1096–1105

Kubo T, Aida Y, Nakamura K, Tsunematsu H, Doi K, Yoshimura A (2002) Reciprocal chromosome segment substitution series derived from japonica and indica cross of rice (Oryza sativa L.). Breed Sci 52:319–325

Liu D, Yan Y, Fujita Y, Xu D (2018) Identification and validation of QTLs for 100-seed weight using chromosome segment substitution lines in soybean. Breed Sci 68(4):442–448

Li XY, Qian Q, Fu ZM, Wang YH, Xiong GS, Zeng DL, Wang XQ, Liu XF, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li JY (2003) Control of tillering in rice. Nature 422:618–621

Li XN, Wang WK, Wang Z, Li KN, Lim YP, Piao ZY (2015) Construction of chromosome segment substitution lines enables QTL mapping for flowering and morphological traits in Brassica rapa. Front Plant Sci 6:432. https://doi.org/10.3389/fpls.2015.00432

Liu JH, Shen JQ, Xu Y, Li XH, Xiao JH, Xiong LZ (2016) *Ghd2,* a *CONSTANS*-like gene, confers drought sensitivity through regulation of senescence in rice. J Exp Bot 67(19):5785–5798

Matsubara K, Yamanouchi U, Wang ZX, Minobe Y, Izawa T, Yano M (2008) *Ehd2*, a rice ortholog of the maize indeterminate1 gene, promotes flowering by up-regulating *Ehd1*. Plant Physiol 148:1425–1435

Matsubara K, Yamanouchi U, Nonoue Y, Sugimoito K, Wang XZ, Minobe Y, Yano M (2011) *Ehd3*, encoding a phd finger-containing protein, is a critical promoter of rice flowering. Plant J 66(4):603–612

Mei HW, Xu JL, Li ZK, Yu XQ, Guo LB, Wang YP, Ying CS, Luo LJ (2006) QTLs influencing panicle size detected in two reciprocal introgressive line (IL) populations in rice (Oryza sativa L.). Theor Appl Genet 112(4):648–656

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4326

Panaud O, Chen X, Mccouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (Oryza sativa L.). Mol Gen Genet 252:597–607

Qi L, Sun Y, Li J, Su L, Zheng XM, Wang XN, Li KM, Yang QW, Qiao WH (2017) Identify QTLs for grain size and weight in common wild rice using chromosome segment substitution lines across six environments. Breed Sci 67(5):472–482

Sui F, Zhao D, Zhu H, Gong Y, Tang Z, Huang XY, Zhang G, Zhao FJ (2019) Map-based cloning of a new total loss-of-function allele of *OsHMA3* causing high cadmium accumulation in rice grain. J Exp Bot 70(10):2857–2871. https://doi.org/10.1093/jxb/erz093

Subudhi PK, Leon TD, Singh PK, Parco A, Cohn MA, Sasaki T (2015) A chromosome segment substitution library of weedy rice for genetic dissection of complex agronomic and domestication traits. PLoS One 10(6):e0130650

Takai T, Nonoue Y, Yamamoto S, Yamanouchi U, Matsubara K, Liang ZW, Lin HX, Ono N, Uga Y, Yano M (2007) Development of chromosome segment substitution lines derived from backcross between indica donor cultivar 'Nona bokra' and japonica recipient cultivar 'Koshihikari'. Breed Sci 57:257–261

Tanabe S, Ashikari M, Fujioka S, Takatsuto S, Yoshida S, Yano M, Yoshimura A, Kitano H, Matsuoka M, Fujisawa Y, Kato H, Iwasaki Y (2005) A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, dwarf11, with reduced seed length. Plant Cell 17:776–790

Tsuji H, Taoka KI, Shimamoto K (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. Curr Opin Plant Biol 14:45–52

Wang E, Wang JJ, Zhu XD, Hao W, Wang LY, Li Q, Zhang LX, He W, Lu BR, Lin HX, Ma H, Zhang GQ, He ZH (2008) Control of rice grain-filling and yield by a gene with a potential signature of domestication. Nat Genet 40(11): 1370–1374

Wei XJ, Xu JF, Guo HN, Jiang L, Chen SH, Yu CY, Zhou ZL, Hu PS, Zhai HQ, Wan JM (2010) *DTH8* suppresses flowering in rice, influencing plant height and yield potential simultaneously. Plant Physiol 153:1747–1758

Wu CY, You CJ, Li CS, Long T, Chen GX, Mary EB, Zhang QF (2008) *RID1*, encoding a Cys2/His2-type zinc finger transcription factor, acts as a master switch from vegetative to floral development in rice. Proc Natl Acad Sci U S A 105: 12915–12920

Wu WX, Zheng XM, Lu GW, Zhong ZZ, Gao H, Chen LP, Wu CY, Wang HJ, Wang Q, Zhou KN, Wang JL, Wu FQ, Zhang X, Guo XP, Cheng ZJ, Lei CL, Lin QB, Liang L, Wang HY,

Ge S, Wan JM (2013) Association of functional nucleotide polymorphisms at *DTH2* with the northward expansion of rice cultivation in Asia. Proc Natl Acad Sci U S A 110(8): 2775–2780

Xue WY, Xing YZ, Weng XY, Zhao Y, Tang WJ, Wang L, Zhou HJ, Yu SB, Xu CG, Li XH, Zhang QF (2008) Natural variation in *ghd7* is an important regulator of heading date and yield potential in rice. Nat Genet 40(6):761–767

Xi ZY, He FH, Zeng RZ, Zhang ZM, Ding XH, Li WT, Zhang GQ (2006) Development of a wide population of chromosome single-segment substitution lines in the genetic background of an elite cultivar of rice (*Oryza sativa* L.). Genome 49:476–484

Xu JJ, Zhao Q, Du P, Xu CW, Wang BH, Feng Q, Liu QQ, Tang SZ, Gum H, Han B, Liang GH (2010) Developing high throughput genotyped chromosome segment substitution lines based on population whole-genome re-sequencing in rice (*Oryza sativa* L.). BMC Genomics 24:656

Yang DW, Ye XF, Zheng XH, Cheng CP, Ye N, Huang FH (2016) Development and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of the whole wild rice genome. Front Plant Sci 7:01737

Yang WJ, Lu ZH, Xiong YF, Yao JL (2017) Genome-wide identification and co-expression network analysis of the *OsNF-Y* gene family in rice. Crop J 5(1):21–31

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12(12):2473–2483

Zhang ZH, Zhu YJ, Wang LS, Fan YY, Zhuang JY (2019) Importance of the interaction between heading date genes *Hd1* and *Ghd7* for controlling yield traits in rice. Int J Mol Sci 20:516

Zhu WY, Lin J, Yang DW, Zhao L, Zhang YD, Zhu Z, Chen T, Wang CL (2009) Development of chromosome segment substitution lines derived from backcross between two sequenced rice cultivars, indica recipient 93-11 and japonica donor Nipponbare. Plant Mol Biol Report 27(2):126–131