Molecular Biology Reports (2022) 49:63–71
https://doi.org/10.1007/s11033-021-06842-8

ORIGINAL ARTICLE

QTL mapping and candidate gene mining of flag leaf size traits in *Japonica* rice based on linkage mapping and genome-wide association study

Jiangxu Wang[1] · Tao Wang[1] · Qi Wang[1] · Xiaodong Tang[1] · Yang Ren[1] · Haiyan Zheng[1] · Kai Liu[1] · Luomiao Yang[2] · Hui Jiang[1] · Yidan Li[1] · Qi Liu[1] · Detang Zou[2] · Hongliang Zheng[1,2]

Received: 31 August 2021 / Accepted: 13 October 2021 / Published online: 22 October 2021

Abstract

Background As one of the most important factors of the *japonica* rice plant, leaf shape affects the photosynthesis and carbohydrate accumulation directly. Mining and using new leaf shape related genes/QTLs can further enrich the theory of molecular breeding and accelerate the breeding process of *japonica* rice.

Methods In the present study, 2 RILs and a natural population with 295 *japonica* rice varieties were used to map QTLs for flag leaf length (FL), flag leaf width (FW) and flag leaf area (FLA) by linkage analysis and genome-wide association study (GWAS) throughout 2 years.

Results A total of 64 QTLs were detected by 2 ways, and pleiotropic QTLs *qFL2* (Chr2_33,332,579) and *qFL10* (Chr10_10,107,835; Chr10_10,230,100) consisted of overlapping QTLs mapped by linkage analysis and GWAS throughout the 2 years were identified.

Conclusions The candidate genes *LOC_Os02g54254*, *LOC_Os02g54550*, *LOC_Os10g20160*, *LOC_Os10g20240*, *LOC_Os10g20260* were obtained, filtered by linkage disequilibrium (LD), and haplotype analysis. *LOC_Os10g20160* (*SD-RLK-45*) showed outstanding characteristics in quantitative real-time PCR (qRT-PCR) analysis in leaf development period, belongs to S-domain receptor-like protein kinases gene and probably to be a main gene regulating flag leaf width of *japonica* rice. The results of this study provide valuable resources for mining the main genes/QTLs of *japonica* rice leaf development and molecular breeding of *japonica* rice ideal leaf shape.

Keywords Japonica rice · QTLs · Flag leaf · Linkage mapping · Genome-wide association study

Introduction

Rice is one of the most important food crops in the word, bearing the lifeline of human food security [1–3]. The rice plant leaf is important part of histogenesis and morphogenesis, and also the main organ for photosynthesis and respiration [4–6]. The photosynthetic energy storage and normal life function of rice are directly affected by the leaf [7]. Meanwhile, leaf size is an important part of ideal rice plant type, as well as an important character of rice yield formation [8]. As the top functional leaf of rice, flag leaf has significant effects on plant related physiological characters and field population structure [9, 10]. Therefore, shaping and screening of rice leaf morphology in the breeding process is an effective way to improve rice quality and yield.

Although leaf size has a great influence on high photosynthetic efficiency, the genetic mechanism of leaf morphological characteristics is still unclear [11]. Mining QTLs by linkage mapping in RILs and GWAS mapping in natural populations to find the candidate genes might be the most efficient way to analyze the genetic basis of rice leaf shape.

In recent years, many QTLs and regulatory genes related to rice leaf morphology have been discovered (http://www.

Jiangxu Wang and Tao Wang contributed equally to this work.

✉ Hongliang Zheng
hongliangzheng@neau.edu.cn

[1] Heilongjiang Academy of Agricultural Sciences, Institute of Crops Tillage and Cultivation, Harbin 150030, China

[2] Present Address: Key Laboratory of Germplasm Enhancement, Physiology and Ecology of Food Crops in Cold Region, Ministry of Education, Northeast Agricultural University, Harbin 150030, China

gramene.org/) [12–15]. These QTLs and genes can change the physiological function of plants by regulating leaf morphology, and have important impacts on the coordination of light energy utilization and "sink-source" relationship. At the same time, their potential impact on rice yield has also been gradually discovered. Tang et al. [16] used CSSL population with 143 individuals, obtained 14 leaf length and 19 leaf width QTLs, and further obtained the rice leaf size gene *Ghd7.1* by fine mapping, mutation test, and allelic variation analysis. Zhang et al. [17] mapped the flag leaf width QTL *qflw7.2* to 27.1 kb by recombinant inbred lines derived from 93 to 11 and Peiai 64 s, and identified 2 candidate genes *LOC_Os07g41180*, *LOC_Os07g41200*. A natural population of 532 individuals was used for genome-wide association analysis and high-throughput leaf scoring, 73 QTLs associated with rice leaves were mapped as a result [18]. At present, there is a clear understanding of the cloned gene *NAL1* located on chromosome 4, which affects the growth of lateral leaf [19]. The *NAL1* mutant is characterized by dwarf and narrow leaf, and the expression level of the genes related to the polar transport of auxin and leaf development in the mutant changed [20]. It was highly expressed in vascular tissue, which played an important role in cell division and cell size regulation, promoting the lateral growth of the leaf. *OsFLW7* regulated the width of flag leaf and increased photosynthetic leaf area [21]. At the same time, *OsFLW7* is an allele of *GL7 / GW7*, which may be related to the regulation of grain traits and the mutant was found to have increased grain length, grain plumpness and yield.

GWAS and linkage analysis are both accurate and effective tools for QTL detection of complex rice traits [22]. The breadth and accuracy of QTL detection could be significantly improved by combining these 2 methods. In this study, 2 sets of RILs populations with high-density bin-map and 295 re-sequenced *japonica* rice accessions were used to conducted linkage/GWAS mapping of flag leaf length, width and area of *japonica* rice. Two novel QTL *qFL2*, *FL10* and five candidate genes related to flag leaf size in *japonica* rice were discovered, providing important references for molecular breeding of *japonica* rice with ideal leaf size and plant type.

Methods

Populations for QTL mapping

Natural population is composed of 295 rice varieties, most of which are temperate *japonica* rice, widely collected from Heilongjiang, Jilin and Liaoning provinces, while foreign varieties mainly originate from Japan, Korea, the Democratic People's Republic of Korea and Russia. This population has been used in previous studies [23]. RILsA contains 195 individuals, obtained from crossing the narrow erect leaf *japonica* rice variety K131 and wide curved leaf upland rice variety HDB. RILsB contains 189 individuals, which was derived from a cross between the long wide flag leaf *japonica* rice variety WD20342 and short narrow flag leaf variety Caidao. All materials were planted in Acheng rice experimental base of Northeast Agricultural University from 2019 to 2020. Each individual was planted in 8 rows with 20 plants in each row. Single plant transplanting was implemented and the spacing of rows and plants was 30 cm × 3 cm. The field management of water and fertilizer followed the basic method of conventional field production.

Phenotypic identification of flag leaf size

In order to reduce the influence of marginal effect on phenotype, for each variety plants the 5th plant in row 4 were selected as the research objects, and calculated that the 5 plants average value of each variety as the phenotypic value and the average value of five plants for each line was calculated. The flag leaf length (FL), width (FW) and area (FLA) were investigated at full heading stage using Tuopu YMJ-D Living Leaf Area Meter (Tuopu Yunnong Technology Co., Ltd). Population phenotypic correlation analysis for QTL mapping was performed by SPSS.

Linkage analysis and genome-wide association study

Two linkage maps for linkage analysis were constructed through 10 K array genotyping by targeted sequencing (GBTS) supported by MOLBREEDING Biotechnology Co., Ltd (Shijiazhuang, China). The RILsA population has been used in a previous study [24]. Nine hundred and seventy-eight bin markers covered 2465.32 cM of the rice genome with an average distance of 2.52 cM constructed the linkage map (Fig. S1). The linkage map of RILsB contains 527 bin markers that covered 1874.85 cM of the rice genome with an average distance of 3.56 cM (Fig. S2). The IciMapping Ver.4.2 [25] based on inclusive composite interval mapping (ICIM) was used to detected the QTLs for rice flag leaf traits. The walking speed was set as 1 cm, and the LOD threshold of ICIM was set as 2.5. To ensure the accuracy of mapping results, we controlled the type 1 error of whole genome detection below 5% by 1000 permutation tests. The natural population for GWAS was deep re-sequenced by Beijing Genomics Institute (BGI www.genomics.org.cn). A total of 788, 396 SNPs meeting the criteria (minimum allele frequency ≥ 5%, deletion rate ≤ 20%) were selected for follow-up analysis, and 295 *japonica* rice varieties' population structure analysis, genetic relationship analysis and linkage disequilibrium analysis have been completed in a previous study [23]. The mixed linear model (MLM) of TASSEL 5.0

[26] was used for genome-wide association analysis and setting the threshold of SNPs significantly associated with flag leaf traits as 5.46×10^{-6} which was calculated by GEC software (http://statgenpro.psychiatry.hku.hk/gec/). If 2 or more SNPs were located in the same LD interval, they were regarded as the same QTL, and the SNP with the smallest p value was treated as the lead SNP. QQman package in R was used to create the Manhattan and Q–Q plots [27].

Haplotype analysis of candidate genes

Considering the LD decay of the whole genome was confirmed in a previous study by 109 kb [23], we selected 218 kb upstream and downstream of the SNP as the target interval to screen candidate genes. Non-synonymous SNPs of all exons were extracted from the "Rice SNP-Seek Database" of The International Rice Informatics Consortium (IRIC) (https: //snp-seek.irri.org/), which were then used for haplotype analysis of candidate genes with DnaSP software [28].

RNA extraction and qRT-PCR analysis

The flag leaves of four parents (K131, HDB, WD20342, Caidao) of RILs populations took 4–6 days to fully extend. From the flag leaf initial growth to full extension, a total of 4 samples were taken, repeated 5 times for each parent. The total RNA was extracted with TranZol Up RNA Kit (TransGen Biotech). HiFiScript cDNA synthesis kit (CoWin Biosciences, Beijing, China) was used to synthesize cDNA. qRT-PCR was performed on Bio-Rad CFX96 system using 2 × Fast qPCR Master Mixture with 3 biological replicates for each sample. House-keeping gene *Actin1* was used to measure the mRNA levels of candidate genes [23] as an internal control. The primers used for qRT-PCR in this study are all shown in Table S1. Relative gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method [29]. Data shown in figures and tables are mean values of three replicates.

Results

Phenotypic analysis

The phenotypic data of flag leaf size of RILs populations and natural population from 2019 to 2020 are shown in Tables S2, S3, and the general trends during the 2 years were basically the same. The 3 leaf size traits of parents of RILs populations showed great phenotypic differences during 2 years and the RILs showed significant variation in FL, FW and FLA. FL and FLA in 2 RILs populations with standard deviation from 4.38 to 7.25, presented stronger variation than FW with the standard deviation from 0.17 to 1.44. The variation characteristics of flag leaf phenotypic in the natural population were similar to those of RILs in 2 years. Most of the absolute values of kurtosis and skewness were near 1, which basically conformed to the normal distribution and showed a typical genetic model of quantitative traits, which was suitable for linkage analysis and GWAS.

Linkage mapping for flag leaf size in *Japonica* rice

We conducted QTL linkage analysis for FL, FW and FLA of 2 RILs populations in 2019 and 2020. A total of 28 QTLs were detected, which were distributed on chromosome 1, 2, 3, 4, 6, 7, 10 and 11 of rice (Table S4). The phenotypic variation explained by a single QTL ranged from 4.97% to 20.88%. *qFLr7-2* and *qFWr2-3* in RILsA; *qFLr3*, *qFWr2-1*, *qFLAr4-2* and *qFWr10* in RILsB were detected in 2 years simultaneously. This represents the stable expression of genetic effects in the corresponding interval. At the same time, *qFLr6-1*, *qFLAr6-1* detected in the RILsA population and *qFLr3*, *qFLAr3-2* detected in the RILsB population were detected to control different traits although they were located in the same interval, and were identified as pleiotropic QTLs.

GWAS for flag leaf size in *Japonica* rice

A natural population which has been deeply re-sequenced and its 788, 396 high quality SNP markers were used to conducted GWAS. Manhattan and Q-Q plots for the GWAS are shown in Figs. 1, 2. A total of 36 SNPs were detected under the threshold of 5.46×10^{-6} which were significantly associated with flag leaf size of *japonica* rice (Tables S5, S6). These SNPs were distributed on all chromosomes of rice except chromosome 11 with the R^2 ranging from 8.87% to 12.81%. The GWAS results showed that Chr10_10,230,100 (*qFWn10-2*), Chr10_10,107,835 (*qFLAn10-1*) located in one LD interval was detected in both years associated with FW and FLA separately. Chr2_33,332,579 (*qFWn2-2*, *qFLAn2-5*) and Chr7_20,475,568 (*qFWn7*, *qFLAn7-2*) were detected in 2019 associated with FW and FLA simultaneously. The above-mentioned results are consistent with those that we obtained in phenotypic analysis.

Identification of pleiotropic QTLs for flag leaf size in *Japonica* rice

In this study, different materials and different analysis methods were used to detect QTLs related to flag leaf size for *japonica* rice in the 2-year experiment QTLs detected by the two methods and whose physical positions of chromosomes coincided were defined as co-location QTLs (pleiotropic QTLs) (Table 1). In co-location QTL *qFL2*, *qFWr2-3* located in C2_33,142,844-C2_35,004,908 was

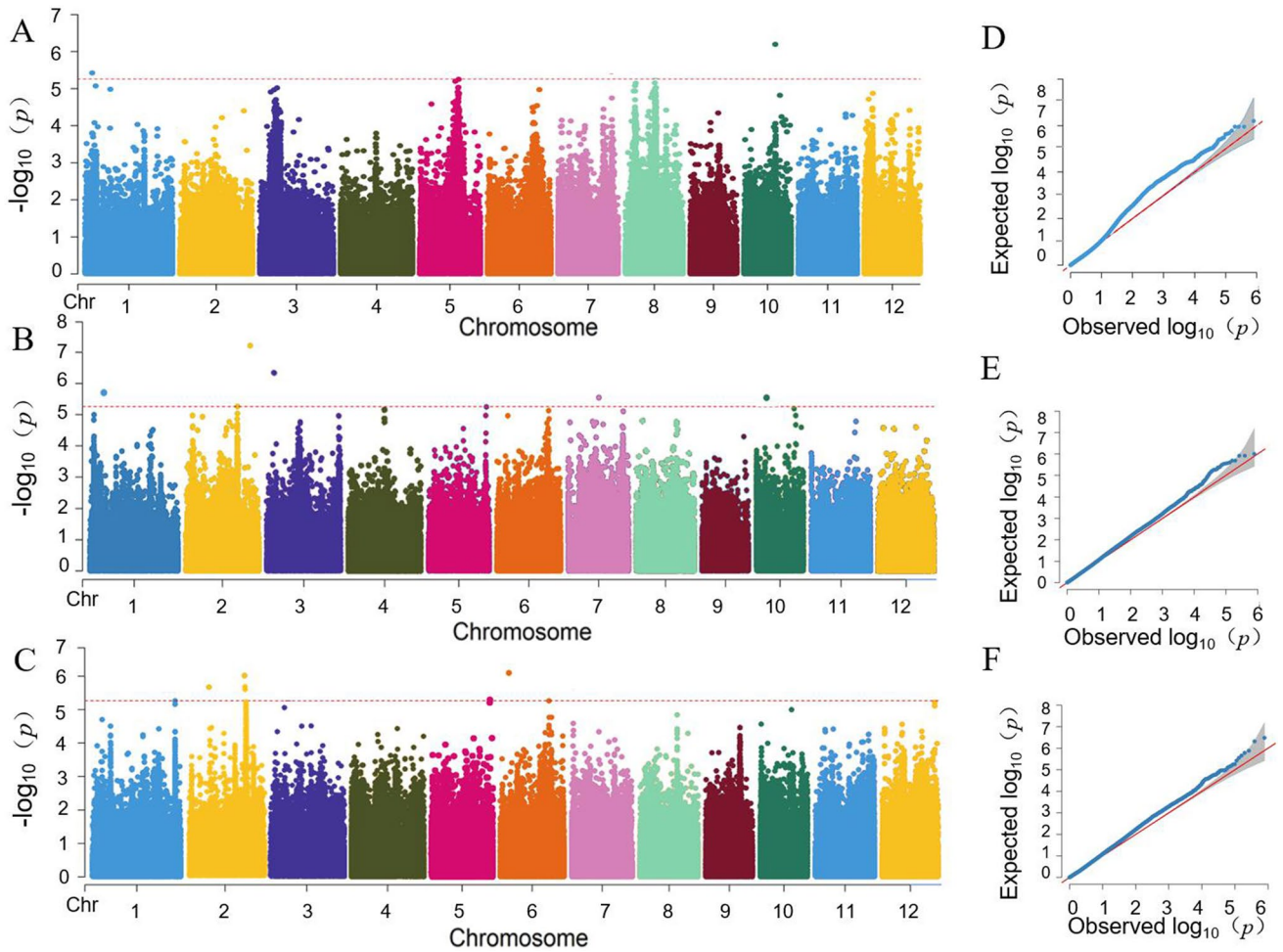

Fig. 1 Manhattan plots and quantile–quantile (Q–Q) plots of genome-wide association studies for FL, FW and FLA in 2019. **A**, Manhattan plot for FL. **B**, Manhattan plot for FW. **C**, Manhattan plot for FLA. **D**, Q–Q plot for FL. **E**, Q–Q plot for FW. **F**, Q–Q plot for FLA

detected in RILsA in both 2019 and 2020. This region contains the lead SNP Chr2_33,332,579 (*qFLAn2-5*, *qFWn2-2*) detected by GWAS and significantly associated with both FW, FLA of *japonica* rice. The other co-location QTL (pleiotropic QTL) *qFL10* contains linkage analysis QTL *qFWr10* located in C10_9,054,066–C10_10,570,732 interval, which was repeatedly detected in 2019 and 2020. According to the physical location of the rice chromosome, *qFLAn10-1* and *qFWn10-2* were both located in *qFWr10* interval, and their physical locations are partially coincident.

These 2 pleiotropic QTLs *qFL2* and *qFL10* were the most important subjects that remained stable through linkage analysis and GWAS in the same interval in this study, indicating that the corresponding interval probably contain the candidate genes of flag leaf size of *japonica* rice. Thus, further research is required.

Candidate gene screening and haplotype analysis

The P of lead SNP Chr2_33, 332, 579 of *qFWn2-2* in *qFL2* is the smallest, which is 3.18E-08. Considering that the LD of the whole genome is 109 kb (Fig. 3A), we selected 109 kb upstream and downstream of this SNP as the target interval to screen candidate genes. There are 37 genes in the target region, including 22 function annotated genes, 5 expression proteins with unknown function, 5 hypothetical proteins and 5 retrotransposon proteins (Table S7). We used SNPs with nonsynonymous mutations in exons to analyze the haplotypes of these genes and found that there were 2 functional annotation genes *LOC_Os02g54254*, *LOC_Os02g54550* that had significant differences in FW among different haplotypes, and the differences in 2019 and 2020 were basically the same (Fig. S3). Table 2, showed that 2 haplotypes of *LOC_Os02g54254* (G/A) and *LOC_Os02g54550* (C/T)

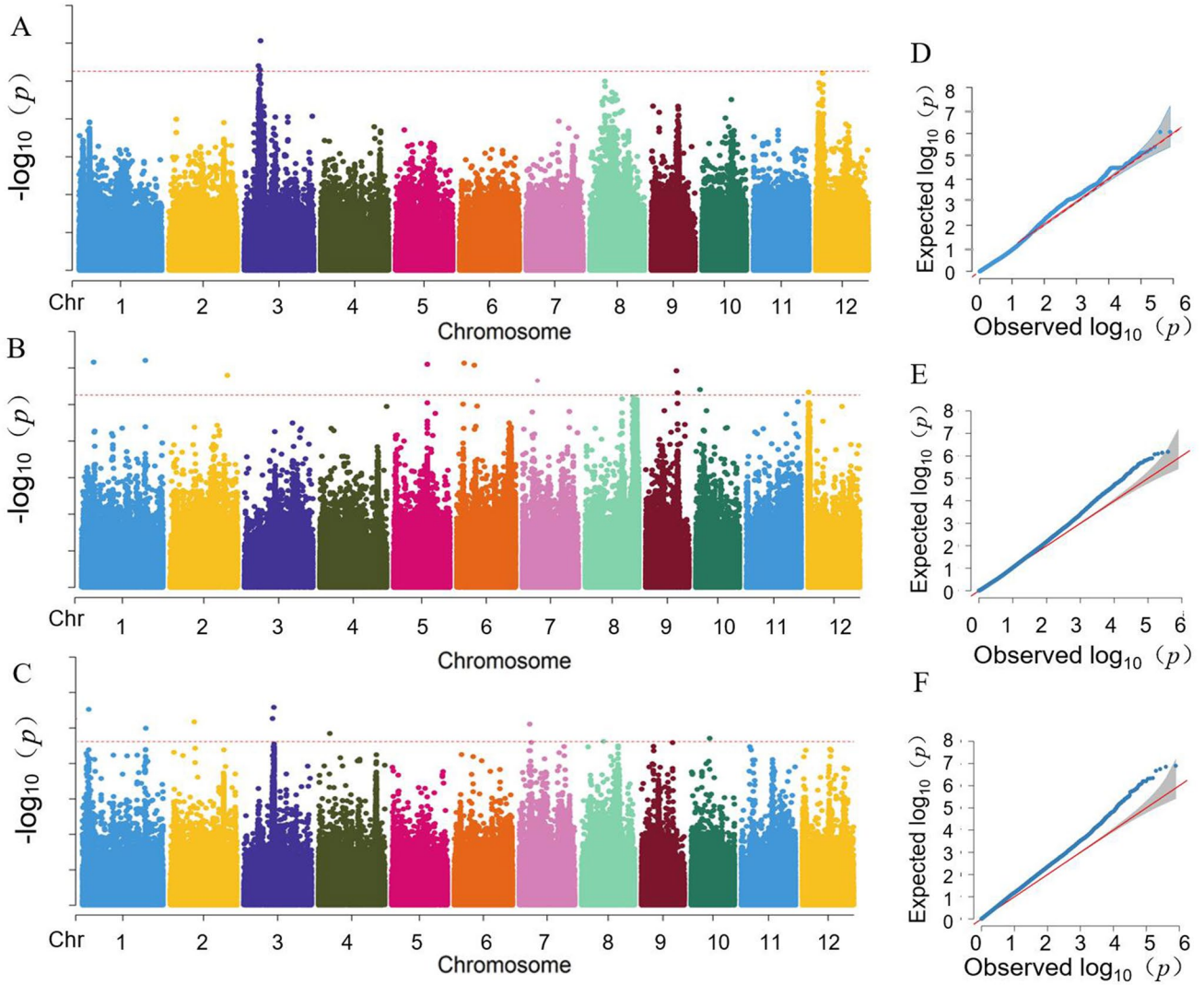

Fig. 2 Manhattan plots and quantile–quantile (Q–Q) plots of genome-wide association studies for FL, FW and FLA in 2020. **A**, Manhattan plot for FL. **B**, Manhattan plot for FW. **C**, Manhattan plot for FLA. **D**, Q–Q plot for FL. **E**, Q–Q plot for FW. **F**, Q–Q plot for FLA

Table 1 The name and distribution of the co-location QTLs

QTL name	Chr	GWAS				Original QTL	Linkage mapping		
		Original QTL	Peak SNP	P	R^2(%)		QTL interval	LOD	R^2(%)
qFL2	2	qFLAn2-5	Chr2_33332579	1.84E-06	9.33	qFWr2-3	C2_33142844–C2_35004908	4.65	9.46
		qFWn2-2	Chr2_33332579	3.18E-08	12.45		C2_33142844–C2_35004908	2.52	7.27
qFL10	10	qFLAn10-1	Chr10_10107835	4.32E-06	8.79	qFWr10	C10_9054066–C10_10570732	2.59	8.15
		qFWn10-2	Chr10_10230100	1.67E-06	10.32		C10_9054066–C10_10570732	2.86	11.26

had significant differences in FW. *qFL10*, a pleiotropic QTL either was composed of stable QTLs detected by linkage analysis and significant SNPs detected by GWAS. There is an overlapping interval between the two way results, that is, the 10.12 Mb–10.22 Mb interval of chromosome 10 (overlapping interval of *qFLAn10-1qFWn10-2*) to be the target interval (Fig. 3B, C). Fifteen genes exist in the target region, including 5 function annotated genes, 4 expression proteins with unknown function and 6 retrotransposon proteins (Table S8, Fig. 3D). SNPs with nonsynonymous mutations in exons were used to analyze the haplotypes of the genes, and 3 functional annotation genes *LOC_Os10g20160*,

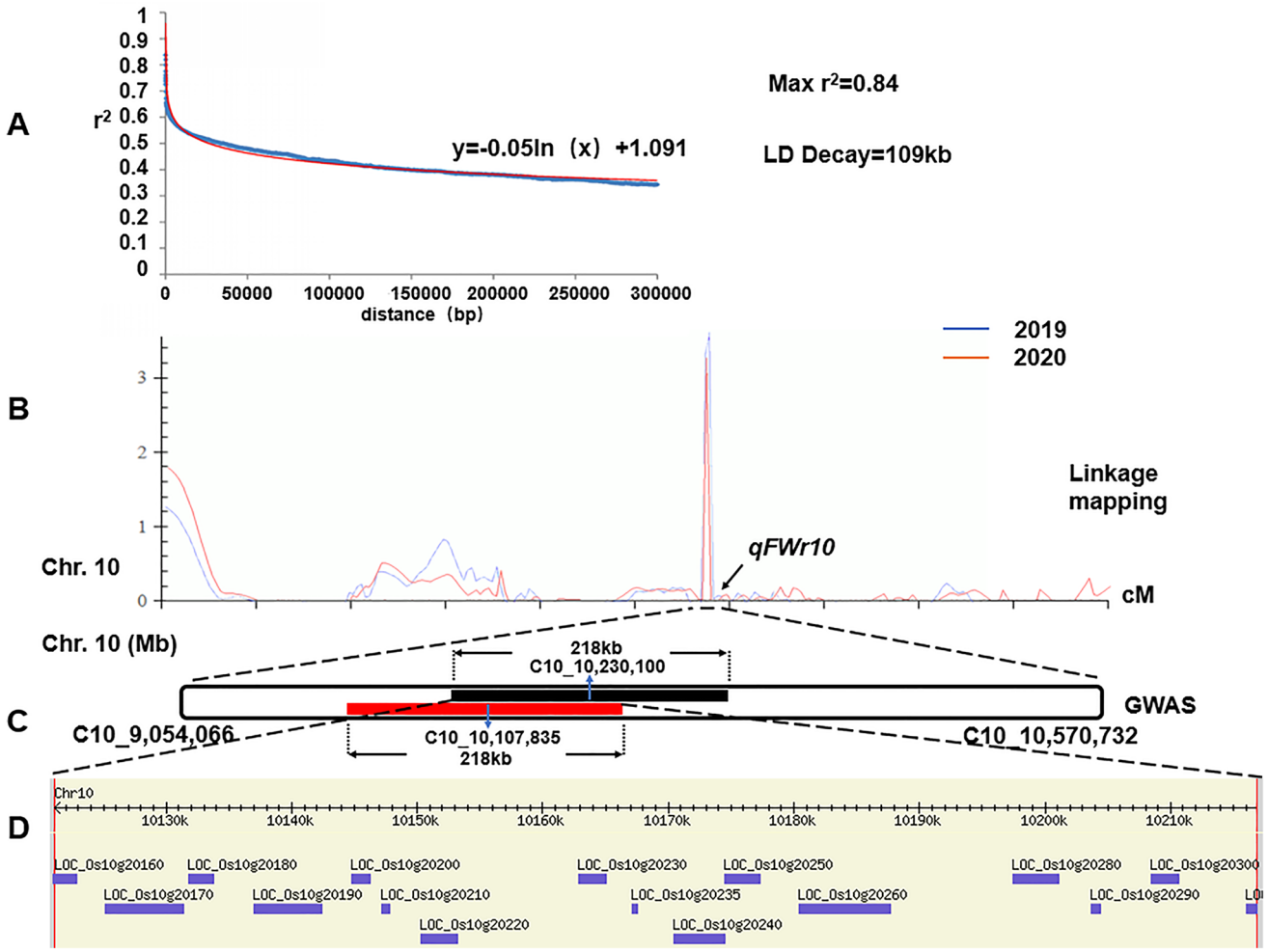

Fig. 3 Identification of candidate genes by linkage mapping and GWAS. **A** LD decay of the whole genome in 295 japonica rice varieties. When r^2 decays to the half, the corresponding physical distance (109 kb) is recorded as the LD attenuation distance of the whole genome. **B** *qFWr10* located in C10_9,054,066–C10_10,570,732 interval, which was repeatedly detected in 2019 and 2020. **C** Overlapping physical location of the lead SNP (C10_10,107,835, C10_10,230,100) on chromosome 10 detected by GWAS. **D** 15 genes in the 218 kb region

Table 2 Candidate gene haplotypes and the number of varieties corresponding to each haplotype

Gene	Hap1/number	Hap2/number	Hap3/number
LOC_Os02g54254	G/274	A/17	
LOC_Os02g54550	C/275	T/14	
LOC_Os10g20160	TGT/217	TAT/30	CGT/18
LOC_Os10g20240	GA/279	AC/11	
LOC_Os10g20260	CCC/255	CCG/17	

LOC_Os10g20240 and *LOC_Os10g20260* were found to have significant differences in FW among different haplotypes, and the differences in 2019 and 2020 were basically the same (Fig. S3). Haplotype analysis revealed that significant differences for FW were observed between hap1 (TGT), hap2 (TAT) and hap3 (CGT) in *LOC_Os10g20160*. *LOC_Os10g20240* and *LOC_Os10g20260* both had 2 haplotypes as hap1 (GA), hap2(AC) and hap1 (CCC), hap2(CCG) which showed significant difference for FW.

Identification of candidate genes based on qRT-PCR

According to the results of haplotype analysis, 5 candidate genes were analyzed by qRT-PCR using 4 RILs parents (K131, HDB, WD20342, Caidao) as templates in 4 growth periods from the flag leaf initial growth to fully extended. The candidate genes and quantitative primers are shown in Table S1. Expressions of *LOC_Os02g54254*, *LOC_Os02g54550*, *LOC_Os10g20240*, *LOC_Os10g20260*, had no obvious regularity and did not increase significantly in a certain period (Fig. S4). On the other hand, *LOC_Os10g20160* presented different expression type, and the expression level of the RILs parents in 4 periods was significantly higher

than that of other genes. *LOC_Os10g20160* (*SD-RLK-45*) belongs to S-domain receptor-like kinases (SD-RLK) family shows preferential in leaf, shoot and seeds [30]. Thus, *SD-RLK-45* is probably the candidate gene of *qFL10*.

Discussion

Leaf type improvement is one of the important methods to increase rice yield [31]. Rationally controlling leaf type traits could enhance lodging resistance, photosynthetic utilization rate, and increase yield per plant. [32, 33]. *Japonica* rice has better cooking and eating quality due to higher amylose content, which is cultivated and consumed in East Asia as the major variety (https://en.wikipedia. org/wiki/Japonica_rice). It is essential to conduct research on the genetic variation of leaf type (size) of *japonica* rice. Although some genes or QTLs regulating flag leaf size were identified by classical mapping and reverse genetics, the number of studies on leaf genetic variation about *japonica* rice is still relatively small [34, 35]. In this study, three *japonica* rice populations were used for linkage mapping and GWAS in 2 years, and 64 leaf shape QTLs were mapped, some of which were overlapped or similar to that in previous studies, and some were novel QTLs. Interval of *qFLr6-2* and *qFLAr3-1* detected by linkage mapping overlapped with *qFLL6* and *qLA3-1* [36] regulating leaf length and leaf area respectively. *qFLL1.2* mapped by Zhang et al. [37] using an RIL population and resequencing genetic map was found to be located within *qFLr1* possessing same function. The recognized narrow leaf gene *NAL1* [20] on chromosome 4 of rice was found to be located within *qFLAr4-4* in this study. *qFWn7* located in the same interval with *OsFLW7* identified by Xu et al. [21]. Known genes *OsBAK1* [38], *SNFL1* [39] and *OsDET1* [40], were found to be located within or nearby the LD interval of *qFWn8*, *qFWn5-2*, *qFLAn1-2* in this study. Among these genes, *OsBAK1* controlling leaf angle and length by regulating brassinosteroid (BRs), has been observed significantly to reduce plant height. The study of *SNFL1* mutant indicated that the length of epidermal cells and the number of longitudinal veins in flag leaves decreased remarkably. *OsDET1* has been proved to regulate ABA signal transduction and play an important role in maintaining rice growth and plant type development.

Using GWAS and linkage analysis in 2 years, we found pleiotropic and stable QTLs *qFL2* and *qFL10*, representing stable genetic effects. In view of the genome-wide LD decay of GWAS, we selected 218 kb upstream and downstream to screen candidate genes, and also served as the overlap region of linkage analysis and GWAS [20]. Based on haplotype analysis, we obtained 5 candidate genes: *LOC_Os02g54254* (*OsLKR/SDH*), *LOC_Os02g54550* (*OsFBX63*), *LOC_Os10g20240* (*OsKNOLLE*), *LOC_Os10g20260* (*CSlF7*), *LOC_Os10g20160* (*SD-RLK-45*). *OsLKR/SDH* were proved to be a bifunctional lysine degrading enzyme [41], *OsFBX63* is a F-box family gene, the function of which hasn't been studied. Syntaxin-related protein *OsKNOLLE* probably plays an important role in regulating abiotic stress resistance [42]. *CSlF7* was confirmed as a Cellulose Synthase family gene [43]. *SD-RLK-45* belongs to *SD-RLK* family and is supposed to be a novel functional gene. Characteristics of expression during leaf development period make *SD-RLK-45* the most likely candidate gene for *qFL10*.

Plant receptor-like protein kinases (*RLKs*) comprise one of the largest and most diverse superfamily of plant proteins with 610 and 1131 members in the *Arabidopsis* and rice genomes, respectively [44]. The *RLKs* gene superfamily played fundamental roles in hormone perception, developmental regulation, innate immunity, adaptation to abiotic stresses, and quantitative yield components [45–47]. S-domain RLKs (*SD-RLKs*) belongs to a subfamily of *RLKs*, with 147 members in rice. [48] Recent studies on rice have confirmed that *OsSRK1* regulates leaf width by promoting cell division in the leaf primordium and *OsSRK1*-overexpression plants exhibited enhancing ABA sensitivity and salt tolerance compared with wild types [49].

In the present study, there were significant differences in flag leaf width of the haplotypes of 5 candidate genes *OsLKR/SDH*, *OsFBX63*, *OsKNOLLE*, *CSlF7* and *SD-RLK-45*. Only the expression of *SD-RLK-45* showed excellent characteristics during flag leaf development, and it's most likely to be the candidate gene of *qFL10*. As a novel gene that has not been systematically studied, additional data is needed to verify the function of *SD-RLK-45* in controlling flag leaf width or size. The overexpression, construction of CRISPR-Cas9 and omics experiments will be the focus of our future studies.

Conclusions

Two RILs and 295 *japonica* rice varieties were collected to identify the flag leaf size phenotypic. Two pleiotropic QTLs *qFL2*, *qFL10* consisted of overlapping QTLs mapped by linkage analysis and GWAS were identified. Based on LD decay distance and pleiotropic interval overlapping, 2 intervals of 218-kb and 100-kb were selected for candidate gene screening. *LOC_Os02g54254*, *LOC_Os02g54550*, *LOC_Os10g20160*, *LOC_Os10g20240*, *LOC_Os10g20260* were identified by haplotype analysis as candidate genes, and qRT-PCR showed *LOC_Os10g20160* probably to be a novel functional gene contributing flag leaf size by regulating flag leaf width of *japonica* rice. The results provide resources for leaf type breeding improvement.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06842-8.

Author contribution JW and TW were the main writer of the whole paper. QW, XT, YR, HZ, KL responsible for field data collection. LY mainly engaged in data statistics and data processing. HJ responsible for sequencing data sorting and analysis. YL, QL proofread the full text. DZ, HZ guided the technical route of this study.

Funding This research was supported by the Heilongjiang Provincial governmental Postdoctoral Foundation of China (LBH-Z16188), the Natural Science Foundation Joint Guide Project of Heilongjiang (LH2019C035) and the Province-Academy Science and Technology Cooperation Project of Heilongjiang (YS20B05). Application R & D Project of Heilongjiang Academy of Agricultural Science (2021YYYF037).

Declarations

Ethical approval The authors of this paper declare that we have no conflict of interest. Molecular Biology Reports is the only journal we submitted. The submitted work is original and haven't been published elsewhere in any form or language. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

References

1. Pane TC, Supriyono Y, Novita D (2021) Supporting food security with rice farming insurance: the farmers' perceptions (case study in cinta damai village, percut sei tuan subdistrict, deli serdang district). IOP Conf Ser 782(2):022044. https://doi.org/10.1088/1755-1315/782/2/022044
2. Barboza LGA, Vethaak AD, Lavorante BR, Lundebye AK, Guilhermino L (2018) Marine microplastic debris: an emerging issue for food security, food safety and human health. Marine Pollut Bull 133:336–348. https://doi.org/10.1016/j.marpolbul.2018.05.047
3. Timmer CP (2014) Food security in Asia and the pacific: the rapidly changing role of rice. Asia Pacific Policy Stud. https://doi.org/10.1002/app5.6
4. Adachi M, Hasegawa T, Fukayama H, Tokida T, Sakai H, Matsunami T, Nakamura H, Sameshima R, Okada M et al (2014) Soil and water warming accelerates phenology and down-regulation of leaf photosynthesis of rice plants grown under free-air co2 enrichment (face). Plant Cell Physiol. https://doi.org/10.1093/pcp/pcu005
5. Zhu C, Zhu J, Cao J, Jiang Q, Liu G, Ziska LH (2014) Biochemical and molecular characteristics of leaf photosynthesis and relative seed yield of two contrasting rice cultivars in response to elevated [CO2]. J Exp Bot 65(20):6049. https://doi.org/10.1093/jxb/eru344
6. Gu J, Yin X, Tjeerd-Jan S, Wang H, Struik PC (2012) Physiological basis of genetic variation in leaf photosynthesis among rice (oryza sativa l.) introgression lines under drought and well-watered conditions. J Exp Bot 14:5137. https://doi.org/10.1093/jxb/ers170
7. He PL, Wang XW, Zhang XB, Jiang YD, Tian WJ, Zhang XQ, Li YY, Sun Y, Xie J, Ni JL, He GH, Sang XC (2018) Short and narrow flag leaf1, a GATA zinc finger domain-containing protein, regulates flag leaf size in rice (*Oryza sativa* L.). BMC Plant Biol. https://doi.org/10.1186/s12870-018-1452-9
8. Rahman MA, Haque ME, Sikdar B, Islam MA, Matin MN (2013) Correlation analysis of flag leaf with yield in several rice cultivars. J Life Earth Sci 8:49–54. https://doi.org/10.3329/jles.v8i0.20139
9. Giuliani R, Koteyeva N, Voznesenskaya E, Evans MA, Cousins AB, Edwards GE (2013) Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (Genus Oryza). Plant Physiol 162:1632–1651. https://doi.org/10.1104/pp.113.217497
10. Zhu X, Song Q, Ort DR (2012) Elements of a dynamic systems model of canopy photosynthesis. Curr Opin Plant Biol 15:237–244. https://doi.org/10.1016/j.pbi.2012.01.010
11. Hoang GT, Gantet P, Nguyen KH, Phung N, Xuan HP (2019) Genome-wide association mapping of leaf mass traits in a vietnamese rice landrace panel. PLoS ONE 14(7):e0219274. https://doi.org/10.1371/journal.pone.0219274
12. Fujino K, Matsuda Y, Ozawa K, Nishimura T, Koshiba T, Fraaije M, Sekiguchi H (2008) Narrow leaf 7 controls leaf shape mediated by auxin in rice. Mol Genet Genom 279:499–507. https://doi.org/10.1007/s00438-008-0328-3
13. Farooq M, Tagle AG, Santos RE, Ebron LA, Fujita D, Kobayashi N (2010) Quantitative trait loci mapping for leaf length and leaf width in rice cv. IR64 derived lines. J Integr Plant Biol 52:578–584. https://doi.org/10.1111/j.1744-7909.2010.00955.x
14. Chen M, Luo J, Shao G, Wei X, Tang S, Sheng Z, Song J, Hu P (2012) Fine mapping of a major QTL for fag leaf width in rice, qFLW4, which might be caused by alternative splicing of NAL1. Plant Cell Rep 31(863–872):1. https://doi.org/10.1007/s00299-011-1207-7
15. Zhang G, Li S, Wang L, Ye W, Zeng D, Rao Y, Peng Y, Hu J, Yang Y, Xu J, Ren D, Gao Z, Zhu L, Dong G, Hu X, Yan M, Guo L, Li C, Qian Q (2014) LSCHL4 from Japonica cultivar, which is allelic to NAL1, increases yield of Indica super rice. Mol Plant 7:1350–1364. https://doi.org/10.1093/mp/ssu055
16. Tang X, Gong R, Sun W et al (2018) Genetic dissection and validation of candidate genes for flag leaf size in rice (Oryza Sativa L.). Theor Appl Genet. https://doi.org/10.1007/s00122-017-3036-8
17. Zhang B, Ye W, Ren D, Tian P, Peng Y, Gao Y, Ruan B, Wang L, Zhang G, Guo L, Qian Q, Gao Z (2015) Genetic analysis of flag leaf size and candidate genes determination of a major QTL for flag leaf width in rice. Rice (New York, NY) 8(1):39. https://doi.org/10.1186/s12284-014-0039-9
18. Yang W, Guo Z, Huang C, Wang K, Jiang N, Feng H, Chen G, Liu Q, Xiong L (2015) Genome-wide association study of rice (Oryza sativa L.) leaf traits with a high-throughput leaf scorer. J Exp Bot 66:5605–5615. https://doi.org/10.1093/jxb/erv100
19. Jiang D, Fang JJ, Lou LM, Zhao JF, Yuan SJ, Yin L, Sun W, Peng LX, Guo BT, Li XY (2015) Characterization of a null allelic mutant of the rice NAL1 gene reveals its role in regulating cell division. PLoS ONE 10(2):e0118169. https://doi.org/10.1371/journal.pone.0118169
20. Li A, Yz B, Fang LB, Qian CB, Jq A (2019) Narrow leaf 1 (NAL1) regulates leaf shape by affecting cell expansion in rice (oryza sativa l). Biochem Biophys Res Commun 516(3):957–962. https://doi.org/10.1016/j.bbrc.2019.06.142
21. Xu J, Wang L, Wang YX, Zeng DL, Zhou MY, Fu X, Ye WJ, Hu J, Zhu L, Ren DY, Gao ZY, Dong GJ, Guo LB, Zhang GH, Qian Q (2017) Reduction of OsFLW7 expression enhanced leaf area and grain production in rice expression enhanced leaf area and grain production in rice. Sci Bull 62(24):1631–1633. https://doi.org/10.1016/j.scib.2017.11.013
22. Famoso AN, Zhao K, Clark RT, Tung CW, Wright MH, Bustamante C et al (2011) Genetic architecture of aluminum tolerance in rice (oryza sativa) determined through genome-wide association analysis and qtl mapping. PLoS Genet 7(8):747–757. https://doi.org/10.1371/journal.pgen.1002221

23. Li N, Zheng H, Cui J, Wang J, Zou D (2019) Genome-wide association study and candidate gene analysis of alkalinity tolerance in japonica rice germplasm at the seedling stage. Rice. https://doi.org/10.1186/s12284-019-0285-y
24. Li X, Zheng H, Wu W, Liu H, Zhao H (2020) QTL mapping and candidate gene analysis for alkali tolerance in japonica rice at the bud stage based on linkage mapping and genome-wide association study. Rice. https://doi.org/10.1186/s12284-020-00412-5
25. Meng L, Li H, Zhang L, Wang J (2015) Qtl icimapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations-sciencedirect. Crop J 3(3):269–283
26. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635. https://doi.org/10.1093/bioinformatics/btm308
27. Turner SD (2014) Qqman: an r package for visualizing gwas results using q-q and manhattan plots. Biorxiv. https://doi.org/10.1101/005165
28. Julio R, Albert FM, Sánchez-DelBarrio JC, Sara GR, Pablo L, Ramos-Onsins SE et al (2017) Dnasp 6: dna sequence polymorphism analysis of large data sets. Mol Biol Evol 12:12. https://doi.org/10.1093/molbev/msx248
29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001
30. Aya K, Suzuki G, Suwabe K, Hobo T, Takahashi H, Shiono K et al (2011) Comprehensive network analysis of anther-expressed genes in rice by the combination of 33 laser microdissection and 143 spatiotemporal microarrays. PLoS ONE 6(10):e26162. https://doi.org/10.1371/journal.pone.0026162
31. Dastan S, Ghareyazie B, Silva J (2020) Selection of ideotype to increase yield potential of gm and non-gm rice cultivars. Plant Sci 279:110519. https://doi.org/10.1016/j.plantsci.2020.110519
32. Ya-Jie HU, Cao WW, Qian HJ, Xing ZP, Zhang HC, Dai QG et al (2015) Effect of planting density of mechanically transplanted pot seedlings on yield, plant type and lodging resistance in rice with different panicle types. Acta Agron Sin. https://doi.org/10.3724/SP.J.1006.2015.00743
33. Clerget B, Domingo AJ, Layaoen HL et al (2016) Leaf emergence, tillering, plant growth, and yield in response to plant density in a high-yielding aerobic rice crop. Field Crop Res 199:52–64. https://doi.org/10.1016/j.fcr.2016.09.018
34. Jiang SK, Zhang XJ, Zheng-Jin XU, Wen-Fu C (2010) Comparison between QTLs for chlorophyll content and genes controlling chlorophyll biosynthesis and degradation in japonica rice. Acta Agron Sin 36(3):376–384. https://doi.org/10.1016/S1875-2780(09)60036-5
35. Hu W, Zhang H, Jiang J, Wang Y, Sun D, Wang X, Hong D (2012) Discovery of a germplasm with large flag leaf angle and its genetic analysis as well as QTL mapping in japonica rice. Chin J Rice Sci 26(1):34–42
36. Shen B, Yu WD, Du JH et al (2011) Validation and dissection of quantitative trait loci for leaf traits in interval RM4923-RM402 on the short arm of rice chromosome. Genet 90(1):39–44. https://doi.org/10.1007/s12041-011-0019-4
37. Zhang B, Ye W, Ren D, Tian P, Peng Y, Gao Y et al (2015) Genetic analysis of flag leaf size and candidate genes determination of a major QTL for flag leaf width in rice. Rice. https://doi.org/10.1186/s12284-014-0039-9
38. Li D, Wang L, Wang M, Xu Y, Luo W, Liu Y et al (2009) Engineering osbak1 gene as a molecular tool to improve rice architecture for high yield. Plant Biotechnol J 7(8):791–806. https://doi.org/10.1111/j.1467-7652.2009.00444.x
39. He P, Wang X, Zhang X, Jiang Y, Tian W, Zhang X (2018) Short and narrow flag leaf1, a GATA zinc finger domain-containing protein, regulates flag leaf size in rice (oryza sativa). BMC Plant Biol. https://doi.org/10.1186/s12870-018-1452-9
40. Zang G, Zou H, Zhang Y, Xiang Z, Huang J, Luo L et al (2016) OsDET1 modulates the aba signaling pathway and aba biosynthesis in rice. Plant Physiol. https://doi.org/10.1104/pp.16.00059
41. Takaiwa F (2010) Differences in transcriptional regulatory mechanisms functioning for free lysine content and seed storage protein accumulation in rice grain. Plant Cell Physiol 51(12):1964–1974. https://doi.org/10.1093/pcp/pcq164
42. Wang F, Zhu C (2011) Heterologous expression of a rice syntaxin-related protein KNOLLE gene (OsKNOLLE) in yeast and its functional analysis in the role of abiotic stress. Genetics 33(11):1251–1257
43. Julian G, Schwerdt K, MacKenzi F et al (2015) Evolutionary dynamics of the cellulose synthase gene superfamily in grasses. Plant Physiol. https://doi.org/10.1104/pp.15.00140
44. Zou X, Qin Z, Zhang C, Liu B, Liu J, Zhang C et al (2015) Overexpression of an s-domain receptor-like kinase extracellular domain improves panicle architecture and grain yield in rice. J Exp Bot 22:7197–7209. https://doi.org/10.1093/jxb/erv417
45. Lehti-Shiu MD, Zou C, Hanada K, Shiu SH (2009) Evolutionary history and stress regulation of plant receptor-like kinase/pelle genes. Plant Physiol 150(1):12–26. https://doi.org/10.1104/pp.108.134353
46. Herder GD, Yoshida S, Antolin-Llovera M, Ried MK, Parniske M (2012) Lotus japonicus e3 ligase seven in absentia4 destabilizes the symbiosis receptor-like kinase symrk and negatively regulates rhizobial infection. Plant Cell 24(4):1691–1707. https://doi.org/10.1105/tpc.110.082248
47. Pan J, Li Z, Wang Q, Yang L, Yao F, Liu W (2019) An s-domain receptor-like kinase, osesg1, regulates early crown root development and drought resistance in rice. Plant Sci. https://doi.org/10.1016/j.plantsci.2019.110318
48. Morillo SA, Tax FE (2006) Functional analysis of receptor-like kinases in monocots and dicots. Curr Opin Plant Biol 9(5):460–469. https://doi.org/10.1016/j.pbi.2006.07.009
49. Jinjun Z, Peina JU, Zhang ZH et al (2020) Ossrk1, an atypical s-receptor-like kinase positively regulates leaf width and salt tolerance in rice. Rice Sci 27(2):57–66