Genetic Basis Underlying Tiller Angle in Rice (*Oryza sativa* L.) by Genome-wide Association Study

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Research Article

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Abstract

Rice tiller angle is a key agronomic trait determining rice grain yield. Several quantitative trait loci (QTLs) affecting rice tiller angle have been mapped in the past decades. little is known about the genetic base of tiller angle in rice, because rice tiller angle is a complex polygenic trait. In this study, we performed genome-wide association study (GWAS) on tiller angle in rice using a population of 164 *japonica* varieties derived from the 3K Rice Genomes Project (3K RGP). We detected a total of 18 QTLs using 1,135,519 single-nucleotide polymorphisms (SNP) based on three GWAS models (GLM, FastLMM and FarmCPU). Among them, two identified QTLs, qTA8.3 and qTA8.4, overlapped with *PAY1* and *TIG1*, respectively, and additional 16 QTLs were identified for the first time. Combined with haplotype and expression analyses, we further revealed that *PAY1* harbors one non-synonymous variation at its coding region, likely leading to variable tiller angle in the population, and that nature variations in the promoter of *TIG1* significantly affect its expression, closely correlating with tiller angle phenotypes observed. Similarly, using qRT-PCR and haplotype analysis, we identified 1 and 7 candidate genes in qTA6.1 and qTA8.1 that were commonly detected by two GWAS models, respectively. In addition, we identified 3 more candidate genes in the remaining 14 novel QTLs after filtering by transcriptome analysis and qRT-PCR. In summary, this study provides new insights into the genetic architecture of rice tiller angle and candidate genes for rice breeding.

Key Message

Novel alleles of two reported tiller angle genes and eleven candidate genes for rice tiller angle were identified by combining GWAS with transcriptomic, qRT-PCR and haplotype analysis.

Introduction

Rice plant architecture is regarded as one of the major agronomical traits that influence grain yield, which is mainly determined by plant height, tiller number, tiller angle and panicle morphology. Tiller angle, mainly controlled by the asymmetric growth of tiller base, is defined as the angle between the main culm and its side tillers, and it is one of the decisive factor for achieving ideal plant architecture in rice (Wang and Li 2008, 2011). In practice, spread-out rice varieties are able to escape from some diseases but occupy too much space. By contrast, extremely compact rice varieties are less efficient in capturing light and are more susceptible to infection by pathogen attack. Thus, a suitable tiller angle is very important for rice yield (Jiang et al. 2012; Huang et al. 2016; Qu et al. 2021).

Previous studies have revealed that rice tiller angle is strongly associated with gravitropism and polar auxin transport (PAT) (Roychoudhry and Kepinski 2015; Zhang et al. 2018; Li et al. 2019). For example, *LAZY1* (*LA1*), the first gene identified in rice, controls tiller angle and encodes a novel plant-specific protein with an unknown function. *LA1* controls shoot gravitropism and tiller angle by regulating PAT, affecting the asymmetric distribution of auxin; and the *la1*-mutant displays enlarged tiller angle in rice (Li et al. 2007). Subsequently, several suppressors of *LA1* (*SOLs*), such as *dwarf 17* (*d17*), *d10*, *d27*, *d14*, and

d3, that are involved in the strigolactones (SLs) biosynthetic or signaling pathway are able to recover the enlarged tiller angle phenotype of *la1* by enhancing gravitropic response (Sang et al. 2014). Recently, it is reported that *LA1*-interacting protein *OsBRXL4* affects its nuclear localization, and that it is essential for function of *LA1* in controlling rice tiller angle. Furthermore, three rice *BRXL* genes (*OsBRXL1*, *OsBRXL4*, and *OsBRXL5*) can act redundantly in generating the rice tiller angle (Li et al. 2019). In addition, *HEAT STRESS TRANSCRIPTION FACTOR 2D* (*HSFA2D*) acts as an upstream positive regulator of *LA1*-mediated asymmetric distribution of auxin, thus, induces the asymmetric expression of *WUSCHEL RELATED HOMEODOMAIN 6* (*WOX6*) and *WOX11*, two transcription factors that specify tiller angle by modulating rice shoot gravitropism (Zhang et al. 2018). Other key genes/quantitative trait loci (QTLs) controlling tiller angle have also been identified and functionally characterized over the past decades, including *Tiller Angle Control 1* (*TAC1*) (Yu et al. 2007), *PROSTRATE GROWTH 1* (*PROG1*) (Jin et al. 2008; Tan et al. 2008), *Loose Plant Architecture 1* (*LPA1*) (Wu et al. 2013), *PLANT ARCHITECTURE AND YIELD 1* (*PAY1*) (Zhao et al. 2015), *TILLER INCLINED GROWTH 1* (*TIG1*) (Zhang et al. 2019), *TAC3* (Dong et al. 2016a), *TAC4* (Li et al. 2021) and *LA2* (Huang et al. 2021). Although these findings provide valuable information regarding tiller angle regulation, the underlying molecular mechanisms and functional relationships among them in rice is largely unknown.

Map-based cloning and association mapping have contributed to understanding of the genetic and molecular bases of many complex agronomic traits. However, conventional cloning method is extremely troublesome (Korte and Farlow 2013; Bhat et al. 2021). Fortunately, advances in next-generation sequencing (NGS) technology and bioinformatics tools, providing large-scale SNP arrays in natural groups in rice become reality (Alexandrov et al. 2015; Wang et al. 2020). Genome-wide association studies (GWAS), as a powerful method for studying the genetics of natural variation based on a linkage disequilibrium mapping approach, have widely been applied to detection of complex agronomic traits in plants (Cockram et al. 2010; Wang et al. 2016, 2020; Luján Basile et al. 2019; Okada et al. 2019; Bai et al. 2021). However, so far, only have *TAC3* and *DWARF2* (*D2*), been identified as rice tiller angle regulators by GWAS (Dong et al. 2016b). Addition attempts to identify rice tiller angle regulator using GWAS detected several genetic loci (Lu et al. 2015; Wu et al. 2019). These GWAS analyses were performed in *indica* and *japonica* varieties or *indica* varieties (Lu et al. 2015; Dong et al. 2016b; Wu et al. 2019), and few studies have been performed within *japonica* varieties that generally have smaller tiller angles than *indica* varieties. To understand the regulatory mechanisms of *japonica* rice tiller angle, more QTLs need to be identified from the *japonica* varieties.

The purpose of this study was to detect genetic loci significantly associated with tiller angle in *japonica*, based on GWAS analysis on a panel of 164 *japonica* varieties selected from the 3K RGP, and to explore favorable SNP alleles and reliable candidate genes that can be used to breed rice with ideal tiller angle. Taken together, our study revealed in total 11 candidate genes and provided the basis for further elucidating mechanisms underlying tiller angle in rice.

Materials And Methods

Plant materials

The 164 *japonica* varieties used in this study were derived from were selected from the 3K RGP (Wang et al. 2018). Detailed information regarding these varieties, including their geographical origin, is shown in Additional File 2: Table S1.

Genotyping data and SNP filtering

The 3 K RGP 4.8mio SNP dataset was downloaded from the Rice SNP-Seek Database (<https://snp-seek.irri.org/>) (Alexandrov et al. 2015). A total of 1,135,519 cleaned SNPs were called in the population. SNPs were filtered utilizing the software PLINK (Purcell et al. 2007) with missing rate < 20% and minor allele frequency (MAF) > 0.05.

Evaluation of tiller angle and population structure

All varieties were grown in the experimental field at density of 17 cm × 20 cm. A completely randomized block design with three replicates was performed. Six plants in the middle of every row were selected for evaluation of tiller angle at maturity stage. The mean value of three replicates was used for analysis. The field experiment was performed in December 2015 to April 2016, Sanya city, Hainan (HN) Province, China (18°15'N, 109°30'E). Principal component analysis (PCA) using PLINK (Purcell et al. 2007) was used to assess the population structure.

Genome-wide association mapping

GWAS was performed on SNPs as described above using the factored spectrally transformed linear mixed models (Fast-LMM) (Lippert et al. 2011), GLM (Wang and Zhang 2021) and FarmCPU (Liu et al. 2016). Suggestive thresholds were calculated using the formula “ $-\log_{10}(1/\text{effective number of independent SNPs})$ ” as described previously (Wang et al. 2016). The SNP with the minimum *P* value was considered the lead SNP. Regions were considered as QTLs when SNPs exceeding the *P* value within a 200-kb interval of the leading SNP. Manhattan plots for the GWAS results were drawn using the R package “qqman” (<https://www.r-project.org/>).

Haplotype analysis and identification of candidate genes

Candidate genes were scanned within the 200 kb region centered on the lead SNP of each QTL (using the reference Nipponbare genome (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/>)). Haplotype analysis of the candidate genes were carried out using non-synonymous SNPs in exons, while the *PAY1* and *TIG1* were carried out for all available SNPs in the promoter region (2 kb), non-synonymous SNPs in exons, introns, and 3' untranslated region were selected from the Rice SNP-Seek Database (<https://snp-seek.irri.org/>). Only haplotypes shared by at least 5 varieties were used for multiple comparisons. Duncan's multiple comparison tests followed by a one-way analysis of variance were completed with the R package “agricolae” (<https://myaseen208.github.io/agricolae/>) (<https://cran.r-project.org/package=agricolae>).

Quantitative Reverse Transcription PCR (qRT-PCR) Analysis

Tiller base from the varieties used in this study, were collected in triplicate at the tillering stage.

Total RNA was extracted using the Trizol reagent (Invitrogen), following the manufacturer's instructions. cDNA was synthesized from total RNA using the Fast-Quant RT Kit (with gDNase; Tiangen). Quantitative real time PCR (qRT-PCR) was performed in a two-step reaction using SuperReal PreMix Color (SYBR Green; Tiangen) on a Roche Light Cycler 2.10 system with three technical replicates. Expression levels were normalized to *OsActin1* (*LOC_Os03g50885*).

The sequences of the candidate genes were downloaded from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml). Primer sequences for candidate genes were downloaded from the qPrimerDB-qPCR Primer Database (<https://biodb.swu.edu.cn/qprimerdb/best-primers-ss>) and designed by NCBI except for *LOC_Os08g05900*, which could not find the appropriate primer. Primer sequences are given in Additional File 2: Table S6.

Results

Natural variation of rice tiller angle

To dissect the genetic structure of rice tiller angle, a total of 164 *japonica* varieties from 3K RGP were selected for tiller angle phenotyping at the heading stage in Hainan, China (Additional File 2: Table S1). The tiller angle observed at the maturity stage in this panel ranged from 6.3° to 35.6°, which exhibited a nearly normal distribution (Fig. 1a), indicating that tiller angle was regulated by many genes with small or moderate effects (Zhang et al. 2018; He et al. 2021). Tiller angles of about half of the panel (46.2%, 73 of 164) were between 18 and 24°, with the median value of 19.4° (Fig. 1a-b). Principal component analysis (PCA) of the 164 varieties using high-confidence SNPs showed that all tested varieties are closely linked, and that there is no obvious structural pattern existed in this panel (Additional File 1: Figure S1), avoiding population structure-induced false positives. Taken together, these results indicate that the rice panel used in this study has abundant genetic diversity and is suitable for GWAS.

GWAS for tiller angle

To dissect all possible genomic loci associated with tiller angle, we performed GWAS with FastLMM, FarmCPU and GLM models using 1,135,519 high-confidence SNPs. The genome-wide suggestive thresholds $P \leq 8.81 \times 10^{-7}$ were calculated using the formula “ $-\log_{10}(1/\text{effective number of independent SNPs})$ ”. Totally, 18 QTLs were identified to be significantly associated with tiller angle when significant SNPs located within 200-kb range (Fig. 1). Among these QTLs, 8, 2, 8 QTLs were identified by Fast-LMM (Lippert et al. 2011), GLM (Wang and Zhang 2021) and FarmCPU (Liu et al. 2016), respectively. Notably, 2 QTLs, qTA8.3 and qTA8.4, identified in this study were co-localized with *PAY1* (Zhao et al. 2015) and *TIG1* (Zhang et al. 2019), respectively (Table 1), confirmed the reliability of our GWAS results. Among the remaining 16 QTLs newly identified, 2 QTLs (qTA6.1 and qTA8.1) were simultaneously identified by both GLM and FastLMM approaches, which are highly valuable for further identification of the causal genes for tiller angle trait.

Table 1
QTLs obtained from genome-wide association study.

GWAS models	QTLs name	Chr	Pos. (bp)	Pvalue	Reported genes	Ref
FarmCPU	qTA1.1	1	1297448	1.64E-07		
FarmCPU	qTA1.2	1	12048457	1.47E-12		
FarmCPU	qTA2.1	2	30414101	1.11E-07		
FarmCPU	qTA3.1	3	8188594	7.51E-07		
FastLMM	qTA3.2	3	19294773	8.14E-07		
GLM	qTA3.3	3	35723855	2.81E-07		
GLM	qTA4.1	4	24368973	4.99E-07		
FarmCPU	qTA5.1	3966839	5.49E-10			
FarmCPU	qTA5.2	5	13304801	8.76E-07		
GLM FastLMM	qTA6.1	6	3234165	5.85E-08 8.21E-07		
GLM FastLMM	qTA8.1	3242743	4.24E-08 2.05E-08			
FarmCPU	qTA8.2	8	15198129	2.43E-10		
FarmCPU	qTA8.3	8	19541427	1.44E-07	<i>PAY1</i>	(Zhao et al. 2015)
GLM	qTA8.4	8	20759700	9.85E-08	<i>TIG1</i>	(Zhang et al. 2019)
FarmCPU	qTA10.1	10	16590118	4.60E-08		
GLM	qTA10.2	10	18120346	6.10E-07		

GWAS models	QTLs name	Chr	Pos. (bp)	Pvalue	Reported genes	Ref
GLM	qTA10.3	10	18931537	1.39E-07		
GLM	qTA10.4	10	20426674	2.29E-07		

PAY1, the candidate gene of qTA8.3

The QTL qTA8.3 was found to contain *PAY1* gene, a single nucleotide change, G to A, at position 19468238 in exon 4 of which affected plant architecture via affecting polar auxin transport activity and altering endogenous indole-3-acetic acid distribution (Zhao et al. 2015). To understand how *PAY1* sequence may affect tiller angle phenotype, we analyzed SNPs in the genomic coding region and 2 kb of upstream promoter region of *PAY1* across 164 varieties, which revealed 2 major haplotypes; tiller angles of varieties containing *PAY1*^{HapA} were significantly lower than those of *PAY1*^{HapB}, five SNP variations including only one in the first exon were observed between these two haplotypes (Fig. 3a-b; Additional File 2: Table S1). However, none of those variations observed in these two haplotypes was the same as the functional mutation previously reported in *PAY1* (Zhao et al. 2015). The SNP variations observed in both promoter region and CDS region of *PAY1* indicated that both may play important role in regulating tiller angle in rice. To further investigate the causative SNP variation in *PAY1* responsible for tiller angle, we used qRT-PCR to examine expression level of *PAY1* between varieties harboring *PAY1*^{HapA} or *PAY1*^{HapB}, and results showed that expression levels of *PAY1* are not associated with haplotypes (Fig. 3c), indicating that the only variation in the first exon, a non-synonymous SNP (T to C) identified in *PAY1* could be a new functional allele that leads to spread phenotypes. In generally, *japonica* varieties always have a relatively smaller tiller angle and show a more compact plant architecture than *indica* varieties (Dong et al. 2016a). Therefore, to further understand the breeding utilization of “T” and “C” alleles in *PAY1* between the *japonica* and *indica* varieties. we investigated the changes of allelic frequencies using 2584 varieties from 3K RGP (Fig. 3d and Additional File 2: Table S2). The results showed that nearly all of the *indica* varieties (98%) contained “C” allele, while most *japonica* varieties (73%) contained “T” allele, indicating that *PAY1* has been selected during *japonica-indica* differentiation.

TIG1, the candidate gene of qTA8.4

Another QTL, qTA8.4, contained *TIG1*, which encodes a TCP family transcriptional activator and variations in the *TIG1* promoter of *indica* varieties (*tig1* allele) decrease expression of *TIG1* in the adaxial side of tiller base and reduce cell length and tiller angle, leading to the transition from inclined tiller growth in wild rice to erect tiller growth during rice domestication (Zhang et al. 2019). To investigate the causative SNP variations in *TIG1* responsible for phenotypic variations for tiller angle, we analyzed SNPs in the genomic coding region and 2 kb of upstream promoter region of *TIG1* across 164 varieties, which revealed 4 major haplotypes with no SNP in CDS region (Fig. 4a; Additional File 2: Table S1). Compared with previously reported SNP variations of *TIG1* in rice (Zhang et al. 2019), SNPs detected in our research were completely new. Moreover, multiple comparison tests of tiller angle showed that tiller angles of

varieties with *TIG1*^{HapA} were significantly higher than those with *TIG1*^{HapC} or *TIG1*^{HapD} (Fig. 4b), indicating that SNP variations identified in promoter regions may play an important role in regulating tiller angle as a new allele. To test whether the relative expression level of different haplotypes of *TIG1* were associated with tiller angle, we used qRT-PCR analysis on samples from tiller base, which revealed that expression level of *TIG1* in *TIG1*^{HapA} varieties was significantly higher than that in *TIG1*^{HapD} varieties (Fig. 4c), in agreement with previously report that *TIG1* positively regulates tiller angle in rice (Zhang et al. 2019).

Candidate genes analysis of qTA6.1 and qTA8.1 by qRT-PCR and haplotype analysis

To identify the candidate genes in two novel QTLs (qTA6.1 and qTA8.1) that were commonly detected by both GLM and FastLMM, we selected candidate genes in a 200-kb region (100 kb flanking the left and right side of the most significantly associated SNPs) using the Nipponbare reference genome (<http://rice.plantbiology.msu.edu>), and found contained 60 genes including 50 functionally annotated genes (31, 19 in qTA6.1 and qTA8.1, respectively.) and 10 retro/transposon/hypothetical proteins (Additional file 2: Table S3). Two analyses were performed further to identify candidate genes for these two QTLs: 1) expression analysis using qRT-PCR analysis in the tiller base of 22 varieties: 11 spread-tiller varieties and 11 compact-tiller varieties; 2) haplotype analyses in CDS region.

For qTA6.1, *LOC_Os06g06990* was the only one showing significantly lower expression level in spread-tiller varieties than compact-tiller varieties as revealed by qRT-PCR analysis (Fig. 5a). Haplotype analysis in CDS region of all candidate genes in the same region showed that 12 genes can be divided into different haplotypes, and that none of identified SNP variations among these genes were associated with significant differences in the tiller angle (Fig. 5c and Additional File 1: Figure S2), indicating that the *LOC_Os06g06990* is likely the most promising candidate gene for qTA6.1.

For qTA8.1, *LOC_Os08g06090* was shown to be significantly and highly expressed in spread-tiller varieties than that in compact-tiller varieties as evidenced by qRT-PCR analysis (Fig. 5b). Additional haplotype analysis in CDS region showed that 6 genes (*LOC_Os08g05840*, *LOC_Os08g05860*, *LOC_Os08g05870*, *LOC_Os08g05890*, *LOC_Os08g05900*, and *LOC_Os08g05910*) can be divided into different haplotypes, all were associated with significant differences in the tiller angle (Fig. 5c). These results indicated that abovementioned seven genes are all potential candidate genes for qTA8.1.

Candidate genes analysis of the remaining 14 novel QTLs by integrating GWAS and transcriptomics

We further analyzed the 100 kb window near the leading SNPs within these remaining 14 QTLs. After removing 142 retro/transposon/hypothetical proteins, 297 annotated genes were identified (Additional file 2: Table S4).

To further narrow down candidate gene numbers, we referred to two publicly available transcriptomic data about tiller angle regulatory genes: one contained 4204 differentially expressed genes (DEGs) between gravistimulated shoots and control shoots without stimulation (Zhang et al. 2018); the other contained 4431 DEGs between *ZnF* transgenic plants and the wild type (Wu et al. 2018). By combining these DEGs and our GWAS results, we finally identified 15 overlapping candidate genes (Fig. 6a). Further qRT-PCR analysis showed that among these 15 candidate genes, expression levels of 3 genes (*LOC_Os01g21590*, *LOC_Os03g15050* and *LOC_Os04g41229*) are significantly lower in spread-tiller varieties than in compact-tiller varieties (Fig. 6b and Additional file 2: Table S5), showing close association with tiller angle. These 3 candidate genes are likely to be a promising candidate gene in qTA1.2, qTA3.1 and qTA4.1, respectively, negatively regulating the tiller angle in rice.

Discussion

Natural variation, including phenotypic and genotypic variation caused by spontaneous mutations and sections, has been maintained in nature by all evolutionary processes (Jiang et al. 2012). Rice tiller angle is controlled by QTLs derived from natural variations (Yu et al. 2007; Jin et al. 2008; Tan et al. 2008; Jiang et al. 2012; Dong et al. 2016a; Zhang et al. 2019). Therefore, dissection of new genetic variation controlling tiller angle is important for rice breeding to use potential source of beneficial alleles. Compared with QTL mapping, GWAS enables the identification of causal loci at high-resolution and is a powerful tool for detecting favorable alleles in natural resources (Ogura and Busch 2015; Bai et al. 2021). In this study, 164 *japonica* varieties were selected from 3K RGP for studying the genetic basis of tiller angle using GWAS as well as other approaches. Our findings provide important novel genetic resource for further gene discovery and breeding of tiller angle in rice.

Phenotype variations and GWAS

The erect plant architecture is favored by human beings because it improves photosynthetic efficiency and increases planting density, which eventually enhances grain yield (Wang and Li 2008, 2011). The genetic basis of rice tiller angle is complex and is associated with many loci, each with small contributions to phenotypic variance (Lu et al. 2015; Wu et al. 2019), our results confirmed these previous findings and showed rice tiller angle trait tends to show a nearly normal distribution in a segregated population (Fig. 1a). The natural variation of rice tiller angle revealed in this study, ranging from 6.3° to 35.6° (Fig. 1a-b), indicated not only that the panel selected in this study is rich in genetic diversity that suitable for GWAS analysis, but also that this panel is useful for direct use as parents in rice breeding for ideal tiller angle.

We used three models to perform GWAS in this study, all generated QTL signals, some of them were repeatedly detected in more than one model while most of them were not (Fig. 2). These results indicated that when performing GWAS, if possible, different models should be used in order to identify as much as possible the QTLs. Our results are consistent with previous report that different GWAS analysis methods possess unique features and complement each other (Liu et al. 2020). In total, three models yielded 18

QTLs for tiller angle; and the identification of two known rice tiller angle genes, *PAY1* and *TIG1*, that co-localized with qTA8.3 and qTA8.4, respectively, indicated the reliability of our GWAS results.

Novel alleles in *PAY1* and *TIG1* affect tiller angle in rice

Some alleles of the genes related to yield-related QTLs were selected during domestication or breeding programs (Jin et al. 2008; Tan et al. 2008; Jiang et al. 2012). The allele mining technique has been successfully employed to find important variations at various locus for understanding mechanisms underlying domestication and for breeding (Huang et al. 2018; Guo et al. 2020; Bai et al. 2021). Present study identified novel alleles of *PAY1* and *TIG1* through GWAS in a *japonica* panel that are associated with variable tiller angle, and provided genetic resource for further understanding mechanisms underlying rice tiller angle. Clearly, the two alleles have different mechanisms regulating rice tiller angle. The SNP in coding region of *PAY1* plays important role in regulating tiller angle (Fig. 3), while SNPs in the promoter region of *TIG1* are associated with variation of tiller angle (Fig. 4). Therefore, learning from domestication, the promoter edited are interesting targets for genome editing to create new alleles for rice breeding as did previously (Swinen et al. 2016; Oliva et al. 2019; Huang et al. 2020). Moreover, differences in allelic frequencies in the two sub-species (Fig. 3d) suggest that *PAY1* has been subjected to selection during rice breeding, which partially explains why the *Indica* varieties usually have a relatively larger tiller angle and display comparatively spread-out plant architecture whereas *japonica* varieties always have a relatively smaller tiller angle and show a more compact plant architecture (Yu et al. 2007; Zhang et al. 2019). Similarly, the major tiller angle gene *TIG1*, is reported to be strongly selected in *indica* varieties by human beings (Zhang et al. 2019). Therefore, our study suggests the idea that rice tiller angle is selected during domestication.

Notably, the previous reported variants in both *PAY1* and *TIG1* were not found in 3K RGP (Zhao et al. 2015; Zhang et al. 2019), the detected SNPs, particularly those in the *TIG1* promoter, in this study, thus, provide valuable information for breeding rice varieties, especially *japonica* rice.

Novel candidate genes

Previous studies have demonstrated that rice tiller angle is strongly related to plant gravitropic responses, which also can be effected by hormones, such as auxin and brassinosteroids (BRs) (Dong et al. 2016a; Liu et al. 2016; Waite and Dardick 2018; Zhang et al. 2018). Using expression and haplotype analysis combined with GWAS, we identified 11 candidate genes for rice tiller angle in this study (Fig. 5a-c and Fig. 6b). Among them, 4 candidate genes (*LOC_Os08g05840*, *LOC_Os08g05870*, *LOC_Os08g05890* and *LOC_Os01g21590*) attracted specific attention based on their functional annotations. Because DNA Topoisomerase 1 (*OsTOP1*) functions as a role in gravitropism by linking transcriptional R-loops with auxin signaling (Shafiq et al. 2017), *LOC_Os08g05840*, encoding DNA topoisomerase 1, likely is involved in tiller angle regulation in rice. *LOC_Os08g05840* may be regulation of tiller angle in rice.

LOC_Os08g05870 and *LOC_Os08g05890* encode an oxysterol-binding protein-related protein 6 and a MSP domain containing protein, respectively. both protein families have been documented to transport sterols and involved in sterol regulation (Reynolds and Hand 2009; Saravanan et al. 2009; Umate 2011),

Considering that sterols also act as precursors for BR hormones, we propose that *LOC_Os08g05870* and *LOC_Os08g05890* are two important components of BR signaling associated with rice tiller angle. *LOC_Os01g21590*, encodes a homeodomain containing protein, whose homologues have been reported to be associated with tiller angle (Zhang et al. 2018; Hu et al. 2020). Considering its significant higher expression in compact-tiller varieties than in spread-tiller varieties (Fig. 6b), *LOC_Os01g21590* is likely connected with tiller angle in rice. Nevertheless, these candidate genes provide useful genetic resources for expand our understanding regarding hormone mediated tiller angle regulation in rice.

In summary, this study provides important genetic information for better understanding the genetic diversity of tiller angle in rice and useful genetic resources for breeding rice varieties with ideal plant architecture.

Declarations

Funding

Not applicable.

Availability of data and material

The association mapping population data can be found in Rice SNP-Seek database (<https://snp-seek.irri.org/>), and transcriptome data can be found in the previously studies supplementary data (Wu et al. 2018; Zhang et al. 2018).

Code availability

Not applicable.

Author contribution statement

DZ and WL designed the study. SB and JH performed the experiments and bioinformatic analysis. ZL and WW contributed plant materials. JS helped to revise the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Figures

a.

b.

Figure 1

Tiller angle of the 164 *japonica* varieties. **a.** Distributions of tiller angle, **b.** Box plot of the phenotypic variation of tiller angle. Boxes show median, and upper and lower quartiles. Whiskers extend to 1.5× the interquartile range, with any remaining points indicated with dots.

Figure 2

Manhattan plots of GWAS for tiller angle in 164 rice varieties. The arrows represent QTLs (red arrows for single GWAS model detected QTLs, green arrows for multiple GWAS models detected QTLs and black arrows for reported genes) when the peak SNP correlation exceeds the significance threshold, $-\log_{10}(P) > 6.06$ ($P < (P \leq 8.81 \times 10^{-7})$)

Figure 3

Haplotype analysis of *PAY1*. **a.** Two haplotypes of *PAY1* based on 4 SNPs observed in all assessed rice varieties. The schematic representation of *PAY1* gene structure (upper) shows the promoter as a white box, exons as green boxes, and introns and intergenic regions as black lines. Thin black lines indicate the genomic position of each SNP. Haplotypes with fewer than 5 varieties are not shown. The SNP in red and bold is a non-synonymous SNP. '\$#' indicates a missense mutation from Trp to Arg. **b–c.** Box plots for **b**

tiller angle based on the two haplotypes for *PAY1* and **c** *PAY1* expression levels in different haplotypes relative to *OsActin1*. Boxes show median, and upper and lower quartiles. Whiskers extend to 1.5× the interquartile range, with any remaining points indicated with dots. * $P < 0.05$ (Welch two sample t-test). **d.** Frequencies of the two different alleles in *PAY1* between the *japonica* and *indica* varieties. n, number of varieties in each panel allele.

Figure 4

Haplotype analysis of *TIG1*. **a.** Four haplotypes of *TIG1* based on 12 SNPs observed in all assessed rice varieties. The schematic representation of *TIG1* gene structure (upper) shows the promoter as a white box, exons as green boxes, and intergenic regions as black lines. Thin black lines indicate the genomic position of each SNP. Haplotypes with fewer than 5 varieties are not shown. Yellow highlight indicates SNP alternatives. **b–c.** Box plots for **b** tiller angle based on the four haplotypes for *TIG1* and **c** *TIG1* expression levels in different haplotypes relative to *OsActin1*. Boxes show median, and upper and lower quartiles. Whiskers extend to 1.5× the interquartile range, with any remaining points indicated with dots. ** $P < 0.01$ (Welch two sample t-test). Letters indicate significant differences, $P < 0.05$ (Duncan’s multiple comparison test)

Figure 5

The candidate genes analysis in qTA6.1 and qTA8.1. **a-b.** The relative expression of *LOC_Os06g06990* and *LOC_Os08g06090* in extreme tiller angle phenotypes relative to *OsActin1*. The green horizontal lines depict the average expression levels in the spread-tiller varieties and compact-tiller varieties, respectively. **c.** Haplotype analyses of the candidate genes Gene structures (left) and different haplotypes (right). Thin black lines indicate the genomic position of each SNP. Haplotypes with fewer than 5 varieties are not shown. Boxes show median, and upper and lower quartiles. Whiskers extend to 1.5× the interquartile range, with any remaining points indicated with dots. * $P < 0.05$, *** $P < 0.001$ (Welch two sample t-test)

Figure 6

Candidate gene identification by integrating GWAS and transcriptomic analysis. **a.** Venn diagram showed that a total 15 overlapping candidate genes were identified. **b.** The relative expression of *LOC_Os01g21590*, *LOC_Os03g15050* and *LOC_Os04g41229* in extreme tiller angle phenotypes relative to *OsActin1*. The green horizontal lines depict the average expression levels in the spread-tiller varieties and compact-tiller varieties, respectively. * $P < 0.05$ (Welch two sample t-test)

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