New Loci Relating to Rice Tiller Angle Traits Discovery Through Genome-Wide Association Analysis

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Abstract: Tiller angle (TA) is one of the most important agronomic traits which directly affected plant architecture, photosynthetic efficiency, and planting density of rice. Although many quantitative trait loci (QTL) and associated genes for TA have been identified, the detection of QTL for TA still required emphasis. In this study, 238 micro-core germplasm populations were used for genome-wide association analysis (GWAS) of the TA. The frequency distribution of TA was shown to be continuous in 2018 and 2019, five QTL designated as *qTA1*, *qTA2*, *qTA6*, *qTA9*, *qTA1*2, and two QTL designated as *qTA1*, *qTA1* were detected in 2018 and 2019, respectively. These QTL explained phenotypic variation ranged from 5.94% to 12.38%. Among them, *qTA2* and *qTA6* are new QTL for TA, and only *qTA1* was detected repeatedly in two years. 253 germplasm from the 3K Rice Genome Project were used for candidate gene and haplotypes analysis, the candidate gene and haplotypes analyzed showed *LOC_Os01g67770*, *LOC_Os02g35180* and *LOC_Os09g38130* were the candidate gene of *qTA1*, *qTA2*, and *qTA9*, respectively. The results of this study provide insight into the genetic improvement of plant architecture in rice.

Keywords: Rice; TA; QTL; GWAS; germplasm; haplotype

Highlights

- A total of 238 micro-core germplasm populations were used for genome-wide association analysis (GWAS) of the TA. *Rice Genome Project were used for candidate gene and haplotypes analysis, the candidate gene and haplotypes analyzed showed LOC_Os01g67770, LOC_Os02g35180 and LOC_Os09g38130 were the candidate gene of qTA1, qTA2, and qTA9, respectively.*

- The results of this study provide insight into the genetic improvement of plant architecture in rice.

Introduction

Rice (Oryza sativa) is the staple food for more than half of the world's population and increasing rice production is crucial to ensuring global food security (Al-Tamimi et al. 2016). Rice TA is defined as the angle between the vertical line and the side tillers with maximum inclination, reflects the spreading degree of rice plant architecture, is a key agronomic trait determining rice grain yield by affecting planting density (Wang et al. 2022; Cai et al. 2023).

Human long-term cultivation practice showed that the construction of rice ideotype can significantly improve rice yield (Wang et al. 2022). TA is affected by both genetic and environmental factors. TA QTL and gene mining is the basis for genetic improvement of plant architecture in rice (Wang et al. 2018; Huang et al. 2011; Zhao et al. 2011).

Previous studies showed that TA is a complex quantitative trait, and TA-related QTL has been explored in rice via different rice genetic populations. Dozens of QTL for TA were detected over recent decades, which are mainly located on chromosomes 1, 2, 3, 5, 7, 8, 9, 11, and 12 of rice, and some major QTL, such as *qTAC8*, *qTAC9*, and *qTA3* were future fine mapping (Li et al. 1999; Qian et

al. 2001; MacMillan et al. 2006; Li et al. 2006; Yu et al. 2007; Jiang et al. 2007; Zhang et al. 2013; Dong et al. 2016; He et al. 2017). But regrettably, only *TAC1*, *PROG1*, *TAC3* and *TIG1were* successively cloned by map-base cloning (Yu et al. 2007; Jiang et al. 2007; Tan et al. 2008; Jin et al. 2008; Dong et al. 2016; Zhang et al. 2019). Researchers have screened many TA mutants from different mutant libraries, and many studies on TA mutants have shown that TA also is controlled by quality genes. TA-related genes have been explored in rice via different mutant plants. Dozens of genes for TA were fine mapping over recent decades, la1, lazy1, Ostil1, tac2, lpa1, onac16-1D, fuct-1, CR-wox6 and CR-wox11, osarf17, la2, tac4 and lta1 (Takeshi et al. 2007; Li et al. 2007; Yoshihara et al. 2007; Mao et al. 2007; Fang et al. 2009; Wu et al. 2013; Sakuraba et al. 2015; Harmoko et al. 2016; Liu et al. 2016; Hu et al. 2020; Li et al. 2020; Chen et al. 2020; Li et al. 2021; Pan et al. 2022), etc. Only *LAZY1*, *OsNAC2*, *LAP1*, *ONAC106*, *FucT*, *WOX6* and *WOX11*, *OsARF17*, *LAZY2*, *LTA1* and *LAZY3* were detected, and these genes are major genes of TA in rice (Takeshi et al. 2007; Li et al. 2007; Yoshihara et al. 2007; Fang et al. 2009; Wu et al. 2013; Sakuraba et al. 2015; Harmoko et al. 2016; Liu et al. 2016; Hu et al. 2020; Li et al. 2020; Chen et al. 2020; Li et al. 2021; Pan et al. 2022; Cai et al. 2023). Most of these cloned QTL and genes regulate TA via playing an important role in regulating gravity perception, auxin transport, or redistribution under gravity stimulation of rice shoots (Takeshi et al. 2007; Li et al. 2007; Yoshihara et al. 2007; Zhang et al. 2018; Li et al. 2019; Furutani et al. 2020; Zhao et al. 2023; Li et al. 2021; Hu et al. 2020;). Such as *TAC1*, *TIG1*, *OsPIN1*, *LAZY1*, and *OsPIN2* regulate rice TA by affecting the asymmetric distribution of auxin (Yu et al. 2007; Jiang et al. 2007; Mao et al. 2007; Dong et al. 2016; Zhang et al. 2019; Hu et al. 2020; Li et al. 2021); downstream of the auxin gravity response gene, *WOX6* and *WOX11* can regulate TA in rice, while *HSFA2D* and *LAZY1* regulate TA through the auxin-mediated asymmetric expression of *WOX6* and *WOX11* (Tan et al. 2008; Jin et al. 2008; Yoshihara et al. 2007; Zhang et al. 2018). Plant phytohormones also affected TA; there is evidence showing that gibberellin (GA) is also involved in regulating TA. *OsGRF7* regulates rice plant architecture by regulating the synthesis and signaling pathways of auxin and gibberellin (Chen et al. 2020). All of these results indicated that the genetic regulation of TA is complex, the distribution of these QTL (genes) and excellent alleles of genes in germplasm is still not clear, and the application of cloned genes in production practice needs to be strengthened. Currently, GWAS is performed using core accessions with strong polymorphisms in target traits, has the advantages of extensive genetic variation, high reliability, high accuracy, and is an effective method to study TA, which provides information to analyze the natural variation and evolutionary change of TA (Wang et al. 2018; Huang et al. 2011; Zhao et al. 2011; Bai et al. 2022;).

In this study, GWAS was performed for TA using 238 micro-core germplasm resources, detected QTL and preliminarily analyzed candidate genes. Then, for new candidate genes, we selected 253 germplasm resources part of 3K Rice Genome Project to confirm the haplotype and the distribution of elite haplotypes of these candidate genes. A new candidate gene, *qTA1*, was discovered by GWAS in two consecutive years. The qPCR results indicated that cytokinin response regulator *qTA1* significantly differential expression in rice stem base, TA of elite haplotype is larger. These results could help geneticists and breeders to better understand the distribution of elite haplotype. Meanwhile, this study also provides germplasm and genetic resources for the genetic improvement of TA in rice.

Materials and Methods

Plant Materials

238 micro-core germplasm populations divided into 6 subgroups including 51 Indica (IND), 61 Tropical Japonica (TRJ), 50 Temperate Japonica (TEJ), 39 Australasia (AUS), 30 Admixture (ADMIX), and 7 Aromatic（AROMATIC）(Zhao et al. 2011). 253 germplasm resources from 3K Rice Genome Project divided into 12 subgroups (Wang et al. 2018). Detailed information regarding these varieties, including their geographical origin, were listed in (Figure 1A,B; Supplement File. A1).
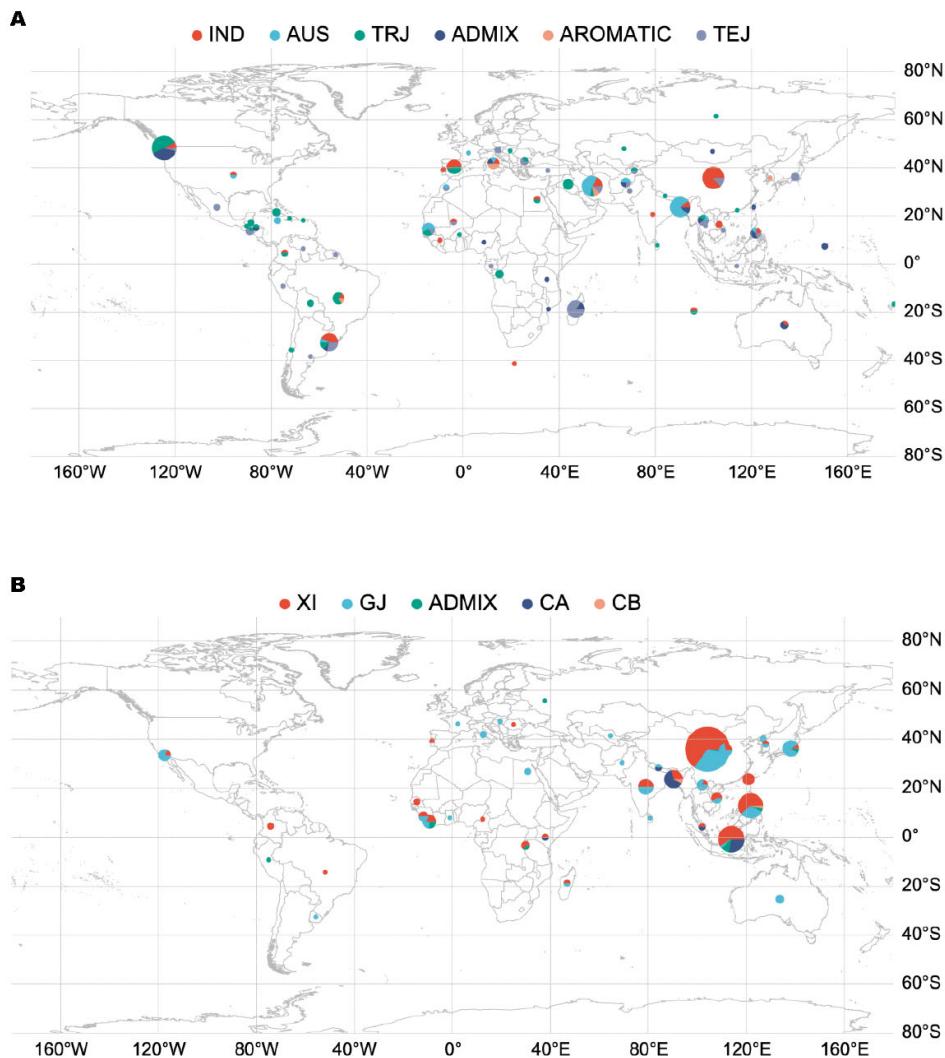
Figure 1. Geographic distribution core germplasm. (**A**) 238 germplasm geographic distribution; (**B**) 253 3K germplasms geographic distribution.

Phenotypic identification of TA

238 micro-core germplasm were planted in Liuyang, Changsha, Hunan Province in 2018 and 2019(E: 113.82, N: 28.32), seeds sowed on May 20 and seedlings transplanted on June 15 to 16; 253 germplasm resources from 3K Rice Genome Project planted in the experimental site of Liuyang in 2020, Six plants per row, one seedling per hill, with a density of 25 cm ×25 cm, and each accession was planted in 4 rows. Field management is carried out by normal field production. TA was measured at the heading stage of each germplasm (Shen et al. 2005), 5 plants were measured for each germplasm, and the average value was taken as the TA value.

GWAS for TA in rice

Population genotype data was downloaded from Rice Diversity (http://www.ricediversity.org/), A total of 34,453 SNP markers were used for GWAS (missing data > 90 %, minor allele frequency > 0.01), GWAS analysis was performed using the mixed linear model (MLM) of the association analysis software TASSEL 5.2 (Bradbury et al., 2007), with $P<1\times10^{-3}$ as the threshold. There were two or more significant SNP loci in the physical location of 200 kb, which was identified as one QTL. The highest R^2 value of SNP loci in the QTL interval represents the contribution

rate of the corresponding associated region (Zhao et al, 2011). Manhattan and Q-Q plots were drawn by R package CMplot.

Candidate gene screening

Referring to the Nipponbare genome annotation (https://rapdb.dna.affrc.go.jp/), 100Kb upstream and downstream of the QTL locus were used as the candidate gene prediction interval, and the predicted genes, conserved hypothetical proteins, reducing transposons and transposons were deleted. At the same time, the relative expression levels of plant expression tissues and plant endogenous hormones in the functional candidate genes in the QTL interval predicted by the rice expression profile database (https://ricexpro) were used to select genes with relatively higher expression levels as candidate genes. the gene with the most significant hit within a local LD block constructed around the QTL was screened as the candidate gene (Shin et al., 2005). The R package 'LD heatmap' was used to draw the heatmap of pairwise LDs. All non-synonymous mutation SNPs in the 2Kb promoter region, exons, introns and downstream 1Kb promoter region of the gene were selected by using the comprehensive database of rice genomic variation and functional annotation (http://ricevarmap.ncpgr.cn/) published by Huazhong Agricultural University. SNP loci with primary allele frequency (Primary Allele Frequency)>80% and deletion rate (DEL Frequency)>8% were screened, high-density association and gene-based haplotype analyses were carried out to detect candidate genes of QTL for TA by using 253 accessions from 3K RGB germplasm. Haplotypes of each candidate gene carried by at least 15 accessions were used for multiple comparisons (Zhao et al. 2011). Duncan's multiple comparison tests (5% significance level) followed by one-way ANOVA were completed with SPSS 25.

RNA extraction and expression analysis

To verify whether the detected QTL-related genes affect the TA. Total RNAs were isolated from stem base of rice at the tillering stage bases using a TRIzol kit (Invitrogen) according to the user's manual. Total RNA was treated with DNase I and used to synthesize cDNA with the HiScript reverse transcriptase System. Quantitative reverse transcription qPCR was implemented with SsoFast EvaGreen Supermix Kit (Bio-Rad) on the CFX96 Real-time system (Bio-Rad) following the manufacturer's instructions. Rice *OsActin* gene (*LOC_Os03g50885*) was used as the internal reference gene and gene specific primers are listed in (Supplement table A1). The $2^{-\triangle\triangle ct}$ method was used to calibrate relative expression levels of target genes with the reference gene (Livak et al. 2001).

Results

Phenotypic variation in TA among 238 micro-core germplasm populations

There were large variations in TA throughout the population in 2018 and 2019. The TA ranges from 9.63 ° to 55.66 ° in 2018 and 14.59 ° to 47.76 ° in 2019, with an average of 27.86 ° and 25.82 °, the coefficient of variation (CV) was 24.23 % and 25.43 %, respectively. The absolute values of population skewness were 0.86 and 0.85, and the kurtosis was 1.42 and 0.67, respectively (Supplement Table A2), which indicated the frequency distribution of TA showed an approximately normal distribution (Figure 2A,B). Correlation analysis showed that TA was significantly positive correlated in two years, and the correlation coefficient was 0.987(Figure 2C), indicated that genetics had a greater impact on TA than environmental factors. Statistical analysis of TA in different subgroups in the two years showed that there was no significant difference in other subgroups except for AUS and AROMATIC in 2018.
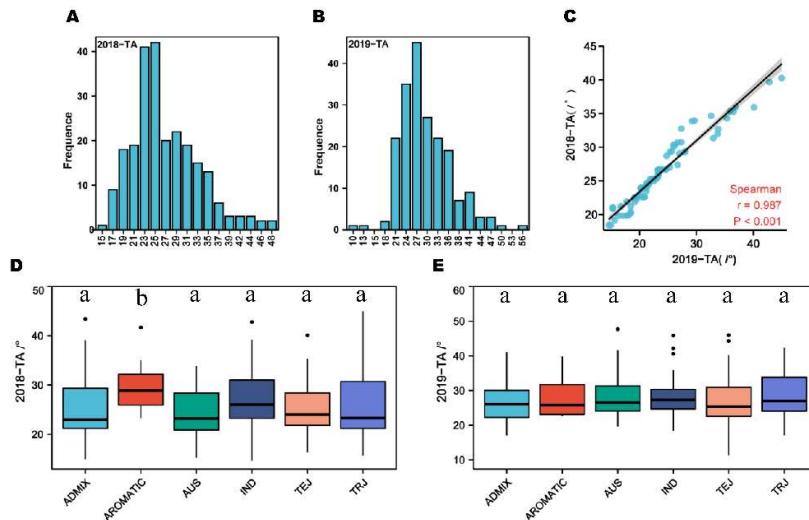
Figure. 2 The variation of TA in 238 rice accessions. (**A**) and (**B**) the distribution of TA values of 238 accessions in 2018 and 2019, respectively; (**C**) Correlation coefficients for TA values of 238 accessions in 2018 and 2019; (**D**) and (**E**) distribution of TA values within subgroups in 2018 and 2019, respectively. Data represent mean ± SD from five replicates. Different lowercase letters indicate significant differences among different subpopulations based on Duncan's new multiple-range test (p <0.05).

GWAS analysis for TA

GWAS analysis was performed of the 238 germplasm populations of TA in 2018 and 2019, based on the threshold (P value =1.0×10^{-3}), a total of 42 SNPs significantly associated with TA were identified, and these SNPs distribute on rice Chr 1, 2, 6, 9, and 12（Table 1）. Based on these significant SNPs, we finally identified 6 QTL for TA. Among these QTL, *qTA1*, *qTA2*, *qTA6*, *qTA9*, *qTA9*-1, *qTA12* were detected in 2018, *qTA1* and *qTA1*-1 was detected in 2019 (Figure 3). These QTL explained phenotypic variation ranged from 5.94% to 12.38% (Table 1). Among them, *qTA2* and *qTA6* are new QTL for TA（Supplement Table 3）, and only *qTA1* was repeated detected in 2018 and 2019, this indicated that *qTA1* was stably expressed in different years. Referring to the previous study, *TAC1*, *TAC3*, and *TIG1* were major genes controlling TA. The P value of the SNP flanked by these genes were 1.7×10^{-3}, 3.2×10^{-2} and 3.9×10^{-1}, below the threshold (P=1.0×10^{-3}) in this study. We furtherly analyze the haplotypes, the results showed that there was no significant difference in TA between different haplotypes, the results showed that the SNPs flanked by these genes did not influence TA, this could be the cause why we did not detect *TAC1*, *TAC3*, and *TIG1* in this study (Supplement Figure A3, Supplement Figure A4, Supplement Figure A5).
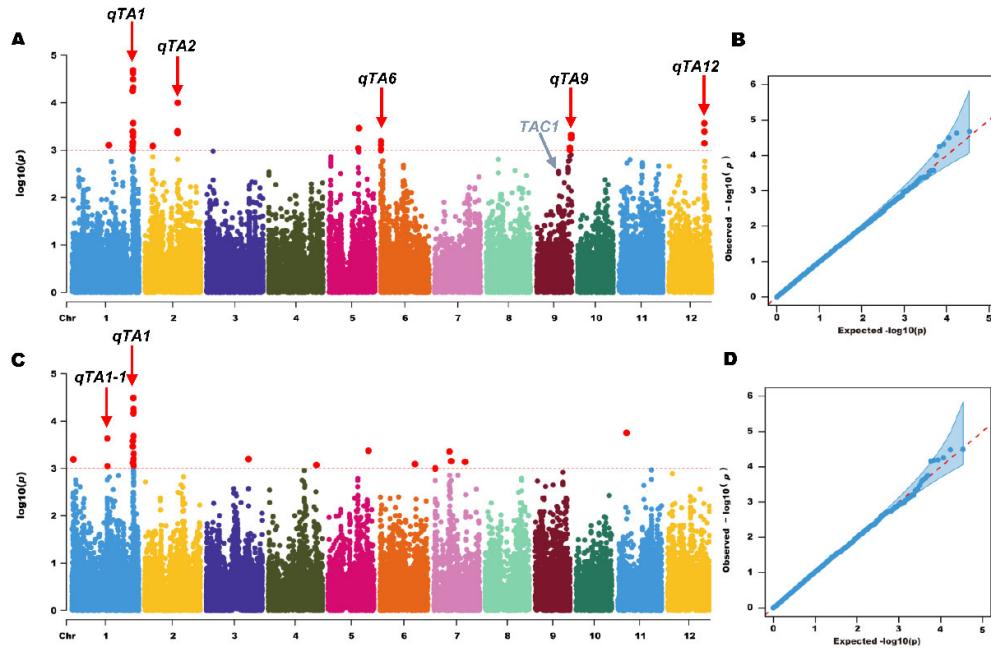
Figure 3. Genome-wide association analysis for TA in rice. (**A**) and (**C**) Manhattan plots of genome-wide association analysis in 2018 and 2019, respectively; (**B**) and (**D**) Q-Q plots of genome-wide association analysis in 2018 and 2019, respectively.

Table 1. List of QTL for TA identified by GWAS.

Treatment	QTL	Chr	SNP	Position	P-value	R²
2018 Liuyang	*qTA1*	1	id1024678	38923066	5.63E-05	12.38%
	qTA2	2	id2008457	21110964	1.01E-04	6.95%
	qTA6	6	id6000160	342503	6.54E-04	7.06%
	qTA9	9	id9007421	21868430	8.87E-04	6.61%
	qTA12	12	id12007801	22996012	2.71E-04	8.00%
	qTA1-1	1	id1013058	22837243	2.32E-04	6.07%
2019 Liuyang	*qTA1*	1	id1025079	39548160	3.29E-05	8.20%

3.3. Candidate gene identification and haplotype analyses for TA

To search for the candidate genes in six QTL, we choose the genes mainly expressed in stem or node based on the expression profile in Rice Expression Database(http://expression.ic4r.org/), and then excluded the genes encoding retrotransposon or transposon protein based on their function annotations (https://www.rmbreeding.cn). 12 annotated genes, including Zinc finger protein, auxin response factor and cytokinin response in *qTA1*, *qTA2*, *qTA6*, *qTA9* and *qTA12*, respectively. were preliminary excavated (Table 2). Then the high-density association and gene-based haplotype analyses were used for identifying the candidate genes by using 253 germplasm resources from 3K Rice Genome Project. We finally mining three candidate genes for *qTA1 qTA2* and *qTA9*. Did not find appropriate candidate genes for *qTA2*, *qTA6* and *qTA9*-1 based on the results of haplotype analyses. In the region of *qTA1*, 7 SNPs were used for high-density association analysis. The annotated gene with the most significant hit was *LOC_Os01g67770* (Figure 4A). three major haplotypes were detected among 253 accessions based on three SNPs in the *LOC_Os01g67770* promoter, five SNPs in the coding region, and one SNP in the 3'-UTR region (Figure 4B). Significant differences for TA were observed

among the three haplotypes. Hap1, Hap2 and Hap3 contain 75, 70 and 90 accessions, respectively. Hap1, had the highest mean TA (25.60 °). And showed a significantly higher mean TA than the Hap2 (Figure 4C and Figure 4D). Hap1 was mainly composed of XI-1A and CA subgroups, and Hap2 was mainly composed of GJ-tmp and GJ- trp subgroups (Figure 4E). In the region of *qTA2*, 8 SNPs were used for high-density association analysis. The annotated gene with the most significant hit was *LOC_Os02g35180* (Figure 5A). Two major haplotypes were detected among 253 accessions based on three SNPs in the *LOC_Os02g35180* promoter, three SNPs in the coding region, one SNP in encoding region, and one SNP in the 3'-UTR region (Figure 5B). Significant differences for TA were observed among the three haplotypes. Hap1, Hap2 contain 112 and 92 accessions, respectively. Hap1, had the highest mean TA (23.62 °). And showed a significantly higher mean TA than the Hap2 (Figure 5C and Fig.5D). Hap1 were mainly composed of XI-1A, XI-1B, and XI-adm subgroups, and Hap2 were mainly composed of XI-1A, XI-1B, and XI-adm subgroups (Figure 5E). In the region of *qTA9*, 8 SNPs were used for high-density association analysis. The annotated gene with the most significant hit was *LOC_Os09g38130* (Figure 6A). Two major haplotypes were detected among 253 accessions based on four SNPs in the *LOC_Os09g38130* promoter, one SNPs in the coding region, one SNP in encoding region, and one SNP in the 3'-UTR region (Figure 6B). Significant differences for TA were observed among the three haplotypes. Hap1, Hap2 contain 112 and 92 accessions, respectively. Hap1, had the highest mean TA (27.03 °). And showed a significantly higher mean TA than the Hap2 and Hap3(Figure 6C and Fig.6D). Hap1 and Hap2 is mainly composed of XI-1A, XI-1B, and XI-adm subgroups, Hap3 is mainly composed of GJ-adm, GJ-tmp and GJ-trp subpopulations (Figure 6E). The above results showed that the elite haplotype of the TA mainly existed in the indica rice subgroup, and the TA haplotype of the indica rice subgroup was the elite haplotype. To further certify the *LOC_Os01g67770*, *LOC_Os02g35180* and *LOC_Os09g38130* are the candidate gene of *qTA1*, *qTA2* and *qTA9*, qRT-PCR were used for analyzed the relative expression level of *LOC_Os01g67770*, *LOC_Os02g35180* and *LOC_Os09g38130* in ten accessions, the results showed that relative expression level of these genes are positively associated with TA, this indicated that *LOC_Os01g67770*, *LOC_Os02g35180* and *LOC_Os09g38130* may be the candidate genes of these QTL (Figure 4, Figure 5, and Figure 6).

Table 2. Candidate genes and function annotations of QTL for TA.

QTL	Candidate gene	Description
	LOC_Os01g67770	Two-component response regulator, response to cytokinin stimulus
qTA1	*LOC_Os01g66890*	Broad Complex BTB domain with TAZ zinc finger and Calmodulin-binding domains
	LOC_Os01g66970	Zinc finger, C3HC4 type domain-containing protein
	LOC_Os02g35140	Auxin response factor 7
qTA2	*LOC_Os02g35150*	Zinc finger CCCH domain-containing protein
	LOC_Os02g35180	*OsRR2* type-A response regulator, response to auxin stimulus
qTA6	*LOC_Os06g01620*	Plant-specific transcription factors maintain bud tip meristem uncertainty
qTA9	*LOC_Os09g38110*	RING-H2 finger protein
	LOC_Os09g38130	Auxin efflux carrier component, response to auxin stimulus
qTA9-1	*LOC_Os09g39400*	Histidine-containing phosphotransfer protein
	LOC_Os09g39660	C2H2 zinc finger protein
qTA12	*LOC_Os12g37430*	PAPA-1-like conserved region family protein/ zinc finger protein
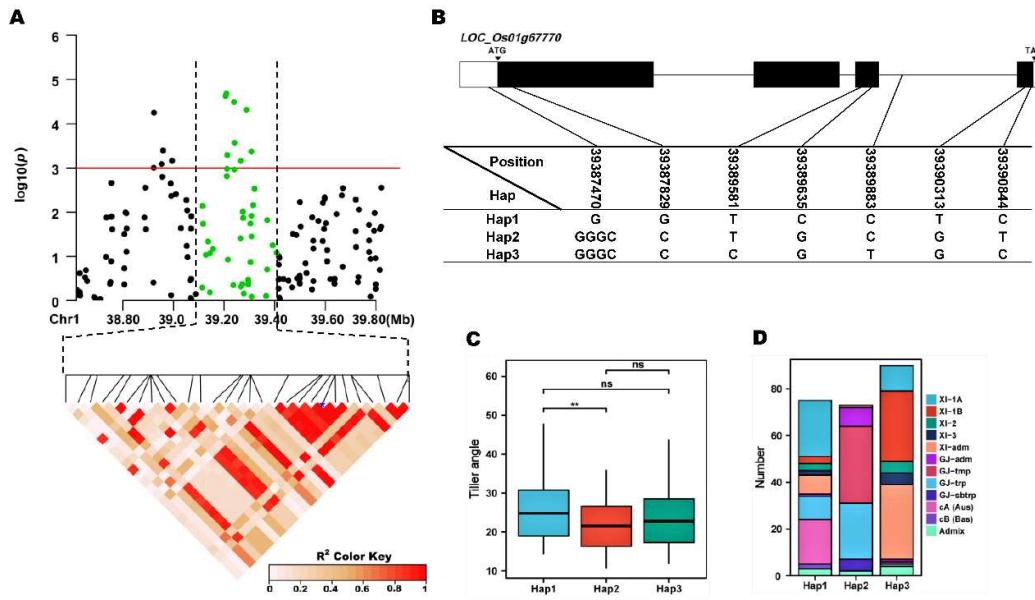
Figure 4. *LOC_Os01g67770* haplotype significance analysis. (**A**) linkage disequilibrium plot for SNPs with -log10(p) value > 3 in *qTA1* on Chr. 1; (**B**) Schematic representation of *LOC_Os01g67770* structure and the positions of 7 SNPs used for haplotype analysis, bar=100bp; (**C**) Comparisons of TA values among accessions with different haplotypes. *p ≤ 0.05, **p ≤ 0.01; (**D**) the subpopulation composition of *LOC_Os01g67770* haplotypes for TA.
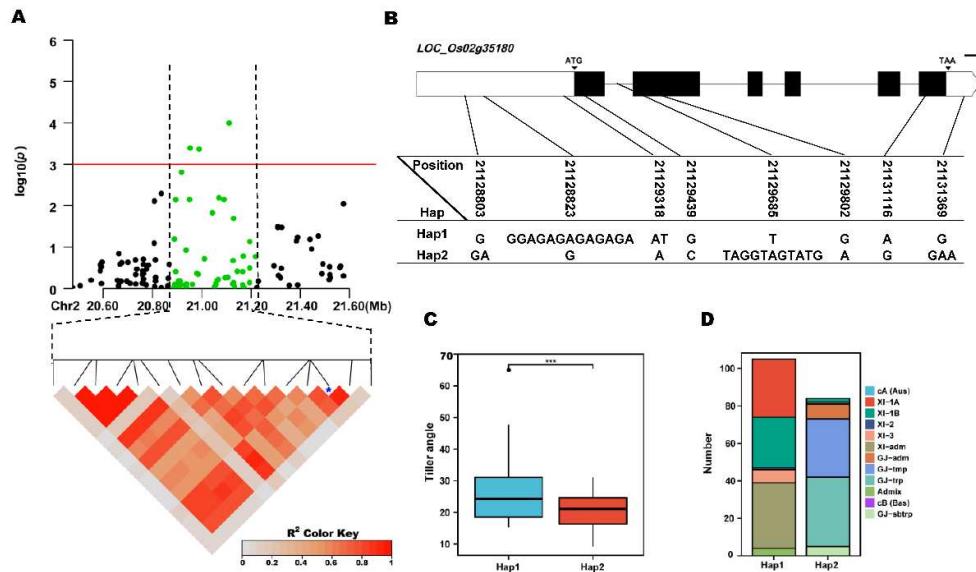
Figure 5. *LOC_ Os02g35180* haplotype significance analysis. (**A**) linkage disequilibrium plot for SNPs with -log10(p) value > 3 in *qTA2* on Chr. 2; (**B**) Schematic representation of *LOC_ Os02g35180* structure and the positions of 8 SNPs used for haplotype analysis, bar=100bp; (**C**) Comparisons of TA values among accessions with different haplotypes. ***p ≤ 0.001; (**D**) the subpopulation composition of *LOC_ Os02g35180* haplotypes for TA.
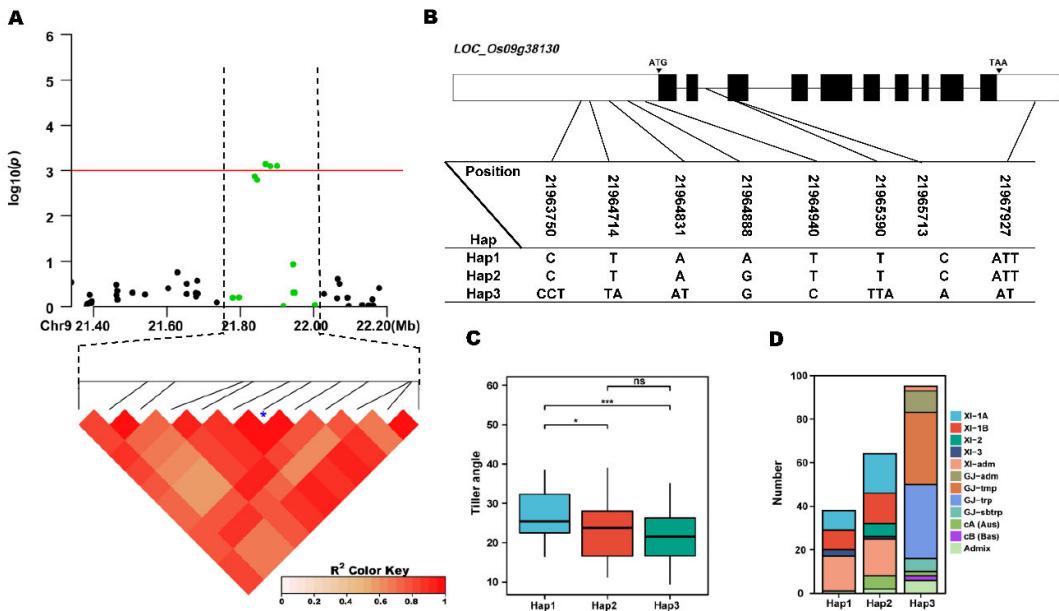
Figure 6. *LOC_Os09g38130* haplotype significance analysis. (**A**) linkage disequilibrium plot for SNPs with -log10(p) value > 3 in *qTA9* on Chr. 9; (**B**) Schematic representation of *LOC_Os09g38130* structure and the positions of 8 SNPs used for haplotype analysis, bar=100bp; (**C**) Comparisons of TA values among accessions with different haplotypes. *p ≤ 0.05, ***p ≤ 0.001; (**D**) the subpopulation composition of *LOC_Os09g38130* haplotypes for TA.
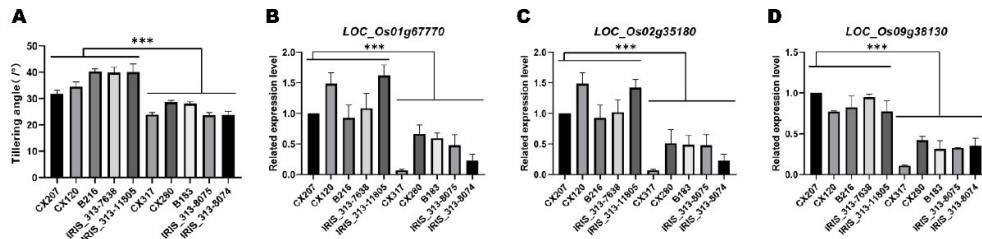
Figure 7. Expression verification the function of candidate genes. (**A**) Comparisons of TA values between accessions with larger TA and accessions with litter TA, *** p ≤ 0.001; (**B**), (**C**) and (**D**) relative expression level of *LOC_Os01g67160*, *LOC_Os02g35180*, and *LOC_Os09g38130* in stem node of accessions. Rice *OsActin* gene (*LOC_Os03g50885*) was used as the internal reference gene, the $2^{-\triangle\triangle ct}$ method was used to calibrate relative expression levels of target genes with the reference gene.

Discussion

TA of rice affected by the genetic factor of accessions and environmental factors, genetic effects explained more of the variation, environment factors, such as the planting density per unit area, field management, Light and temperature etc., also significantly affected TA (Wang et al. 2022). Plant type can be divided into plant morphology and plant type, the generalized plant type refers to the organic combination of the morphological characteristics of the plant body, including roots, stems, leaves, and ears, and the physiological functions directly related to the light energy utilization of the plant population; narrow plant type refers to the morphological characteristics and spatial arrangement of plants (Donald. 1968). The ideal plant type is to cultivate an excellent canopy structure, so that the crop can maximize the capture of solar energy, improve the photosynthetic efficiency of the population, and maximize the economic yield under the limited light conditions during the whole growth period (Wang et al. 2022). Cultivating rice varieties with suitable TA, low shading, large

photosynthetic area and high light energy utilization rate can effectively improve the yield of rice population. Natural variation is the form of variation that is significant in the evolution of developmental processes, genetic analysis of rice TA QTL derived from natural variation is of great significance for genetic improvement of rice plant type (Tan et al. 2008; Jin et al. 2008; Huang et al. 2011; Zhao et al. 2011). 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 (Bai et al. 2011).

Abundant variations of TA in rice germplasm

In this study, our results showed that there was no significant difference in TA among different subpopulations in different years, and no significant difference in the variation amplitude and coefficient of variation of TA among the same subpopulations, this indicated that TA is greatly affected by environmental factors; we detected six QTL for TA in 2018, total explained about 47.79% of phenotypic variance, but just detected two QTL for TA in 2019, total explained 14% of phenotypic variance. these results showed that the expression of QTL for TA easily affected by environment factors.

Comparison of GWAS results with previously reported results

TA plays a vital role in rice production, it can directly affect plant architecture, photosynthetic efficiency, and planting density of rice, so appropriate TA is beneficial for improving rice production (Tan et al. 2008; Jin et al. 2008). Although significant progress in cloning of genes (QTL) involved in regulating TA has been made recently in rice, few of these genes was used in rice breeding for ideal architecture, and the distribution of alleles of TA in germplasm remain largely unknown. Then, we comparison of GWAS results with previously reported results of TA (Supplement Table A3), in our study, *qTA1*(id1024678), *qTA1-1*(id1024648), *qTA2*(id2008457), *qTA6*(id6000160), *qTA9*(id9007808), and *qTA12*(id12007801) for TA were detected in 2018 and 2019. and only *qTA1* were repeatedly detected in two years, *qTA1* explains 12.38% and 5.94% of phenotypic variance in 2018 and 2019, respectively. *QTa1* which controls TA was firstly mapped between RG173-RG532 (Li et al. 1999), which locates on the same region with *qTA1* and *qTA1-1*, suggesting that *QTa1* may be an allele of *qTA1* or *qTA1-1*. *qTA-9*, was mapped between RG662-CT100 (Qian et al. 2001), which locates on the same region with *qTA9* and *qTA9-1*, suggesting that *qTA-9* may be an allele of *qTA9* or *qTA9-1*. *qTA-12*, was mapped between RG461-G148 (Qian et al. 2001), suggesting that *qTA12* may be an allele of *qTA-12*. On the interval of *qTA2* and *qTA6*, we did not find any QTL for TA reported before, this indicated that *qTA2* and *qTA6* may be the new QTL for TA.

Identification of candidate genes for TA

It was reported that the plant endogenous hormones IAA, GA, BR, and SL can affected TA (Wang et al. 2022). *D2*, encoding a key enzyme in BR biosynthesis, positive regulates TA in rice (Dong et al. 2022). *TAC1*, *LAZY1* and *PROG1* regulate TA via involving in auxin polar transport (Yu et al. 2007; Jiang et al. 2007; Takeshi et al. 2007; Li et al. 2007; Yoshihara et al. 2007; Tan et al. 2008; Jin et al. 2008). In this study, consulting the relevant literature, the expression profile in Rice Expression Database and the results of haplotype analyses, *LOC_Os01g67770*, was finally identified as the candidate gene of *qTA1*, it encodes cytokinin-signaling B-type response regulator, three SNPs in the promoter, five SNPs in the coding region, and one SNP in the 3'-UTR region, caused significant difference in TA between three haplotypes. Hap1 is the superior haplotype conferring largest TA. *LOC_Os02g35180*, was identified as the candidate gene of *qTA2*, it encodes a cytokinin-responsive type-A response regulator. three SNPs in the promoter, three SNPs in the coding region, one SNP in encoding region, and one SNP in the 3'-UTR region, caused significant difference in TA between two haplotypes. Hap1 is the superior haplotype conferring largest TA. *LOC_Os09g38130*, identified as the candidate gene of *qTA9*, it encodes an auxin efflux carrier component, four SNPs in the promoter, one SNPs in the coding region, one SNP in encoding region, and one SNP in the 3'-UTR region caused

significant difference in TA between three haplotypes. Hap1 is the superior haplotype conferring largest TA.

In the future, CRISPR/Cas9 could be applied, further verify the function of these genes and apply they in molecular breeding of ideotype.

Conclusions

We detected six QTL for TA by GWAS within 238 rice accessions, and screened 3 new candidate genes for *qTA1*, *qTA2* and *qTA9* vivo high-density association and gene-based haplotype analyses. Then we used the qRT-PCR to further validated the *LOC_Os01g67770*, *LOC_Os02g35180* and *LOC_Os09g38130* are the candidate gene of *qTA1*, *qTA2* and *qTA9*, respectively. The identification of those candidate genes and their elite haplotypes will provide a promising source for molecular breeding of ideotype in rice.

Supplementary Materials:

Author Contributions: Conceptualization, H.J. and Z.H.; methodology, H.J.; software, G.J.; validation, G.J., L.X. Y.Y. and H.X; formal analysis, G.J. and H.X.; investigation, G.J.; resources, H.J.; data curation, G.J.; writing—original draft preparation, G.J.; writing—review and editing, H.J. and Z.H.; visualization, G.J., and L.X.; supervision, Z.H.; project administration, H.J.; funding acquisition, H.J.

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