Dissection of genetic architecture for tiller angle in rice (*Oryza sativa*. L) by multiple genome-wide association analyses

Muhammad Abdul Rehman Rashid [Corresp., 1, 2, 3] , **Rana Muhammad Atif** [4] , **Yan Zhao** [2, 5] , **Farrukh Azeem** [3] , **Hafiz Ghulam Muhu-Din Ahmed** [6] , **Yinghua Pan** [7] , **Danting Li** [7] , **Yong Zhao** [2] , **Zhanying Zhang** [2] , **Hongliang Zhang** [2] , **Jinjie Li** [2] , **Zichao Li** [Corresp. 2]

1 School of Agriculture, Yunnan University, Kunming, Yunnan, China

2 State Key Laboratory of Agrobiotechnology / Beijing Key Laboratory of Crop Genetic Improvement,, College of Agronomy and Biotechnology,, China Agricultural University, Beijing, China

3 Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Faisalabad, Pakistan

4 Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Faisalabad, Pakistan

5 College of Agronomy, Shandong Agricultural University, Taian, Shandong, China

6 Department of Plant Breeding and Genetics, Islamia University, Bahawalpur, Bahawalpur, Pakistan

7 Rice research Institute, Guangxi academy of agricultural Sciences, Guangxi, China

Corresponding Authors: Muhammad Abdul Rehman Rashid, Zichao Li
Email address: marrashid@gcuf.edu.pk, lizichao@cau.edu.cn

Background: The rice plant architecture is determined by spatially and temporally domesticated tiller angle. The deeper insight into the genetic mechanism for rice plant architecture will allow more efficient light capture by increasing the planting density, reproducibility, and the ability to survive in a stressful environment. **Methods:** In this study, a natural population of 795 genotypes further divided into *japonica* and *indica* subpopulations, was evaluated for tiller angle. A significant variation with a wide range was observed. Genome-wide association analysis was performed by the general linear model (GLM), and compressed mix linear model (cMLM) for three populations to disclose the genomic associations. The population Principal components and kinship matrix in 1000 permutations were used to remove the false positives. The candidate genes were evaluated for their functional annotations and specific molecular pathways. The sequencing-based haplotype analysis was further performed to reveal the functional variation among candidate genomic regions. **Results**: As a result, 37 significant QTLs with 93 annotated loci were identified. Among the loci, a known tiller angle controlling locus *TAC1* was also identified. The introduction of the sequence pooling technique was observed fruitful to screen the 12 significant QTLs with 22 annotated loci. For ten of these loci, the functional variations were identified by haplotype analysis. These results were not only providing a better understanding of the genetic bases of rice plant architecture but also provide significant information for future breeding programs.

1 **Dissection of genetic architecture for tiller angle in rice (*Oryza sativa*.**
2 **L) by multiple genome wide association analyses**

3

4 Muhammad Abdul Rehman Rashid[1,2,7],Rana Muhammad Atif [3], Yan Zhao[2,4], Farrukh Azeem[1],
5 Hafiz Ghulam Muhu-din Ahmed[5], Yinghua Pan[6], Danting Li[6], Yong Zhao[2], Zhanying Zhang[2],
6 Hongliang Zhang[2], Jinjie Li[2], Zichao Li[2*]

7 [1] Department of Bioinformatics and Biotechnology, Government College, University Faisalabad,
8 Faisalabad-38000, Pakistan
9 [2] State Key Laboratory of Agrobiotechnology / Beijing Key Laboratory of Crop Genetic
10 Improvement, College of Agronomy and Biotechnology, China Agricultural University, Beijing
11 100193, China.
12 [3] Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Faisalabad-
13 38000, Pakistan
14 [4] College of Agronomy, Shandong Agricultural University, Tai'an-271018, Shandong, China
15 [5] Department of Plant Breeding and Genetics, Islamia University Bahawalpur, Bahawalpur-
16 63100, Pakistan
17 [6] Rice research institute, Guangxi Academy of Agricultural Sciences/Guangxi key laboratory of
18 rice genetics and breeding, Nanning-53007, China
19 [7] School of Agriculture, Yunnan University, Kunming-650205, China.
20
21 [*] Corresponding Author:
22 Zichao Li, Email address: lizichao@cau.edu.cn , Tel: +86-18618489195, ORCID: 0000-0002-
23 3186-1132
24

25 Abstract

26 **Background:** The rice plant architecture is determined by spatially and temporally domesticated
27 tiller angle. The deeper insight into the genetic mechanism for rice plant architecture will allow
28 more efficient light capture by increasing the planting density, reproducibility, and the ability to
29 survive in a stressful environment.
30 **Methods:** In this study, a natural population of 795 genotypes further divided into *japonica* and
31 *indica* subpopulations, was evaluated for tiller angle. A significant variation with a wide range
32 was observed. Genome-wide association analysis was performed by the general linear model
33 (GLM), and compressed mix linear model (cMLM) for three populations to disclose the genomic
34 associations. The population Principal components and kinship matrix in 1000 permutations
35 were used to remove the false positives. The candidate genes were evaluated for their functional
36 annotations and specific molecular pathways. The sequencing-based haplotype analysis was
37 further performed to reveal the functional variation among candidate genomic regions.
38 **Results**: As a result, 37 significant QTLs with 93 annotated loci were identified. Among the
39 loci, a known tiller angle controlling locus *TAC1* was also identified. The introduction of the
40 sequence pooling technique was observed fruitful to screen the 12 significant QTLs with 22

41 annotated loci. For ten of these loci, the functional variations were identified by haplotype
42 analysis. These results were not only providing a better understanding of the genetic bases of rice
43 plant architecture but also provide significant information for future breeding programs.

44

45 **Keywords:** tiller angle, association mapping, rice (*Oryza sativa* L.), candidate genes, genetic
46 architecture.

47

Introduction

49 Among the cereal crops, rice (*Oryza sativa* L.) is one of the most important food staples. It is
50 rolling as a model plant to study the monocots due to its comparatively collinear and smaller genome
51 (Peijin, Dali et al. 2003). To feed the continuously growing human population, the increasing crop yield is
52 always an objective of any research activity. The main culm and tillers play seminal role to determine the
53 rice plant architecture and finally the yield (Wang and Li 2005). Plant architecture referred to the three-
54 dimensional arrangement of arial parts of plants, is a major factor contributing to the total plant yield
55 (Reinhardt and Kuhlemeier 2002, Wang and Li 2006). The plant architecture can be determined by the
56 variation in tillering, plant height, leaf shape and arrangement and panicle morphology (Wang and Li
57 2005). Tiller angle affects the ability of survival and to trap maximum light for photosynthesis. The erect
58 to optimum spreading tillers of plants have been evolved during rice domestication from wild species to
59 cultivated varieties while a relatively wider tiller angle in *indica* sub-species has been observed than in
60 *japonica*. In dense planting, a wider tiller angle may enable the plant to escape some diseases by reducing
61 the humidity. Contrastingly, it will cover more space and increase the leaf shading and can cause
62 reduction in photosynthesis. The dense and upright leaves have also been reported to improve the leaf
63 nitrogen accumulation for grain filling (Wang and Li 2008).

64

65 Likewise other yield related traits, tiller angle also showed a complex genetic behavior. In
66 previous studies different quantitative trait loci (QTLs) and genes have been identified for tiller angle as
67 *Ta* (Li. Zhikang, H et al. 1999), *OsHOX1*, *OsHOX28* (Hu, Li et al. 2020), *qTA-9a* (Qian, He et al. 2001),
68 *qTA-9b* (Qian, He et al. 2001), *qTA9-2* (Shen S Q, Zhuang J Y et al. 2005) and *qTA-9* (Yu, Liu et al.
69 2005), and *qTAC-8* (He, Shao et al. 2017)have been mapped. The *TAC1* (Yu, Lin et al. 2007) have been
70 mapped and cloned as well, while the *TAC3* together with *TAC1* and *D2* have been known for significant
71 variation in tiller angle (Dong, Zhao et al. 2016, Li, Sun et al. 2021). A study revealed the effect of
72 overexpression of CpG-binding protein gene *OsMBD707* causing a larger tiller angle (Qu, Zhang et al.
73 2021). Several mutants such as *La* (Singh, Multani et al. 1996), *La2* (Peijin, Dali et al. 2003), *er*
74 (Takahashi M, Kinoshita T et al. 1968), *D20* (Kinoshita T, Takahashi M et al. 1974) and *Spk(t)* (Maiyata
75 M, Komori T et al. 2005) were also observed to affect the tiller angle. The TCP transcriptional factor
76 controlled by *TIG1* found to affect the tiller angle in rice (Zhang, Tan et al. 2019). Another QTL mapping
77 study for tiller angle performed by RIL population identified the 24 alleles on 12 QTLs (Zeng, Chen et al.
78 2019).

79 Various genetics studies for plant growth regulators including the *TAC4* (Li, Sun et al. 2021) and
80 *OsmiR167a* gene (Li, Li et al. 2020) have also been reported to control tiller angle by regulation and
81 endogenous distribution of auxin contents. Furthermore, *LAZY1* is known to control the tiller angle by
82 gravitropism (Li, Wang et al. 2007, Yoshihara, Spalding et al. 2013, Zhu, Hu et al. 2020) and asymmetric
83 distribution of Auxin (Zhang, Yu et al. 2018), and *LAZY2* by regulation of starch biosynthesis in gravity-

84 sensing cells (Huang, Wang et al. 2021). The phytochrome-interacting factor- like protein *OsPIL15* has
85 also been reported to integrate the light and gravtropism to regulate the tiller angle in rice (Xie, Zhang et
86 al. 2019). The hormonal homeostasis in combination with environmental factors has also been studied to
87 involve in regulation of tiller angle (Lomax 1997). Recently, strigolactone hormone has been reported to
88 regulate tiller angle by shoot gravtropism in rice through auxin biosynthesis inhibition (Sang, Chen et al.
89 2014). Several tiller angle governing loci in rice have been identified by both classical and molecular
90 methods. Although the mapping, cloning and functional genetics studies laid a solid ~~bases~~ *basis* to understand
    s are still unclear.
91 the plant architecture development ~~but~~ exact mechanism is still not clear.
92
    throughput
93 The genome-wide association (GWA) study with the high through put sequencing technology has
94 appeared as an efficacious tool to identify the QTL/gene associated with complex agronomic traits. The
95 GWA-mapping took the advantage of maximum recombinant events to identify the trait association to the
96 narrower genomic region. The genome-wide distribution of single nucleotide polymorphic (SNP) markers
97 enhanced the power of GWA mapping studies. In recent years, the GWA study has gained the attention of
98 rice researchers to identify different agronomically important traits including flowering time and yield
99 traits (Huang, Wei et al. 2010, Huang, Zhao et al. 2012), stress related traits as salinity (Kumar, Singh et
100 al. 2015) and drought (Zaniab Al-Shugeairy, Adam H. Price et al. 2015) but no specified GWA study has
    basis
101 performed to dissect the genetic ~~bases~~ of tiller angle. This study was aimed to investigate the genome-
102 wide regions associated with tiller angle in rice. It will not only extend our understanding about the
    in a   *will*   *toward improving*
103 genetic nature of tiller angle in model plant but also contribute to the improving yield potential in
104 breeding programs.
105

Materials & Methods

107 *Plant and field material*

108 The 795 geographically diverse accessions from 3000 Rice genome project (3KRGP) (Li, Wang
109 et al. 2014, Li, Fu et al. 2014) were used in this study. The genetic homozygosity was ensured by grown
    descent
110 plants through single seed ~~decants~~ for three years. All the plant material was cultivated in two blocks at *a*
111 bird-net protected experimental farm of China Agricultural University, Sanya city, Hainan (18° N, 109°
112 E) province of China. Three lines of one meter length and seven seed per line were grown. Five guarded
113 plants from middle line were selected for phenotyping. All the standard cultural practices were kept
114 constant.
    [stopped editing here -- needs revisions to improve clarity]

115 *Phenotyping*

116 The tiller angle (TA) of five guarded plants from each block was recorded as the angle between
117 extreme right and extreme left tiller (Chen, Fan et al. 2012) by goniometer. And, an average value of ten
118 individual plants was considered as final TA for further analysis (Supplementary Table 1).

119   *Genotyping and population Structure*

120   The second generation of whole-genome high-throughput sequence with ~13fold coverage was
121   obtained by Illumina sequencing and the sequence reads were aligned against rice reference genome. The
122   direct comparison was performed with corresponding regions of *japonica* cv. Nipponbare reference
123   sequence to produce 10 million raw SNPs. The screening for >50 percent missing SNPs resulted in 3.3
124   million SNPs for 795 genotypes. These SNPs covered the 373 Mb (mega-base) of whole rice genome
125   with an average SNP density of 12.3 SNPs per kb (kilo-base). All the selected SNP markers distributed
126   over the 12 chromosomes were used for detection of population structure. The principal components and
127   kinship matrix were calculated by GAPIT program (http://www.maizegenetics.net/GAPIT). On the basis
128   of principal components and kinship matrix, whole population was divided into two sub-populations;
129   *japonica* and *indica*. Then, the principal components and kinship matrix were re-estimated for individual
130   sub-population to use as covariates in the mixed linear model (MLM). The physical position of known
131   genes and candidate loci were acquired from rice online data base (http://rapdb.dna.affrc.go.jp).

132   *Statistical analysis for phenotypes*

133   The analysis of variances (ANOVA) was performed to estimate the genotypic variance (Vg),
134   error variance (Ve) and interaction variances by using the STATISTICA software (StatSoft 1995, Tulsa,
135   OK, USA). The phenotypic data descriptive and Q-Q plots were analyzed by SPSS version 19
136   (http://www-01.ibm.com/software/analytics/spss/). The broad sense heritability (H^2) and repeatability (R^2)
137   (Sukumaran, Dreisigacker et al. 2015) were estimated by using genotypic variance (Vg), error variance
138   (Ve) and interaction variances according in following formula:

139
$$R^2 = \frac{\sigma^2 G}{\sigma^2 G + \dfrac{\sigma^2 (G \times E)}{b} + \dfrac{\sigma^2 E}{br}}$$

140   Where R^2 is the estimate of repeatability, $\sigma^2 G$ is the genetic variance, $\sigma^2 G \times E$ is the genotype to blocks
141   interaction variance, and $\sigma^2 E$ is the residual variance, r is the number of replications, and b is the number
142   of blocks. All the related variances were estimated by ANOVA.
143   The data normality was confirmed by Q-Q plot drawn by SPSS program and also in GAPIT analysis. The
144   best linear unbiased predictions (BLUPs) were estimated in GAPIT analysis using R-program.

145   *Genome wide Association analysis*

146   The mean values of ten plants for each genotype were used as final tiller angle. Two models
147   (GLM, cMLM) were applied on three populations (Whole population, *indica* and *japonica* sub-
148   populations) by GAPIT software to identify the SNPs association with tiller angle. In first model, the
149   population structure (PCAs) was incorporated to accomplish the general linear model (GLM). In second
150   model, population structure and kinship matrix respectively were included as fixed and random effect to
151   conduct compressed mix linear model (cMLM). The 1000-permutations test by MLM was performed for
152   detection of false SNP signals.

153 *Sequence pooling, Candidate Genes identification and Haplotyping*

154 The QTLs regions were defined by SNP significance at threshold of –log10 (P) <4 which were
155 further screened by deletion of SNPs not reside in CDS or promoter regions of annotated loci. For further
156 identification of significant loci, a new sequence based pooling strategy was applied. The 20 genotypes
157 with highest phenotypic value and 20 genotypes with lowest phenotypic value were selected separately
158 from two subpopulations. The difference of major and minor allele frequencies in four pools were
159 compared by χ^2-test at α = 0.05. The SNPs with significantly different major and minor allele frequency
160 were selected to define significantly associated loci. Then all the significant and non-significant SNPs in
161 promoter and CDS region of selected loci were used to construct a map and to evaluate their functional
162 haplotypes.

163

164 **Results**

165 *Phenotypic Variation of tiller Angle*

166 The highly divergent worldwide collection of 795 rice genotypes showed significant variation for
167 tiller angle ranging from 20.1 to 82.7 degrees (Supplementary Table 1). The mean tiller angle for
168 *japonica* sub-population (38.05±7.75) was significantly lower than that of *indica* sub-population
169 (41.84±9.8) and whole populations (40.55±9.3) (Table 1). An identical and positively semi-skewed
170 frequency distribution was observed for all three populations (Fig. 1, Table 1). The two-way analysis of
171 variance (ANOVA) showed the significantly high variation among genotypes (Table 2). The high broad
172 sense heritability (h^2) and the repeatability factor (R^2) demonstrated the genotypes as the main source of
173 variation for tiller angle which was consistent in three populations. The *japonica* cultivars showed
174 relatively lower heritability but the higher repeatability confirmed the genotypic effect as the main cause
175 of variation.

176 *Population Structure and Relative Kinship*

177 The evaluation of 795 genotypes against 3.3million SNPs by PCA and kinship matrix resulted in
178 a strong population structure. The PC1 showed the maximum variation which divided the whole
179 population into two clearly distinct groups. The same results were observed by phylogenetic tree and
180 kinship matrix (Fig. 2). The group-I was composed of 289 *japonica* accessions while the group-II framed
181 the 506 *indica* genotypes and named as *japonica* and *indica* subpopulations respectively (Supplementary
182 Table 1). The other structural features were consistent as reported before (Zhang, Zhang et al. 2009,
183 Zhang, Zhang et al. 2011).

184 *Association Mapping and QTL analysis*

185 Firstly, the SNPs association was estimated by GLM and MLM models for *japonica*, *indica*
186 subpopulations and whole population separately (Fig. 3). Then, a 1000-time permutation with randomized
187 phenotype was performed by MLM and the threshold –log10 (p) ≥ 4 was set for calling the significant

188 associations. It is suggested that more association signals in a common region are caused by linkage
189 disequilibrium (LD) decay while the lower LD decay rate can reduce the power of association analysis for
190 complex traits. The genome-wide r^2 reduction for *japonica* and *indica* subpopulations to 0.28 and 0.25
191 with LD decay rate 167kb and 123kb has been reported previously (Huang, Wei et al. 2010, Huang, Zhao
192 et al. 2012). Therefore, in this study, the genomic region containing minimum three significant SNPs
193 within 170kb range was defined as a QTL. In this way, the 37 QTLs were defined by 336 significant
194 SNPs (Supplementary Table 2 & 3). Among these QTLs, 13 and 9 QTLs were specifically identified in
195 *indica* and *japonica* subpopulations while 15 non-common QTLs were observed in whole population
196 (Fig. 4, Table 3).
197   Notably, *TAC1*, a previously identified gene was found as a candidate of *qTA9-6*. The *TAC1* was
198 previously mapped for tiller angle in rice by using F_2 segregating population originated from a cross
199 between *indica* genotype and *japonica* introgressed line (Yu, Lin et al. 2007). Its molecular study also has
200 revealed the functional single nucleotide polymorphism (Jiang, Tan et al. 2012). Here in our results, two
201 and three significant SNPs were found in promoter and in genomic region of *TAC1* respectively.

202 *Candidate Genes evaluation*

203   To evaluate the available candidate loci in identified QTLs, the SNPs in physical range of
204 annotated loci or their promoter were observed. The 97 and 43 significantly associated signals were
205 identified in promoter and coding sequence (CDS) regions of 93 annotated loci. Those may consider as
206 candidate loci for the respective 28 QTLs.
207   We also introduced and adopted the sequence pooling approach for screening the significant
208 genes. For this purpose, high phenotype pool and low phenotype pools were constructed by selecting the
209 20 genotypes from right and left tails of frequency distribution lines of *indica* and *japonica*
210 subpopulations separately (Supplementary Table 4). The major and minor allele frequencies of high and
211 low pools were compared and significance was estimated according to χ^2 (chi-square) test. Finally, we
212 sieved the 28 SNPs with significant difference in major and minor alleles to reveal the 22 candidate loci
213 for tiller angle (Supplementary Table 5). Among them, 13 and 7 loci were specific for *indica* and
214 *japonica* subpopulations while two loci commonly presented by both subpopulations.

215 *Haplotypes evaluation for significant loci*

216   We further evaluated the 22 candidate loci in 12 QTLs for functional haplotypes. The haplotypes
217 evaluation by using CDS and promoter residing SNPs was conducted and ten functional genes on the
218 bases of protein change and expression variation were observed for tiller angle (Fig.5, Supplementary Fig.
219 1). The *Os10g0412700* showed the maximum range of tiller angle (37.50-48.14) among haplotypes while
220 *Os01g0621600* have maximum haplotypes variation on the basis of three SNPs (Fig. 5).
221   For the known gene *TAC1*, three SNPs in CDS region were observed as the functional to develop
222 four and three haplotypes in *indica* and *japonica* sub-populations respectively (Fig. 6, Supplementary
223 Table 1). The *TAC1* has already known to cause the functional variation for tiller angle. The identification
224 of its superior haplotype would be beneficial for selection of compact plants and worth further studies.

225

226 **Discussion**

227   The plant morphological traits have important contribution to the total yield and resistance
228   against lodging. The elite architecture may include short stature, thick and strong culm, fewer
229   unproductive tillers, more number of panicles with more grains per panicle and erect type of leaves
230   (Khush 1995). Most of these traits including tiller angle in rice is proposed to control by quantitative trait
231   loci. Different tiller angle governing QTLs have been identified by studies based on different mapping
232   material. In this study, we performed the GWAS analysis using a huge natural population. A wide range
233   of tiller angle of cultivated species was observed in diverse population. Furthermore, the 13X deep
234   sequence data provided a high resolution for fine-scale mapping in GWAS. The deep sequence data
235   assembled with a sufficient number of land races also acquired the advantage of maximum genetic
236   diversity which was targeted to explore in GWAS. In this study, an effort was made to improve the
237   mapping resolution, imputation efficiency and identification of new alleles by addition of new SNPs in
238   the worldwide collection of accessions for genome wide association study (Huang, Wei et al. 2010).

239   No doubt GWAS is an effective tool to explore the complex genetic variation of rice genome but
240   it is still pretentious because of resulting in identification of false positives (Atwell, Huang et al. 2010).
241   The computation of structural associations and genetic control are two approaches to avoid the
242   identification of false positive signals in human and plants (Devlin and Roeder 1999, Marchini, Cardon et
243   al. 2004, Yu, Pressoir et al. 2006). In this study, the whole association panel was divided into two
244   subpopulations and separately re-evaluated as *indica* and *japonica* to control the population structure
245   based false positives. In addition, the population structure and genetic relatedness among individuals were
246   uncovered by PCA and kinship matrix in GAPIT program that may helpful to control the type I error
247   (false positives). Finally, we used the GLM method with Q-matrix (PCA) and compressed mix linear
248   model (cMLM) with Q and K (kinship) matrixes for three structural panels; whole population, *indica* and
249   *japonica* subpopulations, as it has been suggested to reduce the false positives (Yang, Yan et al. 2010,
250   Yang, Gao et al. 2011). Along with structural differentiation into three panels and adoption of two
251   different models to avoid false positives, 1000 permutation test was also performed with whole
252   population in cMLM and a significance threshold of $-\log 10$ (P) ≤ 4 was set associations (Fig. 3).

253   LD determination is very important to examine the resolution of association analysis (Flint-
254   Garcia, Thornsberry et al. 2003). Different studies have been performed to evaluate the LD decay in rice,
255   and 167 kb and 123 kb LD decay rate in *japonica* and *indica* subpopulations have already been reported
256   previously (Huang, Wei et al. 2010, Huang, Zhao et al. 2012). Therefore, to maintain the power of GWAS
257   resolution in present study, the criteria of minimum three SNPs in 170 kb LD range was strictly followed
258   for QTL declaration.

259   To date, some tiller angle related QTLs/genes have been reported by linkage mapping.
260   Contrastingly, association mapping not only provided a higher mapping resolution but also helped to
261   detect the candidate loci (Li, Qian et al. 2003, Yu, Lin et al. 2007, Jin, Huang et al. 2008). Therefore,
262   GWAS has widely been performed to disclose the genetic variation of morphological traits in rice
263   (Huang, Wei et al. 2010, Kumar, Singh et al. 2015). Similarly in our study, a total 336 significant SNPs
264   classified into 37 QTLs which according to Rice Genome Annotation Project database include 93
265   annotated loci were identified for tiller angle (Supplementary Table 2). Among these QTLs a known gene
266   *TAC1* was observed in *qTA9-6* which can validate the performance of GWAS. Other known tiller angle
267   related genes were not observed in association signals that may due to over missing or insufficient SNPs
268   availability in specific genomic region. Another possible reason was type II error (false negatives) due to
269   strict threshold.

270   We introduce a sequence pooling strategy to identify the significant loci. The major and minor
271 allele frequencies were compared between the genotypes in high and low phenotype pools. Twenty-eight
272 SNPs in 12 QTLs which included the 22 annotated loci were screened in this way. To identify the
273 functional variations in these loci, all the SNPs included in CDS and promoter were used and functional
274 haplotypes were evaluated. The single significant gene per QTL and its functional variation will be
275 validated by linkage population and genetic transformation in future studies.

276   As the significant difference in tiller angle between *japonica* and *indica* genotypes has been
277 observed and reported, this study demonstrated the feasibility to perform the genome wide association
278 analysis for a morphological trait which is vulnerable to be affected by population structure. Because of
279 substantial resolution power, it also revealed that the dissection of complex quantitative traits can be
280 initiated from QTL mapping that can further confirmed by linkage mapping and genetic transformation of
281 specific genomic region. The genome wide association study for tiller angle in rice populations provided
282 the significant loci. The manipulation of these loci can improve the plant architecture and can be used for
283 ideotype breeding. These results provided the striking information for development of further breeding
284 programs for lodging resistance and higher yield.

Conclusions

287   The improvements in rice plant architecture especially the tiller angle allows the more
288 efficient light capture by increasing the planting density. The deep insight into the genetic basis
289 of plant architecture will help to plan the future breeding programs. Current study revealed 37
290 significant QTLs with 93 annotated loci governing the tiller angle in rice. The accuracy of results
291 was proved by the identification of a known tiller angle controlling locus *TAC1*. The sequence
292 pooling technique was observed helpful to screen the 12 significant QTLs with 22 annotated loci.
293 The functional variations were estimated by haplotype analysis and the genotypes were screened
294 for significant haplotypes. These results deepened our understanding about genetic bases of tiller
295 angle in rice. These results can play a significant role in future breeding programs.

Acknowledgements

References

306 Atwell, S., Y. S. Huang, B. J. Vilhjalmsson, G. Willems, M. Horton, Y. Li, D. Meng, A. Platt, A. M.
307   Tarone, T. T. Hu, R. Jiang, N. W. Muliyati, X. Zhang, M. A. Amer, I. Baxter, B. Brachi, J.
308   Chory, C. Dean, M. Debieu, J. de Meaux, J. R. Ecker, N. Faure, J. M. Kniskern, J. D. Jones, T.
309   Michael, A. Nemri, F. Roux, D. E. Salt, C. Tang, M. Todesco, M. B. Traw, D. Weigel, P.

310   Marjoram, J. O. Borevitz, J. Bergelson and M. Nordborg (2010). "Genome-wide association
311   study of 107 phenotypes in Arabidopsis thaliana inbred lines." <u>Nature</u> **465**(7298): 627-631.
312 Chen, Y., X. Fan, W. Song, Y. Zhang and G. Xu (2012). "Over-expression of OsPIN2 leads to increased
313   tiller numbers, angle and shorter plant height through suppression of OsLAZY1." <u>Plant</u>
314   <u>Biotechnol J</u> **10**(2): 139-149.
315 Devlin, B. and K. Roeder (1999). "Genomic control for association studies." <u>Biometrics</u> **55**(4): 997-1004.
316 Dong, H., H. Zhao, W. Xie, Z. Han, G. Li, W. Yao, X. Bai, Y. Hu, Z. Guo and K. Lu (2016). "A novel
317   tiller angle gene, TAC3, together with TAC1 and D2 largely determine the natural variation of
318   tiller angle in rice cultivars." <u>PLoS genetics</u> **12**(11): e1006412.
319 Flint-Garcia, S. A., J. M. Thornsberry and B. IV (2003). "Structure of linkage disequilibrium in plants*."
320   <u>Annual review of plant biology</u> **54**(1): 357-374.
321 He, J., G. Shao, X. Wei, F. Huang, Z. Sheng, S. Tang and P. Hu (2017). "Fine mapping and candidate
322   gene analysis of qTAC8, a major quantitative trait locus controlling tiller angle in rice (Oryza
323   sativa L.)." <u>PLoS One</u> **12**(5): e0178177.
324 Hu, Y., S. Li, X. Fan, S. Song, X. Zhou, X. Weng, J. Xiao, X. Li, L. Xiong and A. You (2020). "OsHOX1
325   and OsHOX28 redundantly shape rice tiller angle by reducing HSFA2D expression and auxin
326   content." <u>Plant physiology</u> **184**(3): 1424-1437.
327 Huang, L., W. Wang, N. Zhang, Y. Cai, Y. Liang, X. Meng, Y. Yuan, J. Li, D. Wu and Y. Wang (2021).
328   "LAZY2 controls rice tiller angle through regulating starch biosynthesis in gravity-sensing cells."
329   <u>New Phytologist</u>.
330 Huang, X., X. Wei, T. Sang, Q. Zhao, Q. Feng, Y. Zhao, C. Li, C. Zhu, T. Lu, Z. Zhang, M. Li, D. Fan,
331   Y. Guo, A. Wang, L. Wang, L. Deng, W. Li, Y. Lu, Q. Weng, K. Liu, T. Huang, T. Zhou, Y.
332   Jing, W. Li, Z. Lin, E. S. Buckler, Q. Qian, Q. F. Zhang, J. Li and B. Han (2010). "Genome-wide
333   association studies of 14 agronomic traits in rice landraces." <u>Nat Genet</u> **42**(11): 961-967.
334 Huang, X., Y. Zhao, X. Wei, C. Li, A. Wang, Q. Zhao, W. Li, Y. Guo, L. Deng, C. Zhu, D. Fan, Y. Lu,
335   Q. Weng, K. Liu, T. Zhou, Y. Jing, L. Si, G. Dong, T. Huang, T. Lu, Q. Feng, Q. Qian, J. Li and
336   B. Han (2012). "Genome-wide association study of flowering time and grain yield traits in a
337   worldwide collection of rice germplasm." <u>Nat Genet</u> **44**(1): 32-39.
338 Jiang, J., L. Tan, Z. Zhu, Y. Fu, F. Liu, H. Cai and C. Sun (2012). "Molecular evolution of the TAC1 gene
339   from rice (Oryza sativa L.)." <u>J Genet Genomics</u> **39**(10): 551-560.
340 Jin, J., W. Huang, J. P. Gao, J. Yang, M. Shi, M. Z. Zhu, D. Luo and H. X. Lin (2008). "Genetic control
341   of rice plant architecture under domestication." <u>Nat Genet</u> **40**(11): 1365-1369.
342 Khush, G. S. (1995). "Breaking the yield frontier of rice." <u>GeoJournal</u> **35**(3): 329-332.
343 Kinoshita T, Takahashi M and M. K. (1974). "Character expression and inheritance node of three kinds of
344   dwarf rice." <u>Res Bull Farm Hokkaido Univ</u> **19**: 64-75.
345 Kumar, V., A. Singh, S. V. Mithra, S. L. Krishnamurthy, S. K. Parida, S. Jain, K. K. Tiwari, P. Kumar, A.
346   R. Rao, S. K. Sharma, J. P. Khurana, N. K. Singh and T. Mohapatra (2015). "Genome-wide
347   association mapping of salinity tolerance in rice (Oryza sativa)." <u>DNA Res</u> **22**(2): 133-145.
348 Li, H., H. Sun, J. Jiang, X. Sun, L. Tan and C. Sun (2021). "TAC4 controls tiller angle by regulating the
349   endogenous auxin content and distribution in rice." <u>Plant biotechnology journal</u> **19**(1): 64-73.
350 Li, J.-Y., J. Wang and R. S. Zeigler (2014). "The 3,000 rice genomes project: new opportunities and
351   challenges for future rice research." <u>J Gigascience</u> **3**(1): 2047-2217X-2043-2048.
352 Li, P., Y. Wang, Q. Qian, Z. Fu, M. Wang, D. Zeng, B. Li, X. Wang and J. Li (2007). "LAZY1 controls
353   rice shoot gravitropism through regulating polar auxin transport." <u>Cell Res</u> **17**(5): 402-410.

354 Li, X., Q. Qian, Z. Fu, Y. Wang, G. Xiong, D. Zeng, X. Wang, X. Liu, S. Teng and F. Hiroshi (2003).
355   "Control of tillering in rice." Nature **422**(6932): 618-621.
356 Li, Y., J. Li, Z. Chen, Y. Wei, Y. Qi and C. Wu (2020). "OsmiR167a-targeted auxin response factors
357   modulate tiller angle via fine-tuning auxin distribution in rice." Plant biotechnology journal
358   **18**(10): 2015-2026.
359 Li, Z., B.-Y. Fu, Y.-M. Gao, W.-S. Wang, J.-L. Xu, F. Zhang, X.-Q. Zhao, T.-Q. Zheng, Y.-L. Zhou, G.
360   Zhang, S. Tai, J. Xu, W. Hu, M. Yang, Y. Niu, M. Wang, Y. Li, L. Bian, X. Han, J. Li, X. Liu, B.
361   Wang, K. L. McNally, M. E. B. Naredo, S. M. Q. Mercado, M. C. Rellosa, R. A. Reaño, G. L. S.
362   Capilit, F. C. d. Guzman, J. Ali, N. R. S. Hamilton, R. P. Mauleon, N. N. Alexandrov and H.
363   Leung (2014). "The 3,000 rice genomes project." Gigascience **3**: 7.
364 Li. Zhikang, A. H, Paterson, R.M. Shannon Pinson and J. W. Stansel (1999). "RFLP facilitated analysis
365   of tiller and leaf angles in rice (Oryza sativa L.)." Euphytica **109**: 79-84.
366 Lomax, T. L. (1997). "Molecular genetic analysis of plant gravitropism." Gravit Space Biol Bull **10**(2):
367   75-82.
368 Maiyata M, Komori T, Yamamoto T, Ueda T, Yano M and N. N. (2005). "Fine scale and physical
369   mapping of Spk(t) controlling spreading stub in rice." Breeding Sci, **55**: 237-239.
370 Marchini, J., L. R. Cardon, M. S. Phillips and P. Donnelly (2004). "The effects of human population
371   structure on large genetic association studies." Nat Genet **36**(5): 512-517.
372 Peijin, L., Z. Dali, L. Xinfang, X. Dan, G. Dai, L. Jiayang and Q. Qian (2003). "Mapping and
373   characterization of a tiller-spreading mutant lazy-2 in rice " Chinese Science Bulletin **48**(24):
374   2715-2717.
375 Qian, Q., P. He, S. Teng, D. L. Zeng and L. H. Zhu (2001). "[QTLs analysis of tiller angle in rice (Oryza
376   sativa L.)]." Yi Chuan Xue Bao **28**(1): 29-32.
377 Qu, M., Z. Zhang, T. Liang, P. Niu, M. Wu, W. Chi, Z.-Q. Chen, Z.-J. Chen, S. Zhang and S. Chen
378   (2021). "Overexpression of a methyl-CpG-binding protein gene OsMBD707 leads to larger tiller
379   angles and reduced photoperiod sensitivity in rice." BMC plant biology **21**(1): 1-14.
380 Reinhardt, D. and C. Kuhlemeier (2002). "Plant architecture." EMBO Rep **3**(9): 846-851.
381 Sang, D., D. Chen, G. Liu, Y. Liang, L. Huang, X. Meng, J. Chu, X. Sun, G. Dong, Y. Yuan, Q. Qian, J.
382   Li and Y. Wang (2014). "Strigolactones regulate rice tiller angle by attenuating shoot
383   gravitropism through inhibiting auxin biosynthesis." Proc Natl Acad Sci U S A **111**(30): 11199-
384   11204.
385 Shen S Q, Zhuang J Y, Bao J S, Zheng K L, Xia Y W and S. Q. Y. (2005). "Analysis of QTLs with
386   additive, epistasis and G×E interaction effects of the tillering angle trait in rice." J Agric Biotech
387   **13**(1): 16–20 (in Chinese with English abstract).
388 Singh, K., D. S. Multani and G. S. Khush (1996). "Secondary trisomics and telotrisomics of rice: origin,
389   characterization, and use in determining the orientation of chromosome map." Genetics **143**(1):
390   517-529.
391 Sukumaran, S., S. Dreisigacker, M. Lopes, P. Chavez and M. P. Reynolds (2015). "Genome-wide
392   association study for grain yield and related traits in an elite spring wheat population grown in
393   temperate irrigated environments." Theor Appl Genet **128**(2): 353-363.
394 Takahashi M, Kinoshita T and T. K. (1968). "Character expression and causal genes of some mutants in
395   rice." Fac Agric Hokkaido Univ **54**: 496-512.
396 Wang, Y. and J. Li (2005). "The plant architecture of rice (Oryza sativa)." Plant Mol Biol **59**(1): 75-84.
397 Wang, Y. and J. Li (2006). "Genes controlling plant architecture." Curr Opin Biotechnol **17**(2): 123-129.

398 Wang, Y. and J. Li (2008). "Rice, rising." Nat Genet **40**(11): 1273-1275.

399 Xie, C., G. Zhang, L. An, X. Chen and R. J. P. Fang (2019). "Phytochrome-interacting factor-like protein
400   OsPIL15 integrates light and gravitropism to regulate tiller angle in rice." Planta **250**(1): 105-114.

401 Yang, X., S. Gao, S. Xu, Z. Zhang, B. M. Prasanna, L. Li, J. Li and J. Yan (2011). "Characterization of a
402   global germplasm collection and its potential utilization for analysis of complex quantitative traits
403   in maize." Molecular Breeding **28**(4): 511-526.

404 Yang, X., J. Yan, T. Shah, M. L. Warburton, Q. Li, L. Li, Y. Gao, Y. Chai, Z. Fu, Y. Zhou, S. Xu, G. Bai,
405   Y. Meng, Y. Zheng and J. Li (2010). "Genetic analysis and characterization of a new maize
406   association mapping panel for quantitative trait loci dissection." Theor Appl Genet **121**(3): 417-
407   431.

408 Yoshihara, T., E. P. Spalding and M. Iino (2013). "AtLAZY1 is a signaling component required for
409   gravitropism of the Arabidopsis thaliana inflorescence." Plant J **74**(2): 267-279.

410 Yu, B., Z. Lin, H. Li, X. Li, J. Li, Y. Wang, X. Zhang, Z. Zhu, W. Zhai, X. Wang, D. Xie and C. Sun
411   (2007). "TAC1, a major quantitative trait locus controlling tiller angle in rice." Plant J **52**(5): 891-
412   898.

413 Yu, C. Y., Y. Q. Liu, L. Jiang, C. M. Wang, H. Q. Zhai and J. M. Wan (2005). "[QTLs mapping and
414   genetic analysis of tiller angle in rice (Oryza sativa L.)]." Yi Chuan Xue Bao **32**(9): 948-954.

415 Yu, J., G. Pressoir, W. H. Briggs, I. Vroh Bi, M. Yamasaki, J. F. Doebley, M. D. McMullen, B. S. Gaut,
416   D. M. Nielsen, J. B. Holland, S. Kresovich and E. S. Buckler (2006). "A unified mixed-model
417   method for association mapping that accounts for multiple levels of relatedness." Nat Genet
418   **38**(2): 203-208.

419 Zaniab Al-Shugeairy, Adam H. Price and D. Robinson (2015). "Genome Wide Association Mapping for
420   Drought Recovery Trait in Rice (Oryza Sativa L.)." International Journal of Applied Agricultural
421   Sciences **1**(1): 11-18.

422 Zeng, Y., Y. Chen, Z. Ji, Y. Liang and C. Yang (2019). "Twenty-four alleles at twelve quantitative trait
423   loci act additively to control tiller angle in cultivated rice." Plant Growth Regulation **88**(2): 195-
424   203.

425 Zhang, D., H. Zhang, M. Wang, J. Sun, Y. Qi, F. Wang, X. Wei, L. Han, X. Wang and Z. Li (2009).
426   "Genetic structure and differentiation of Oryza sativa L. in China revealed by microsatellites."
427   Theor Appl Genet **119**(6): 1105-1117.

428 Zhang, H., D. Zhang, M. Wang, J. Sun, Y. Qi, J. Li, X. Wei, L. Han, Z. Qiu, S. Tang and Z. Li (2011). "A
429   core collection and mini core collection of Oryza sativa L. in China." Theor Appl Genet **122**(1):
430   49-61.

431 Zhang, N., H. Yu, H. Yu, Y. Cai, L. Huang, C. Xu, G. Xiong, X. Meng, J. Wang and H. Chen (2018). "A
432   core regulatory pathway controlling rice tiller angle mediated by the LAZY1-dependent
433   asymmetric distribution of auxin." The Plant Cell **30**(7): 1461-1475.

434 Zhang, W., L. Tan, H. Sun, X. Zhao, F. Liu, H. Cai, Y. Fu, X. Sun, P. Gu and Z. Zhu (2019). "Natural
435   variations at TIG1 encoding a TCP transcription factor contribute to plant architecture
436   domestication in rice." Molecular plant **12**(8): 1075-1089.

437 Zhu, M., Y. Hu, A. Tong, B. Yan, Y. Lv, S. Wang, W. Ma, Z. Cui and X. Wang (2020). "LAZY1 controls
438   tiller angle and shoot gravitropism by regulating the expression of auxin transporters and
439   signaling factors in rice." Plant Cell Physiology **61**(12): 2111-2125.

440

Table 1(on next page)

Descriptive statistics of tiller angle in different populations.

* r^2 = repeatability ** Two-tail T-test significance at $P \geq 0.01$.

Table 1: Descriptive statistics of tiller angle in different populations.

Populations	N	Range	Mean ± SD	CV%	h²(%)	Skw	Kur	r²*
Whole population	795	20.10-82.70	40.55 ± 9.33	23.01	73	0.92	1.28	0.91
Indica	514	21.90-82.70	41.84 ± 9.80	23.42	74	0.85	1.01	0.91
Japonica	281	20.10-69.50	38.05 ± 7.75**	20.37	45	0.87	1.34	0.92

Skw: Skewness
Kur: Kurtosis
* r² = repeatability
 ** Two-tail T-test significance at P ≥ 0.01.

1

Table 2(on next page)

Analysis of variance in whole population for tiller angle.

**: Significance at α= 0.01, ns: non-significant, b: blocks, g: genotypes

1   **Table 2**: **Analysis of variance in whole population for tiller angle.**

Sources of variation	Degree of freedom	Sum Squares	Mean Squares	F-value
Replications	4	1746.26	436.57	10.56**
Treatment	1589	816231.99	513.68	12.43**
Genotypes	794	693201.20	873.05	21.12**
Block	1	43.19	43.19	1.05^{ns}
B × G	794	122987.61	154.90	3.75**
Error	6360	262935.34	41.34	
Total	7949	1080913.60		

2   **: Significance at $\alpha = 0.01$, ns: non-significant, b: blocks, g: genotypes

Table 3(on next page)

The list of QTLs identified in different models of association mapping for tiller angle

* These QTLs were also identified and confirmed by Non-Syn GWAS.

1 **Table 3: The list of QTLs identified in different models of association mapping for tiller**
2 **angle.**

GWAS-QTL	Chromosome	Model-population	Peak SNP	Flanking SNPs	
				Right	Left
qTA1-1	1	MLM/GLM-whole	22379048	21952887	22436318
qTA1-2	1	MLM/GLM-whole	24887286	24781381	25029148
qTA2-1	2	MLM/GLM-whole	829685	829685	870392
qTA2-2	2	MLM/GLM-Jap	8709646	8686587	8709646
qTA2-3	2	GLM-ind	16631825	16631825	16766562
qTA2-4	2	MLM-whole	18056292	17094194	18789028
qTA2-5	2	GLM-ind	18789028	18702054	18789028
qTA2-6	2	MLM-ind	29311370	29221199	29407400
qTA3-1	3	MLM-whole	25422986	25263068	25423816
qTA3-2	3	MLM-whole	26277666	26263239	26313715
qTA3-3	3	GLM-whole	33199574	33198645	33199696
qTA3-4	3	GLM-jap	35863137	35856297	35918334
qTA4-1	4	GLM-whole	723871	654721	724006
qTA4-2	4	MLM/GLM-whole	972323	921112	972402
qTA4-3	4	GLM-whole	1902732	1886204	1979550
qTA5-1	5	MLM-ind	17795916	17795916	18061792
qTA5-2	5	MLM-ind	18668045	18485378	18709357
qTA5-3	5	MLM-ind	19108377	19108377	19210385
qTA6	6	MLM-ind	26092692	26086626	26127844
qTA8-1	8	MLM-ind	20690040	20689044	20696741
qTA8-2	8	GLM-whole	20802316	20802316	20995468
qTA8-3	8	MLM-ind	28327637	28326448	28438469
qTA9-1	9	GLM-jap	1193941	1070773	1193941
qTA9-2	9	MLM/GLM-Jap	2070634	1313684	2207902
qTA9-3	9	MLM/GLM-Jap	2516331	2407587	2621693
qTA9-4	9	MLM/GLM-Jap	2980680	2936852	3180164
qTA9-5	9	GLM-jap	3383978	3383859	3486825
qTA9-6	9	*MLM-whole	20739050	20721611	20749797
qTA10-1	10	*MLM/GLM-whole	14414010	14191834	14733631
qTA10-2	10	GLM-jap	20273073	20268702	20295300
qTA10-3	10	MLM/GLM-Jap	22799648	22774612	22836250
qTA11-1	11	GLM-whole	4826905	4826905	4829616
qTA11-2	11	GLM-ind	13090765	13090765	13171938
qTA12-1	12	MLM-ind	3329842	3258584	3539326
qTA12-2	12	MLM-ind	9262194	9262194	9337250
qTA12-3	12	MLM-ind	9606800	9606800	9755631
qTA12-4	12	MLM/GLM-whole	21905686	21901194	22047563

3 * These QTLs were also identified and confirmed by Non-Syn GWAS.

Figure 1

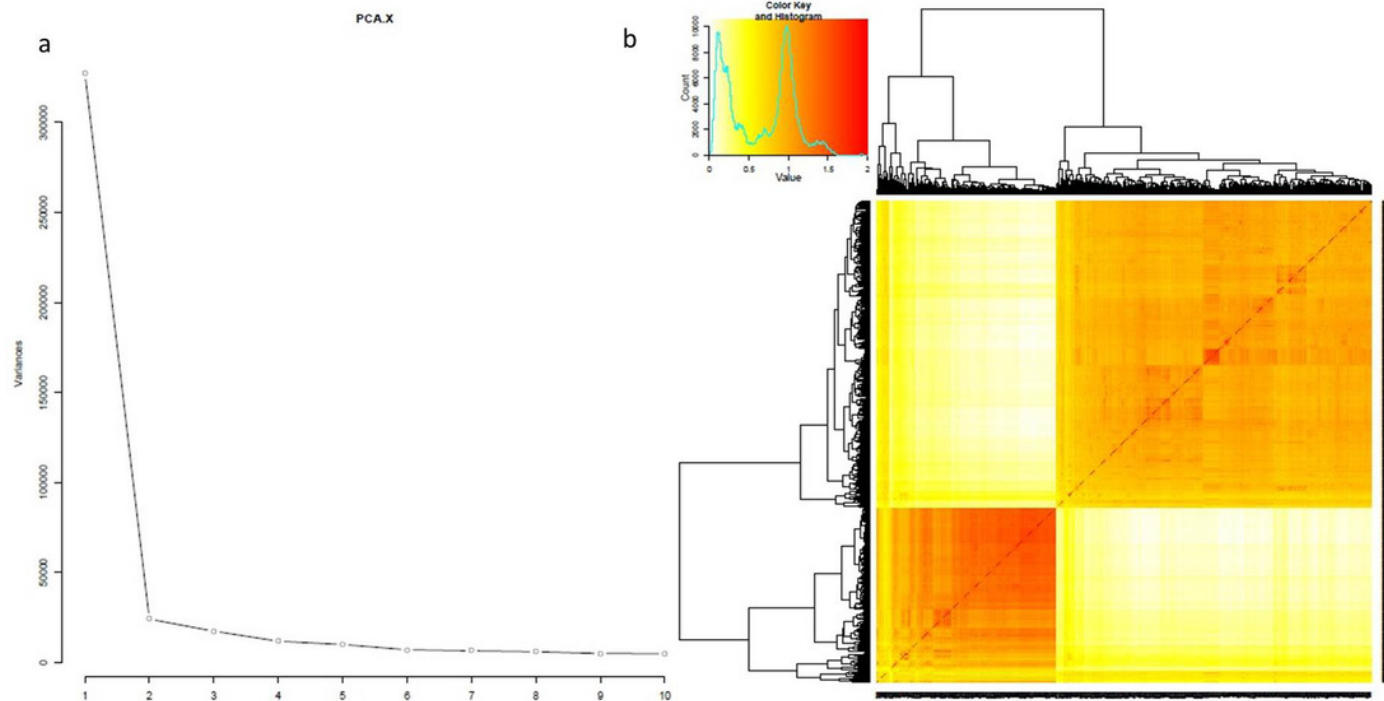Frequency distribution and Q-Q plots.

(a, b) whole population (c, d) for *indica* sub-population and (e, f) *japonica* sub-population.
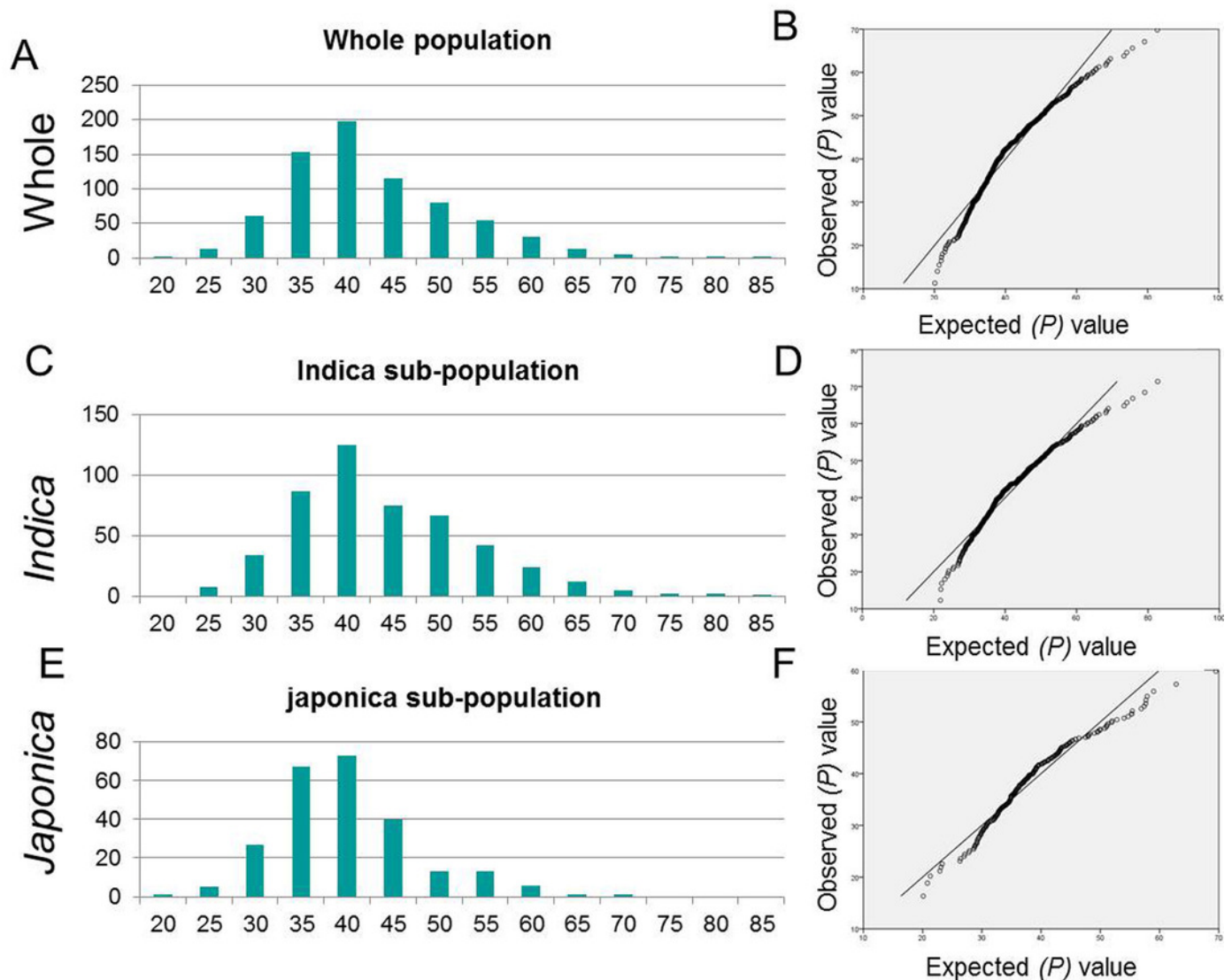
Figure 2

Population structure analysis for 795 genotypes
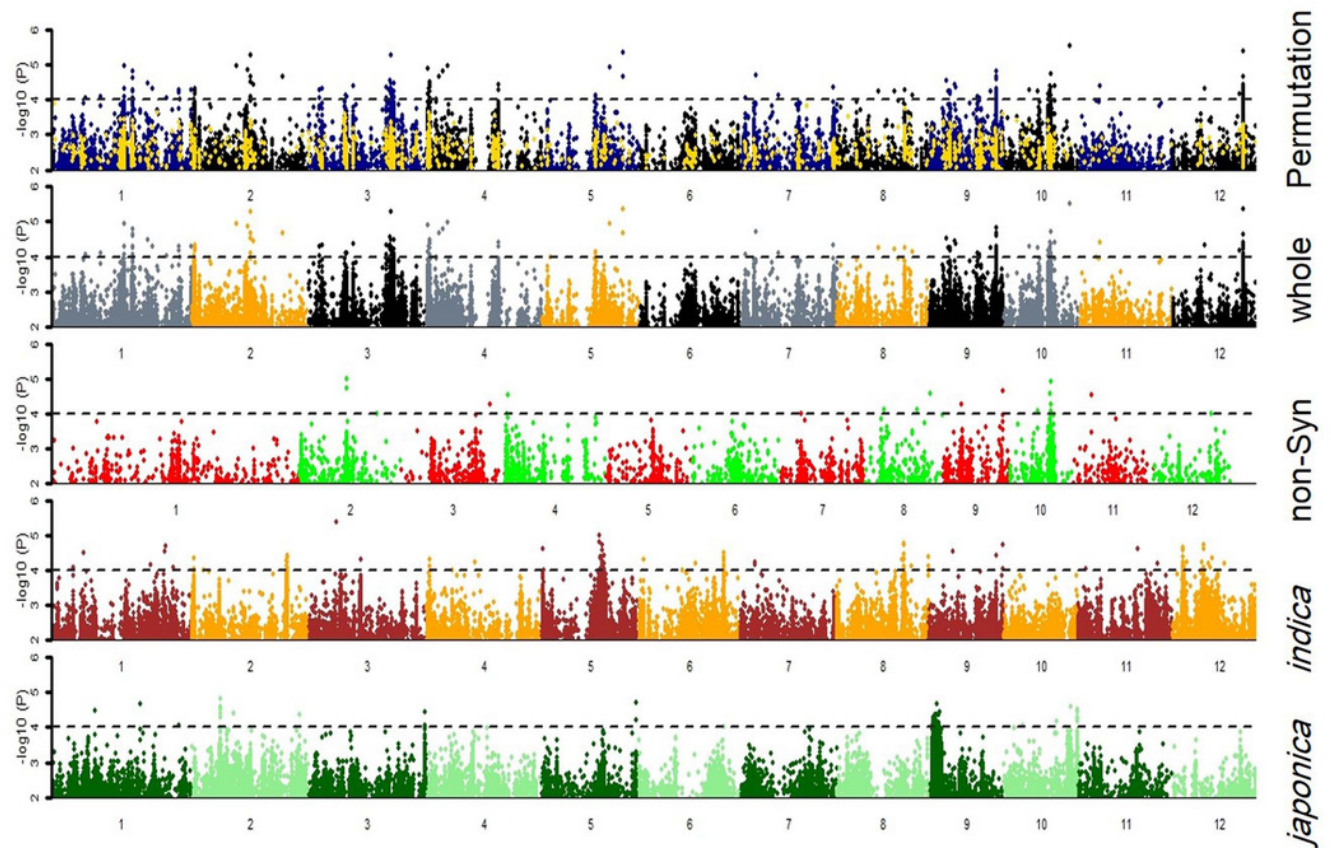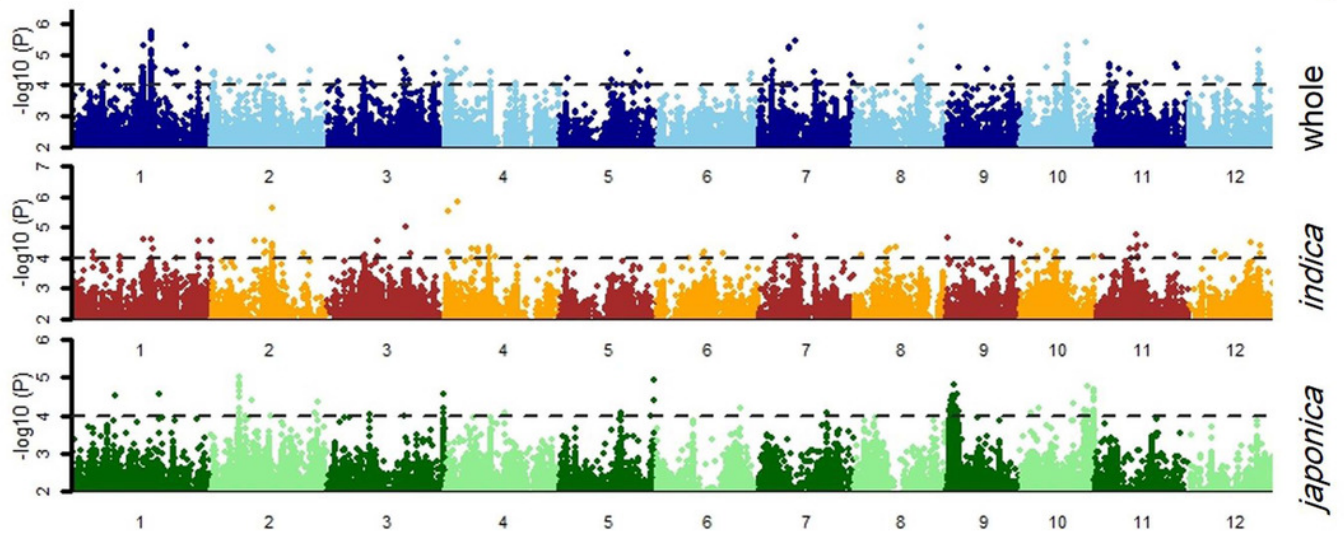
(a) PCAs, and (b) kinship

Figure 3

Manhattan plots for Tiller angle for SNPs in the whole population, and *indica* and *japonica* subpopulations SNPs obtained by (A) cMLM and (B) GLM, and Non-Synonymous SNPs by (A) cMLM.

The blue and black dots in the permutation plot indicate the original P-Value of SNPs while Golden dots indicate the 1000-permutation P-value.
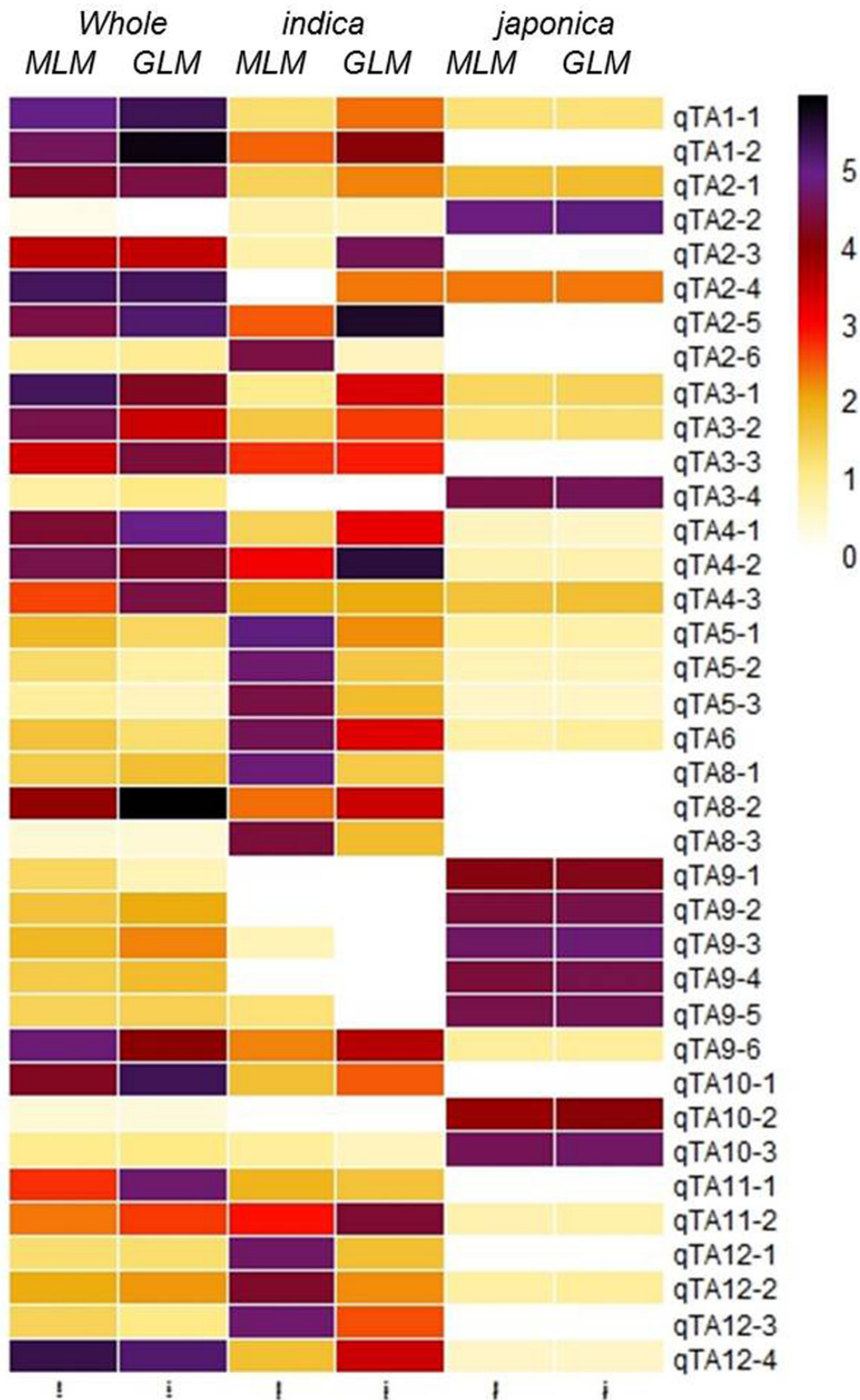
Figure 4

Heatmap of 37 GWA-QTLs for –log10(P) of peak SNPs in different association models

Figure 5

Haplotypes of two significant loci for tiller angle in *indica* and *japonica* subpopulations.

Sub population	haplotype	Os01g0621600 CDS 24786734	Os01g0621600 CDS 24786776	Os01g0621600 Promoter 24789507	N	TA	Sub population	haplotype	Os10g0412700 CDS 14399366	Os10g0412700 promoter 14402688	N	TA
Indica	hap1	A	C	A	175	44.08	Indica	hap1	C	A	39	48.14
	hap2	T	C	A	14	38.93		hap2	C	G	320	42.13
	hap3	T	T	A	132	39.42		hap3	T	G	58	38.01
	hap4	T	T	G	34	38.89						
Japonica	hap1	A	C	A	24	39.82						
	hap2	T	C	A	10	38.23	Japonica	hap2	C	G	64	39.87
	hap3	T	T	A	25	37.59		hap3	T	G	156	37.50
	hap4	T	T	G	148	37.21						

Figure 6

Haplotypes of known gene *TAC1* significant loci for tiller angle in *indica* and *japonica* subpopulations

sub-population	Haplotype	20733643	20733730	20734345	No. of cultivars	Tiller Angle(°)
Indica	Hap-1	A	T	A	133	38.94[a]
	Hap-2	A	C	A	22	39.60[b]
	Hap-3	A	C	G	5	40.44[b]
	Hap-4	T	C	G	344	43.04[b]
Japonica	Hap-1	A	T	A	208	37.82[a]
	Hap-2	A	C	A	13	37.91[a]
	Hap-4	T	C	G	52	39.05[a]