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

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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.

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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 was
32 observed. Genome-wide association analysis was performed by the general linear model (GLM),
33 and compressed mix linear model (cMLM) for three populations to disclose the genomic
34 associations. The population principal components (PCs) 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 loci,
39 a known tiller angle controlling locus *TAC1* was also identified. The introduction of the sequence
40 pooling technique was observed fruitful to screen the 12 significant QTLs with 22 annotated loci.

41 For ten of these loci, the functional variations were identified by haplotype analysis. These results
42 were not only providing a better understanding of the genetic basis of rice plant architecture but
43 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. Its role as
50 a model plant to study the monocots is due to its comparatively collinear and smaller genome (Peijin, Dali
51 et al. 2003). To feed the continuously growing human population, the increasing crop yield is always an
52 objective of any research activity. The main culm and tillers play a seminal role to determine the rice plant
53 architecture and finally the yield (Wang and Li 2005). Plant architecture referred to the three-dimensional
54 arrangement of arial parts of plants, is a major factor contributing to the total plant yield (Reinhardt and
55 Kuhlemeier 2002, Wang and Li 2006). The plant architecture can be determined by the variation in tillering,
56 plant height, leaf shape and arrangement and panicle morphology (Wang and Li 2005). Tiller angle affects
57 the ability of survival and to trap maximum light for photosynthesis. The erect to optimum spreading tillers
58 of plants have been evolved during rice domestication from wild species to cultivated varieties while a
59 relatively wider tiller angle in *indica* sub-species has been observed than in *japonica*. In dense planting, a
60 wider tiller angle may enable the plant to escape some diseases by reducing the humidity. Contrastingly, it
61 will cover more space and increase the leaf shading and can cause reduction in photosynthesis. The dense
62 and upright leaves have also been reported to improve the leaf nitrogen accumulation for grain filling (Wang
63 and Li 2008).

64

65   As with other yield related traits, tiller angle also showed a complex genetic behavior. In previous
66 studies different quantitative trait loci (QTLs) and genes have been identified for tiller angle as *Ta* (Li.
67 Zhikang, H et al. 1999), *OsHOX1*, *OsHOX28* (Hu, Li et al. 2020), *qTA-9a* (Qian, He et al. 2001), *qTA-9b*
68 (Qian, He et al. 2001), *qTA9-2* (Shen S Q, Zhuang J Y et al. 2005) and *qTA-9* (Yu, Liu et al. 2005), and
69 *qTAC-8* (He, Shao et al. 2017)have been mapped. The *TAC1* (Yu, Lin et al. 2007) have been mapped and
70 cloned as well, while the *TAC3* together with *TAC1* and *D2* have been known for significant variation in
71 tiller angle (Dong, Zhao et al. 2016, Li, Sun et al. 2021). A study revealed the effect of overexpression of
72 CpG-binding protein gene *OsMBD707* causing a larger tiller angle (Qu, Zhang et al. 2021). Several mutants
73 such as *La* (Singh, Multani et al. 1996), *La2* (Peijin, Dali et al. 2003), *er* (Takahashi M, Kinoshita T et al.
74 1968), *D20* (Kinoshita T, Takahashi M et al. 1974) and *Spk(t)* (Maiyata M, Komori T et al. 2005) were also
75 observed to affect the tiller angle. The TCP transcriptional factor controlled by *TIG1* was found to affect
76 the tiller angle in rice (Zhang, Tan et al. 2019). Another QTL mapping study for tiller angle performed by
77 RIL population identified the 24 alleles on 12 QTLs (Zeng, Chen et al. 2019).
78   Various genetic studies for plant growth regulators including the *TAC4* (Li, Sun et al. 2021) and
79 *OsmiR167a* gene (Li, Li et al. 2020) have also been reported to control tiller angle by regulation and
80 endogenous distribution of auxin contents. Furthermore, *LAZY1* is known to control the tiller angle by
81 gravitropism (Li, Wang et al. 2007, Yoshihara, Spalding et al. 2013, Zhu, Hu et al. 2020) and asymmetric
82 distribution of Auxin (Zhang, Yu et al. 2018), and *LAZY2* by regulation of starch biosynthesis in gravity-
83 sensing cells (Huang, Wang et al. 2021). The phytochrome-interacting factor- like protein *OsPIL15* has

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

104 **Materials & Methods**

105 *Plant and field material*

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

112 *Phenotyping*

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

116 *Genotyping and population Structure*

117 The second generation of whole-genome high-throughput sequence with ~13fold coverage was
118 obtained by Illumina sequencing and the sequence reads were aligned against rice reference genome. The
119 direct comparison was performed with corresponding regions of *japonica* cv. Nipponbare reference

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

129   *Statistical analysis for phenotypes*

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

136
$$R^2 = \frac{\sigma^2G}{\sigma^2G + \dfrac{\sigma^2(G \times E)}{b} + \dfrac{\sigma^2E}{br}}$$

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

142   *Genome wide Association analysis*

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

150   *Sequence pooling, Candidate Genes identification and Haplotyping*

151   The QTLs regions were defined by SNP significance at threshold of –log10 (P) <4 which were
152   further screened by deletion of SNPs not reside in CDS or promoter regions of annotated loci. For further

153 identification of significant loci, a new sequence based pooling strategy was applied. The 20 genotypes
154 with highest phenotypic value and 20 genotypes with lowest phenotypic value were selected separately
155 from two subpopulations. The difference of major and minor allele frequencies in four pools were compared
156 by χ^2-test at $\alpha = 0.05$. The SNPs with significantly different major and minor allele frequency were selected
157 to define significantly associated loci. Then all the significant and non-significant SNPs in promoter and
158 CDS region of selected loci were used to construct a map and to evaluate their functional haplotypes.
159

Results

161 *Phenotypic Variation of tiller Angle*

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

171 *Population Structure and Relative Kinship*

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

179 *Association Mapping and QTL analysis*

180   Firstly, the SNPs association was estimated by GLM and MLM models for *japonica*, *indica*
181 subpopulations and whole population separately (Fig. 3). Then, a 1000-time permutation with randomized
182 phenotype was performed by MLM and the threshold $-\log10$ (p) ≥ 4 was set for calling the significant
183 associations. It is suggested that more association signals in a common region are caused by linkage
184 disequilibrium (LD) decay while the lower LD decay rate can reduce the power of association analysis for
185 complex traits. The genome-wide r^2 reduction for *japonica* and *indica* subpopulations to 0.28 and 0.25 with
186 LD decay rate 167kb and 123kb has been reported previously (Huang, Wei et al. 2010, Huang, Zhao et al.
187 2012). Therefore, in this study, the genomic region containing minimum three significant SNPs within
188 170kb range was defined as a QTL. In this way, the 37 QTLs were defined by 336 significant SNPs

189 (Supplementary Table 2 & 3). Among these QTLs, 13 and 9 QTLs were specifically identified in *indica*
190 and *japonica* subpopulations while 15 non-common QTLs were observed in whole population (Fig. 4, Table
191 3).
192   Notably, *TAC1*, a previously identified gene was found as a candidate of *qTA9-6*. The *TAC1* was
193 previously mapped for tiller angle in rice by using F_2 segregating population originated from a cross
194 between *indica* genotype and *japonica* introgressed line (Yu, Lin et al. 2007). Its molecular study also has
195 revealed the functional single nucleotide polymorphism (Jiang, Tan et al. 2012). Here in our results, two
196 and three significant SNPs were found in promoter and in genomic region of *TAC1* respectively.

197 *Candidate Genes evaluation*

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

210 *Haplotypes evaluation for significant loci*

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

220

Discussion

222   The plant morphological traits have important contribution to the total yield and resistance against
223 lodging. The elite architecture may include short stature, thick and strong culm, fewer unproductive tillers,
224 more number of panicles with more grains per panicle and erect type of leaves (Khush 1995). Most of these
225 traits including tiller angle in rice are proposed to govern by quantitative trait loci. Different tiller angle
226 governing QTLs have been identified by studies based on different mapping material. In this study, we
227 performed the GWAS analysis using a huge natural population. A wide range of tiller angle of cultivated

228 species was observed in diverse population. Furthermore, the 13X deep sequence data provided a high
229 resolution for fine-scale mapping in GWAS. The deep sequence data assembled with a sufficient number
230 of land races also acquired the advantage of maximum genetic diversity which was targeted to explore in
231 GWAS. In this study, an effort was made to improve the mapping resolution, imputation efficiency and
232 identification of new alleles by addition of new SNPs in the worldwide collection of accessions for genome
233 wide association study (Huang, Wei et al. 2010).
234   No doubt GWAS is an effective tool to explore the complex genetic variation of rice genome but
235 it is still pretentious because of resulting in identification of false positives (Atwell, Huang et al. 2010). The
236 computation of structural associations and genetic control are two approaches to avoid the identification of
237 false positive signals in human and plants (Devlin and Roeder 1999, Marchini, Cardon et al. 2004, Yu,
238 Pressoir et al. 2006). In this study, the whole association panel was divided into two subpopulations and re-
239 evaluated separately as *indica* and *japonica* to control the population structure based false positives. In
240 addition, the population structure and genetic relatedness among individuals were uncovered by PCA and
241 kinship matrix in GAPIT program that may helpful to control the type I error (false positives). Finally, we
242 used the GLM method with Q-matrix (PCA) and compressed mix linear model (cMLM) with Q and K
243 (kinship) matrixes for three structural panels; whole population, *indica* and *japonica* subpopulations, as it
244 has been suggested to reduce the false positives (Yang, Yan et al. 2010, Yang, Gao et al. 2011). Along with
245 structural differentiation into three panels and adoption of two different models to avoid false positives,
246 1000 permutation test was also performed with whole population in cMLM and a significance threshold of
247 $-\log10 (P) \leq 4$ was set to reveal the true associations (Fig. 3).
248   LD determination is very important to examine the resolution of association analysis (Flint-Garcia,
249 Thornsberry et al. 2003). Different studies have been performed to evaluate the LD decay in rice, and 167
250 kb and 123 kb LD decay rate in *japonica* and *indica* subpopulations have already been reported previously
251 (Huang, Wei et al. 2010, Huang, Zhao et al. 2012). Therefore, to maintain the power of GWAS resolution
252 in present study, the criteria of minimum three SNPs in 170 kb LD range was strictly followed for QTL
253 declaration.
254   To date, some tiller angle related QTLs/genes have been reported by linkage mapping.
255 Contrastingly, association mapping not only provided a higher mapping resolution but also helped to detect
256 the candidate loci (Li, Qian et al. 2003, Yu, Lin et al. 2007, Jin, Huang et al. 2008). Therefore, GWAS has
257 widely been performed to disclose the genetic variation of morphological traits in rice (Huang, Wei et al.
258 2010, Kumar, Singh et al. 2015). Similarly in our study, a total 336 significant SNPs classified into 37
259 QTLs which according to Rice Genome Annotation Project database include 93 annotated loci were
260 identified for tiller angle (Supplementary Table 2). Among these QTLs a known gene *TAC1* was observed
261 in *qTA9-6* which can validate the performance of GWAS. Other known tiller angle related genes were not
262 observed in association signals that may due to over missing or insufficient SNPs availability in specific
263 genomic region. Another possible reason was type II error (false negatives) due to strict threshold.
264   We introduce a sequence pooling strategy to identify the significant loci. The major and minor
265 allele frequencies were compared between the genotypes in high and low phenotype pools. Twenty-eight
266 SNPs in 12 QTLs which included the 22 annotated loci were screened in this way. To identify the functional
267 variations in these loci, all the SNPs included in CDS and promoter were used and functional haplotypes
268 were evaluated. The single significant gene per QTL and its functional variation will be validated by linkage
269 population and genetic transformation in future studies.
270   As the significant difference in tiller angle between *japonica* and *indica* genotypes has been
271 observed and reported, this study demonstrated the feasibility to perform the genome wide association

272 analysis for a morphological trait which is vulnerable to population structure. Because of substantial
273 resolution power, it also revealed that the dissection of complex quantitative traits can be initiated from
274 QTL mapping that can further confirmed by linkage mapping and genetic transformation of specific
275 genomic region. The genome wide association study for tiller angle in rice populations provided the
276 significant loci. The manipulation of these loci can improve the plant architecture and can be used for
277 ideotype breeding. These results provided the striking information for development of further breeding
278 programs for lodging resistance and higher yield.
279

280 ## Conclusions

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

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297

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432

Descriptive statistics of tiller angle in different populations.

* r^2 = repeatability ** Two-tail T-test significance at P ≥ 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.

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

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.

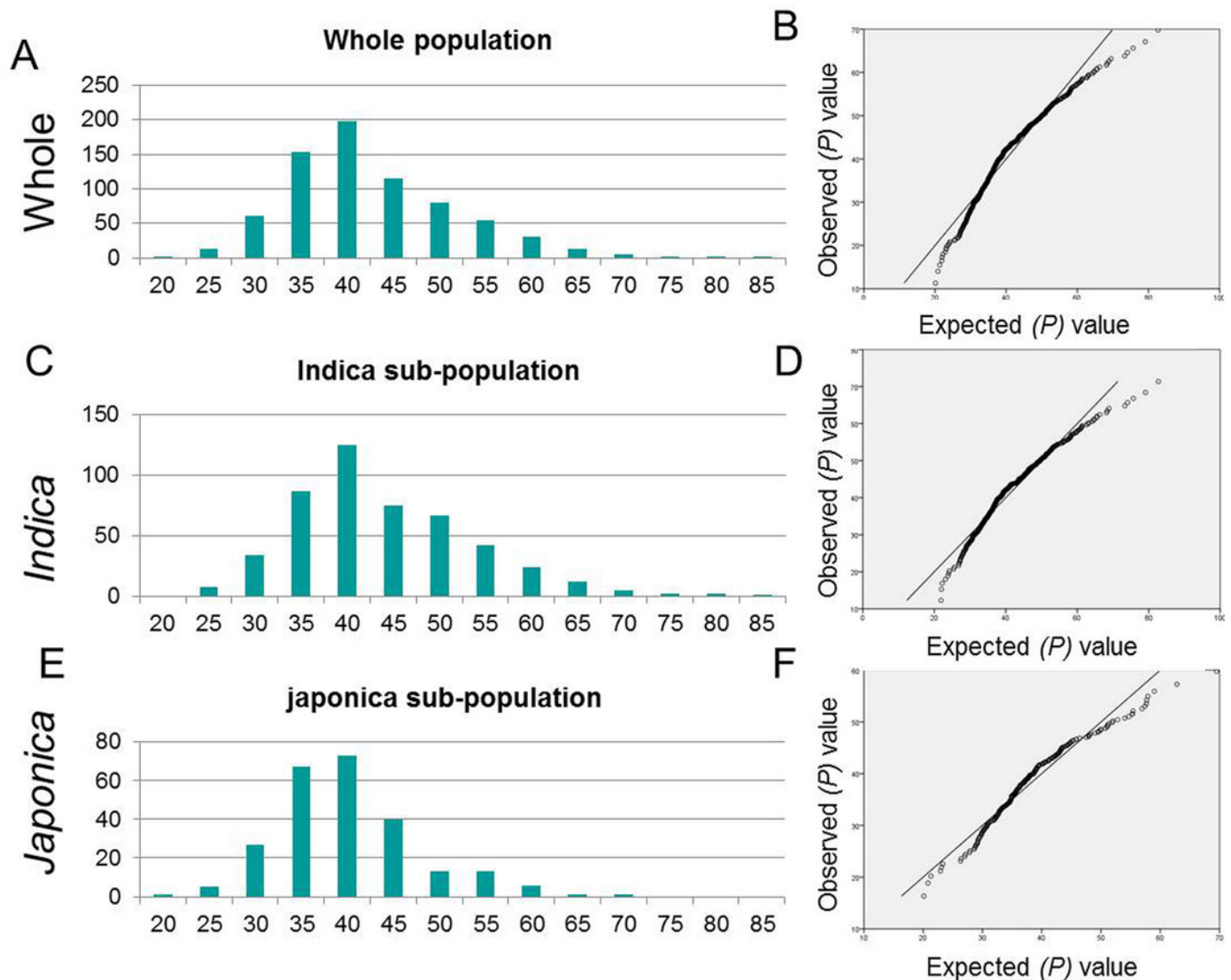



Figure 2

Population structure analysis for 795 genotypes

(a) PCAs, and (b) kinship

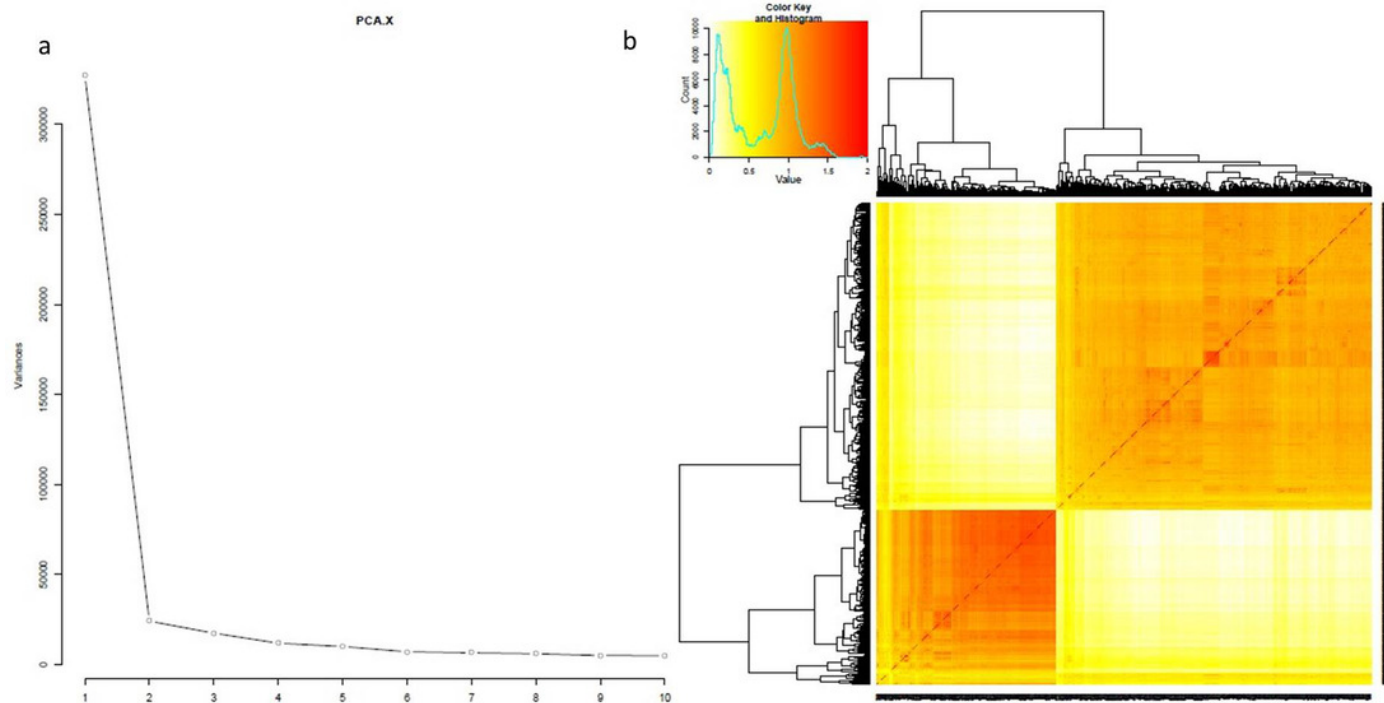



Figure 3

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.

A

Permutation

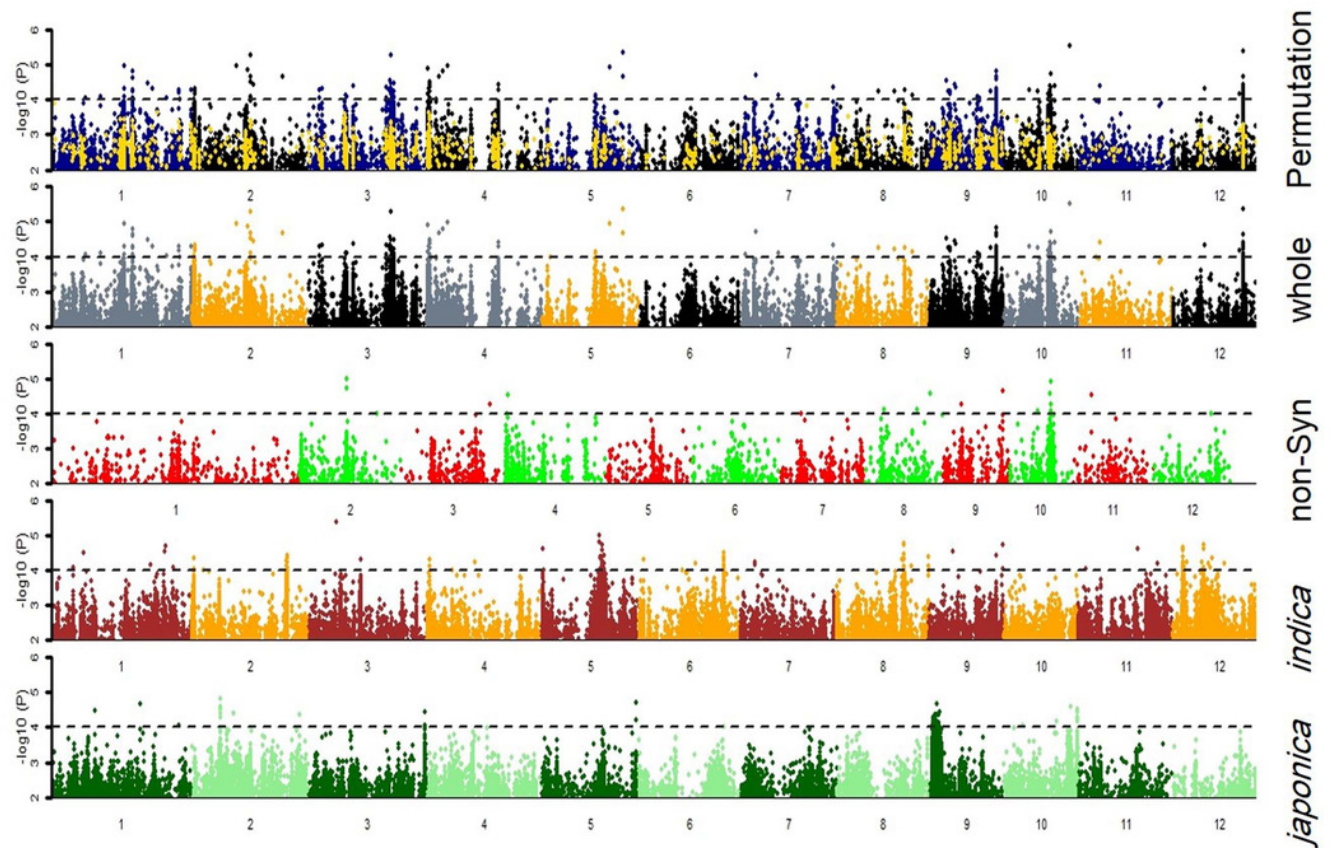
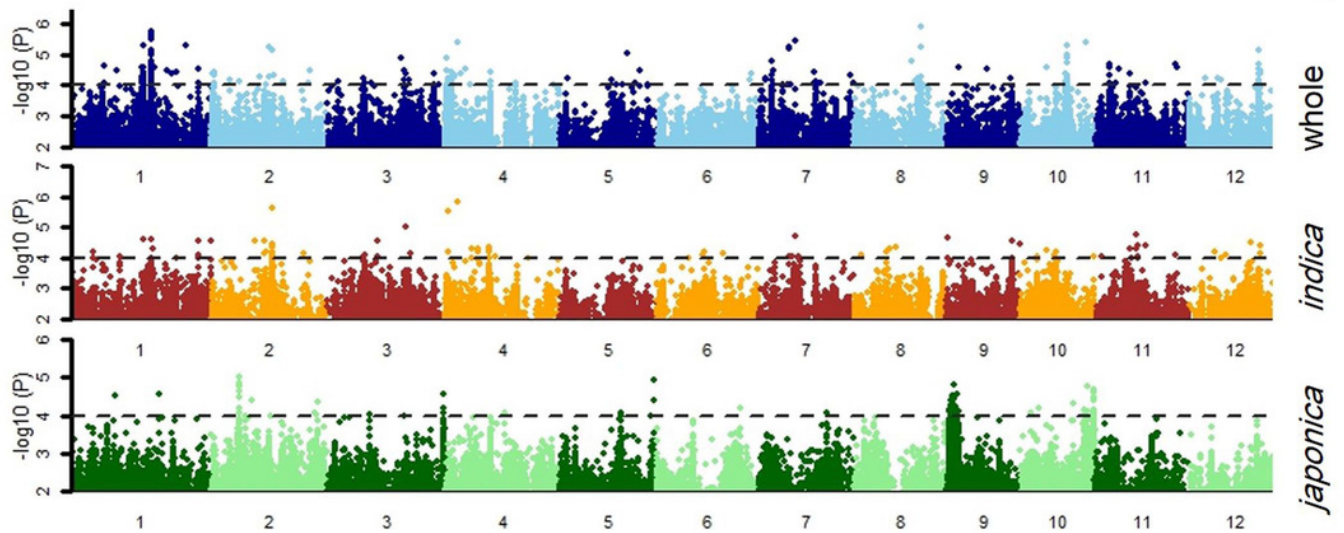
whole

non-Syn

indica

japonica

B

whole

indica

japonica

Figure 4

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

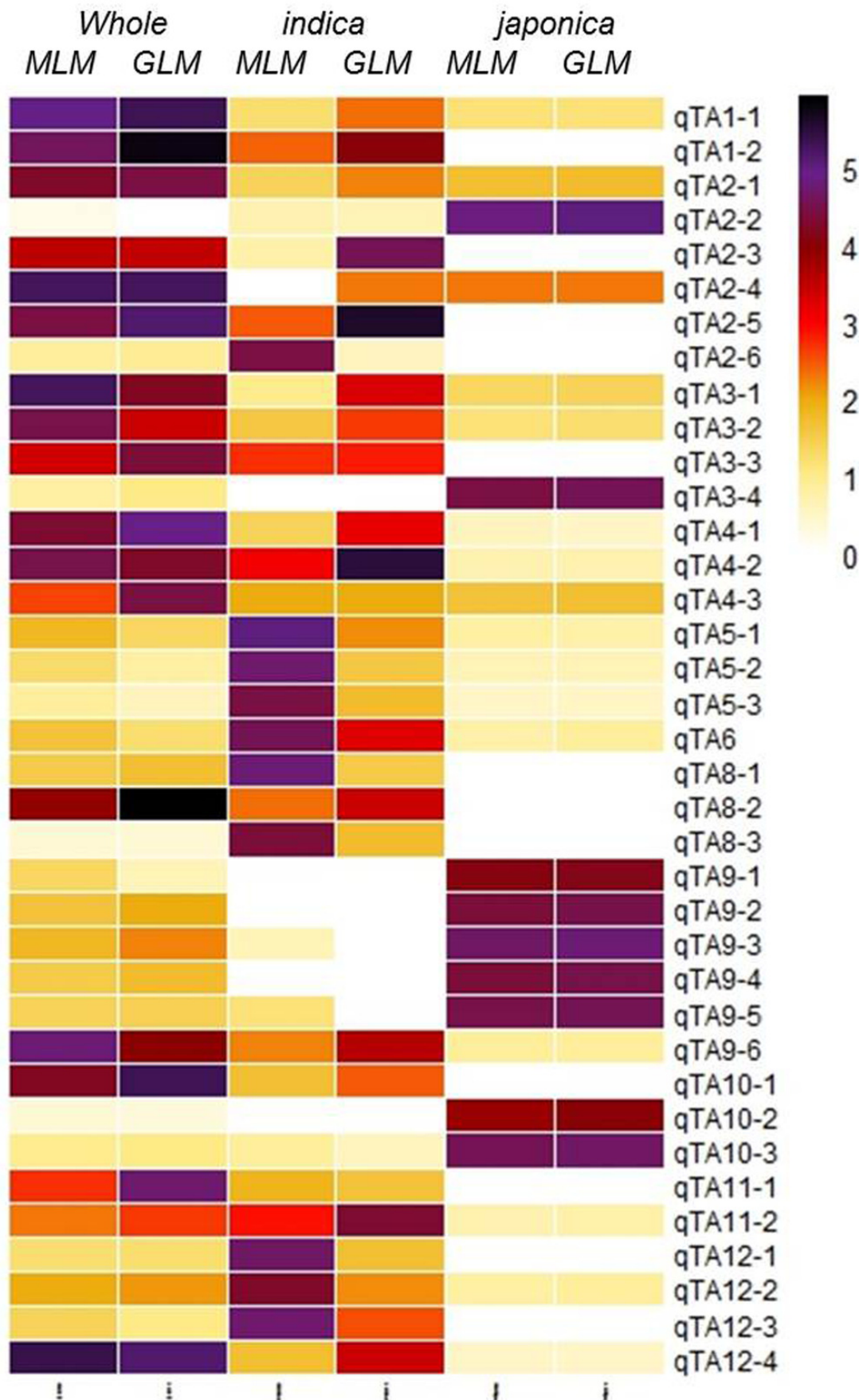



Figure 5

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
Indica	hap2	T	C	A	14	38.93	Indica	hap2	C	G	320	42.13
Indica	hap3	T	T	A	132	39.42	Indica	hap3	T	G	58	38.01
Indica	hap4	T	T	G	34	38.89						
Japonica	hap1	A	C	A	24	39.82						
Japonica	hap2	T	C	A	10	38.23	Japonica	hap2	C	G	64	39.87
Japonica	hap3	T	T	A	25	37.59	Japonica	hap3	T	G	156	37.50
Japonica	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]