Unveiling the genetic loci for a panicle developmental trait using genome-wide association study in rice

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Abstract

Panicle size has a high correlation with grain yield in rice. There is a bottleneck to identify the additional quantitative trait loci (QTL) for panicle size due to the conventional traits used for QTL mapping. To identify more genetic loci for panicle size, a panicle developmental trait (LNTB, the length from panicle neck-knot to the first primary branch in the rachis) related to panicle size was identified and the genetic loci were investigated using genome-wide association study (GWAS). Three populations including two subspecies, Indica and Japonica and the whole population were used to perform GWAS by the linear mixed model. Nineteen significant associations were totally identified in the whole population. One locus was examined in both Indica and Japonica. Four associations identified in the present study were near the cloned panicle related genes. *Hd16* (*Heading date 16*) and *DST* (*Drought and salt tolerance*) were examined for LNTB. These results indicate that LNTB was also an ideal trait for GWAS to identify the underlying QTLs for panicle size. The associations not related to the cloned genes need to be further validated by the bi-parental populations. Dissecting the favorable alleles of these associations could be helpful for breeding by marker assisted selection in rice.

Key words: Genome-wide association study, LNTB, Panicle size, Single nucleotide polymorphism.

INTRODUCTION

Rice is an important crop to human as a food supply. It is the staple food for most of people in Asia. Due to the increase in the population, and the decrease in the cultivated land, commonly the focus is on grain yield improvement by the researchers and breeders. Panicle size is highly related to grain yield in rice. This is a complex trait that could be evaluated by the three components, panicle length, the number of secondary branches and spikelets per panicle (SPP). The formation and elongation of the inflorescence occurs in the phase of transition from vegetative to the reproductive growth in rice. After the formation of inflorescence meristem, a bract primordium is produced enclosing the inflorescence meristem, which suppresses its growth (Ikeda *et al.,* 2005). In general, there is a small protrusion at the base of panicle axis, which is a vestige of the bract. The distance between the protrusion (bract) and the base of the first primary branch in the rachis was defined as LNTB (Figure 1). LNTB has a positive correlation with the panicle length and/or density of the primary panicle branches to a large extent. It is a panicle developmental trait associated with grain yield.

Panicle length is popular as a trait for quantitative trait loci (QTL) identification. It is a complex trait determined by several QTLs and environments. Up to date, dozens of QTLs have been identified for panicle length by positive genetic mapping analysis using the populations derived from the bi-parental crosses (www.gramane.org). A few associations for panicle length have also been identified by association mapping based on the association population (Zhao *et al.,* 2011). *Ghd7*, *Ghd7.1* and *Ghd8* were reported to regulate panicle

length except for heading date (Xue *et al.,* 2008; Yan *et al.,* 2011; Yan *et al.,* 2013). *DEP1* (*Dense and Erect Panicle 1*), *DEP2* (*Dense and Erect Panicle 2*), *DEP3* (*Dense and Erect Panicle 3*) were found to affect grain density and yield (Huang *et al.,* 2010; Li *et al.,* 2010; Qiao *et al.,* 2011). Besides, several genes regulating panicle length have been identified by mutants; Such as, *APO1* (A*berrant Panicle Organization 1*), *APO2* (A*berrant Panicle Organization 2*), *SP1* (*Short Panicle1*) (Ikeda *et al.,* 2005; Rao *et al.,* 2008; Ikeda-Kawakatsu *et al.,* 2012; Li *et al.,* 2009). Together, these cloned genes are important for understanding the panicle architecture. However, the genetic network on their regulation of panicle growth and development is still less understood. LNTB is an internode in panicle axis and shows a high correlation to panicle axis length and/or the density of the primary branches. It is a powerful strategy to identify the genetic loci for LNTB using genome-wide association study (GWAS). The statistical method of linear mixed model (LMM) also known as mixed linear model (MLM) is popularly employed in GWAS since it could avoid the false positive associations (Yu *et al.,* 2006; Zhang *et al.,* 2010; Chen *et al.,* 2014). The association results here could enrich the genetic constitution of panicle length and the density of primary branches, and even the underlying grain yield, which could provide a few potential favorable haplotypes for molecular breeding.

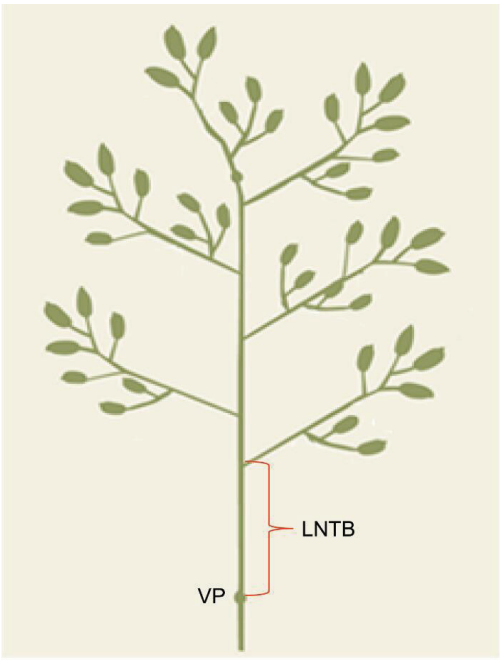

Figure 1: The model diagram of rice panicle: The small vestige protrusion (VP) in panicle axis represented the neck-node. The length form neck-node to the first primary branch in rachis is defined as LNTB.

MATERIALS AND METHODS

Plant materials and field experiments

A diverse worldwide rice collection of 529 accessions (containing 295 Indica, 156 Japonica rice and 78 others) reported by Chen *et al.* (2014) and Bai *et al.* (2016) was used. The population was planted in a bird-net-equipped field of the experimental farm of Huazhong Agricultural University on May, 2014 in Wuhan, China. Seven of 25-day old seedlings of each line were transplanted into one row in the field with a distance of 16.5 cm between the plants within a row and 26.4 cm between rows. Field trials were carried out following the randomized complete block design with two replications each year. Field management was conducted according to the standard agronomic practices (Bai *et al.,* 2016). Five plants in the middle of each row were used individually to measure LNTB.

Measurement of phenotype

Three panicles per plant were randomly selected to measure LNTB in the maturity time of the 529 accessions. Five middle plants in a line were chosen for LNTB measurements. The LNTB were measured by a ruler and the mean value of the five plants for each accession was used for GWAS analysis.

Single nucleotide polymorphism (SNP) database

The SNP data of the 529 rice accessions were reported in the previous study (Chen *et al.,* 2014). The detailed information is available in the website (http://ricevarmap.ncpgr.cn). The physical locations of SNP markers were cited in TIGR Rice Genome Annotation project (version 6.1).

Statistical analyses

Linkage disequilibrium (LD) was estimated by using standardized disequilibrium coefficients (D'), and squared allele-frequency correlations (r^2) for pairs of SNP loci were determined according to the TASSEL program (Chen *et al.,* 2014). LD plots were generated in Haploview (Barrett *et al.,* 2005). Three SNPs (in the 3'UTR) were identified around *DST* and extracted from the dataset (http://ricevarmap.ncpgr.cn) to perform the haplotype analysis.

Genome-wide association study

A total of 3,916,415 SNPs (considering the SNPs with minor allele frequencies of ≥ 0.05 and varieties with minor allele frequencies of ≥ 6 in a population) were used for GWAS in the whole population (Chen *et al.,* 2014). LMM was used to make association analysis by running the FaST-LMM program (Lippert *et al.,* 2011). Using a method described by Li *et al.* (2012), the effective number of independent SNPs was calculated

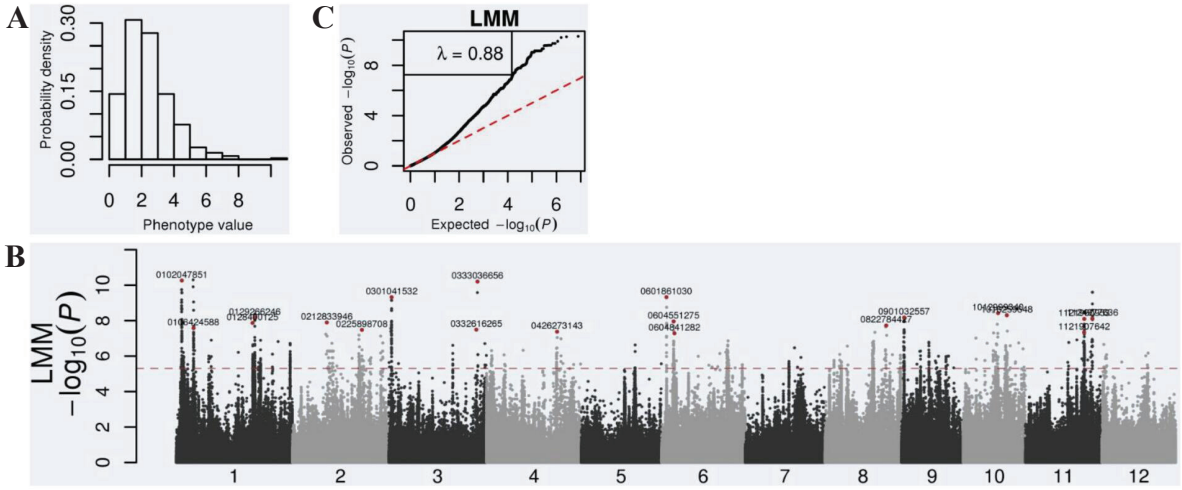

Figure 2: Genome-wide association study of LNTB in the whole population: **A:** The phenotypic distribution of LNTB in the whole population. **B:** Manhattan plots of LMM for LNTB in the whole population. **C:** Quantile-quantile plot of the linear mixed model (LMM) for LNTB.

Table 1. The variation of LNTB in the two subpopulations.

Population	Mean ± SD (cm)	Range (cm)	*P* value
Indica	1.6 ± 0.7	0.2-3.6	6.4E-59
Japonica	3.8 ± 1.4	1.1-10.1	

as 757,578 for the whole population. The suggestive *P* values were specified as 1.3×10^{-6} in the whole population. The thresholds were set as $P=1.2\times10^{-6}$ to identify significant association signals by LMM. To obtain independent association signals, multiple SNPs exceeding the threshold in a 5-Mb sliding window were clustered by r^2 of LD≥0.25 and the SNPs with the minimum *P*-values in a cluster were considered as lead SNPs. The detailed method is described in the previous study (Chen *et al.,* 2014; Wang *et al.,* 2015).

RESULTS

The phenotypic variation in three panels

Two major subpopulations, Japonica and Indica, were classified from the 529 rice accessions (Chen *et al.,* 2014). Broad variations for LNTB were observed in the whole collection varieties (Figure 2A). We also observed large variations in LNTB in both Indica and Japonica subpopulations (Table 1). Interestingly, there was a significant difference (*P*=6.4E-59) between these two subpopulations (Table 1). The mean value for LNTB was 1.6 cm in Indica, whereas, it was 3.8 cm in Japonica subpopulation. The minimum value for LNTB was 0.2 cm and 1.1 cm in Indica and Japonica, respectively. The maximum value for LNTB in Japonica was more than three times of that in Indica.

GWAS for LNTB

As there was a large variation for LNTB in whole and two subpopulations (Figure 2A and Table 1), we performed GWAS for LNTB in the two subpopulations Indica and Japonica individually and the whole population, using LMM to identify significant associations (Figure 2B-C). Since the LD decay in cultivated rice was extended from 100 kb to 200 kb (Mather *et al.,* 2007; McNally *et al.,* 2009), the 100-kb region of the upstream (-) and/or downstream (+) of the association SNPs were considered as possible candidate regions for LNTB. In this study, 19 associations were totally identified (Table 2). Seventeen of them were identified in the whole population by LMM (Table 2), which were distributed on the 12 rice chromosomes except for chromosomes 5, 7 and 12. One association was identified both in the subpopulations of Indica and Japonica, respectively. Three associations were identified on chromosomes 1 and 3 (Table 2). Two associations were identified on chromosome 2, 6, 10 and 11. Four associations were near the cloned panicle size related genes (Table 2).

Table 2. Genome-wide association signals for LNTB detected using the linear mixed model (LMM).

Pop	Lead SNP	Chr.	P_{LMM}	Var (%)	Neighboring QTL/Locus	Reference
Whole	Sf0102047851	1	5.5E-11	21.1		
Whole	Sf0106424588	1	2.6E-08	3.6		
Whole	Sf0129266246	1	6.7E-09	16.6	*SPL28* (-97)	Qiao et al. 2010
Whole	Sf0212833946	2	1.3E-08	17.7		
Whole	Sf0225898708	2	3.3E-08	15.4		
Whole	Sf0301041532	3	4.8E-10	25.2		
Whole	Sf0332616265	3	3.2E-08	13.1	*DST* (22)	Li et al. 2013
Whole	Sf0333036656	3	6.3E-11	17.1	*Hd16* (-37)	Hori et al.2013
Whole	Sf0426273143	4	4.2E-08	8.5		
Whole	Sf0601861030	6	4.7E-10	19.5		
Whole	Sf0604841282	6	5.2E-08	9.4		
Whole	Sf0822784427	8	1.9E-08	1.8		
Whole	Sf0901032557	9	6.7E-09	10.7		
Whole	Sf1012999340	10	3.8E-09	19.4	*EBL1* (-77)	Iwamoto and Takano 2011
Whole	Sf1016259648	10	5.0E-09	17.5		
Whole	Sf1121960953	11	8.0E-09	15.3		
Whole	Sf1124977636	11	7.7E-09	11.3		
Indica	Sf0914475949	9	1.2E-06	17.7		
Japonica	Sf0224281327	2	5.2E-07	17.9		

Pop: Populations; Chr: Chromosome; Var: Variation.

On the other hand, the associations identified in the whole population explained the phenotypic variation of 3.6% to 25.2% (Table 2). The most significant association (*P*=5.5E-11) was detected on chromosome 1 with a contribution of 21.1% to the phenotypic variation (Table 2). The two associations identified in subpopulations of Indica and Japonica could all explain more than 17% of the phenotypic variation (Table 2).

Panicle size related genes examined for association with LNTB

It was reported that the heading date related genes, such as *Ghd7*, *Ghd7.1* and *Ghd8*, could regulate panicle size as well as heading date (Xue *et al.,* 2008; Yan *et al.,* 2011; Yan *et al.,* 2013). In this study, an association SNP (Sf0333036656) near *Hd16* (heading date 16) (~ 37 kb) was significantly identified for LNTB (*P*=6.3E-11), which could explain more than 17% of the phenotypic variation (Table 2). The results suggested that *Hd16* may participate in the regulation of LNTB, which is in correspondence with the report on its regulation on plant height and panicle size (Hori *et al.,* 2013). Besides, the sf0332616265 SNP located on chromosome 3 was examined (*P*=3.2E-08), and found to be about 22 kb away from *DST* (drought and salt tolerance) regulating the number of primary branch and grain number per panicle (Figure 3). In order to further validate the association of *DST*, we identified the SNPs around *DST* (5' UTR, ORF and 3'UTR) based on the database (http://ricevarmap.ncpgr.cn). Three SNPs (sf0332638340, sf0332638658 and sf0332638807) located on the 3'UTR were identified, which were subsequently classified into 3 haplotypes (Table 3). Hap 2 showed the highest value of LNTB compared to those of two other haplotypes (Hap1 and Hap3), and Hap1 displayed the lowest value. There was a significant difference in LNTB between each of the two haplotypes (Table 3). These results suggested that *DST* was responsible for LNTB as well as panicle size.

On the other hand, the SNP linked to *SPL28* (spotted leaf 28) encoding a clathrin-associated adaptor protein complex 1 was identified for LNTB. A short panicle and decreased plant height was reported in the *spl28* mutant (Qiao *et al.,* 2010). In addition, it was reported

Table 3: Haplotype analysis of *DST* based on the available SNPs located on the 3' UTR.

Haplotypes	SNPs*	Accessions		LNTB (cm)
		Indica	*Japonica*	
Hap1	TGA	293	0	1.8±0.8C
Hap2	AGG	1	98	4.0±1.6A
Hap3	ACG	0	55	3.3±1.0B

*: indicated the genotypes of the SNPs of sf0332638340, sf0332638658 and sf0332638807. The phenotype values of LNTB is presented as mean±SD. Letters are ranked by Duncan test at P<0.01.

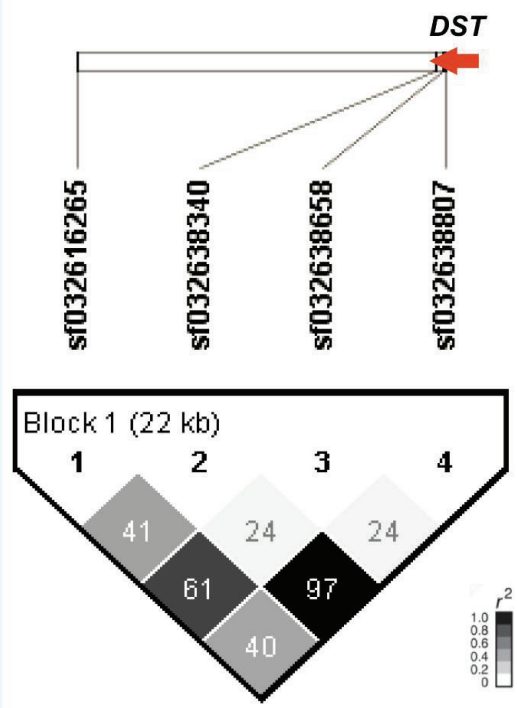

Figure 3: Linkage disequilibrium (LD) among the SNPs located on *DST* and an associated SNP. A LD block was identified around *DST*. The red arrow shows *DST* and its direction of translation. The intensity of the color in each box corresponds to the r^2 value. The value of each box indicates the r^2 values between pairs of SNPs multiplied by 100.

that *EBL1* (OsEREBP1-LIKE 1) regulates internode elongation in rice (Iwamoto and Takano 2011). Interestingly, we identified an association between SNP (sf1012999340) and *EBL1*. This may suggest that the genes for internode elongation are involved in the regulation of LNTB.

DISCUSSION

Enriching the genetic loci for panicle size

Panicle size is a complex trait controlled by a number of QTLs. Panicle length and SPP are popularly used for QTL mapping using the populations derived from a cross between two parents. A few QTLs have been identified in the previous study (www.gramene.org) which cannot explain completely the whole variation in a number of rice varieties for panicle size. In this study, we investigated the values for LNTB in 529 rice accessions, which is a panicle developmental trait. There was a large variation in the whole population (Figure 2A). These results suggested that LNTB is probably controlled by multiple QTLs. Furthermore, it was one internode of panicle axis, which could represent the growth and development of panicle (rachis elongation and primary branches formation) to some extent. Totally, 19 associations for LNTB were identified using LMM. Four panicle size related genes were examined in the candidate region of the associations. It was reported that *Ghd7* and *Ehd1* regulate panicle size except for heading date (Xue *et al.,* 2008; Endo-Higashi and Izawa 2011). *Hd16* was reported to specifically phosphorylate *Ghd7* that participates in the regulation of *Ehd1* (Hori *et al.,* 2013) may suggest that *Hd16* regulates panicle size. Interestingly, *Hd16* was found to be associated with LNTB in the present study (Table 2). Especially, *DST* (controlling the number of primary branches and grain number per panicle) (Li *et al.,* 2013), was associated to LNTB (Tables 2 and 3). Together, these results indicated that LNTB was an ideal trait to identify more genes/QTLs for panicle size. Furthermore, 15 loci were associated without linkage to a cloned panicle size related gene. Validation of these results by a bi-parental population would provide more insight into panicle growth and development.

Difference for LNTB between Indica and Japonica

It is well known that there are two major subspecies in cultivated rice (*O. sativa*), which differ in several traits, such as drought tolerance, cold sensitivity, grain shape, germination and so on (Oka 1998). We

also found a significant difference in LNTB between these two subspecies. Genetic diversity has also been reported between the two based on the restriction fragment length polymorphism markers (Wang and Tanksley 1989; Zhang *et al.,* 1992). Recently, it was reported that there were different origins of evolution and/or domestication for Indica and Japonica (Huang *et al.,* 2012). Interestingly, *Hd16* was examined for association to LNTB with a very significant *P* value (*P*=6.3E-11). We investigated the SNPs located in the exon, 5' and 3' UTR of *Hd16* for haplotype analysis. Two haplotypes were identified harboring Indica and Japonica rice cultivars (data not shown). However, there was a significant difference in LNTB between the two subpopulations (Table 1). Altogether, it was further validated that *Hd16* was significantly associated with LNTB. Besides, there was a larger variation for LNTB in intra-subspecies, especially in Japonica population (Table 1). It might be explained partially by the significant difference for LNTB between the haplotypes Hap 2 and Hap 3 of *DST* which mainly harbored Japonica varieties (Table 2). However, the less association was identified in subpopulations of either Indica or Japonica than in the whole population. This may be attributed to some extent to the lower variation in intra-subpopulation compared to the whole population.

Application in rice breeding

LNTB is an index to reflect panicle size that is highly related to grain yield in rice. In the present study, totally, 19 associations were identified. Some associations linked to the cloned panicle genes, for instance, *DST* was associated with LNTB. The LNTB was significantly higher in Hap 2 of *DST* (mostly in Japonica varieties) compared to that in Hap1, which is mostly present in Indica varieties. It is suggested that the panicle size in Indica could be improved by the Hap 2 allele cloned from Japonica. In addition, the associations without linkage to the cloned panicle genes can probably be utilized for rice breeding upon their validation using the bi-parental populations. On the other hand, LNTB may be related to panicle enclosure to some extent. The SNP near *EBL1* affecting panicle enclosure (Iwamoto and Takano 2011), was identified to be associated with LNTB (Table 2). However, the panicle enclosure could result in the sterility and the loss of grain yield to a large extent. Hence, the further validation of these associations here might facilitate to better understand the genetic basis of grain yield in rice.

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CONFLICT OF INTERESTS

The authors declared that there is no conflict of interest concerning the publication of this paper.

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