Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension
Volume 18 Number 1 (January 2019) pp. 22-29
ISSN 1119-7455

DISSECTING THE QUANTITATIVE VARIATIONS OF THRESHABILITY IN RICE (*Oryza sativa* L.)

Charles A.O.J.

Department of Botany, University of Dares Salaam Box 35091, Dares Salaam Tanzania

*Corresponding author's email: jcharles@udsm.ac.tz

ABSTRACT

In rice, low threshability retains grains on the panicles especially in japonica rice (O. sativa subsp. Japonica) and high threshability makes it easier for harvesting in indica. The genetic mechanisms underlying moderate threshability in rice remain largely unclear. A set of reciprocal Backcross Inbred Lines BILs including 226 and 229 lines from indica rice (O. sativa subsp. indica) and japonica rice (O. sativa subsp. japonica) backgrounds, respectively, and 262 recombinant inbred lines RILs developed from same cross between an indica, were adopted for testing threshability in two seasons. Percentages of dislodged grains were estimated at the maturity, harvesting and after sun drying. A total of 265 SNP markers developed based on sequencing and evenly distributed throughout the 12 chromosomes were used for genotyping assay. Inclusive composite interval mapping (ICIM) was used to dissect genomic regions and interactions affecting quantitative variation of threshability. A total of 13 QTLs were found to be conferring quantitative variations of threshability, which were distributed on all chromosomes. Two known major genes (QTLs) qSH1 and sh4 were also present. New additive QTLs, qThr1-a, qThr2, qThr3 qThr7, qThr8, qThr10, qThr11 and qThr12 were found as well. Twenty-four epistatics QTLs of which eight were common in both japonica and indica backgrounds were detected in two consecutive seasons. The overall threshability explained by all additive and interacting QTLs (R^2) were 70% and 1 for indica, 15 and 8% for japonica and 17.4 and 10.6% for RIL backgrounds, respectively. Findings have preliminarily revealed a relatively complex mechanism underlying the quantitative variations of threshability in rice, which was controlled not only by major loci but also affected by epistasis and genetic background effects.

Key words: epistasis, genetic background effect, quantitative variation, rice (*Oryza sativa* L.), threshability

INTRODUCTION

Threshing is a technique of separating grains from the panicle. The easy by which grains are removed from the panicle is called threshability. Threshability depends on the strength of spikelet attachment to the panicle (Kumar and Sharma, 1982). It varies depending on the variety, moisture content and the degree of maturity of the grain (FAO, 1994). Easy threshability or inhibition for difficult threshing (ITH) was first reported by Prasada rao and Misro (1986). At first, seed shattering was thought to be controlled by some major simple genes such as *sh1* in chromosome 1 (Oba *et al.,* 1990), *Sh3*, *sh4* and *SHA1* in chromosome 4 (Eiguchi and Sano1990; Lin *et. al.,* 2007) and recessive gene *sh-h* in chromosome7 (Ji *et. al.,* 2006). However, further studies have revealed that seed shattering is controlled by many quantitative trait loci (QTLs) widely distributed in rice genome (Eiguchi and Sano, 1990; Cai and Morishima 2000; Konishi *et. al.,* 2006; Li *et.al.,* 2006; Onishi *et al.,* 2007; Zhang *et al*., 2009; Qin *et al.,* 2010; He *et al.,* 2011). Three hypotheses have been reported regarding the direction of evolution of non-shattering trait: 1. Lin *et al.* (2007) reported that selection for non-shattering trait during rice domestication occurred prior to O. sativa subsp. indica, commonly referred to as indica rice and –O. sativa subsp. japonica commonly referred to as Japonica rice differentiation. 2. Tan *et al.* (2008) reported that non-shattering is a positive selection driven trait, the direction of introgression, is either from *Japonica* to *Indica* or *vice versa*. 3. Konishi *et al.* (2006) and Tao Sang (2009) supported the hypothesis that non-shattering occurred independently in *Japonica* and *Indica* types. Konishi *et al.* (2006) detected two QTLs on chromosomes 11 and 12 with alleles from *Indica* and three QTLs on chromosomes 1, 2 and 5 with favorable alleles coming from *Japonica* all contributing to non-shattering. Of these QTLs *qSH1* located on chromosome 1 explained 68.6% of the total phenotypic variation in the background. They subsequently demonstrated that a single-nucleotide polymorphism (SNP) in the 5' regulatory region of the *qSH1* gene caused non-

Please cite as: Charles A.O.J. (2019). Dissecting the quantitative variations of threshability in rice (*Oryza sativa* L.). *Agro-Science,* **18 (1),** 22–29. **DOI:** https://dx.doi.org/10.4314/as.v18i1.4

shattering owing to the absence of abscission layer formation. Genetic Model of Regulatory Network Specifying Abscission zone Development in Rice was characterized by the submissions of Zhou *et al.* (2012) who stated that the expression of *SHAT1gene* that encodes transcriptional factor *APETALA2* in the abscission zone (AZ) is required for seed shattering. The expression of SHATI gene however is regulated by transcription factor *SH4*. Mutant *sh4* leads g to non-shattering. Persistent and concentrated expression of active *SHAT1* and *SH4* in the AZ during the early spikelet developmental stages is required for conferring AZ identification.*qSH1* function downstream of *SHAT1* and *SH4*, thus promoting AZ differentiation. However, during the course of selection for non-shattering varieties, a wide range in the degree of grain threshability across rice varieties has emerged. Some cultivars are easy to thresh, some are moderate and some are hard to thresh especially the *Japonica* varieties. This variability has been associated to different morphological degree of the abscission layer which occurs at the juncture between the sterile lemma and pedicel among varieties (Jin *et al.,* 1995). Most farmers do threshing manually especially by beating against a hard surface. Thus, hard or non-threshability retains more grains on the straws whereas easy threshability grains fall down during or before harvesting. Hence, both hard and easy threshability would increase yield loss significantly. It has been reported that small head-feeding combined harvester is favourable in the harvesting of hard or non-threshing varieties, while the large combined harvester is usually used for the moderate threshing varieties (Ji *et al.,* 2006). However small-scale farmers who are the majority in Africa cannot secure harvesters. Thus it would be appropriate to grow rice varieties with moderate threshability. Study on the FI segregation indicated complete dominance of hard threshability over easy threshability (Kumar and Sharma, 1982). The genetic architecture of moderate threshability however largely remains unclear. Using two sets of backcross inbred lines (BILs) and one set of recombinant inbred lines (RILs), QTL analysis was performed to understand additive QTLs, epistasis and genetic background effect affecting hard, moderate and hard threshability in *Japonica and Indica* rice varieties.

MATERIAL AND METHODS

Planting Material

'Minghui 63' (MH63) is a non-threshing *Indica* variety and elite restorer line which occupies largest planting area and has contributed significantly to yield increase in most hybrid rice breeding in China as a male parent. It was crossed with '02428' a *Japonica* type, a hard threshing and widely adapted as a wide compatibility variety (WCV). The F_1 hybrids were simultaneously backcrossed to both parents to produce two BC_1F_1 population. The F_1 hybrids were self-pollinated to generate F_2 population. The F_1 were backcrossed to each parent to produce BC_2F_1. Both the BC_2F_1 and F_2 population were advanced by single seed descent from year to year to produce BC_2F_8 and F_6 population, respectively. Finally, two sets of reciprocal inbred lines, consisting of 226 lines (BC_2F_8) with the MR63 background (MH63_ILs), 229 lines (BC_2F_8) with the 02428 backgrounds (02428_ILs) and 262 ($F_{2:8}$) recombinant inbreed lines (RILs) were selected.

Threshing

All Backcross Inbred Lines (BILs) and Recombinant Inbred Lines (RILs) were grown at the experimental farms of the Chinese Academy of Agricultural Science (CAAS) at Hainan, China in 2013 and 2014. The field experiments were arranged in a completely randomized block design with two replications. At the maturity, harvesting and after sun drying, percentage of dislodged grains were estimated according to standard evaluation systems for rice (IRR, 2002). Rice plant was considered mature when grains were hard and difficult to divide with thumbnail. Rice plant was considered to be in harvesting stage when grains were very hard, cannot be dented by thumbnail and plant is dead and collapsing. After harvesting five panicles per genotype were dried in the sun or 10 days before threshing. Four people, each person at a time grasped a panicle by hand and firmly pulled over to dislodge grains. The strength required to dislodge grains from the panicles estimated the degree of grains attachment which is equivalent to threshability. Estimates were recorded as follows, 1: Difficulty (less than 1%), 3: moderately difficulty (1-5%), 5: intermediate (6-15%) 7: Loose (26-50%) 9: Easy (51-100). Such method has previously been reported by Lee *et al.,* (2005) who stated that dislodging panicles by hand gripping, accurately detect degree of threshability. This activity was done three times on farm site after maturity and during bulk harvesting and in the working room using 5 panicles that were dried in the sun for 10 days before threshing. Threshing immediately after maturity stage was regarded as high moisture content, during harvesting as moderate moisture content and after sun-drying grains for 10 days and kept in the working room as low moisture content.

SNP Genotyping

Minghui63 "(MH63)" an *indica* rice variety and '02428' a *japonica* rice variety were submitted to whole genome re-sequencing. A total of 265 high-quality SNP were evenly distributed along the genome and were picked for SNP chip design by Illumnia Corp, USA and genotyping assay by Xing-Wang Deng's Lab in Peking University.

Linkage Map Construction
For the genotyping, 265 high-quality SNPs were used for a consensus linkage map construction by ICI-Mapping Version 4.2 (Wang *et al.,* 2013). The total length of the consensus map was 1132.9 centiMorgan cM, which covered 12 rice chromosomes with an average distance of 4.27 centiMorgan among adjacent markers.

Normality and Analysis of Variation
The experimental data were analyzed using SAS institute version 9.2 (2009).

QTL Mapping
Additive and epistasis QTLs affecting threshability were identified by Inclusive Composite Interval Mapping (ICIM) and Inclusive Composite Interval Mapping of digenic epistatics (ICIM-EPI) in ICIMapping ver. 4.0 presented in Cent-Morgan (cM) using the Kosambi function (Wang *et al.,* 2013). The thresholds of Logarithm of the odds ratio (LOD) of 3 and 1.5 were used for declaring additive QTL in all the Backcross Inbred Lines (BILs) and in Recombinant Inbred Lines (RILs), respectively. LOD 5 was used for declaring Epistasis QTLs in all populations. Edit plus software was used to identify SSR markers corresponding to SNP markers within a distance less than 1000kb to locate their physical positions.

RESULTS

Moisture content and population distribution
During grain ripening in cereal, decreased moisture content and increased dry weight has been reported (Hyde 1971). Thus in this study threshing immediately after maturity stage was regarded as high moisture content, during harvesting as moderate moisture content and after harvesting and sun-drying grains for 10 days as low moisture content. This study found it was hard, easy and very easy to thresh at the maturity, harvest and after harvesting and sun-drying for 10 days, respectively. Analysis of threshability for RILs population indicated that degree of grain maturity had influence on threshability at $P \leq 0.05$ whereby repeatability was 95.5%. However, this was not the case for the MH63-IL and 02428-IL populations where threshing at maturity, harvesting and after sun-drying all had no significant influence on threshability at $P \leq 0.05$ with repeatability of 74% and 50% respectively. The mixed result might have been attributed by different timing of threshing. For example in RIL population threshing timing among maturity stage, harvesting and after sun-drying clearly marked different moisture content, while for MH63-IL and 02428-IL, timing for threshing among three stages were close to each other thus did not depict the differences in moisture content. SAS statistical tests for normality and normal probability plot (QQ plot) at maturity, harvesting and after sun-drying stage for all populations revealed that the locations of first quantile, (Q1) mean, median, and third quintile (Q3) indicated a bell-shaped distribution. The mean and median was very close. These result illustrated that threshability was normally distributed (Fig1).

Additive QTLs
In *Indica* background, *qThr1*-b located on chromosome 1 flanked by markers M29 and M30 explaining over 80% of the threshability variations of the major QTL detected in all the three stages for two years (Fig. 2). This QTL is exactly the same or very similar to *sh1, qSHT-1* or *qSH1* reported previously by Nagao and Takahashi (1963), Cai and Morishima (2000) and Konishi *et al.* (2006), respectively. Some QTLs detected in maturity, harvesting and sun-drying were *qThr11* and *qThr12* flanked with markers M240/M242 and M263/M264 on chromosome 11 and 12, respectively. QTLs like *qThr1-a*, *qThr2*, *qThr6* and *qThr9* were detected after sun-drying, while *qThr3*, *qThr5* and *qThr10* were detected at harvesting stage. QTL *qThr* 8 was detected at maturity stage while *qThr7* after sun-drying and harvesting stages (Table 1). All additive QTLs explained 61.8%, 47.2% and 70% of threshability variations after sun-drying, maturity and harvesting stages, respectively (Table 2). In 02428_IL *a japonica* background QTLs *qThr1*-b, *qThr2* and *qThr9* on chromosome 1, 2 and 9 respectively were detected in all the three different maturity stages. The *qThr9* located between markers M206 and M207 explaining over 74% of threshability variation was the major QTL in this population (Fig. 1).

Other QTLs such as *qThr3*, *qThr5*, *qThr10* and *qThr11* on chromosomes, 3, 5 10 and 11 were identified after sun-drying stage only. QTLs *qTh1-a*, and *qThr12* were detected at maturity and after sun-drying, whereas *qThr4* and *qThr7* were detected at harvesting and after sun-drying stages. QTL mapped in chromosome 5, *qThr5,* was similar to the *Qal5-1* gene which is responsible for abscission layer formation in rice (Qin *et al.,* 2010). Total threshability variations (R^2) explained by additive QTLs in 02428_IL *a japonica* background was 10.8%, 12.2% and 15.3% for Maturity, harvesting and after sun-drying stages, respectively (Table 2). In RIL population, QTL analysis detected two major QTLs; *qThr1-b* and *qThr3* located on chromosome 1 and 3 respectively (Table 1). These two major QTLs contributed to 15 and 8% threshability variations, respectively (Table 2). Some minor QTLs (LOD < 3.0) such as *qThr6*, *qThr7*, *qThr8* and *qThr12* (not indicated) on chromosome 6, 7, 8 and 12 were detected by interval mapping and single marker analysis methods only.

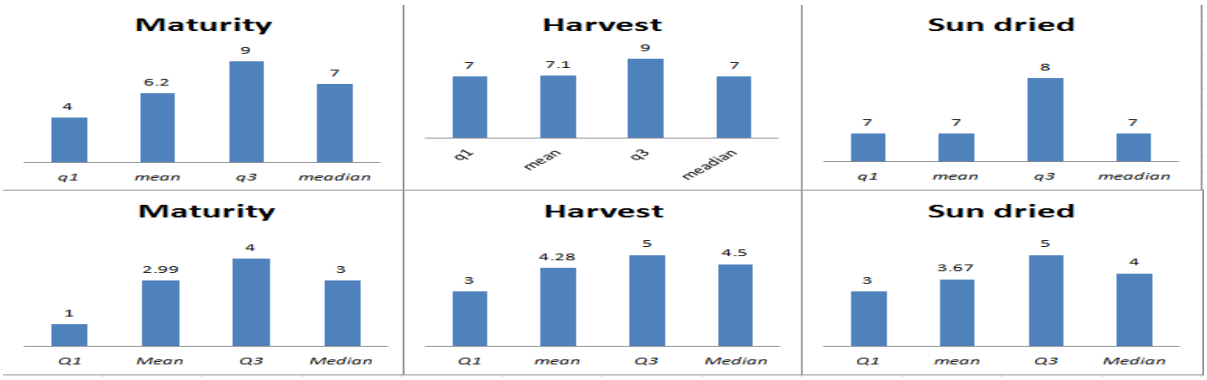

Figure 1: Histogram summarizes normal distribution from MH63_IL (upper row) and 02428_IL (lower row) at maturity, harvesting and sun dried stage derived from a box plot. For normally distributed population its 25th percentile (Q1) and 75th percentile are symmetrical, its 50th percentile (median) and mean are exactly or nearly the same and median Q3 and almost form a bell shape

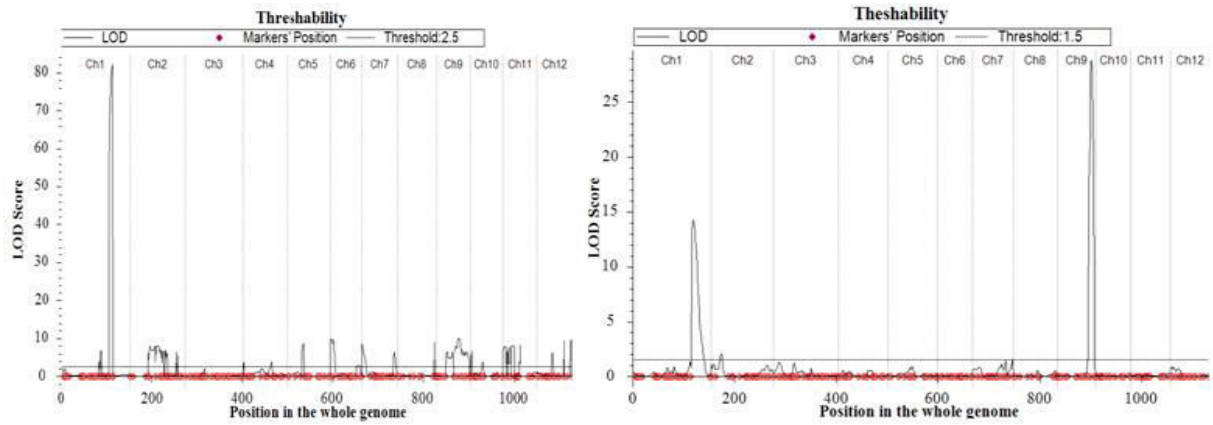

Figure 2 for ICIM additive mapping showing major additive QTLs in chromosomes 1 and 9 in the whole genome in *indica* (left) and *japonica* (right)backgrounds respectively

Table 1: Additive QTLs affecting threshability in rice as confirmed by Inclusive Composite Interval Mapping (ICIM) in three populations at three different harvesting stages; maturity $^{(m)}$, harvesting $^{(h)}$ and sun dried stage $^{(d)}$

QTL	Interval	Ch	Pop.	LOD	PEV	add	phy.pos	Loci report	REF
qThr1-a$^{(m,h,d)}$	M6-M7	1	MH63-IL	21	69	-2.55	5762721-10287685		
qThr1-a$^{(m,h,d)}$	M6-M7	1	02428-IL	4	23	-1.28			
qThr1-b$^{(m,h,d)}$	M29-M30	1	MH63-IL	82	80	-2.78	34260883-36369760		
qThr1-b$^{(m,h,d)}$	M31-M32	1	02428-IL	19	72	-2.62	36952999-42298039	*sh1, qSHT-1, qSH1*	a, b, d
qThr1-b$^{(m,h,d)}$	M29-M30	1	RIL	7	15	-1.41	34260883-36369760		
qThr2$^{(.m,h,d)}$	M36-M37	2	02428-IL	3	18	-0.97			
qThr2$^{(m,)}$	M36-M37	2	MH63-IL	26	36	-3.21	4385365-8735694		
qThr2$^{(m,h)}$	M36-M38	2	RIL	1.7	2.9	-0.48			
qThr3$^{(m,h,d)}$	M57-M58	3	RIL	4	9	-0.81	442367-2546314		
qThr3$^{(m,h)}$	M62-M63	3	MH63-IL	13	36	-3.25	7612707-8854594		
qThr4$^{(m,h,d)}$	M106-M107	4	MH63-IL	23	36	-3.18	25012772-27293771	*Sh3,SHA1*	e, f
qThr4$^{(m)}$	M106-M108	4	RIL	2	3.6	-0.89			
qThr5$^{(d)}$	M134-M135	5	02428-IL	4	9	-0.89	28117409-29470947	*Qal5-1*	g
qThr5$^{(h)}$	M129-M130	5	MH63-IL	10	9	-3.08			
qThr6$^{m,H,D)}$	M139-M140	6	RIL	1.6	3.1	-0.61			
qThr7$^{(m,h,d)}$	M174-M175	7	02428-IL	4	15	-0.94	25436357-26738335		
qThr7$^{(m,h,d)}$	M156-M157	7	MH63-IL	27	37	-3.18	1142811-2000576		
qThr7$^{(m,h)}$	M156-M158	7	RIL	1.6	2.7	-0.6			
Thr8$^{(m,h,d)}$	M180-M181	8	MH63-IL	8	9	-2.46	1537588-3027524	*qSHT8, qSH8*	a ,c
Thr8$^{(h,d)}$	M180-M182	8	RIL	1.5	2.7	-0.44			
qThr9$^{(m,h,d)}$	M206-M207	9	MH63-IL	20	36	-3.25	20982434-21923812		
qThr9$^{(m,h,d)}$	M206-M207	9	02428-IL	29	74	-2.51			
qThr10$^{(m,h,d)}$	M221-M223	10	MH63-IL	24	37	-3.05	15274964-19504134		
qThr10$^{(d)}$	M210-M211	10	02428-IL	4	13	-0.76	1242485-3551423		
qThr11$^{(d)}$	M240-M241	11	02428-IL	4	9	-0.55	13578131-14038359		
qThr11$^{(m,h,d)}$	M228-M229	11	MH63-IL	19	36	-3.24	570684-1793375		
qThr12$^{(m,h,d)}$	M264-M265	12	MH63-IL	21	36	-3.25	24440442-25291547		
qThr12$^{(d)}$	M249-M250	12	02428-IL	4	9	-0.55	1858522-2741858		

References: a= Cai and Morishima 2000, b=Konishi *et al.,* (2006) c=Li *et al.,* (2006), d= Nagao and Takahashi (1963), e= Eiguchi and sano (1990) and f=Lin *et al.,* (2007), g = Qin *et al.,* (2010)

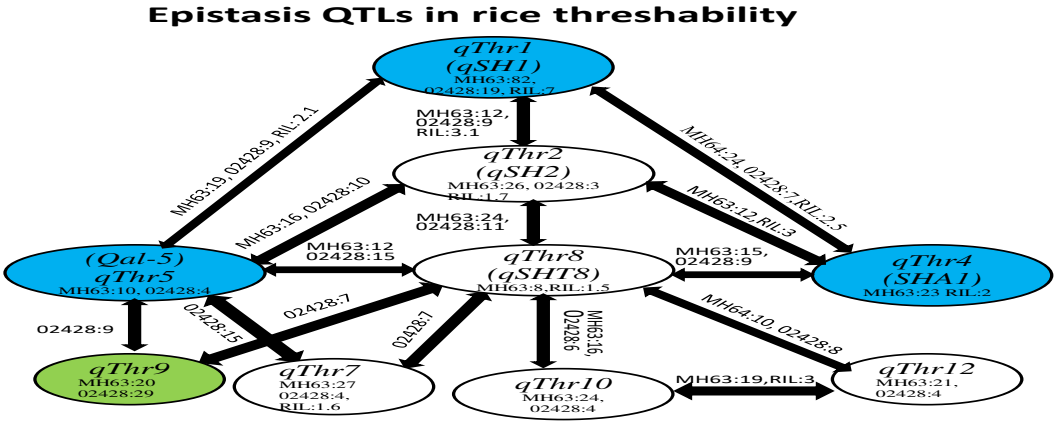

Figure 3: The epistasis networks between major QTLs in rice threshability in 02428 a *japonica*, MH63 an *indica* and RIL backgrounds. The numbers indicates LOD for QTL detection

Table 2: Retained markers and their coefficients in stepwise regression (STP). ADD and EPI are the total phenotypic variation explained by all additive and epistasis QTLs respectively

Stage	MH63_IL		02428_IL		RIL	
	ADD	EPI	ADD	EPI	ADD	EPI
Maturity	47.2	0	10.8	0	13.6	5.7
Harvesting	70	1.9	12.2	7.9	16.4	10.6
Sun-dried	61.8	0	15.3	6.1	9.1	0

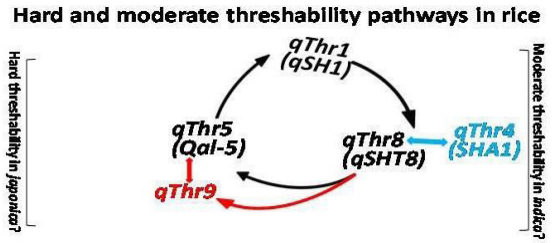

Figure 4 *qSH1* main non-shattering gene offers threshability in both *indica* and *japonica.* Both qThr4 and *SHA1* may contribute to moderate threshability in *indica.* However the presence of another major non-shattering gene *qThr9 in japonica* or the interaction of this gene with *qSH1* indirectly via *qThr5* or *qThr8* leads to hard threshability in *japonica.*

Epistasis QTLs

In MH63_IL population, 14 epistasis QTLs (*qEPs*) were detected (Fig. 3), of which 8 *qEPs* were detected in both *indica* and *japonica* backgrounds. However, total phenotypic variations (R^2) explained by these *qEPs* were 0 for after sun-drying and maturity stages and 1.9% for harvesting stage (Table 2). This result would suggest that epistasis QTLs had no significant impact on threshability in *Indica* background. In 02428-ILs population 18 epistasis QTLs (*qEP*) were detected (Fig. 3), 9 *qEPs* were common in both *Indica* and *Japonica* backgrounds. Total threshability variations (R^2) explained by these *qEPs* were 7.9%, 6% and 0 for harvesting, after sun-drying and maturity stages, respectively (Table 2). These figures suggest that both additive and epistasis QTLs affect threshability in *Japonica* background. For RIL population there was only 1 epistasis QTL located between chromosomes 1 and chromosome3 ($qEP_{1\text{-}3}$). The overall threshability variation explained by this epistasis QTL was10.7%.

DISCUSSION

This study shows shattering habit as a polygenic and complex trait that evolves additive and epistasis QTLs networks but also genetic background dependent. Many of QTLs detected in this study coincided with previous reports that applied in other methods such as tensile strength force gauge (Kennard *et al.,* 2002; Ji *et al.,* 2006; Konishi *et al.,* 2006; Onishi *et al.,* 2007; Qin *et al.,* 2010), free shedding off or bagging (Xiong *et al.,* 1999, Bres-Patry *et al.,* 2001; Cai and Morishima, 2000; Lin *et al.,* 2006), shaking harvested panicles then calculating the percentage of shattered seed to the total seed weight (Gu *et al.,* 2005) and combination of both hand gripping and digital device (Qin *et al.,* 2010). In all the three populations, all significant QTLs showed the same direction of additive gene effect as that expected from the phenotypic difference between the parents. A major QTL *qThr1-a* in chromosome1 linked to markers M29 and M30 located between 34260883 and36369760bp in MH63_IL and RIL populations and between 36952999 and42298039bp in 02428_IL (*Japonica*) background was detected. Judging from its physical position, this QTL is identical to *qSH1* which has been mapped (Cai and Morishima, 2000) and cloned (Konishi *et al.,* 2006). It was a major QTL in MH63_IL population (Fig. 2), with LOD 80, 19 and 7 explaining 80% 72% and 15% of threshability variation (R^2) in *Indica Japonica* and RILs (Table.1). Another major QTL in this study was *qThr9* located in chromosome 9 between 20982434bp and 21923812bp (Fig. 2). This QTLs had the largest phenotypic effect in *japonica* background. From integrated rice science data base, more than 41 QTL controlling shattering in rice are listed (http://www.shigen.nig.ac.jp/rice/oryzabase/).

However from that list only one QTL *(qSH9)* detected from a cross between Lemont/Teqing cross was reported in Chr.9 between markers RG451 and RZ404 (Zhong *et al*., 1999). From the IRRI data base RG451 and RZ404 are located between 76.0 and 82.7cM while *qThr9* in this study is located between 63.42cM and72.62 cM.

This QTL or its interactions with other QTLs seems to be the source of hard threshability in *japonica*. Another important threshability gene responsible for non-shattering in rice was QTL *qThr4* in chromosome 4 between 25012772 and 17293771 bp. This gene was named as *sh4* (Li *et al.,* 2006) or *SHA1* (Lin *et al.,* 2007). The overall addictive effect of this QTL was 36% in *Indica* and 3.6% in RIL population. Tao Sang (2009) suggests that *qSH1* was selected during the domestication of *Japonica* rice, but was subsequently eliminated from most of the *Japonica* cultivars after *sh4* was introduced from cultivars of different origins, because the combination of *qSH1* and *sh4* could have made threshing too difficult and laborious. On the other hand, Zhou *et al*. (2012) showed that *qSH1* function downstream of *SHAT1* gene and *sh4* to eliminate abscission layer formation. Our study, however, suggests that *qSH1* and *sh4* do not appear in all rice varieties to exert non-shattering in rice, instead are genetic background dependent. This can be supported by the fact that both *qSH1* and *sh4* were detected in *indica* and RIL population but not in 02428_IL a *japonica* background. Therefore, hard-threshability in the *japonica* could not have risen from the combination of *qSH1* and *sh4* as previous reported. The data also suggest that, the presence of *qSH1* and *sh4* in the MH63_IL *indica* variety do not result into hard threshability either because *indica* used in this study is a moderate threshing variety. Depending on genetic background, *sh4* or *qSH1* can work independently or dependently to confer non-shattering and/or hard threshing in rice. The other important QTL detected in this study was *qThr8* previously reported as *qSTH8* or *qSD8* (Li *et al.,* 2006; Gu *et al.,* 2005) and *qThr2*. These QTLs seem to play transcriptional role in coordinating stimulus reception and the response QTLs identified further below. In all the five threshability tests (maturity, harvesting and after sun-drying in 2013 and harvesting and after sun-drying stage*s* in 2014), *qThr8* was detected in *indica* and RIL population but not in *japonica* while *qThr2* was detected in chrmosome2 in all populations. On chromosome 2, Nakamura *et al*. (1999) reported that QTL *sh2* is expressed from the early stage of grain development and forms the abscission layer at the base of sterillemma. The same QTL was also reported by Zhong *et al.* (1999) between the markers KG139 and C624x, while Konishi *et al.* (2006) identified *qSH2* using SNP makers. Judging from their positions these two QTLs slightly differ from *qThr2* detected in this study. Another important QTL detected was *qThr5* located in chromosome 5. Qin *et al.* (2010) named this QTL as *Qal-5*. It was reported to affect pulling strength when pulling gauge was applied to detach grains from the pedicle. It shows a strong interaction with *qThr9* in *japonica* background only. It is proposed that this interaction might be one of the primary sources of hard threshability in *japonica* background. Another important QTL was *qThr7* detected in all populations. On the same region QTL with large phenotypic effects on key domestication traits, harvest and planting, including a reduction in seed shattering and seed dormancy and the synchronization of seed maturation has been reported (Li *et al.,* 2006). They explained that selection for higher yield was probably responsible for the fixation of mutations at a cluster of QTL on chromosome 7 and a few other chromosomal locations that could have substantially improved plant architecture and panicle structure, resulting in fewer erect tillers and longer and more highly branched panicles in cultivated rice. Other QTLs that were detected in all the three populations their physical positions are indicated in Table 1. From integrated rice science data base more than 41 QTLs controlling shattering in rice are listed (http://www.shigen.nig.ac.jp/rice/oryzabase/). In most cases flanking markers surrounding these QTLs, are not listed, making it difficult to make comparison. However, with SNP markers, physical positions of all QTLs detected in this study are indicated, making it easier for references.

Epistasis and Hard Threshability in *Japonica*

Phenotypic gene expression and genetic variations in different species are affected by epistasis (Wu *et al.,* 2000; Cao *et al.,* 2001; Yuan *et al.,* 2003; Liu *et al.,* 2007; Große-Brinkhaus *et al.,* 2010) and genetic background (Liao *et al.,* 2001; Zheng *et al.,* 2011). For instance, genetic architecture of carcass composition and meat quality are mainly composed of complex network of interacting genes rather than the sum of individual QTL effects (Große- Brinkhaus *et al.,* 2010). In rice, epistasis has been reported in aluminum tolerance (Wu *et al.,* 2000), plant height (Cao *et al*, 2001), heading date (Liu *et al.,* 2007), grain weight and shape (Zheng *et al.,* 2011), heterosis (Jiang *et al.,* 2012) growth and productivity (Zhang *et al.,* 2013), and also in rice shattering (Qin *et al.,* 2010). In this study MH63-ILs *Indica* type, 14 epistasis QTLs were detected (Fig. 3) of which 3 were common in *Indica*, *Japonica* and RIL backgrounds, 6 in *indica* and *japonica,* 2 in *indica* and RIL and 3 were found in *japonica* only. Total phenotypic variation (R^2) explained by these QTLs interactions are presented (Table 3). In *indica* background QTLs interactions did not explain threshability variation (R^2) maturity and after sun-drying stages. QTL interactions however explained 1.9% of threshability variation in harvesting stages compared to 61.8, 47.2 and 70% of the total phenotypic variation (R^2) explained by additive QTLs. These results would suggest epistasis QTLs had no influence on threshability in *indica* background. In 02428-IL *Japonica* background, the overall total phenotypic variation explained by all additive QTLs were 10.8, 12.2 and 15.3% for maturity, harvesting and after sun-drying stages respectively. While epistasis

QTLs contributed to 7.9 and 6.1% of the phenotype variations at harvesting and after sun-drying stages respectively. The high proportion of phenotype variation due to epistasis QTL explained how QTL×QTL interactions were important in *japonica* population. For RIL population the total phenotypic variation explained by all additive QTLs were 13.6, 16.4 and 9.1 at maturity, harvesting and sun-drying stages, while phenotypic variation explained by all epistasis QTLs were 5.7 and 10.6 at maturity and harvesting stages, respectively. These results further illustrated the importance of epistasis in threshability. The epistasis network shown in Fig. 3 proposed the possible pathway for the hard threshability in *japonica*. However, it should be noted that some interactions involving minor QTLs and those occurring between non-main effect QTLs are not involved in this pathway. In addition, only 2 QTLs *qSH1* and *SHA1* have been cloned and characterized. Moderate and hard threshability pathways are presented in Fig. 4. Interaction network shows that *qThr1* which was a major QTL plays a central role in threshability in both *indica* and *japonica.* It interacts with some intermediate QTLs including *qThr2*, *qThr5* and *qThr4* in all populations. However *qThr4* is not a main effect of QTL in *japonica* background. Thus, interaction in *japonica* might be environmental or human error. Intermediate *qThr2, qThr4* and *qTh5* interact with *qThr8.* Below *qThr8* there are *qThr12*, *qThr10, qTh9* and *qThr7* which were detected in all populations. Interestingly, *qThr8* interacts with a set of different QTLs in both populations in a unique way. In *japonica* background, it interacted with QTLs, *qThr7* and *qThr9.* These *QTLs* in turn interacted with an upstream *qThr5 (Qal-1*). *Qal-1* has been reported to affect pooling strength when detaching grains from pedicle (Qin *et al.,* 2010); while in *qThr8* interacted with downstream QTLs *qThr10* and *qThr12* both in *japonica* and *indica* in similar ways. However, *qThr10* interacted with *qThr12* in *indica* background only. It is therefore proposed that interactions involving *qThr8* with *qThr5*, *qthr7* and *qThr9* indicated in Fig. 4 might explain hard threshability in *japonica* background. Similarly, in *indica*, *qThr8* interacted with *qThr4, qThr10 and qThr12*. In addition, *qThr10* further interacted with *qThr12* in *indica* background only. These unique interactions could explain moderate threshability in *indica*. *QTLs qThr9* might play a response role in *japonica* background while *qTh12* or *q*Th10 play the same role in *indica* background. Otherwise molecular characterization of QTLs especially *qThr5, qTrh8, qThr9* and *qThr12* would provide more understanding for genetic mechanism that might be involved in hard and moderate threshability.

CONCLUSION

There is a wide range in the degree of grain threshability. Some cultivars are easy to thresh while, others are hard to thresh. Hard threshability retains more grains on the panicles and too easy threshability leads to shattering before or during harvesting, thus moderate threshability is economical. Degree of grain maturity affects threshability, it is relatively hard to thresh immediately after mature stage compared to harvesting stage and after sun-drying of harvested panicles. The normal distribution of threshability trait, the QTLs and QTL interactions detected in this study together suggest that threshability is a polygenic trait. A major QTL located in chromosome 1 that was cloned by Konishi *et al.* (2006) was detected in all the three populations. In this study, *qThr1* (*qSH1*) played a central role as stimulus perception. However, *qThr2*, *qThr8*, *qTh5* (*Qal-1)* and *qThr4* (*SHA1)* are regarded as transcriptional factors. QTL *qThr4* (*SHA1),* a transcription factor in chromosome 4 (Lin *et al.,* 2007) offering non threshability, was only detected in *Indica* population, suggesting that *qSH1* and *sh4* do not always work as a functional unit and that hard threshability in *Japonica* is not always due to the presence of both *qSH1* and *SHA1* as previously thought (Sang, 2009), instead are genetic background dependent. From the transcriptional factors, responses were generated by QTLs *qThr9, qThr7, qThr10* and *qTh12.* Response was relayed back via *qThr12* in *indica* and *qThr5 (Qal-1)* in *japonica.* Other minor QTL interactions involving *qThr1* and *qThr7* in both *indica* and *japonica* were also detected. It is concluded that the major gene controlling threshability in *indica* was *qThr1* located between M29 and M30 in chromosome 1 at position 34260883 and 36369760bp while in 02428_IL *Japonica* background major QTLs controlling threshability is *qThr9* located between M206 and M207 in chromosome 9 at position 20982434 and 21923812bp and *qThr1*.

ACKNOWLEDGMENTS

I acknowledge Dr. Zhi-kang Li from Chinese Academy of Agricultural Sciences for funding the data collection and Dr. Tian-qing Zheng for providing study material.

REFERENCES

Bres-Partry C., Lorieux M., Clement G., Bangratz M. and Ghesquiere A. (2001). Heredity and genetic mapping of domestication-related traits in temperate *Japonica* weedy rice. *Theor. Appl. Genet.,* **102**, 118−126

Cai H.W. and Morishima H. (2000) Genomic regions affecting seed shattering and seed dormancy in rice. *Theor. Appl. Genet.*, **100**, 840–846

Cao G., Zhu J. He C. Gao Y. Yan J. and Wu P. (2001). Impact of epistasis and QTL×environment interaction on the developmental behavior of plant height in rice (*Oryza sativa* L.). *Theor. Appl. Genet*. **103**, 153–160

Eiguchi M. and Sano Y. (1990). A gene complex responsible for seed shattering and panicle spreading found in common wild rice. *Rice Genet Newslett.,* **7**, 105–107

F**AO (1994)** Post-harvest operations and management of food grains. M-17.ISBN 92-5-103108-8

Fukuta Y. and Yagi T. (1998). Mapping of a shattering resistance gene in a mutant line SR-5 induced from an *Indica* rice variety, Nan-jing11. *Breed. Sci*., **48**, 345−348

Große-Brinkhaus C., Jonas E., Buschbell H. *et al*. (2010). Epistatic QTL pairs associated with meat quality and carcass composition traits in a porcine Duroc × Pietrain population. *Genetics Selection Evol.*, **42**, 39-52

Gu X.Y., Shahryar F.K., Gary A.H., Barry L.H. and Michael E.F. (2005). Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (*Oryza sativa*). *Theor Appl Genet*., **110**, 1108–1118

IRRI (2002). Standard evaluation systems for rice. p7

Ish K. and Sharma, H. L. (1982). Inheritance of grain threshability in rice. *Euphytica*, **31 (3),** 815-816

Ji H.S., Chu S.H., Jiang W.Z., Cho Y.I., Hahn J.H., Eun, M.Y., McCouch S.R. and Koh H.J. (2006). Characterization and mapping of a shattering mutant in rice that corresponds to a block of domestication genes. *Genetics*, **173**, 995−1005

Jiang J.H., Liu Q.M., Lu C**.** *et al.* (2012). Genetic basis dissection of heterosis in *Japonica* rice (*Oryza sativa* L.). *Acta. Agron. Sin*., 38,**12**, 2147-2161

Jin I.D., Bae Y.H. and Inouye J. (1995). Formation and development of abscission layer between pedicel and rachilla, and chages in grain shedding during ripening in African rice Oryza glaberrima steud. *Korean J. Crop. Sci*., **40,** 103−112

Kennard W.C., Phillips R.L. and Porter R.A. (2002). Genetic dissection of seed shattering, agronomic and color traits in American wild rice (*Zizania palustris var. interior* L.) with a comparative map. *Theor. Appl. Genet.,* **105**, 1075−1086

Konishi S., Takeshi I., Shao Y.L., Kaworu E., Yoshimichi F., Takuji S. and Masahiro Y. (2006). A SNP caused loss of seed shattering during rice domestication. *Science,* **312**, 1392-1396

Kumar I. and Sharma H.L. (1982) Inheritance of grain threshability in rice. *Euphytica,* **31**, 815-816

Lee S.J., Oh C.S., Suh J.P., McCouch S.R. and Ahn S.N. (2005). Identification of QTLs for domestication-related and agronomic traits in an *Oryza sativa* × *O. rufipogon* BC_1F_7 population. *Plant breed*, **124**, 209−219

Li C., Zhou A. and Sang T. (2006). Rice domestication by reducing shattering. *Science*, **311**, 1936-1939

Liao C.Y., Wu P. Hu B. and Yi K.K. (2001). Effects of genetic background and environment on QTLs and epistasis for rice (Oryza sativa L.) panicle number. *Theor Appl Genet*., **103**, 104–111

Lin Z., Megan E.G., Xianran L. *et al.* (2007). Origin of seed shattering in rice (*Oryza sativa L.*). *Planta*, **226**,11–20

Liu G., Yang J. Xu H. and Zhu J. (2007). Influence of epistasis and QTL × environment interaction on heading date of rice (*Oryza sativa* L.). *J. Genet and Geno.,* 34 (**7**), 608-615

Mary B.H. (1971). Increase in dry weight and decrease in moisture in wheat kernels during ripening. *J. Stro Pro. Res.*, **7 (4),** 299-301

Nagao S. and Takahashi M. (1963). Trial construction of 12 linkage groups in Japanese rice. *J Fac. Agr Hokkaido Univ.,* **53,** 72–130

Nakamura A., Komatsu S., Xia, B.S and Hirano H. (1995). Linkage analysis of gene loci for seed glutelin (Glu-1), semidwarf (sd-1) and shattering habit (sh-2) in rice (*Oryza sativa* L.). *Breeding Science,* **45**,185-188

Oba S., Kikuchi F. and Maruyama K. (1990). Genetic analysis of semi-dwarfness and grain shattering of Chinese rice variety "Ai-Jio- Nana-Te". *Japan J. Breed.,* **40**, 13–20

Onishi K., Takagi K. Kontani M. Tanaka T.and Sano Y. (2007). Different patterns of genealogical relationships found in the two major QTLs causing reduction of seed shattering during rice domestication. *Genome,* **50**, 757–766

Prasada R.U. and Misro B. (1986). Inheritance and interrelationships of genes governing morphological and chemical characters in *Oryza var. javanica*. *Oryza*, **23**, 217-223

Qin Y., Suk-Man K., Xinhua Z., *et al.* (2010). Identification for quantitative trait loci controlling grain shattering in rice. *Genes & Genomics*, **32**, 173-180

Tan L., Li X., Liu F., Sun X. and Li C. (2008). Control of a key transition from prostrate to erect growth in rice domestication. *Nat Genet*., **40**, 1360–1364

Tao S. (2009). Genes and mutations underlying domestication transitions in grasses. *Plant Physiol*., **149**, 63–70

Wang J., Li H., Zhang L. and Meng L. (2012). Users' Manual of QTL ICI Mapping Version 4.2. The Quantitative Genetics Group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

Wu P., Liao C.Y., Hu B., Yi K.K., JinW.Z., Ni J.J. and He C. (2000). QTLs and epistasis for aluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages. *Theor Appl Genet.,* **100**, 1295–1303

Xiong L.Z., Liu K.D., Dai X.K., Xu C.G. and Zhang Q.F. (1999). Identification of genetic factors controlling domestication-related traits of rice using an F_2 population of a cross between *Oryza sativa* and *O. rufipogon. Theor. Appl. Genet*., **98**, 243−251

Yan Z., Danfeng L., Canyang L. *et al.* (2012). Genetic control of seed shattering in rice by the APETALA2 transcription factor shattering abortion. *Plant Cell*, **24**, 1034–1048

Yuan A.P., Cao L.Y., Zhuang J.Y., Li R.Z., Zheng K.L., Zhu J. and Cheng S.H. (2003). Analysis of additive and AE interactions effects of QTL controlling plant height, heading date and panicle number in Rice (*Oryza sativa* L.). *Acta Genetika Sinica*, **30 (10),** 899-906. (In Chinese, abstract in English)

Zhang F., Yu-zhu J., Si-Bin Y., *et al.* (2013). Three genetic systems controlling growth, development and productivity of rice (*Oryza sativa* L.): a re-evaluation of the 'Green Revolution'. *Theor Appl Genet***.,** **126**, 1011-1024

Zhang L.B., Zhu Q., Wu Z.Q., Ross-Ibarra J. and Gaut B.S. (2009). Selection on grain shattering genes and rates of rice domestication. *New Phytol*., **184**,708–720

Zheng T.Q., Wang Y., Ali A.J., *et al.* (2011). Genetic effects of background-independent loci for grain weight and shape identified using advanced reciprocal introgression lines from Lemont × Teqing in rice. *Crop Sci.,* **51**, 2525–2534

Zhong D.B., Yu S.B., Xu J.L., Luo L.J. and Li Z.K. (1999. QTLs for grain shattering in the Lemont/Teqing RI population. *RGN* **16**, 71-74

Ziwen H., Weiwei Z., Haijun W., *et al.* (2011). Two evolutionary histories in the genome of rice: the roles of domestication genes. *PLoS Genet*., **6**, 1-10