Full Length Article

Analysis of Genomic Regions Affecting Cooking and Eating Quality in a Recombinant Inbred Population of Rice

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Abstract

A population of recombinant inbred lines (RILs) derived from the cross between *Japonica* rice Nipponbare (NIP) and *Indica* rice Zhongjiazao17 (YK 17) was used to analyse the genomic regions for amylose content (AC), gel consistency (GC), gelatinization temperature (GT) and protein content (PC). Sums of 33 quantitative trait loci (QTLs) were detected on all chromosomes except chromosome 8, 10 and 12, respectively with single QTL explaining 1.56–89.51% of the phenotypic variation. From total, 15 of them were identified in all three environmental conditions. The marker interval RM7158-RM3414 harbours most of the detected QTLs and it may host the *Waxy* (*Wx*) gene along with *Alk* are the major determinant for cooking and eating quality (ECQs). Four QTLs (*qAC6, qGT6.2, qPC6* and *qPC7*) showed highly significant additive × environment interaction providing evidence of environment effect. Moreover, 7 epistatic QTL pairs including 3 for AC, 3 for GT and 1 for PC were also detected. The *Wx* gene sequencing results showed that the promoter region of *Wx* gene in YK17 contains three Indels along with two SNPs on Exon 9 and Exon 10 in comparison to NIP. The results confirmed that the *Wx* gene is the major determinant for cooking and eating quality in rice. These findings can help to develop understanding for the genetic mechanism of ECQs and allelic variation in YK17 *Wx* gene will be utilized for future grain quality improvement programs. © 2019 Friends Science Publishers

Keywords: Rice quality; QTL detection; Recombinant inbred lines (RILs); Allelic variation; Rice (*Oryza sativa* L.)

Introduction

Rice (*Oryza sativa* L.) as an important cereal ensures food security for more than half of the world population. Earlier rice breeders were much concerned regarding yield as the demand will increase 40% by the year 2030 due to projection in population (Khush, 2005). Whereas, the major shift in living standard of consumers made them conscious about quality food around the globe (Xie *et al.,* 2014). Therefore, improvement in grain quality attributes is fundamental along with yield for any rice breeding program. Grain quality attributes can be categorised into appearance, milling, eating and cooking, and nutritional (Wu *et al.,* 2015). Many of these quality traits are quantitative in nature with complex genetic mechanism and largely influenced by genotype, environmental conditions and their interactions (Wang *et al.,* 2007; Jin *et al.,* 2018). Among the grain quality attributes eating and cooking qualities (ECQs) have an integral importance. The ECQs are determined by their physio-chemical characteristics: amylose content (AC) (Juliano, 1971) gel consistency (GC) (Cagampang *et al.,* 1973) gelatinization temperature (GT) (Little, 1958) and protein content (PC). The recent advancements in the field

of rice molecular biology, has enabled rice breeders to dissect polygenic traits into single Mendelian quantitative trait loci (QTLs).

Amylose content is a key chemical attribute affecting ECQs and regarded as an authentic indicator in determining appearance and texture of cooked rice (Lanceras *et al.,* 2000). GC is an indicator for textural properties of cooked rice (Cuevas and Fitzgerald, 2012). It is being employed to distinguish among varieties having similar AC class and is negatively correlated with AC (Cruz and Khush, 2000; Cuevas and Fitzgerald, 2012). The amylopectin structure of starch granules of rice determines the behaviour of gelatinization (Umemoto *et al.,* 2002). The amylopectin synthesis mechanism is much complicated and so far, not yet been well understood how amylopectin cluster-structure is formed, although several models have been proposed (Myers *et al.,* 2000; Nakamura, 2002). Generally, protein content (PC) of rice grain is considered as a key component to determine nutritional quality but the synergetic relationship of PC with AC affects the taste of cooked rice. Moreover, physico-chemical properties of cooked rice are affected by PC (Ye *et al.,* 2010). Rice with high protein content is hard, less elastic, less viscous and worse in taste after being cooked (Aluko *et al.,* 2004).

Many researchers have reported major genes/QTLs controlling ECQs in rice through employing different mapping populations like RILs (Bao *et al.*, 2003), CSSLs (Zhang *et al.*, 2013) and DH population (Bao *et al.*, 2000). The mapping of genomic regions has shown that *Waxy* (*Wx*) gene located on chromosome 6 encoding granule bound starch synthase I (*GBSSI*) is an important determinant of AC (Aluko *et al.*, 2004; Fan *et al.*, 2005; Wang *et al.*, 2007). However, AC inheritance in rice endosperm is rather complex because the expression of the *Wx* gene is also affected by genetic background (He *et al.*, 1999; Mikami *et al.*, 2000; Tian *et al.*, 2009) and environmental factors (Larkin and Park, 1999), all of which make it difficult to accurately evaluate the genetic effects of different alleles at the *Wx* locus. Mikami *et al.* (2008) and Tian *et al.* (2009), identified four common *Wx* alleles, *wx*, Wx^b, Wx^{in}, and Wx^a, and one rare Wx^{op} allele. At present, many *Wx* alleles that correspond to various classes of AC have been reported. The five common haplotypes are *wx*, Wx^1, Wx^{g1}, Wx^{g2}, and Wx^{g3}, which corresponds to glutinous, low, intermediate, high I, and high II classes of apparent AC, respectively (Teng *et al.*, 2012). These haplotypes are suitable for selecting lines with the desired AC in breeding program, no matter the source of the germplasm. In case of GC, Bao *et al.* (2000) reported that two minor effect QTLs were responsible however, some recent studies disclosed that *Wx* gene is also a principle gene for GC (Tian *et al.*, 2005). Nevertheless, three classes of enzymes *i.e.*, starch synthase (SS), starch branching enzymes (SBE) and starch debranching enzyme (DBE) with multiple isoforms play their role in amylopectin synthesis (Gao *et al.*, 2003; Zheng-xun *et al.*, 2018). Moreover, it has been reported that amylopectin structure is determined by starch synthesis genes (SSGs). Several independent studies confirmed *Alk* gene at the same locus as of soluble starch synthase IIa (*SssIIa*) located at chromosome 6 is responsible for GT (Waters *et al.*, 2006). Several SNPs in the *SssIIa* gene have been identified (Umemoto *et al.*, 2004; Masouleh *et al.*, 2012). These various haplotypes of *SssIIa* exert different effects on starch composition and structure in *Indica* and *Japonica* rice varieties (Tian *et al.*, 2009; Gao *et al.*, 2011; Zhang *et al.*, 2017). PC, generally display normal frequency distribution but several reports have shown the influence of minor effect QTLs (Peng *et al.*, 2014). Several studies have shown major influencing QTLs located on chromosome 1 and 6, respectively. A major gene *qPC1* encoding a putative amino acid transporter *OsAAP6*, functioning as positive regulator of PC was cloned and functionally characterised (Yang *et al.*, 2018).

There is a continually increasing demand for better quality rice grain, which vary by germplasm and environment, as determined by in terms of the physico-chemical properties of rice starch. Therefore, present study, investigated physio-chemical properties of a recombinant inbred lines (RILs) population derived from inter-subspecific cross between *Japonica* rice NIP and *Indica* super rice YK 17, to identify QTLs associated with ECQs during three cropping seasons. The detected QTLs were analysed further to understand epistatic effects and their interaction with environment. To unearth the allelic variations between YK17 and NIP, *Wx* gene a major determinant of ECQs was genome sequenced between both parent and some lines with ECQs corresponding to both parents. The identification of QTLs and allelic variations between both parents and corresponding lines could be utilized to facilitate the improvement of rice quality by marker assisted selection.

Materials and Methods

Plant Materials and Field Experiments

A recombinant inbred lines (RILs) plant population containing 193 lines were developed by single-seed-descent method (SSD) from a cross between genomic sequenced *Japonica* rice NIP with *Indica* super rice YK17. Field experiments were established in Hangzhou (HZ) (30° N latitude) in 2015 and 2016 whereas, Hainan province (HN) (18.4° N latitude) in 2015. In HZ 2015 and HZ 2016 field trials, seeds were sown in May and seedlings were transplanted in June, while in HN 2015 field trial, seeds were sown in November and seedlings were transplanted in December. Each plot of plant population consisted of three rows of 21 plants at a spacing pattern of 25 cm (between rows) by 20 cm (within rows). The field trials were arranged in a randomized complete block design (RCBD) with three replications. Irrigation, fertilizer application and other management measures followed normal field production practices. At maturity, each plot of RILs was harvested in bulk, and dried naturally. The dried rice grains were stored for three months at room temperature prior to the evaluation of physio-chemical properties.

Quality Traits Evaluation

From each individual line fully filled grains were utilized to evaluate grain quality. Hulls were removed from 125 g of grains by using Satake testing husker (THU-35A Satake Engineering, Japan) and debranned with a McGill number 2 mill (seedburo Equipment, U.S.A.). Milled rice flour samples were obtained by grinding milled rice grains to pass through a 0.42 mm screen on an Udy cyclone mill (Cyclotec 1093 sample mill, Tecator, Sweden). The milled flour samples were sieved through a 100-mesh sieve to get uniform granule size. Following standard procedure were followed for the traits under investigation.

Amylose content (AC): About 50 mg rice flour was weighted with three repeats in 50 mL flask. To make the floor wet for uniform digestion 0.5 mL of 95% ethanol was utilized, 4.5 mL of 1 *M* NaOH was added into the flask covered with stopper and was put overnight for complete digestion. On completion of digestion the flasks were filled

up to the mark with deionized water (dH$_2$O). Standard amylose solutions (26.5, 16.2, 10.4 and 1.5%) were prepared as checks by following the same above mentioned method. The prepared samples were analysed through rapid flow auto analyzer (AA3, SEAL, Germany).

Gel consistency (GC): Three repeats were prepared according to the standard method (Cagampang *et al*., 1973). Briefly, 100 mg of rice flour (on the base of 12% moisture) was weighted in culture tubes of 10–110 mm. The quantity of 0.2 mL of 95% ethanol having 0.025% of thymol blue to overcome clumping of rice flour during gelatinization process. 2 mL of 0.2 N KOH was added and vortexed thoroughly. The water bath was prepared, and tubes covered with marbles were boiled vigorously for the time of 8 min. On completion, the tubes were kept at room temperature for 5 min and then moved into ice-water bath for 20 min. The tubes were than taken out and laid down horizontally on a table surface at temperature 25ºC for 1 h. The length of the gel was measured as the distance travelled from the bottom to the front of the tube. The length of the gel provides an insight to the behaviour of GC; the longer the distance, the softer the gel.

Gelatinization temperature (GT): The evaluation of alkali spreading value (ASV) is an indirect method to measure GT (Little, 1958). Three replications were prepared with each replication of six intact grains put in a weighing boat, to which 10 mL of 1.7% KOH was added and covered. By using forcep, the grains were separated and incubated 30ºC for 23 h for spreading. By visual scoring, the spreading value was scored on a scale of 1 to 7. A low ASV corresponds to a high GT, conversely, a high ASV indicates a low GT.

Protein content (PC): The protein content (PC) was measured using the micro-Kjeldahl pre-treatment method, protein was converted to ammonium nitrogen by sulfuric acid digestion and checked the absorbance value of the blue production of reaction with natrium salicylicum and hypochlorous acid at wavelength of 660 nm. The nitrogen contents were measured by employing Rapid Flow Auto Analyzer (AA3, SEAL, Germany). A conversion factor of 5.95 was used to extract PC from calculated nitrogen content of milled rice flour.

QTL Analysis

QTLs controlling ECQs were mapped using Windows QTL Cartographer Version 2.5 (WinQTLCart 2.5) (Wang, 2007) with the composite interval mapping (CIM) and an LOD value of 2.5 was set as threshold for the detection of putative QTLs. QTLs with epistatic effects and QTL-by-environment interaction (QEs) effects were analysed using QTL Network-2.1 (Yang *et al.,* 2008) with the mixed-model-based composite interval mapping (MCIM).

Statistical Analysis

All data were analysed using S.P.S.S. 22.0 and Microsoft Excel 2016.

Sequence Analysis of Cooking and Eating Quality Related Gene

The cloned and functional characterized *Wx* gene (*LOC_Os06g04200*) a major determinant for ECQs of rice grain, gene sequence was taken from genomic database (www.gramene.org) along with promoter, totalling 7032 bp, were amplified and sequenced in 10 lines of 'YK17 X NIP' RIL population and YK17 with reference to genome sequenced NIP. The overlapped primers were designed for polymerase chain reaction using program Prime Primer 5.

Results

Phenotypic Variation in Parents and the RIL Lines for ECQs

Over three rice growing seasons, the variation in parents and RIL population was significant and wide. A bimodal frequency distribution of phenotypic data was observed for AC and GT, indicating the influence of a major gene for these two traits along with some minor modifying genes (Table 1 and Fig. 1). Analysis of variance (ANOVA) based on a fixed model revealed highly significant variance between the three environments (HZ 2015, HN 2015 and HZ 2016) in the RIL population (Table 2).

Correlation Analysis of Traits

The analysis of correlation showed correlation among all traits under investigation ranged from -0.036 to 0.747 (Table 3). In HZ 2015 and HN 2015, highly significant negative correlation was exhibited by AC to GC whereas, non-significant correlation was observed in HZ 2016. The correlation coefficients of AC with GT displayed highly significant positive correlation in all three (HZ 2015, HN 2015 & HZ 2016) population growing seasons. Meanwhile, the correlation coefficients of GT in HZ 2015 and HZ 2016 exhibited higher values as compared to HN 2015. In HZ 2015, GC and GT showed highly negatively significant correlation and reached -0.496, while in HZ 2016 was only -0.115. Meanwhile, PC exhibited highly positive significant correlation to AC, GC and GT in HZ 2016 whereas, GT was non-significant in HN 2015.

QTL Analysis

A total of 33 QTLs related to AC, GC, GT and PC were identified by employing WinQTLCart 2.5 based on CIM in relation to the phenotypic performance of the RIL population (Table 3). These QTLs were detected on all chromosomes except chromosomes 8, 10 and 12, with single QTL explaining 1.56–89.51% of phenotypic variation. Among the detected 33 QTLs, 4 QTLs for AC, 6 for GC, 6 for GT and 1 for PC, were identified on
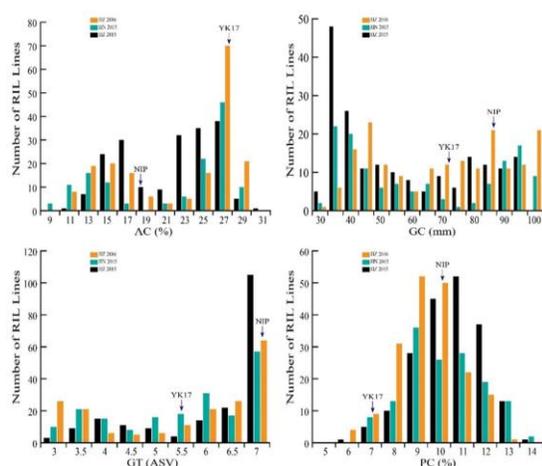
Fig. 1: Phenotypic distribution of AC, GC, GT and PC in the *japonica* rice NIP × YK17 RIL population across three growing conditions. Mean value NIP and YK17 from three environments were shown above with arrow
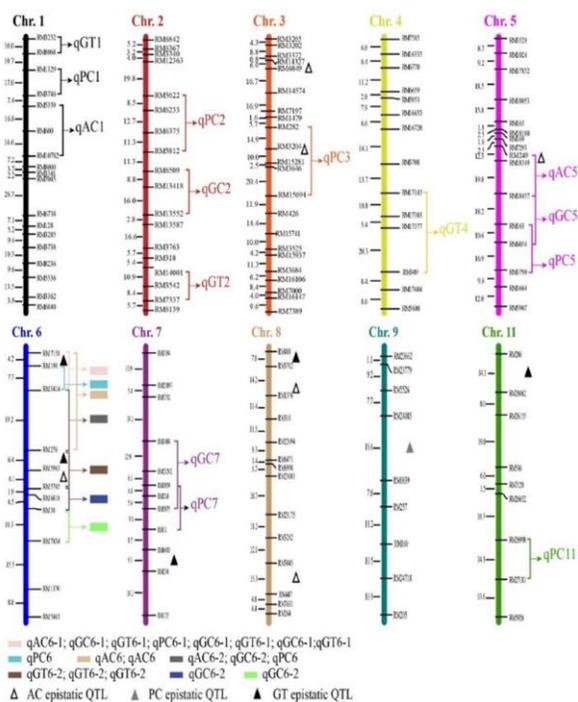

qAC6-1; qGC6-1; qGT6-1; qPC6-1; qGC6-1; qGT6-1; qGC6-1;qGT6-1
qPC6   qAC6; qAC6   qAC6-2; qGC6-2; qPC6
qGT6-2; qGT6-2; qGT6-2   qGC6-2   qGC6-2
△ AC epistatic QTL   ▲ PC epistatic QTL   ▲ GT epistatic QTL

Fig. 2: Location of main and epistatic effect QTLs for AC, GC, GT and PC on the genetic linkage map

Fig. 3: *Wx* gene structural and natural variation between alleles from NIP and YK17. The gene model containing 14 exons in red; CDS length of 3.8 kb is shown horizontally along the top. The promoter length of 2 kb is shown with dotted line. The Indels in promoter region are indicated with triangle; SNPs at exon 9 and 10 are indicated with arrows. The position of Indel and SNP is linked with dotted lines

Chromosome 6 under three population growing conditions (Table 4 and Fig. 2).

QTL for Amylose content (AC): The QTLs for AC, *qAC5* and *qAC6-2* were detected only in HZ 2015 whereas, *qAC1* detected in HN 2015. The QTLs *qAC5, qAC6-2* and *qAC1* contained 1.76, 74.46 and 1.56% of total phenotypic variation, respectively. The allele of *qAC5* from NIP in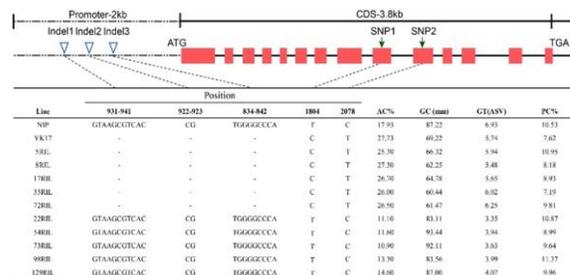creased the AC about 0.63% whereas; the allele of *qAC1* from YK17 reduced the AC about -0.82%. The major QTL, *qAC6* bordered by markers RM7158-RM276 on chromosome 6 was detected repeatedly between these markers under HN 2015 and HZ 2016 growing conditions. The phenotypic variation explained by *qAC6* was 89.51% in HN 2015 and 82.16% in HZ 2016, respectively. The allele of *qAC6* came from NIP increased AC 6.57% in HN 2015 and 5.70% in HZ 2016, respectively. The long distance between the peak positions of the two QTLs (*qAC6-1* and *qAC6-2)* in HZ 2015 indicated that these are unlikely to be the same QTL even the additive effect of both came from *Japonica* rice NIP. The *qAC6* peak of LOD score covered the *Wx* gene region, predicting *qAC6* may correspond to the *Wx* gene.

QTL for Gel consistency (GC): Nine QTLs for GC were detected under all three growing seasons on chromosomes 2, 5, 6 and 7, respectively. The contribution of phenotypic variation explained by individual QTLs ranged from 3.15–54.65%. Among these QTLs, *qGC2 and qGC6-2* was significant only in HZ 2015, *qGC6-2* in HN 2015 whereas, *qGC5, qGC6-2* and *qGC7* were detected only in HZ 2016. The phenotypic variation explained by these six QTLs were 3.15% of *qGC2,* 20.11% of *qGC6-2,* 3.70% of *qGC6-2,* 4.14% of *qGC5,* 6.59% of *qGC7,* 5.30% of *qGC6-2,* respectively. The allele of *qGC2, qGC6-2* and *qGC7* came from *Indica* rice YK17, which reduced GC by -4.07, -11.16 and -5.74 mm, respectively. The positive additive effect of, *qGC6-2, qGC6-2* and *qGC5* came from *Japonica* rice NIP. The major QTL, *qGC6-1* mapped on chromosome 6 between RM7158-RM3414 was detected over all three population growing conditions. The allele from *Indica* rice YK17 reduced the GC about -15.78 mm, -19.53 mm and -14.64 mm with the explained variation of 47.59, 54.65 and 44.8%, respectively and overlapped with *Wx* locus.

QTL for gelatinization temperature (GT): Total ten QTLs were identified for GT over three population growing conditions. A pair of QTLs (*qGT6-1* and *qGT6-2*) were detected repeatedly on all three locations and each pair

Table 1: Performance of cooking and eating quality traits of the parents and their RIL population in three cropping seasons

Year	Traits	Parents (mean ± SD)			RIL Population			
		Nipponbare	YK17	*P* value	Range	Mean ± SD	Skewness	Kurtosis
HZ 2015	AC (%)	19.19 ± 0.13	26.35 ± 0.38	0.002	10.89-29.35	20.29 ± 4.64	-0.59	0.08
	GC (mm)	81.33 ± 4.16	70.33 ± 0.01	0.013	30.00-94.67	54.52 ± 21.80	0.38	-1.06
	GT (ASV)	7.00 ± 0.00	6.6 ± 0.93	0.015	3.33-7.00	6 .00 ± 1.28	-1.32	1.16
	PC (%)	10.00 ± 0.01	7.79 ± 0.14	0.001	5.95-13.75	10.02 ± 1.42	-1.46	7.15
HN 2015	AC (%)	15.96 ± 0.26	28.50 ± 0.54	0.001	7.42-27.55	20.94 ± 6.61	-0.16	-1.68
	GC (mm)	90.33 ± 1.15	66.33 ± 1.53	<0.0001	30.00-97.00	60.46 ± 24.39	0.20	-1.30
	GT (ASV)	7.00 ± 0.00	5.50 ± 0.87	<0.0001	3.00-7.00	5.40 ± 1.36	-0.41	-1.19
	PC (%)	10.91 ± 0.27	7.45 ± 0.06	0.003	6.19-13.80	9.68 ± 1.65	-0.82	-0.87
HZ 2016	AC (%)	18.65 ± 0.21	28.35 ± 0.49	0.001	9.40-28.60	21.23 ± 6.06	-0.97	0.21
	GC (mm)	90.00 ± 1.73	71.00 ± 2.00	0.0002	30.00-97.00	66.86 ± 20.93	-0.59	-0.12
	GT (ASV)	6.81 ± 1.04	5.14 ± 2.02	0.001	3.00-7.00	5.44 ± 1.57	-0.96	0.12
	PC (%)	10.67 ± 0.00	7.62 ± 0.00	<0.0001	5.02-12.38	8.93 ± 1.40	-2.12	5.94

Data are presented as the mean ± standard deviation (SD)

AC amylose content, GC gel consistency, GT gelatinization temperature, PC protein content

Table 2: ANOVA results based on a fixed-effect model of the 193 RILs in three environments

Trait[1]	SoV[2]	*Df*[3]	MS[4]	*F*-value	*P*-value
AC	Lines	192	216.34	101.74[***]	<.0001
	Environments	2	152.12	18876.20[***]	<.0001
	Error	4	0.008		
GC	Lines	192	3130.63	205.10[***]	<.0001
	Environments	2	21404.034	8515.29[***]	<.0001
	Error	4	2.51		
GT	Lines	192	513.10	369.59[***]	<.0001
	Environments	2	2316.53	3495.39[***]	<.0001
	Error	4	0.66		
PC	Lines	192	9.57	104.30[***]	<.0001
	Environments	2	174.41	272605[***]	<.0001
	Error	4	0.001		

[1] AC, amylose content; GC, gel consistency; GT, gelatization temperature; PC, protein content

[2] SoV, Source of Variations; Lines, Seasons, Replications and Error

[3] *df*, degree of freedom

[4] MS, mean square

[***], significant at 0.001 *P*-value

Table 3: Coefficients of pairwise correlation for cooking and eating quality related traits from a RIL population derived from the cross of NIP × YK17

	AC	GC	GT
GC	-0.531[**a]		
	-0.443[**b]		
	-0.140[c]		
GT	0.747[**]	-0.496[**]	
	0.287[**]	-0.247[**]	
	0.740[**]	-0.115	
PC	-0.207[**]	0.316[**]	-0.164[*]
	0.433[**]	0.405[**]	-0.036
	0.420[**]	0.423[**]	0.365[**]

[a, b, c] represents Hangzhou in 2015, Hainan in 2015 and Hangzhou in 2016, respectively.

[*] and [**] are significant at 0.05 and 0.01 levels, respectively

mapped in same chromosomal interval, the average proportions of phenotypic variance explained by each pair were 25.38% of HZ 2015, 15.85% of HN2015 and 25.05% of HZ 2016, respectively, the additive effect for each pair were contributed both from *Japonica* rice NIP and *Indica* rice YK17. Moreover, *Indica* rice YK17 contributed additive effect to four from three pair of QTLs, demonstrating may have similarities in genetic mechanism. Four QTLs, *qGT4* detected in HZ 2015, *qGT1* and *qGT2* detected in HN 2016, whereas, *qGT9* was significant only in HZ 2016. The QTLs *qGT4*, *qGT1*, *qGT2* and *qGT9*

explained 3.85, 3.86, 3.10 and 4.42%, respectively. The positive additive effect of *qGT4* and *qGT2* was contributed from allele of *Japonica* rice NIP whereas, the allele for *qGT1* and *qGT9* came from *Indica* rice YK17.

QTL for protein content (PC): A major gene and five QTLs were identified for PC in three population growing seasons. The major gene *qPC6* located on chromosome 6 explained 9.73% of average phenotypic variation with average additive effect of 0.55%. The allele from *Indica* rice YK17 reduced *qPC6* in HZ 2015, HN 2015 and HZ 2016, respectively. The other QTLs, *qPC1*, *qPC2* and *qPC11*

Table 4: QTLs for cooking and eating quality related traits in RIL population of NIP × YK17

Trait	QTL	Candidate gene	Chr.	Interval	LOD	Var. %	Add.
HZ 2015							
AC	qAC5		5	RM249-RM18457	2.54	1.76	0.63
	qAC6-1	Wx	6	RM7158-RM3414	48.83	65.86	4.01
	qAC6-2	Wx	6	RM3414-RM276	17.28	74.46	4.23
GC	qGC2	SBE III	2	RM6509-RM13552	2.75	3.15	-4.07
	qGC6-1	Wx	6	RM7158-RM3414	28.72	47.59	-15.78
	qGC6-2	Wx	6	RM3414-RM276	10.64	20.11	-11.16
GT	qGT4		4	RM17143-RM349	3.11	3.85	0.26
	qGT6-1	Alk	6	RM7158-RM3414	25.07	41.65	0.89
	qGT6-2	Alk	6	RM276-RM5745	8.00	9.11	-0.54
PC	qPC1		1	RM1329-RM3746	5.10	9.38	-0.61
	qPC2		2	RM6233-RM5812	2.95	5.04	0.33
	qPC6	Wx	6	RM7158-RM3414	7.69	14.08	-0.57
	qPC11		11	RM27181-RM5926	2.87	5.94	-0.35
HN 2015							
AC	qAC1		1	RM5359-RM10782	2.62	1.56	-0.82
	qAC6	Wx	6	RM7158-RM276	59.48	89.51	6.57
GC	qGC6-1	Wx	6	RM7158-RM3414	25.95	54.65	-19.53
	qGC6-2	Wx	6	RM30-RM7434	2.89	3.70	5.00
GT	qGT1		1	RM3252-RM8068	3.14	3.86	-0.27
	qGT2		2	RM14001-RM7377	2.54	3.10	0.26
	qGT6-1	Alk	6	RM7158-RM3414	15.74	28.12	-0.92
	qGT6-2	Alk	6	RM276-RM5745	2.85	3.58	-0.34
PC	qPC3		3	RM282-RM15694	5.37	12.34	0.62
	qPC6	Wx	6	RM3414-RM276	2.53	7.26	-0.53
HZ 2016							
AC	qAC6	Wx	6	RM7158-RM276	48.37	82.16	5.70
GC	qGC5		5	RM18457-RM6054	3.54	4.14	4.30
	qGC6-1	Wx	6	RM7158-RM3414	28.20	44.8	-14.64
	qGC6-2	Wx	6	RM5745-RM30	4.14	5.30	5.33
	qGC7		7	RM3484-RM5875	5.53	6.59	-5.74
GT	qGT6-1	Alk	6	RM7158-RM3414	15.93	31.2	0.93
	qGT6-2	Alk	6	RM276-RM5745	4.52	6.85	-0.50
	qGT9		9	RM6839-RM160	3.16	4.42	-0.34
PC	qPC6	Wx	6	RM190-RM3414	3.08	7.85	-0.41
	qPC7		7	RM3859-RM11	2.63	5.78	0.34

were significant in HZ 2015, *qPC3* in HN 2015, and *qPC7* in HZ 2016, respectively. The total phenotypic variation explained by *qPC2, qPC3* and *qPC7* was 5.04, 5.78 and 12.34%, respectively. The positive additive effects of all these QTLs were derived from allele of NIP, respectively.

Detection of QTLs with 'Additive × Environment' and Epistasis Interactions

To get more in depth understanding of genetic architecture of ECQs attributes, the digenic epistatic effects of AC, GC, GT and PC were estimated. Seven QTLs (*qAC6, qGC5, qGC6, qGT6.1, qGT6.2, qGT9* and *qPC6)* were detected by joint analysis of AC, GC, GT and PC of three population growing seasons having significant additive × environment interaction, respectively (Table 5). Four QTLs (*qAC6, qGT6.2, qPC6* and *qPC7*) were highly significant for additive × environment interaction. One QTL, *qPC7* was not detected by CIM analysis but the additive × environment interaction results showed highly significant providing evidence of environment effect (Table 5). The phenotypic contribution rate was relatively higher, which suggested that the QTLs expression of AC, GT and PC were largely influenced by the environmental conditions. To elaborate

understanding for genetic components of these attributes, four locus bi-allelic epistatic interactions were estimated except for GC (Table 6). The epistatic interaction also played an important role in determining rice grain quality. The attributes AC, GT and PC showed several pairs of epistatic loci, however, GC exhibited non-significant epistasis effect indicating M-QTLs controlling GC. AC exhibited three pairs of epistatic loci, which elucidated 1.43, 1.69 and 2.14% of phenotypic variation, respectively. Three pairs of epistatic loci were detected for GT and accounted for 1.20, 1.56 and 1.56% of the phenotypic variation, respectively. Meanwhile, one pair of epistatic loci were scanned for PC with explained phenotypic variation of 2.22%.

The Sequence Analysis of *Wx* Gene Controlling Cooking and Eating Quality Traits

The sequence results of *Wx* gene showed several SNPs and Indels in the genomic region of YK17 in comparison to the Genome sequenced NIP (Fig. 3). These detected SNPs and Indels of YK17 were consistent with the selected investigated RIL population lines i.e. 5RIL, 8RIL, 17RIL, 35RIL and 72RIL. Whereas, selected RIL population lines *i.e.,* 22RIL, 54RIL, 73RIL, 98RIL and 129RIL were

Table 5: QTLs with additive × environment interaction for AC, GC, GT and PC detected in RIL population under three trials

Trait/QTL	Marker Interval	Marker (Position, c*M*)	Range	A[a]	AE1	AE2	AE3	R[2] (%)
AC								
*qAC6**	RM7158-RM190	4	3.0-5.2	5.1432	-1.017**	0.9683**	0.0686**	80.75
GC								
*qGC5**	RM18457-RM163	105.7	96.7-115.9	3.8907	-0.0001	0.0001	-	2.86
*qGC6**	RM190-RM3414	4.2	3.0-5.2	-15.7712	-	-0.0001	0.0001	46.99
GT								
*qGT6.1**	RM7158-RM190	3	2.0-5.2	0.4904	0.0003	-0.0004	0.0001	11.44
*qGT6.2**	RM276-RM5963	36.1	33.1-39.5	-0.7537	0.0486**	-0.0491**	0.0014**	27.01
*qGT9**	RM257-RM160	45.4	42.6-51.4	-0.1665	0.0003	-	-0.0003	1.32
PC								
*qPC6**	RM7158-RM190	2	0.0-7.2	-0.4127	-0.0276**	0.0046*	0.0235**	7.2
qPC7	RM3859-RM214	57.4	52.3-65.8	0.2993	-0.0761**	0.1687**	-0.0909**	3.79

A[a], Significant additive effects contributed by QTL mapped in the environments
AE1, AE2, AE3, represent the additive effects of significant QTL× environment interaction in three locations (HZ 2015, HN 2015 and HZ 2016)
*QTL were also detected in CIM analysis

Table 6: Epistatic interaction for AC, GC, GT and PC detected in RIL population under three trials

Trait	Chr	Marker Interval	Chr	Marker Interval	aa$_{ij}$	R[2]%
AC	3	RM14327-RM6849	8	RM3702-RM1376	-0.685	1.43
	3	RM14327-RM6849	6	RM5963-RM5745	0.745	1.69
	3	RM3204-RM15281	8	RM3845-RM447	0.837	2.14
GT	6	RM7158-RM190	6	RM276-RM5963	0.502	1.20
	6	RM276-RM5963	7	RM6403-RM234	0.181	1.56
	8	RM408-RM3702	11	RM286-RM26062	0.181	1.56
PC	5	RM249-RM18349	9	RM24085-RM6839	-0.229	2.22

AAij: Additive × additive epistatic effect, positive value indicates parental type>recombinant type; negative value indicates parental type<recombinant type

genetically consistent with NIP. Notably, there were two SNPs at exon 9 and exon 10. The SNP of exon 9 does not alter amino acid whereas, the SNP at exon 10 altered amino acid from proline to serine whereas, the 2kb promoter region of *Wx* gene shown three Indels.

Discussion

Rice grain quality is evaluated by complex attributes, including grain appearance, milling, eating and cooking, and nutritional aspects (Wu *et al.,* 2015). All of these attibutes are multigenic in nature and being influenced by major or minor genes along with their epistatic and enviornmental interactions effects. The analysis for major contributing genes/QTLs is very important to understand grain quality traits (Fiaz *et al.*, 2019). The interaction among major or minor genes may holds a significant effect in determination of these attributes. Sometimes, major or minor genes interaction is equally affected by the variation in the environmental conditions (He *et al.,* 2006). From the past few decades, the goal of high yield is greatly fulfilled through breeding for semi dwarf cultivar and hybrids of compromised grain quality, which ultimately needs the attention of researchers (Leng *et al.,* 2014). In present investigation, AC, GC, GT and PC were analysed for their QTLs, epistatic QTLs and QEs association under all three population growing seasons.

AC is an important determinant for rice eating quality and the QTL *qAC6* was detected on the corresponding position of *Wx* locus and confirmed the results derived from several other mapping populations. The QTL *qAC6-1* and

qAC6-2 are of significantly important to understand allelic diversity within *Wx* gene region. GC is fundamental to determine the softness of high amylose rice accessions and a good indicator for texture of cooked rice. Our findings showed AC exhibited a highly negative significant relation to GC a confirmation of previous findings (Li *et al.,* 2003). The chromosome 2 harboured QTL *qGC2* which seemed to be flanked with the same locus confirming starch branching enzyme III (*SBE III*). The enzyme *SBE III* was mapped on Chromosome 2 responsible to amylopectin biosynthesis and structure. It was also observed that GC largely influenced by amylopectin instead of amylose (Fan *et al.,* 2005). However, the significant QTL was detected repeatedly on chromosome 6 showing *Wx* is the major determinant for GC confirming the findings by other researchers (He *et al.,* 2006; Su *et al.*, 2011). GT is recognized as one of the key attributes responsible for the determination of cooking quality. In various studies, researchers have testified that chromosome 6 harboured the major genomic region for GT parallel to alkali degeneration locus (*Alk*), which encodes a soluble starch synthase II (Takeuchi *et al.,* 2007). In present investigation, QTL *qGT6-1* was detected at locus similar to reported gene *Alk*. However, the QTLs *qGT6-1* and *qGT6-2* are worthy to understand the existence of allelic diversity in *Alk* gene. The natural existence of allelic diversity in *Alk* gene have significant effects on starch composition and structure (Umemoto *et al.,* 2004; Gao *et al.,* 2011; Zhang *et al.,* 2017). PC an integral component that influences ECQs along with grain's nutritional quality (Aluko *et al.,* 2004). Earlier several studies to detect QTL for PC have been conducted and reported PC had either weak or no correlation with other rice

grain quality characteristics (Zheng *et al.*, 2008). However, our findings shown highly significant to significant correlation of PC with other studied quality attributes except in HN 2015. The repeated detection of *qPC6* confirmed the existence of QTL corresponding to the *Wx* genomic region. The *qPC1*, a major QTL for PC on chromosome 1 was detected only under HZ 2015 population growing season and was not detected repeatedly. The discrepancy of present results may be due to the differences of germplasm evaluated and location of experiment. In the present study, an in-depth understanding of genetic basis for ECQs was obtained by analysing the and epistatic QTLs. The additive × environment interactions had shown phenotypic contribution rate relatively higher, suggested that the QTLs expression of AC, GT and PC were largely influenced by the environmental conditions except for GC, showed dominant influence of QTL. For epistatic interaction AC, GT and PC were observed in epistatic interaction excluding GC. The sum effects of QTLs were higher in comparison to corresponding epistatic QTLs suggesting that the QTL is the primary genetic basis for these traits.

The *Wx* gene locus contained three functional alleles: Wx^a, Wx^b and Wx^{op} found mainly in *Japonica*, *Indica* and *Waxy* rice, respectively (Hoai *et al.*, 2014). Rice cultivars with the Wx^a allele accumulate more GBSSI protein at grain filling stage and hence have more AC content than Wx^b predominating cultivars. However, Wx^a and Wx^b are not enough to foster all the observed variation in ECQs. Teng *et al.* (2012) identified wx, Wx^t, Wx^{g1}, Wx^{g2}, and Wx^{g3} haplotypes with potential to provide suitable criteria for selecting lines with the desired AC in rice grain quality improvement programs. The degree of GBSS activities of these five different *Wx* alleles were in the order $Wx^{g3} > Wx^{g2} > Wx^{g1} > Wx^t > wx$ and the differences were at the significance level among different AC groups, supporting the classification of the five functional alleles. Therefore, GBSS activity profiles of the *Wx* alleles laid a foundation for identification of different *Wx* alleles and provided valuable insights into the physiological mechanism underlying the allelic variation at the *Wx* locus. For the *Wx* alleles reported previously, the wx, Wx^a, and Wx^b alleles were identical to wx, Wx^t, and Wx^{g1} alleles. In present study, the gene sequence analysis result had shown many allelic variations of YK17 an *Indica* super rice with reference to genome sequenced NIP and these variations were constant with RIL population lines possessing ECQs like NIP and YK17 (Fig. 3 and Table 6). Notably, the male parent YK17 and RILs with corresponding ECQs possess three Indels in the promoter region of *Wx* gene and two SNPs in the coding region at exon 9 and exon 10. The SNPs of exon 9 and exon 10 are also reported in previous studies (Umemoto *et al.*, 2004). The SNP of exon 9 does not alter amino acid whereas; the SNP at exon 10 altered amino acid from proline to serine. The Wx^{g2} allele has a C-to-T substitution in exon 10, detected simultaneously in YK17 and RILs of similar ECQs. Teng *et al.* (2012) demonstrated that the exon

10 SNP was the cause of the AC phenotypic diversity between the high I and high II AC lines, and used it to subdivide the Wx^a allele into high I AC Wx^{g2} and high II AC Wx^{g3} alleles. The Indels in the promoter region are of significant importance and the outcomes of these allelic variations will enhance the study on the genetic evolution of the *Wx* locus and play an integral role to understand the mechanism for the improvement of ECQs in rice.

Conclusion

There were significant implications of minor QTLs, epistatic QTLs and additive × environment interactions along with QTLs on rice grain quality improvements programs. The information will help overcome the problems associated with eating and cooking quality attributes which is particularly severe in hybrids and early season rice cultivar of the multiple cropping systems of China. Moreover, marker assisted selection will be easy and useful in breaking the unfavourable association of genes, such as in the case of present study the major QTLs are detected on chromosome 6 enabling the combination of all favourable alleles in a single individual, which would be only possible through modern breeding methods. Moreover, the existence of allelic diversity in *Wx* gene of YK17 can be explored further to find out more functional alleles on *Wx* locus.

Acknowledgements

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