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QTL MAPPING FOR GRAIN APPEARANCE QUALITY TRAITS USING DOUBLED HAPLOID POPULATION OF RICE UNDER DIFFERENT ENVIRONMENTS

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Abstract

Grain quality is an intrigue attribute with great importance as yield to any rice breeding program. In this study, quantitative trait loci (QTLs) analysis was performed for rice grain appearance quality traits, a double haploid (DH) population derived from Zhongjiazao 17, a super rice variety, crossed with tropic *Japonica* variety D50. The seven appearance quality traits of DH population grown under three different environmental conditions were measured based on a linkage map containing 170 SSR markers. Total of 73 QTLs were identified covering all chromosome except chromosome 12. The main focus of the study was to identify QTLs that were stable under all different environments. However, four QTLs including *GL3*, *GW3*, *LWR3* and *TGW3* found in the surrounding of pericentromeric region of chromosomes 3. These QTLs, found responsible for grain length and grain width. *GL3*, a major gene with *qGS3* was repeated in all three growth conditions, particularly interval RM6929-RM15490 was detected and stable in all three environments with phenotypic variations of 34.97%, 21.32% and 33.0%. In addition, for digenic interaction, a total of 8 QTLs on five traits were identified for additive environment interaction. Conclusively, QTLs *qPGC-1*, *qTGW-3* and *qCS-1* had high phenotypic variation and additive effect that could improve the Grain appearance quality and such findings are valuable for future map-based cloning of the new detected QTLs and improving grain appearance quality and yield.

Key words: Appearance quality; QTL mapping; double haploid population (DH); Rice grain.

Introduction

It is imperative to increase the yield of rice because it's a major source of food for more than a half of the world population (Ishimaru *et al*., 2013). The demand of premium quality rice is increasing and becoming priority for rice production system due to rise in consumers' living standards (Tahmina *et al*., 2020, Sheng *et al*., 2018). Rice grain quality is composed of complex attributes including milling, appearance, cooking and eating, and nutritional aspects. However, the simultaneous improvement of yield and grain quality traits is a great challenge for rice breeders (Gao *et al*., 2016).

Grain shape, mainly evaluated by grain length (GL), grain width (GW), grain thickness (GT) and length width ratio (LWR), is closely associated with grain weight and plays a pivotal role in grain appearance quality (Lin & Wu, 2003; Yoon *et al*., 2006). Chalkiness is another important attribute related to grain appearance that greatly influences consumer acceptance in domestic and international markets. Meanwhile, the phenotypic variations have extensively been observed among cultivars, species, and subspecies of *Oryza sativa* L. Recently, several classical and modern plant breeding efforts have been undertaken to identify QTLs/genes responsible for rice grain appearance quality. Several studies have confirmed that grain appearance traits i.e., GL, GW, LWR, GT, thousand grain weight (TGW), percentage of grain chalkiness (PGC) and chalkiness score (CS) are quantitative in nature (Saho *et al*., 2010; Bai *et al*., 2010; Oh *et al*., 2011). Additionally, hundreds of QTLs for grain appearance have been identified in various QTL analyses (Tahmina *et al*., 2020; Fiaz *et al*., 2019) and scattered throughout the whole rice genome (Wan *et al*., 2006). Fan *et al*., (2006) cloned *qGS3*, a major QTL located on chromosome 3 controlling grain length and weight and encoding a putative transmembrane protein which negatively regulates grain size. *GW2*, encoding a RING-type E3 ubiquitin ligase, is a QTL governing of grain width and weight. It is reported that the loss of function in *GW2* resulted in enhanced grain width, weight and yield (Song *et al*., 2007). Another major QTL *GS7/qSS7* associated with grain length have been cloned by Wang *et al*., (2012) on chromosome 7. Similarly, on the basis of loss of function, another gene (*qSW5/GW5*) associated with grain shape has been cloned and mapped on chromosome 5. The analyses indicated that a 1212-bp deletion in this gene was associated with increased grain width. Moreover, *GS5*, cloned in the adjacent region of *qSW5/GW5*, encodes a putative serine carboxypeptidase and functions as a positive regulator of grain size (Li *et al*., 2011). *GW8* is also a positive regulator for grain width and yield, interestingly, high expression of this gene promotes cell division and grain filling, with positive consequences on grain width and yield in rice (Wang *et al*., 2012). Additionally, Ishimaru *et al*., (2013), cloned and functionally analyzed the *TGW6* locus for TGW, which the results indicated that *TGW6* encodes an IAA-glucose hydrolase, which enhances rice grain weight and increases yield when it loses function.

Appearance quality

For grain chalkiness, *chalk5* has been previously cloned. It does encode a vacuolar H^+-translocatingpyrophosphatase (V-PPase), and its elevated expression increases the chalkiness of the endosperm (Li *et al*., 2014). A recent study validated another QTL (*qPGC8-2*) of grain chalkiness on chromosome 8 (Yang *et al*., 2021) The transgenic lines with suppression of the isoamylase gene (*ISA1*, *LOC_Os08g40930*), residing on the region of *qPGC8-2*, and produced grains with 20% more chalkiness than the control (Sun *et al*., 2015). Further more, Zhou *et al*., (2009), identified a PGWC QTL, *qPGWC-7*, to a 44-kb DNA fragment that contained 13 predicted genes. These advances enhanced the understanding of the genetic and molecular basis of grain appearance quality and grain weight.

In present study, we investigated the grain appearance properties using DH population derived from an early season *Indica* rice cultivar Zhongjiazao17 (YK17) and a tropic *Japonica* rice cultivar D50. The genetic linkage map was employed to map the locus underlying seven parameters of appearance quality in different environments. The identification of QTLs related to grain appearance is important to utilize in the improvement of rice appearance quality by marker assisted selection. Thus; the main purpose of this study was (i) to find stable QTLs (*qGL-3, qGW-7, qTGW-3 and qPGC-5*) related to rice appearance quality from the DH population grown under different environments, (ii) to investigate the grain appearance properties using DH population derived from a combination between early season *Indica* rice cultivar (Zhongjiazao17, YK17) and a tropic *Japonica* rice cultivar (D50), (iii) to employ the genetic linkage map to understand the different parameters of appearance quality under three different environments.

Material and Methods

Plant materials and field conditions: A double haploid (DH) population consisting of 101 lines was constructed from a cross between *Indica* super rice YK17 and tropic *Japonica* rice D50. Field trails were conducted in Hangzhou (HZ), China in 2017 and 2018, whereas in Hainan province (HN), China only in 2017. The field trials in HZ 2017 and HZ 2018, grains were sown in May and seedlings were transplanted in June, while the field trials in HN 2017, the grains were sown in November and seedlings were transplanted into field in December. Each plot consisted of four rows of 24 plants at a spacing pattern of 25 by 20 cm between and within rows respectively. In triplicates randomized complete block (RCB) were designed and the management practice was same as normal field management in all the three different environments. Each plot of DH population was harvested at maturity and dried naturally at room temperature.

Phenotypic evaluation: Five yield-related traits including grain length (GL, in millimeters), grain width (GW, in millimeters), ratio of grain length to width (LWR), grain-thickness (GT, in millimeters) and 1000-grain weight (TGW, in grams) the percentage of grain with chalkiness (PGC) and chalkiness score CS were examined on the grains collected from eight randomly selected plants from the middle of the rows of each plot. Ten whole rice grains were chosen randomly from each plant for trait measurement.

QTL analysis: QTLs controlling appearance quality were mapped using Windows QTL Cartographer Version 2.5 (Win QTL Cart 2.5) (Wang, 2007) with the composite interval mapping (CIM), and LOD value of 2.5 was set as threshold for the detection of putative QTLs. QTL-by-environment interaction (QEs) effects were analyzed using QTL Network-2.1, with the mixed-model-based composite interval mapping (MCIM) (Wang, 2007).

Data analysis

The SPSS 20 statistical package was used to analyze the data by student's *t-test*, and correlation co-efficient of traits. However, frequency distribution was calculated by using MS Excel software.

Results

Phenotypic performance of appearance quality traits of Parents and DH population: The appearance traits of parents and DH population were described in Table 1. Over three rice growing seasons, the variation in parents and DH population have showed highly significant differences among all the traits under investigation. It was found that D50 had higher phenotypic variation for GL, and LWR, whereas YK17 had significant variation for GW, TGW, GT and CS. All the seven traits studied in present research, ranged widely and indicated that these traits were quantitatively inherited and suitable for QTL analysis (Figs. 1 and 2).

Correlations among seven traits controlling grain appearance quality: The results of phenotypic correlation analysis were presented in Table 2. All the traits under investigation showed correlation ranging between -0.0273 to 0.8936. There was highly significant correlation of GL with GW, LWR, TGW and PGC, except GT and CS which were non-significant under all three environments. However, GW was positively significant with all traits in all environments but negatively associated with LWR. The correlation of LWR with GT, PGC and CS was negatively significant in all environmental conditions. However, LWR showed non-significant correlation with TGW in all environments. Meanwhile, the correlation of GT with other traits such as PGC and CS was highly positive but significantly negative to TGW in all environments. A strong positive correlation of TGW with PGC and CS was recorded under all three environments. Similarly, highly positive correlation was found between PGC and CS under all three environmental conditions.

QTL analysis of appearance quality traits: Total 73 QTLs were identified in the present study for 7 appearance quality traits GL, GW, LWR, GT, TGW, PGC and CS by employing Win QTL Cart 2.5 based on CIM method. The QTLs identification is summarized in Table 3. These QTLs were detected on all chromosomes except chromosomes 12 with single QTL explaining 6.11-34.97% of phenotypic variation. Among the detected 73 QTLs, 11 QTLs for GL, 11 for GW and 9 for LWR, 10 for GT, 14 for TGW, 8 for PGC and 9 QTLs for CS were detected. Some of these QTLs were repeatedly detected across the three environments such as *qGL3-3, qGL-10, qGW3, qGW-7, qGW-10, qLWR-7, qLWR-10, qGT-1, qGT-3, qPWC-1* and *qCS-3*.The QTL positions and their biometrical parameters were shown in Table 3.

Table 1. Performance of appearance quality traits of the parents and their DH population in three cropping seasons.

Traits	Parents (mean ± SD)			DH population			
	YK17	D50	*p*-value	Mean±SD	Range	Skewness	Kurtosis
				E1HZ 2017			
GL (mm)	7.94 ± 0.126	9.93 ± 0.084	0.001**	9.013 ± 0.65	7.42-10.3	-0.602	0.104
GW (mm)	2.73 ± 0.053	2.34 ± 0.015	0.002**	2.501 ± 0.13	2.06-2.73	-0.464	1.182
LWR (mm)	3.41 ± 3.992	3.85 ± 5.201	0.210	4.36 ± 0.447	2.77-4.58	0.029	0.039
GT (mm)	1.96 ± 0.012	1.86 ± 0.013	0.031*	1.90 ± 0.04	1.72-1.98	-1.483	3.357
TGW (g)	25.1 ± 0.18	23.4 ± 0.307	0.028*	25.72 ± 3.87	14.7-38.7	0.338	0.998
PGC (%)	96.5 ± 2.121	47.2 ± 8283	0.022*	71.89 ± 24.3	13.5-98.6	-1.410	2.864
CS (cm)	45.83 ± 6861	16.5 ± 2298	0.015*	39.7 ± 12.53	14.2-67.3	0.134	-0.64
				E2 HN 2018			
GL (mm)	7.89 ± 0.053	9.95 ± 0.069	0.000**	9.01 ± 0.631	7.35-10.27	-0.567	8.563
GW (mm)	2.60 ± 0.014	2.22 ± 0.123	0.026*	2.09 ± 0.115	2.15-2.77	-0.496	1.002
LWR (mm)	3.62 ± 4.046	4.09 ± 5.085	0.221	3.52 ± 0.352	2.77-4.48	-0.013	0.025
GT (mm)	1.92 ± 0.025	1.80 ± 0.027	0.020*	1.90 ± 0.05	1.75-1.97	-1.311	2.380
TGW (g)	27.0 ± 0.153	23.1 ± 0.203	0.000**	26.9 ± 3.593	15.0-39.25	-0.096	1.814
PGC (%)	82.7 ± 1.524	43.7 ± 5.036	0.001**	63.7 ± 25.05	8.0-96.71	-0.700	-0.65
CS (cm)	48.6 ± 56.85	35.8 ± 45.57	0.012*	30.53 ± 14.3	2.98-57.34	-0.277	-1.10
				E3 HZ 2018			
GL (mm)	7.86 ± 0.037	9.98 ± 0.028	0.000**	9.01 ± 0.667	7.47-10.30	-0.522	0.062
GW (mm)	2.64 ± 0.006	2.24 ± 0.042	0.001**	2.48 ± 0.143	2.03-2.79	-0.088	0.937
LWR (mm)	3.52 ± 3.985	3.78 ± 5.193	0.189	3.64 ± 0.394	2.77- 4.79	0.051	0.525
GT (mm)	2.04 ± 0.022	1.90 ± 0.04	0.001**	2.021 ± 0.12	1.76- 2.0	-0.689	0.240
TGW (g)	24.7 ± 0.808	22.5 ± 0.604	0.005**	27.62 ± 3.98	16.2-39.1	0.305	0.923
PGC (%)	83.7 ± 2.525	58.7 ± 9.292	0.013*	71.1 ± 22.14	7.0-97.7	-0.934	0.170
CS (cm)	51.2 ± 59.68	28.4 ± 41.14	0.014*	40.19 ± 13.6	2.7-75.4	-0.379	0.220

Data are presented as the mean ± standard deviation (SD), GL, Grain length, GW, Grain width, LWR, Length width ratio, GT, Grain thickness, TGW, 1000-grain weight, PGC, Percentage of grain with chalkiness, CS, Chalkiness score

Table 2. Coefficient of pairwise correlation for appearance quality traits from a DH population derived from YK17×D50 under three different environments.

Traits	Environment	GL (mm)	GW (mm)	LWR (mm)	GT (mm)	TGW (g)	PGC (%)
GW (mm)	E1-HZ 2017	-0.3429**					
	E2-HN 2017	-0.4010**					
	E3-HZ 2018	-0.2849**					
LWR (mm)	E1-HZ 2017	0.8642**	-0.7646**				
	E2-HN 2017	0.8936**	-0.7656**				
	E3-HZ 2018	0.8481**	-0.7440**				
GT (mm)	E1-HZ 2017	-0.2248	0.8410**	-0.2464**			
	E2-HN 2017	-0.2163	0.6869**	-0.2185**			
	E3-HZ 2018	-0.2410*	0.4426**	-0.2233**			
TGW (g)	E1-HZ 2017	0.3157**	0.2796**	-0.5288	-0.0810**		
	E2-HN 2017	0.2832**	0.4354**	-0.0101	0.1384**		
	E3-HZ 2018	0.3182**	0.2580**	-0.0365	-0.2074**		
PGC (%)	E1-HZ 2017	-0.2116**	0.3423**	-0.3356**	0.5638**	0.1519**	
	E2-HN 2017	-0.2523**	0.4304**	-0.3913**	0.2959**	0.2186**	
	E3-HZ 2018	-0.2337**	0.3274**	-0.3506**	0.3760**	0.2722**	
CS (cm)	E1-HZ 2017	0.0851	0.1974**	-0.222*	0.4328**	0.497**	0.6140**
	E2-HN 2017	0.0130	0.2159**	-0.214*	0.2823**	0.3463**	0.8787**
	E3-HZ 2018	-0.0273	0.2493**	-0.253**	0.3327**	0.4240**	0.6603**

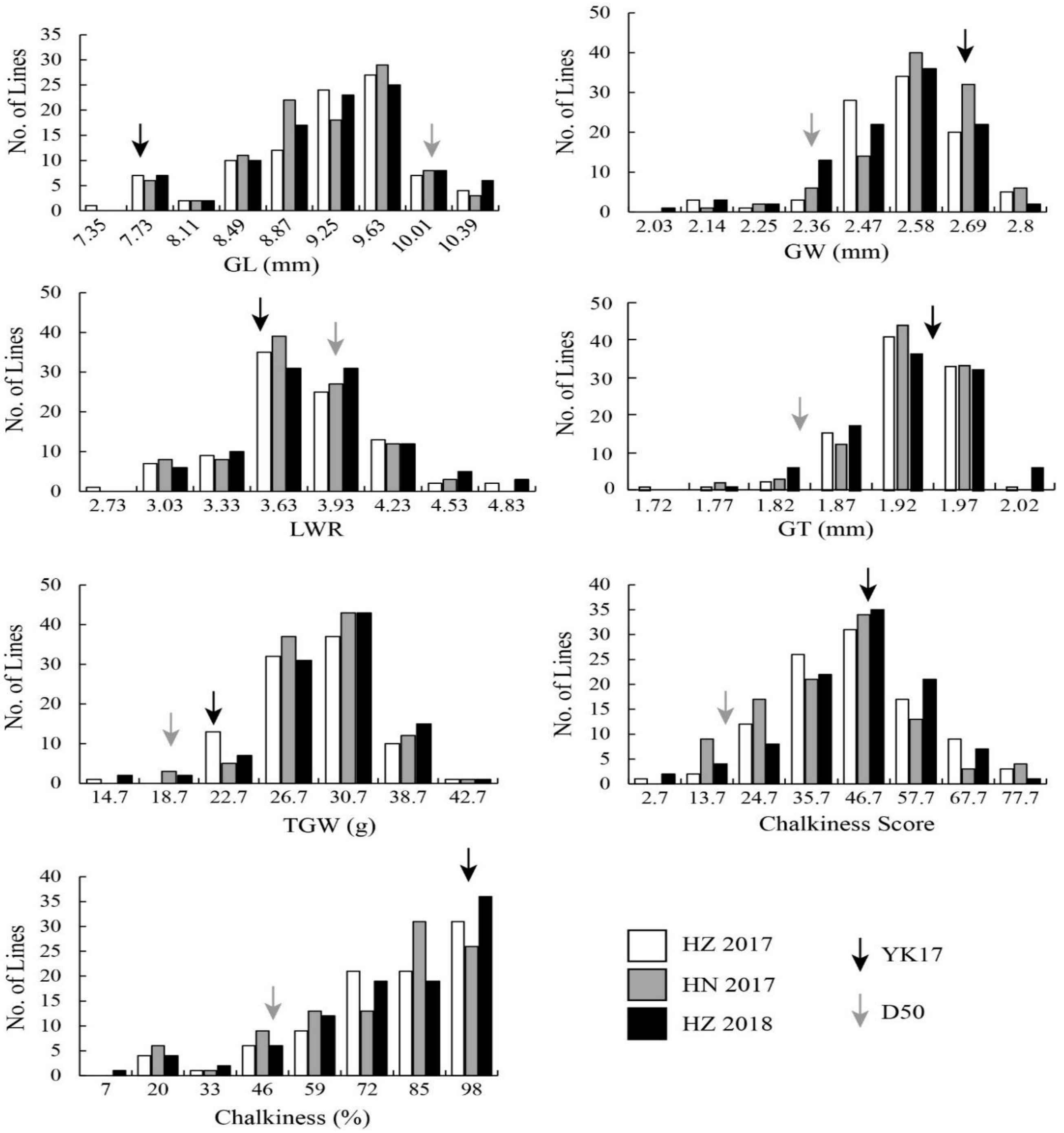

Fig. 1. Phenotypic distribution of seven appearance quality traits (GL, GW, LWR, GT, TGW, PGC and CS) in the YK17 ×D50 DH population across three growing conditions. Mean value of YK17and D50 from three environments showed above with arrow.

QTLs for GL: Total 11 QTLs for GL were identified, with four QTLs being significant in each environment. the QTLs *qGL-2,qGL-3*, *qGL-6* and *qGL-10* were detected in Environment 1 (E1) and located on chromosomes 2, 3 ,6, and 10, their phenotypic variations were 13.75%, 34.97%,8.26% and 19.97% respectively. 2 QTLs, *qGL-2* and *qGL-6* exerted negative effect whereas, another 2 QTLs showed positive effect, which suggested the QTLs derived from female parent YK17 had negative effects on the appearance quality. The major QTL *qGL-3* flanked by marker interval RM6959-RM15490 on chromosomes 3, was detected repeatedly under three environments, the additive affect came from D50 were 0.39mm in E1, 0.34mm in E2 and 0.41mm in E3. In addition, 3 QTLs, *qGL-4, qGL-8 and qGL-9* were identified in E2, and their total phenotypic variance was explained as 6.70%, 7.23% and 10.36%, respectively. Interestingly, the additive effect of these QTLs from YK17 reduced phenotypic variations of 0.17mm, 0.21mm, and 0.41mm, respectively. Furthermore, one more stable QTL *qGL-10* was detected on chromosome 10, repeated under all three environmental conditions, with the highest LOD value of 8.66. However, the phenotypic variation of *qGL-10* was 19.17% in E1, 7.01% in E2 and 8.66% in E3. The additive effect of *qGL-10* came from D50 increased 0.29 mm in E1, 0.21mm in E2 and 0.20mm in E3, respectively.

Table 3. QTLs for appearance quality related traits in DH population in three different planting seasons.

Traits	QTL	Chr.	Marker interval	LOD	Var %	Add.
			E1			
GL	*qGL-2*	2	RM341-RM13418	7.92	13.75	-0.23
	qGL-3	3	RM6929-RM15490	11.75	34.97	0.39
	qGL-6	6	RM3805-RM19521	3.76	8.26	-0.23
	qGL-10	10	RM1375-RM25741	7.38	19.17	0.29
GW	*qGW-3*	3	RM14898-RM6914	2.52	7.60	-0.03
	qGW-4	4	RM7585-RM16335	3.46	12.85	0.04
	qGW-7	7	RM2752-RM-234	4.66	15.22	-0.05
	qGW-10	10	RM311 -RM25366	3.20	9.70	0.45
LWR	*qLWR-3*	3	RM6929-RM15490	8.02	26.67	0.19
	qLWR-4	4	RM7585-RM16335	4.00	12.31	-0.15
	qLWR-7	7	RM2752-RM234	5.86	15.87	0.14
GT	*qGT-1*	1	RM8071-RM129	4.37	13.81	-0.01
	qGT-3	3	RM7197- RM6929	2.50	7.18	-0.01
	qGT-5	5	RM1024-RM17960	9.17	26.96	-0.03
	qTGW-1	1	RM8068-RM1329	2.64	6.11	1.04
TGW	*qTGW-3*	3	RM15490-RM3601	4.48	13.69	1.62
	qTGW-5	5	RM1024-RM17863	3.27	9.43	-1.79
	qTGW-7	7	RM234-RM248	8.95	24.99	-2.15
	qTGW-10	10	RM311-RM1375	3.28	7.37	1.23
	qTGW-11	11	RM6680-RM27230	3.35	10.00	-1.36
PGC	*qPGC-1*	1	RM10316-RM8071	4.09	30.18	-19.35
	qPGC-3	3	RM3199-RM7389	9.27	31.18	9.57
	qPGC-9	9	RM24537-RM6854	3.36	20.17	12.70
CS	*qCS-1*	1	RM7405-RM11307	9.74	24.76	-7.80
	qCS-3	3	RM3684-RM1373	3.02	7.06	3.48
	qCS-5	5	RM1024-RM18448	3.68	7.52	-5.17
			E2			
GL	*qGL-3*	3	RM6929-RM15490	7.92	21.32	0.34
	qGL-4	4	RM16852-RM3839	2.61	6.70	-0.17
	qGL-8	8	RM22418-RM23174	2.63	7.23	-021
	qGL-9	9	RM24537-RM6797	3.15	10.36	-041
	qGL-10	10	RM25664-RM25798	2.85	7.01	0.21
GW	*qGW-3*	3	RM14898-RM6929	3.24	8.27	-0.04
	qGW-4	4	RM7585-RM16335	3.95	12.26	0.05
	qGW-7	7	RM2752-RM118	5.69	17.25	-0.07
	qGW-10	10	RM25366-RM1375	3.56	9.63	0.043
LWR	*qLWR-3*	3	RM6929-RM3601	4.93	17.11	0.05
	qLWR-7	7	RM2752-RM118	4.71	13.11	0.03

Table 3. (Cont'd.).

Traits	QTL	Chr.	Marker interval	LOD	Var %	Add.
GT	*qGT-1*	1	RM10782-RM7405	6.89	15.55	-0.34
	qGT-5.1	5	RM1024 -RM17960	2.69	5.75	-0.01
	qGT-5.2	5	RM18448-RM163	2.53	7.51	-0.41
TGW	*qTGW-1*	1	RM10782-RM11307	2.52	6.86	-0.10
	qTGW-3	3	RM5488-RM15490	6.69	32.26	1.95
	qTGW-5	5	RM1024-RM17863	3.09	10.43	-1.86
	qTGW-9	9	RM6491-RM6797	3.74	10.28	1.89
PGC	*qPGC-1*	1	RM7405-RM11307	2.72	11.63	-9.28
	qPGC-5	5	RM17960-RM18448	5.06	19.30	15.9
CS	*qCS-3*	3	RM3684-RM1373	3.26	16.46	5.41
	qCS-8.1	8	RM22448-RM3262	3.19	9.55	-5.28
	qCS-8.2	8	RM23174-RM3262	2.91	9.47	-4.98
			E3			
GL	*qGL-3*	3	RM6929-RM15490	9.72	33.0	0.41
	qGL-7.1	7	RM3859-RM11	3.76	9.36	-0.26
	qGL-7.2	7	RM234-RM118	3.66	10.09	0.21
	qGL-10	10	RM1375-RM25741	3.54	8.66	0.20
GW	*qGW-3*	3	RM7585-RM16335	4.05	13.83	-0.05
	qGW-7	7	RM2752-RM234	2.53	8.85	-0.04
	qGW-10	10	RM311-RM25366	3.48	11.77	0.45
LWR	*qLWR-3*	3	RM6929-RM3601	9.79	29.61	0.22
	qLWR-4	4	RM7585-RM16335	3.09	8.87	-0.12
	qLWR-7	7	RM3805-RM19620	3.25	8.04	-0.13
	qLWR-8	8	RM3702-RM22418	2.87	10.18	-0.14
GT	*qGT-1*	1	RM212-RM3520	3.44	11.48	-0.01
	qGT-5.1	5	RM1024-RM17960	3.30	4.39	-0.02
	qGT-5.2	5	RM17960-RM18448	2.60	12.53	-0.02
	qGT-9	9	RM4854-RM24537	3.29	33.57	0.04
TGW	*qTGW-1*	1	RM8068-RM1329	4.07	15.36	1.80
	qTGW-3	3	RM15490-RM3601	6.78	22.76	2.11
	qTGW-5.1	5	RM1024-RM17863	4.19	11.98	-2.10
	qTGW-5.2	5	RM3170-RM5907	2.83	6.70	-1.19
PGC	*qPGC-1*	1	RM7405-RM11307	3.42	16.26	-10.45
	qPGC-3	3	RM1024-RM18663	4.39	17.66	-13.97
	qPGC-5	5	RM3765-RM5463	2.48	8.45	-7.28
CS	*qCS-1*	1	RM7405-RM11307	4.11	14.64	-6.00
	qCS-3	3	RM3684-RM16115	2.81	7.43	4.69

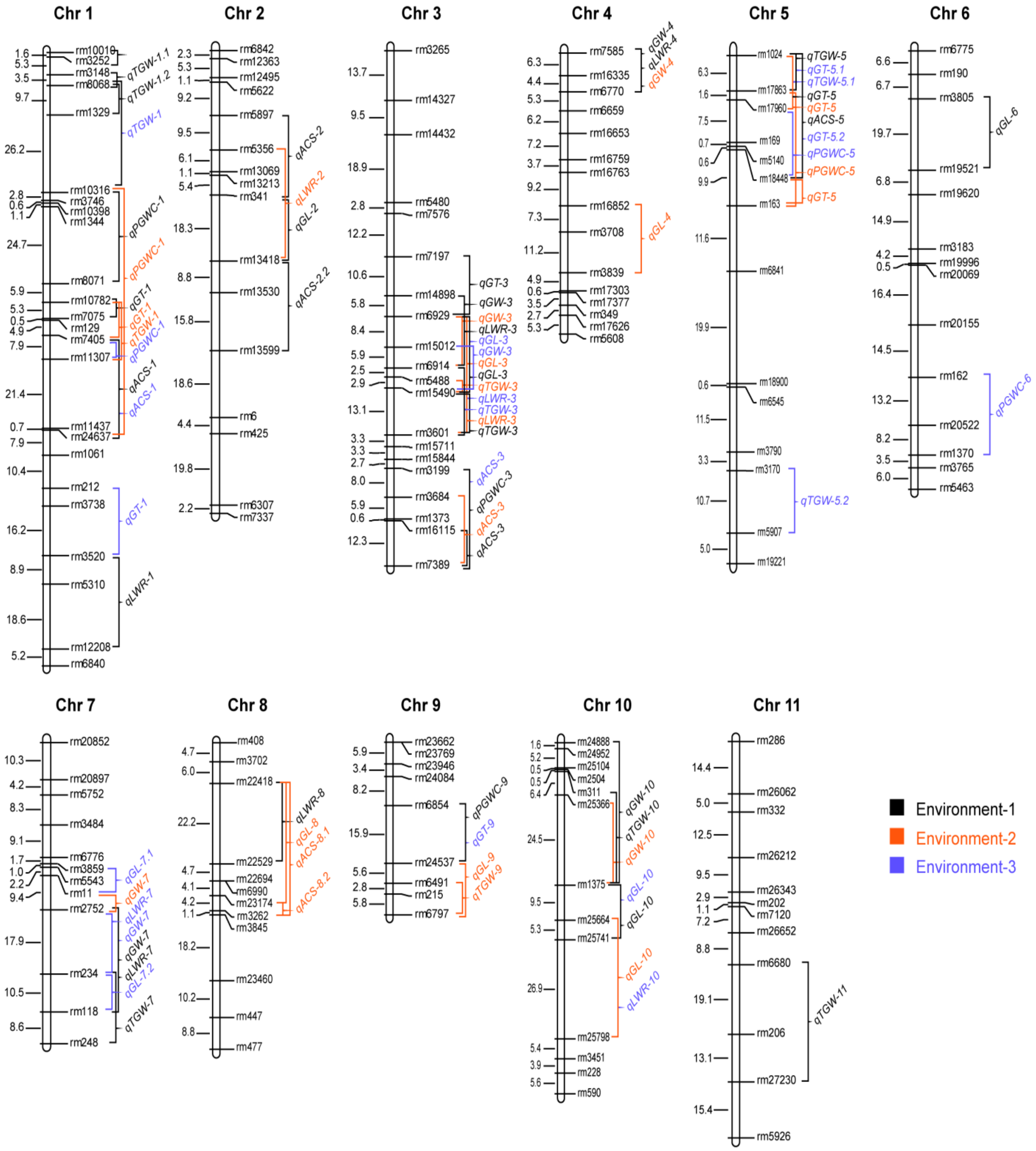

Fig. 2. Locations of main and epistatic effect QTLs for GL, GW, LWR, GT, TGW, PGC and CS on the genetic linkage map.

QTLs for GW: In present study, different QTLs for GW were identified in different environmental conditions. The QTL, *qGW-3* was major QTL detected on the chromosomes 3 under all three environmental conditions, located between the markers RM14898-RM6914, RM18071-RM11307 and RM7585-RM16335 in E1, E2 and E3. The phenotypic variations of *qGW-3* were 7.60%, 11.97% and 13.83% in E1, E2 and E3, the additive effect of *qGW-3* came from YK17, and the variations of GW under E1, E2 and E3 were recorded as -0.03mm, -0.04mm and -0.05mm, respectively. In addition, three QTLs *qGW-4*, *qGW-7, and qGW-10* were detected in E1 and E2, located on chromosomes 4, 7 and 10, with the phenotypic variation of 12.85%, 15.22% and 9.70% in E1 and E2, respectively. The additive effects came from D50 were about 0.04mm and 0.45mm whereas, the other -0.05mm came from YK17. The major QTLs *qGW-7* was found between the same marker intervals RM2752-RM118 in E1, E2 and E3, respectively. The LOD value was 5.69 and the phenotypic variations of *qGW-7* were 15.22% in E1, 17.25% in E2 and 8.85% in E3. The negative additive effect of *qGW-7* came from YK17 with -0.05mm, -0.07 and -0.04mm in E1, E2 and E3, respectively.

QTLs for LWR: Total 9 QTLs, for LWR were identified, The QTL *qLWR-3*, was located between the markers RM14898-RM6914 in E1, and RM6929-RM3601 in E2 and E3, respectively. The highest LOD value was 9.79 and the phenotypic variation was 26.67% in E1, 17.11% in E2 and 29.61%in E3, respectively. The additive effect came from D50 which increased LWR 0.19% in E1, 0.05% in E2 and 0.22% in E3. The QTL, *qLWR-7* was detected on chromosomes 7 that harbored between the markers RM2752-RM118 in E1, E2 and RM3805-RM19620 in E3. Moreover, the contribution rate was observed as 15.22% in E1, 13.11% in E2 and 8.04% in E3. The additive effects came from D50 which was increased by 0.14% in E1, 0.03% E2 and 0.13% in E3. Additionally, some other detected QTLs including *qLWR-4* in E1 and *qLWR-8* in E3, which the phenotypic variation of *qLWR-4* and *qLWR-8* were 12.31% and 10.18% in E1 and E3, respectively. The additive effects from YK17 were -0.15% in E1, and -0.14% in E3.

QTLs for GT: Total 10 QTLs for GT were identified, among these QTLs, *qGT-1* and *qGT-5* were repeatedly detected in all three environments. The QTL *qGT-1* was detected between the marker RM8071-RM129 in E1 and RM10782-RM7405 in E2 and RM212-RM3520 in E3. The phenotypic contribution of *qGT-1* was 13.81% in E1, 15.55% in E2, 11.48% in E3, and its additive effect came from YK17 with variations of -0.01 mm in E1, -0.04 mm in E2 and -0.01mm in E3, respectively. Another QTL, *qGT-3,* was detected in E1 and its phenotypic variation was recorded as 13.81%, however, the negative additive effect came from YK17 with variation of -0.01mm. Similarly, a stable QTL *qGT-5-1* was found between the markers RM1024-RM17960 in E1, E2 and E3, respectively. Whereas, another QTL (*qGT-5-2*) was identified both in E2 and E3 on chromosome 5 with the phenotypic variance of 7.51% and 12.53%, the additive effect came from YK17. Furthermore, another QTL, *qGT-9*, was detected in E3 and its phenotypic variation was found as 33.57%. Moreover, it was noted that the positive additive effect came from D50 which increased variation with 0.04mm.

QTLs for TGW: Total 14 QTLs such as *qTGW-1, qTGW-7, qTGW-9, qTGW-10 and qTGW-11* on chromosomes 1, 3, 5, 7, 9, 10 and 11, were identified. The maximum LOD value was recorded as 8.95. The single QTL showed 6.11% to 33.75% of phenotypic variation and had 1.80 mm to 2.11 mm of additive effect. *qTGW-1* and *qTGW-3* were detected under all three environments. The *qTGW-1* was located between the markers RM8068-RM1329 in E1, RM10782-RM11307in E2 and E3, whereas the phenotypic variations were 6.11% in E1, 6.86% in E2 and 15.36 % in E3. The additive effect came from D50 were 1.04g in E1 and 1.80g in E3, whereas the additive effect came from YK17 with -0.10g in E2. In addition, *qTGW-3* was identified with the same interval of RM15490-RM3601 under all three environments; the phenotypic variations explained by *qTGW-3* were 13.69%, 32.26% and 22.76%, and the additive effect from D50 increased variation of TGW with 1.62g, 1.95g and 2.11g in E1, E2 and E3, respectively. Furthermore, another QTLs of *qTGW-5* were detected under all environments between the markers RM1024-RM17863, the total phenotypic variation explained by 38.54% in E1, E2 and E3, whereas the negative additive effect came from YK17 with -1.79g in E1, -1.86g in E2 and -2.11g in E3, respectively.

QTLs for PGC: Total 8 QTLs including *qPGC-1, qPGC-3, qPGC-5, qPGC-9* on chromosomes 1, 3, 5 and 9 were identified. The QTLs *qPGC-1* was repeated in all environments between the marker interval RM10316-RM8071 in E1 and RM7405-RM11307 in E2 and E3. In addition, *qPGC-5* were detected in E2 and E3 between the marker RM17960-RM18448, RM3765-RM5463 , with the phenotypic variation of 19.30% in E2 and 8.45% in E3, the additive effect came from YK17 with variation of -7.28% to -15.9%. In addition, 2 QTLs *qPGC-3* and *qPGC-9* were detected in E1, the contribution rate were 31.18% and 20.17%, the additive effect, which was calculated that the positive allele of *PGC-3* came from YK17, but *qPGC-9* came from D50 respectively.

QTLs for CS: Total 8 QTLs of CS were identified, of which the QTL, *qCS-1*, was detected between the marker interval RM7405-RM24637 in E1 and RM7405-RM11307 in E3. The phenotypic variations of *qCS-1* were 24.76% in E1 and 14.64% in E3, however its additive effect of *qCS-1* came from YK17, were -7.80% and -6.0%, respectively. Another QTL, *qCS-3*, was detected repeatedly under all three environments, which harbored between the marker interval RM16115-RM7389, RM3684-RM1373 and RM3684-RM16115 with explained phenotypic variation of 7.06%, 16.46% and 7.43 % in E1, E2 and E3, respectively. The additive effect came from D50 increasing the variation with 3.48% in E1, 5.41% in E2 and 4.69 % in E3. Additionally, *qCS-5* was detected in E1 bordered between the marker interval RM1024-RM18448, with phenotypic variation of 12.18 %, and the additive effect came from YK17 with -5.17%. Similarly, two other QTLs, *qCS-8.1* and *qCS-8.2,* were detected only in E2, with phenotypic variation of 9.55% and 9.47%. The negative additive effect came from YK17 decreased the variation with -5.28% and -4.98%, respectively.

Detection of QTLs with additive × environment and epistasis interactions: Total 8 QTLs for 5 traits, including GL, LWR, TGW, PGWC and CS, the additive × environment interaction were detected under all three environments (Table 4). The phenotypic contribution rate was less than their main effects. These QTLs were distributed on six different chromosomes. However, all the identified QTLs were non-significant under all three environments. The epistatic interaction of all traits was estimated but only one pair of bi-allelic epistatic interaction for PGC was identified which had highly significant epistatic effect that covered chromosome 1 and 6. The contribution rate of epistatic QTL was 6.24% (Table 5).

Table 4.QTL with additive x environment interaction for appearance qualities detected in DH population in three trials.

Trait/QTL	Marker interval	Marker (Position, cM)	Range	AE1/R^1	AE2 /R^2	AE3/R^3
qGL-1	RM10782-RM7075	84.4	79.5-90.2	-0.0001/0.0	0.0000/0.0	0.00000/0.0
qLWR-1	RM10782-RM7075	82.4	79.5-86.4	0.0000/0.0	-0.0001/0.0	0.0001/0.0
qLWR-2	RM5897-RM5356	17.9	14.7-21.9	0.0051/0.0	0.0111/0.01	-0.0162/0.01
qTGW-7	RM234-RM118	66.1	59.2-71.1	-0.6767/0.072	0.3610/0.020	0.3257/0.017
qPGC-9	RM23946-RM24084	6.9	4.0-11.3	-0.0001/0.0	0.0001/0.0	0.00010.0
qPGC-11	RM6680-RM206	61.4	59.6-67.4	0.0001/0.0	0.0000/0.0	-0.00010.0
qCS-1	RM11307-RM11437	107.0	95.1-116.0	-0.0001/0.0	0.0000/0.0	0.0000/0.0
qCS-3	RM3199-RM3684	119.6	114.9-129.5	-0.0000/0.0	0.0000/0.0	0.0000/0.0

Table 5. Epistatic interaction of QTLs for appearance qualities in DH population under three different planting seasons.

Traits	Chr.	Marker interval	Chr.	Marker interval	aa_{ij}	R^2 %
PGC	1	RM1329-RM10316	6	RM20522-RM1370	-6.2203	6.24

Discussions

Appearance is an important property of rice grain quality that defines its market value, and is closely related to grain quality and yield (Fiaz*et al*., 2019). Although, rice grain quality has negative correlation with yield but it is considered as a crucial aspect in breeding programs (Xu*et al*., 2013). Grain quality traits are quantitative in nature and being influenced by major and minor QTLs/genes along with their epistatic and environmental effects.

It is important to understand the interaction of major and minor QTLs on grain quality traits, as sometimes a major or a minor QTL can be affected by variation in the environmental condition (He *et al*., 2006, Leng *et al*., 2014). In present study, GL, GW, LWR, GT, TGW, PGC and CS were analyzed for their M-QTLs, epistatic QTLs and QEs association under three different environmental conditions.

GS is a comprehensive trait which includes GL, GW and GT. QTLs affecting different GS traits were detected on the same segments of chromosome as reported in previous studies. Firstly, GL is one of the most important agronomic traits because it positively regulates grain weight and influence on yield (Zuo *et al*., 2014; Huang *et al*., 2013). The two QTLs, *qGL3* and *qGL10* were found stable under all population growing seasons. The *qGL3* allele employs a strong effect not only on GL but also a slight influence on TGW which indicates that it may be co-localized with a thousand-grain weight QTL, *qTGW3,* as reported by Tang *et al*., 2013. *GS3* had highest phenotypic variation of 34.97% which showed its potential role in improving grain quality. In addition, the *qGL10* was identified in all three environments which needed further investigation to figure out its possible role to influence GL. One QTL, *qGW-7* associated with GW harbored within markers RM2752-RM234 on chromosome 7 which was found as a major QTL for grain width on the basis of its consistent mapping in three environments and due to contribution rate with maximum of 17.25% total phenotypic variations. Furthermore, LWR is another important trait which measure the grain appearance quality in contribution with GL and GW. In current study, different QTLs related to LWR were identified but two QTLs, *qLWR3* and *qLWR7* were recorded as the major QTLs being repeatedly mapped in all environments with high phenotypic variance. These results were in accordance with the previously reported findings (Li *et al*., 2011). In our study, we also identfied QTL *qGT5* located on chromosomes 5 controlling the grain thickness. Interestingly, two QTLs for TGW (*qTGW5* and *qTGW5.1*) were found in the adjacent region of *qGT5.* The presence of both *qGT5* and *qTGW5* in the same genomic region on chromosome 5, indicating that *qGT5* has great influence on thousand grain weight (Yang *et al*., 2001; Yuan *et al*., 2016).

Similarly, chalkiness is an important trait of rice appearance. In current study, two potential QTLs, *qPGC1* and *qPGC5*, for PGC were identified. Strong effect of *qPGC-5,* located between RM17863-RM163 intervals on chromosome 5 with high additive effect, which was also reported previously at chalkness. These two QTLs could be explored further in improving the grain quality, (Li *et al*., 2014). The CS is an important indicator to determine the area of chalkiness covering rice grain. One QTL, *qCS3* was also identified as a major QTL detected under all environmental conditions. Similar results were found by (Gao *et al*., 2016) which identified 19 QTLs controlling chalkiness of rice using RIL population. Moreover, a cluster of QTLs was observed between RM2503-RM1126 on chromosome 10. The site was found common for four QTLs of *qGL10*, *qGW10*, *qGT10* and *qTGW10*. Noticeably, all these four QTLs steadily played very strong effects on the phenotype in different environments of the three years' experiments.

Previous studies have found that the main effects of QTLs accounted for a higher percentage of the variance than epistasis interactions (Kepiro *et al*., 2008). On the other hand, some studies demonstrated that epistasis also played an important role in the genetic basis for some important traits. In current study, total 8 QTLs of 5 traits i.e., GL, LWR, TGW, PGWC and CS were detected for additive x environment interaction, except for GW and GT, under all three environments (Table 4). 2 QTLs, *qGL-1* and *qLWR-2* were found similar to identified by Wan *et al*., (2006) and Lou *et al*., (2009). However, some differences among our findings and previously reported studies were observed which might be the outcome of environment and local field condition. One

QTL of epistatic interaction was identified located on chromosomes 6. The phenotypic contribution was less than their main QTLs. The epistatic interactions of all traits were estimated but only one pair of bi-allelic epistatic interaction for PGC was identified which had highly significant epistatic effect that covered chromosome 1 and 6, respectively. The contribution rate 6.24% of epistatic QTL has accounted for a higher percentage of the variance than epistasis interactions (Kepiro *et al*., 2008 Xing *et al*., 2002). Contrarily, other reports demonstrated that epistasis also played an important role in the genetic basis for some important traits in rice (Yu *et al*., 2008; Wang *et al*., 2012). In order to obtain a better understanding of the genetic basis for grain appearance quality and grain weight in YK17 and D50 populations, the epistasis QTLs and QTL × environmental interactions were also analyzed. An increasing number of studies revealed that the performance of some traits in rice was affected by QTL × environment interactions (Wang *et al*., 2012). The interaction had shown phenotypic contribution rate relatively lower, suggesting that the QTL expression of GL, LWR, TGW, PGC and CS were less influenced by the environmental conditions except for GW and GT, showed influence of M-QTLs was dominant. For epistatic interaction, only PGC was observed in epistatic interaction excluding GL, GW, GT, LWR, TGW and CS. The sum effects of M-QTLs were higher in comparison to corresponding epistatic QTLs, suggesting that the M-QTLs were the primary basis for these traits.

In this study, it is worth noting that main QTLs for grain appearance quality on intervals of RM6929-RM15490 on chromosome 3, RM1024-RM17960 on chromosomes 5 and RM2752-RM234 on chromosomes 7 were located. These locations suggested that these regions were important in grain appearance quality; hence more emphasis should be given to these intervals in future studies.

Conclusion

In this study, we identified 73 additive QTLs, 8 additive x environments QTLs and 1 QTL associated with epistasis interaction for grain appearance quality and grain weight. The identified additive QTLs were distributed on the 11 chromosomes, 12 of these QTLs was identified in all three environmental conditions in this study. Moreover, we found that major QTLs were detected mostly on chromosome 3, 5, 7 and 10. The QTLs including, *qGL-3 qGL-10 qGW-3, qGW-7, qLWR-3-1, qGT-1, qTGW-1, qTGW-3, qTGW-5*, *qPGC-1* and *qCS-1* were repeated under all the three environmental conditions. Among these, three QTLs *qPGC-1*, *qTGW-3* and *qCS-1* had high phenotypic variation and additive effect that could improve the grain appearance quality. Most of the QTLs reported in previous studies, also validated in this research. These QTLs will help in exploring fine mapping and molecular mechanisms and it could be used in developing molecular markers for improving rice appearance quality.

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