Theor Appl Genet (2005) 110: 1334–1346
DOI 10.1007/s00122-005-1976-x

ORIGINAL PAPER

X. Y. Wan · J. M. Wan · J. F. Weng · L. Jiang
J. C. Bi · C. M. Wang · H. Q. Zhai

Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments

Received: 15 October 2004 / Accepted: 21 February 2005 / Published online: 5 April 2005

Abstract Rice appearance quality, including traits specifying grain dimension and endosperm chalkiness, represents a major problem in many rice-producing areas of the world. In this study, the genetic basis of six appearance quality traits of milled rice was dissected into quantitative trait loci (QTL) main effects, and the stability of these QTLs was assessed in a population of 66 chromosome segment substitution lines (CSSLs) across eight environments. The CSSLs showed transgressive segregation for many of the traits, and significant correlations were detected among most of the traits. Twenty-two QTLs were identified on eight chromosomes, and numerous QTLs affecting related traits were mapped in the same regions, probably reflecting pleiotropic effects. Nine QTLs, namely *qGL-1*, *qGL-3*, *qGW-5*, *qLWR-3*, *qLWR-5*, *qPGWC-8*, *qPGWC-9*, *qACE-8*, and *qDEC-8*, were consistently detected across the eight environments. The additive main effect and multiplicative interaction (AMMI) analysis showed that genotype (G) × environment (E) interaction was significant for all six traits, with the first three *iPCA* terms accounting for over 80% of the G × E variance. Both D_I values and the *iPCA1-iPCA2* biplots showed that the CSSLs harboring the nine QTL alleles were more stable than those carrying any of the additional 13 QTL alleles, thereby confirming their environmental stability and pointing to their appropriateness as targets for marker-assisted selection for high-quality rice varieties.

Communicated by D.J. Mackill

X. Y. Wan · J. M. Wan · J. F. Weng
L. Jiang · J. C. Bi · C. M. Wang
National Key Laboratory for Crop Genetics
and Germplasm Enhancement,
Jiangsu Plant Gene Engineering Research Center,
Nanjing Agricultural University,
Nanjing, 210095, China

J. M. Wan (✉) · H. Q. Zhai
Institute of Crop Science,
Chinese Academy of Agriculture Sciences,
Beijing, 100081, China
E-mail: wanjm@caas.net.cn
Tel.: +86-10-62186628
Fax: +86-10-62186628

Introduction

Rice appearance quality is determined mainly by grain length (GL), grain width (GW), length-width ratio (LWR), percentage of grains with chalkiness (PGWC), area of chalky endosperm (ACE), and degree of endosperm chalkiness (DEC). For improvement of milling, eating, and cooking quality, the endosperm of high-quality rice varieties should be free of chalkiness, since chalky grains have a lower density of starch granules than vitreous ones and are therefore more prone to breakage during milling (Del Rosario et al. 1968). Also, since both longitudinal and transverse cracks occur easily in chalky kernels when the grain is steamed or boiled, chalkiness reduces the palatability of cooked rice (Nagato and Ebata 1959). On the other hand, rice cultivars with chalkiness are useful for the production of special food, an example of which is Japanese sake. Similarly, preferences for rice grain shape vary across consumer groups. Long and slender grain varieties are preferred by consumers in most Asian countries, including China, India, Pakistan, and Thailand, and in the USA, while short and bold grain cultivars are preferred in Japan and Sri Lanka. Therefore, breeding for the appropriate grain shape and endosperm opacity needs to be considered in the context of market requirements.

Rice appearance quality traits are quantitatively inherited (Tan et al. 2000). The identification of quantitative trait loci (QTLs) for appearance quality and the elucidation of their genetic control are necessary for the development of marker-assisted selection (MAS) strategies aimed at improving breeding efficiency. Using a number of primary mapping

populations, Huang et al. (1997) detected 12 QTLs for GL, GW, and LWR, while He et al. (1999) identified three QTLs affecting PGWC and ACE. Redoña and Mackill (1998) found that GL, GW, and LWR were controlled primarily by two loci, one each on chromosomes 3 and 7, whereas Tan et al. (2000) detected a major GL QTL on chromosome 3 and a GW QTL on chromosome 5. However, since all the above studies were conducted in a single environment, the stability of the resultant QTLs could not be evaluated. This characteristic is critical for determining the utility of a QTL for marker-assisted breeding.

In the present study, 66 chromosome segment substitution lines (CSSLs), derived from three backcrosses of IR24 (*indica*) to Asominori (*japonica*) (Asominori/IR24//3*Asominori), were used for QTL identification across eight environments. The use of CSSLs, as opposed to primary mapping populations, has distinct advantages for QTL identification. Most importantly, genetic interactions between donor alleles are limited to those between genes on homozygous substituted tracts since each CSSL carries one or a few donor segments in the near-isogenic background of a recurrent genotype, thus reducing the effects of interferences from genetic background (Howell et al. 1996). Second, for the fine mapping and positional cloning of a QTL, a secondary F_2 population can be derived from a further backcross between a selected CSSL and the recurrent parent (Frary et al. 2000; Yano et al. 2000). Third, F_2 populations generated from intercrosses between different CSSLs can be used to analyze epistatic interactions between pairs of QTLs (Lin et al. 2000). Finally, CSSLs form the germplasm basis for the pyramiding of multiple QTLs in a plant breeding program (Li 2001).

The objectives of this study were to detect stable QTLs affecting GL, GW, LWR, PGWC, ACE, and DEC, in order to develop MAS assays for appearance quality, and to construct secondary F_2 populations via backcrossing for the fine mapping and positional cloning of stable QTLs.

Materials and methods

Population development

Seventy-one recombinant inbred lines (RILs), derived from the cross Asominori × IR24, were developed by single-seed descent (Tsunematsu et al. 1996). To produce a series of CSSLs in a largely Asominori genetic background, 19 selected RILs composed of more than 60% Asominori genotype were crossed and backcrossed with Asominori, without selection, until the BC_3F_1 generation. Sixty-six individuals were selected at the BC_3F_1 generation on the basis of a whole genome survey [116 restriction fragment length polymorphism (RFLP) loci]. These 66 lines, denoted L1–L66, have representation of the whole IR24 genome, except for the 9.8-cM region defined by the interval C1468-G1015 on chromosome 3 (Kubo et al. 1999; Wan et al. 2004).

Phenotypic data collection

The CSSLs and the parental varieties were grown in eight environments involving four locations (Table 1). Each entry plot consisted of ten rows of ten plants each, grown in a randomized block design with two replications of each entry per environment. After drying (to about 13.5% of the moisture content), the grain was stored at room temperature for 3 months and then milled using the Yamamoto Rice-Pal VP-30T with the following procedure: 200 g of purified paddy rice per CSSL was put once into the miller and milled for 60 s (Yamamoto et al. 1995). The milled rice thus obtained was used for measuring the appearance quality traits.

The quality traits GL, GW, and LWR were measured following the method of Tan et al. (2000). GL and GW were estimated from the mean of 20 grains. LWR, which represents the shape of the grain, was given by the ratio GL/GW. PGWC, ACE, and DEC were evaluated

Table 1 The test environments in which the Asominori/IR24 CSSL population was evaluated

Code	Number of replications	Environments
E1	2	Nanjing Agricultural University, Nanjing, China, N 31.2 Â°, E 118.4 Â°, May–October, 2001
E2	2	Experimental Farm of Jinhu County, Huai'an, Jiangsu, China, N 32.7 Â°, E 119.6 Â°, May–October, 2001
E3	2	Experimental Farm of Donghai County, Lianyungang, Jiangsu, China, N 35.1 Â°, E 118.4 Â°, June–November, 2001
E4	2	Rice Breeding Base of Lingshui County, Sanya, Hainan, China, N 18.2 Â°, E 108.9 Â°, December, 2001–May, 2002
E5	2	Nanjing Agricultural University, Nanjing, China, N 31.2 Â°, E 118.4 Â°, May–October, 2002
E6	2	Experimental Farm of Jinhu County, Huai'an, Jiangsu, China, N 32.7 Â°, E 119.6 Â°, May-October, 2002
E7	2	Experimental Farm of Donghai County, Lianyungang, Jiangsu, China, N 35.1 Â°, E 118.4 Â°, June–November, 2002
E8	2	Rice Breeding Base of Lingshui County, Sanya, Hainan, China, N 18.2 Â°, E 108.9 Â°, December, 2002–May, 2003

according to He et al. (1999) and NSPRC (1999). To separate chalky from vitreous grains, we assess 100 grains per entry on a chalkiness visualizer constructed at the China National Rice Research Institute (NSPRC 1999); on the basis of these observations, we calculated PGWC. Twenty chalky grains were then selected at random, and the ratio of the area of chalkiness to the area of the whole endosperm for each grain was evaluated by human visual assessment on the chalkiness visualizer. The values were averaged and used as values for ACE. DEC was calculated as the product PGWC × ACE.

All six traits were measured on three replications per sample.

Linkage map construction

Genomic DNA was extracted by the CTAB method (Murray and Thompson 1980). The DNA clones

Table 2 Summary statistics of phenotypic performance of the CSSL population and its parents for six quality traits in eight environments (*SD* standard deviation)

Traits[a]	Environments	Parents		Population		
		Asominori	IR24	Range	Mean ± SD	CV (%)[b]
GL (mm)	E1	5.3	5.9	4.9–5.8	5.3 ± 0.2	3.7
	E2	5.3	5.9	4.9–5.8	5.3 ± 0.2	3.5
	E3	5.3	6.0	4.9–5.8	5.4 ± 0.2	3.5
	E4	5.2	5.8	4.8–5.8	5.2 ± 0.2	3.8
	E5	5.3	5.8	4.9–5.8	5.3 ± 0.2	3.8
	E6	5.3	5.9	5.0–5.9	5.3 ± 0.2	3.7
	E7	5.3	6.0	4.9–5.8	5.4 ± 0.2	3.6
	E8	5.3	5.9	4.9–5.8	5.3 ± 0.2	3.6
GW (mm)	E1	2.7	2.4	2.3–2.8	2.6 ± 0.1	4.3
	E2	2.8	2.4	2.3–2.9	2.7 ± 0.1	4.4
	E3	2.8	2.5	2.3–3.0	2.7 ± 0.1	4.8
	E4	2.8	2.5	2.3–2.8	2.6 ± 0.1	4.0
	E5	2.8	2.5	2.3–2.8	2.6 ± 0.1	4.2
	E6	2.7	2.4	2.3–2.9	2.7 ± 0.1	4.3
	E7	2.8	2.5	2.2–3.0	2.7 ± 0.1	5.4
	E8	2.8	2.5	2.3–2.8	2.6 ± 0.1	4.1
LWR	E1	2.0	2.4	1.8–2.4	2.0 ± 0.1	6.5
	E2	1.9	2.5	1.8–2.3	2.0 ± 0.1	6.4
	E3	1.9	2.4	1.7–2.4	2.0 ± 0.1	6.9
	E4	1.9	2.3	1.8–2.4	2.0 ± 0.1	6.4
	E5	1.9	2.4	1.8–2.4	2.0 ± 0.1	6.2
	E6	1.9	2.4	1.7–2.3	2.0 ± 0.1	6.6
	E7	1.9	2.4	1.7–2.3	2.0 ± 0.1	7.0
	E8	2.0	2.3	1.8–2.4	2.0 ± 0.1	6.5
PGWC (%)	E1	25.0	20.0	1.3–85.9	28.4 ± 22.8	80.4
	E2	26.5	18.0	1.5–84.0	26.9 ± 22.5	83.6
	E3	29.0	18.0	1.0–91.5	34.3 ± 27.0	78.8
	E4	27.0	16.0	0.5–94.5	32.6 ± 26.2	80.1
	E5	29.0	21.5	3.8–92.4	33.0 ± 24.7	75.0
	E6	28.3	18.3	2.5–90.5	33.3 ± 24.5	73.5
	E7	28.5	14.8	1.5–94.8	34.5 ± 26.9	78.1
	E8	25.0	16.0	0.5–88.5	32.4 ± 25.9	80.2
ACE (%)	E1	4.1	9.2	2.2–18.7	7.4 ± 3.5	47.1
	E2	5.3	8.4	1.1–24.3	7.6 ± 5.7	75.1
	E3	4.5	8.1	0.5–31.3	7.8 ± 6.6	84.4
	E4	5.8	10.8	0.5–20.0	5.2 ± 3.9	76.0
	E5	4.3	8.2	1.2–28.2	8.2 ± 5.3	64.9
	E6	5.7	10.4	1.5–23.1	8.5 ± 5.9	69.0
	E7	4.2	9.1	1.2–23.8	6.7 ± 5.0	74.3
	E8	4.3	8.8	0.3–19.6	4.8 ± 4.0	83.3
DEC (%)	E1	1.0	1.8	0.1–15.9	2.6 ± 3.5	137.1
	E2	1.4	1.5	0.1–20.5	2.8 ± 4.3	154.9
	E3	1.3	1.5	0.0–20.1	3.4 ± 4.9	141.8
	E4	1.6	1.7	0.0–17.5	2.4 ± 3.8	159.5
	E5	1.3	1.8	0.1–16.8	3.1 ± 3.7	119.5
	E6	1.6	1.9	0.1–20.7	3.8 ± 5.0	130.6
	E7	1.2	1.4	0.0–21.7	3.2 ± 4.4	140.6
	E8	1.1	1.4	0.0–17.0	2.2 ± 3.6	162.4

[a] GL, Grain length; GW, grain width; LWR, length-width ratio; PGWC, percentage of grains with chalkiness; ACE, area of chalky endosperm; DEC, degree of endosperm chalkiness. See Materials and methods for details of parameter calculations

[b] CV, Coefficient of variation

mapped by Tsunematsu et al (1996) were used as probes. DNA labeling, hybridization, and signal detection were conducted using the ECL detection system (Amersham, UK). For the whole genome survey, 116 RLFP loci distributed over the framework map were used in the BC_3F_1 generation. A linkage map was constructed with 85 RFLP markers evenly distributed over all 12 chromosomes using MAPMAKER/EXP 3.0 (Lander et al. 1987). The overall map length was 1,275.4 cM with an average distance of 15.0 cM between adjacent markers, as reported by Kubo et al. (1999).

Data analysis

Tests of homogeneity of variance were conducted to determine whether data from multiple environments could be pooled to conduct a combined analysis of variance (ANOVA) across environments. For the combined analyses, the variance was partitioned into genotype (CSSL), year, site, year × site, Rep (year × site), CSSL × year, CSSL × site, and year × CSSL × site. Phenotypic correlations among traits were estimated using the mean phenotypic values of the CSSL population, combined across the eight environments.

Following classical quantitative genetics theory (Falconer 1981), the phenotypic value of a CSSL (y_{ihk}) is described by the genetic model:

$$y_{ihk} = \mu + G_i + E_h + GE_{ih} + \varepsilon_{ihk}, \quad (1)$$

where y_{ihk} is the trait value for the i^{th} CSSL ($I = 1, 2,..., 66$) of the k^{th} field replication in the h^{th} environment, μ is the CSSL population mean, G_i is the genotypic effect (fixed) of the i^{th} CSSL, $E_h \sim N_{(0,\sigma_E^2)}$ is the random effect of the h^{th} environment ($h = 1, 2,...,8$), $GE_{ih} \sim N_{(0,\sigma_{GE}^2)}$ is the interaction effect between the i^{th} CSSL and the h^{th} environment, and $\varepsilon_{ihk} \sim N_{(0,\sigma_E^2)}$ is the random residual effect.

The QTL parameters were estimated by composite interval mapping, a combination of interval mapping with multiple regression analysis (Zeng 1994). To obtain empirical thresholds of the experiment, 1,000 permutations were run by randomly shuffling the trait values (Churchill and Doerge 1994). Based on the permutation test, a LOD value of 3.0 was used for claiming a significant main-effect QTL. The QTL mapping was performed based on the data in each environment using the JMP VER. 3.1 software package (SAS Institute 1994).

The QTL effects were evaluated by a *t*-test to test the presence of significant differences between the phenotypic values of Asominori and those of CSSLs harboring QTL alleles derived from IR24.

The stability of a QTL was estimated using the additive main effects and multiplicative interaction (AMMI) model (Crossa et al. 1990; Gauch and Zobel 1988) by analyzing the genotype × environment interaction (GEI) for individual traits and thereby generating stability parameters in several dimensions. The AMMI analysis was performed using software described by Vargas and Crossa (2000). The stability parameter (D_i) of the i^{th} CSSL is calculated by:

$$D_i = \sqrt{\sum_{c=1}^{3} \lambda_c \theta_{ic}^2} \quad (2)$$

(Zhang et al. 1998; Vargas et al. 1999) where λ_c is the singular value of the c^{th} principal components analysis (PCA) axis and θ_{ic} is the principal component values for the c^{th} PCA axis of the i^{th} CSSL.

The smaller the D_i value, the more stable the i^{th} CSSL is across multi-environments. When the first two *iPCA* values account for a majority of the total GEI sum of squares (SS), the AMMI biplot with the abscissa of *iPCA1* and the ordinate of *iPCA2* can also be applied to evaluate the stability of the i^{th} CSSL.

Results

Variation of phenotypic traits

Table 2 shows the phenotypic variation of the CSSLs and their parents for the six quality traits across the

Table 3 Analysis of variance for six appearance quality traits[a] in the CSSL population across two years and four sites

Sources	*df*	GL		GW		LWR		PGWC		ACE		DEC	
		MS	*F* values	MS	*F* values	MS	*F* values	MS	*F* values	MS	*F* values	MS	*F* values
Year	1	0.258	62.86**	0.017	11.60**	0.005	3.96*	2117.55	66.94**	101.29	10.19**	21.46	15.95**
Site	3	0.683	166.16**	1.000	680.87**	0.171	129.97**	970.04	30.67**	520.80	52.41**	56.42	41.94**
Year × Site	3	0.066	16.05**	0.003	2.38	0.016	11.90**	697.15**	22.04**	63.09	6.35**	24.54	18.24**
Rep (Year × site)	8	0.105	25.62**	0.008	5.15**	0.012	9.13**	336.91**	10.65**	30.68	3.09**	5.54	4.12**
CSSL	65	0.556	135.31**	0.203	138.36**	0.258	196.00**	9220.84	291.50**	267.06	26.87**	244.72	181.90**
CSSL × Year	65	0.005	1.10	0.001	0.79	0.001	0.87	122.39	3.87**	18.01	1.81**	5.19	3.86**
CSSL × Site	195	0.010	2.48**	0.007	4.51**	0.004	3.18**	180.87	5.72**	29.38	2.96**	8.01	5.95**
CSSL × Year* Site	195	0.002	0.52	0.001	0.95	0.001	0.89	69.90	2.21**	13.96	1.41**	2.38	1.77**
Error	520	0.004		0.001		0.001		31.63		9.94		1.35	

*, **Significant at $P \leq 0.05$ and 0.01, respectively

[a] Abbreviations are the same as in Table 2

Table 4 QTLs affecting the six quality traits detected using the CSSL population across eight environments

Trait[a]	QTLs	Chromosome	Marker interval	Parameter[b]	E1	E2	E3	E4	E5	E6	E7	E8	QTL-CSSLs
GL	*qGL-1*	1	R210-C955	LOD	5.3	4.0	7.4	5.9	5.6	5.0	7.1	6.6	L1,L2,L4,L9
				PVE	17.7	10.7	20.6	15.4	18.2	18.4	24.3	20.3	
				AE	−0.15	−0.11	−0.17	−0.15	−0.16	−0.15	−0.18	−0.16	
	qGL-3	3	R19-C1677	LOD	5.9	8.7	5.7	9.7	4.7	5.0	5.2	6.8	L16,L17,L18,
				PVE	24.5	51.1	21.6	49.3	19.5	35.5	18.5	42.1	L46
				AE	0.29	0.27	0.24	0.26	0.27	0.25	0.24	0.24	
	qGL-2	2	C601-R3393	LOD		3.9	5.0				3.0		L13,L14,L61
				PVE		12.4	14.0				10.6		
				AE		−0.15	−0.16				−0.14		
	qGL-4	4	XNpb331-C335	LOD		3.1	6.2	4.8			4.2	3.8	L25,L26,L27
				PVE		7.0	12.7	15.1			9.8	11.1	
				AE		−0.12	−0.16	−0.15			−0.15	−0.12	
GW	*qGW-5*	5	R3166-R569	LOD	5.4	7.9	7.5	5.2	5.2	7.2	6.6	5.3	L28,L29
				PVE	23.2	33.9	30.9	18.7	29.6	32.6	27.8	19.2	
				AE	−0.16	−0.16	−0.19	−0.11	−0.17	−0.19	−0.23	−0.11	
	qGW-1	1	XNpb113-C112	LOD			3.4						L7,L9,L37
				PVE			10.6						
				AE			−0.13						
	qGW-6	6	C991-XNpb12	LOD	3.9								L9,L11,L19,
				PVE	13.7								L37,L38,L39,
				AE	−0.08								L61
LWR	*qLWR-3*	3	R19-C1677	LOD	3.9	4.2	4.3	4.1	3.6	3.7	3.6	4.0	L16,L17,L18,
				PVE	17.3	24.0	23.3	15.0	21.0	21.7	21.8	20.7	L46
				AE	0.15	0.15	0.15	0.15	0.14	0.15	0.15	0.14	
	qLWR-5	5	R3166-R569	LOD	6.1	9.0	6.3	6.6	6.9	6.6	6.1	5.4	L28,L29
				PVE	23.5	32.2	26.6	24.6	28.9	31.9	23.7	19.6	
				AE	0.18	0.18	0.17	0.19	0.16	0.15	0.19	0.17	
	qLWR-1	1	R210-C955	LOD	3.2			3.3			4.0	3.4	L1,L2,L4,L9
				PVE	11.7			13.4			18.2	15.3	
				AE	−0.10			−0.08			−0.12	−0.10	
	qLWR-2	2	XNpb67-XNpb132	LOD			3.2						L7,L10,L11,
				PVE			12.2						L12
				AE			−0.14						
PGWC	*qPGWC-8*	8	G1149-R727	LOD	7.3	6.1	6.2	5.9	5.3	5.2	6.4	5.4	L49,L50,L51
				PVE	21.7	17.5	27.2	23.7	19.6	29.8	27.2	22.9	
				AE	25.9	22.0	33.1	30.2	26.8	21.8	33.7	27.9	
	qPGWC-9	9	XNbp36-XNpb103	LOD	5.0	3.9	4.7	3.0	3.1	3.1	4.4	3.7	L52,L53,L64
				PVE	12.6	11.7	19.0	12.6	11.2	17.8	19.9	15.8	
				AE	32.9	31.4	22.3	16.8	33.8	32.7	20.9	19.3	
	qPGWC-1	1	C2340-C1370	LOD			4.3	5.3			3.1	4.4	L5,L6,L7,L8
				PVE			18.5	18.3			16.3	18.7	
				AE			27.1	29.3			19.7	27.2	
ACE	*qACE-8*	8	G1149-R727	LOD	6.2	8.6	3.2	14.1	4.5	4.3	6.5	12.6	L49,L50,L51
				PVE	31.5	39.2	20.2	40.6	24.5	22.0	29.3	34.3	
				AE	5.6	7.2	5.9	6.1	4.9	5.8	5.6	5.8	
	qACE-2	2	G1340-R459	LOD							3.3		L7,L10
				PVE							20.7		
				AE							3.9		
	qACE-9	9	XNbp36-XNpb103	LOD				3.9	3.0		3.5		L52,L53,L64
				PVE				11.1	14.5		15.7		
				AE				4.8	4.0		3.9		

DEC	*qDEC-8*	8	G1149-R727	LOD	17.2	13.4	8.0	20.0	10.5	10.3	14.1	23.8	L49,L50,L51
				PVE	38.8	48.5	23.6	40.0	37.8	34.8	33.2	42.8	
				AE	5.9	6.9	6.7	6.3	5.5	7.0	7.6	5.8	
	qDEC-9	9	XNbp36-XNpb103	LOD	4.3	4.6	6.2	8.1		4.0	5.2	8.3	L52,L53,L64
				PVE	5.1	8.7	13.3	13.4		15.6	15.3	11.7	
				AE	3.2	5.2	6.4	5.7		7.1	4.6	5.1	
	qDEC-1a	1	R210-C955	LOD	3.4			3.3			4.2	4.7	L1,L2,L4,L9
				PVE	5.2			6.4			12.9	8.4	
				AE	1.8			2.7			2.5	2.0	
	qDEC-1b	1	C2340-C1370	LOD								4.8	L5,L6,L7,L8
				PVE								5.8	
				AE								1.7	
	qDEC-2	2	G1340-R459	LOD		3.3						3.8	L7,L10
				PVE		7.3						5.5	
				AE		2.6						1.7	

[a] Abbreviations are the same as in Table 2[b]PVE and AE represent the percentage of phenotypic variation explained and the additive effect of the QTL, respectively. These were both estimated from mean trait values of individual CSSLs in the individual environments

eight environments. Significant differences were observed for all of the traits between the parental cultivars. The CSSL population segregated transgressively for PGWC, ACE, and DEC. The mean trait value of the CSSLs was largely consistent across the different environments, except that ACE and DEC both showed lower phenotypic values in 2001, 2002, and in Hainan, indicating that the Hainan environment (N 18.2 Â°, E 108.9 Â°) tends to lessen the occurrence of chalkiness. The coefficients of variation varied considerably among the traits, ranging from 3.7% for GL to 143.3% for DEC. For GL, GW and LWR, the ANOVA indicated highly significant effects due to genotype (CSSL), year, site, year × site, Rep (year × site), and CSSL × site for all traits, but non-significant ones due to the year × CSSL and year × site × CSSL interactions (Table 3).

Significant or highly significant correlations were observed between GL and GW (−0.297*), GL and LWR (0.766**), GW and LWR (−0.835**), PGWC and ACE (0.691**), PGWC and DEC (0.876**), and ACE and DEC (0.885**). In addition, GW was positively correlated with PGWC, ACE, and DEC, but no correlations were significant between GL and any of the three chalkiness traits, suggesting that the width of rice grains is closely associated with the occurrence of chalkiness.

QTL analysis

Twenty-two QTLs for the six traits were identified in the eight environments and mapped to eight chromosomes with LOD values between 3.0 and 23.8 (Table 4, Fig. 1). Of the 11 GL, GW, and LWR QTLs, five (*qGL-1, qGL-3, qGW-5, qLWR-3*, and *qLWR-5*) were consistently detected across all eight environments. *qGL-1* and *qGL-3* were mapped to chromosomes 1 and 3 with the average percentage of phenotypic variation explained (PVE) of 18.2% and 32.8%, respectively. The IR24 allele at the *qGL-3* locus increased GL by an average of 0.26 mm, while the positive effect of the *qGL-1* locus was contributed by the Asominori allele. *qGW-5* was mapped to chromosome 5 and accounted for 27.0% of the phenotypic variation, with the IR24 allele providing a negative effect of 0.17 mm. LWR was mostly controlled by loci on chromosomes 3 and 5; *qLWR-3* and *qLWR-5* coincided with the major GL and GW QTLs. On average, *qLWR-3* and *qLWR-5* explained 20.6% and 26.4% of the total variation, respectively. The positive effects at both loci were contributed by IR24 alleles. Six additional QTLs (*qGL-2, qGL-4*, *qGW-1, qGW-6, qLWR-1*, and *qLWR-2*) were identified in one to five environments, with a PVE of 7.0–18.2%.

Three PGWC, ACE, and DEC QTLs (*qPGWC-8, qACE-8*,and *qDEC-8*), were mapped in the interval G1149-R727 on chromosome 8 across all eight environments and accounted, on average, for 23.7%, 30.2%, and 37.4% of the phenotypic variation, respectively. In

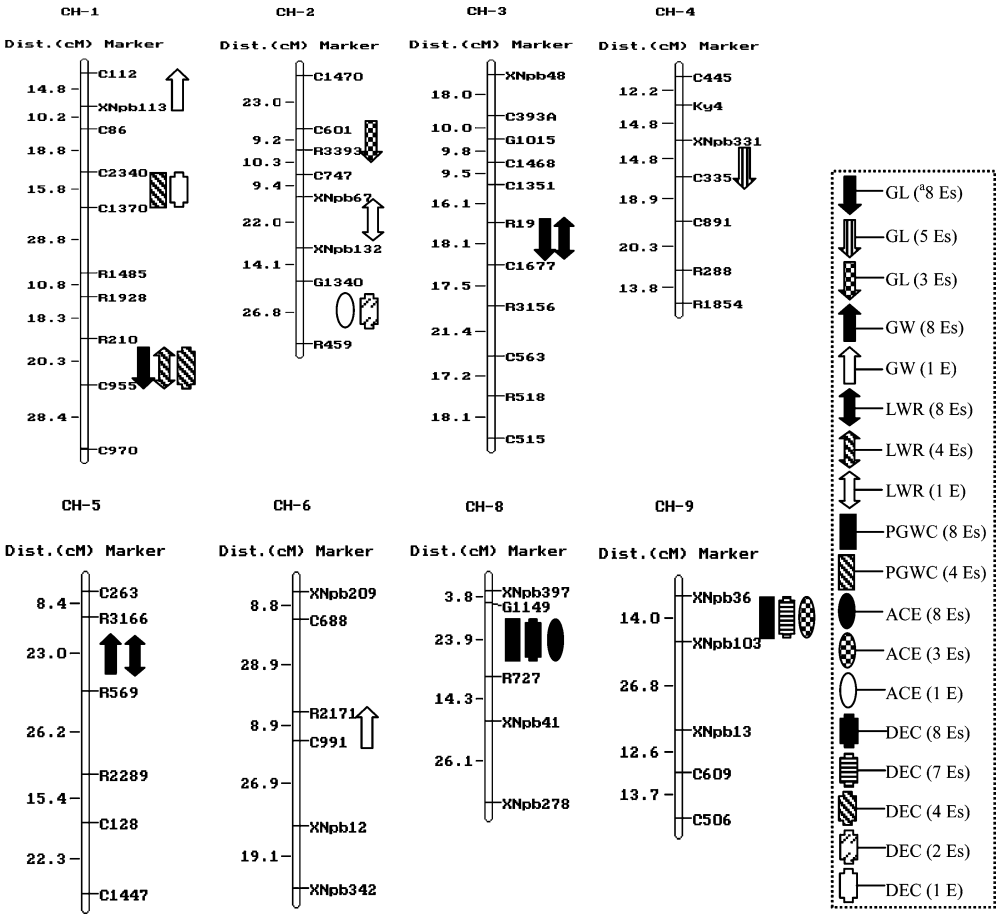

Fig. 1 Map locations of identified QTLs affecting grain length (*GL*), grain width (*GW*), length-width ratio (*LWR*), percentage of grains with chalkiness (*PGWC*), area of chalky endosperm (*ACE*), and degree of endosperm chalkiness (*DEC*) of Asominori × IR24 CSSL population, detected in the eight environments. *8 Es* represents QTLs detected in eight environments, such as in E1, E2, E3, E4, E5, E6, E7, and E8. The same terminology is used for all of the following QTLs; i.e., *1 Es* is one environment; *2 Es*, two environments, etc

the interval XNpb36-XNpb103 on chromosome 9, *qPGWC-9* was consistently identified in all eight environments, while *qACE-9* and *qDEC-9* were only expressed in three and seven environments, respectively. The positive effects of these six QTLs were all contributed by IR24 alleles. Five QTLs (*qPGWC-1, qACE-2*, *qDEC-1a, qDEC-1b*, and *qDEC-2*) were identified in one to four environments, with a PVE of 5.2–20.7%.

Effects of QTLs with high repeatability

Among the 22 QTLs detected, nine (*qGL-1, qGL-3, qGW-5, qLWR-3*, *qLWR-5, qPGWC-8, qPGWC-9, qACE-8*, and *qDEC-8*) were detected across all eight environments (Table 4). *qGL-1* was located in the interval R210-C955. Four CSSLs (L1, L2, L4, and L9) carried IR24 alleles in this chromosome region (Table 4). *qGL-3* and *qLWR-3* were both mapped in the interval R19-C1677. Four CSSLs (L16, L17, L18, and L46) carried IR24 alleles at R19 and C1677. Similarly, two or three CSSLs carried one of the *qGW-5, qLWR-5, qPGWC-8, qPGWC-9, qACE-8*, and *qDEC-8* alleles (Table 4). *t*-tests demonstrated significant differences between the phenotypic values of Asominori and each CSSL carrying one of the nine QTL alleles (Table 5), indicating that the effects of these QTLs were significant and repeatable across the eight environments. However, the other 13 QTLs were environment-specific, as significant effects were detected in only one to seven environments.

Stability of QTL effects

The AMMI analysis demonstrated significant GEI for all six traits (Table 6), indicating that QTL-by-environment interactions (QEI) were significant for each trait studied. It was therefore necessary to analyze the stability of the CSSLs harboring the detected QTL alleles and to assess the stability of individual QTLs across the eight environments. The first three *iPCA* terms of each trait accounted for over 80% of the GEI SS, indicating that the stability of the CSSLs can be precisely evaluated with the D_i values calculated from the first three *iPCA* values (Table 6). For GL QTLs, the average D_i value of four CSSLs (L1, L2, L4, and L9) harboring the *qGL-1* allele was 0.13, and those of the CSSLs carrying the *qGL-2*, *qGL-3* and *qGL-4* alleles were 0.24, 0.13 and 0.31, respectively (Table 7). Using the abscissa of *iPCA1* and the ordinate of *iPCA2* in the AMMI biplot, we observed that eight CSSLs (L1, L2, L4, L9, L16, L17, L18, and L46) harboring the *qGL-1* and *qGL-3* alleles were closer to the origin, while six CSSLs (L13, L14,

Table 5 *t*-test for the differences of phenotypic values between Asominori and the CSSL carrying any one of the QTL alleles with high repeatability in eight environments

Target QTLs and CSSLs		E1[a]		E2		E3		E4		E5		E6		E7		E8	
		Means[b]	*P*	Means	*P*	Means	*P*	Means	*P*	Means	*P*	Means	*P*	Means	*P*	Means	*P*
qGL-1	AsominoriAso	5.3		5.3		5.3		5.2		5.3		5.3		5.3		5.3	
	L1	5.0	0.034*[c]	5.0	0.023*	5.1	0.004**	5.0	0.025*	5.0	0.006**	5.1	0.017*	5.1	0.003**	5.1	0.010**
	L2	5.0	0.031*[d]	5.1	0.033*	5.1	0.005**	5.0	0.017*	5.0	0.006**	5.0	0.012*	5.0	0.003**	5.0	0.012*
	L4	5.1	0.027*[e]	5.1	0.038*	5.1	0.003**	5.0	0.005**	5.1	0.007**	5.0	0.027*	5.1	0.003**	5.1	0.008**
	L9	5.0	0.01**[f]	5.0	0.039*	5.0	0.001**	4.9	0.011*	4.9	0.003**	5.0	0.058*	4.9	0.002**	5.0	0.006**
qGL-3	Asominori	5.3		5.3		5.3		5.2		5.3		5.3		5.3		5.3	
	L16	5.8	0.007**	5.8	0.004**	5.8	0.006**	5.8	0.004**	5.8	0.002**	5.9	0.004**	5.8	0.011*	5.8	0.006**
	L17	5.8	0.006**	5.8	0.004**	5.8	0.001**	5.8	0.001**	5.8	0.003**	5.8	0.008**	5.8	0.014*	5.8	0.006**
	L18	5.7	0.025*	5.7	0.007**	5.7	0.008**	5.6	0.002**	5.6	0.010**	5.7	0.013*	5.7	0.016*	5.7	0.010**
	L46	5.8	0.015*	5.8	0.012*	5.7	0.001**	5.8	0.002**	5.8	0.009**	5.8	0.008**	5.8	0.015*	5.8	0.002**
qGW-5	Asominori	2.7		2.8		2.8		2.8		2.8		2.7		2.8		2.8	
	L28	2.4	0.001**	2.3	0.002**	2.4	0.001**	2.3	0.022*	2.3	0.003**	2.4	0.009**	2.4	0.013*	2.3	0.004**
	L29	2.3	0.000**	2.4	0.005**	2.4	0.002**	2.3	0.006**	2.3	0.003**	2.4	0.001**	2.4	0.001**	2.4	0.010**
qLWR-3	Asominori	2.0		1.9		1.9		1.9		1.9		1.9		1.9		2.0	
	L16	2.3	0.003**	2.3	0.007**	2.2	0.016*	2.3	0.006**	2.3	0.023*	2.3	0.002**	2.2	0.008**	2.2	0.008**
	L17	2.3	0.003**	2.2	0.008**	2.2	0.000**	2.3	0.006**	2.3	0.009**	2.2	0.004**	2.2	0.017*	2.3	0.003**
	L18	2.3	0.015*	2.2	0.008**	2.3	0.003**	2.3	0.020*	2.2	0.009**	2.2	0.003**	2.3	0.003**	2.3	0.014*
	L46	2.3	0.005**	2.3	0.008**	2.3	0.007**	2.3	0.008**	2.3	0.032*	2.3	0.011*	2.3	0.001**	2.3	0.017*
qLWR-5	Asominori	2.0		1.9		1.9		1.9		1.9		1.9		1.9		2.0	
	L28	2.3	0.007**	2.3	0.005**	2.4	0.010**	2.3	0.014*	2.3	0.015*	2.3	0.007**	2.3	0.001**	2.3	0.002**
	L29	2.4	0.001**	2.3	0.006**	2.3	0.014*	2.4	0.007**	2.4	0.011*	2.3	0.007**	2.3	0.005**	2.4	0.002**
qPGWC-8	Asominori	25.0		26.5		29.0		27.0		29.0		28.3		28.5		25.0	
	L49	85.0	0.003**	84.0	0.011*	90.0	0.003**	87.0	0.003**	89.5	0.002**	90.5	0.011*	91.0	0.000**	88.5	0.002**
	L50	84.3	0.006**	84.0	0.003**	83.0	0.004**	81.0	0.001**	82.6	0.002**	83.3	0.004**	85.0	0.005**	80.5	0.001**
	L51	83.5	0.005**	75.0	0.001**	91.5	0.002**	94.5	0.000**	90.8	0.004**	90.5	0.008**	94.8	0.002**	87.3	0.014*
qPGWC-9	Asominori	25.0		26.5		29.0		27.0		29.0		28.3		28.5		25.0	
	L52	77.8	0.005**	71.5	0.000**	84.0	0.004**	86.5	0.000**	84.1	0.005**	79.3	0.023*	87.5	0.004**	83.8	0.003**
	L53	76.7	0.003**	76.5	0.003**	78.5	0.006**	74.5	0.003**	77.4	0.001**	79.0	0.003**	76.8	0.005**	77.3	0.004**
	L64	85.9	0.003**	81.5	0.000**	89.5	0.003**	94.0	0.000**	92.4	0.005**	88.8	0.015*	92.3	0.002**	87.0	0.014*
qACE-8	Asominori	4.1		5.3		4.5		5.8		4.3		5.7		4.2		4.3	
	L49	18.7	0.026*	24.3	0.015*	22.4	0.017*	20.0	0.047*	18.9	0.042*	23.1	0.016*	23.8	0.006**	19.2	0.004**
	L50	18.5	0.056*	18.6	0.005**	21.5	0.004**	18.7	0.008**	18.9	0.042*	18.1	0.041*	20.4	0.005**	19.6	0.039*
	L51	14.4	0.016*	15.6	0.012*	15.3	0.013*	14.6	0.025*	16.8	0.022*	17.1	0.020*	14.2	0.011*	14.8	0.002**
qDEC-8	Asominori	1.0		1.4		1.3		1.6		1.2		1.6		1.2		1.1	
	L49	16.0	0.022*	20.5	0.029*	20.1	0.008**	17.5	0.041*	16.8	0.019*	20.7	0.001**	21.7	0.006**	17.0	0.001**
	L50	16.8	0.036*	15.6	0.005**	17.9	0.003**	15.1	0.002**	15.7	0.041*	15.0	0.013*	17.4	0.012*	15.8	0.031*
	L51	12.1	0.011*	11.7	0.006**	14.0	0.005**	13.8	0.011*	15.3	0.023*	15.6	0.028*	13.4	0.002**	12.9	0.008**

*, **Significant levels of *t*-tests at $P \leq 0.05$ and 0.01, respectively

[a]E1–E8, The eight environments as in Table 1

[b]Mean represents the trait average values of two replicates in the field experiment

[c, d, e, f] Values denote the significant difference between Asominori and CSSL1, CSSL2, CSSL4, or CSSL9 in the E1 (2000, Nanjing) at $P \leq 0.05$, $P \leq 0.05$, $P \leq 0.05$, and $P \leq 0.01$, correspondingly. The same parameters are true for each of the QTLs in the table

Table 6 Analysis of genotype-by-environment interaction using the AMMI model for six appearance quality traits among the CSSLs harboring 22 QTL alleles detected in eight environments

Traits	Sources	Error	Environment	Genotype	Environment × Genotype	*iPCA1*	*iPCA2*	*iPCA3*
GL	*df*	127	7	15	105	21	19	17
	SS	0.35	0.18**	32.25**	0.43*	0.18**	0.10*	0.07
	SS%					40.9	22.9	16.8
GW	*df*	95	7	11	77	17	15	13
	SS	0.08	0.11**	2.77**	0.42**	0.20**	0.10**	0.05**
	SS%					46.9	24.1	11.4
LWR	*df*	127	7	15	105	21	19	17
	SS	0.1	0.07**	9.29**	0.35**	0.14**	0.08**	0.07**
	SS%					40	22.8	20.5
PGWC	*df*	95	7	11	77	17	15	13
	SS	3116.12	3834.73**	115207.79**	5774.64**	3639.57**	1337.52**	421.02
	SS%					63	23.2	7.2
ACE	*df*	79	7	9	63	15	13	11
	SS	524.01	212.57**	4180.39**	775.73**	353.03**	203.93**	119.6
	SS%					45.5	26.3	15.4
DEC	*df*	135	7	16	112	22	20	18
	SS	328.51	247.33**	8070.35**	974.74**	374.73**	305.91**	151.51**
	SS%					38.4	31.4	15.5

*, **Significant levels of *t*-tests at $P \leq 0.05$ and 0.01, respectively

[a]Abbreviations are the same as in Table 2

[b]SS%, the percentage of SS (sum of squares) of *iPCA1-3* to that of enivronment × genotype

L61, L25, L26, and L27) carrying the *qGL-2* and *qGL-4* alleles were far from this point (Fig. 2A). These results showed that the stability of *qGL-1* and *qGL-3* was higher than that of *qGL-2* and *qGL-4* across the eight environments. Using the same inference, *qGW-5* was the most stable among the three GW QTLs (Table 7, Fig. 2B), and the stability of *qLWR-3* and *qLWR-5* was higher than that of *qLWR-1* and *qLWR-2* (Table 7, Fig. 2C).

The average D_i value of four CSSLs (L5, L6, L7, and L8) harboring the *qPGWC-1* allele was 3.90, and those of the CSSLs carrying the *qPGWC-8* and *qPGWC-9* alleles were 1.44 and 1.21, respectively (Table 7). The four CSSLs (L5, L6, L7, and L8) were far from the origin in the *iPCA1-iPCA2* biplot (Fig. 2D). Therefore, *qPGWC-8* and *qPGWC-9* were more stable than *qPGWC-1* across the eight environments. Similarly, the effects of *qACE-8* and *qDEC-8* were the most stable of the eight ACE and DEC QTLs (Table 7, Fig. 2E, F).

In summary, the nine QTLs (*qGL-1, qGL-3, qGW-5, qLWR-3, qLWR-5*, *qPGWC-8, qPGWC-9, qACE-8*, and *qDEC-8*) were relatively stable across the eight environments based on the presence and direction of significant QTL effects, the consistency of QTL detection, and the AMMI analysis of CSSLs harboring consistent QTL alleles.

Clustering of QTLs

The QTLs for related traits detected in at least three environments were frequently identified in the same genome regions (Table 4, Fig. 1). The GL, LWR and DEC, QTLs were clustered in the interval defined by R210 and C955 on chromosome 1. The IR24 allele decreased GL and LWR but increased DEC. Similarly, common GL and LWR QTLs were detected in the interval R19-C1677 on chromosome 3; in both cases, the IR24 allele had a positive effect on the traits. In addition, the region defined by markers R3166 and R569 on chromosome 5 harbored GW and LWR QTLs, although these act in opposite directions. Additionally, the PGWC, ACE, and DEC QTLs could be simultaneously mapped in the interval G1149-R727 and XNpb36-XNpb103 on chromosomes 8 and 9, respectively. The IR24 alleles at both loci had the positive effects on the three traits.

Discussion

Of the 22 QTLs identified in this study, nine (41%) were relatively stable across the eight environments (Table 4), unlike the outcome reported in other studies [29.7%, 1.5%, 0%, and 0% in Li et al. (2003), Hittalmani et al. (2003), Teulat et al. (2003), and Campbell et al. (2003), respectively]. The high percentage of stable QTLs found in the present study may be due to one or a combination of the following factors. (1) The stable QTLs were responsible for major effects and were associated with high LOD scores (average 6.8) and PVE (average 25.7%). As suggested by Tanksley (1993) and Zhuang et al. (1997), QTLs with major effects are more likely to be stable across multiple environments. (2) The heritability of GL, GW, and LWR all exceeded 95%, while those of PGWC, ACE, and DEC were over 83% (Lin et al. 2001; Xing et al. 2001). Highly heritable traits tend to be more repeatable and stable across multiple environments (Paterson et al. 1991). (3) Each CSSL carries a small number of IR24 segments in a largely Asominori background, and thus genetic interactions between IR24 alleles are minimized.

Table 7 The stability parameters of all the CSSLs carrying 22 QTL alleles detected across eight environments

Traits	QTLs	D_i values of parents and the target CSSLs harboring any of the 22 QTL allele				
GL		Parents	Aso	IR24		
		D_i	0.17	0.09		
	qGL-1	CSSL	L1	L2	L4	L9
		D_i	0.11	0.10	0.14	0.15
	qGL-3	CSSL	L16	L17	L18	L46
		D_i	0.13	0.16	0.05	0.20
	qGL-2	CSSL	L13	L14	L61	
		D_i	0.28	0.32	0.14	
	qGL-4	CSSL	L25	L26	L27	
		D_i	0.17	0.44	0.33	
GW		Parents	Aso	IR24		
		D_i	0.17	0.25		
	qGW-5	CSSL	L28	L29		
		D_i	0.13	0.08		
	qGW-1	CSSL	L7	L9	L37	
		D_i	0.21	0.14	0.26	
	qGW-6	CSSL	L9	L11	L19	L38
		D_i	0.14	0.23	0.39	0.20
		CSSL	L39	L61		
		D_i	0.40	0.19		
LWR		Parents	Aso	IR24		
		D_i	0.12	0.27		
	qLWR-3	CSSL	L16	L17	L18	L46
		D_i	0.11	0.15	0.16	0.15
	qLWR-5	CSSL	L28	L29		
		D_i	0.11	0.14		
	qLWR-1	CSSL	L1	L2	L4	L9
		D_i	0.23	0.17	0.12	0.31
	qLWR-2	CSSL	L7	L10	L11	L12
		D_i	0.15	0.30	0.34	0.20
PGWC		Parents	Aso	IR24		
		D_i	1.77	2.11		
	qPGWC-8	CSSL	L49	L50	L51	
		D_i	1.22	2.14	0.95	
	qPGWC-9	CSSL	L52	L53	L64	
		D_i	0.79	1.86	0.98	
	qPGWC-1	CSSL	L5	L6	L7	L8
		D_i	2.51	4.12	4.42	4.53
ACE		Parents	Aso	IR24		
		D_i	0.95	1.22		
	qACE-8	CSSL	L49	L50	L51	
		D_i	1.11	1.02	0.53	
	qACE-9	CSSL	L52	L53	L64	
		D_i	2.37	2.67	1.97	
	qACE-2	CSSL	L7	L10		
		D_i	2.63	1.63		
DEC		Parents	Aso	IR24		
		D_i	0.64	0.74		
	qDEC-8	CSSL	L49	L50	L51	
		D_i	0.80	0.60	0.65	
	qDEC-9	CSSL	L52	L53	L64	
		D_i	2.19	2.02	2.18	
	qDEC-1a	CSSL	L5	L6	L7	L8
		D_i	2.78	0.94	1.32	0.98
	qDEC-1b	CSSL	L1	L2	L4	L9
		D_i	0.94	1.31	1.66	0.74
	qDEC-2	CSSL	L7	L10		
		D_i	1.32	1.23		

Of the stable QTLs, *qGL-1, qPGWC-9, qACE-8*, and *qDEC-8* are reported here for the first time, while the remaining five QTLs (*qGL-3*, *qGW-5*, *qLWR-3*, *qLWR-5*, and *qPGWC-8*) are located in the vicinity of QTLs affecting these traits detected in the other mapping populations (Huang et al. 1997; Redoña and Mackill 1998; He et al. 1999; Tan et al. 2000). These five QTLs each accounted for a significant proportion of the overall phenotypic variation, with an average PVE of 42.0%, 44.0%, 24.6%, 31.8%, and 22.8%, respectively across various studies. This is taken to mean that the effects of these five QTLs are more stable not just across environments, but also across varied genetic backgrounds. Nine CSSLs (L16, L17, L18, L46, L28, L29,

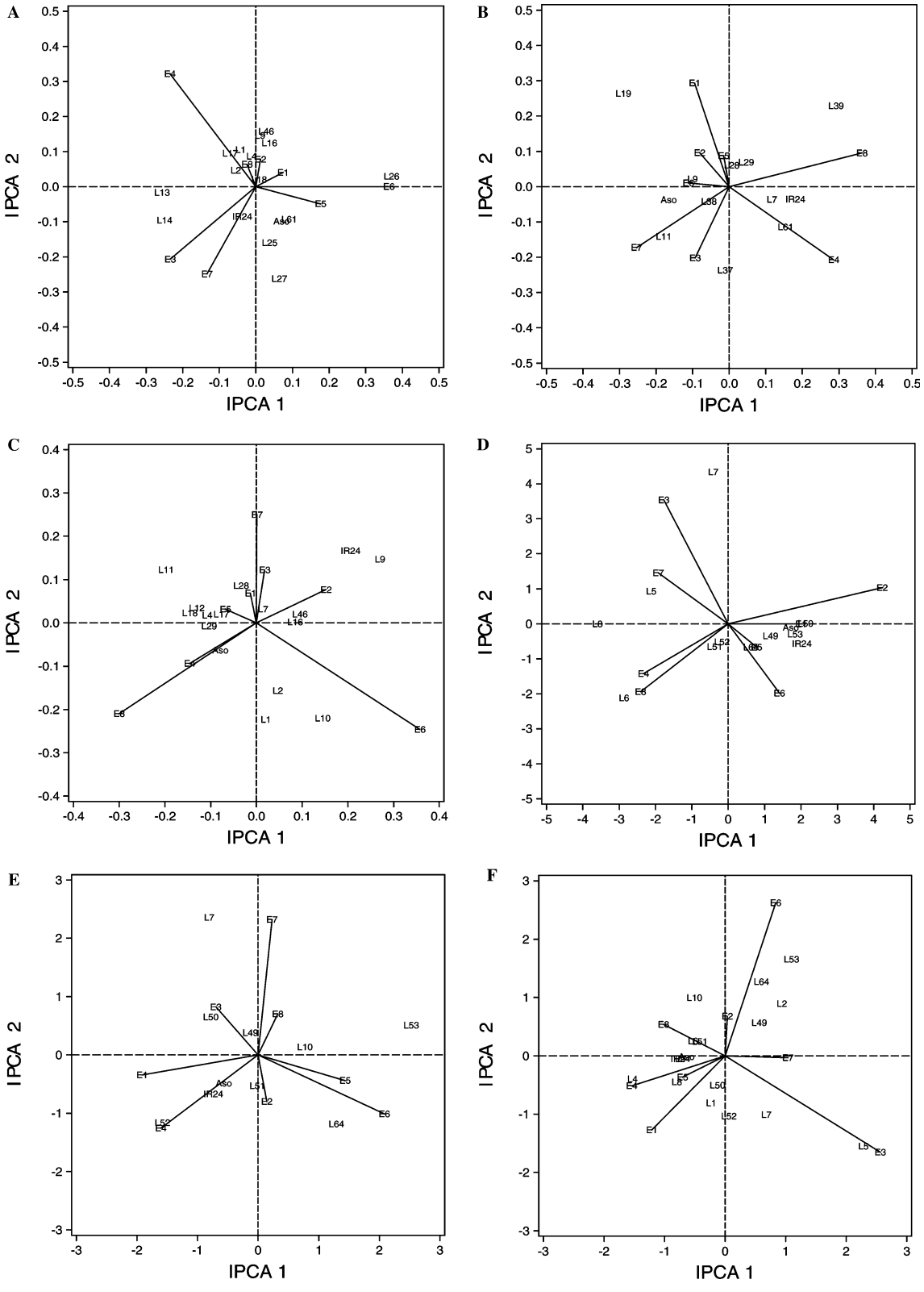

Fig. 2 The AMMI biplots for six appearance quality traits drawn using the phenotypic values of target CSSLs carrying any of the 22 QTL alleles detected in eight environments. AMMI biplots: **A** GL, **B** GW, **C** LWR, **D** PGWC, **E** ACE, **F** DEC. A detailed description of E1–E8 is give in Table 1

L49, L50, and L51), all harboring one of these five QTL alleles, have been backcrossed to Asominori, and nine secondary F_2 populations are currently being exploited for the fine mapping and positional cloning of these QTLs.

Appearance quality traits are quantitatively inherited. It is thus difficult for breeders to efficiently improve rice grain appearance using conventional methods due to a lack of discrete phenotypic segregation in rice progeny. Moreover, before maturity, the phenotypic identification of appearance traits can not be carried out during conventional breeding. Therefore, it is particularly helpful for enhancing the efficiency of selection and shortening the course of breeding to use markers closely linked to these above five QTLs to screen target genotypes directly for related traits in early generations.

Classical quantitative genetics assumes that trait correlations are the result of either pleiotropic effects or the tight linkage of genes. As numerous QTLs affecting related traits were mapped to similar genomic regions (Fig. 1), pleiotropy was the most probable genetic basis of the high trait correlations, which ranged from −0.835** between GW and LWR to 0.885** between ACE and DEC. Where pleiotropy is implicated, a coincidence of location and direction of genetic effect is expected for positively related traits. The results obtained here are in good agreement with this expectation. The detection of chromosomal segments harboring clusters of QTLs and the directions of the effects of these intervals were only slightly affected by environmental factors (Table 4, Fig. 1).

Three stable QTLs (*qPGWC-8*, *qACE-8*, and *qDEC-8*) were mapped in the interval G1149-R727 on chromosome 8 (Fig. 1). Interestingly, three stable QTLs (*qAC-8*, *qTD-8*, and *qIVOE-8*) affecting, respectively, the amylose content, tenderness, and palatability of cooked rice have also been detected in the same region (Wan et al. 2004). In addition, Jiang (2002) and Fujita et al. (1999) mapped the soluble starch synthase III (*SSSIII*) gene and isoamylase (*ISA*) gene in the V115-R1813 and C10122S-G1149 intervals on chromosome 8, respectively. Since these two regions overlap the G1149-R727 interval in high-density maps (Causse et al. 1994; McCouch et al. 2002), these QTL clusters appear to be associated with the synthesis and structure of amylose and amylopectin controlled by the *SSSIII* and *ISA* genes (Smith et al. 1997; Nakamura 2002). The elucidation of the molecular effects of starch properties on the determination of rice quality will require fine mapping and positional cloning of the QTL clusters using secondary F_2 populations between target CSSL and Asominori.

Acknowledgements We are extremely grateful to Prof. A. Yoshimura, Kyushu University, Japan, for kindly providing the CSSL population and genotype data. We thank http://www.smartenglish.co.uk for linguistic correction. This research is supported by the grants from the National High Technology Research and Development Program of China (No. 2003AA222131; 2003AA207020), the National Natural Science Foundation of China (No.30270811).

References

Campbell BT, Baenzigar PS, Gill KS, Eskridge KM, Budak H, Erayman M, Dweikat I, Yen Y (2003) Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci 43:1493–1505

Causse MA, Fulton TM, Cho YG, Ahn SN, Wu KS, Xiao JH, Yu ZH, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Crossa J, Gauch HG, Zobel RW (1990) Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. Crop Sci 30:493–500

Del Rosario AR, Briones VP, Vidal AJ, Juliano BO (1968) Composition and endosperm structure of developing and mature rice kernel. Cereal Chem 45:225–235

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. London New York

Frary An, Nesbitt TC, Frary Am, Grandillo S, Knaap EVD, Cong B, Liu JP, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: A quantitative trait locus key to the evolution of Tomato fruit size. Science 289:85–88

Fujita N, Kubo A, Francisco PB, Nakakita M, Harada K, Minaka N, Nakamura Y (1999) Purification, characterization, and cDNA structure of isoamylase from developing endosperm of rice. Planta 208:283–293

Gauch HG, Zobel RW (1988) Predictive and postdictive success of statistical analysis of yield trails. Theor Appl Genet 76:1–10

He P, Li SG, Qian Q, Ma YQ, Li JZ, Wang WM, Chen Y, Zhu LH (1999) Genetic analysis of rice grain quality. Theor Appl Genet 98:502–508

Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar HE, Zhuang JY, Zheng KL, Liu GF, Wang GC, Sidhu JS, Srivantaneeyahul S, Singh VP, Bagali PG, Prasanna HC, Mclaren G, Khush GS (2003) Identification of QTL for growth-yield and grain yield-related traits in rice across nine locations of Asia. Theor Appl Genet 107:679–690

Howell PM, Marshall DF, Lydiate DJ (1996) Towards developing intervarietal substitution lines in *Brassica napus* using marker-assisted selection. Genome 39:348–358

Huang N, Parco A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu JC, Subudhi P, Angeles ER, Khush GS (1997) RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a doubled haploid rice population. Mol Breed 3:105–113

Jiang HW (2002) The cloning and characterization of the gene for starch synthesis in rice endosperms and studies on the molecular physiological effects of high temperature on rice grain quality forming. PhD thesis, Zhejiang University, Hangzhou China

Kubo T, Nakamura K, Yoshimura A (1999) Development of a series of Indica chromosome segment substitution lines in Japonica background of rice. Rice Genet Newsl 16:104–106

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li ZK (2001) QTL mapping in rice: a few critical considerations. Rice genetics . In: Khush GS, Brar DS, Hrady B (eds) Proc 4th Int Rice Genet Symp. IRRI, Los Banos, Philippines, pp 153–171

Li ZK, Yu SB, Lafitte HR, Huang L, Courtois B, Hittalmani S, Vijayakumar CHM, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S, Khush GS (2003) QTL × environment interactions in rice. I. Heading date and plant height. Theor Appl Genet 108:141–153

Lin HX, Yamamoto T, Sasaki T, Yano M (2000) Characterization and detection of epistatic interactions of 3 QTLs, *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice using nearly isogenic lines. Theor Appl Genet 101:1021–1028

Lin JR, Wu MG, Shi CH (2001) Analysis on genetic effects of appearance quality traits in *japonica* hybrid rice. Chin J Rice Sci 15:93–96

McCouch SR, Teytelman L, Xu YB (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nagato K, Ebata M (1959) Studies on white –core rice kernel II. On the physical properties of the kernel. Proc Crop Sci Soc Jpn 28:46–50

Nakamura Y (2002) Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: rice endosperm as a model tissue. Plant Cell Physiol 43:718–725

NSPRC (National Standard of People Republic of China) (1999) High quality paddy, GB/T17891-1999, Standards Press of China

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127:181–197

Redoña ED, Mackill DJ (1998) Quantitative trait locus analysis for rice panicle and grain characteristics. Theor Appl Genet 96:957–963

SAS Institute (1994) JMP statistics and graphics guide: version 3: SAS Institute, Cary, N.C.

Smith AM, Denyer K, Martin C (1997) The synthesis of the starch granule. Annu Rev Plant Physiol. Plant Mol Biol 48:67–87

Tan YF, Xing YZ, Li JX, Yu SB, Xu CG, Zhang QF (2000) Genetic bases of appearance quality of rice grains in Shanyou 63, an elite rice hybrid. Theor Appl Genet 101:823–829

Tanksley SD (1993) Mapping ploygenes. Annu Rev Genet 27:205–233

Teulat B, Zoumarou-Wallis N, Rotter B, Salem MB, Bahri H, This D (2003) QTL for relative water content in field-grown barley and their stability across Mediterranean environments. Theor Appl Genet 108:181–188

Tsunematsu H, Yoshimura A, Harushima Y, Nagamura Y, Kurata N, Yano M, Sasaki T, Iwata N (1996) RFLP framework map using recombinant inbred lines in rice. Breed Sci 46:279–284

Vargas MH, Crossa J (2000) The AMMI analysis and graphing the biplot. Universidad Autónoma Chapingo, Biometrics and Statistics Unit, CIMMYT, Mexico. http://www.cimmyt.cgiar.org/biometrics

Vargas M, Crossa J, van Eeuwijk FA, Ramírez ME, Sayre K (1999) Using partial least squares regression, factorial regression, and AMMI models for interpreting genotype × environment interaction. Crop Sci 39:955–967

Wan XY, Wan JM, Su CC, Wang CM, Shen WB, Li JM, Wang HL, Jiang L, Liu SJ, Chen LM, Yasui H, Yoshimura A (2004) QTL detection for eating quality of cooked rice in a population of chromosome segment substitution lines. Theor Appl Genet 110:71–79

Xing YZ, Tan YF, Xu CG, Hua JP, Sun XL (2001) Mapping quantitative trait loci for grain appearance traits of rice using a recombinant inbred line population. Acta Bot Sin 43:840–845

Yamamoto R, Horisue N, Ikeda R (1995) Rice breeding manual. Miscellaneous Publication of the National Agriculture Research Centre in Japan. No. 30, October

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna Lisa, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd-1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12:2473–2483

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zhang Z, Lu C, Xiang ZH (1998) Analysis of variety stability based on the AMMI model in silkworm. Sci Agric Sin 31:62–68

Zhuang JY, Lin HX, Lu J, Qian HR, Hittamani S, Huang N, Zheng KL (1997) Analysis of QTL × environment interaction for yield components and plant height in rice. Theor Appl Genet 95:799–808