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Three main genetic regions for grain development revealed through QTL detection and meta-analysis in maize

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Abstract Grain weight is one of the three direct yield components, being developed through a dynamic process of grain filling in maize. In this study, 258 recombinant inbred lines derived from a cross between a dent corn and a popcorn inbred were evaluated for grain fresh and dry weight at 10, 20, 30, and 40 days after pollination (DAP) and the activities of ADP-Glc pyrophosphorylase (AGPP), granule-bound starch synthase (GBSS), and soluble starch synthase (SSS) at 30 DAP. Grain-filling rate (GFR) and increasing rate of fresh weight (FWIR) were calculated during all periods. Quantitative trait locus (QTL) mapping was conducted for all traits. Meta-QTL (mQTL) was revealed by meta-analysis using BioMercator. Totally, 161 QTL were detected for six traits. QTL on chromosomes 1, 7, and 10 were detected in most cases, with 43, 54, and 28 QTL, respectively. For each trait, 1–4 QTL were detected but no QTL for GBSS. Three mQTL at bins 7.02–7.03, 1.03–1.04, and 10.05–10.06 included 47, 24, and 23 QTL detected in this study. Together with 28 QTL for grain weight detected in our previous research, they included 53, 28, and 25 QTL, respectively. Five identified expressed sequence tags (EST), five candidate genes with related functions, and QTL for grain weight in other research were co-located in these regions. It is worth concentrating further research on these regions to develop near-isogenic lines (NILs) of common QTL and their chromosome segment substitution lines (CSSL). Also, cloning and function validation for co-located EST and candidate genes could facilitate identification of genes for grain development and final weight.

Keywords Grain weight · Grain-filling rate · Starch synthesis enzyme · Common QTL · Co-location · Meta-QTL analysis

Introduction

Grain weight is one of the three direct yield components, being developed through a dynamic process of grain filling in maize (*Zea mays* L.). Its genetic basis needs to be extensively revealed through all developmental stages. After cell division, differentiation, and expansion, grain weight at mature harvest time is determined by grain-filling rate and duration (Takai et al. 2005). Final grain weight is largely a genetically determined trait (Reddy and Daynard 1983; Seka and Cross 1995; Seka et al. 1995; Borrás et al. 2009), though grain development is affected by many environmental and physiological factors, such as drought (Ouattar et al. 1987),

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temperature (Wilhelm et al. 1999), nitrogen content (Ta and Weiland 1992), assimilate availability (Borrás et al. 2004), related enzyme activity (Jones and Brenner 1987; Thévenot et al. 2005), kernel water content (Ouattar et al. 1987; Borrás et al. 2003), and kernel number (Hartung et al. 1989; Borrás and Otegui 2001). Ample variations in grain weight, and grain-filling rate and duration have been observed among commercial hybrids (Gambín et al. 2007) and diverse inbreds (Borrás et al. 2009). Different genotypes exhibit a wide range of grain growth patterns (Borrás et al. 2009). Both rate and duration of grain filling are important in grain development (Ercole and Alessandro 1981; Wang et al. 1999; Takai et al. 2005). However, improvement in grain-filling rate should receive more attention than long grain-filling duration in areas such as the Huanghuihai Corn Belt in China, where the growth season for maize is limited (Liu et al. 2011).

In previous research, QTL mapping for grain weight at final harvest time has been extensively conducted using three kinds of maize germplasm: normal maize (Stuber et al. 1987, 1992; Veldboom and Lee 1994; Austin and Lee 1996, 1998; Austin et al. 2000; Mihaljevic et al. 2004; Blanc et al. 2006; Yan et al. 2006; Ma et al. 2007), high-oil maize (Song 2003), and popcorn (Li et al. 2007, 2011). According to public data in Gramene (http://www.gramene.org/), 186 QTL for grain weight had been reported until 31 May 2010, distributed on all ten maize chromosomes. However, very few studies have been carried out on dynamic QTL of grain weight, and grain-filling rate and duration across different developmental stages. Recently, Capelle et al. (2010) detected QTL for fresh grain weight (GFW) and dry grain weight (GDW) at 30, 40, 60, and 80 DAP using an intermated RIL population derived from two normal maize inbreds F2 and F252. Liu et al. (2011) detected QTL for GDW at 16, 23, 32, 40, and 45 DAP, and QTL for grain-filling rate (GFR) during four periods using 203 RIL from a cross between two normal maize inbreds, Huang-C and Xu178.

Starch constitutes 70–75% of grain dry weight in maize (Boyer and Hannah 2001). Therefore, starch concentration plays an important role in grain weight potential, and the starch synthesis pathway could be an important site of regulation. ADP-Glc pyrophosphorylase (AGPP), starch synthase (granule-bound starch synthase, GBSS; soluble starch synthases, SSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) are four kinds of important enzymes in starch synthesis and accumulation (Prioul et al. 1990, 1994; Slattery et al. 2000). An effective way to determine main QTL for objective traits is to use genetic variability in enzyme activity and carbohydrate concentration. QTL co-located with genes encoding for related functions could be considered as candidate genes (Prioul et al. 1997; Thévenot et al. 2005). Such an approach has been successfully used for carbon metabolism in leaves (Causse et al. 1995; Pelleschi et al. 1999). Thévenot et al. (2005) detected QTL for enzyme activities and soluble carbohydrates involved in starch accumulation at 15, 25, and 35 DAP using 100 RIL from a cross between a late dent line MBS and an early flint line F-2.

Since the results of QTL detection were greatly influenced by different populations, generations, and environments (Austin and Lee 1996; Li et al. 2007), direct comparisons of QTL data across different studies were difficult. Integration of QTL detected across independent studies could be realized through meta-QTL analysis, and true QTL with more accurate confidence intervals and small target regions for candidate genes could be provided (Goffinet and Gerber 2000; Arcade et al. 2004). This method has been used in QTL integration for grain yield components (Wang et al. 2009) and flowering time (Chardon et al. 2004) in maize, and in other crops (Shi et al. 2009).

In this study, a cross between two contrasting genotypes, an early popcorn inbred N04 with small grain size and a late dent inbred Dan232 with large grain size, was used to develop 258 RIL (Li et al. 2011). QTL mapping was conducted for grain dry weight and fresh weight at four stages (10, 20, 30, and 40 DAP), grain-filling rate in dry and fresh weight during six periods, and activities of three enzymes (AGPP, GBSS, and SSS) at 30 DAP. In addition, meta-QTL was analyzed for QTL of all traits detected herein, and together with QTL for grain dry weight at mature harvest time obtained in our previous studies using the same population (Li et al. 2011) and its $F_{2:3}$ and BC_2F_2 generations (Li et al. 2007). Our main objectives were to (1) reveal the characteristics of dynamic QTL for grain weight and grain-filling rate, (2) relate QTL for grain weight and grain-filling rate with the activities of

starch synthesis-related enzymes, (3) identify consistent QTL across different generations, environments, and related traits, and (4) determine main genetic regions for grain development worthy of elucidation in further research in maize.

Materials and methods

Plant materials and field experiment

The population used in this study consisted of 258 F_9 RIL derived by the single-seed descent method from a cross between two contrasting inbred lines, Dan232 and N04. Dan232 is a late dent corn inbred with large-size grain, derived from Lu 9 Kuan × Dan340. N04 is an early popcorn inbred with small-size grain, derived from a Chinese popcorn variety BL03 (Li et al. 2007, 2011).

The 258 F_9 RIL and both parents were evaluated using α-designs with three-row plots and two replications at Zhengzhou, Henan, China in 2008 and 2009. The rows were 4 m long with 0.67 m spacing between rows. Plots were planted by hand at density of 60,000 plants per ha. Standard cultivation management practices were used at each environment. All plants were self-pollinated within each plot by hand when more than 80% silks appeared.

Trait evaluation

Three to five ears with uniform grain set were harvested at 10, 20, 30, and 40 DAP. To increase the representativeness and uniformity of the sampled kernels, only kernels on the middle two-thirds of each ear were shelled manually and bulked within plot. Some of the samples collected at 30 DAP were immediately frozen in liquid nitrogen and stored at −80°C for measurement of enzyme activity. This date was chosen because previous studies have shown that the activities of AGPP, GBSS, and SSS peaked at 25–35 DAP (Prioul et al. 1990, 1994; Thévenot et al. 2005; Yang 2010).

One-hundred-grain fresh weight (GFW, g) was tested with two replications at each time point for each plot. After being naturally dried, 100-grain dry weight (GDW, g) was tested accordingly. Grain-filling rate (GFR, g/day) during six periods was calculated, including 10–20 DAP (12 DAP), 10–30 DAP (13 DAP), 10–40 DAP (14 DAP), 20–30 DAP (23 DAP), 20–40 DAP (24 DAP), and 30–40 DAP (34 DAP). Since the GFW for some lines at 40 DAP was lower than at 20 DAP and/or 30 DAP, increasing rate of fresh weight (FWIR, g/day) was calculated for five periods, 10 DAP, 12 DAP, 13 DAP, 14 DAP, and 23 DAP. Trait measurements averaged over two replications were used as the preliminary data in further analysis.

The activities of three enzymes AGPP, GBSS, and SSS at 30 DAP were measured as described by Douglas et al. (1988) and Nakamura and Yuk (1989).

Phenotypic data analysis

Analysis of variance for each trait and correlation coefficients among traits were calculated using the statistical software package SPSS12.0 with random statistical model. Heritability and the confidence intervals of the measured traits were computed according to Knapp et al. (1985). Heritability (H_B^2) was calculated as $H_B^2 = 1 - 1/F$, where $F = MS_g/MS_{ge}$. $MS_g = nr\sigma_g^2 + r\sigma_{ge}^2 + \sigma_e^2$, $MS_{ge} = r\sigma_{ge}^2 + \sigma_e^2$, where σ_g^2 is the variance of family, σ_{ge}^2 is the variance of interaction between family and environment, σ_e^2 is the error variance, r is the number of replications, and n is the number of locations.

Simple sequence repeat (SSR) analysis and QTL analysis

A total of 207 simple sequence repeat (SSR) markers were used to genotype the 258 RIL using the same method described by Li et al. (2007). The linkage maps covered 10 maize chromosomes with total length of 2,408.8 cM and average interval of 11.6 cM (Li et al. 2011).

Composite interval mapping (CIM) was used to map QTL and estimate their effects for each trait (Zeng 1993, 1994). Model 6 of the Zmapqtl procedure in QTL Cartographer version 2.5 (Wang et al. 2011) was employed, specifying the five markers identified by stepwise regression that explained most of the variation for a given trait as genetic background parameters and a window size of 10 cM on either side of the markers flanking the test site. To identify an accurate significance threshold for each trait according to the traditionally used alpha of 5%, an empirical threshold was determined for CIM using 1,000 permutations (Churchill and Doerge 1994).

QTL position was assigned to relevant regions at the point of a maximum likelihood odds ratio (LOD) score. QTL confidence interval was calculated by subtracting one LOD unit on each side from the maximum LOD position. Based on the results of QTL mapping, interactions among detected QTL were analyzed using MIM in WinQTLCart (Wang et al. 2011).

Meta-QTL analysis with BioMercator

In our previous research, three generations ($F_{2:3}$, BC_2F_2, and RIL) were derived from the same cross between Dan232 and N04. Using two genetic linkage maps constructed using $F_{2:3}$ and RIL populations, QTL for GDW at mature harvest time have been detected in three populations (Li et al. 2007, 2011). The field experiments in previous research were conducted under two or four environments in Henan, China.

Since QTL for the same or related traits detected in different experiments and mapped to the same or similar chromosome regions might be several estimates of the position of a single QTL, algorithms for meta-analysis were used to estimate the numbers and positions of their meta-QTL (mQTL) (Goffinet and Gerber 2000) using BioMercator 2.1 software (Arcade et al. 2004). The merged genetic linkage map was obtained by projecting $F_{2:3}$ map onto RIL map consisting of 237 SSR markers, and was 2,452.2 cM long with average marker interval of 10.35 cM (Li et al. 2011). According to data for multiple individual QTL, a modified Akaike's information criterion (AIC) was calculated to select among models with varying numbers of mQTL. The model with the lowest test statistic was the most probable model. In each model, a confidence interval was calculated for each mQTL.

Results

Trait analysis of variance, performance, heritability, and correlation in RIL population

Variances for genotype (σ^2_g) and genotype × year interactions (σ^2_{ge}) were significant for all traits, and those for year (σ^2_e) were significant for most traits (Table 1). Except GFR at 12 DAP and 34 DAP, heritability estimates for other traits were middle to high, with ranges from 0.60 to 0.88.

All traits differed greatly between the two parents. The popcorn inbred N04 had lower values than the dent corn inbred Dan232 for all traits (Table 1). According to the values of skewness and kurtosis, all traits showed normal distributions and transgressive segregations in the RIL population. The coefficients of variation (CV) were high, ranging from 15.3% for GDW at 20 DAP to 46.3% for FWIR at 23 DAP.

For correlations among traits, almost the same tendency was observed for data in 2008 and 2009 and in combined analysis. According to the results in 2008 (Table 2), significant positive correlations were shown in all cases except 11, where the correlations were insignificant, being GFR at 34 DAP with GDW at 30 DAP, GFR at 24 DAP and 34 DAP, FWIR at 13 DAP, 14 DAP, and 23 DAP, and the activity of AGPP with GDW at 10 DAP, FWIR at 23 DAP, and the activities of AGPP and GBSS with GFR at 34 DAP, and the activity of GBSS with GFR at 24 DAP.

QTL identification for GDW and GFW at four stages after pollination

Since the variance components for genotype × year interactions (σ^2_{ge}) were significant for all traits, QTL mapping was conducted for data for each year. For comparison, combined analyses using means across 2 years were also conducted. A total of 34 QTL for GDW were detected at four stages (Table 3, Supplementary Table 1), being located on chromosomes 1, 3, 4, 5, 7, 9, and 10. Individual QTL explained phenotypic variances from 4.8% to 23.5%, with 23 QTL over 10% and 10 QTL over 15%. The positive alleles of all QTL came from the dent corn parent Dan232 except QTL on chromosomes 3 and 4. QTL on chromosome 7 were consistently detected at all stages under each environment and in combined analysis. They were located in the same marker interval umc2057–umc1567 (bin 7.02–7.03), except at umc1068–umc1066 (bin 7.01–7.02) at 10 DAP in 2008. QTL on chromosome 10 were all detected at four stages in 2009 and in combined analysis, and at 20 DAP in 2008, being located in the same marker interval umc1677–umc2122 (bin 10.05–10.06). On chromosome 1 in the same marker interval phi001–umc2227 (bin 10.05–10.06), QTL were detected at

Table 1 Combined analysis of variances and heritabilities (H_B^2) for all traits, and their performance for two parents and in the RIL population

Trait	Stage (DAP)	Parent		RIL population									
		Dan232	N04	Range	Average	CV%	Skewness	Kurtosis	σ_g^2	σ_e^2	σ_{ge}^2	H_B^2	H_B^2 95% CI
GFW	10	9.18	5.35	4.92–12.73	8.05	16.25	0.47	0.41	7.03**	1.92	6.03**	0.86	0.82–0.89
	20	28.37	10.67	11.50–27.40	18.73	16.75	0.22	–0.10	8.04**	0.26	4.65**	0.88	0.85–0.90
	30	40.11	11.85	11.87–33.46	22.52	18.89	0.04	–0.21	7.14**	8.63*	6.32**	0.86	0.82–0.89
	40	41.42	11.96	9.22–38.48	22.46	23.45	0.40	0.48	5.61**	18.08**	6.60**	0.82	0.77–0.86
GDW	10	1.09	0.97	0.73–1.95	1.25	16.09	0.46	0.27	3.99**	13.77**	3.70**	0.75	0.68–0.80
	20	8.41	5.20	5.16–11.01	7.46	15.25	0.26	–0.27	5.85**	6.99	3.47**	0.83	0.78–0.87
	30	19.73	8.19	7.24–18.29	12.83	17.02	0.02	–0.26	5.41**	17.66**	4.75**	0.82	0.76–0.86
	40	23.16	9.01	7.60–24.47	15.38	19.15	0.14	0.19	4.48**	0.98	4.40**	0.78	0.71–0.83
FWIR	10	0.92	0.54	0.49–1.27	0.81	16.24	0.47	0.41	5.73**	24.11*	3.03**	0.83	0.78–0.86
	12	1.92	0.53	0.51–1.71	1.07	21.25	0.28	–0.03	5.16**	41.55**	4.94**	0.81	0.75–0.85
	13	1.55	0.33	0.28–1.22	0.73	24.80	0.02	–0.19	5.31**	2.88**	3.99**	0.81	0.76–0.85
	14	1.08	0.22	0.13–0.97	0.48	33.02	0.43	0.52	2.48**	8.68**	3.29**	0.60	0.49–0.68
	23	1.17	0.12	0.001–0.95	0.40	46.31	0.27	0.13	3.20**	0.08	3.85**	0.69	0.60–0.76
GFR	12	0.73	0.42	0.43–0.92	0.62	16.32	0.27	–0.32	1.86**	2.21	2.19**	0.46	0.31–0.58
	13	0.93	0.36	0.31–0.84	0.58	18.02	0.01	–0.24	7.03**	1.92	6.03**	0.86	0.82–0.89
	14	0.74	0.27	0.22–0.77	0.47	20.38	0.15	0.21	6.80**	0.37	2.46**	0.85	0.81–0.88
	23	1.13	0.30	0.07–0.99	0.54	25.65	–0.06	0.47	5.21**	7.41*	4.46**	0.81	0.75–0.85
	24	0.74	0.19	0.12–0.75	0.39	29.49	0.27	0.26	5.15**	16.06**	5.58**	0.81	0.75–0.84
	34	0.34	0.08	0.002–0.83	0.28	57.99	0.77	0.77	1.78**	4.96*	2.55**	0.44	0.28–0.56
AGPP	30	3.05	2.17	0.97–4.76	2.47	27.59	0.23	–0.10	–	–	–	–	–
GBSS	30	3.31	1.79	0.71–5.87	2.66	25.09	0.10	1.80	–	–	–	–	–
SSS	30	4.17	2.85	1.43–6.57	3.57	26.63	0.16	–0.26	–	–	–	–	–

CI confidence interval, *GFW* 100-grain fresh weight, *GDW* 100-grain dry weight, *FWIR* increasing rate of fresh weight, *GFR* grain-filling rate, *AGPP* ADP-Glc pyrophosphorylase, *GBSS* granule-bound starch synthase, *SSS* soluble starch synthases, *CV* coefficient of variance

* and ** Indicate significance at 0.05 and 0.01 levels, respectively

Table 2 Phenotypic correlations among all traits according to the data in 2008

Trait	Stage	GDW				GFW			
		10 DAP	20 DAP	30 DAP	40 DAP	10 DAP	20 DAP	30 DAP	40 DAP
GDW	20 DAP	0.60**	1.00						
	30 DAP	0.41**	0.78**	1.00					
	40 DAP	0.34**	0.71**	0.84**	1.00				
GFW	10 DAP	0.81**	0.63**	0.53**	0.46**	1.00			
	20 DAP	0.51**	0.84**	0.75**	0.70**	0.74**	1.00		
	30 DAP	0.33**	0.69**	0.95**	0.77**	0.54**	0.79**	1.00	
	40 DAP	0.24**	0.55**	0.71**	0.97**	0.43**	0.69**	0.76**	1.00
GFR	12 DAP	0.46**	0.99**	0.79**	0.64**	0.55**	0.83**	0.70**	0.56**
	13 DAP	0.32**	0.76**	0.99**	0.76**	0.48**	0.77**	0.95**	0.72**
	14 DAP	0.24**	0.61**	0.75**	0.99**	0.42**	0.69**	0.78**	0.97**
	23 DAP	0.15*	0.40**	0.87**	0.63**	0.32**	0.52**	0.87**	0.63**
	24 DAP	0.09^{ns}	0.30**	0.55**	0.93**	0.26**	0.46**	0.62**	0.93**
	34 DAP	0.0^{ns}	0.21**	0.12^{ns}	0.64**	0.17*	0.30**	0.19**	0.64**
FWIR	10 DAP	0.81**	0.63**	0.53**	0.46**	10.0**	0.74**	0.54**	0.44**
	12 DAP	0.25**	0.79**	0.77**	0.69**	0.46**	0.94**	0.81**	0.68**
	13 DAP	0.12^{ns}	0.59**	0.91**	0.74**	0.30**	0.71**	0.96**	0.75**
	14 DAP	0.05^{ns}	0.44**	0.65**	0.94**	0.22**	0.56**	0.71**	0.97**
	23 DAP	0.11^{ns}	0.30**	0.67**	0.49**	0.18**	0.23**	0.68**	0.48**
AGPP	30 DAP	0.11^{ns}	0.29**	0.43**	0.33**	0.19**	0.31**	0.44**	0.32**
GBSS	30 DAP	0.16**	0.21**	0.29**	0.17**	0.20**	0.22**	0.28**	0.18**
SSS	30 DAP	0.15*	0.25**	0.37**	0.31**	0.25**	0.33**	0.40**	0.31**

Trait	Stage	GFR						FWIR					AGPP	GBSS
		12 DAP	13 DAP	14 DAP	23 DAP	24 DAP	34 DAP	10 DAP	12 DAP	13 DAP	14 DAP	23 DAP	30 DAP	30 DAP
GDW	20 DAP													
	30 DAP													
	40 DAP													
GFW	10 DAP													
	20 DAP													
	30 DAP													
	40 DAP													

Table 2 continued

Trait	Stage	GFR						FWIR					AGPP	GBSS
		12 DAP	13 DAP	14 DAP	23 DAP	24 DAP	34 DAP	10 DAP	12 DAP	13 DAP	14 DAP	23 DAP	30 DAP	30 DAP
GFR	12 DAP	1												
	13 DAP	0.77**	1											
	14 DAP	0.62**	0.76**	1										
	23 DAP	0.42**	0.89**	0.65**	1									
	24 DAP	0.32**	0.58**	0.94**	0.59**	1								
	34 DAP	0.23**	0.12^{ns}	0.65**	0.04^{ns}	0.72**	1							
FWIR	10 DAP	0.55**	0.48**	0.42**	0.32**	0.26**	0.17*	1						
	12 DAP	0.82**	0.77**	0.69**	0.53**	0.47**	0.31**	0.46**	1					
	13 DAP	0.62**	0.93**	0.75**	0.89**	0.63**	0.16*	0.30**	0.78**	1				
	14 DAP	0.47**	0.66**	0.95**	0.62**	0.94**	0.66**	0.22**	0.62**	0.74**	1			
	23 DAP	0.31**	0.68**	0.52**	0.85**	0.47**	0.08^{ns}	0.18**	0.29**	0.74**	0.51**	1.00		
AGPP	30 DAP	0.30**	0.43**	0.34**	0.39**	0.27**	0.09^{ns}	0.19**	0.35**	0.43**	0.30**	0.27**	1.00	
GBSS	30 DAP	0.20**	0.28**	0.17**	0.23**	0.10^{ns}	-0.003^{ns}	0.20**	0.20**	0.25**	0.15*	0.19**	0.28**	1.00
SSS	30 DAP	0.25**	0.37**	0.31**	0.35**	0.27**	0.16*	0.25**	0.32**	0.37**	0.28**	0.24**	0.71**	0.44**

GFW 100-grain fresh weight, *GDW* 100-grain dry weight, *FWIR* increasing rate of fresh weight, *GFR* grain-filling rate, *AGPP* ADP-Glc pyrophosphorylase, *GBSS* granule-bound starch synthase, *SSS* soluble starch synthases, *ns* not significant

* and ** Indicate significance at 0.05 and 0.01 levels, being 0.12 and 0.16, respectively

Table 3 QTL detected for GDW and GFW at four stages in combined analysis, and for the activities of AGPP and SSS at 30 DAP in 2008

Trait	Stage (DAP)	QTL	Marker interval	Bin loci[a]	Position	LOD[b]	R^2(%)[c]	A[d]
GDW	10	qcGDW1-7-1	umc2057–umc1567	7.02–7.03	75.2	5.0	11.3	0.07
		qcGDW1-10-1	umc1677–umc2122	10.05–10.06	75.7	3.8	10.8	0.07
	20	qcGDW2-1-1	phi001–umc2227	1.03–1.04	129.3	5.7	23.5	0.55
		qcGDW2-7-1	umc2057–umc1567	7.02–7.03	75.2	9.1	16.5	0.47
		qcGDW2-10-1	umc1677–umc2122	10.05–10.06	75.7	6.4	14.6	0.44
	30	qcGDW3-1-1	phi001–umc2227	1.03–1.04	131.3	4.2	15.9	0.87
		qcGDW3-7-1	umc2057–umc1567	7.02–7.03	75.2	10.4	20.5	0.99
		qcGDW3-10-1	umc1677–umc2122	10.05–10.06	75.7	3.1	7.1	0.58
	40	qcGDW4-1-1	phi001–umc2227	1.03–1.04	131.3	4.5	16.0	1.20
		qcGDW4-5-1	umc1502–umc1941	5.05–5.06	162.7	3.7	9.0	0.91
		qcGDW4-7-1	umc2057–umc1567	7.02–7.03	75.2	7.8	16.1	1.21
		qcGDW4-10-1	umc1677–umc2122	10.05–10.06	73.7	3.0	6.3	0.75
GFW	10	qcGFW1-1-1	umc1976–bnlg1803	1.02	32.5	4.8	7.6	–0.36
		qcGFW1-1-2	bnlg1007–umc1403	1.02–1.03	51.1	4.0	8.7	–0.39
		qcGFW1-1-3	umc2083–umc1281	1.05–1.06	205.7	3.9	6.1	0.33
		qcGFW1-7-1	umc2057–umc1567	7.02–7.03	75.2	4.8	10.3	0.43
		qcGFW1-10-1	umc1677–umc2122	10.05–10.06	75.7	3.1	7.7	0.37
	20	qcGFW2-1-1	umc1906–umc2083	1.05–1.06	199.8	8.6	13.6	1.17
		qcGFW2-7-1	umc2057–umc1567	7.02–7.03	75.2	8.7	17.7	1.33
		qcGFW2-10-1	umc1938–umc2163	10.03–10.04	56.1	3.4	5.5	0.75
	30	qcGFW3-1-1	phi001–umc2227	1.03–1.04	131.3	4.0	17.2	1.78
		qcGFW3-5-1	umc1502–umc1941	5.05–5.06	162.7	3.2	7.2	1.15
		qcGFW3-7-1	umc2057–umc1567	7.02–7.03	75.2	8.7	18.0	1.82
	40	qcGFW4-1-1	phi001–umc2227	1.03–1.04	131.3	3.6	13.3	1.92
		qcGFW4-5-1	umc1502–umc1941	5.05–5.06	162.7	4.2	9.8	1.66
		qcGFW4-7-1	umc2057–umc1567	7.02–7.03	75.2	6.8	14.2	1.99
AGPP	30	qAGPP3-7-1	umc2057–umc1567	7.02–7.03	75.2	6.3	14.6	–1.18
SSS	30	qSSS3-7-1	umc2057–umc1567	7.02–7.03	75.2	4.5	10.0	–1.42

GDW 100-grain dry weight, *GFW* 100-grain fresh weight, *AGPP* ADP-Glc pyrophosphorylase, *SSS* soluble starch synthases

[a] Bin locations for the flanking markers

[b] LOD, the likelihood odds ratio

[c] R^2, percent of phenotypic variance explained by each QTL

[d] The additive effects of QTL; positive values indicated that alleles from Dan232 increased the trait scores

20, 30, and 40 DAP in 2009, and in combined analysis. On chromosome 5, QTL were detected in umc1478–bnlg565 (bin 5.01–5.02) at 30 DAP in 2008 and at 10 DAP in 2009, and in umc1502–umc1941 (bin 5.05–5.06) at 40 DAP in 2008, and in combined analysis. QTL on chromosomes 3, 4, and 9 were only detected in one case.

For GFW, a total of 37 QTL were detected at four stages (Table 3, Supplementary Table 1), being located on chromosomes 1, 5, 7, 9, and 10. Individual QTL explained phenotypic variances from 4.8 to 21.3%, with 20 QTL over 10% and 5 QTL over 15%. The positive alleles of all QTL came from the dent corn parent Dan232 except the QTL at bins 1.02, 1.02–1.03, and 3.03–3.04. QTL on chromosome 7 were consistently detected at all stages under both environments and in combined analysis. They were located in the same marker interval umc2057–umc1567 (bin 7.02–7.03), except at bnlg2233–umc1068 (bin 7.02) at 10 DAP in 2008. QTL at bin

1.05–1.06 and QTL on chromosome 10 were detected at 10 DAP and 20 DAP, while those QTL at bin 1.03–1.04 and on chromosome 5 were detected at 30 DAP and 40 DAP in most cases. QTL on chromosome 9 was only detected at 10 DAP in 2009.

QTL identification for GFR and FWIR during six periods

Totally, 44 QTL for GFR were detected, located on chromosomes 1, 3, 4, 5, 7, and 10 (Table 4,

Table 4 QTL detected for GFR and FWIR during six periods in combined analysis

Trait	Stage (DAP)	QTL	Marker interval	Bin loci[a]	Position	LOD[b]	R^2 (%)[c]	A[d]
GFR	12	qcGFR12-1	phi001–umc2227	1.03–1.04	129.3	6.0	25.4	0.51
		qcGFR12-7	umc2057–umc1567	7.02–7.03	75.2	8.4	15.7	0.41
		qcGFR12-10	umc1677–umc2122	10.05–10.06	75.7	6.4	15.0	0.39
	13	qcGFR13-1	phi001–umc2227	1.03–1.04	129.3	4.1	17.5	0.88
		qcGFR13-7	umc2057–umc1567	7.02–7.03	75.2	9.0	17.5	0.88
		qcGFR13-10	umc1677–umc2122	10.05–10.06	73.7	3.1	6.6	0.54
	14	qcGFR14-1	phi001–umc2227	1.03–1.04	131.3	4.5	15.7	1.17
		qcGFR14-5	umc1502–umc1941	5.05–5.06	162.7	4.1	9.9	0.93
		qcGFR14-7	umc2057–umc1567	7.02–7.03	75.2	7.6	15.8	1.17
		qcGFR14-10	umc1677–umc2122	10.05–10.06	71.7	3.0	5.8	0.71
	23	qcGFR23-5	umc1221–umc1502	5.04–5.05	147.2	3.9	7.5	0.38
		qcGFR23-7	umc2057–umc1567	7.02–7.03	75.2	6.2	13.8	0.51
	24	qcGFR24-5	umc1502–umc1941	5.05–5.06	156.7	3.0	6.0	0.59
		qcGFR24-7	bnlg2233–umc1068	7.02	18.0	3.2	10.0	0.77
	34	qcGFR34-5	bnlg565–phi109188	5.02–5.03	75.4	3.9	16.9	0.67
		qcGFR34-10	umc1677–umc2122	10.05–10.06	70.3	3.0	12.3	–0.57
FWIR	10	qcFWIR10-1-1	bnlg1007–umc1403	1.02–1.03	51.1	4.0	8.8	–0.039
		qcFWIR10-1-2	umc2083–umc1281	1.05–1.06	205.7	3.9	6.1	0.033
		qcFWIR10-7-1	umc2057–umc1567	7.02–7.03	75.2	4.8	10.1	0.042
		qcFWIR10-10-1	umc1677–umc2122	10.05–10.06	75.7	3.2	7.9	0.037
	12	qcFWIR12-1-1	umc1906–umc2083	1.05–1.06	199.8	6.4	10.2	0.073
		qcFWIR12-5-1	bnlg565–phi109188	5.02–5.03	73.4	3.1	11.1	0.076
		qcFWIR12-7-1	umc2057–umc1567	7.02–7.03	77.2	5.6	10.8	0.075
	13	qcFWIR13-1-1	phi001–umc2227	1.03–1.04	129.3	4.2	17.0	0.075
		qcFWIR13-5-1	umc1478–bnlg565	5.01–5.025	45.5	3.4	7.6	0.051
		qcFWIR13-7-1	umc2057–umc1567	7.02–7.03	77.2	4.4	9.0	0.054
	14	qcFWIR14-1-1	phi001–umc2227	1.03–1.04	127.3	3.9	13.6	0.059
		qcFWIR14-5-1	umc1502–umc1941	5.05–5.06	162.7	3.7	9.0	0.048
		qcFWIR14-7-1	umc2057–umc1567	7.02–7.03	73.2	3.7	7.0	0.042
		qcFWIR14-9-1	phi065–umc2121	9.03–9.04	111.4	3.0	6.6	–0.041
	23	qcFWIR23-2-1	mmc0381–umc1992	2.08	237.5	3.7	5.9	0.048
		qcFWIR23-5-1	umc1221–umc1502	5.04–5.05	147.2	3.0	6.4	0.049
		qcFWIR23-7-1	bnlg339–umc1070	7.03	101.7	3.1	5.2	0.044

FWIR increasing rate of fresh weight, *GFR* grain-filling rate

[a] Bin locations for the flanking markers

[b] LOD, the likelihood odds ratio

[c] R^2, percent of phenotypic variance explained by each QTL

[d] The additive effects of QTL, positive values indicated that alleles from Dan232 increased the trait scores

Supplementary Table 2). Individual QTL explained phenotypic variances from 4.4 to 25.4%, with 26 QTL over 10% and 14 QTL over 15%. Except two QTL on chromosome 3, the positive alleles of all other QTL came from the dent corn parent Dan232. The distributions and locations of GFR QTL were very similar to those for GDW. On chromosome 7, 14 GFR QTL were detected, with 13 in the same marker interval umc2057–umc1567 (bin 7.02–7.03) and one in bnlg2233–umc1068 (bin 7.02). Twelve QTL were detected on chromosome 10. They were located in four marker intervals, with eight QTL in umc1677–umc 2122 (bin 10.05–10.06), two in umc1319–umc1567 (bin 10.01–10.02), one in umc1576–umc2034 (bin 10.02), and one in phi059–umc2067 (bin 10.02–10.03). Nine QTL detected on chromosome 1 were all located in the same marker interval phi001–umc2227 (bin 1.03–1.04). Six QTL were detected on chromosome 5, being located in four marker intervals (bnlg656–phi109188, umc1389–umc1162, umc1221–umc1502, and umc1502–umc1941) related with bin 5.02–5.06 (5.02–5.03, 5.03–5.04, 5.04–5.05, and 5.05–5.06). In addition, two and one QTL were detected on chromosomes 3 and 4, respectively.

For FWIR, 44 QTL located on chromosomes 1, 2, 5, 7, 9, and 10 were detected (Table 4, Supplementary Table 2). Individual QTL explained phenotypic variances from 5.2 to 21.0%, with 16 QTL over 10% and 4 QTL over 15%. Except six QTL at bins 1.02, 1.02–1.03, 9.03–9.04, and 10.07, the positive alleles of other QTL came from the dent corn parent Dan232. QTL for FWIR on chromosome 7 were detected under both environments and in combined analysis. They were located in three marker intervals at bins 7.02, 7.02–7.03, and 7.03. The QTL on chromosome 1 were detected in 11 cases, being located in four marker intervals at bins 1.02, 1.02–1.03, 1.03–1.04, and 1.05–1.06. In nine cases, QTL on chromosome 5 were detected in four marker intervals at bins 5.01–5.06 (5.01–5.025, 5.02–5.03, 5.04–5.05, and 5.05–5.06). In addition, QTL on chromosomes 2, 9, and 10 were detected in one, three, and three cases, respectively.

QTL identification for the activities of AGPP, GBSS, and SSS

Only two QTL were detected for the activities of three enzymes, one each for AGPP and SSS. No QTL were detected for GBSS (Table 3, Supplementary Table 1). They were located in the same marker interval umc2057–umc1567 (bin 7.02–7.03). Phenotypic variances explained by individual QTL were 6.34 and 4.48%. All their positive alleles came from the popcorn parent N04.

Digenic epistasis among QTL for GFW, GDW, GFR, and FWIR

For the four traits, 59 pairs of digenic interactions were identified, being 14, 13, 17, and 15 pairs for GFW, GDW, GFR, and FWIR, respectively (data not shown). Phenotypic variance explained by the interaction between umc1307 and umc1867 at bin 9.0–9.01 and umc2163–umc1677 at bin 10.04–10.05 was 11.2% for GFR in 2009. However, the contribution values of other digenic interactions were all low, ranging from 0.1 to 4.8%. These results suggested that the contributions of digenic interactions to grain weight and grain-filling rate were minimal.

Meta-QTL analysis for all traits in this study and GDW at maturity in our previous research

In this study, 161 QTL were detected for GDW, GFW, GFR, and FWIR and the activities of two enzymes. In our previous studies using the same two parents, 28 QTL for GDW at maturity were detected, 22 QTL using the same RIL population under four environments, and three QTL using its $F_{2:3}$ and BC_2F_2 populations under two environments. Altogether, 189 QTL were detected. For both 161 QTL and the total 189 QTL, 13 distinct QTL clusters (mQTL) were found (Table 5). These mQTL were located on six chromosomes, three on chromosomes 1 and 5, two on chromosomes 3, 7, and 10, and one on chromosome 9.

For the 161 QTL detected in this study, 155 QTL were located in those cluster regions, accounting for 96.3%. One mQTL included 11.9 QTL on average, with a variation of 2–47 QTL for one to six traits. For the totally detected 189 QTL, 178 QTL were located in those cluster regions, accounting for 94.2%. One mQTL included 13.7 QTL on average, with a variation of 2–53 QTL for one to six traits. In both cases, many more QTL were included in mQTL7-2 (47/53 QTL for six traits), mQTL1-2 (24/28 QTL for four traits), and mQTL10-2 (23/25 QTL for four

Table 5 Meta-QTL identified by meta-analysis for all traits in this study and GDW at maturity using RIL, $F_{2:3}$, and BC_2F_2 populations in our previous research

mQTL	AIC[a]	Position (cM)	Confidence interval (cM)	Adjacent marker	QTL number	Related trait	QTL integrated[b]
mQTL1-1	325.2/376.5	45.43	40.62–50.24	bnlg1007–umc1403	7/7	GFW, FWIR	q8GFW1-1-1, q9GFW1-1-1, qcGFW1-1-1, qcGFW1-1-2, q8FWIR10-1-1, q9FWIR10-1-1, qcFWIR10-1-1
mQTL1-2	325.2/376.5	130.65	129.61–131.69	phi001–umc2227	24/28	GDW, GFR, GFW, FWIR	q9GDW2-1-1, q9GDW3-1-1, q9GDW4-1-1, qcGDW2-1-1, qcGDW3-1-1, qcGDW4-1-1, q8GFW3-1-1, q9GFW3-1-1, qcGFW3-1-1, qcGFW4-1-1, q8GFR13-1-1, q9GFR12-1-1, q9GFR13-1-1, q9GFR14-1-1, q9GFR24-1-1, q9GFR34-1-1, qcGFR12-1-1, qcGFR13-1-1, qcGFR14-1-1, q8FWIR13-1-1, q9FWIR13-1-1, q9FWIR14-1-1, qcFWIR13-1-1, qcFWIR14-1-1, q7wGDW-1-1, q7xGDW-1-1, q7zGDW-1-1, qzcGDW-1-1
mQTL1-3	325.2/376.5	199.62	196.55–202.69	umc1906–umc2083	12/14	GDW, GFW, FWIR	q8GFW1-1-2, q8GFW2-1-1, q9GFW1-1-2, q9GFW2-1-1, qcGFW1-1-3, qcGFW2-1-1, q8FWIR10-1-2, q8FWIR12-1-1, q9FWIR10-1-2, q9FWIR12-1-2, qcFWIR10-1-2, qcFWIR12-1-1, q100GW1-1, qB100GW1-1
mQTL3-1	30.9/30.9	41.48	34.14–48.82	umc2049–bnlg1647	2/2	GDW, GFR	q9GDW2-3-1, q9GFR12-3-1
mQTL3-2	30.9/30.9	87.04	71.35–102.73	umc2259–bnlg1452	2/2	GDW, GFR	q9GFW2-3-1, q8GFR24-3-1
mQTL5-1	191.2/266.9	43.98	39.54–48.41	umc1478–bnlg565	4/10	GDW, GFR, FWIR	q8GDW3-5-1, q9GDW1-5-1, q9FWIR12-5-1, qcFWIR13-5-1, q7wGDW-5-1, q7xGDW-5-1, q7zGDW-5-1, qzcGDW-5-1, qB100GW5-1, q100GW5-1
mQTL5-2	191.2/266.9	73.79	70.52–77.05	bnlg565–phi109188	4/6	GFR, FWIR	q9FWIR13-5-1,q9FWIR14-5-1,qcGFR34-5-1,qcFWIR12-5-1,q7xGDW-5-2,q7zGDW-5-2
mQTL5-3	191.2/266.9	161.05	158.29–163.81	umc1502–umc1941	16/17	GDW, GFW, GFR, FWIR	q8GDW4-5-1, qcGDW4-5-1, q8GFW2-5-1, q8GFW4-5-1, q9GFW4-5-1, qcGFW3-5-1, qcGFW4-5-1, q8GFR14-5-1, q8GFR24-5-1, qcGFR14-5-1, qcGFR23-5-1, qcGFR24-5-1, q8FWIR14-5-1, q9FWIR23-5-1, qcFWIR14-5-1, qcFWIR23-5-1, q8zGDW-5-2
mQTL7-1	382.1/419.0	18.24	13.11–23.37	bnlg2333/bnlg2160–umc1068	5/5	GDW, GFW, GFR, FWIR	q8GDW1-7-1, q8GFW1-7-1, qcGFR24-7-1, q9FWIR14-7-1, q8FWIR10-7-1
mQTL7-2	382.1/419.0	75.58	74.55–76.62	umc2057–umc1567	47/53	GDW, GFW, GFR, FWIR, AGPP, SSS	q8GDW2-7-1, q8GDW3-7-1, q8GDW4-7-1, q9GDW1-7-1, q9GDW2-7-1, q9GDW3-7-1, q9GDW4-7-1, qcGDW1-7-1, qcGDW2-7-1, qcGDW3-7-1, qcGDW4-7-1, q8GFW2-7-1, q8GFW3-7-1, q8GFW4-7-1, q9GFW1-7-1, q9GFW2-7-1, q9GFW3-7-1, q9GFW4-7-1, qcGFW1-7-1, qcGFW2-7-1, qcGFW3-7-1, qcGFW4-7-1, q8GFR12-7-1, q8GFR13-7-1, q8GFR14-7-1, q8GFR23-7-1, q8GFR24-7-1, q9GFR12-7-1, q9GFR13-7-1, q9GFR14-7-1, q9GFR23-7-1, qcGFR12-7-1, qcGFR13-7-1, qcGFR14-7-1, qcGFR23-7-1, q8FWIR12-7-1, q8FWIR13-7-1, q8FWIR14-7-1, q9FWIR10-7-1, q9FWIR12-7-1, q9FWIR13-7-1, qcFWIR10-7-1, qcFWIR12-7-1, qcFWIR13-7-1, qcFWIR14-7-1, q8RAGPP3-7-1, q8RSSS3-7-1, q7wGDW-7-1, q7xGDW-7-1, q7zGDW-7-1, q8zGDW-7-1, qzcGDW-7-1, q100GW7-1

Table 5 continued

mQTL	AIC[a]	Position (cM)	Confidence interval (cM)	Adjacent marker	QTL number	Related trait	QTL integrated[b]
mQTL9-1	35.6/35.6	105.12	97.79–112.44	umc2337–phi065	5/5	GDW, GFW, FWIR	q9GDW1-9-1, q9GFW1-9-1, q8FWIR14-9-1, q9FWIR10-9-1, qcFWIR14-9-1
mQTL10-1	235.2/260.4	23.83	17.13–30.54	umc2034–phi059/umc2069	4/4	GFR	q9GFR14-10-1,q9GFR24-10-1,q9GFR34-10-1,q9GFR34-10-2
mQTL10-2	235.2/260.4	75.11	73.03–77.19	umc1677–umc2122	23/25	GDW, GFW, GFR, FWIR	q8GDW2-10-1, q9GDW1-10-1, q9GDW2-10-1, q9GDW3-10-1, q9GDW4-10-1, qcGDW1-10-1, qcGDW2-10-1, qcGDW3-10-1, qcGDW4-10-1, q9GFW1-10-1, q9GFW2-10-1, qcGFW1-10-1,qcGFW2-10-1, q8GFR12-10-1,q9GFR12-10-1,q9GFR13-10-1, q9GFR14-10-2, qcGFR12-10-1, qcGFR13-10-1, qcGFR14-10-1, qcGFR34-10-1, q9FWIR10-10-1, qcFWIR10-10-1, q7xGDW-10-1, qzcGDW-10-2

[a] *AIC* Akaike's information criterion; data before slash indicate items related with mQTL for QTL detected in this study, and data after slash indicate items related with mQTL for QTL detected herein and in our previous studies

[b] *Underlined* QTL indicate QTL for GDW at maturity detected in our previous research using RIL (Li et al. 2011), $F_{2:3}$ and BC_2F_2 populations (Li et al. 2007)

traits). mQTL1-3, mQTL5-1, and mQTL5-3 included 12/14 QTL for three traits, 10/4 QTL for three traits, and 16/17 QTL for four traits, respectively. Other mQTL included 2–7 QTL for one to four traits each.

Discussion

Three main genetic regions for grain weight and QTL co-location with EST identified from endosperms of the same two parents

Although three populations ($F_{2:3}$, BC_2F_2, and RIL) used in our present and previous research were derived from the same parents, the field experiments were not all conducted at the same locations and in the same years, and two linkage maps were constructed and used for QTL mapping. Therefore, the QTL experiments were considered as independent when using the BioMercator software. Through 189 QTL totally detected for GDW, GFW, GFR, FWIR, AGPP, and SSS in this study, and for GDW at maturity in our previous studies using the same RIL population (Li et al. 2011), and its $F_{2:3}$ and BC_2F_2 generations (Li et al. 2007) derived from the same two parents, three main genetic regions on chromosomes 1, 7, and 10 were clearly found in determining grain weight and development. The same two parents (Dan232 and N04) were also used to identify differentially expressed EST at 10 and 20 DAP in our previous study (Liu et al. 2010b). For the totally identified 160 unique EST, 70 were assigned to 39 chromosome bins distributed over all ten maize chromosomes. Eleven EST were found to co-localize with previously detected QTL for grain weight. Eight EST were co-localized with six mQTL in this study: mQTL1-1, mQTL1-2, mQTL3-2, mQTL5-2, mQTL7-1, and mQTL7-2. Two EST (PE12C5 and PE15C3) were located in the same marker interval phi001–umc2227 as mQTL1-2. Another two EST (PM44C3 and DM27D9) were co-located with mQTL7-2 in the marker interval umc2057–umc1567.

The most important one was the marker interval umc2057–umc1567 at bin 7.02–7.03, in which 54 QTL were integrated. mQTL7-2 included 47 QTL detected in this study (11 QTL for both GDW and GFW, 13 for GFR, and 10 for FWIR) and seven QTL for GDW detected using the same RIL population, and its $F_{2:3}$ and BC_2F_2 generations. Notably, both one

QTL for the activities of AGPP and SSS and two EST (PM44C3 and DM27D9) were located in this marker interval. Thévenot et al. (2005) considered that QTL co-located with genes encoding for related functions might be considered as candidate genes. PM44C3 and DM27D9 were deduced to encode opaque-2 modifier and alanine aminotransferase (*AlaAT*), respectively. Opaque-2 modifiers increase zein synthesis and alter its spatial distribution in maize endosperm. *AlaAT* could increase the biomass and grain yield significantly through enhancing nitrogen uptake efficiency in rice (Shrawat et al. 2008). According to information from MaizeSequence (http://www.maizesequence.org/index.html), one candidate gene, elongation initiation factor 2 (*eIF2*), is located in silico in this region. eIF2 is a phosphoprotein, and *eIF2a* regulates phosphorylation through several intermediate isoforms that correlated with the increase and subsequent reduction in protein synthetic activity during seed development (Le et al. 1998).

In the marker interval phi001–umc2227 at bin 1.03–1.04, 24 QTL were detected in this study, including 6 QTL for GDW, 4 for GFW, 9 for GFR, and 5 for FWIR. In our previous study, four QTL for GDW at maturity were also commonly detected using the same RIL population (Li et al. 2011). In addition, one QTL for GDW was detected near bin 1.04–1.05 (umc2025–bnlg1811 and bnlg1811–bnlg1884) in both $F_{2:3}$ and BC_2F_2 generations. Two EST (PE12C5 and PE15C3) and three candidate genes (Glycine rich protein, *Grp*; PhytochromeB1, *phyB1*; and Pdc3 pyruvate decarboxylase3, *pdc3*) were also located in this marker interval. PM12C5 encodes zinc finger (C3HC4-type RING finger) family protein. Zinc finger proteins are transcription factors, most of which regulate important biological processes, such as flower development, light-regulated morphogenesis, pathogen responses, and kernel growth and development in maize (Colasanti et al. 1998; Kozaki et al. 2004; Bluhm et al. 2008; Royo et al. 2009). PE15C3 encodes guanosine triphosphate (GTP) binding protein, which participates in a wide range of biological process, including signal transduction, protein synthesis and secretion, and cellular proliferation (Takatsuji 1998; Flaherty and Woloshuk 2004). *Grp* encodes an important structural protein in plant cell wall, and the growth of cell wall influences cell elongation. *phyB1* encodes a kind of light receptor in plants. Sawers et al. (2005) suggest that phytochrome signaling pathways are attractive targets for manipulating grain yield in cereal grasses.

Now, the full-length complementary DNA (cDNA) sequences for three co-located EST (PM44C3, PE12C5, and PE15C3) have been obtained in our library, and their same putative functions have been reflected (Liu et al. 2010a; unpublished data). For the full-length cDNA sequences of PE12C5 and PE15C3, the GenBank accession numbers are GQ131520.1 and EU816421, being designated *ZmC4HC3* and *ZmArf2,* respectively. Preliminary expression analyses have shown their different expression levels during endosperm development and among different tissues. Based on the comparisons for nucleotide and amino acid sequences of *ZmArf2* between the popcorn inbred N04 and the dent corn inbred Dan232, eight nucleotides differed and five amino acids changed between the two inbreds (Liu et al. 2010a).

Totally, 25 QTL were detected in the marker interval umc1677–umc2122 at bin 10.05–10.06, including 23 detected in this study (9 for GDW, 4 for GFW, 8 for GFR, and 2 for FWIR) and 2 QTL for GDW at maturity detected in the same RIL population (Li et al. 2011). mQTL10-3 was co-located with a candidate gene ribosomal protein S11 (*Rps11*). *Rps11* is a major component of ribosome and involved in the process of protein biosynthesis (Lebrun and Freyssinet 1991).

Clearly, the three marker intervals for mQTL7-2, mQTL1-2, and mQTL10-2 are worthy of greater attention in further research. Wang et al. (2009) also obtained meta-QTL for grain weight in these regions. Construction of near-isogenic lines (NIL) for common QTL for GDW located at these regions, and chromosome segment substitution lines (CSSL) for these regions are in progress in our present research. Since QTL for grain-weight-related traits in these regions were consistently detected across different generations, environments, and developmental stages/periods, marker-assisted selection (MAS) could be used to improve grain weight in maize. Moreover, full-length cDNA cloning of co-located EST (DM27D9, PE12C5, PE15C3, PM44C3, and PM44C3), and homologous cloning of co-located candidate genes (*eIF2*, *Grp*, *phyB1*, *pdc3*, and *Rps11*), and their function validations in further research might help to reveal grain-weight-related genes in these regions.

QTL detected at different stages and coincidence with related traits

Grain weight at harvest is controlled by a series of genes expressed systemically during different developmental stages. To reveal its dynamic genetic mechanism, QTL for grain weight at four stages and grain-filling rate during all periods in dry and fresh content were detected in this study. Among all the 159 QTL for GDW, GFW, GFR, and FWIR, QTL on chromosomes 1, 5, 7, and 10 were detected almost for all traits and across almost all stages/periods. They could play important roles during the whole process of grain development. For these QTL, MAS could be done for any trait at any stage. Considering measurement convenience, GDW at harvest was the best choice.

However, QTL on chromosomes 2, 3, 4, and 9 were shown to be specific for traits and developmental stages/periods. On chromosome 2, QTL was only detected for FWIR during 20–30 DAP. On chromosome 3, QTL for GDW and GFW at 20 DAP, for GFR during 10–20 DAP and 20–40 DAP were found. On chromosome 4, QTL were detected for GDW at 10 DAP and GFR during 20–40 DAP. On chromosome 9, QTL for GDW and GFW at 10 DAP and for FWIR during 0–10 DAP and 10–40 DAP were found. Clearly, most specific QTL were related with early developmental stages/periods, especially 10–20 DAP, consistent with some kinds of genes functioning only at early developmental stages in endosperm, such as cell division. According to previous research on endosperm development, cell numbers reached maximum at 12–21 DAP (Crane 1964; Linn 1977; Tollenaar 1978; Kiesselbach 1980; Jones 1985; Olsen 2004). For the two parent inbred lines in this study, endosperm microstructures at 3, 5, 7, 10, 15, 20, and 25 DAP showed that 10 DAP endosperms were in the stage of cell division (Liu et al. 2010b). Therefore, QTL on chromosomes 3, 4, and 9 might influence grain weight through controlling cell division. However, this should be proved in further research.

Through analyzing starch synthase genes in the databases of whole genome and full-length cDNA during grain filling in rice, Hirose and Terao (2004) divided the ten starch synthase genes into three groups of early, late and steady expressers. In our present study, QTL on chromosome 7 at bin 7.02–7.03 were detected across all stages and for all traits, which could be considered as steady expressers for both grain weight and grain-filling rate. Accordingly, QTL on chromosomes 3, 4, and 9 were only detected for related traits at 10 and 20 DAP, which were similar to early expressers of the starch synthesis-related genes. QTL for GFW on chromosome 5 only detected at 20, 30, and 40 DAP might be late expressers. In the study of QTL detection for grain weight and grain-filling rate by Liu et al. (2011), no QTL were consistently detected across four stages (16, 23, 32, and 40/45 DAP). Thévenot et al. (2005) found one common QTL at bin 7.04 for grain fresh weight across three stages (15, 25, and 35 DAP). Interestingly, this common QTL was located near bin loci 7.02–7.03 as in this study, in which QTL was detected in all cases (for grain weight at all stages and grain-filling rate during all periods in fresh/dry content). This further reflects the importance of QTL detected on chromosome 7 in controlling grain development and final grain weight. QTL on chromosomes 2, 3, 4, 5, and 9 shown to be specific for various stages/periods were consistent with the fact that different sets of regulatory genes were encountered during different stages/periods (Thévenot et al. 2005). Ohdan et al. (2005) found that the expression of genes encoding for starch biosynthetic enzymes in developing rice endosperm was largely developmental specific. Specific QTL for both stages/periods and related traits demonstrated that QTL detection for developmental traits should be conducted simultaneously in the view of time course.

In conclusion, three main chromosome regions, bins 1.03–1.04, 7.02–7.03, and 10.05–10.06, were consistently found to be related with QTL for grain weight and grain-filling rate in this study. Some identified EST and candidate genes with related functions were co-located with these common QTL. Further research should be concentrated on revealing QTL/genes for grain development and final weight both through development of near-isogenic lines of common QTL and their chromosome segment substitution lines, and through cloning and function validation of co-located EST and candidate genes.

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