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QTL mapping for the color, carotenoids and polyphenol oxidase activity of flour in recombinant inbred lines of wheat

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Abstract

Wheat flour color, which is highly related to carotenoids and polyphenol oxidase activity, is important in the assessment of flour quality, particularly the quality of whole wheat flour. In the present study, quantitative trait locus (QTL) mapping for the lutein content (LUC), beta-carotene content (BCC), *L** value (Fl*), *a** value (Fa*), *b** value (Fb*) and polyphenol oxidase activity (PPOA) of the whole wheat flour was conducted using a set of recombinant inbred lines (RILs) derived from the cross 'Chuan 35050 × Shannong 483' in three different environments and their mean values (MV). A total of 77 QTLs were located on 20 chromosomes for all of the investigated traits. A single QTL in an environment explained 3.9 (*QFb*-7B*) to 48.7% (*QBcc-4D*) of the phenotypic variation. The positive alleles for 36 of the QTLs were obtained from Chuan 35050, whereas the positive alleles of 41 QTLs came from Shannong 483. There were 9, 17, 13, 12, 13 and 13 QTLs found for LUC, BCC, Fl*, Fa*, Fb* and PPOA, respectively. Fifteen QTLs were detected in at least two environments or in at least one environment and the MV. Seventeen QTL clusters were mapped on 12 chromosomes. Of the QTL clusters, three clusters (C4, C8, C13), formed by the relatively stable QTLs, were more important, and the markers (*wPt-5587* on 2B for Fl* and Fa*, *wPt-6498* on 5B for LUC and Fb*, *wPt-671568* on 6A for Fl* and Fb*) near these loci should be useful in marker-assisted selection (MAS).

Keywords: Common wheat, flour color, carotenoids, polyphenol oxidase (PPO), quantitative trait locus (QTL).
Abbreviations: LUC (lutein content), BCC (beta-carotene content), Fl* (*L** value), Fa* (*a** value), Fb* (*b** value), PPOA (polyphenol oxidase activity), QTL (quantitative trait locus), RIL (recombinant inbred line).

Introduction

Common wheat (*Triticum aestivum* L., 2n=42, AABBDD genome) is one of the most important staple crops worldwide. The grain of wheat is milled into flour and is subsequently processed into a variety of foods that have important roles in human nutrition, such as bread, steamed bread and noodles. However, the products prepared from refined wheat flour are nutritionally poor and do not adequately meet the requirements for many macro and micro nutrients (Skrbic and Filipcev, 2008). Whole wheat flour, which contains more fiber and more nutrients than refined flour, receives increasing demand year by year, due to its properties of decreasing the risks of heart disease, hypertension, colon cancer, diabetes and obesity (Miller et al., 2000; Slavin, 2000; Adam et al., 2003; Liu, 2007). Flour color is important in the assessment of flour quality for the production of many end products. The color of whole wheat flour is darker than refined flour, as it is derived from the complete wheat grain. Color readings of flour are usually determined for colorimetric measures by the *L** value (lightness, Fl*), *a** value (red-green chromaticity, Fa*) and *b** value (yellow-blue chromaticity, Fb*) into the CIE (Commission Internationale I'Eclairage) scale. There is generally a high correlation between the Fb* and the yellow pigment content (YPC), and the YPC is primarily influenced by the level of carotenoids (Mares and Campbell, 2001). In addition to their role in providing color, carotenoids are important as antioxidants in human health and nutrition (Palozza and Krinsky, 1992), are valuable in the prevention of atherosclerosis (Dwyer et al., 2001), maintenance of immune function (Hinds et al., 1997) and in eye health (Moeller et al., 2000). The major carotenoids in wheat flour are lutein and beta-carotene (Panfili et al., 2004). Lutein has been implicated in the prevention of macular degeneration (Landrum and Bone, 2004), and beta-carotene is a precursor to vitamin A (Yeum and Russell, 2002). Carotenoid content varies greatly among different varieties of wheat (Adom et al., 2003). The color of flour is also associated with polyphenol oxidase (PPO), which catalyzes the oxidation of phenols to produce dark-colored products that are undesirable for flour and end products (Feillet et al., 2000; Simeone et al., 2002; Fuerst et al., 2006). These flour color-related traits are genetically quantitative, often influenced by the environment and exhibit high genotype × environment interaction. Quantitative trait locus (QTL) analysis has provided an effective approach to dissect complicated quantitative traits into their component loci and allow the study of their relative effects on a specific trait (Doerge, 2002). A large number of QTLs have been focused on flour color (Mares and Campbell, 2001; Kuchel et al., 2006; He et al., 2008; Blanco et al., 2011; Roncallo et al., 2012), PPO activity (Simeone et al., 2002; Raman et al., 2005; He et al., 2007; Beecher et al., 2012), and YPC (Patil et al., 2008; Blanco et al., 2011;

Table 1. Phenotypic performance for the investigated traits of RILs and their parents in the three environments and their mean values (MV)

Trait	Environment [a]	Parent		RIL population				
		Chuan 35050	Shannong 483	Min	Max.	Average	SD	Heritability (h_B^2 %)
LUC	E1	0.37	0.58	0.20	2.99	0.95	0.57	27.1
(μg/g)	E2	0.36	0.55	0.21	2.13	1.00	0.43	
	E3	0.42	0.62	0.23	1.82	0.89	0.38	
	MV	0.38	0.58	0.21	2.32	0.95	0.46	
BCC	E1	0.04	0.15	0.03	0.27	0.10	0.05	37.1
(μg/g)	E2	0.04	0.16	0.02	0.34	0.11	0.08	
	E3	0.04	0.14	0.02	0.22	0.09	0.05	
	MV	0.04	0.15	0.02	0.28	0.10	0.06	
Fl*	E1	84.70	84.82	82.34	87.68	85.07	0.92	63.7
	E2	84.60	84.76	83.45	87.39	84.92	0.76	
	E3	84.02	84.14	82.65	87.36	84.65	0.82	
	MV	84.44	84.57	82.81	87.48	84.88	0.83	
Fa*	E1	0.97	0.85	0.33	1.34	0.84	0.17	38.8
	E2	0.78	0.48	0.19	1.07	0.56	0.19	
	E3	0.96	0.69	0.32	1.10	0.63	0.15	
	MV	0.90	0.67	0.28	1.17	0.68	0.17	
Fb*	E1	11.57	11.82	9.88	13.55	12.13	0.73	72.2
	E2	12.79	13.61	10.57	15.36	13.69	1.12	
	E3	12.40	13.32	10.31	15.22	13.35	1.05	
	MV	12.25	12.92	10.25	14.71	13.06	0.96	
PPOA	E1	85.90	84.97	35.33	155.60	90.22	28.27	29.4
($U \cdot g^{-1} \cdot min^{-1}$)	E2	72.47	62.03	47.40	137.80	80.13	23.60	
	E3	71.53	69.20	40.20	132.00	72.01	17.95	
	MV	76.63	72.07	40.98	141.80	80.78	23.28	

[a] E1, E2, E3 were Heze 2008, Tai'an 2009, and Heze 2009, respectively.

Roncallo et al., 2012). To the best of our knowledge, the QTLs for carotenoids, including lutein and beta-carotene, were rarely reported (Howitt et al., 2009; Blanco et al., 2011).The objectives of the present study were to identify QTLs associated with the color, carotenoids and PPOA of whole wheat flour using a population of recombinant inbred lines (RILs) derived from two Chinese winter wheat varieties.

Results

Phenotypic variation

The two parents, Chuan 35050 and Shannong 483, showed differences for LUC, BCC, Fa*, Fb* and PPOA, and a small difference for Fl* (Table 1). For the RILs, the results of ANOVA showed that the variance for either genotype or environment effects on all six investigated traits were significant ($p \leq 0.01$). There were transgressive segregations for almost all of the traits in the three environments, and continuous distributions were obtained for all of the traits in the three environments, demonstrating the quantitative nature of polygenic inheritance. The heritability (h_B^2) for the investigated traits ranged from 27.1 (LUC) to 72.2% (Fb*); the h_B^2 values for Fl* and Fb* were over 50.0% (Table 1). The simple correlation coefficients showed that the LUC had significant positive correlations with the BCC, Fb* and PPOA (Table 2). The Fl* had significant negative correlations with all the traits except for BCC. A significant negative correlation was found between the BCC and Fa*, and a significant positive correlation was found between the Fb* and PPOA.

Analysis of QTLs

A total of 77 QTLs for the six traits were located on 20 chromosomes (all chromosomes except for 1D) in the three environments and MV (Table 3; Fig. 1). Of these QTLs, 15 QTLs were detected in at least two environments or in at least one environment and the MV, and the other 62 QTLs were found only in one treatment or MV. A single QTL in an environment explained 3.9 (*QFb*-7B* in E2) to 48.7% (*QBcc-4D* in E3) of the phenotypic variation. The highest LOD was 11.9 for Fl* in the MV (*QFl*-6A.1*). The additive effects for 36 QTLs were positive, indicating that the positive alleles came from Chuan 35050. The remaining 41 QTLs were negative, with the positive alleles came from Shannong 483. Fifteen QTLs were detected in at least two environments or at least one environment and MV (Table 3; Fig. 1), indicating that these QTLs were relatively stable. For

LUC, nine QTLs were found on eight chromosomes: 1B, 3A, 3B, 3D, 5B, 5D, 6B and 7D (Table 3; Fig. 1). *QLuc-5B* was detected in E1, E2 and MV, explaining 5.7 ~ 13.8% of the phenotypic variation (Table 3), with Shannong 483 increasing the QTL effect. Seventeen QTLs for BCC were detected on 12 chromosomes: 2A, 3A, 3B, 4B, 4D, 5A, 5D, 6A, 6B, 6D, 7A and 7B (Table 3; Fig. 1). *QBcc-6A.2* and *QBcc-7B* were all detected in E2 and MV, explaining 12.1 ~ 15.1% and 25.1 ~ 25.7% of the phenotypic variation, respectively (Table 3). The positive alleles of the two QTLs originated from Chuan 35050. For Fl*, 13 QTLs were detected on nine chromosomes: 1A, 1B, 2B, 2D, 3B, 5B, 5D, 6A and 7B (Table 3; Fig. 1). *QFl*-2B* was found in E1, E2 and MV, *QFl*-2D.2* in E1 and E3, *QFl*-6A.1* in all the environments and MV, explaining 9.0 ~ 19.9%, 18.9 ~ 22.2% and 7.3 ~ 27.6% of the phenotypic variation, respectively (Table 3). The positive alleles of *QFl*-2B* and *QFl*-2D.2* originated from Shannong 483, whereas the positive allele of *QFl*-6A.1* from Chuan 35050. Twelve QTLs for Fa* were found on nine chromosomes: 1B, 2B, 3A, 4B, 5A, 5D, 6A, 6B and 7B (Table 3; Fig. 1). *QFa*-2B.1* were found in E3 and MV, *QFa*-5D.1* were found in E1 and MV, and *QFa*-6B* were found in E3 and MV, explaining 6.1 ~ 21.9%, 6.9 ~ 7.5% and 7.1 ~ 9.9% of the phenotypic variation, respectively (Table 3). The positive allele of *QFa*-5D.1* originated from

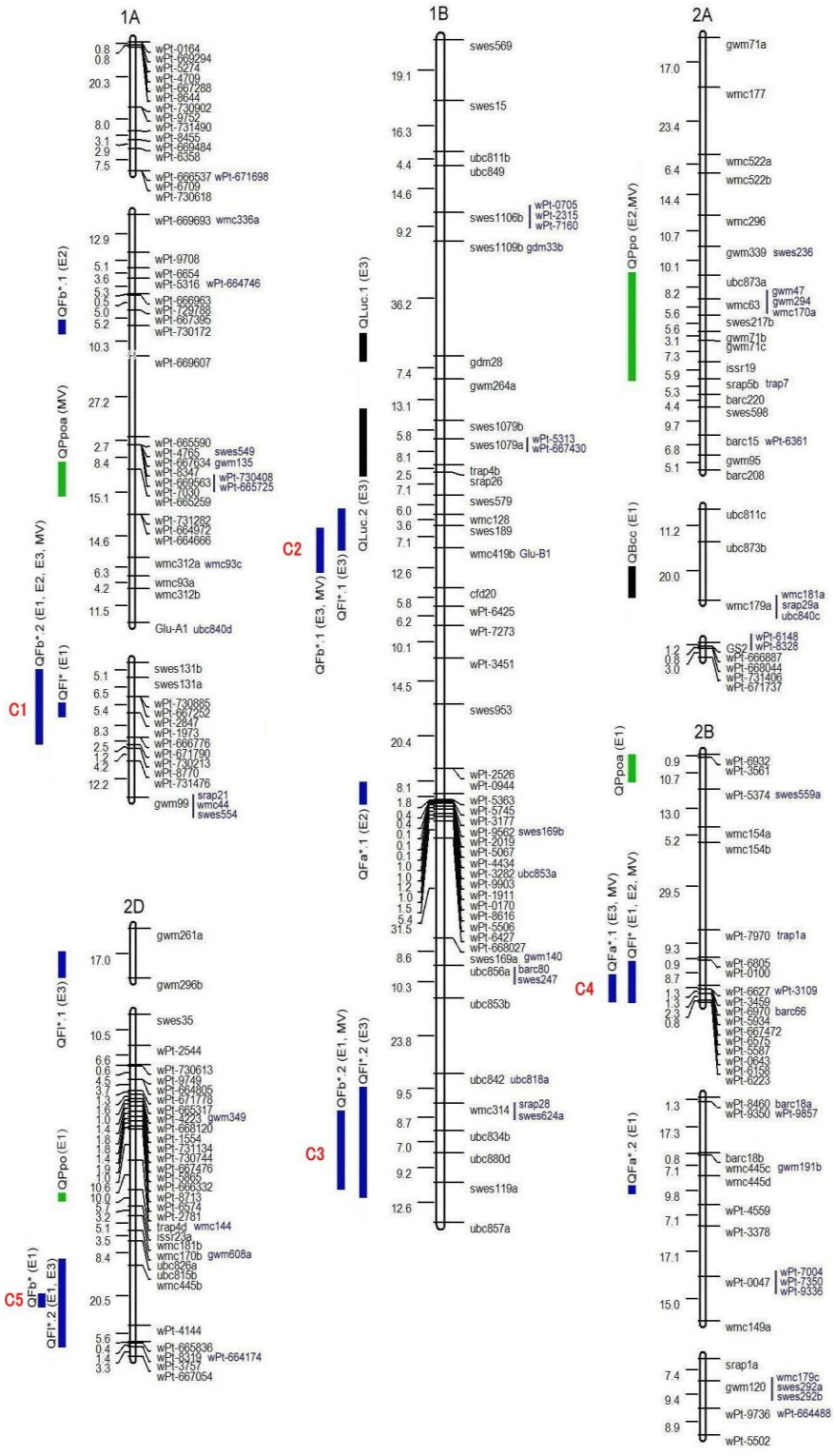

Fig 1. Locations of 77 QTLs for the investigated traits based on RILs derived from the cross 'Chuan 35050 × Shannong 483'. QTLs are indicated on the left side of each chromosome, and the names of markers are indicated on the right side. The intervals of the QTLs were LOD ≥ 2.0 with LOD peak values higher than 2.5. QTL clusters are indicated on the left side of the chromosomes as C1 ~ C17.

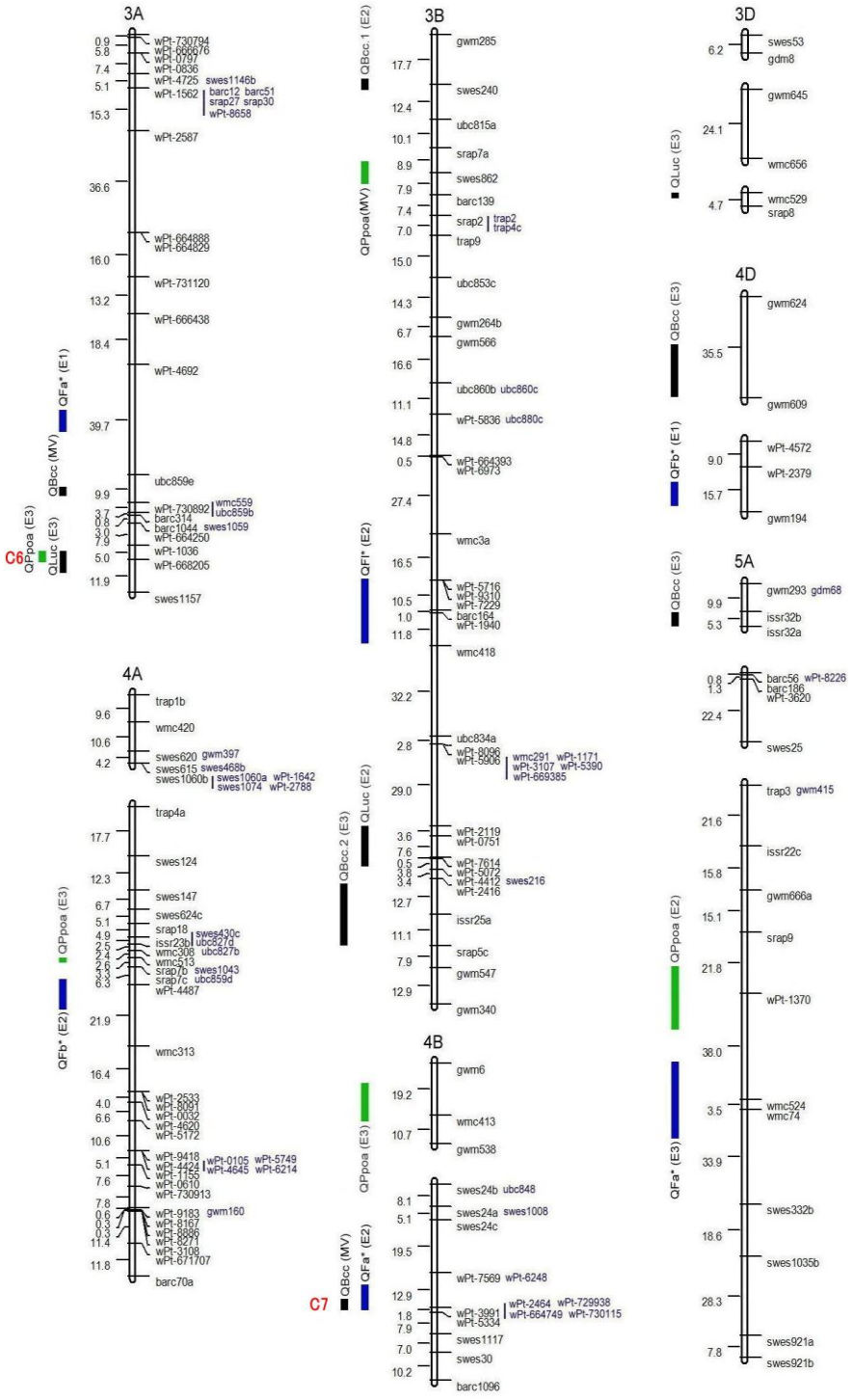

Fig1. Continued.

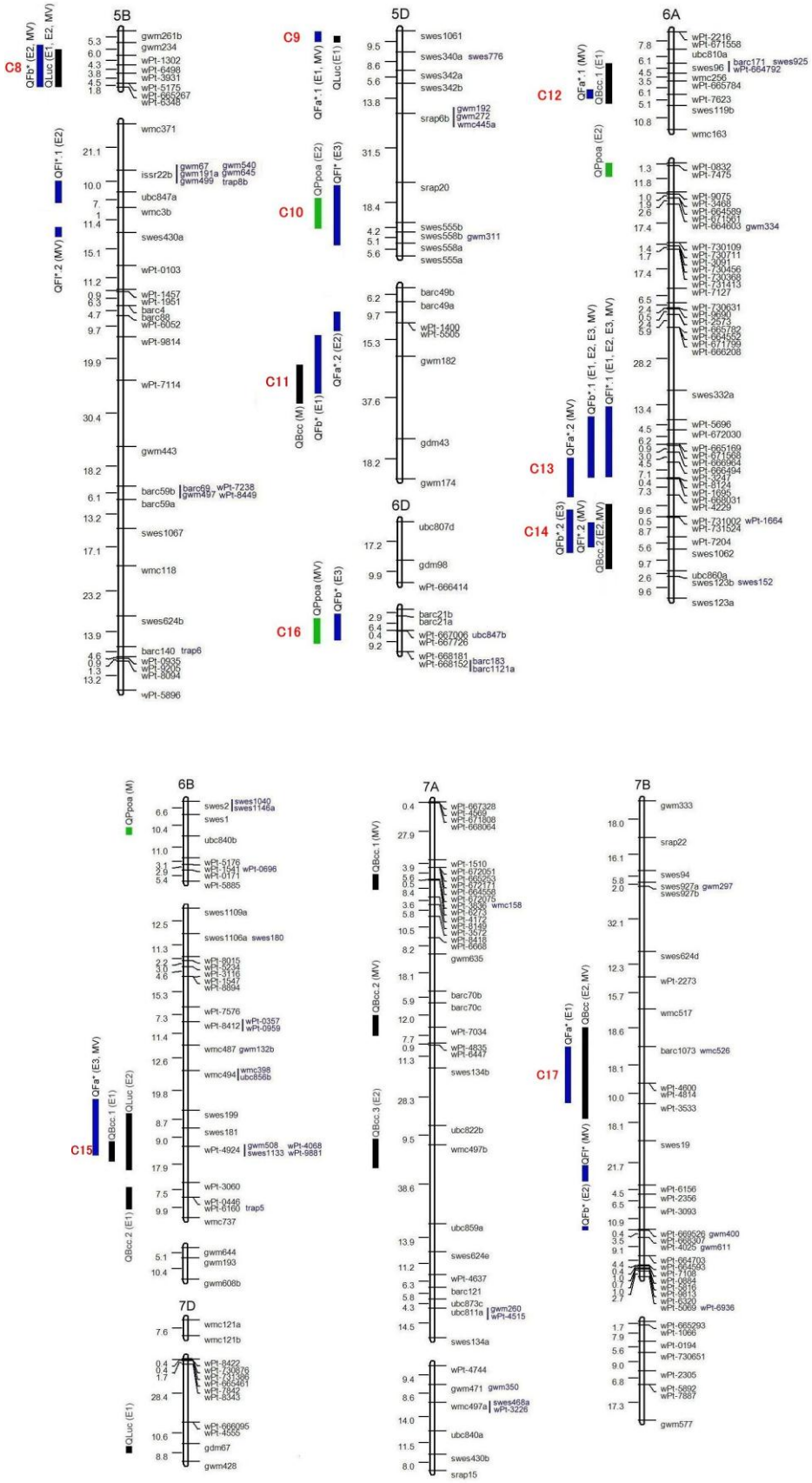

Fig 1. Continued.

Table 2. Simple correlation coefficients (*r*) between investigated traits.

Trait	LUC	BCC	Fl*	Fa*	Fb*
BCC	0.271**				
Fl*	-0.419**	0.165			
Fa*	-0.086	-0.456**	-0.512**		
Fb*	0.647**	0.015	-0.753**	0.148	
PPOA	0.181*	0.098	-0.187*	-0.122	0.180*

*indicates significance at the $p \leq 0.05$ level; ** indicates significance at the $p \leq 0.01$ level.

Shannong 483, whereas the positive alleles of *QFa*-2B.1* and *QFa*-6B* were from Chuan 35050. Thirteen QTLs for Fb* were detected on 10 chromosomes: 1A, 1B, 2D, 4A, 4D, 5B, 5D, 6A, 6D and 7B (Table 3; Fig. 1). *QFb*-1A.2* and *QFb*-6A.1* were detected in all the environments and MV, explaining 8.9 ~ 11.9% and 6.7 ~ 16.3% of the phenotypic variation, respectively. *QFb*-1B.1* were detected in E3 and MV, *QFb*-1B.2* were detected in E1 and MV, and *QFb*-5B* were detected in E2 and MV; explaining 5.3 ~ 5.7%, 5.8 ~ 11.6% and 12.1 ~ 13.8% of the phenotypic variation, respectively (Table 3). The positive alleles of *QFb*-1A.2*, *QFb*-1B.1*, *QFb*-5B* and *QFb*-6A.1* originated from Shannong 483, whereas the positive allele of *QFb*-1B.2* from Chuan 35050. For PPOA, 13 QTLs were detected on 13 chromosomes: 1A, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 5D, 6A, 6B and 6D (Table 3; Fig. 1). *QPpoa-2A* was found in E2 and MV, explaining 13.6 ~ 14.8% of the phenotypic variation, and the positive allele were obtained from Chuan 35050 (Table 3). Despite the wide distribution of the QTLs throughout the wheat genome, 17 QTL clusters (C1 ~ C17) were identified on 12 chromosomes, specifically on 1B (2 clusters), 5D (3 clusters) and 6A (3 clusters) (Fig.1). These QTL clusters involved all of the investigated traits and were formed by 37 QTLs. Clusters were frequently detected between Fl* and Fb* (4 clusters), BCC and Fa* (3 clusters).

Discussion

To date, only a small number of studies on QTL locations for individual carotenoids have been reported (Howitt et al., 2009; Blanco et al., 2011); in contrast, a large number of QTLs for flour color (Mares and Campbell, 2001; Kuchel et al., 2006; He et al., 2008; Blanco et al., 2011; Tsilo et al., 2011; Roncallo et al., 2012), PPOA (Simeone et al., 2002; Raman et al., 2005; He et al., 2007; Beecher et al., 2012) and YPC (Patil et al., 2008; Blanco et al., 2011; Roncallo et al., 2012) have been detected. In the present study, 77 QTLs for six traits (LUC, BCC, Fl*, Fa*, Fb* and PPOA) of whole wheat flour were located (Table 3; Fig. 1). A QTL detected in multiple environments is a relatively stable QTL (Collins et al., 2008). In this study, fifteen QTLs for the six traits were detected in at least two environments or one environment and MV (Table 3; Fig. 1), indicating that these QTLs were relatively stable. Of these stable QTLs, three QTLs (*QFl*-6A.1*, *QFb*-1A.2* and *QFb*-6A.1*) were detected in all environments and MV, two QTLs (*QLuc-5B* and *QFl*-2B*) were detected in two of the environments and MV, and one QTL (*QFl*-2D.2*) was detected in two of the environments, indicating that they were more 'stable' QTLs (Table 3). In this study, most of the QTLs were located in new marker regions for using different mapping population and molecular markers, whereas a few QTLs were located in marker regions adjacent to those identified in previous studies. The relatively stable QTL, *QPpoa-2A*, was located at the marker *gwm294* on chromosome 2A in our population (Fig. 1), which corresponds with the QTL for PPOA detected by Raman et al. (2005). We mapped *QBcc-5A* for BCC near *gwm293* on chromosome 5A (Fig. 1); the QTL for YPC was also reported near a similar position by Blanco et al. (2011). We found a relatively stable QTL, *QFb*-5B* for Fb*, near *gwm234* on 5B (Fig. 1); Tsilo et al. (2011) also identified the QTL for Fb* near *gwm234*. Roncallo et al. (2012) identified QTLs for YPC at *Barc1073* on 7B; we also detected the QTL near the same markers for BCC on 7B (Fig. 1). Pozniak et al. (2007) have mapped the *Psy1* gene, codes for the enzyme phytoene synthase 1, on the chromosome 7B. In this study, *QBcc-7B* was located at *Barc1073* on the 7B, which confirming the association of *Psy1* with the synthesis of carotenoids in this region. The *Psy2* gene, codes for the enzyme phytoene synthase 2, was localized at *wPt-1302* on group 5 chromosomes of durum wheat through genetic and physical mapping (Blanco et al., 2009). Interestingly, *QLuc-5B* and *QFb*-5B* were co-located at *wPt-1302* on chromosome 5B in this study, confirming the association of the *Psy2* with part of the synthesis of carotenoids in the region. In wheat, a large number of QTL clusters have been mapped in the same genomic regions (McCartney et al., 2005; Quarrie et al., 2005, 2006; Li et al., 2007; Sun et al., 2009; Zhang et al., 2010; Guo et al., 2011). In this study, clusters C4, C8 and C13 were formed by the relatively stable QTLs, and their R^2 were relatively high, which indicated that the three loci were more important and the markers around these loci should be useful in MAS. Cluster C4 in marker region *wPt-5587-wPt-6223* on chromosome 2B involved two QTLs (*QFl*-2B* and *QFa*-2B.1*) with a single QTL explaining 9.0 ~ 19.9% and 6.1 ~ 21.9% of the phenotypic variation (Table 3; Fig. 1). The two QTLs were detected in at least one environment and MV. The additive effects of *QFl*-2B* and *QFa*-2B.1* were negative and positive, respectively, indicating that Shannong 483 and Chuan 35050 increasing the QTL effects, respectively. The negative relationships between the two QTLs corresponded with the significant negative correlations between Fl* and Fa* (r = -0.512) (Table 2). Cluster C8 in marker region *wPt-6498-wPt-3931* on chromosome 5B involved two QTLs (*QLuc-5B* and *QFb*-5B*), with a single QTL explaining 5.7 ~ 13.8% and 12.1 ~ 13.8% of the phenotypic variation (Table 3; Fig. 1). These two QTLs were detected in at least one environment and MV; their additive effects were all negative, with Shannong 483 increasing the QTL effects. The positive relationships between the two QTLs corresponded with the significant positive correlations between LUC and Fb* (r = 0.647) (Table 2). This result agreed with previous reports that the two traits had a strong positive correlation (Humphries et al., 2004). Cluster C13 in marker region *wPt-5696-wPt-668031* on chromosome 6A involved three QTLs (*QFl*-6A.1*, *QFa*-6A.2* and *QFb*-6A.1*), with a single QTL explaining 7.3 ~ 27.6%, 16.9% and 6.7 ~ 16.3% of the phenotypic variation (Table 3; Fig. 1). *QFl*-6A.1* and *QFb*-6A.1* were detected in all the environments and MV, whereas *QFa*-6A.2* only in MV. The positive allele of *QFl*-6A.1* originated from Chuan 35050, whereas *QFa*-6A.2* and *QFb*-6A.1* from Shannong 483. The relationships between the QTLs corresponded with the significant negative correlations between Fl* and Fa* (r = -0.512), Fl* and Fb* (r = -0.753) and the positive correlation between Fa* and Fb* (r = 0.148).

Table 3. Additive QTLs for the investigated traits in the three environments and their mean values (MV).

Trait	QTL	Environment	Marker interval [a]	Site [b] (cM)	LOD	Additive effect [c]	R^2 (%)
LUC	*QLuc-1B.1*	E3	*gdm28-gwm264a*	0	2.8	0.115	6.8
	QLuc-1B.2	E3	*swes1079b-swes1079a*	0	5.7	-0.169	13.2
	QLuc-3A	E3	*wPt-668205-swes1157*	0	4.2	-0.188	9.6
	QLuc-3B	E2	*wPt-2119-wPt-0751*	3	3.2	0.127	7.1
	QLuc-3D	E3	*wmc529-srap8*	0	2.7	-0.156	7.6
	QLuc-5B	E1	*wPt-6498-wPt-3931*	3	4.3	-0.290	13.8
		E2	*wPt-3931-wPt-665267*	0	2.7	-0.144	5.7
		MV	*wPt-3931-wPt-665267*	*0*	*3.9*	*-0.128*	*8.0*
	QLuc-5D	E1	*swes1061-swes340a*	3	2.7	-0.195	9.9
	QLuc-6B	E2	*swes181-wPt-4924*	6	5.3	-0.167	13.4
	QLuc-7D	E1	*gdm67-gwm428*	2	2.7	-0.652	24.9
BCC	*QBcc-2A*	E1	*ubc873b-wmc179a*	16	2.9	0.016	9.7
	QBcc-3A	MV	*ubc859e-wPt-730892*	6	2.7	0.030	12.6
	QBcc-3B.1	E2	*swes240-ubc815a*	0	2.9	0.020	5.3
	QBcc-3B.2	E3	*issr25a-srap5c*	4	6.0	-0.028	27.1
	QBcc-4B	MV	*wPt-3991-wPt-5334*	0	2.8	-0.013	6.2
	QBcc-4D	E3	*gwm624-gwm609*	29	4.7	0.037	48.7
	QBcc-5A	E3	*issr32b-issr32a*	4	2.7	0.017	10.1
	QBcc-5D	MV	*gwm182-gdm43*	13	4.8	0.033	34.6
	QBcc-6A.1	E1	*wmc256-wPt-665784*	0	7.1	-0.034	19.4
	QBcc-6A.2	E2	*wPt-731524-wPt-7204*	3	4.9	0.030	12.1
		MV	*swes1062-ubc860a*	5	3.2	0.019	15.1
	QBcc-6B.1	E1	*wPt-4924-wPt-3060*	5	2.8	-0.017	10.3
	QBcc-6B.2	E1	*wPt-6160-wmc737*	0	3.7	-0.017	9.6
	QBcc-6D	MV	*wPt-666414-barc21b*	15	2.5	-0.032	35.8
	QBcc-7A.1	MV	*wPt-4172-wPt-8149*	0	3.0	-0.014	6.8
	QBcc-7A.2	MV	*wPt-7034-wPt-4835*	0	4.1	0.017	9.9
	QBcc-7A.3	E2	*wmc497b-ubc859a*	0	3.3	-0.026	8.6
	QBcc-7B	E2	*wPt-4814-wPt-3533*	2	9.5	0.048	25.7
		MV	*wPt-4814-wPt-3533*	4	8.7	0.027	25.1
Fl*	*QFl*-1A*	E1	*wPt-2847-wPt-1973*	5	4.0	0.421	10.6
	QFl-1B.1*	E3	*swes189-wmc419b*	4	2.9	0.241	7.2
	QFl-1B.2*	E3	*ubc880d-swes119a*	3	4.3	-0.283	10.4
	QFl-2B*	E1	*wPt-6223-wPt-8460*	0	3.9	-0.300	9.0
		E2	*wPt-6223-wPt-8460*	10	4.6	-0.349	19.9
		MV	*wPt-5587-wPt-0643*	0	7.3	-0.294	14.8
	QFl-2D.1*	E3	*gwm261a-gwm296b*	15	3.3	0.244	8.2
	QFl-2D.2*	E1	*wmc445b-wPt-4144*	8	4.8	-0.535	22.2
		E3	*wPt-3757-wPt-667054*	2	7.7	-0.501	18.9
	QFl-3B*	E2	*wPt-1940-wmc418*	2	4.2	-0.258	10.5
	QFl-5B.1*	E2	*issr22b-ubc847a*	9	3.1	-0.212	7.5
	QFl-5B.2*	MV	*wmc3b-swes430a*	11	2.5	-0.159	4.6
	QFl-5D*	E3	*swes555b-swes558b*	0	4.3	-0.249	8.8
	QFl-6A.1*	E1	*wPt-672030-wPt-671568*	5	4.9	0.330	12.0
		E2	*wPt-3247-wPt-1695*	3	9.2	0.483	27.4
		E3	*wPt-5696-wPt-672030*	3	3.3	0.223	7.3
		MV	*wPt-666964-wPt-666494*	1	11.9	0.502	27.6
	QFl-6A.2*	MV	*wPt-7204-swes1062*	1	4.1	-0.274	8.4
	QFl-7B*	MV	*swes19-wPt-6156*	16	2.6	0.242	8.2

[a] Marker interval means the interval of LOD peak value for QTLs.
[b] Site means the distance of the LOD peak value for the QTL after the first marker in the marker interval.
[c] Positive additive effect is the increased effect contributed by Chuan 35050; the negative additive effect by Shannong 483

Table 3. Continued.

Trait	QTL	Environment	Marker interval [a]	Site [b] (cM)	LOD	Additive effect [c]	R^2 (%)
Fa*	*QFa*-1B.1*	E2	*wPt-5363-wPt-5745*	0	3.1	0.088	6.6
	QFa-2B.1*	E3	*wPt-5587-wPt-0643*	1	3.0	0.041	6.1
		MV	*wPt-6223-wPt-8460*	11	4.1	0.065	21.9
	QFa-2B.2*	E1	*wmc445d-wPt-4559*	4	2.5	0.048	7.1
	QFa-3A*	E1	*wPt-4692-ubc859e*	20	2.6	-0.098	28.0
	QFa-4B*	E2	*wPt-7569-wPt-3991*	11	3.2	0.054	7.4
	QFa-5A*	E3	*wPt-1370-wmc524*	30	4.0	0.098	25.7
	QFa-5D.1*	E1	*swes1061-swes340a*	0	3.2	-0.051	7.5
		MV	*swes1061-swes340a*	2	2.6	-0.038	6.9
	QFa-5D.2*	E2	*wPt-5505-gwm182*	0	6.0	0.134	14.2
	QFa-6A.1*	MV	*wPt-7623-swes119b*	0	2.9	0.059	6.5
	QFa-6A.2*	MV	*wPt-668031-wPt-4229*	6	6.4	-0.059	16.9
	QFa-6B*	E3	*swes181-wPt-4924*	4	3.9	0.054	9.9
		MV	*swes199-swes181*	4	2.5	0.039	7.1
	QFa-7B*	E1	*barc1073-wPt-4814*	12	4.7	-0.069	14.7
Fb*	*QFb*-1A.1*	E2	*wPt-730172-wPt-669607*	0	3.4	0.426	5.7
	QFb-1A.2*	E1	*wPt-2847-wPt-1973*	3	3.9	-0.357	11.9
		E2	*wPt-1973-wPt-671790*	7	4.4	-0.535	8.9
		E3	*swes131a-wPt-2847*	2	5.2	-0.506	10.5
		MV	*swes131a-wPt-2847*	0	6.8	-0.368	9.3
	QFb-1B.1*	E3	*wmc419b-cfd20*	0	3.5	-0.264	5.3
		MV	*wmc419b-cfd20*	0	5.0	-0.252	7.3
	QFb-1B.2*	E1	*ubc880d-swes119a*	0	6.3	0.263	11.6
		MV	*ubc880d-swes119a*	0	4.3	0.223	5.8
	QFb-2D*	E1	*wmc445b-wPt-4144*	12	2.7	0.390	16.3
	QFb-4A*	E2	*wPt-4487-wmc313*	4	3.0	-0.475	9.1
	QFb-4D*	E1	*wPt-2379-gwm194*	9	3.6	-0.370	16.5
	QFb-5B*	E2	*wPt-6498-wPt-3931*	1	7.5	-0.661	13.8
		MV	*wPt-3931-wPt-665267*	3	7.3	-0.411	12.1
	QFb-5D*	E1	*wPt-5505-gwm182*	9	4.1	-0.375	13.7
	QFb-6A.1*	E1	*wPt-666964-wPt-666494*	0	7.8	-0.313	14.7
		E2	*wPt-671568-wPt-666964*	0	4.4	-0.314	6.7
		E3	*wPt-666964-wPt-666494*	1	9.1	-0.543	16.3
		MV	*wPt-3247-wPt-1695*	4	9.3	-0.353	16.0
	QFb-6A.2*	E3	*wPt-4229-wPt-731002*	6	4.4	0.407	9.2
	QFb-6D*	E3	*wPt-667006-wPt-667726*	0	3.1	0.227	4.1
	QFb-7B*	E2	*wPt-668307-wPt-4025*	0	2.7	-0.409	3.9
PPOA	*QPpoa-1A*	MV	*wPt-665259-wPt-664666*	2	3.7	6.447	10.5
	QPpoa-2A	E2	*ubc873a-wmc63*	7	5.4	10.000	14.8
		MV	*issr19-srap5b*	2	4.9	8.085	13.6
	QPpoa-2B	E1	*wPt-6932-wPt-3561*	0	3.4	8.400	8.4
	QPpoa-2D	E1	*issr23a-wmc181b*	2	2.6	7.800	6.9
	QPpoa-3A	E3	*wPt-668205-swes1157*	0	3.0	-8.400	8.4
	QPpoa-3B	MV	*swes862-barc139*	0	3.3	-5.259	7.4
	QPpoa-4A	E3	*srap7b-srap7c*	0	3.5	14.200	9.8
	QPpoa-4B	E3	*gwm6-wmc413*	14	3.1	-6.700	13.8
	QPpoa-5A	E2	*wPt-1370-wmc524*	13	2.8	14.800	31.9
	QPpoa-5D	E2	*srap20-swes555b*	13	3.0	-7.500	9.3
	QPpoa-6A	E2	*wPt-7475-wPt-9075*	0	3.5	-7.100	8.6
	QPpoa-6B	MV	*swes1-ubc840b*	8	2.5	-4.963	6.8
	QPpoa-6D	MV	*barc21a-wPt-667006*	6	3.5	5.459	8.3

Materials and methods

Plant materials and field arrangement

The population used for QTL mapping consisted of a set of 131 RILs derived from the cross: 'Chuan 35050 × Shannong 483' (Li et al., 2007). Chuan 35050 has been cultivated in the South-Western Winter Wheat Region of China. Shannong 483 was derived from 'Ai-Meng-Niu' and was grown in the Huang-Huai Winter Wheat Region. 'Ai-Meng-Niu' is one of the most widely used germplasms in Chinese wheat breeding programs and was used to develop more than 16 released cultivars, which have been planted in more than 30 million hectares. The 131 RILs and their parents were grown in three environments (Heze 2008, E1; Tai'an 2009, E2; and Heze 2009, E3) in Shandong Province of China. Tai'an (116°20′~117°59′ east longitude, 35°38′~36°28′ north latitude) and Heze (114°48″~116°24″east longitude, 30°39″~35°53″north latitude) were parts of Huang-Huai Winter Wheat Region, in the middle and southwest of Shandong province, respectively. The RILs were planted in randomized blocks designed with two replicates. A six-row

plot 2 m long and 25 cm apart was used, and 70 seeds were planted in each row. The experimental fields had loamy soil, and the grain yield was approximately 7,500 kg / ha.

Trait measurement

A 200 g sample of grain from each line in each environment was milled using a Perten Experimental Mill (Model-3100, Switzerland). Color analysis (Fl*, Fa* and Fb*) of whole flour samples was performed using a Minolta Color Meter 310 (Minolta Camera Co. Ltd., Japan). Carotenoids were isolated using the method described by Chen and Peng (1995). The carotenoid pigments were extracted from 5 g of flour using 8 mL of a 10 : 7 : 6 : 7 (v / v / v / v) mixture of hexane, acetone, ethanol and toluene and 6 mL of 40 % KOH in methanol. To prevent carotenoid oxidation, 0.8 mL of 0.1 % BHT (butylated hydroxytoluene) was used. The solution was saponified at room temperature in the dark for 16 h. Next, the sample was supplemented with 8 mL of hexane and 8 mL of 10% Na_2SO_4. The upper phase was collected and the lower phase was twice rinsed with 8 mL of hexane. All supernatants were evaporated at 40°C under a nitrogen stream and dissolved in 2 mL of methanol : dichloromethane (45 : 55, v / v). The extracts were quantified at 25°C by a UPLC (ACQUITY Ultra Performance LC, Waters corporation, USA) equipped with a BEH C18 column (1.7 µm, 2.1 × 50 mm). The carotenoid pigments were eluted by methanol : MTBE (89 : 11, v / v) at a flow rate of 1 mL / min and a sample size of 5 µL. The absorbance was measured at a wavelength of 450 nm. Lutein content (LUC) and beta-carotene content (BCC) were identified and calculated based on the lutein and the beta-carotene true standard (Sigma Corporation, USA). The procedure for estimating PPOA followed the method described by Morris and Anderson (2001) with the following minor modifications: 1.0 mL of 120 mM catechol (1-2-benzenediol) in 50 mM MOPS [3-(N-morpholino) propane sulfonic acid] buffer (pH 6.5) was added to 250 mg whole wheat flour, and the mixture was constantly rotated in a 10 mL centrifuge tube for 0.5 h at room temperature to allow the reaction to take place. Absorbance (A_{415}) was measured against a solvent blank with 1.0 mL of incubated solution at 415 nm using a T6 New Century UV-VIS spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.) running the UV-WIN (a WINDOWS platform) software package.

The catechol solution was made fresh daily. One unit of PPOA was defined as a change of 1 absorbance (A_{415}) unit / $min^{-1} \cdot g^{-1} \cdot 10^{-3}$ in a 1 cm path at 415 nm.

Data and QTL analysis

The analyses of variance (ANOVA) and simple correlation coefficients between traits were calculated using the SAS software (Knapp et al., 1985). The broad-sense heritability (h_B^2) was calculated using the GLM procedure in SAS using a model where the three environments were regarded as three replications, and the genotype × environment interaction was the error term. An enriched genetic map (Wang et al., 2011) was used for the QTL analysis. The map comprised 719 loci, 561 of which were assigned to 21 chromosomes, giving a total map length of 4,008.4 cM, with a marker density of 7.15 cM; and 158 loci were mapped to the most likely intervals. Among the 719 loci, the majority of marker loci were DArTs (361), SSRs (170) and EST-SSRs (100); and 88 other molecular and biochemical loci. Windows QTL Cartographer 2.5 (Wang et al., 2007) software was used to perform the QTL mapping. Composite-interval mapping (CIM) was selected to search for the QTL of each trait separately for each environment and their mean value (MV). The parameter setup 'model 6 standard analysis' was used with a walk speed of 1 cM, 'forward and backward' regression for the selection of the markers to control for the genetic background, up to five control markers, and a blocked window size of 10 cM to exclude closely linked control markers at the testing site. The threshold for declaring the presence of a significant QTL for each trait combination was defined by 1000 permutations at $p \leq 0.05$ (Churchill and Doerge, 1994). For all six traits, a QTL was claimed to be significant at a LOD peak value of 2.5. We defined a QTL cluster as two or more significant QTLs with overlapping confidence intervals, which we defined as map distances corresponding to LOD ≥ 2.0 (Stoll et al., 2000; Guo et al., 2011).

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