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Impact of epistasis and QTL × environmental interaction on the mass filling rate during seed development of soybean

ZHENFENG JIANG[1], YINGPENG HAN[1], WEILI TENG[1], YONGGUANG LI[1], XUE ZHAO[1], ZHONGCHEN ZHANG[1], WEIQUN MAN[2] AND WENBIN LI[1]*
[1] *Key Laboratory of Soybean Biology in Chinese Education Ministry* (*Key Laboratory of Biology and Genetics and Breeding for Soybean in Northeast China, Ministry of Agriculture*), *Northeast Agricultural University, Harbin 150030, People's Republics of China*
[2] *Agricultural Academy of Heilongjiang Province, Harbin 150041, People's Republics of China*

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Summary

Seed filling rate of soybean has been shown to be a dynamic process in different developmental stages affected by both genotype and environment. The objective of the present study was to determine additive, epistatic and quantitative trait loci (QTLs) × environment interaction (QE) effects of the QTL underlying a seed filling rate of soybean. One hundred and forty-three recombinant inbred lines (RILs) derived from the cross of Charleston and Dongnong 594 were used with 2 years of field data (2004 and 2005). Eleven QTLs with significantly unconditional and conditional additive (*a*) effect and/or additive × environment interaction (*ae*) effect at different filling stages were identified. Of them six QTLs showed positive *a* effects and four QTLs had negative *a* effects on the seed filling rate during seed development. *aa* and *aae* effects of 12 pairs of QTLs were identified by unconditional mapping from the initial stage to the final stage. Thirteen pairs of QTLs underlying the seed filling rate with *aa* and *aae* effects were identified by conditional mapping. QTLs with *aa* and *aae* (additive × additive × environment) effects appeared to vary at different filling stages. Our results demonstrated that the mass filling rate in soybean seed were under genetic and environmental control.

1. Introduction

Seed filling is the final stage of soybean growth and marks the translocation of assimilation such as carbohydrate and amino acids from the reserve pools (leaf and stem) to the sink (caryopses) (Schussler *et al*., 1984; Egli & Bruening, 2004). The rate and duration of seed filling determine the final seed weight, a key component of the total seed yield while maintaining high yield is a major goal of soybean breeding. The filling period is critical for grain yield and the yield potential is largely based on high biomass accumulation if no water stress exists (Yoshida, 1972; Nicolas *et al*., 1985*a*, *b*; Palta *et al*., 1994; Plaut *et al*., 2004). In today's crop production systems with their high yield outputs, improvement in a grain filling rate has become vitally important and more challenging than ever.

* Corresponding author: Soybean Research Institute (Key Laboratory of Soybean Biology in Chinese Education Ministry), Northeast Agricultural University, Harbin 150030, People's Republics of China. Tel: (+86)451-55190778. Fax: (+86)451-55103336. E-mail: wenbinli@neau.edu.cn

It was well documented that the high filling rate could increase crop yield (Jones *et al*., 1979; Smith & Nelson, 1986*a*; Hunt *et al*., 1991). For example, Wiegand & Cueller (1981) reported that the grain filling rate was positively associated with the final grain weight in wheat (Hunt *et al*., 1991). In rice, a study showed that the grain filling rate was highly correlated with actual panicle weight and 100-grain weight (Jones *et al*., 1979). Although the seed filling duration was different for a given genotype of soybean, the variations of a seed filling rate accounted for the major part of the seed weight variation among different environments (Munier-Jolain & Ney, 1998). Lines with high filling rate might help produce new varieties with high yield potential.

In soybean, the seed yield was positively associated with seed filling period, and a significant difference of seed filling period was found in various soybean genotypes (Smith & Nelson, 1986*b*; Pfeiffer & Egli, 1988). The seed filling rate of soybean was partially determined by genetic factors, while the duration of seed filling was more easily influenced by

environmental factors, such as temperature, oxygen, photoperiod, ABA and water (Egli *et al*., 1978; Thorne, 1981; Schussler *et al*., 1984; Munier-Jolain & Ney, 1998; Egli, 2004). Therefore, a high seed filling rate of soybean genotype could be referred to as a breeding index for yield and quality improvement.

Epistasis is termed as the interaction between one pair of loci located in the same or different chromosome, and referred to the effect of one locus on a particular phenotype depends on the genotype at a second locus (Cockerham & Zeng, 1996; Carlborg *et al*., 2006). The mechanism of epistasis to the genomic control of complex traits is more complicated to be detected than individual gene effects and might decrease the individual quantitative trait locus (QTL) effects. If epistasis is ignored, individual locus might not be detected and the QTL contribution to the phenotype was neglected, which could lead to an incorrect application to molecular selection assist (MSA) and weaken the ability in QTL identification and reduce the economic gain than predicted (Carlborg *et al*., 2006). In order to gain more accurate and unbiased understanding estimates of the genetic background of economically important traits, epistatic effects should be included in QTL mapping studies (Jannink, 2008). Carlborg & Haley (2004) showed that epistasis is a common response to selection in breeding programmes. Several studies in soft winter wheat based on either first- or second-moment statistics have demonstrated a significant contribution of epistasis to grain yield and flowering time (Goldringer *et al*., 1997).

Epistasis in soybean was particularly important as lots of multiple allelisms were observed in soybean chromosomes, and the duplicate copies of genes were likely to interact with each other (Schmutz *et al*., 2010). However, non-allelic interaction had been observed between loci controlling important traits such as oil content, protein content, yield and related traits in soybean (Croissant & Torrie, 1971; Han *et al*., 2008; Martin *et al*., 2009; Reif *et al*., 2011).

Many genetic factors were involved in seed filling of soybean. The identification of these genetic factors at different developmental stages is important for a substantially improving seed filling rate. Epistatic QTL underlying important traits, such as seed weight, protein and oil contents expressed in different seed developmental stages of soybean had been reported (Han *et al*., 2008; Jiang *et al*., 2010) rather than seed mass filling rate.

The objectives of our work were to analyse the dynamic behaviour of seed filling rate at different filling stages and to detect QTL with additive and epistatic effects as well as their QTL × environment (QE) interaction effects using the statistical model of Zhu (1995) for analysing conditional genetic effects.

2. Materials and methods

(i) *Plant materials*

The mapping population consisted of 143 F_2 derived F_5 recombinant inbred lines (RILs) that were advanced by single-seed descent from the cross of 'Charleston' (provided by Dr R. L. Nelson, Illinois State University of USA) and 'Dongnong 594' (developed by the Northeast Agriculture University, Harbin, China). The RILs and their parents were grown at Harbin during the summers of 2004 and 2005 as two environmental treatments in a randomized complete block. Rows were 3 m long and 0·7 m wide with a distance of 6 cm between plants. Three row plots were used. Pods were picked from the fifth to seventh nodes of main stems every 10 days from 30 days after flowering (30D) until physiological maturity (80D). The 30D sample represented the R3 stage (initial stage) and the 80D sample represented the R8 stage (final stage) of growth with intervening stages at about 10D intervals. Seeds were pre-dried for 30 min in an oven at 105 °C and then continuously dried until the seed weight was stable at 50–70 °C. All dried samples were weighed (Teng *et al*., 2009). Seed filling rate was calculated as follows: $(W_t - W_{(t-1)})/10$, and represented by Dt/D$(t-1)$ stage (Li *et al*., 2006).

(ii) *Random Amplification of Polymorphic DNA (RAPD) analysis*

Total DNA of each RIL was isolated from freeze-dried leaf tissue by the CTAB method as described by Doyle & Doyle (1990). RAPD analysis was carried out with 1200 random decamer primers obtained from Operon Technologies Inc. (Alameda, CA, USA). A 20 μl of reaction mixture containing 2 μl of genomic DNA (15 ng/μl), 1·5 μl of $MgCl_2$ (25 mM), 0·3 μl of dNTPs (10 mM), 2 μl of 10 × PCR buffer, 2 μl of RAPD primer (2 μM), 0·2 μl of *Taq* polymerase (10 units/μl) and 12 μl of H_2O. The PCR programme consisted of 2 min at 94 °C, and 41 cycles of 1 min at 94 °C, 1 min at 36 °C and 1 min at 72 °C. The final extension step of 10 min was carried out at 72 °C. PCR products were separated on 1·5 % (w/v) agarose gel and stained with ethidium bromide and UV fluorescence.

(iii) *Simple Sequence Repeat (SSR) analysis*

SSR analysis was performed with 600 pairs of primers developed by Song *et al*. (2004). PCR was performed in a 20 μl reaction mixture containing 2 μl of genomic DNA (25 ng/μl), 1·5 μl of $MgCl_2$ (25 mM), 0·3 μl of dNTP mixtures (10 mM), 2 μl of 10 × PCR buffer, 2 μl of SSR primer (2 μM), 0·2 μl of *Taq* polymerase (10 units/μl) and 12 μl of H_2O. The amplification

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profiles were 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 47 °C, 30 s at 72 °C, then 5 min at 72 °C. PCR products were mixed with loading buffer (2·5 mg/ml Bromophenol Blue, 2·5 mg/ml Diphenylamine Blue, 10 mM EDTA and 95% (v/v) formamide), and denatured for 5 min at 94 °C. Denatured DNA was placed on ice for 5 min and separated on 6% (w/v) denaturing polyacrylamide gel by electrophoresis. DNA bands were visualized using silver straining (Trigizano & Caetano-Anolles, 1998).

(iv) *Statistical analysis*

QTLs with additive and additive × additive epistatic effects, as well as their environmental interaction effects in the RIL population, were mapped by QTLMapper version 1.6 (Wang *et al*., 1999*a*). For unconditional analysis of seed filling rate at 80D stage, the phenotypic value of the ***k***th RIL in environment ***h*** can be partitioned by the following mixed linear model (Zhu, 1999):

$$\begin{aligned} y_{hk} = {} & \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} \\ & + \mu_{E_{hk}} e_{E_h} + \mu_{A_i E_{hk}} e_{A_i E_h} + \mu_{A_j E_{hk}} e_{A_j E_h} \\ & + \mu_{AA_{ij} E_{hk}} e_{AA_{ij} E_h} + \sum_{f(h)} \mu_{M_{fk(h)}} e_{M_{f(h)}} \\ & + \sum_{l(h)} \mu_{MM_{lk(h)}} e_{MM_{l(h)}} + \varepsilon_{hk}. \end{aligned} \tag{1}$$

The meaning of each parameter is the same as that described by Wang *et al*. (1999*a*) and Luo *et al*. (2001): where μ is the population mean; a_i and a_j are the additive effects (fixed effects) of two putative loci Q_i and Q_j, respectively; aai_{ij} is the additive × additive epistatic effect (fixed effect) between the two loci; $x_{A_{ik}}$,$x_{A_{jk}}$ and $x_{AA_{ijk}}$ are the coefficients of these genetic main effects; e_{E_h} is the random effect of environment h with a coefficient $u_{E_{hk}}$; $e_{A_i E_h}$ (or e_{AjE_h}) is the random additive × environment interaction effect with a coefficient $\mu_{A_i E_{hk}}$ (or $\mu_{A_j E_{hk}}$) for Q_i (or Q_j); $e_{AA_{ij}E_h}$ is the random epistasis × environment interaction effect with a coefficient $\mu_{AA_{ij}E_{hk}}$; $e_{M_{f(h)}}$ is the random effect of marker f nested within the hth environment with a coefficient $\mu_{M_{fk(h)}}$, $e_{MM_{l(h)}}$ is the random effect of the lth marker × marker interaction nested within the hth environment with a coefficient $\mu_{MM_{lk(h)}}$; ε_{hk} is the random residual effect. The marker factors $e_{M_{f(h)}}$ and $e_{MM_{l(h)}}$ in the model are used to absorb additive and epistatic effects of background QTL for controlling the noise. The QTL detected by this unconditional mapping method would indicate the cumulative gene effects from the initial time (30D) to the final time (80D).

Conditional QTL analysis was conducted with the phenotypic value at time t, given the phenotypic behaviour at time $(t-1)$, using QTLMapper version 1.6 (Wang *et al*., 1999*a*). Similar to that in Equation (1), the conditional value $y_{hk(t/t-1)}$ can be partitioned as

$$\begin{aligned} y_{hk(t/(t-1))} = {} & \mu_{(t/(t-1))} + a_{i(t/(t-1))} x_{A_{ik}} + a_{j(t/(t-1))} x_{A_{jk}} \\ & + aa_{ij(t/(t-1))} x_{AA_{ijk}} + \mu_{E_{hk}} e_{E_h(t/(t-1))} \\ & + \mu_{A_i E_{hk}} e_{A_i E_h(t/(t-1))} + \mu_{A_j E_{hk}} e_{A_j E_h(t/(t-1))} \\ & + \mu_{AA_{ij} E_{hk}} e_{AA_{ij} E_h(t/(t-1))} + \sum_{f(h)} \mu_{M_{fk(h)}} e_{M_{f(h)(t/(t-1))}} \\ & + \sum_{l(h)} \mu_{MM_{lk(h)}} e_{MM_{l(h)(t/(t-1))}} + \varepsilon_{hk(t/(t-1))}, \end{aligned} \tag{2}$$

with all the parameters defined as conditional effects. The QTL detected by conditional mapping will reflect the net expression of genes during the time period from time $(t-1)$ to time t, independent of the genetic effects before time $(t-1)$.

The conditional phenotypic value $y_{hk(t/(t-1))}$ of filling behaviour was obtained by the mixed model approaches for the conditional genetics of developmental quantitative traits (Zhu, 1995). The likelihood-ratio threshold was chosen at $\alpha = 0{\cdot}01$ for claiming putative QTL, of which their genetic effects were further tested by a t-test with the jack-knifing resampling procedure. QTLs were presented when genetic main effects (a and aa) or QE interaction effects (ae and aae) were significantly different from zero ($P \leqslant 0{\cdot}01$).

Broad-sense heritability of seed filling rate was computed as $h^2 = \sigma_g^2/((\sigma_g^2 + \sigma_e^2)/n)$, where σ_g^2 and σ_e^2 are the estimates of genetic and residual variance, which were, respectively, derived from the expected mean squares of the variance and n is the number of replications (Blum *et al*., 2001).

3. Results

(i) *Phenotypic variation*

The mean seed filling rates of Charleston and Dongnong 594 were significant difference at different developmental stages except for the 60D/50D stage in 2005 in which the parents possessed the same seed filling rate. The mean seed filling rate of the two parental cultivars showed an increase at the first four measuring stages including initial stage, 40D/30D stage, 50D/40D stage and 60D/50D stage in the two environments (2004 and 2005) except that Charleston showed a decline at 60D/50D stage in 2005. The highest seed filling rate was observed at 60D/50D stage for the two parents except that parent Dongnong 594 reached the highest seed filling rate at 50D/40D stage and then decreased and reached the lowest value at 80D/70D stage in 2005. To show the mean seed filling rate, the seed filling rate at the final development stage (R8) was analysed, and the result showed that Charleston was low than that of Dongnong 594 across the years 2004 and 2005 (Fig. 1).

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Table 1. *Statistical analysis of seed filling rate (g/d·100-seed) for the parents and the RIL population at different developmental stages over 2 years at Harbin, China*

		Parents		RIL population					
Developmental stages (days)[a]	Years	Charleston	Dongnong594	Range	Means ± S. D.	CV (%)	Skew	Kurt	Broad-sense heritability
Initial	2004	0·04	0·05	0·01–0·09	0·04 ± 0·01	37·87	1·18	0·96	0·80
	2005	0·02	0·03	0·01–0·12	0·04 ± 0·02	58·67	0·72	−0·02	
40D/30D[b]	2004	0·10	0·11	0·02–0·70	0·20 ± 0·09	43·76	1·072	1·85	0·68
	2005	0·18	0·30	0·16–0·84	0·36 ± 0·11	30·48	0·79	1·16	
50D/40D	2004	0·15	0·35	0·08–0·83	0·34 ± 0·13	39·47	0·76	1·07	0·46
	2005	0·45	0·71	0·11–0·94	0·51 ± 0·18	35·09	0·13	−0·62	
60D/50D	2004	0·36	0·83	0·02–0·90	0·44 ± 0·19	42·26	0·16	−0·22	0·65
	2005	0·59	0·59	0·02–1·01	0·56 ± 0·20	35·30	0·04	−0·33	
70D/60D	2004	0·32	0·56	0·01–0·74	0·31 ± 0·15	46·85	0·19	−0·05	0·86
	2005	0·15	0·23	0·005–0·70	0·19 ± 0·17	91·22	1·00	0·21	
80D/70D	2004	0·15	0·34	0·002–0·64	0·21 ± 0·13	64·03	0·53	−0·21	0·43
	2005	0·13	0·08	0·001–0·35	0·09 ± 0·08	86·01	1·05	1·26	
Final	2004	0·15	0·24	0·14–0·29	0·20 ± 0·03	13·58	0·14	0·12	0·49
	2005	0·20	0·25	0·17–0·34	0·23 ± 0·03	11·24	0·45	0·94	

RIL, recombinant inbred line; CV, coefficient of variation; SD, standard deviation.
[a] 30, 40, 50, 60, 70, 80D represents 30, 40, 50, 60, 70 and 80 days after flowering, with maturity at 80D; initial indicates the initial stage (from flowering to 30 days), and final indicates the final stage (from initial time to 80 days).
[b] 40D/30D indicates the stage from 30 days to 40 days, and so on.

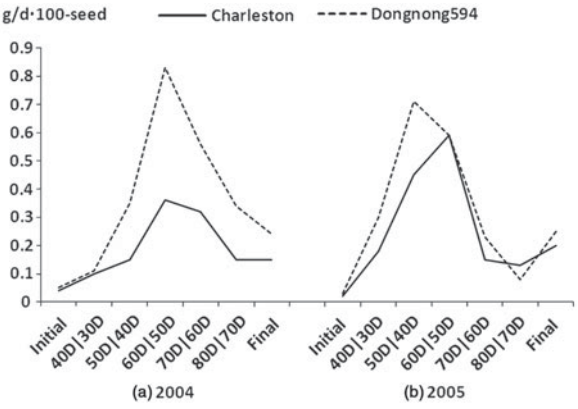

Fig. 1. Seed filling rate for the parents and the RIL population at different developmental stages in 2004 and 2005 at Harbin, China.

Individual RILs also varied significantly in their mean seed filling rate. Some RILs had higher mean seed filling rate, while the others had lower mean seed filling rate. In contrast, the variation of mean seed filling rate for each RIL was not significant across the 2 years (data not shown). Therefore, RILs performance was consistent and G × E interaction was limited. Most of the skewness and kurtosis values of seed filling rate were less than 1·0 at different growth stages measured in the two environments, indicating that segregation pattern of seed filling rate fit a normal distributing model. Broad-sense heritability of seed filling rate from the initial phase to the final phase was 0·70, 0·68, 0·46, 0·65, 0·86, 0·43 and 0·49, respectively (Table 1).

(ii) *Analysis of* a *and* ae *effects during seed filling*

Eleven QTLs of seed filling rate with significant *a* and/or *ae* effects at different seed developmental stages were identified in 2004 and 2005 and were mapped on seven linkage groups (LGs) using unconditional and conditional mapping (Table 2). Of them, six QTLs showed positive additive (*a*) effects, four QTLs with negative additive (*a*) effects and one QTL with positive or negative effects at different stages. 'Dongnong 594' (higher seed filling rate) contributed the alleles QFRC2_2, QFRF_1 and QFRN_1 that increase seed filling rate at different filling stages, against the alleles QFRA1_1, QFRA1_2 and QFRG_1 that decreased seed filling rate. QFRA1_3 decreased seed filling rate at 60D/50D stage, while it increased filling rate at the final stage, suggesting that the impact of some QTLs was different at different filling stages. QFRC2_2 showed a positive additive effect across the developmental stages and explained 3·68–9·49% of the phenotypic variation. QFRG_1 showed a negative additive effect across four different developmental stages (60D/50D, 70D/60D, 80D/70D and final stage) and explained 1·71–2·36% of the phenotypic variation. Other QTLs showed either positive or negative *a* effect at different developmental stages and explained the phenotypic variation from 1·6 to 5·9%.

Seven QTLs were identified to have significant *ae* effects at different seed filling stages in 2004 and 2005 (Table 2). QFRC2_2 had a significant *ae* effect across initial stage, 30D/20D, 40D/30D and 50D/40D stage. QFRC2_2 had a significant *ae* effect at initial

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Table 2. *Estimated additive (a) and additive × environment interaction (ae) effects of QTL underlying seed filling rate at different developmental stages of soybean seed in 2004 and 2005*

QTL	Maker Interval	Stage	a_i[a]	H^2[b] (a_i)	ae_i[c](2004)	ae_i (2005)	H^2[d](ae_i)
QFRA1_1	satt276–satt042	60D/50D	−0·012**	3·28	0·4*	−0·4*	5·6
QFRA1_2	satt449–satt300	70D/60D	−0·01**	3·68	−0·1*	0·1*	6·78
		40D/30D	−0·019**	1·6			
QFRA1_3	satt042–satt155	70D/60D			0·6	0·4	13·12
		80D/70D	−0·006**	2·82			
		Final	0·011**	2·37			
QFRC1_1	OPBF12_5–satt164	80D/70D	0·009**	6·35	−0·4	0·4*	8·6
		Final	0·009**	6·35	−0·4	0·4*	7·65
QFRC2_1	satt277–sat_076	50D/40D	0·012**	4·45			
QFRC2_2	satt460–satt134	Initial	0·005**	5·67	−0·7	0·7**	12·22
		50D/40D	0·054**	7·15	−0·11	0·09**	6·81
		60D/50D	0·015**	5·12	0·8	−0·8**	5·29
		70D/60D	0·01**	3·68	0·6	−0·6**	3·03
		80D/70D	0·011**	9·49			
		Final	0·011**	9·49			
QFRD1b_1	sat_135–OPD16_60	Initial	−0·004**	3·63			
QFRF_1	sat_120–sat_103	70D/60D	0·014**	7·22	0·3	−0·3*	9·1
		80D/70D	0·007**	3·84	0·6	0·1	5·3
		Final	0·007**	3·31			
QFRF_2	satt335–sat_120	50D/40D	0·049**	5·89			
QFRG_1	OPJ06_70–sat_094	60D/50D	−0·009**	1·84			
		70D/60D	−0·008**	2·36			
		80D/70D	−0·005**	1·85			
		Final	−0·005**	1·71			
QFRN_1	OPK17_40–OPBA08_5	80D/70D	0·005**	1·66	0·17**	0·25**	5·3
		Final	0·005**	1·59			

*$P < 0·01$.
**$P < 0·05$.
[a] a_i is the additive effects of the test points *i*.
[b] h^2a_i is the percentages of the phenotypic variations explained by a_i.
[c] ae_i is the effects of the environmental interaction of locus *i*.
[d] $h^{2}ae_i$ is the percentages of the phenotypic variations explained by ae_i.

stage; 50D/40D, 60D/50D and 70D/60D stage. A significant *ae* effect was detected in QFRC1_1 (at 80D/70D stage and final stage) and QFRF_1 (at 50D/40D and 60D/50D stage). Four QTLs (QFRA1_1, QFRA1_2, QFRA1_3 and QFRN_1) possessed significant *ae* effect at only one filling stage, suggesting that the impact of QTL varied at different developmental stages. One QTL (at QFRA1_3 at 70D/60D stage) had a significant *ae* effect rather than a significant *a* effect and accounted for 13·12% of the phenotypic variation. Three QTLs (QFRA1_3 at 70D/60D stage, QFRF_1 at 80D/70D stage and QFRN_1 at 80D/70D stage) were identified to have a significantly positive *ae* effect on seed filling rate in both 2004 and 2005, accounting for 5·3–13·12% of the phenotypic variation. Other QTLs showed either positive or negative *ae* effects on seed filling rate in both years and explained 3·03–12·22% of the phenotypic variation.

Seven QTLs (QFRA1_1 at 60D/50D stage; QFRA1_2 at 70D/60D stage; QFRC1_1 at 80D/70D stage; QFRC1_1 at the final stage; QFRC2_2 at 50D/40D, 60D/50D, 70D/60D and initial stages; QFRF_1 at 70D/60D and 80D/70D stages; QFRN_1 at 80D/70D stage) were identified with both *a* and *ae* effects.

(iii) *Analysis of* aa *and* aae *effects during seed filling*

Both *aa* (epistasis) and *aae* (epistasis × environment) effects were analysed using QTLMapper version 1.6 (Wang *et al*., 1999*a*). Twenty-three epistatic pairwise of *aa* QTLs or *aae* QTLs were identified in different seed filling stages (Table 3). Of them, *aa* and *aae* effects of 12 pairs of QTLs were identified by unconditional mapping from the initial stage to the final stage (12 pairs of QTLs with *aa* effects and two pairs of QTLs with *aae* effects). Thirteen pairs of QTLs underlying seed filling rate with *aa* and *aae* effects were identified by conditional mapping. Of them 11 pairs of QTLs were detected as *aa* effects and two pairs of QTLs were detected as *aae* effects. Five pairs of epistatic QTLs were detected across two different stages (QFRC1_2 and QFRF_4 at 80D/70D and final stages; QFRC2_2 and QFRC2_1 at initial and 50D/40D stages; QFRE_1 and QFRC1_4 at 80D/70D and final stages; QFRE_4 and QFRN_2 at 80D/70D and

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Table 3. *Estimated epistatic (aa) and epistasis × environment interaction (aae) effects of QTL underlying seed filling rate at different developmental stages of soybean in 2004 and 2005*

QTL	Maker interval	QTL	Maker interval	Stage	aa_{ij}[a]	H^2[b](aa_{ij})	aae_{ij}[c](2004)	aae_{ij}(2005)	H^2[d](aae_{ij})
QFRA1_2	satt449–satt300	QFRO_1	satt094–satt358	40D/30D	0·011*	6·15			
QFRA2_1	OPI14_55–satt547	QFRI_2	satt292–satt330	40D/30D	−0·008*	3·25	0·9**	−0·9**	8·23
QFRB1_1	satt426–satt509	QFRC1_1	OPBF12_5–satt164	60D/50D	0·049**	5·9			
QFRB1_2	satt509–satt251	QFRA2_2	sct_067–satt390	50D/40D	−0·059**	8·09			
QFRC1_2	satt164–OPAO19_1	QFRF_4	satt218–satt522	80D/70D	0·008**	3·44			
				final	0·008**	3·44	0·05**	−0·05**	0·15
QFRC2_3	satt202–satt460	QFRC2_4	sat_076–OPN09_12	50D/40D	0·048**	5·36	−0·02*	0·02*	1·06
QFRC2_2	satt460–satt134	QFRC2_1	satt277–sat_076	initial	−0·004**	2·89	0·13*	−0·13*	0·93
				50D/40D	−0·043*	3·04	0·21**	−0·21**	5·8
		QFRC2_5	OPN09_12–satt457	initial	−0·003**	1·63	0·03**	−0·03**	0·79
		QFRC2_4	sat_076–OPN09_12	70D/60D	0·006*	0·83	−0·09**	0·09**	0·5
QFRD1b_2	sat_069–satt459	QFRC1_3	OPAO19_1–OPM04_90	80D/70D	0·045**	9·83			
QFRD2_1	OPAS18_1–satt372	QFRI_3	satt330–OPBE13_7	60D/50D	0·018**	6·14			
QFRD2_2	satt413–sat_086	QFRJ_1	sat60D/50D1–satt414	50D/40D	0·010*	1·62			
QFRE_1	satt355–satt452	QFRC1_4	satt195–sat_042	80D/70D	−0·007**	2·63			
				final	−0·007**	2·63			
QFRE_2	satt117–sat_112	QFRA2_3	satt341–OPI14_55	50D/40D	−0·014*	3·18	0·012**	−0·012**	4·68
QFRE_3	satt263–satt117	QFRO_2	satt094–satt358	70D/60D	0·009**	2·69			
QFRE_4	satt452–satt263	QFRN_2	OPBA08_5–GMABAB	80D/70D	0·004**	0·86			
				final	0·004**	0·86			
QFRF_2	sct_188–satt335	QFRC2_6	satt134–satt289	60D/50D	0·053**	6·9	−0·051**	0·051**	12·78
QFRG_2	sat_117–satt191	QFRC2_2	satt202–satt460	40D/30D	−0·042**	6·81	0·03**	−0·03**	6·95
QFRG_3	sat_088–sat_105	QFRM_1	OPT14_90–OPR11_65	60D/50D	0·011**	2·29			
QFRI_1	sct_189–satt440	QFRA1_3	OPT14_50–sat70D/60D5	40D/30D	−0·032**	3·95			
QFRL_1	sat_099–sat_113	QFRA2_4	satt538–sct_067	70D/60D	−0·048**	7·52	0·064**	−0·064**	12·72
QFRL_2	satt229–sat_099	QFRA1_2	satt449–satt300	40D/30D			0·023**	−0·023**	4·08
		QFRC2_2	satt460–satt134	80D/70D	−0·012*	7·73			
				final	−0·012**	7·73			

*$P<0·01$.
**$P<0·05$.
[a] aa_{ij} is the additive-by-additive interaction between points i and j.
[b] h^2aa_{ij} is the percentages of the phenotypic variations explained by aa_{ij}.
[c] aae_{ij} is epistatic effect of the environmental interaction of locus i, j.
[d] h^2aae_{ij} is the percentages of the phenotypic variations explained by aae_{ij}.

final stages; QFRL_2 and QFRC1_2 at 80D/70D and final stages). One pair of epistatic QTLs (QFRL_2 and QFRA1_2) was detected with only *aae* effect at 40D/30D stage. Other pairs of QTLs were identified in only one filling stage with *aa* or *aae* effects. However, QFRC2_2 was detected to interact with other three different QTLs (QFRC2_1 at initial and 50D/40D stages, QFRC2_4 at 50D/40D stage and QFRC2_5 at initial stage). The epistatic effects of these QTLs explained 0·83–3·04 % of the phenotype variation. QFRL_2 interacted with two other QTLs (QFRA1_2 at 40D/30D stage; QFRC2_2 at 80D/70D and final stages) and explained 4·08–7·73 % of the phenotypic variation. Three pairs of QTLs (QFRC1_2 and QFRF_4, QFRE_1 and QFRC1_4, QFRE_4 and QFRN_2, QFRL_2 and QFRC2_2) were detected in the late filling stages (all at 80D/70D and final stages), and explained the proportion of phenotype variation by epistatic interactions from 0·86 to 7·73 %. Six pairs of epistatic QTLs (QFRC1_2 and QFRF_4; QFRE_1 and QFRC1_4; QFRE_4 and QFRN_2; QFRL_2 and QFRC2_2; QFRC2_2 and QFRC2_1; QFRC2_2 and QFRC2_5) with *aa* or *aae* effects were detected only in initial stage or final stage and explained the proportion of phenotype variation from 0·86 to 7·73 % (Table 3).

aae was an important component of the total QE interaction effects. Of the identified 23 digenic interactions in this study for seed filling rate, 16 had only *aa* effects that explained 0·83–9·83 % of the phenotype variation by epistatic interactions, and one had only *aae* effect that explained 4·08 % of the phenotype variation. Other pairs had both *aa* and *aae* effects and explained the proportion of phenotype variation by epistatic interactions from 0·83 to 7·52 % (Table 3).

4. Discussion

Many studies have shown that seed filling rate is the key genetic factor influencing seed yield (Jones *et al*., 1979; Smith & Nelson, 1986*a*, *b*). In crops, seed filling rate and duration could account for the most variation of seed weight (Nass & Reiser, 1975). Our results indicated that the seed filling rate of soybean was under developmental genetics and environmental control. Similar results were reported in rice (Takai *et al*., 2005), wheat (Wang *et al*., 2009) and maize (Wang *et al*., 1999*b*; Liu *et al*., 2011).

Most previous studies of QTLs were limited on the analyses of individual QTLs rather than the interaction between QTLs (Specht *et al*., 2001; Hyten *et al*., 2004), resulting in the underestimation of genetic variance and the overestimation of individual QTL effects (Carlborg & Haley, 2004). Considerable loss in genetic response to marker-assisted selection (MAS) at late generations had been observed (Liu *et al*., 2004) due to the negligence of interaction between QTLs. Twenty-eight pairs of QTLs with epistasis effects at different developmental stages were detected in our study. One pair showed only *aae* effect at different seed filling stages and others showed both *aa* and *aae* effects, suggesting that epistasis was important and should be considered in breeding programmes for increasing seed-filling rate in soybean.

Unconditional and conditional QTL mapping provided an effective way to evaluate the dynamic expression of quantitative traits during soybean development. *aa* and *aae* effects of the conditional QTL underlying seed weight and linolenic acid content have been determined using conditional and unconditional QTL strategy in soybean (Teng *et al*., 2009; Han *et al*., 2011). In the present study, epistatic effect and QTL × environmental interaction of mass filling rate during soybean seed development were determined. Ten QTLs were identified to possess *a*, *ae*, *aa* or *aae* effects at the final filling stage of seed that explained 0·15–9·49 % of the phenotypic variation, while other 34 QTLs were identified to have *a*, *ae*, *aa* or *aae* effects from initial to 80D/70D stages and explained 0·5–13·12 % of the phenotypic variation. Our findings indicated that *aa* and *aae* effects existed mostly for a short time period, so that a pair of QTLs was hardly detected during consistent seed filling stages. This was implied by the fact that *aa* and *aae* effects were mostly contributed by transient gene expression.

Some studies showed that epistatic variance accounted for a large proportion of the genetic variance of quantitative traits in mapping population (Wilfert & Schmid-Hempel, 2008). In our study, the interaction between QFRD1b_1 and QFRC1_3 (*aa* effect) at 80D/70D stage explained the largest proportion of phenotypic variation (9·83 %), followed by the interaction between QFRB1_2 and QFRA2_1 at 50D/40D stage (explained 8·09 % of the phenotypic variation), and then by the interaction between QFRL_2 and QFRC2_2 at the final stage (explained 7·73 % of the phenotypic variation). The phenotypic variation explained by these *aa* effects were almost equal to the proportion of the phenotypic variation explained by *a* effect, such as, QFRC2_2 at 80D/70D stage that explained the largest proportion of phenotypic variation (9·49 %). Because of the obvious contribution by epistatic interaction, QTL with significant epistatic effect should be considered in breeding programme for increasing seed filling rate or seed weight in soybean.

QE interaction was an important component affecting quantitative traits. Understanding QE interaction is of importance to the MAS. Usually, QE interaction effect is treated as random effect. This implied that QTL would be affected by different environments. The mixed model approaches for QTL mapping provided an unbiased prediction on QE

interaction when the experiment was conducted under multiple environments (Zhu, 1999), which could enhance the efficiency of the analyses for QTL × environment interaction. In the present work, nine QTLs had only *a* effect at different filling stages, and one QTL (QFRA1_3 at 70D/60D stage) had only *ae* effect, while other QTLs had both *a* and *ae* effects at different seed filling stages. The QTL with only QE effects were mainly determined by environments and was ineffective for MAS. For example, QFRA1_3 at 80D/70D and final stages (with only *a* effect) explained 2·37–2·82% of the phenotypic variation, while the same QTL QFRA1_3 at 70D/60D stage (with *ae* effect) explained 13·12% of the phenotypic variation. This result described an environmentally sensitive phenomenon of QTL and could not be desirable for MAS. QTL QFRG_1 showed only *a* effect at 60D/50D, 70D/60D, 80D/70D and final stages in this study, suggesting that this QTL was stable in different environments and could be a desirable loci to improve seed filling rate of soybean by MAS.

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Declaration of interest

None.

References

Blum, A., Klueva, N. & Nguven, H. (2001). Wheat cellular thermo-tolerance is related to yield under heat stress. *Euphytica* **117**, 117–123.

Carlborg, O. & Haley, C. S. (2004). Epistasis: too often neglected in complex trait studies? *Nature Reviews Genetics* **5**, 618–625.

Carlborg, O., Jacobsson, L., Ahgren, P., Siege, P. & Andersson, L. (2006). Epistasis and the release of genetic variation during long-term selection. *Nature Genetics* **38**, 418–420.

Cockerham, C. C. & Zeng, Z. B. (1996). Design with marker loci. *Genetics* **143**, 1437–1456.

Croissant, G. L. & Torrie, J. H. (1971). Evidence of non-additive effects and linkage in two hybrid populations of soybeans. *Crop Science* **11**, 675–677.

Doyle, J. J. & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.

Egli, D. B., Legget, J. E. & Wood, J. M. (1978). Influence of soybean seed size and position on the rate and duration of filling. *Agronomy Journal* **70**, 127–130.

Egli, D. B. & Bruening, W. P. (2004). Water stress, photosynthesis, seed sucrose levels and seed growth in soybean. *Journal of Agricultural Sciences* **142**, 1–8.

Goldringer, I., Brabant, P. & Gallais, A. (1997). Estimation of additive and epistatic genetic variances for agronomic traits in a population of doubled-haploid lines of wheat. *Heredity* **79**, 60–71.

Han, Y., Teng, W., Sun, D., Du, Y., Qiu, L., Xu, X. & Li, W. (2008). Impact of epistasis and QTL × environment interaction on the accumulation of seed mass of soybean (*Glycine max* L. Merr.). *Genetical Research* **90**, 481–491.

Han, Y., Xie, D., Teng, W., Zhang, S., Chang, W. & Li, W. (2011). Dynamic QTL analysis of linolenic acid content in different developmental stages of soybean seed. *Theoretical and Applied Genetics* **122**, 1481–1488.

Hunt, L. A., van der Poorten, G. & Pararajasingham, S. (1991). Postanthesis temperature effects on duration and rate of grain filling in some winter and spring wheats. *Canadian Journal of Plant Science* **71**, 609–617.

Hyten, D. L., Pantalone, C. E., Sams, A. M. Saxton, D., Landau-Ellis, T. R., Stefaniak, T. R. & Schmidt, M. E. (2004). Seed quality QTL in a prominent soybean population. *Theoretical and Applied Genetics* **109**, 552–561.

Jannink, J.-L. (2008). QTL × genetic background interaction: predicting inbred progeny value. *Euphytica* **161**, 61–69.

Jiang, Z., Han, Y., Teng, W., Zhang, Z., Sun, D., Li, Y. & Li, W. (2010). Identification of QTL underlying the filling rate of protein at different developmental stages of soybean seed. *Euphytica* **175**, 227–236.

Jones, D. B., Peterson, M. L. & Geng, S. (1979). Association between grain filling rate and duration and yield components in rice. *Crop Science* **19**, 641–644.

Li, S. B., Zhang, Z. H., Hu, Y., Li, C. Y., Jiang, X., Mao, T., Li, Y. S. & Zhu, Y. G. (2006). Genetic dissection of developmental behavior of crop growth rate and its relationships with yield and yield related traits in rice. *Plant Science* **170**, 911–917.

Liu, P., Zhu, J. & Lu, Y. (2004). Marker-assisted selection in segregating generations of self-fertilizing crops. *Theoretical and Applied Genetics* **109**, 370–376.

Liu, Z. H., Ji, H. Q., Cui, Z. T., Wu, X., Duan, L. J., Feng, X. X. & Tang, J. H. (2011). QTL detected for grain-filling rate in maize using a RIL population. *Molecular Breeding* **27**, 25–36.

Luo, L. J., Li, Z. K., Mei, H. W., Shu, Q. Y., Tabien, R., Zhong, D. B., Ying, C. S., Stansel, J. W., Khush, G. S. & Paterson, A. H. (2001). Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. *Genetics* **158**, 1755–1771.

Martin, S. K., Xie, F. T., Zhang, H. J., Zhang, W. & Song, X. (2009). Epistasis for quantitative traits in crosses between soybean lines from China and the United States. *Crop Science* **49**, 20–28.

Munier-Jolain, N. G. & Ney, B. (1998). Seed growth rate in grain legumes II. Seed growth rate depends on cotyledon cell number. *Journal of Experimental Botany* **49**, 1971–1978.

Nass, H. G. & Reiser, B. (1975). Grain filling period and grain yield relationships in spring wheat. *Canadian Journal of Plant Science* **55**, 673–678.

Nicolas, M. E., Lambers, H., Simpson, R. J. & Dalling, M. J. (1985*a*). Effect of post-anthesis drought on cell

division and starch accumulation in developing wheat grains. *Annals of Botany* **55**, 433–444.

Nicolas, M. E., Lambers, H., Simpson, R. J. & Dalling, M. J. (1985*b*). Effect of drought on metabolism and partitioning of carbon in two wheat varieties differing in drought-tolerance. *Annals of Botany* **55**, 727–747.

Palta, J. A., Kobata, T., Turner, N. C. & Fillery, I. R. (1994). Remobilization of carbon and nitrogen in wheat as influenced by post-anthesis water deficits. *Crop Science* **34**, 118–124.

Pfeiffer, T. W. & Egli, D. B. (1988). Heritability of seed-filling period estimates in soybean. *Crop Science* **28**, 921–925.

Plaut, Z., Butow, B. J., Blumenthal, C. S. & Wrigley, C. W. (2004). Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated temperature. *Field Crops Research* **86**, 185–198.

Reif, J. C., Maurer, H. P., Korzun, V., Ebmeyer, E., Miedaner, T. & Würschum, T. (2011). Mapping QTLs with main and epistatic effects underlying grain yield and heading time in soft winter wheat. *Theoretical and Applied Genetics* **123**, 283–292.

Schmutz, J., Cannon, S. B. & Schlueter, J. (2010). Genome sequence of the palaeopolyploid soybean. *Nature* **463**, 178–183.

Schussler, J. R., Brenner, M. L. & Brun, W. A. (1984). Abscisic acid and its relationship to seed filling in soybeans. *Plant Physiology* **76**, 301–306.

Smith, J. R. & Nelson, R. L. (1986*a*). Selection for seed-filling period in soybean. *Crop Science* **26**, 466–469.

Smith, J. R. & Nelson, R. L. (1986*b*). Relationship between seed-filling period and yield among soybean breeding lines. *Crop Science* **26**, 469–472.

Song, Q. J., Marek, L. F., Shoemaker, R. C., Lark, K. G., Concibido, V. C., Delannay, X., Specht, J. E. & Cregan, P. B. (2004). A new integrated genetic linkage map of the soybean. *Theoretical and Applied Genetics* **109**, 122–128.

Specht, J. E., Chase, K., Macrander, M., Graef, G. L., Chung, J. & Markwell, J. P. (2001). Soybean response to water: a QTL analysis of drought tolerance. *Crop Science* **41**, 493–509.

Takai, T., Fukuta, Y., Shiraiwa, T. & Horie, T. (2005). Time-related mapping of quantitative trait loci controlling grain-filling in rice (*Oryza sativa* L.). *Journal of Experimental Botany* **56**, 2107–2118.

Teng, W., Han, Y., Du, Y., Sun, D., Zhang, Z., Qiu, L., Sun, G. & Li, W. (2009). QTL analyses of seed weight during the development of soybean (*Glycine max* L. Merr.). *Heredity* **102**, 372–380.

Thorne, J. H. (1981). Morphology and ultrastructure of maternal seed tissues of soybean in relation to the import of photosynthesis. *Plant Physiology* **67**, 1016–1025.

Trigizano, R. N. & Caetano-Anolles, G. (1998). Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. *Horticultural Technology* **8**, 413–423.

Wang, D. L., Zhu, J., Li, Z. K. & Paterson, A. H. (1999*a*). Mapping QTLs with epistatic effects and QTL environment interactions by mixed linear model approaches. *Theoretical and Applied Genetics* **99**, 1255–1264.

Wang, G. L., Kang, M. S. & Moreno, O. (1999*b*). Genetic analyses of grain-filling rate and duration in maize. *Field Crops Research* **61**, 211–222.

Wang, R. X., Hai, L., Zhang, X. Y., You, G. X., Yan, C. S. & Xiao, S. H. (2009). QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai × Yu8679. *Theoretical and Applied Genetics* **118**, 313–325.

Wiegand, C. L. & Cueller, J. A. (1981). Duration of grain filing and kernel weight of wheat as affected by temperature. *Crop Science* **21**, 95–101.

Wilfert, L. & Schmid-Hempel, P. (2008). The genetic architecture of susceptibility to parasites. *BMC Evolutionary Biology* **8**, 187.

Yoshida, S. (1972). Physiological aspects of grain yield. *Annual Review of Plant Physiology* **23**, 437–464.

Zhu, J. (1995). Analysis of conditional genetic effects and variance components in developmental genetics. *Genetics* **141**, 1633–1639.

Zhu, J. (1999). Mixed model approaches of mapping genes for complex quantitative traits. *Journal of Zhejiang University* (*Natural Science edition*) **33**, 327–335.