RESEARCH ARTICLE

Quantitative trait loci analysis of individual and total isoflavone contents in soybean seeds

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Abstract

Soybean isoflavones play diverse roles in human health, including cancers, osteoporosis, heart disease, menopausal symptoms and pabulums. The objective of this study was to identify the quantitative trait loci (QTL) associated with the isoflavones daidzein (DC), genistein (GeC), glycitein (GlC) and total isoflavone contents (TIC) in soybean seeds. A population of 184 $F_{2:10}$ recombinant inbred lines derived from a 'Xiaoheidou' ×'GR8836' cross was planted in pot and field conditions to evaluate soybean isoflavones. Twenty-one QTL were detected by composite interval mapping. Several QTL were associated with the traits for DC, GeC, GlC and TIC only. *QDGeGlTIC4_1* and *QDGlTIC12_1* are reported first in this study and were associated with the DC, GeC, GlC and TIC traits simultaneously. The QTL identified have potential value for marker-assisted selection to develop soybean varieties with desirable isoflavone content.

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Introduction

Soybean (*Glycine max* L. Merr.) is one of the world's most important oilseed crops and comprises ∼20% oil and 40% protein. Soybean seeds have received considerable attention for their high isoflavone concentrations (1.0–3.0 $\mu g \cdot mg^{-1}$) (Wang and Murphy 1994; Cardinal *et al*. 2007). The three main isoflavone components, dadzein (DC), genistein (GeC) and glycitein (GlC) made up ∼95% of total isoflavones in soybean seeds (Kudou *et al*. 1991; Latunde-Dada *et al*. 2001). Isoflavone had pharmacological activities in preventing ovarian, breast, colon and prostate cancers as well as osteoporosis and cardiovascular diseases (Naim *et al*. 1976; Weidenborner *et al*. 1990; Aedin *et al*. 2000; Tikkanen and Adlererеutz 2000; Watanabe *et al*. 2002; Lo *et al*. 2007). Manipulation of crop characteristics using molecular biology is now possible. Previous studies have demonstrated that the main isoflavone compounds are synthesized in a reaction catalysed by several key enzymes from a branch of the general phenylpropanoid pathway, and that isoflavone synthase (IFS), phenylalanine ammonialyase (PAL), chalcone synthase (CHS), chalcone isomerase and certain CytP450s are involved in the GeC and DC biosynthetic pathways (Akashi *et al*. 1999; Jung *et al*. 2000; Yu *et al*. 2003). Compared with transgenic technology, molecular markers may be easier to utilize in a breeding programme. The two genetic *IFS* loci polymorphisms (*IFS1* and *IFS2*) have been mapped and associated with isoflavone concentration (Cheng *et al*. 2008) and several quantitative trait loci (QTL) for isoflavones are located on the same linkage group as the *CHS* family genes (Kassem *et al*. 2004; Matsumura *et al*. 2005; Primomo *et al*. 2005; Sangeeta *et al*. 2007). For example, the QTL for GlC share the same chromosome (Chr) 5, 9, 1 and 11 as *CHS2*, *CHS6*, *CHS7* and *CHS8*, respectively (Matsumura *et al*. 2005; Sangeeta *et al*. 2007). Thus, these QTL might be important.

Other QTL among various mapping populations have been mapped for DC, GeC, GlC and TIC (Njiti *et al*. 1999; Meksem *et al*. 2001; Kassem *et al*. 2004, 2006; Primomo *et al*. 2005; Sangeeta *et al*. 2007; Yang *et al*. 2008, 2011; Gutierrez-Gonzalez *et al*. 2009; Liang *et al*. 2009; Murphy *et al*. 2009; Zeng *et al*. 2009; Zhang *et al*. 2012). In total, 18 major QTL for DC have been mapped on Chr1, Chr3, Chr5, Chr7, Chr8, Chr13 and Chr14, and these explain 3.4% to

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50.2% of the phenotypic variation. Twenty-one QTL for GlC are located on Chr1, Chr3, Chr5, Chr7, Chr11 and Chr18; these explain 3.4–50.2% of the genetic variation. Eighteen QTL for GeC are present on Chr1, Chr3, Chr5, Chr7, Chr11, Chr13 and Chr18 and these explaine 9.3% to 29.5% of the phenotypic variation, respectively. Twenty major QTL for TIC are mapped on Chr5, Chr6, Chr7, Chr8, Chr12 and Chr13; these explain 4.1–32.3% of the genetic variation.

Soybean isoflavone content is a quantitative trait (Hoeck *et al*. 2000) and is regulated by environmental factors, including crop year, planting date, fertilizer, water and temperature (Tsukamoto *et al*. 1995; Bennett *et al*. 2004; Caldwell *et al*. 2005; Lozovaya *et al*. 2005; Murphy *et al*. 2009). The effects of genotype, environment, and the genotype × environment interaction are significant (Wang and Murphy 1994; Hoeck *et al*. 2000; Lee *et al*. 2003; Primomo *et al*. 2005; Murphy *et al*. 2009). The genetic basis of regulation of the amount of isoflavones is not well-understood, due to the tremendous variability in isoflavone content in seeds harvested from different environments (Njiti *et al*. 1999; Meksem *et al*. 2001; Kassem *et al*. 2004, 2006; Primomo *et al*. 2005; Zeng *et al*. 2009; Gutierrez-Gonzalez *et al*. 2009; Yang *et al*. 2011; Meng *et al*. 2011). Several QTL have been detected across different environments (Primomo *et al*. 2005; Zeng *et al*. 2009); however, they are insufficient for use in a breeding programme. Thus, it is important to identify soybean isoflavone QTL in different environments and populations.

The main objective of this study was to identify QTL associated with loci-conditioned variations in DC, GlC, GeC and TIC used a recombinant inbred lines (RILs) population derived from 'Xiaoheidou' and 'GR8836' grown in field-cultured and pot-cultured environments. The parents were derived from varieties that displayed wide genetic diversity in terms of isoflavone content.

Materials and methods

Plant materials

The mapping population contained 184 RILs of $F_{2:10}$ advanced by single-seed-descent and derived from a 'Xiaoheidou' × 'GR8836' cross. The parents, Xiaoheidou (♀, with low individual as well as total isoflavone content in seeds: DC, 1.85 $\mu g \cdot mg^{-1}$; GeC, 0.21 $\mu g \cdot mg^{-1}$; GlC, 1.26 $\mu g \cdot mg^{-1}$; TIC, 3.32 $\mu g \cdot mg^{-1}$) is a Chinese landrace and GR8836 (PI534647, maturity group III, ♂, with high individual and total isoflavone content in seeds: DC, 2.04 $\mu g \cdot mg^{-1}$; GeC, 0.44 $\mu g \cdot mg^{-1}$; GlC, 1.95 $\mu g \cdot mg^{-1}$; TIC, 4.43 $\mu g \cdot mg^{-1}$) was introduced from USA.

Field and greenhouse experiments

A set of 184 RILs were grown together with parents in a field-culture environment at the Jilin University Experimental Station, Changchun, China (43°54′N, 125°19′E), following a randomized complete block design with three replicates. The frost-free period was 145 days. The farming soil style was phaeozem which contained 120.19 $\mu g \cdot mg^{-1}$ rapidly available nitrogen, 17.33 $\mu g \cdot mg^{-1}$ rapidly available phosphorus and 153.74 $\mu g \cdot mg^{-1}$ rapidly available potassium. The previous crop was corn. The population was sown on 2 May 2010 in a field containing 5-m long rows with a row width of 0.65 m and was thinned to a uniform density with a space of 12.5 cm between plants two weeks after emergence. Weeds and pests were controlled routinely. The same RIL population was sown with three replicates on 20 April 2010 in plastic barrels (30 cm in diametre) contained ~6 kg local soil, each pot contained three plants. Management of plants was similar to field-culture environment, except that irrigation was used as needed for the pot-cultured condition. Seed samples were harvested from single plants until complete ripeness. No symptoms of fertilizer deficiency were identified in plants under two conditions.

Isoflavone extraction and determination

Sample preparation: Soybean seed powder (100 mg) was dissolved in 4 mL of 80% methanol (Dingguo, Beijing, China) in distilled water and stirred in an ultrasonic cleaning bath (Kunshan KQ3200DE, Jiangsu, China) for 30 min at 80°C and then left overnight at room temperature. The supernatant was filtered through a 0.45-μm filter and transferred to a 5-mL high-performance liquid chromatography (HPLC) volumetric flask. A 20 μL aliquot of the filtrate was subjected to HPLC analysis.

Chromatographic conditions: A C_{18} column (Shimadzu LC-20A, Tokyo, Japan; 150 × 4.6 mm, 5.0 μm) was used for all separations at a column temperature of 40°C. The linear gradient system consisted of solvent A (HPLC-grade methanol) and solvent B (0.4% orthophosphoric acid in distilled water). The solvent flow rate was 1.0 $mL \cdot min^{-1}$ and UV absorption was measured at 254 nm.

Genetic analysis: Young trifoliate leaves of the parents and each recombinant inbred line were collected from seedlings in the pot-cultured condition. Total DNA was purified as described by Yuan *et al*. (2002). SSR primers developed by Song *et al*. (2004) were screened from Soybase (2005). Polymerase chain reaction (PCR) amplification was performed as described by Zeng *et al*. (2009). The PCR products were separated in 6% (w/v) denaturing polyacrylamide gels and visualized by silver staining (Trigizano and Caetano-Anolles 1998).

Frequency distribution and statistical parameters for the parental and RIL populations were analysed by using the SPSS 13.0 (SPSS, Chicago, USA) and Excel 2003 (Microsoft, Redmond, USA) software. A linkage map contained 87 SSR markers was constructed using Mapmaker/Exp 3.0b (Lander *et al*. 1987) and the Kosambi mapping function (Kosambi 1944). The commands 'group',

'map', 'try', and 'compare' were used to build the linkage groups. The type I error detection ratio was set to 5%. The Haldane mapping function (Haldane 1919) was used with a minimum LOD score of 2.5 and a maximum distance of 50 cM (Promomo *et al*. 2005). QTL were identified by composite interval mapping (Zeng 1993, 1994).

Results

Phenotypic analysis of individual and total isoflavone contents

Ranges, means, standard deviations, coefficients of variation (CV), skewness, kurtosis, and broad-sense heritability for seed isoflavone contents of the parents and RIL population across the two environments (field-cultured and pot-cultured) are presented in table 1. 'GR8836' had significantly higher values than those of 'Xiaoheidou' for all isoflavone contents across both environments, indicated that the two parents differed in the genes controlling individual and total isoflavone contents. In fact, isoflavone content was significantly higher in pot-cultured than in the field-cultured environment, and the reason may be consistent with the large environmental interaction generally associated with isoflavone content in soybean seeds (Wang and Murphy 1994; Hoeck *et al*. 2000; Lee *et al*. 2003; Primomo *et al*. 2005; Murphy *et al*. 2009). Frequency distribution of field cultured and pot cultured conditions for the RILs population was determined for the samples and both displayed a continuous distribution (table 1; figure 1). DC was higher than GeC and GlC was the lowest in soybean seeds. Widely transgressive segregations were detected in all research environments.

The CV values that were not significant were <0.5 for the isoflavone content means. Broad-sense heritability estimates for DC, GeC, GlC and TIC across field-cultured and pot-cultured environments were 0.54, 0.66, 0.61, 0.52, 0.44, 0.49, 0.56, and 0.69 (table 1), individually. Broad-sense heritability estimates for the isoflavone content were similar to those reported previously by Primomo *et al*. (2005) and Zeng *et al*. (2009), which ranged from 0.35 to 0.57. However, heritability estimates reported by Meksem *et al*. (2001) and Yang *et al*. (2011) were higher than the values obtained here.

Both skewness and kurtosis values for relative traits were <1.0, and all isoflavone contents were normally distributed. Positive values for skewness (table 1; figure 1) indicated that all distributions were skewed towards 'GR8836'. An analysis of variance of isoflavone content indicated a significant ($P < 0.001$) genotypic variation for DC, GeC, GlC and TIC among RILs. Significant variation was also detected between the different environments ($P < 0.001$).

Linkage analysis

A total of 667 SSR markers were screened between the two parents and 232 SSR primers had polymorphisms. A

Table 1. Statistical analysis of DC, GeC, GlC and TIC of parents and the 184 $F_{2:10}$ RILs population across field-cultured and pot-cultured environments.

Traits	Environment	Parents		RILs population							Variability source					
		GR8836 ($\mu g \cdot mg^{-1}$) (♂)	Xiaoheidou ($\mu g \cdot mg^{-1}$) (♀)	Min ($\mu g \cdot mg^{-1}$)	Max ($\mu g \cdot mg^{-1}$)	Mean ($\mu g \cdot mg^{-1}$)	SD ($\mu g \cdot mg^{-1}$)	CV ($\mu g \cdot mg^{-1}$)	Skewness	Kurtosis	H^2	Genetic			Environment	
												DF	P value		DF	P
DC	Field-cultured[a]	2.35	1.47	0.89	5.05	2.50	0.32	0.23	0.85	0.96	0.54	183	0.001***		1	0.001***
	Pot-cultured[b]	3.10	2.07	1.16	5.78	2.80	0.65	0.29	0.93	0.88	0.66	183	0.001***			
GeC	Field-cultured	0.24	0.16	0.12	0.83	0.28	0.01	0.29	0.46	0.14	0.61	183	0.001***		1	0.001***
	Pot-cultured	0.29	0.20	0.18	1.39	0.59	0.05	0.36	1.00	0.94	0.52	183	0.001***			
GlC	Field-cultured	2.19	1.22	0.56	4.63	2.44	0.23	0.20	0.48	0.91	0.44	183	0.001***		1	0.001***
	Pot-cultured	2.14	1.57	1.33	4.45	2.76	0.48	0.25	0.25	–0.38	0.49	183	0.001***			
TIC	Field-cultured	4.78	2.85	1.60	10.13	5.23	1.08	0.20	0.79	0.93	0.56	183	0.001***		1	0.001***
	Pot-cultured	5.53	3.84	3.16	10.79	6.15	2.35	0.25	0.54	0.18	0.69	183	0.001***			

SD, standard deviation; CV, coefficient of variation; H^2, broad-sense heritability; DC, daidzein; GeC, genistein; GlC, glycitein; TIC, total isoflavone contents; RILs, recombinant inbred lines DF, degree of freedom. [a]Field-cultured environment in Changchun in 2010. [b]Pot-cultured environment in Changchun in 2010. *** Significantly different at $P < 0.001$.

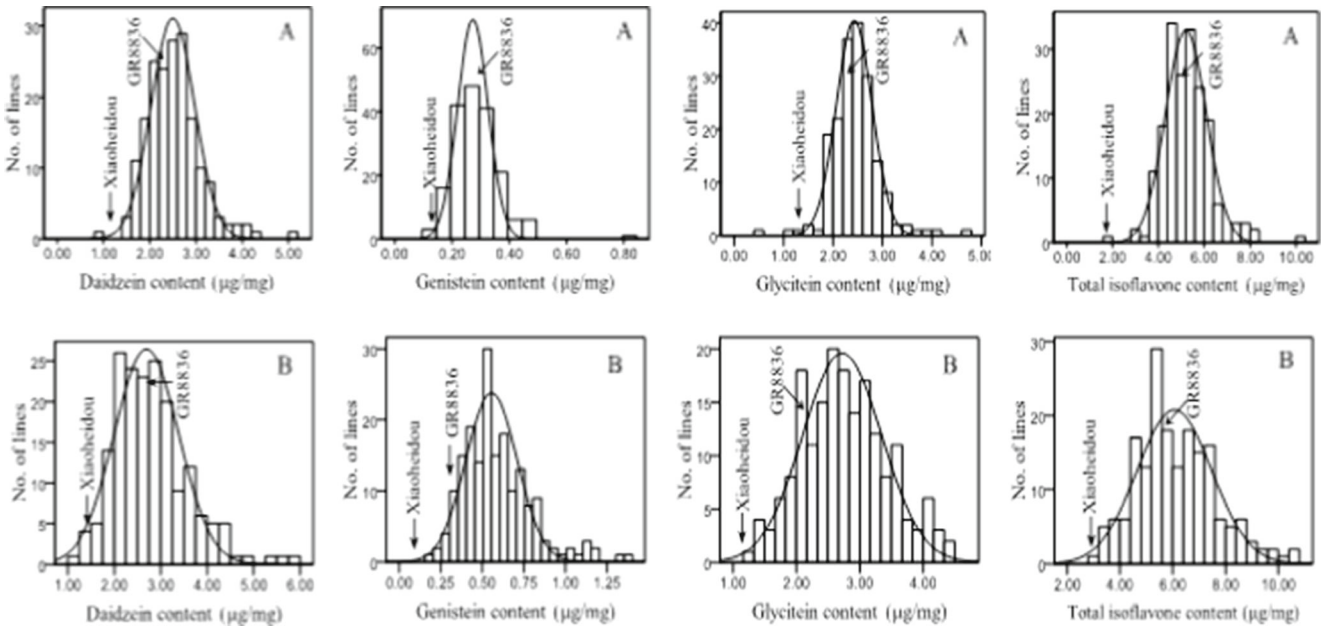

Figure 1. Frequency distribution of daidzein, genistein, glycitein and total isoflavone contents in soybeans seeds among 184 $F_{2:10}$ RIL derived from a cross between the cultivars 'Xiaoheidou' and 'GR8836' in field-cultured environments (A) and pot-cultured environments (B). Values next to the *x*-axis are the upper limit of each category. Parental values are indicated for 'Xiaoheidou' and 'GR8836'.

geneticlinkage map that covered 20 chromosomes and contained 87 SSR markers was constructed (Lander *et al*. 1987). Total length of the map was 1733 cM with an average distance between markers of 25.1 cM. The markers were initially grouped and anchored based on a consensus map (Song *et al*. 2004).

Isoflavone QTL mapping

Twenty-one QTL associated with DC, GeC, GlC and TIC, including many novel regions, were identified in the field-cultured and pot-cultured environments (table 2; figure 2). The QTL were located on eight chromosomes

Table 2. QTL associated with DC, GeC, GlC and TIC in soybean seeds in the field-cultured and pot-cultured environments.

Trait	Environment	QTL	Chr.	Near marker	LOD value	AAE	R^2 / %
DC	Field cultured[a]	*QDTIC9_1*	9	Sat_319	2.85	0.20	5.81
	Pot cultured[b]	*QDGlTIC3_1*	3	Satt009	2.63	–0.25	4.87
		QDGeGlTIC4_1	4	Satt524	2.78	0.39	6.70
		QDTIC4_2	4	Sat_140	4.17	0.24	7.37
		QDGlTIC12_1	12	Satt353	2.57	0.23	5.11
GeC	Field cultured	*QGeC13_1*	13	Satt395	2.77	0.02	5.26
	Pot cultured	*QDGeGlTIC4_1*	4	Satt524	3.48	0.11	7.87
		QGeC7_1	7	Satt323	2.92	0.07	5.78
		QGeC10_1	10	Satt479	4.01	0.09	8.83
GlC	Field cultured	*QGlC4_1*	4	Satt150	3.98	0.18	5.53
		QGlC17_1	17	Satt488	2.92	0.14	5.90
	Pot cultured	*QDGlTIC3_1*	3	Satt009	3.27	–0.24	7.29
		QDGeGlTIC4_1	4	Satt524	2.97	0.29	4.87
		QDGlTIC12_1	12	Satt353	2.97	0.29	4.92
		QGlC17_1	17	Satt488	2.77	0.14	5.90
TIC	Field cultured	*QDTIC9_1*	9	Sat_319	2.69	0.38	5.32
		QDGlTIC12_1	12	Satt353	2.69	0.40	4.87
	Pot cultured	*QDGlTIC3_1*	3	Satt009	2.68	–0.53	5.75
		QDGeGlTIC4_1	4	Satt524	3.40	0.59	6.63
		QDTIC4_2	4	Sat_140	2.76	0.42	5.09
		QDGlTIC12_1	12	Satt353	2.58	0.60	4.48

Chr, chromosome number; AAE, additive allelic effect; R^2, the proportion of phenotypic data explained by the marker locus
[a]Field-cultured environment in Changchun in 2010
[b] Pot-cultured environment in Changchun in 2010

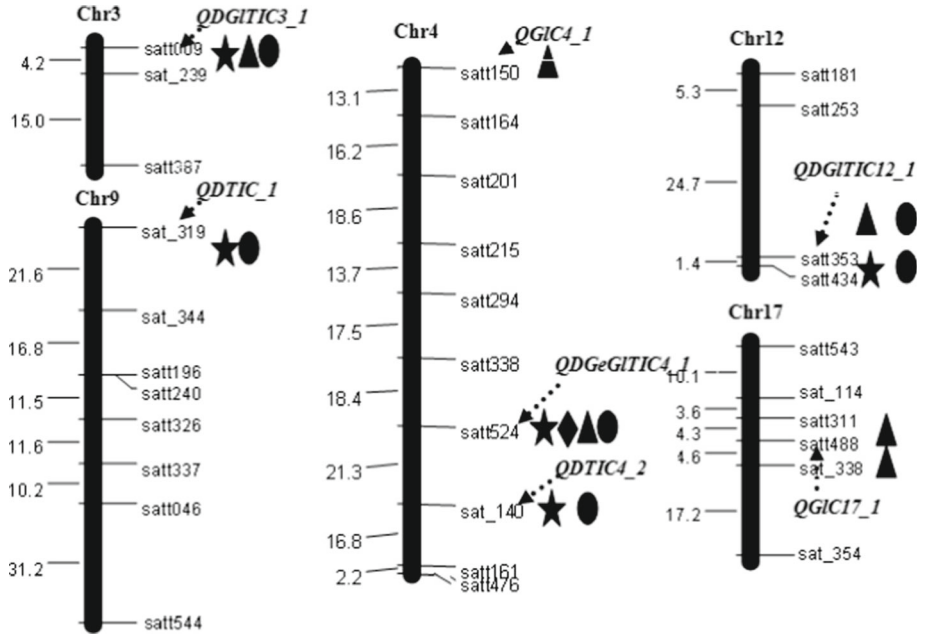

Figure 2. Locations of some of the chromosomes with major QTL for DC, GeC, GlC and TIC in field-cultured and pot-cultured environments in Changchun in 2010. ★ Daidzein content, ▲ glycitein content, ● genistein content and ♦ total isoflavone content. Chromosome number is indicated at the top of the linkage group diagram. QTL names, marker names and distances for the interval are given. Genetic distances are from the RIL function of Mapmaker/EXP 3.0b (Lander *et al*. 1987). Linkage groups were named using the consensus map (Song *et al*. 2004) and coincided with the chromosome number on Soybase website (http://www.soybase.org/).

(Chr3, Chr4, Chr7, Chr9, Chr10, Chr12, Chr13 and Chr17). The total explained phenotypic variation for specific isoflavone content was 4.48–8.83%. Nine of the 10 QTL effects were positive that was contributed by 'GR8836' and only one QTL named *QDGlTIC3_1* had a negative effect that was donated by 'Xiaoheidou'.

Five QTL (table 2) associated with DC were mapped on Chr3, Chr4, Chr9 and Chr12. Of them, two QTL for DC, *QDGeGlTIC4_1* and *QDTIC4_2*, both located on Chr4, explained 6.70% and 7.73% of the phenotypic variation. Four QTL for GeC were mapped on Chr4, Chr7, Chr10 and Chr13. Six QTL for GlC were detected on Chr3, Chr4, Chr12 and Chr17. Of them, two QTL, *QGlC4_1* and *QDGeGlTIC4_1* both located on Chr4, explained 5.53% and 4.87% of the phenotypic variation in the two environments, respectively. One QTL, *QGlC17_1* for GlC located on Chr17, was identified in both field-cultured and pot-cultured conditions and was associated with Satt488. Six QTL (table 2) for TIC were detected on Chr3, Chr4, Chr9, and Chr12, which could explain 4.48–6.63% of the phenotypic variations. Of them, two QTL, *QDGeGlTIC4_1* and *QDTIC4_2* for TIC both located on Chr4, explained 6.63% and 5.09% of the genetic variation, individually.

Most of the QTL were clustered in genomic regions, particularly on Chr3, Chr4, Chr9 and Chr12 (table 2; figure 2). One novel QTL, *QDGeGlTIC4_1* associated with Satt524, was identified for DC, GeC, GlC and TIC, simultaneously. Two QTL, *QDGlTIC12_1* associated with Satt353 and *QDGlTIC3_1* with Satt009, were identified for DC, GlC and TIC, respectively. Another two QTL, *QDTIC4_2* with Sat_140 and *QDTIC9_1* with Sat_319, were detected across only one environment for DC and TIC, respectively. Two major QTL, *QGlC17_1* for GlC and *QDGlTIC12_1* for TIC were detected in both culture environments. One QTL, *QGlC17_1* for GlC, explained 6.7% of the phenotypic variation in both environments. Another QTL, *QDGlTIC12_1* for TIC, was also detected in both environments and it explained 4.87% of the phenotypic variation in the field-cultured environment and 4.48% in the pot-cultured environment, individually.

Discussion

Individual and total isoflavone contents in the pot-cultured environment were markedly higher than those in the field-cultured environment. The reason might be the interaction between genetic and multiple environmental factors (Tsukamoto *et al*. 1995; Bennett *et al*. 2004; Caldwell *et al*. 2005; Lozovaya *et al*. 2005; Murphy *et al*. 2009), as we detected a significant environmental effect ($P < 0.001$). Differences between the two environments were water availability and early sowing, which have been demonstrated to benefit the accumulation of individual and total isoflavone contents (Bennett *et al*. 2004; Caldwell *et al*. 2005; Lozovaya *et al*. 2005). Thus, it could be inferred that irrigation and/or an early sowing date should be considered to produce high isoflavone content soybeans.

Notably, two QTL, *QDGeGlTIC4_1* located on Chr4 associated with Satt524 and *QDGlTIC12_1* located on Chr12 with Satt353, are first reported here. The two QTL were associated with the DC, GeC, GlC and TIC traits simultaneously, suggesting that these QTL are linked to the same gene or represent the action of clustered genes. If so, it may be inferred that these QTL are linked to the upstream genes in the isoflavone biosynthetic and regulatory pathways. Genes linked to new intervals were identified on the SoyBase website. *QDGeGlTIC4_1* might associate with Glyma04g42110 which was noted as a R2R3-MYB transcription factor. Previous studies had proved that the R2R3-MYB transcription factor gene family could increase the expression abundance of some key enzyme genes in flavonid biosynthesis in transgenic *Arabidopsis* plants (Stracke *et al*. 2001). It can be inferred that Glyma04g42110 is a candidate gene for *QDGeGlTIC4_1*. No candidate gene associate with *QDGlTIC12_1* was found to relate to the synthesis of isoflavone in the adjacent region of Satt353.

Stability of the QTL across environments and genetic backgrounds is key to determine if they can be used in a breeding programme (Brummer *et al*. 1997). In this study, three QTL were consistent with previously mapped results. The QTL *QDGlTIC3_1* for DC, GeC and TIC associated with Satt009 was first mapped by Liang *et al*. (2009). *QGeC7_1* for GeC associated with Satt323 detected in the present study was ~20 cM away from the QTL loci associated with Satt540 detected by Zeng *et al*. (2009) and Primomo *et al*. (2005). *QDTIC4_2* corresponded to Sat_140 and was similar to the QTL *QGC4* for GeC detected by Yang *et al*. (2011). In addition, three QTL of *QGlC4_1*, *QGlC17_1* and *QDGlTIC12_1* were detected simultaneously in pot cultured and field cultured conditions and were significantly different ($P < 0.001$) in the present study. However, these QTL were not detected in other study populations, suggested that they may be unique to the 'Xiaoheidou' and 'GRRR36' parents, which have a distant genetic relationship. All these stable QTL should be considered to narrow down the genomic regions and identify related genes for future research.

Previous studies have shown that genomic regions associated with soybean isoflavone content are always linked to other agronomic (seed yield, weight, maturity, lodging and height) and quality (oil and protein content) traits (Wang *et al*. 2000; Meksem *et al*. 2001; Kassem *et al*. 2004; Primomo *et al*. 2005). In this study, the location of *QDGlTIC3_1* was shared by oil content, iron efficiency, reaction to *Sclerotinia sclerotiorum* and flower number; the location of *QDGeGlTIC4_1* was shared by oil content and protein content; and the locus of *QDGlTIC12_1* was shared by somatic embryos per explant and protein content (Qi *et al*. 2011; Lin *et al*. 1997; Zhang *et al*. 2010; Song *et al*. 2010). Such linkages should be considered in a breeding programme.

Several QTL located on the same chromosome as key enzymes or transcription factor genes have been reported (Cheng *et al*. 2008; Kassem *et al*. 2004; Primomo *et al*. 2005; Matsumura *et al*. 2005). In the present study, three QTL, named *QDGlTIC12_1*, *QDTIC9_1* and *QDGlTIC3_1*, were matched with the candidate genes, *CHS, bHLH* and those of the *DFR2* family, which are related to isoflavone accumulation. Although these assumptions should be confirmed by further studies, these QTL may facilitate improvement of soybean lines in terms of their isoflavone content.

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References

Aedin C., Bryn H. and Rosa M. 2000 Isoflavones, lignans and stilbenes – origins, metabolism and potential importance to human health. *J. Sci. Food. Agric.* **80**, 1044–1062.

Akashi T., Aoki T. and Ayabe S. 1999 Cloning and functional expression of a cytochrome P450 cDNA encoding 2-hydroxyisoflavanone synthase involed in biosynthesis of the isoflavonoid skeleton in licorice. *Plant Physiol.* **121**, 821–828.

Bennett J. O., Yu O., Heatherly L. G. and Krishnan B. 2004 Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. *J. Agric. Food Chem.* **52**, 7574–7579.

Brummer E. C., Graef G. L., Orf J., Wilcox J. R. and Shoemaker R. C. 1997 Mapping QTL for seed protein and oil content in eight soybean populations. *Crop Sci.* **37**, 304–310.

Caldwell C. R., Britz S. J. and Mirecki R. M. 2005 Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean (*Glycine max* (L.) *Merrill*) grown in controlled environments. *J. Agric. Food Chem.* **53**, 1125–1129.

Cardinal A. J., Burton J. W., Camacho-Roger A. M., Yang J. H., Wilson R. F. and Dewey R. E. 2007 Molecular analysis of soybean lines with low palmitic acid content in the seed oil. *Crop Sci.* **47**, 304–310.

Cheng H., Yu O. and Yu D. Y. 2008 Polymorphisms of *IFS1* and *IFS2* gene are associated with isoflavone concentrations in soybean seeds. *Plant Sci.* **175**, 505–512.

Gutierrez-Gonzalez J. J., Wu X., Zhang J., Lee J. D., Ellersieck M., Shannon J. G. *et al.* 2009 Genetic control of soybean seed isoflavone content: importance of statistical model and epistasis in complex traits. *Theor. Appl. Genet.* **119**, 1069–1083.

Haldane J. B. S. 1919 The combination of linkage values and the calculation of distances between the loci of linked factors. *J. Genet.* **8**, 299–309.

Hoeck J. A., Fehr W. R., Murphy P. A. and Welke G. A. 2000 Influence of genotype and environment on isoflavone contents of soybean. *Crop Sci.* **40**, 48–51.

Jung W., Yu O., Lau S. M., O'Keefe D. P., Odell J., Fader G. and McGonigle B. 2000 Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. *Nat. Biotechnol.* **18**, 208–212.

Kassem M. A., Meksem K., Iqbal M. J., Njiti V. N., Banz W. J., Winters T. A. *et al.* 2004 Definition of soybean genomic regions that control seed phytoestrogen amounts. *J. Biomed. Biotechnol.* **1**, 52–60.

Kassem M. A., Shultz J., Meksem K., Cho Y., Wood A., Iqbal M. J. and Lightfood D. A. 2006 An updated 'Essex' by 'Forrest' linkage map and first composite interval map of QTL underlying six soybean traits. *Theor. Appl. Genet.* **113**, 1015–1026.

Kosambi D. D. 1944 The estimation of map distances from recombination values. *Ann. Eugen.* **12**, 172–175.

Kudou S., Fleury Y., Welti D., Magnolato D., Uchida T., Keisuke K. and Okubo K. 1991 Malonyl isoflavone glycosides in soybean seeds (*Glycine max. Merrill*.) *Agric. Biol. Chem.* **55**, 2227–2233.

Lander E. S., Green P., Abrahamson J., Barlow A., Daly M. J., Lincoln S. E. and Newburg L. 1987 Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**, 174–181.

Latunde-Dada A. O., Cabello-Hurtado F., Czittrich N., Didierjean L., Schopfer C., Hertkorn N. *et al.* 2001 Flavonoid 6-hydroxylase from soybean (*Glycine max* L.), a novel plant *P-450* monooxygenase. *Biol. Chem.* **276**, 1688–1695.

Lee S. J., Yan W., Ahn J. K. and Chung I. M. 2003 Effects of year, site, genotype and their interactions on various soybean isoflavones. *Field Crops Res.* **81**, 181–192.

Liang H., Wang S., Yu Y., Lian Y., Wang T., Wei Y. *et al.* 2009 QTL mapping of isoflavone, oil and protein content in soybean. *Sci. Agric. Sin.* **42**, 2652–2660.

Lin S., Cianzio S. R. and Shoemaker R. C. 1997 Mapping genetic loci for iron deficiency chlorosis in soybean. *Mol. Breed.* **3**, 219–229.

Lo F. H., Mak N. K. and Leung K. N. 2007 Studies on the anti-tumor activities of the soy isoflavone daidzein on murine neuroblastoma cells. *Biomed. Pharmaeother.* **61**, 591–595.

Lozovaya V. V., Lygin A.V., Ulanov A. V., Nelson R. L., Dayde J. and Widholm J. M. 2005 Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. *Crop Sci* **45**, 1934–1940.

Matsumura H., Watanabe S., Harada K., Senda M., Akada S., Kawasaki S. *et al.* 2005 Molecular linkage mapping and phylogeny of the chalcone synthase multigene family in soybean. *Theor. Appl. Genet.* **110**, 1203–1209.

Meksem K., Njiti V. N., Banz W. J., Iqbal M. J., Kassem M. M., Hyten D. L. *et al.* 2001 Genomic regions that underlie soybean seed isoflavone content. *J. Biomed. Biotechnol.* **1**, 38–44.

Meng F., Han Y., Teng W., Li Y. and Li W. 2011 QTL underlying the resistance to soybean aphid (Aphis glycines Matsumura) through isoflavone-mediated antibiosis in soybean cultivar 'Zhongdou 27'. *Theor. Appl. Genet* **123**, 1459–1465.

Murphy S. E., Lee E. A., Woodrow L., Seguin P., Kumar J., Rajcan I. and Ablett G. R. 2009 Genotype × environment interaction and stability for isoflavone content in soybean. *Crop Sci.* **49**, 1313–1321.

Naim M., Gestetner B., Bondi A. and Birk Y. 1976 Antioxidative and antihemolytic activity of soybean isoflavone. *J. Agric. Food Chem.* **22**, 806–811.

Njiti V., Meksem K., Lightfood D. A., Banz W. J. and Winters T. A. 1999 Molecular markers of phytoestrogen content in soybeans. *J. Med. Food* **2**, 165–167.

Primomo V. S., Poysa V., Ablett G. R., Jackson C. J., Gijzen M. and Rajcan I. 2005 Mapping QTL for individual and total isoflavone content in soybean seeds. *Crop Sci.* **45**, 2454–2464.

Qi Z., Wu Q., Han X., Sun Y., Du X., Liu C. *et al.* 2011 Soybean oil content QTL mapping and integrating with meta-analysis method for mining genes. *Euphytica* **179**, 499–514.

Sangeeta D., Mark G., Pat M. and Mana F. 2007 Transcriptome analysis reveals a critical role of *CHS7* and *CHS8* genes for isoflavonoid synthesis in soybean seeds. *Plant Physiol.* **143**, 326–338.

Song Q. J., Marek L. F., Shoemaker R. C., Lark K. G., Concibido V. C., Delannay X. *et al.* 2004 A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* **109**, 122–128.

Song X., Han Y., Teng W., Sun G. and Li W. 2010 Identification of QTL underlying somatic embryogenesis capacity of immature embryos in soybean (*Glycine max (L.) Merr.*) *Plant Cell Rep.* **29**, 125–131.

Stracke R., Werber M. and Weisshaar B. 2001 The R2R3-MYB gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* **4**, 447–456.

Tikkanen M. J. and Adlerereutz H. 2000 Dietary soy-derived isoflavone phytoestrogens: Could they have a role in coronary heart disease prevention? *Biochem. Pharmacol.* **60**, l–5.

Trigizano R. N. and Caetano-Anolles G. 1998 Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. *Hort. Technol.* **8**, 413–423.

Tsukamoto C., Shimada S., Igita K., Kudou S., Kokubun M., Okubo K. and Kitamura K. 1995 Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agric. Food Chem.* **43**, 1184–1192.

Wang C., Sherrard M., Pagadala S., Wixon R. and Scott R. A. 2000 Isoflavone content among maturity group 0 to II soybeans. *J. Am. Oil Chem. Soc.* **77**, 483–487.

Wang H. and Murphy P. 1994 Isoflavone composition of American and Japanese soybeans in lowa: Efferents of variety, crop year, and location. *J. Agric. Food Chem.* **42**, 1674–1677.

Watanabe S., Uesugi S. and Kikuchi Y. 2002 Isoflavones for prevention of cancer, cardiovascular diseases, gynecological problems and possible immune potentiation. *Biomed Pharmacother* **56**, 302–312.

Weidenborner M., Hindorf H., Jha H. C., Tsotsonos P. and Egge H. 1990 Antifungal activity of isoflavones in different reduced stages on *Rhizoctonia solani* and *Sclerotium rolfsii*. *Phytochemistry* **29**, 801–803.

Yang K., Moon J. K., Jeong N., Back K., Kim H. M. and Jeong S. C. 2008 Genome structure in soybean revealed by a genomewide genetic map constructed from a single population. *Genomics* **9**, 52–59.

Yang K., Moon J. K., Jeong N., Chun H. K., Kang S. T., Back K. and Jeong S. C. 2011 Novel major quantitative trait loci regulating the content of isoflavone in soybean seeds. *Genes Genomics* **33**, 685–692.

Yu O., Shi J., Hession A. O., Maxwell C. A., Mcgonigle B. and Odell C. A. 2003 Metabolic engineering to increase isoflavone biosynthesis in soybean seed. *Phytochemistry* **63**, 753–763.

Yuan J., Njiti V. N., Meksem K., Iqbal M. J., Triwitayakorn K., Kassem M. A. *et al.* 2002 Quantitative trait loci in two soybean recombinant inbred line populations segregating for yield and disease resistance. *Crop Sci.* **42**, 271–277.

Zeng G., Li D., Han Y., Teng W., Wang J., Qiu L. and Li W. 2009 Identification of QTL underlying isoflavone contents in soybean

seeds among multiple environments. *Theor. Appl. Genet.* **118**, 1455–1463.

Zeng Z. B. 1993 Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proc. Natl. Acad. Sci. USA* **90**, 10972–10976.

Zeng Z. B. 1994 Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.

Zhang D., Cheng H., Wang H., Zhang H. Y., Liu C. Y. and Yu D. Y. 2010 Identification of genomic regions determining flower and pod numbers development in soybean (*Glycine max L.*) *J. Genet. Genomics* **37**, 545–556.

Zhang J., Ge Y., Sun J., Han F., Yu F., Yan S. and Yang H. 2012 Identification of QTL for isoflavone components among multiple environment in soybean seeds. *Sci. Agric. Sin* **45**, 3909–3920.

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