Research Communication

Analysis of Quantitative Trait Loci for Protein and Lipid Contents in Soybean Seeds Using Recombinant Inbred Lines

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Improvement of the quality and quantity of soybean seed constituents is one of the most important objectives in soybean breeding. Although the quality of seed constituents has been studied extensively, information on the quantity is still limited. In order to analyze the genetic basis of these traits, recombinant inbred lines (RILs) derived from a cross between *Glycine max* (L.) Merrill variety Misuzudaizu and variety Moshidou Gong 503 were planted in two environments and evaluated for seed protein and lipid contents. Protein and lipid contents were determined by NIR transmittance spectroscopy using an intact single seed. The broad sense heritability of the traits ranged from 0.73 to 0.79 in our RIL population. Single-factor analysis of variance, interval mapping and composite interval mapping were used to detect significant associations between the traits and genetic markers. A total of 17 QTLs, 10 for proteins and 7 for lipids, which were significant in at least one environment were identified. Each QTL explained the total phenotypic variation for protein and lipid contents in the range from 3.4% to 29.7% and 6.1% to 10.1%, respectively. Among all the detected QTLs, three for the protein content and three for the lipid content were detected in both environments. The negative correlation between protein and lipid contents was also confirmed. Epistatic interactions were detected in this study, as another source of genetic variation in our population. The results obtained in our study may serve as a base for analyzing the genetic control of protein and lipid contents and may eventually enable to change the seed constituents.

Key Words: *Glycine max* (L.) Merrill, quantitative trait loci, protein, lipid, recombinant inbred lines, epistatic interaction.

Introduction

In soybean breeding, in addition to increasing seed yield, improvement of the quality and quantity of soybean seed constituents is important. The genes encoding each of the major subunit types of soybean seed storage protein, glycinin and β-conglycinin, have been isolated and characterized (Nielsen 1996). Similarly, many genes for fatty acid synthesis have already been identified and isolated (Yadav 1996). Moreover, mutant lines which can be used as breeding materials and research tools for studying the regulation of the subunit composition of glycinin and β-conglycinin as well as lipid biosynthesis, have been identified (Nielsen 1996).

Based on these findings, some successful attempts have been made to improve the protein and fatty acid composition in soybean seeds. Nevertheless, the information about the genetic control of the protein and lipid contents in soybean seeds is limited. The typical negative relationship between seed protein and lipid contents has remained unsolved and limited the success achieved. It has been suggested that the flux of carbon into carbohydrates, proteins and lipids is tightly regulated in the developing seed (Nielsen 1996). The synthesis of malonyl-CoA, the first step of fatty acid synthesis, is catalyzed by acetyl-CoA carboxylase (ACCase). In soybean, plastidic ACCase plays an important role in the accumulation of lipids in developing seeds (Kozaki 1999). Plastidic ACCase is composed of three functional components: biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC) and a carboxyltransferase (CT) complex consisting of two pairs of α and β subunits. Phosphoenolpyruvate carboxylase (PEPCase) catalyzes the carboxylation of phosphoenolpyruvate to oxalacetic acid which increases the number of carbon skeletons of amino acids. It was reported that the PEPCase activity of soybean seeds is positively correlated with the seed protein content and negatively correlated with the lipid content in seeds (Sugimoto et al. 1989). A better understanding of the genetic and regulatory functions of ACCase and PEPCase in the control of the relative amounts of proteins and lipids in seeds may enable to manipulate the negative correlation between these constituents.

Molecular markers have been used for mapping the
genes controlling the seed protein and lipid contents in soybean. Diers et al. (1992) studied the soybean seed protein and oil contents in a population of F\textsubscript{2}-derived lines developed in crosses between G. max and G. soja. Quantitative trait loci (QTLs) were clustered on two linkage groups. In an intraspecific cross of two soybean cultivars ‘Minsoy’ and ‘Noir 1’, Mansur et al. (1993) reported the existence of two regions associated with seed oil and an unlinked marker to seed protein. Mansur et al. (1996) continued mapping using recombinant inbred lines derived from the same cross combination and found that both protein and oil contents were controlled by QTLs linked to the same RFLP markers, namely T155 and A239. Meanwhile, using two different environments and soybean populations, Lee et al. (1996) identified QTLs associated with seed protein and oil contents which were significantly detected across several locations and were population-specific. A similar study was performed by Brummer et al. (1997). Instead of two, they used eight soybean populations to evaluate QTLs across different genetic backgrounds and different environments. Sebolt et al. (2000) evaluated the two G. soja QTL alleles in the G. max background. They identified markers linked to the QTL allele from G. soja on linkage group I that was significantly associated with higher protein and lower oil concentrations than the G. max alleles. Using a G. max population derived from a cross of two early maturing varieties, QTLs associated with seed protein and oil contents showed environmental interactions (Csanadi et al. 2001).

In the present study, QTLs for protein and lipid contents in soybean seeds were identified based on Near Infrared (NIR) transmittance spectroscopy by using recombinant inbred lines (RILs) planted in two environments, and scored with RFLP and microsatellite (SSR) markers, including the pea clones of ACCase, the rice cDNA and soybean clones of PEPCase.

Materials and Methods

Plant material and phenotypic evaluation of seed composition

A population of recombinant inbred lines (RILs) was derived from a cross between two contrasting phenotypes of Glycine max (L.) Merrill for protein and lipid contents in the seed. Misuzuzaizu was a low-content variety, while Moshidou Gong 503 was a high-content variety for both constituents. A total of 156 RILs from the F\textsubscript{2} generation of selfing were planted in the field of Matsudo campus, Chiba University, Japan, during the summer of 1998 to obtain F\textsubscript{8} seeds (Matsudo trial). In the following year (summer 1999), approximately 15 seeds from each line were collected and planted in the field of Kashiwa farm (about 10 km northeast of Matsudo campus) to produce F\textsubscript{8} seeds (Kashiwa trial).

Ten seeds from each line were collected randomly for protein and lipid content measurements. The measurements were performed on intact single seeds nondestructively at the Nondestructive Evaluation Laboratory (National Food Research Institute, Tsukuba, Japan) using NIRSystems 6500 (Foss NIRSystems, Silver Spring, MD, USA) (Tajuddin et al. 2002). The spectral data were generated at 2-nm intervals in the wavelength region from 700 nm to 1100 nm, with 50 scans per sample. The original data of the seed protein and lipid concentrations from each line in the Matsudo and Kashiwa trials were used for statistical analysis and QTL detection.

Linkage map construction

Genomic DNA was extracted from the leaf tissues of each selected individual according to the CTAB method (Murray and Thompson 1980). The DNA was digested with eight restriction enzymes, Apat, BamHI, BglII, DraI, EcoRI, EcoRV, HindIII and KpnI. Electrophoresis, Southern blotting and hybridization procedures were performed as previously described by Yamanaka et al. (2000).

SSR markers were developed by USDA (Cregan et al. 1999), Dupont Corporation and Chiba University (Hossain et al. 2000). PCR reaction mixtures contained 30 ng of soybean genomic DNA, 200 μM of each dNTP, 0.1 unit of Hotstar Taq (Qiagen, Hilden, Germany), 3.0 μl of forward and reverse primers and 1.0 μl of Qiagen 10× PCR buffer. PCR amplifications were conducted using a MJ Research model PTC-100™ thermocycler (MJ Research, Inc., Watertown, USA). The PCR was performed under the following conditions: 15 min at 95°C, then 33 cycles of 1 min 92°C, 1 min at 48°C, and 1 min at 72°C. PCR products (3.0 μl/lane) were separated on 10% polyacrylamide gel, and then polymorphisms were detected after ethidium bromide staining.

The cDNA clones of pea, BC, BCCP and α-CT, were supplied by Dr. Y. Nagano, Nagoya University, and cDNA of rice PEPCase clone, osppcr, was supplied by Dr. T. Sugimoto, Kobe University. The clones were hybridized to genomic DNA by the same procedures as those described previously (Yamanaka et al. 2000) to detect RFLP. The oligonucleotide primer pairs for amplification of the PEPCase gene were obtained from the sequences information of a conserved region published by Sugimoto et al. 1992. The primers were 5′-GTCATCATTATTTGCGGGGCTGTG-3′ (forward) and 5′-TCCACTAGGCTTTCTCTTTGCTGG-3′ (reverse). Cycle of denaturation at 95°C for 30 sec, primer annealing at 62°C for 2 min, and extension by Ex-Taq (TaKaRa) at 72°C for 1 min were repeated for a total of 25 cycles. The products of PCR were then cleaved with the restriction enzymes Alul, Asnl, AvalI, BamHI, BglII, BsmI, BsrRI, Ddel, DraI, EcoRI, EcoRV, HaeIII, HhaI, Hinfl, HindIII, HinfI, HpaII, MboII, Mspl, MvaI, Rsal, ScrFI, TaqI, XbaI and XhoI to produce cleaved amplified polymorphic sequences.

From the F\textsubscript{2} linkage map (Yamanaka et al. 2001), which was derived from the same cross combination as the RILs in this study, RFLP and SSR markers were selected to be evenly distributed to all the linkage groups. The genotypes of these markers were scored against 156 progenies of F\textsubscript{8} generation to construct a RIL linkage map (Watanabe et
The linkage map was constructed by using the computer program MAPMAKER/EXP 3.0 (Lincoln et al. 1993) at a minimum given log of likelihood (LOD)-value of 3.0 as the threshold to assign RFLP and SSR loci to linkage groups.

**QTL analysis**

The presence of QTLs and their effects were identified by three methods; single-factor analysis of variance (ANOVA), interval mapping (Lander and Botstein 1989) and composite interval mapping, as a combination of interval mapping and multiple regression (Zeng 1993). Both interval mapping and composite interval mapping methods were applied using QTL Cartographer version 1.15 (Basten et al. 2001). A minimum LOD-value of 2.0 was chosen to confirm the presence of a QTL in a given genomic region. The LOD-value peak was used to estimate the most likely QTL position on the RFLP linkage map. In addition, two-factor analysis of variance and EPISTAT program (Lark et al. 1995) were used to detect epistatic interaction between all pairs of QTLs, and QTLs with other marker loci.

**Results and Discussion**

**Variation in seed constituents in RILs**

Seed protein and lipid contents in the Matsudo and Kashiwa trials were distributed continuously (Fig. 1), indicating that these two traits were quantitative traits. The frequency distribution of both traits in the Matsudo and Kashiwa trials fitted to a normal distribution (p < 0.05) when tested with goodness of fit (Gomez and Gomez 1984). For the lipid content, transgressive segregants indicating that at least one of the parents had the alleles for opposite effects on the lipid content were observed. On the other hand, transgressive segregants for the protein content were only observed for a higher value in either environment.

Range, mean, standard deviation and parental values for protein and lipid contents determined in the Matsudo and Kashiwa trials are presented in Table 1. The two parental lines were significantly different (p < 0.001) for both constituents in both environments. Protein and lipid contents were higher in Moshidou Gong 503 than in Misuzudaizu in both environments. Protein content in the RIL population ranged from 36.55% to 49.54% in the Matsudo trial and from 37.44% to 49.94% in the Kashiwa trial. The mean protein contents for the Matsudo and Kashiwa trials were 43.55% and 43.84%, respectively.
respectively. The lipid content in the RIL population ranged from 18.33% to 26.49% in the Matsudo trial and from 17.13% to 25.56% in the Kashiwa trial. The mean lipid contents for the Matsudo and Kashiwa trials were 22.54% and 21.27%, respectively.

The contents of protein and lipid were highly heritable, with broad-sense heritability for the protein and lipid contents being 0.79 and 0.78 in the Matsudo trial, and 0.74 and 0.73 in the Kashiwa trial, respectively.

As expected, the seed protein content showed a negative, but low genetic correlation with the seed lipid content (−0.27 in Matsudo trial and −0.29 in Kashiwa trial), which is in agreement with the general findings that the protein content is negatively correlated with the lipid content in soybean seed. Moreover, correlations between both trials were higher for the protein content (0.81) than for the lipid content (0.60), suggesting that the lipid content was more easily affected by the environment compared to the protein content.

**Detection and localization of QTLs**

Based on the RIL F₈ generation, a genetic linkage map was constructed consisting of 300 RFLP and SSR markers, including 5 phenotypic markers (Watanabe et al. 2002). The map covered a distance of 2,472.7 cM of the soybean genome comprising 22 linkage groups and corresponded well, with most of the markers having the same order, to previously published soybean maps (the USDA/Iowa State University integrated linkage map, Cregan et al. 1999). The average distance between two adjacent marker loci was 9.22 cM.

### QTLs for protein content

Detection and localization of QTLs for the protein content using single-factor ANOVA and interval mapping identified 6 QTLs that were significant in at least one environment. These QTLs were located near the markers Satt239 on linkage group (LG) 23, Satt301–Satt310 on LG19, Satt384 on LG4, I on LG1-2, Satt281 and A703a on LG3. Detection using composite interval mapping confirmed the presence of QTLs around the markers Satt250 on LG17 at a LOD-value of 2.20 and it explained 6.3% of the phenotypic variation (Fig. 2). The QTLs for protein content individually explained about 6.1% to 10.1% of the total phenotypic variation (Table 1). Using this method, an additional QTL associated with the seed lipid content was identified in the Matsudo trial. This QTL, designated as LIP7, was located near the marker Satt250 on LG17 at a LOD-value of 2.20 and it explained 6.3% of the phenotypic variation (Fig. 2). The total variations accounted for 39.3% in the Matsudo trial and 38.7% in the Kashiwa trial. Based on composite interval mapping, the region near the marker Satt071 on LG12 and the region between Satt281–A063 on LG3. QTL analysis using composite interval mapping confirmed the presence of QTLs near the markers A-A111 on LG1-2, A519 on LG2-2, Satt127–Satt239 on LG23, K384 on LG7, Satt308 on LG17, GM214b on LG15 + 25, Satt071 on LG12 and in the region between Satt281–A063 on LG3. QTL analysis using composite interval mapping confirmed the presence of QTLs near the markers A-A111 on LG1-2 (designated as LIP1), A519 on LG2-2 (LIP2), Satt127–Satt239 on LG23 (LIP3), K384 on LG7 (LIP4), Satt308 on LG17 (LIP5) and GM214b on LG15 + 25 (LIP6) (Fig. 2). The QTLs for the lipid content individually explained about 6.1% to 10.1% of the total phenotypic variation (Table 3). Using this method, an additional QTL associated with the seed lipid content was identified in the Matsudo trial. This QTL, designated as LIP7, was located near the marker Satt250 on LG17 at a LOD-value of 2.20 and it explained 6.3% of the phenotypic variation (Fig. 2). The total variations accounted for 39.3% in the Matsudo trial and 38.7% in the Kashiwa trial. Based on composite interval mapping, the region near the marker Satt071 on LG12 and the region between Satt281–A063 on LG3 did not show any significant association with the seed lipid content.

### QTLs detected in both environments

Among the QTLs for the protein content, only three QTLs, PRO1 on LG23, PRO2 on LG19 and PRO3 on LG4 showed a strong association in both Matsudo and Kashiwa trials (Table 2 and Fig. 2). The PRO1 accounted for the largest phenotypic variation, i.e., more than 20% and the additive effect of Moshidou Gong 503 in contrast to the Misuzudaizu.
Analysis of QTLs for protein and lipid contents

among the QTLs associated with the lipid content, three QTLs, *LIP1*, *LIP2* and *LIP3*, were significantly associated in both Matsudo and Kashiwa trials (Table 3 and Fig. 2). The *LIP1* QTL (near the marker *I-A111* on LG1-2) accounted for most of the phenotypic variation, i.e. around 10% and showed an additive effect of −0.55 and −0.43% in the Matsudo and Kashiwa trials, respectively (Table 3).

As shown in this study, some QTLs were easily

Table 2. QTLs for protein content based on composite interval mapping

<table>
<thead>
<tr>
<th>QTL</th>
<th>LG</th>
<th>Marker</th>
<th>Trial</th>
<th>Distance (cM)</th>
<th>LOD</th>
<th>Variance explained (%)</th>
<th>Additive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRO1</strong></td>
<td>LG23</td>
<td>Satt239</td>
<td>M</td>
<td>1.0</td>
<td>9.27</td>
<td>20.9</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td>0.0</td>
<td>15.97</td>
<td>29.7</td>
<td>1.22</td>
</tr>
<tr>
<td><strong>PRO2</strong></td>
<td>LG19</td>
<td>Satt310</td>
<td>M</td>
<td>2.0</td>
<td>3.24</td>
<td>7.4</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Satt501</td>
<td>4.0</td>
<td>3.08</td>
<td>5.5</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>PRO3</strong></td>
<td>LG4</td>
<td>Satt384</td>
<td>M</td>
<td>0.0</td>
<td>3.56</td>
<td>6.8</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td>2.0</td>
<td>4.56</td>
<td>8.2</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>PRO4</strong></td>
<td>LG1-2</td>
<td><em>I</em></td>
<td>M</td>
<td>0.0</td>
<td>2.59</td>
<td>4.9</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>PRO5</strong></td>
<td>LG3</td>
<td>Satt281</td>
<td>K</td>
<td>2.0</td>
<td>3.71</td>
<td>6.2</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>PRO6</strong></td>
<td>LG1-2</td>
<td>A104</td>
<td>M</td>
<td>2.4</td>
<td>3.31</td>
<td>7.5</td>
<td>−0.67</td>
</tr>
<tr>
<td><strong>PRO7</strong></td>
<td>LG9</td>
<td>Satt156</td>
<td>M</td>
<td>4.1</td>
<td>2.19</td>
<td>5.6</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>PRO8</strong></td>
<td>LG8</td>
<td>GM195</td>
<td>K</td>
<td>9.0</td>
<td>2.94</td>
<td>8.4</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>PRO9</strong></td>
<td>LG10 + 26a</td>
<td>Q026</td>
<td>K</td>
<td>6.0</td>
<td>2.11</td>
<td>4.2</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>PRO10</strong></td>
<td>LG5</td>
<td>A378</td>
<td>K</td>
<td>2.4</td>
<td>2.12</td>
<td>3.4</td>
<td>0.41</td>
</tr>
</tbody>
</table>

1) The environments in which QTLs were detected are indicated (M: Matsudo trial in 1998; K: Kashiwa trial in 1999).
2) Distance to the nearest marker.
3) Moshidou Gong 503 effect in contrast to Misuzudaizu.
influenced by the environment. The same phenomenon was observed in previous studies (Lee et al. 1996, Brummer et al. 1997). With respect to the environmental effect, Brummer et al. (1997) differentiated two types of QTLs identified as follows: environmentally stable QTLs, which were detected in both environments, and environmentally sensitive QTLs, which were found in only one environment. The environmentally stable QTLs may be suitable for marker-assisted strategies for breeding programs.

Table 2 and Table 3 show that the region of the markers Satt127–Satt239 on LG23 was associated with higher protein and lower lipid concentrations. These findings are consistent with the negative phenotypic correlation of −0.27 recorded in the Matsudo trial and −0.29 in the Kashiwa trial between these traits in the RIL population. The negative correlation between the protein and lipid contents that was verified by molecular markers was also reported by other researchers (Lark et al. 1994, Sebolt et al. 2000, Csanadi et al. 2001).

An interesting phenomenon was observed on LG23. The QTL LIP3, which was identified in both Matsudo and Kashiwa trials, was located in the same region as that of the QTL PRO1. The LOD-value profile showed the same association pattern for the seed protein and lipid contents in the region of the markers GM222b and GM126, indicating the presence of a close linkage between the two loci (Fig. 3). It is likely that these markers were associated with the variations controlled by the same gene or two different genes closely located. A higher density of markers, especially in the region on LG23, would be necessary to determine whether these protein and lipid QTLs represent the same gene or different genes which are closely linked.

Since the QTLs in this study displayed a small proportion of variation, the presence of these QTLs should be confirmed using nearly isogenic lines in each region. We are currently developing nearly isogenic lines in the region of the markers Satt127–Satt239 of LG23 to reveal the gene action around this region. This information may contribute to the analysis of the genetic control of the protein and lipid contents in soybean seeds and to map-based cloning of responsible genes, which might enable to change the seed constituents.

**Relationship between QTLs and loci of ACCase and PEPCase**

Pea cDNA clones (BC, BCCP and α-CT) were obtained by reverse transcription polymerase chain reaction (PCR) using RNA from pea seedlings as templates (Kozaki et al. 2000). These cDNAs, as well as the rice cDNA clone, were hybridized to the soybean genomic DNA blots and polymorphic loci were mapped using the enzymes EcoRI (for BC and α-CT) and EcoRV (for BCCP and osppcr). The BC locus was mapped at a distance of 0.2 cm from GM254 on LG1-1 (A1). The BCCP locus was located at a distance of 2.7 cm from K390a on LG8, while the α-CT and osppcr loci were located at a distance of 3.3 cm from Satt199 on LG5.
and 7.1 cM from A748 on LG3, respectively. None of these cDNAs were located near our QTLs.

Using the Southern hybridization method, a soybean PEPCase clone (GmPepc7) had been mapped on LG6 (H) using the F2 population (Yamanaka et al. 2001). Since the PEPCase genes in soybean display a high homology in their coding region to each other (Hata et al. 1998), the PEPCase cloning method may occur at multiple loci in the genetic map. In this study, attempts were made to map the soybean PEPCase clone using the cleaved amplified polymorphic sequence method. Among all the tested restriction enzymes, all of these three enzymes produced identical genotypes in RILs. The mapping results showed that the PEPCase clone in this study was located at a single locus that was the same as that previously mapped on LG6 (H) by Yamanaka et al. (2001). However, the PEPCase locus was not associated with either trait in the present study. It appears that the primers used to obtain the PEPCase clone corresponded to GmPepe7.

Comparison with previous results

Comparison of the mapping results for the protein and lipid contents in this study with the results reported previously showed some agreement. Out of 17 QTLs detected in this study, four were located in similar regions to those in other studies. In LG1–2, the region between the markers I–A111 was significantly associated with the protein (PRO4) and lipid (LIP1) contents. In the same region on the linkage group (A2 in the USDA/Iowa St. Univ. map), Mansur et al. (1993) also detected a strong QTL for the seed lipid content with 36% of the variance explained.

The marker Satt127 on LG23 was strongly associated with the lipid content (LIP3). Brummer et al. (1997) who studied eight intraspecific soybean populations over three years, identified the marker A144 on the same linkage group (I in the USDA/Iowa St. Univ. map) that showed a high correlation with the protein content, with 27.5% of the variance explained for the 3-year average. Marker A144 was located at a distance of 2.7 cM from Satt127 on the USDA/Iowa St. Univ. map and between Satt127 and Satt239 (Cregan et al. 1999). Moreover, Sebolt et al. (2000) observed that the marker Satt127 was significantly associated with the protein concentration, seed yield, maturity date, and plant height in backcross populations with introgressed G. soja QTL alleles into the G. max background. Still in the same region, Diers et al. (1992) located QTLs for protein, oil and seed yield and suggested that this region may be specific to G. soja.

Additionally, Diers et al. (1992) detected QTLs for the protein and oil contents on LG4 (E in the USDA/Iowa St. Univ. map, previously A) in the same region as that of PRO3 in this study. Among the eight markers significantly associated with seed-weight that were identified by Maughan et al. (1996), one marker, K384, on linkage group J was strongly associated with the lipid content (LIP4 on LG7) in the present study.

Epistatic interaction among QTLs and markers

Since the magnitude of the QTLs detected was low and accounted for a small proportion of the variation, two-factor ANOVA and EPISTAT were performed to reveal epistasis. Epistatic interactions were tested between all the pairs of QTLs and with other marker loci that did not have significant effects on the traits (non-effect loci). In total, 6,300 combinations were assayed and 140 combinations showed a significant interaction covering all the linkage groups. In addition, 83 of these combinations (60%) were detected for the lipid content alone. Two kinds of interaction, namely in one environment and in both environments were identified.

The epistatic interaction between the QTLs and the non-effect loci that were detected in both environments only are listed in Table 4. There were significant interactions among the QTLs in the Matsudo and Kashiwa trials between PRO1 on LG23 and PRO5 on LG3 (p < 0.035). Furthermore, epistatic interaction among protein QTLs in one environment only was detected between PRO1 and PRO2 on LG19 (p < 0.021). QTL for the lipid content LIP1 on LG1-2 showed a significant interaction with LIP4 on LG 7 (p < 0.05) in the Matsudo trial and with LIP6 on LG 15 + 25 (p < 0.038) in the Kashiwa trial. These results show that epistatic

Table 4. Interaction among QTLs and, QTLs and the non-effect loci detected in both environments

<table>
<thead>
<tr>
<th>Locus 1</th>
<th>Locus 2</th>
<th>F-test(1)</th>
<th>MC-test(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTL</td>
<td>LG</td>
<td>Marker</td>
<td>LG</td>
</tr>
<tr>
<td>PRO1</td>
<td>23</td>
<td>PRO5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Satt173</td>
<td>15+25</td>
</tr>
<tr>
<td>PRO2</td>
<td>19</td>
<td>A715</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A681</td>
<td>5</td>
</tr>
<tr>
<td>LIP1</td>
<td>1-2</td>
<td>A593</td>
<td>11+14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A516</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A234</td>
<td></td>
</tr>
<tr>
<td>LIP2</td>
<td>2-2</td>
<td>GM260</td>
<td>17</td>
</tr>
</tbody>
</table>

1) F-test for the four subgroups of the two marker alleles.
2) Monte Carlo simulation using EPISTAT program (Lark et al. 1995).
*: p < 0.05, **: p < 0.01
interaction between genes is another source of genetic variation in this RIL population.

Acknowledgements

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