Identification of Quantitative Trait Loci Underlying Plant Height and Seed Weight in Soybean

Yu-lin Liu, Ying-hui Li, Jochen C. Reif, Michael F. Mette, Zhang-xiong Liu, Bo Liu, Shan-shan Zhang, Long Yan, Ru-zhen Chang, and Li-juan Qiu*

Abstract
For clarifying the genetic base of the variation of yield-related traits in soybean [Glycine max (L.) Merr.], we mapped quantitative trait loci (QTLs) for plant height and seed weight using a recombinant inbred line population derived from a cross between Chinese elite line Zhongpin03-5373 and cultivar Zhonghuang13. We detected 11 QTLs for plant height and 18 QTLs for seed weight across six diverse environments. These included three pairs of plant height- vs. seed weight-related QTLs located in close proximity to each other, with two pairs, qPH-7 vs. qSW-7-2 and qPH-19-2 vs. qSW-19, sharing the same direction of additive effects. Individual QTLs explained 2.02 to 47.60% of the variation in plant height and 2.13 to 14.35% in seed weight. Two and five of the major QTLs discovered for plant height and seed weight, respectively, that were stable across environments in our study have been reported previously. Among them, four QTLs, qPH-13, qSW-11, qSW-12-2, and qSW-18, were not involved in digenic epistatic interaction in our biparental population, indicating that these QTLs will be useful for marker-assisted selection and should be targeted for the future identification of candidate genes. Moreover, eight QTLs for both plant height and seed weight were newly identified in our population.

SOYBEAN is one of the most important sources of oil and plant protein worldwide and has a high nutritive value (Wilcox, 2004). To keep pace with the increasing global demand for soybean products, grain yield of soybean has to be improved substantially (Rosegrant et al., 2001). Grain yield is a complex trait and prone to substantial genotype × environment interactions. Plant height and seed weight largely influence the harvest index and are therefore two of the main yield-related traits in soybean (Wang et al., 2004; Wilcox and Sediyama, 1981).

Marker-assisted selection (MAS) has been advocated as an important tool in breeding to enhance the selection gain per time unit (Allen, 1994). An important prerequisite for MAS is to identify quantitative trait loci (QTLs) associated with the target trait, including the estimation of main and epistatic effects as well as of QTLs × environment interaction effects (Orf et al., 1999). Several QTL mapping studies have been conducted to dissect the genetic architecture of plant height and seed weight in soybean. Until now, more than 130 QTLs and 120 QTLs have been identified on all chromosomes

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Abbreviations: CP09, environment in Changpin in 2009; CP10, environment in Changpin in 2010; CP11, environment in Changpin in 2011; EI, epistatic interaction; ICS-CAAS, Institute of Crop Science, Chinese Academy of Agricultural Sciences; LOD, logarithm of the odds; MAS, marker-assisted selection; PH, plant height; QTL, quantitative trait locus; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; SYa09, environment in Sanya in 2009; SYa10, environment in Sanya in 2010; SYi10, environment in Shunyi in 2010; ZH, Zhonghuang13; ZP, Zhongpin03-5373.
except Gm14 for plant height and Gm01 for seed weight, respectively (Grant et al., 2010).

The presence of epistasis severely impacts the power to map QTLs and also the accuracy to predict the phenotypic performance in MAS. For instance, Wang et al. (1999) have shown that the precision of QTL mapping would be greatly enhanced if epistatic interactions were considered in the QTL mapping model (Wang et al., 1999). Presence of epistasis for plant height has been previously reported in a pioneering study from Lark et al. (1995) based on a population of 224 recombinant inbred lines (RILs). In contrast, epistasis was not detected for plant height in a soybean population consisting of 111 F2-derived lines (Lee et al., 1996). Most of other previous QTL studies on plant height and seed weight ignored epistasis and focused exclusively on mapping QTLs with significant main effects.

Plant height and seed weight are also impacted by interactions of genotype × environment, which could severely impact the power of QTL detection. Moreover, knowledge on specific QTL × environment interactions can guide the search for varieties adapted to particular environments. To the best of our knowledge, however, QTL × environment interactions have not been studied for plant height and seed weight in soybean so far.

Our current study is based on a biparental soybean population comprising 254 RILs, which has been evaluated for plant height and seed weight in multilocation field trials and genotyped with 508 molecular markers. We mapped specific and overlapping QTLs controlling plant height and seed weight across multiple environments based on a genetic linkage map and evaluated the contributions of epistasis effects to plant height and seed weight variance. The identified molecular markers with linkage to QTLs possess potential for application in MAS for soybean plant height.

Materials and Methods

Mapping Population

The initial cross was made between Zhongpin03-5373 (ZP) (developed by the Institute of Crop Science, Chinese Academy of Agricultural Sciences [ICS-CAAS]) as female parent and Zhonghuang13 (ZH) (provides by Prof. Lianzheng Wang, ICS-CAAS, Beijing, China) as male parent and a total of 254 F1 plants derived from the cross were produced in 2007. The derived lines from F2 plants were propagated by selfing in bulk from F2 to F4 and then in single-seed descent from F4 to F8. Genomic DNA was extracted from young leaves of 10 seedlings per F4 line using the DNAquick Plant System (Tiangen Biotech [Beijing] Co., Ltd.).

Trait Evaluation and Analysis

We evaluated F5-derived families in six environments at three experimental stations of the Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), including at Changping (40.2° N, 116.2° E) in Beijing in 2009 (CP09), 2010 (CP10), and 2011 (CP11), in Sanya (18.2° N, 109.5° E) in Hainan Province in 2009 (SYa09) and in 2010 (SYa10), and at Shunyi (40.1° N, 116.7° E) in Beijing in 2010 (SYI10). The experiments were conducted from mid June to mid October in Beijing and from early January to early March in Hainan Province. The 254 RILs and their parents were planted in a completely randomized design with three replications. Each plot comprised a row with 0.55 m width and 1.5 m length with a space between two plants of 0.10 m.

Ten individuals in the middle of each row were randomly selected and plant height (in cm) and 100-seed weight (in g) were measured. Plant height was defined as the length between the cotyledon node and the peak of the main stem. The measurement was averaged over the 10 individuals. After drying, the seeds from each individual plot were collected together in bulk and random samples of three times 100 seeds were selected to measure seed weight. The measurement was averaged across the three replications.

The variation parameters of plant height and seed weight, such as partial, kurtosis, and standard deviation, were estimated using software IciMapping V3.1 (Li et al., 2008b). Heritability for plant height and also seed weight in six environments respectively were estimated as follows: $H^2 = S_G/(S_G + SG*E/n + S_e/n \times r)$, in which $H^2$ is the broad-sense heritability, $S_G$ is the genotypic variance, $S_G*E$ is the variance of genotype × environment, $S_e$ is the error, $n$ is the number of genotypes, and $r$ is the number of replicates (Hanson et al., 1956).

Molecular Markers and Quantitative Trait Locus Mapping

A total of 508 molecular markers, including 313 single nucleotide polymorphisms (SNPs), 167 simple sequence repeats (SSRs), four expressed sequence tag-SSRs, and 24 indels (Supplemental Table S1) were used in this study. The marker selection and linkage map construction had been described in our companion study (Y.H. Li and L.J. Qiu, unpublished data, 2013). Inclusive composite interval mapping (Li et al., 2007) implemented in QTL IciMapping V3.1 (Li et al., 2008b) was used to detect putative QTLs in each environment. We used an additive genetic model. The walk speed was set to 1 cM and the probability for markers entering into the model in stepwise regression was set to 0.001. Logarithm of the odds (LOD) score peaks higher than 2.5 were used to declare the presence of a putative QTL in a given genomic region. The QTLs detected in all individual environments were named common QTLs while those detected only in subsets of environments were denoted as environment-specific QTLs. We defined QTLs as coincident when the presence of a putative QTL in a genomic region. The functionalities of mapping of additive and digenic epistasis genes from multi-environmental trials of QTL IciMapping V3.1 (Li et al., 2008b) was used to detect QTLs × environment interactions, with a LOD score threshold of 2.5 set to identify a putative QTL.
Moreover, the functionalities of mapping of additive and digenic epistasis genes in biparental populations were used to detect epistatic interactions between markers with significant associations with LOD < 4.0. We followed the nomenclature suggested by McCouch et al. (1997) to name the QTLs detected in our study by adding a serial number separated by a dash after the chromosomal number if there were more than one QTL on a chromosome (McCouch et al., 1997).

Results

Phenotypic Evaluations

Plant height and seed weight were surveyed in a RIL population derived from ZP × ZH in six environments. The two parental lines significantly differed with respect to their plant height and seed weight. Parent ZH consistently was shorter and possessed higher seed weight compared to parent ZP (Supplemental Table S2). Both plant height and seed weight showed continuous normal distributions (Fig. 1), and their skewness and kurtosis values were less than 1.0 for the RIL populations in all environments, suggesting a quantitative inheritance pattern. The heritabilities ($H^2$) estimated across the six environments were 55.4 and 53.2% for plant height and seed weight, respectively.

Polymorphic Marker Selection and Genetic Map Construction

The genetic map constructed from our data comprised 508 molecular markers (Supplemental Table S3) and had a total length of 2635.63 cM with an average marker spacing of 5.4 cM (Fig. 2). All loci were assigned to 20 soybean chromosomes by aligning the included markers to the soybean reference genome (Glyma1.01) and the integrated soybean genetic map (Consensus Map 4.0) (Hyten et al., 2010). A total of 21 gaps larger than 20 cM in length were present on 17 chromosomes, except for Gm17, Gm18, and Gm07. Of these, only two gaps, located...
Figure 2. Soybean genetic linkage map with locations of quantitative trait loci (QTLs) detected for plant height and seed weight. Black and blank bars indicated QTL for plant height and seed weight, respectively.
Quantitative Trait Loci Identified for Plant Height

The genomewide scan revealed the presence of 11 putative QTLs for plant height located on nine chromosomes (Table 1; Fig. 2 and 3A). One out of the eleven QTLs, \( qPH-13 \), was common to all six environments while 10 popped up only in a subset of environments. Among these environment-specific QTLs, six, \( qPH-6 \), \( qPH-8 \), \( qPH-12 \), \( qPH-16 \), \( qPH-17-1 \), and \( qPH-19-1 \), were detected only in one out of six environments while four, \( qPH-7 \), \( qPH-14 \), \( qPH-17-2 \), and \( qPH-19-2 \), were reproducible in two to five environments. In accordance with the single location analyses, we detected in the screen across locations three QTLs with significant environment interactions (Table 1).

Individual QTLs explained 2.02 to 47.60% of the phenotypic variation of plant height. We identified two major QTLs explaining average proportions of more than 10% of the phenotypic variation. One of these QTLs, \( qPH-13 \), had previously been described (Josie et al., 2007) and explained between 28.43 to 47.60% of the phenotypic variation for plant height in the individual environments, suggesting that it is a major and stable QTL for plant height and will be a great value for MAS.

The flanking markers of \( qPH-13 \), Map-2496 and satt554, are located on Gm13 and span an interval amounting to 0.85 Mb according to the soybean reference genome (Glyma1.0). In the four high latitude environments (CP09, SYi10, CP10, and CP11), \( qPH-13 \) had the largest LOD scores (30.92, 31.21, 39.78, and 44.04) (Fig. 3; Table 1) and explained on average 40.86% of the plant height variation. In low latitude environments (SYa09 and SYa10), QTL \( qPH-13 \) explained only 28.43 and 28.74% of the plant height variation. Consequently, our results indicated that QTL \( qPH-13 \) has to be considered in MAS differentially for low and high latitude environments.

Table 1. Quantitative trait loci (QTLs) associated with plant height and their additive and environment interaction effects detected by a logarithm of the odds (LOD) threshold of 2.5 in a recombinant inbred line population across six environments.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chromosome</th>
<th>Genetic position (cM)</th>
<th>Marker internal</th>
<th>Physical position (bp) of markers</th>
<th>Internal genome length of markers (Mb)</th>
<th>Single environment</th>
<th>A × E² (LOD)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qPH-6</td>
<td>Gm06</td>
<td>31</td>
<td>Map-0990 and satt281</td>
<td>4,366,947–6,524,146</td>
<td>2.16</td>
<td>CP11: 3.15</td>
<td>2.88</td>
</tr>
<tr>
<td>qPH-7</td>
<td>Gm07</td>
<td>80</td>
<td>satt175 and Map-1315</td>
<td>15,307,093–16,467,274</td>
<td>1.16</td>
<td>SYa09: 3.31</td>
<td>1.90</td>
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<tr>
<td>qPH-8</td>
<td>Gm08</td>
<td>33</td>
<td>Map-1496 and Map-1501</td>
<td>14,645,679–15,204,729</td>
<td>0.56</td>
<td>CP11: 2.85</td>
<td>1.90</td>
</tr>
<tr>
<td>qPH-12</td>
<td>Gm12</td>
<td>98</td>
<td>satt253 and Map-2220</td>
<td>18,644,128–33,880,278</td>
<td>3.62</td>
<td>SYa09: 5.62</td>
<td>4.42</td>
</tr>
<tr>
<td>qPH-13</td>
<td>Gm13</td>
<td>121</td>
<td>Map-2496 and satt554</td>
<td>37,227,372–38,075,387</td>
<td>0.85</td>
<td>SYa09: 18.76</td>
<td>19.12</td>
</tr>
<tr>
<td>qPH-14</td>
<td>Gm14</td>
<td>30</td>
<td>Map-2603 and Map-2606</td>
<td>5,473,602–6,013,169</td>
<td>0.54</td>
<td>SYa09: 2.61</td>
<td>0.91</td>
</tr>
<tr>
<td>qPH-16</td>
<td>Gm16</td>
<td>93</td>
<td>Map-3130 and Map-3141</td>
<td>30,993,460–31,954,379</td>
<td>0.96</td>
<td>SYa09: 4.90</td>
<td>0.89</td>
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<tr>
<td>qPH-17-1</td>
<td>Gm17</td>
<td>15</td>
<td>Map-3249 and satt458</td>
<td>4,918,617–6,053,125</td>
<td>1.13</td>
<td>SYa09: 2.98</td>
<td>1.75</td>
</tr>
<tr>
<td>qPH-17-2</td>
<td>Gm17</td>
<td>73</td>
<td>Map-3300 and satt389</td>
<td>13,322,792–14,024,961</td>
<td>0.60</td>
<td>SYa09: 2.71</td>
<td>0.65</td>
</tr>
<tr>
<td>qPH-19-1</td>
<td>Gm19</td>
<td>16</td>
<td>Map-3790 and satt446</td>
<td>1,063,390–1,634,198</td>
<td>0.57</td>
<td>CP10: 3.09</td>
<td>1.82</td>
</tr>
<tr>
<td>qPH-19-2</td>
<td>Gm19</td>
<td>131</td>
<td>Map-3972 and Map-3990</td>
<td>44,538,144–45,225,855</td>
<td>0.69</td>
<td>SYa10: 19.34</td>
<td>4.79</td>
</tr>
</tbody>
</table>

1Quantitative trait loci detected in different environments within same, adjacent, or overlapping marker intervals were designated as the same QTL.
3PVE, phenotypic variation explained. Average proportion of variation explained.
4The positive value indicates that Zhonghuang13 contributed the allele to an increase in plant height and negative additive effects indicated that Zhongpin03-5373 contributed the allele to an increase in plant height.
5A × E effect, the additive and dominance × environment effect.

The genomewide scan revealed the presence of 11 putative QTLs for plant height located on nine chromosomes (Table 1; Fig. 2 and 3A). One out of the eleven QTLs, \( qPH-13 \), was common to all six environments while 10 popped up only in a subset of environments. Among these environment-specific QTLs, six, \( qPH-6 \), \( qPH-8 \), \( qPH-12 \), \( qPH-16 \), \( qPH-17-1 \), and \( qPH-19-1 \), were detected only in one out of six environments while four, \( qPH-7 \), \( qPH-14 \), \( qPH-17-2 \), and \( qPH-19-2 \), were reproducible in two to five environments. In accordance with the single location analyses, we detected in the screen across locations three QTLs with significant environment interactions (Table 1).

Individual QTLs explained 2.02 to 47.60% of the phenotypic variation of plant height. We identified two major QTLs explaining average proportions of more than 10% of the phenotypic variation. One of these QTLs, \( qPH-13 \), had previously been described (Josie et al., 2007) and explained between 28.43 to 47.60% of the phenotypic variation for plant height in the individual environments, suggesting that it is a major and stable QTL for plant height and will be a great value for MAS. The flanking markers of \( qPH-13 \), Map-2496 and satt554, are located on Gm13 and span an interval amounting to 0.85 Mb according to the soybean reference genome (Glyma1.0). In the four high latitude environments (CP09, SYi10, CP10, and CP11), \( qPH-13 \) had the largest LOD scores (30.92, 31.21, 39.78, and 44.04) (Fig. 3; Table 1) and explained on average 40.86% of the plant height variation. In low latitude environments (SYa09 and SYa10), QTL \( qPH-13 \) explained only 28.43 and 28.74% of the plant height variation. Consequently, our results indicated that QTL \( qPH-13 \) has to be considered in MAS differentially for low and high latitude environments.
Epistatic interactions were evaluated performing a full two-dimensional genome scan. A total of 11 epistatic interactions were detected using LOD values of 4.0 as threshold (Table 2). The proportion of phenotypic variation explained by all epistatic QTLs ranged from 2.92 to 14.16%. No common epistatic interactions were detected across six environments. Except epistatic interaction (EI)-plant height (PH)-7 and EI-PH-10, 9 of the 11 epistatic interactions did not involve any of the main effect QTLs identified in this study.

Quantitative Trait Loci Identified for Seed Weight

We identified 18 putative QTLs for seed weight located on 14 of the 20 soybean chromosomes (Fig. 2 and 3B; Table 3). Seven QTLs exhibited significant interaction with the environments (Table 3). Individual QTLs explained 2.13 to 14.35% of the phenotypic variation of seed weight. The highest proportion of explained phenotypic variance was observed for qSW-2-1, which was only identified in one environment (Supplemental Fig. S1; Table 3). Moreover, QTLs qSW-19 and qSW-12-2 explained 9.93 and 7.29% of the phenotypic variation. Quantitative trait locus qSW-12-2 was largely affected by the latitude, with only one third of the phenotypic variance explained in low (3.36%) compared to that in high latitude environments (9.26%). This suggests that qSW-12-2 has to be considered in MAS differentially for low and high latitude environments. A two-dimensional genome scan revealed 10 significant pairwise epistatic interactions for seed weight (Table 4). In total, 40% of the pairwise interactions involved loci with significant main effects for seed weight. The proportion of phenotypic variation explained by all epistatic QTLs ranged from 3.84 to 8.88%. No common epistatic interactions were detected across six environments.

Discussion

Quantitative Trait Loci for Plant Height

A series of QTLs had already been known for plant height as an important yield-related trait in soybean. The 11 QTLs that were identified in our study were compared to those from databases and references (Gao et al., 2013; Wang et al., 2011) in a meta-analysis. The major robust QTL for plant height qPH-13 with large and stable effects showing peak LOD scores of phenotypic variation from 28.43 to 47.60% across six environments was found on Gm13 within marker interval Map-2496 to Satt554. Previous studies had identified several QTLs for plant height in or near this chromosomal region using RILs.
with different genetic backgrounds (Fehr et al., 2004; Jarvik et al., 1999; Josie et al., 2007; Reinprecht et al., 2006). For example, in a study using RILs derived from soybean varieties Essex and Forrest, a major QTL, *qPH*, also flanked by Satt554 and an amplified fragment length polymorphism marker CCA19, could explain 25.0% of the phenotypic variation of plant height (Josie et al., 2007). Moreover, the corresponding QTL in our study, *qPH-13*, was not found to be involved in digenic epistatic interaction in this biparental population, making it a suitable target for MAS candidate gene identification. Marker Map-2496 is located in the coding region of gene Glyma13 g35870.1, which encodes a Met-10-like protein (Pfam:02475) with transferase activity (GO:0016740). The marker interval between Map-2496 and Satt554 was 0.85 Mb, in which 245 SNPs were detected between ZP and ZH by resequencing (Li et al., 2013). Eight of these SNPs were located in coding regions, including four nonsynonymous and four synonymous SNPs. This polymorphic information is useful for future fine mapping of the plant height QTL within this region.

Plant height QTLs residing in the same region as *qPH-19-2* explaining 5.14 to 19.42% of phenotypic variation in our study had been reported previously for two intraspecific RIL populations derived from reciprocal crosses of soybean cultivars Minsoy and Noir 1 (Mansur et al., 1996) and one interspecific RIL population derived from cultivated line Tokei 780 (*G. max*) and the wild accession Hidaka 4 (*Glycine soja* Siebold & Zucc.) (Liu et al., 2007). The results showed that they were major QTLs, which explained 31.6, 32.5, and 13.4% of the variation in plant height. Furthermore, QTL *qPH-19-2* coincides with the positions of flowering time–related QTLs located between markers Sat-099 (nucleotide 43523541 to 43523584 of the soybean reference genome) and Satt229 (nucleotide 47049074 to 47049139) (Funatsuki et al., 2005; Githiri et al., 2007). This might indicate that plant height is also affected by flowering time.

In addition, QTL *qPH-7* linked to marker Satt175 was identified previously as a plant height–related QTL in three backcross-derived populations (Guzman et al., 2007). Other studies also mapped the QTLs at the same position as *qPH-6* in interval Map-0980 to satt281 (Gao et al., 2013; Wang et al., 2011). The remaining seven minor QTLs were not yet reported, suggesting that they were novel QTLs for plant height.

The plant height of parental line ZP was consistently taller than that of ZH in all six environments in our study. However, additive effects of QTLs controlling plant height came from both parents (Supplemental Fig. S1; Table 1), which might be the transgression of parental plant height in some of the RILs in our population. Eight alleles of the 11 QTLs serving as short plant height in some of the RILs in our population.

### Table 2. Estimated epistatic interaction (EI) effects of markers for plant height (PH) detected by a logarithm of the odds threshold of 4.0 in six environments.

<table>
<thead>
<tr>
<th>Code</th>
<th>Environ†</th>
<th>Chromosome 1 Marker internal</th>
<th>Chromosome 2 Marker internal</th>
<th>PVE (%)‡</th>
<th>Add1§</th>
<th>Add2¶</th>
<th>Add1 × Add2#</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI-PH-1</td>
<td>SYa09</td>
<td>Gm03</td>
<td>satt530 and sat_166</td>
<td>Gm15</td>
<td>Map-2710 and Map-2758</td>
<td>14.16</td>
<td>–0.19</td>
</tr>
<tr>
<td>EI-PH-2</td>
<td>SYa09</td>
<td>Gm04</td>
<td>Map-0696 and Map-0703</td>
<td>Gm19</td>
<td>Map-3972 and Map-3990</td>
<td>6.01</td>
<td>–0.43</td>
</tr>
<tr>
<td>EI-PH-3</td>
<td>SYi10</td>
<td>Gm02</td>
<td>satt703 and satt459</td>
<td>Gm06</td>
<td>Gm06-79 and satt371</td>
<td>3.55</td>
<td>–0.14</td>
</tr>
<tr>
<td>EI-PH-4</td>
<td>SYi10</td>
<td>Gm05</td>
<td>Map-0782 and Map-0751</td>
<td>Gm14</td>
<td>satt416 and satt560</td>
<td>3.42</td>
<td>0.14</td>
</tr>
<tr>
<td>EI-PH-5</td>
<td>SYi10</td>
<td>Gm06</td>
<td>Gm06-43 and satt433</td>
<td>Gm10</td>
<td>Map-1899 and Map-1902</td>
<td>2.92</td>
<td>0.59</td>
</tr>
<tr>
<td>EI-PH-6</td>
<td>CP10</td>
<td>Gm02</td>
<td>Sat_211 and InDel020</td>
<td>Gm05</td>
<td>satt276 and Map-0742</td>
<td>3.63</td>
<td>–0.41</td>
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<tr>
<td>EI-PH-7</td>
<td>SYa10</td>
<td>Gm19</td>
<td>satt229 and Map-4029</td>
<td>Gm20</td>
<td>Map-4147 and satt650</td>
<td>7.61</td>
<td>0.25</td>
</tr>
<tr>
<td>EI-PH-8</td>
<td>CP11</td>
<td>Gm01</td>
<td>sat_110 and Map-0109</td>
<td>Gm06</td>
<td>satt357 and Gm06-73</td>
<td>3.79</td>
<td>0.45</td>
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<tr>
<td>EI-PH-9</td>
<td>CP11</td>
<td>Gm03</td>
<td>Map-0373 and Map-0375</td>
<td>Gm20</td>
<td>Map-4202 and Map-4215</td>
<td>3.07</td>
<td>0.88</td>
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<tr>
<td>EI-PH-10</td>
<td>CP11</td>
<td>Gm08</td>
<td>sat_250 and Map-1537</td>
<td>Gm19</td>
<td>Gm19-38 and Map-3829</td>
<td>3.54</td>
<td>–0.12</td>
</tr>
</tbody>
</table>

†CP10, environment in Changpin in 2010; CP11, environment in Changpin in 2011; SYa09, environment in Sanya in 2009; SYa10, environment in Sanya in 2010; SYi10, environment in Shunyi in 2010.

‡PVE, phenotypic variation explained. Phenotypic variation explained by epistatic quantitative trait locus (QTL) effects.

§Add1, estimated additive effect of the first QTL.

¶Add2, estimated additive effect of the second QTL.

#Estimated additive × additive effect of QTL at the two scanning positions.

PVE, percentage variation explained; Phenotypic variation explained by epistatic quantitative trait loci (QTL) effects.

Additive effects additive effect of the first QTL.

Estimated additive additive effect of the second QTL.
Table 3. Quantitative trait loci (QTLs) associated with seed weight and their additive and environment interaction effects detected by a logarithm of the odds (LOD) threshold of 2.5 in a recombinant inbred line population across six environments.

<table>
<thead>
<tr>
<th>QTL†</th>
<th>Chromosome</th>
<th>Genetic position (cM)</th>
<th>Marker internal</th>
<th>Physical position (bp) of markers</th>
<th>Internal genome length of markers (Mb)</th>
<th>Single environment A × E‡ (LOD)</th>
<th>PVE (%)§</th>
<th>Additive¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>qSW-2-1</td>
<td>Gm02</td>
<td>9</td>
<td>Map-0187 and Map-0192</td>
<td>808,209–2,146,864</td>
<td>1.34</td>
<td>SYa09</td>
<td>7.03</td>
<td>10.03</td>
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<tr>
<td>qSW-2-2</td>
<td></td>
<td>39</td>
<td>sat216 and Map-0236</td>
<td>4,052,976–7,936,673</td>
<td>3.88</td>
<td>SYI10</td>
<td>4.89</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41</td>
<td>Map-0236 and sat_211</td>
<td>7,936,673–8,742,607</td>
<td>0.81</td>
<td>SYa09</td>
<td>4.54</td>
<td>5.55</td>
</tr>
<tr>
<td>qSW-3</td>
<td>Gm03</td>
<td>94</td>
<td>Map-0522 and Map-0534</td>
<td>40,111,085–40,910,014</td>
<td>0.80</td>
<td>CP10</td>
<td>3.23</td>
<td>3.08</td>
</tr>
<tr>
<td>qSW-4</td>
<td>Gm04</td>
<td>119</td>
<td>satt338 and Map-0721</td>
<td>46,964,891–48,070,478</td>
<td>1.11</td>
<td>SYI10</td>
<td>4.52</td>
<td>4.13</td>
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<tr>
<td>qSW-5</td>
<td>Gm05</td>
<td>56</td>
<td>satt276 and Map-0742</td>
<td>3,304,036–3,442,439</td>
<td>0.14</td>
<td>CP10</td>
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<td>3.32</td>
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<tr>
<td></td>
<td></td>
<td>56</td>
<td>satt276 and Map-0742</td>
<td>3,499,950–4,836,670</td>
<td>1.34</td>
<td>SYa09</td>
<td>2.94</td>
<td>4.70</td>
</tr>
<tr>
<td>qSW-7</td>
<td>Gm07</td>
<td>32</td>
<td>Gm07-19 and InDel085</td>
<td>3,499,950–4,836,670</td>
<td>1.34</td>
<td>SYa09</td>
<td>2.94</td>
<td>4.70</td>
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<tr>
<td></td>
<td></td>
<td>45</td>
<td>satt540 and Map-1233</td>
<td>4,963,031–5,605,451</td>
<td>0.64</td>
<td>SYa10</td>
<td>4.40</td>
<td>5.28</td>
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<tr>
<td>qSW-7</td>
<td>Gm09</td>
<td>17</td>
<td>Map-1616 and Map-1630</td>
<td>1,445,978–3,157,784</td>
<td>1.71</td>
<td>SYI10</td>
<td>4.49</td>
<td>4.56</td>
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<tr>
<td></td>
<td></td>
<td>25</td>
<td>Map-1630 and Map-1640</td>
<td>3,157,784–5,296,880</td>
<td>2.14</td>
<td>SYa10</td>
<td>5.86</td>
<td>8.32</td>
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<tr>
<td>qSW-8</td>
<td>Gm10</td>
<td>96</td>
<td>Barcsoyssr_10_1261 and Map-1890</td>
<td>42,184,560–42,452,641</td>
<td>0.27</td>
<td>SYI10</td>
<td>2.65</td>
<td>2.13</td>
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<td>qSW-11</td>
<td>Gm11</td>
<td>83</td>
<td>Map-2061 and Map-2055</td>
<td>30,741,018–29,572,665</td>
<td>1.17</td>
<td>SYa09</td>
<td>6.29</td>
<td>7.18</td>
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<td></td>
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<td>28</td>
<td>Map-1630 and Map-1640</td>
<td>3,157,784–5,296,880</td>
<td>2.14</td>
<td>SYa10</td>
<td>5.86</td>
<td>8.32</td>
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<tr>
<td>qSW-12</td>
<td>Gm12</td>
<td>5</td>
<td>satt353 and sat_200</td>
<td>1,682,557–267,522</td>
<td>1.42</td>
<td>SYI10</td>
<td>3.15</td>
<td>2.94</td>
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<td></td>
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<td>10</td>
<td>sat_200 and Barcsoyssr_12_100</td>
<td>267,522–2,488,292</td>
<td>2.22</td>
<td>SYa09</td>
<td>6.07</td>
<td>8.47</td>
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<tr>
<td>qSW-13</td>
<td>Gm13</td>
<td>118</td>
<td>Map-2492 and Map-2496</td>
<td>36,636,547–37,227,372</td>
<td>0.59</td>
<td>CP10</td>
<td>5.32</td>
<td>4.09</td>
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<tr>
<td>qSW-17</td>
<td>Gm17</td>
<td>39</td>
<td>satt372 and satt002</td>
<td>7,811,367–9,088,841</td>
<td>1.28</td>
<td>SYI10</td>
<td>2.65</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
<td>satt002 and Map-3288</td>
<td>9,088,841–9,409,139</td>
<td>0.32</td>
<td>CP09</td>
<td>4.08</td>
<td>5.53</td>
</tr>
<tr>
<td></td>
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<td>42</td>
<td>satt002 and Map-3288</td>
<td>9,088,841–9,409,139</td>
<td>0.32</td>
<td>CP09</td>
<td>4.08</td>
<td>5.53</td>
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<td>qSW-18</td>
<td>Gm18</td>
<td>104</td>
<td>Map-3684 and satt612</td>
<td>56,349,766–56,453,831</td>
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<td>SYa09</td>
<td>6.11</td>
<td>7.43</td>
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<td>108</td>
<td>satt612 and sct_199</td>
<td>56,453,831–58,093,530</td>
<td>1.64</td>
<td>SYa10</td>
<td>5.13</td>
<td>7.83</td>
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<tr>
<td></td>
<td></td>
<td>116</td>
<td>sct_199 and sct_187</td>
<td>58,093,530–60,463,057</td>
<td>2.37</td>
<td>SYI10</td>
<td>7.97</td>
<td>7.50</td>
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<tr>
<td>qSW-19</td>
<td>Gm19</td>
<td>127</td>
<td>Map-3948 and Map-3960</td>
<td>42,373,670–43,459,813</td>
<td>1.09</td>
<td>SYa09</td>
<td>7.11</td>
<td>8.90</td>
</tr>
</tbody>
</table>

(Cont’d)
environment. Therefore, it should be possible to produce offspring with desirable plant height by pyramiding different alleles.

The knowledge of epistatic interactions is essential to understand the genetic architecture and gene networks underlying complex traits (Würschum et al., 2011). However, their contribution to the determination of plant height in soybean has not been investigated sufficiently. So far, only Lark et al. (1995) has reported significant epistatic interactions for plant height in soybeans. With 11 epistatic interactions, we identified more than seven indicated by Lark et al. (1995). However, a subset of seven amounting to in total 63.6% of all epistatic interactions controlled only less than 4% of variation. Only the remaining four interactions showed substantial epistatic effects controlling 7.65 to 14.2% of variation. Quantitative trait locus qPH-6 (Map-0980 to satt281) was the only plant height QTLs identified in our study involved in an epistatic interaction (EI-PH-10). Furthermore, marker satt229 was involved in epistatic interaction EI-PH-7. Both EI-PH-7 and EI-PH-10 might deserve for further analysis of their effects in near-isogenic lines. Since multiple epistatic interactions of small sizes can lead to substantial cumulative epistatic effects, the clarification of epistatic interaction involving plant height QTLs will be helpful to improve soybean breeding.

### Quantitative Trait Loci for Seed Weight

Seed weight is another important yield-related trait in soybean. In our study using RILs derived from ZP and ZH, a total of 18 QTLs for seed weight were identified. Among these, two major common QTLs of qSW-12-2 and qSW-19 were confirmed in different genetic backgrounds by previous reports. One QTL qSW-12-2 had been discovered in five F2 populations (Gao et al., 2007) and two interspecific BC1F2 populations (Li et al., 2008a), explaining 8.6 to 14.7% of the phenotypic variation in seed weight. The other QTL qSW-19 had been identified in three RIL populations derived from soybean cultivars (Jarvik et al., 1999). Furthermore, three reproducible QTLs across five environments in our study had also been confirmed by previous studies, including qSW-11 in RILs derived from soybean cultivars Minsoy and Noir 1 (Specht et al., 2001), qSW-17-1 in both interspecific RIL (Liu et al., 2007) and intraspecific RIL (Teng et al., 2008) populations, and qSW-18 in F1 and F2 populations (Mian et al., 1996). Moreover, eight new QTLs for seed weight were detected in our study, located in Gm02, 04, 07, 09, 10, 13, and 17, respectively. Among them, qSW-2-2, qSW-7-2, qSW-9, and qSW-17-2 were detected in more than two environments, explaining 2.13 to 9.10% of the phenotypic variation of seed weight.

Quantitative trait locus qSW-19 interacted epistatically with two QTLs located on chromosomes of Gm13 and Gm18, respectively, suggesting this QTL is difficult to be used for MAS. In contrast, three of the robust QTLs, qSW-11, qSW-12-2, and qSW-18, were not involved in epistatic interactions. Seed weight-increasing alleles were inherited from the ZH at 12 of the 18 QTLs detected in our study.

We also found some proximity QTLs for plant height and seed weight. For example, QTLs qPH-7 vs. qSW-7-2 clustered in the interval Satt173 to Satt697 on Gm07, qPH-13 vs. qSW-13 in the interval Map-2492 to Satt554 on Gm13, and qPH-19-2 vs. qSW-19 in the interval Map-3948 to Satt229 on Gm19. Two co-localizing pairs of QTLs shared the same direction of additive effects, with positive alleles being contributed by ZP for qPH-7 vs. qSW-7-2 and ZH for qPH-19-2 vs. qSW-19. Such coincident QTLs have been previously reported in crops, including rice (Oryza sativa L.) (Cai and Morishima, 2002), wheat (Triticum aestivum L.) (Sun et al., 2009), sorghum [Sorghum bicolor (L.) Moench] (Lin et al., 1995), and soybean (Lin et al., 1995). The markers around these stable co-located QTLs will be useful for MAS.

### Supplemental Information Available

Supplemental material is available at http://www.crops.org/publications/tpg.

Supplemental Figure S1. Quantitative trait locus (QTL) genome scans of additive effects for plant height
Table 4. Estimated epistatic interaction (EI) effects of markers for seed weight (SW) detected by a logarithm of the odds threshold of 4.0 in six environments.

<table>
<thead>
<tr>
<th>Code</th>
<th>Environment</th>
<th>Chromosome 1</th>
<th>Marker internal</th>
<th>Physical position (bp) of markers Chromosome 1</th>
<th>Marker internal</th>
<th>Physical position (bp) of markers Chromosome 2</th>
<th>PVE (%)‡</th>
<th>Add1§</th>
<th>Add2¶</th>
<th>Add1 × Add2#</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI-SW-1</td>
<td>SYa09</td>
<td>Gm02</td>
<td>satt216 and Map-0236</td>
<td>4,052,976–7,936,673 Gm16</td>
<td>Map-2971 and Map-2976</td>
<td>1,600,606–2,611,170</td>
<td>6.32</td>
<td>−0.04</td>
<td>0.15</td>
<td>0.82</td>
</tr>
<tr>
<td>EI-SW-2</td>
<td>SYa09</td>
<td>Gm04</td>
<td>sat_140 and satt607</td>
<td>5,221,477–8,093,801 Gm14</td>
<td>Map-2576 and Map-2595</td>
<td>3,545,054–4,706,907</td>
<td>8.00</td>
<td>0.28</td>
<td>−0.03</td>
<td>−0.94</td>
</tr>
<tr>
<td>EI-SW-3</td>
<td>CP10</td>
<td>Gm16</td>
<td>sat_412 and sct_001</td>
<td>18,435,677–26,989,225 Gm19</td>
<td>Map-3829 and satt523</td>
<td>4,400,362–7,155,427</td>
<td>6.31</td>
<td>0.20</td>
<td>−0.09</td>
<td>−0.67</td>
</tr>
<tr>
<td>EI-SW-4</td>
<td>SYa10</td>
<td>Gm01</td>
<td>Map-0021 and Map-0033</td>
<td>4,283,812–5,312,128 Gm11</td>
<td>Map-1977 and Map-1985</td>
<td>3,688,303–4,865,983</td>
<td>5.94</td>
<td>−0.29</td>
<td>0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>EI-SW-5</td>
<td>SYa10</td>
<td>Gm08</td>
<td>ESSR197 and satt187</td>
<td>7,563,893–9,199,916 Gm20</td>
<td>Map-4202 and Map-4215</td>
<td>38,729,088–40,272,063</td>
<td>5.29</td>
<td>0.30</td>
<td>0.18</td>
<td>0.76</td>
</tr>
<tr>
<td>EI-SW-7</td>
<td>SYa10</td>
<td>Gm17</td>
<td>Map-3269 and satt372</td>
<td>6,713,645–7,811,432 Gm19</td>
<td>satt229 and Map-4029</td>
<td>47,049,074–49,906,987</td>
<td>8.88</td>
<td>0.60</td>
<td>0.35</td>
<td>0.97</td>
</tr>
<tr>
<td>EI-SW-8</td>
<td>CP11</td>
<td>Gm07</td>
<td>Map-1217 and Map-1220</td>
<td>1,034,953–2,264,388 Gm18</td>
<td>Map-3572 and Map-3573</td>
<td>33,478,470–36,976,377</td>
<td>4.04</td>
<td>−0.01</td>
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<td>0.50</td>
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<tr>
<td>EI-SW-9</td>
<td>CP11</td>
<td>Gm13</td>
<td>Map-2470 and Map-2479</td>
<td>29,287,679–33,302,470 Gm19I-2 and satt527</td>
<td>42,435,667–42,835,278</td>
<td>42,435,667–42,835,278</td>
<td>4.26</td>
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<td>0.52</td>
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<tr>
<td>EI-SW-10</td>
<td>CP11</td>
<td>Gm18</td>
<td>satt570 and Gm18-52</td>
<td>3,162,724–3,512,526 Gm19</td>
<td>Map-3948 and Map-3960</td>
<td>42,373,670–43,459,813</td>
<td>3.84</td>
<td>−0.03</td>
<td>0.52</td>
<td>0.54</td>
</tr>
</tbody>
</table>

†CP10, environment in Changpin in 2010; CP11, environment in Changpin in 2011; SYa09, environment in Sanya in 2009; SYa10, environment in Sanya in 2010.
‡PVE, phenotypic variation explained. Phenotypic variation explained by epistatic quantitative trait locus (QTL) effects.
§Add1, estimated additive effect of the first QTL.
¶Add2, estimated additive effect of the second QTL.
#Estimated additive × additive effect of QTL at the two scanning positions.

References


Acknowledgments

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and seed weight in each of six environments and environment × QTL interactions.

Supplemental Table S1. Details for 24 indel markers developed in this study.

Supplemental Table S2. Statistical analysis of plant height and seed weight for parents and the recombinant inbred line (RIL) population at different environments.

Supplemental Table S3. Information for the soybean genetic linkage map constructed in this study by using 508 molecular markers.


