Mapping quantitative trait loci for yield-related traits in soybean (Glycine max L.)

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Development of soybean cultivars with high seed yield is a major focus in soybean breeding programs. This study was conducted to identify genetic loci associated with seed yield-related traits in soybean and also to clarify consistency of the detected QTLs with QTLs found by previous researchers. A population of 135 F2:3 lines was developed from a cross between a vegetable soybean line (MJ0004-6) and a landrace cultivar from Myanmar (R18500). They were evaluated in the experimental field of Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand in a randomized complete block design with two replications each in 2011 and 2012 growing seasons. The two parents exhibited contrasting characteristics for most of the traits that were mapped. Analysis of variance showed that the main effects of genotype and environment (year) were significant for all studied traits. Genotype by environment interaction was also highly significant for all the traits. The population was genotyped by 149 polymorphic SSR markers and the genetic map consisted of 129 SSR loci which converged into 38 linkage groups covering 1156 cM of soybean genome. There were 10 QTLs significantly associated with seed yield-related traits across two seasons with single QTLs explaining between 5.0% to 21.9% of the phenotypic variation. Three of these QTLs were detected in both years for days to flowering, days to maturity and 100 seed weight. Most of the detected QTLs in our research were consistent with earlier QTLs reported by previous researchers. However, four novel QTLs including SF1, SF2 and SF3 on linkage groups L and N for seed filling period and PN1 on linkage group D1b for pod number were identified in the present study.

Key Words: soybean, yield-related traits, simple sequence repeat, quantitative trait loci.

Introduction

Improvement of seed yield is the most important objective in soybean breeding. Seed yield is the result of the expression and association of several plant growth components including seed size traits, days to flowering, days to maturity, seed filling period and number of pods per plant. They are all complex quantitative traits highly influenced by environment. Complex inheritance patterns and strong environmental effects decrease the accuracy in phenotypic estimation of these traits. Molecular markers can be utilized for identifying chromosomal regions that contain genes controlling complex traits. In addition, molecular markers can assist selection of superior genotypes by using the markers that are tightly linked to the target genes (Ribaut and Hoisington 1998). Thus it is important to study the genes/QTLs governing these traits. Microsatellites or simple sequence repeats (SSRs) are extensively used to genotype soybean germplasm collections due to their hypervariability, multi-allelic, co-dominant inheritance, high reproducibility, relative abundance, extensive genome coverage (including organellar genome), chromosome specific location, amenability to automation and high throughput genotyping (Parida et al. 2009). Moreover, 40–60% of the soybean sequences are repetitive (Danesh et al. 1998).

Markers should be validated by testing their effectiveness in determining the target phenotype in independent populations and in different genetic backgrounds, which is referred to as ‘marker validation’ (Collard et al. 2005). Stable and validated QTLs are useful in marker-assisted selection (MAS) programs (Song et al. 2004). Genetic background of mapping populations is an important factor for not detecting common QTLs in different populations. For instance, Orf et al. (1999) found only a few common QTLs across three populations. Thus, conducting QTL studies across several environments and in different genetic background mapping populations is essential to achieve stable and validated QTLs.

Seed weight (seed size) is an important yield component
of soybean and is generally positively correlated with seed yield (Burton 1987). Seed size is also important for production of soy food products, including tofu, natto, miso, soy sprouts and edamame (Hoeck et al. 2003). Small-seeded soybeans are desirable for high quality soybean sprouts and natto production, whereas large-seeded ones are desirable for tofu, edamame and miso production (Wilson 1995). Days to flowering and maturity are useful for developing soybean cultivars with a wider geographical adaptation. Expression of the traits is a function of day length, temperature during the growing seasons, and plant genotype (Whigham and Minor 1978). A range of soybean adaptation depends mainly on difference in day length perception that affects the length of time required for reproductive periods (Cober et al. 1996). Seed yield is closely related to seed filling period (Curtis et al. 2000, Smith and Nelson 1987) and number of pods per plant (Board and Tan 1995).

To date, a number of QTLs for important seed yield-related traits in soybean have been identified, including seed weight (Hoeck et al. 2003, Hyten et al. 2004, Mansur et al. 1996, Panthee et al. 2005), days to flowering and maturity and seed filling period (Cheng et al. 2011, Komatsu et al. 2012, Liu et al. 2011, Tasma et al. 2001, Zhang et al. 2004), and number of pods per plant (Sun et al. 2006, Zhang et al. 2010). Although many QTLs associated with seed yield-related traits have been detected earlier, but only a few QTLs have been validated or confirmed.

The objectives of the present study were to identify QTLs affecting seed yield-related traits from the F2:3 progeny derived from two very diverse parents, and to find out novel QTLs for these mentioned traits.

Materials and Methods

Plant materials
The population used in this study was developed from a cross between two soybean genotypes R18500 and MJ0004-6. R18500 is a small-seeded line from Myanmar collected from Nyaung Kar Yar Village by Chtone Bo Research Farm. MJ0004-6 is a vegetable soybean breeding line developed from a cross between #75 (commercial vegetable soybean cultivar of Taiwan) and Chamame (Japanese vegetable soybean variety) by Chiang Mai Field Crop Research Center. The parental lines were greatly different with respect to seed weight and number of pods per plant. A total of 135 F2:3 lines and the two parents were sown in a field of Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand (14°01′N, 99°59′E, 7.5 m ASL) in a randomized complete block design with two replications in 2011 and 2012 growing seasons (November 2011–March 2012 and November 2012–March 2013). Each plot has one row of 2 m long with constituting 7 plants in each row (totally 14 plants for each line considering two replications) with a space of 20 cm between the adjacent plants.

Trait measurement
The traits were measured on the F2:3 population. Days to flowering was recorded as number of days from planting to 50% of the plants in the plot were flowering. Days to maturity was counted from planting date to maturity date. The period between days to flowering and days to maturity was recorded as seed filling period. Number of pods per plant was counted at harvest time from six random plants (three plants from each replication) per genotype. One hundred seed weight was determined from weight of 100 randomly chosen seeds per plot. Pearson phenotypic correlation coefficients among traits and analysis of variance of traits were calculated using R program (R Core Team 2013).

Heritability of traits
The broad-sense heritability was calculated using the following formula (Nyquist 1991):

\[ h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2 + \sigma_e^2} \]

Where \( h^2 \) is the broad-sense heritability, \( \sigma_g^2 \) is the genetic variance, \( \sigma_{ge}^2 \) is the genotype by environmental interaction variance, \( \sigma_e^2 \) is the pooled error variance, \( e \) is the number of environments, and \( r \) is the number of replications for the experiment.

DNA extraction and SSR analysis
Genomic DNA was extracted from young leaves of the two parents and each F2 line using modified CTAB method recommended by Lodhi et al. (1994). The DNA was quantified against a lambda DNA on 1.0% agarose gel stained with ethidium bromide and diluted to 10 ng/μl. Based on the sequences published on the SoyBase website (http://soybase.agron.iastate.edu/), 506 SSR primer pairs were synthesized for genotyping the F2 population. The PCR amplification was performed using 2 μl of dH2O, 1 μl of 10X PCR Buffer, 2 μl of 1 mM of each dNTP, 2 μl of each SSR primer, 0.8 μl of 25 mM MgCl2, 0.2 μl of Taq DNA polymerase and 2 μl of 10 ng/μl template DNA. The initial step of thermal cycle profile was at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 47°C for 30 s, and 72°C for 1 min with a final extension at 72°C for 1 min. The PCR products were then separated on 5% polyacrylamide gel and visualized by silver staining.

Map construction and QTL detection
We used the program JoinMap 3.0 (Van Ooijen 2006) to construct linkage between the markers, setting a minimum LOD score of 3.0 and the maximum genetic distance of 50 cM. Map distances were calculated using the mapping function of Kosambi (1944). QTL analysis was performed following composite interval mapping method (CIM) (Zeng 1994) using the software WinQTLCartographer 2.5 (Wang et al. 2007). Initially, a minimum LOD score of 2.0 was set to declare the presence of a QTL in a particular genomic region. In the second step, 1000 permutations (Churchill and
Doerge 1994) were performed on each trait with a significance level of 0.05 for getting genome-wide critical threshold value for the experimentwise type I error rate. The QTL was considered significant when its LOD score was found higher than the threshold value in at least one of the two seasons or the average of both seasons.

Results

Phenotypic variation in parents and the population

Except for days to flowering, the two parents, MJ0004-6 and R18500, showed significant difference in all traits measured (Table 1). Except for days to maturity, all of the traits were approximately normally distributed (Fig. 1) suggesting that they were polygenically inherited. Transgressive segregation was observed in all traits except for 100 seed weight.

Significant correlation coefficients among the traits in the F$_{2:3}$ population ranged from 0.18 to 0.83 (Table 2). Positive correlations were detected among days to maturity, seed filling period and 100 seed weight in both seasons revealing that longer reproductive period increases seed weight.

Analysis of variance and heritability of traits

Based on the results of the analysis of variance (Table 3), the effects of Year and interaction (Year × Line) were highly significant for all traits. The broad-sense heritabilities found in this population ranged from 0.66 to 0.83 indicated that selection response would give moderately high genetic gain.

Linkage map construction

The two parents were screened with 506 SSR markers. Two hundred and thirty-two (46%) polymorphic markers were identified. Of these, 149 markers were used in the initial linkage map construction. According to the relative positions of these markers on the reference genetic maps (http://soybase.agron.iastate.edu/), 129 markers showing clear polymorphic bands and distributing evenly on the soybean genome were used to genotype the F$_{2:3}$ population. These markers were mapped onto 38 linkage groups. Coverage ratio of the linkage map constructed in this study to the whole soybean genome was 45.8% (1156 cM out of 2523.6 cM (Song et al. 2004)). Twenty polymorphic markers remained unlinked. Linkage groups were designated with names corresponding to the integrated public soybean genetic map (Song et al. 2004). Five linkage groups were consistent with the consensus map of Cregan et al. (1999), namely B1, D1a, H, I and N. While A1, A2, B2, C1, D1b, D2, G, J, K, L, M and O were splitted into 2 sub-groups, and C1, E and F were divided into 3 sub-groups. Most markers constructed in the linkage groups and the map position in this study corresponded well with the soybean composite map (http://soybase.agron.iastate.edu/), with some variation in linkage distance among the markers.

QTL analysis

All QTLs detected are listed in Table 4 and their locations are marked on the genetic map (Fig. 2). Using CIM, a total of 10 different QTLs were detected across all traits in both seasons with single QTLs explaining between 5.0% to 21.92% of the phenotypic variations. The QTLs were distributed on 7 linkage groups (B1, B2, D1a, D1b, G, L and N). One or more QTLs were identified on each linkage group, with linkage group L having the most (4 QTLs) and other linkage groups having one QTL each. Two major QTLs were found for days to flowering on linkage groups B1 and B2. The QTL $\text{DF1}$ was detected based on only mean values of two years, but not on either 2011 or 2012. The largest QTL ($\text{DF2}$) was detected in the satt070-satt534 interval on linkage group B2 with the LOD score of 4.1 explaining 14.4% of the phenotypic variation, and was contributed by MJ0004-6. This QTL was detected

| Traits                 | Years | Parents | Difference between parents | F$_{2:3}$ population |
|------------------------|-------|---------|---------------------------|---------------------|
|                        |       | MJ0004-6 | R18500 |                  | Range | Mean ± SD |
| Days to flowering      | 2011  | 31       | 33    | 2                  | 31–39 | 33.5 ± 1.6 |
|                        | 2012  | 28.2     | 30    | 1.8                | 28–32 | 29.8 ± 1.2 |
|                        | Combined | 29.6   | 31.5  | 1.9                | 29.5–34.5 | 31.6 ± 1.1 |
| Days to maturity       | 2011  | 92       | 89    | 3                 | 89–95 | 90.9 ± 1.8 |
|                        | 2012  | 86       | 83    | 3                 | 83–89 | 84.6 ± 1.9 |
|                        | Combined | 89     | 86    | 3                 | 86–91.2 | 87.7 ± 1.5 |
| Seed filling period    | 2011  | 61       | 56    | 5                 | 50–63 | 57.4 ± 1.9 |
|                        | 2012  | 57.8     | 53    | 4                 | 51–61 | 54.8 ± 2.1 |
|                        | Combined | 59.4 | 54.5  | 4                 | 51.5–59.8 | 56.1 ± 1.5 |
| Pods per plant         | 2011  | 26       | 50    | 24                 | 9.7–83.3 | 36.8 ± 13.3 |
|                        | 2012  | 22       | 46    | 24                 | 13.5–72.0 | 37.1 ± 11.5 |
|                        | Combined | 24     | 48    | 24                 | 19.6–72.2 | 37.0 ± 10.3 |
| 100 seed weight        | 2011  | 36.8     | 11.1  | 25.7               | 13.4–30.1 | 21.0 ± 3.1 |
|                        | 2012  | 32.1     | 12.5  | 19.6               | 13.5–29.0 | 19.9 ± 3.1 |
|                        | Combined | 34.4 | 11.8  | 22.6              | 15.2–28.2 | 20.5 ± 2.6 |

*,**,*** Significant at 0.05, 0.01 and 0.001 levels, respectively.

Only data of F$_{2:3}$ lines that are available in both seasons are combined.

Table 1. Descriptive statistics of the evaluated quantitative traits in the parents and F$_{2:3}$ population
in both years and can be considered as a stable QTL. One stable QTL (DM) was detected in the sat143-satt523 region for days to maturity on linkage group L, where the alleles of MJ0004-6 decreased the trait. CIM analysis located three QTLs (SF1, SF2 and SF3) for seed filling period on linkage groups L and N. In SF1 and SF2 on linkage group L, the alleles of MJ0004-6 decreased the trait. Two major QTLs were mapped for number of pods per plant on linkage groups D1b and G explaining 14.3% and 17.2% of variation in 2011, respectively. At these QTLs the alleles of MJ0004-6
decreased number of pods per plant. No QTLs were found for number of pods per plant in 2012. Two seed weight QTLs were identified on linkage groups D1a and L. One stable QTL (SW1) was detected in the satt502-satt603 interval on linkage group D1a explaining 8.1% and 6.0% of phenotypic variation in 2011 and 2012, respectively, which the alleles from MJ0004-6 increased the trait. The QTL analyses through CIM in individual seasons demonstrated that 8 QTLs were detected in 2011 and 4 QTLs were identified in 2012. Three stable QTLs including one locus each for days to flowering (DF2), days to maturity (DM) and 100 seed weight (SW1) were mapped in both seasons.

Discussion

The parents of the F$_2$ mapping population were chosen based on high variation in agronomic traits. Except for days to maturity, the traits showed good fit to the normal distribution. Transgressive segregation was observed in all traits except for 100 seed weight, suggesting the possibility of identifying positive alleles in the superior parent and the negative alleles in the inferior parent (de Vicente and Tanksley 1993).

The broad-sense heritability estimates for the traits ranged from 0.66 (pods per plant) to 0.83 (seed weight). These values agree with those reported in soybean (Cheng et al. 2011, Hoeck et al. 2003, Hyten et al. 2004, Panthee et al. 2005, 2007). Panthee et al. (2007) observed 47% broad-sense heritability for days to flowering, 65% for seed filling period and 21% for maturity date. Hoeck et al. (2003), Hyten et al. (2004) and Panthee et al. (2005) observed 47% broad-sense heritability for days to flowering, 65% for seed filling period and 21% for maturity date. Hoeck et al. (2003), Hyten et al. (2004) and Panthee et al. (2005)

Table 2. Pearson phenotypic correlation coefficients among yield-related traits in 2011 and 2012 (italic)

| Trait                   | Days to flowering | Days to maturity | Seed filling period | Pods per plant |
|-------------------------|-------------------|------------------|---------------------|----------------|
| Days to maturity        | 0.37***           | 0.18*            |                     |                |
| Seed filling period     | -0.46***          | 0.65***          | -0.39***            | -0.26***       |
| Pods per plant          | 0.01              | 0.10             | 0.09                |                |
| 100 seed weight         | -0.03             | 0.28**           | 0.30***             | -0.26**        |
|                         |                   |                  |                     |                |
| *,**,** Significant at 0.05, 0.01 and 0.001 levels, respectively. |

Table 3. Analysis of variance and broad-sense heritabilities of traits

| Source       | Days to flowering | Days to maturity | Seed filling period | Pods per plant | 100 seed weight |
|--------------|-------------------|------------------|---------------------|----------------|----------------|
| df           | Mean square       | F value          | df                  | Mean square    | F value         |
| Line         | 130               | 4.76             | 128                 | 8.36           | 3.62*           |
| Year         | 1                 | 1762.45          | 128                 | 8.88           | 2.56*           |
| Line × Year  | 130               | 2.83             | 128                 | 6.33           | 2.74*           |
| Residuals    | 262               | 1.40             | 258                 | 2.31           | 1.40            |
| h$^2$        | 0.73              |                  | 0.69                |                | 0.67            |

* Significant at 0.001 level.

Table 4. QTLs identified for yield-related traits in the F$_2$ mapping population derived from MJ0004-6 × R18500

| Trait                   | QTL     | LG     | Marker interval | Position (cM) | Years | LOD  | Additive$^a$ effect | Dominance effect | PVE$^b$ (%) |
|-------------------------|---------|--------|-----------------|---------------|-------|------|---------------------|-----------------|-------------|
| Days to flowering       | DF1     | B1     | satt251-satt426 | 23.8          | 2011  | 3.7  | -0.55              | -0.50           | 12.0        |
|                         | DF2     | B2     | satt070-satt534 | 35.3          | 2011  | 3.6  | -0.75              | -0.57           | 12.9        |
| Days to maturity        | DM      | L      | satt143-satt523 | 4.1           | 2011  | 6.4  | -1.16              | 0.17            | 17.9        |
| Seed filling period     | SF1     | L      | satt143-satt523 | 0.3           | 2011  | 2.0  | -0.63              | -0.11           | 5.0         |
|                         | SF2     | L      | satt313-satt182 | 39.0          | 2011  | 4.3  | -1.27              | 0.50            | 19.1        |
|                         | SF3     | N      | sat_033-satt387 | 84.3          | 2012  | 4.7  | 0.57               | -1.76           | 21.9        |
| Pods per plant          | PN1     | D1b    | satt041-satt290 | 15.0          | 2011  | 4.0  | -6.87              | -3.73           | 14.3        |
|                         | PN2     | G      | satt394-satt504 | 42.0          | 2011  | 4.2  | -0.03              | 11.27           | 17.2        |
| 100 seed weight         | SW1     | D1a    | satt502-satt603 | 0.5           | 2011  | 3.0  | 1.21               | -0.18           | 8.1         |
|                         | SW2     | L      | satt523-satt313 | 12.3          | 2011  | 4.0  | -1.58              | 0.05            | 12.3        |

$^a$ The additive effect of an allele from MJ0004-6 is shown.

$^b$ Phenotypic variance explained.
reported high heritability values for seed weight (95%, 93% and 71%, respectively). In the present study, we also observed high heritability (83%) for seed weight.

The QTL (DF2) detected on linkage group B2 for days to flowering was stable in both years and was corresponding with the QTL found by Reinprecht et al. (2006) using ANOVA method in one of the two year trial and only at one location out of three. Since a single marker regression does not provide precise information about the position of QTLs and their effects, this QTL needs to be validated by using composite interval mapping method in the future studies. DF1 was detected based on mean values of 2011 and 2012. Nevertheless, this QTL is 10.1 cM from satt197 identified by Gai et al. (2007) and could be inferred as the real QTL. Zhang et al. (2004) indicated that the major gene(s) controlling flowering time may be located on linkage groups C2 and/or B1, however we did not find any QTLs for days to flowering on linkage group C2 in the present investigation.

Considering the QTLs reported in SoyBase (http://soybase.agron.iastate.edu/), linkage groups C2, L and M were most frequently associated with days to maturity. One stable QTL (DM) on linkage group L was found for days to maturity in this study. This QTL is very close to the QTLs reported by Lee et al. (1996) and Tasma et al. (2001). This linkage group was also detected to have some important QTLs for days to maturity reported by Mansur et al. (1996), Orf et al. (1999), Specht et al. (2001), Wang et al. (2004). Moreover, earlier reports indicated that maturity locus E3 was mapped on linkage group L (Cregan et al. 1999). This leads to the conclusion that linkage group L should be considered in MAS programs for days to maturity in soybean.

For seed filling period, several QTLs were identified on linkage groups A1, A2, B1, C1, C2, F, G, I, J, F, L, M and O in previous studies (Cheng et al. 2011, Cregan et al. 1999, Keim et al. 1990, Komatsu et al. 2012, Li et al. 2008, Mansur et al. 1993, Orf et al. 1999, Xin et al. 2008). In the present study, three QTLs for seed filling period were mapped on linkage groups L and N. SF3 on linkage group N is a novel QTL found in our study as no seed filling period QTLs have been reported on this linkage group. This QTL with the LOD score of 4.7 explaining 21.9% of variation in 2012 is the largest QTL and alleles contributed by MJ0004-6 enhance the trait. SF1 and SF2 located on linkage group L are slightly distant to the qRP-l-1 identified as one of the
major loci by Cheng et al. (2011), thus they may be independent and can be considered as two novel QTLs. The markers associated with the QTLs for post-flowering period on linkage group L in this study, revealed that they should be used in MAS programs. This conclusion is confirmed by Cheng et al. (2011), Keim et al. (1990), Mansur et al. (1993, 1996), Orf et al. (1999).

Several QTLs on linkage groups A1, A2, B1, C1, C2, D1b, E, G, I, J, L, N and O were reported in SoyBase (http://soybase.agron.iastate.edu/) for pod number at different developmental stages. Zhang et al. (2010) concluded that most QTLs controlling pod numbers were not expressed at the mature stage, so to breed high-yield cultivars, a new approach would be to identify major QTLs that facilitated high yield at different developmental stages before maturity, rather than using the QTLs detected at the mature stage. PN1 was detected on linkage group D1b in the satt041-sat3290 region to locate at 40.5 cM from the QTL qpn-Chr2 identified by Zhang et al. (2010), indicating that our detected QTL marks a novel QTL associated with pod number in soybean. Although PN2 is very close to the QTL qpn-Chr18 reported by Zhang et al. (2010), but our QTL is an overdominant QTL with an extremely high level of dominance effect/additive effect ratio (376) in 2011. Due to a small chance to detect such a strong overdominant QTL in advanced generations such as the RIL population used by Zhang et al. (2010), therefore, PN2 in our study could not directly confirm the qpn-Chr18 reported by Zhang et al. (2010) based on the significant additive effect.

There were two QTLs associating with seed weight in this population located on two linkage groups D1a and L. Both of QTLs detected for this trait in our research were strongly corresponded to the earlier reports. SW1 on linkage group D1a is a major QTL that is stably detected in both seasons. This QTL is corresponded to the QTL identified by Hyten et al. (2004). SW2 was mapped closely to the QTLs identified by Csanadi et al. (2001), Hoeck et al. (2003) and Maughan et al. (1996). Although many seed weight QTLs distributing on all linkage groups have been reported in SoyBase (http://soybase.agron.iastate.edu/), linkage groups F, G and L were most frequently associated with seed weight in soybean. We also found a major QTL on linkage group L in our investigation.

The high level of overdominance that was detected at two QTLs (PN2 and SF3) in our study may be attributable to a pseudo-overdominance effect (Moll et al. 1964). These QTLs may locate in regions of the genome containing several loci that contribute to the overall effect. In particular, loci in repulsion linkage (which are at their maximum effect in the F2 and F3 generations) could account for some of the overdominance effects. The additive effect of such genes in repulsion linkage would partly cancel and reduce the effect of each others, which exaggerate the overall dominance effect against the underestimated additive effect (Semel et al. 2006, Tar’ an et al. 2002). Large proportion of overdominance gene effects of QTLs for yield and yield components, number of pods per plant and days to flowering were reported in corn (Veldboom and Lee 1996a, 1996b) and in common bean (Tar’ an et al. 2002).

Co-located QTLs for days to maturity, seed filling period and 100-seed weight on linkage group L, and the correlations among these traits (Table 2) suggesting that increasing days to maturity and seed filling period would possibly result in increasing seed weight. Our result is in agreement with the earlier reports (Boote 1981, Curtis et al. 2000, Hanson 1985, Johnson and Bernard 1962, Kantolic and Slaffer 2001, 2005, 2007, Smith and Nelson 1987) concluding that increasing the length of seed filling period is an effective means for improving yield, since it has a positive correlation with many yield determining factors.

QTL analysis conducted in a single environment is likely to underestimate the number of QTL for a certain trait. This is the reason that QTL analysis should be done across several environments (Paterson et al. 1991). In addition, stable and confirmed QTLs are more desirable to be used in MAS (Song et al. 2004). High additive effect and explained phenotypic variance, and good consistency across different environments are the main factors to conduct more successful MAS breeding programs. Considerable attention should be given to chromosomes B2, D1a and L due to their stable QTLs for days to flowering, days to maturity and 100 seed weight. Linkage group L harbors major QTLs for days to maturity, seed filling period and seed weight in this study as reported previously by the other researchers. Thus markers on this linkage group can be simultaneously considered in MAS breeding programs. Most of the detected QTLs in our research were consistent with earlier detected QTLs found by previous researchers. Yet, we have identified four additional QTLs including SF1, SF2 and SF3 on linkage groups L and N for seed filling period and PN1 on linkage group D1b for pod number. None of novel QTLs identified in this study were stable, indicating significant genotype by environment effects as concluded through analysis of variance of traits in two years (Table 3). The failure to detect these QTLs in both seasons could have been due to the relatively small number of replicates used in the trial. In this condition, QTLs with small effects would not be detected at the threshold levels set for declaration of the QTL (Shah et al. 1999). These novel QTLs need to be validated across different environments and in different genetic backgrounds in the future.

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