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ORIGINAL PAPER

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Fine mapping of the *FT1* locus for soybean flowering time using a residual heterozygous line derived from a recombinant inbred line

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Abstract Fine-mapping of loci related to complex quantitative traits is essential for map-based cloning. A residual heterozygous line (RHL) of soybean (*Glycine max*) derived from a recombinant inbred line (RIL) was used for fine-mapping *FT1*, which is a major quantitative trait locus (QTL) responsible for soybean flowering time. The residual heterozygous line RHL1-156 was selected from the RILs that were derived from two distantly related varieties, *Misuzudaizu* and *Moshidou Gong 503*. The genome of RHL1-156 contains a heterozygous segment (approximately 17 cM) surrounding the *FT1* locus but is homozygous in other regions, including three other loci affecting flowering time. A large segregating population of 1,006 individuals derived by selfing of RHL1-156 included two homozygous genotypes for the nearest marker of *FT1* whose flowering time differed by 26 days. No continuous range of phenotypes was observed, in contrast to the F_2 population, suggesting that a single *FT1* locus affected the flowering time in the RHL1-156 line. Linkage analysis revealed that the *FT1* locus mapped as a single Mendelian factor between two tightly linked DNA markers, Satt365 and GM169, at distances of approximately 0.1 cM and 0.4 cM, respectively. Our results show that a RHL derived from RILs can be used to fine-map a QTL and that RHLs can be an efficient tool for a systematic fine-mapping of QTLs.

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Introduction

Quantitative trait locus (QTL) analysis allows the identification of genome regions controlling quantitative traits (Tanksley 1993). With the advent of molecular markers and the development of QTL mapping procedures, genetic and correlation analyses of quantitative traits have become possible and have contributed to a better understanding of the genetic basis of a large number of agriculturally and biologically important quantitative traits in many plant species. One technique that has been widely used to isolate the genes that have been identified as QTLs is map-based cloning. To clone genes by this method, investigators adopt a fine-mapping strategy using a series of near-isogenic lines (NILs), introgression lines, or chromosome-substitution lines. The analysis of NILs using molecular markers is an effective way to detect QTLs (Osborn et al. 1987). The ability to detect a QTL is very often affected by other QTLs also segregating in the population (Knapp and Bridges 1990). Although variation in the genetic background is important for QTL detection, we suggest that a line which has a heterozygous segment surrounding a QTL (we denote such a line as a residual heterozygous line, RHL) can also be used for improving QTL mapping for use in map-based cloning. A similar term, heterogeneous inbred family (HIF) for the selfed population of a RHL was adopted by Tuinstra et al. (1997).

In rice (*Oryza sativa* L.), 14 QTLs affecting flowering time (or heading date) have been identified using F_2 progeny, several advanced backcross progenies, and backcross inbred lines (reviewed by Yano et al. 2001). Some genes involved in flowering time have been

isolated by map-based cloning (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002). The control of flowering time is also a very important objective in soybean (*Glycine max* L.) breeding because it is critical for adapting cultivars to different cultivation areas and growing seasons. At the present time, little is known about the control of flowering time in soybean at the molecular level, and not one flowering-time gene has yet been cloned because of the complexity of the soybean genome, namely the abundance of homologous regions derived from genome duplication (Shoemaker et al. 1996). However, the recent development of a soybean linkage map based on DNA markers has enabled the mapping of QTLs for flowering time and maturity (Lee et al. 1996; Yamanaka et al. 2001). In addition, some simple sequence repeat (SSR) markers flanking the maturity loci have been identified (Molnar et al. 2003), and associations between the orthologous genes for flowering time of *Arabidopsis* and the loci for maturity have also been reported (Tasma and Shoemaker 2003).

In an earlier investigation (Yamanaka et al. 2000, 2001), we identified four QTLs for flowering time in soybean—*FT1*, *FT2*, *FT3*, and *FT4*—using the linkage map of an F_2 population derived from a cross between vars. *Misuzudaizu* and *Moshidou Gong 503*. We suggested that the major QTL for flowering time, *FT1*, corresponded to the flowering and maturity locus *E1* and showed digenic interactions with *FT2* and *FT3*. In addition, many QTLs for leaflet morphology and regions of segregation distortion were identified in the same F_2 population. The QTLs for flowering time, several seed traits, and traits related to yield were validated in the recombinant inbred lines (RILs) derived from this F_2 population (Watanabe et al. 2004).

In the study reported here, we developed a RHL selected from the RILs as a mapping material for identifying DNA markers tightly linked to the major flowering-time QTL (*FT1*) and for use in future map-based cloning. In addition, we demonstrate the potential of using a RHL derived from the RILs for fine-mapping of QTLs in soybean.

Materials and methods

Plant materials

We used 210 F_2 plants derived from a cross between two soybean (*Glycine max*) varieties, *Misuzudaizu* and *Moshidou Gong 503*, of which 190 had been used in previous studies (Yamanaka et al. 2000, 2001). A single line (named RHL1-156) from the 156 RILs (F_8) was identified as being heterozygous around the *FT1* locus based on the genotypes of the DNA markers used for previous maps (Yamanaka et al. 2001; Watanabe et al. 2004). A segregating population of 1,006 individuals (F_{10}) was derived by selfing 18 F_9 plants which had been identified as heterozygous around the *FT1* locus by scoring the genotype of the restriction fragment length polymorphism (RFLP) marker, GmN93, which is closely linked to *FT1* (Fig. 1). This population was sown in a nursery bed on May 24, 2001, transplanted to the field on June 4, 2001, cultivated by standard methods, and used for fine-mapping of *FT1*.

DNA markers

RFLP (Shoemaker and Specht 1995; Yamanaka et al. 2000) and SSR markers (Cregan et al. 1999; Hossain et al. 2000) were used for mapping *FT1*. The DNA marker GmN93 was derived from a cDNA clone encoding an early nodulin GmN93 (Yamanaka et al. 2000). GM169 is a cDNA clone whose amino acid se-

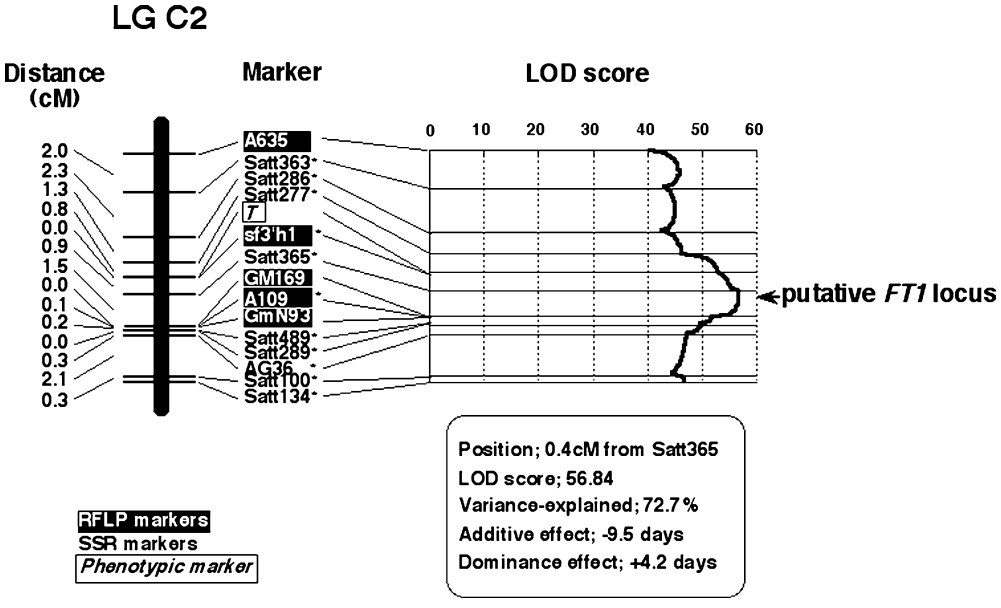

Fig. 1 QTL likelihood maps for flowering time in the middle of linkage group C2 using 210 F_2 plants. Markers denoted with an *asterisk* are the DNA markers newly mapped in the present study

quences are homologous to those of the signal transduction particle receptor-like protein of *Arabidopsis* (accession name AY133612; e-value: 2e-37). In order to convert the dominant morphological marker for pubescence color, *T*, to a co-dominant DNA marker, we developed an RFLP marker by using the cDNA clone for soybean flavonoid 3′-hydroxylase (sf3′h1) as a probe, which was isolated by Toda et al. (2002). We integrated new 11 DNA markers, including *sf3′ h1* (shown in Fig. 1), into the DNA markers previously mapped by adding 20 new individuals to the F_2 population. Eight of these DNA markers were also used for fine-mapping *FT1* in 1,006 plants. Genotyping of each marker was performed as previously described by Yamanaka et al. (2001). To analyze the genome composition of the RHL1-156 line, we checked the genotypes of this line by utilizing the known genotypes of 526 markers in the F_2 map and 300 markers in the RIL map (Yamanaka et al. 2001; Watanabe et al. 2004).

Linkage map and QTL analysis

A molecular linkage map around *FT1* was generated using 210 F_2 plants and 1,006 plants derived from RHL1-156 applying the same methodology as that reported by Yamanaka et al. (2001). In the present study, we carried out interval mapping (0.1-cM intervals) using MAPMAKER/QTL ver. 1.1 (Lander and Botstein 1989; Lincoln et al. 1993) to analyze the location and genetic effect in the F_2 population.

Fine-mapping of *FT1*

The genotypes of the DNA markers surrounding the *FT1* locus were identified, and each homozygous plant was classified into an early- or late-flowering genotype. In addition, the date of actual first flowering was recorded for each of the 1,006 individuals in the segregating population. Linkage analysis was carried out on the basis of these results.

Results

Interval mapping of *FT1* using 210 F_2 plants

A linkage map with 15 markers and a total length of 11.8 cM around the *FT1* region was constructed based on the genotype data obtained from 210 F_2 plants (Fig. 1). The average distance between markers in this region was 0.9 cM. This linkage map has 11 new DNA markers and has a higher density than that previously constructed by Yamanaka et al. (2001) using 190 F_2 individuals. A single putative QTL for flowering time was identified on this map based on 0.1-cM interval mapping. The maximum LOD score was 56.84, which occurred at approximately 1.5 cM, and was between Satt365 and GM169 (GM169 was mapped at the same position as the DNA marker A109), indicating that the *FT1* QTL was most likely located between Satt365 (accession name BH126553) and GM169. The additive effect of *FT1* was −9.5 days, and the dominance effect of *FT1* was +4.2 days. The amount of phenotypic variation explained by *FT1* was 72.7% based on calculations using MAPMAKER/QTL. This map position of *FT1* (*E1*) corresponded well to the previous reports of its position (Orf et al. 1999; Tasma et al. 2003).

Genome composition of RHL1-156

To determine the location of *FT1* more precisely, we identified an RHL—RHL1-156—in which the region around *FT1* is heterozygous and other regions of the genome are homozygous by selection from the RIL population (Watanabe et al. 2004). Based on the genotypes of the markers in the F_2 population and the RILs (Yamanaka et al. 2001; Watanabe et al. 2004), we were able to identify the genome constitution of RHL1-156 (Fig. 2). RHL1-156 has five pairs of chromosomes without any crossovers, four pairs with one crossover, six pairs with two crossovers, and two pairs with three crossovers. Three regions of the chromosomes could not be classified because the genotype data were lacking in the regions on linkage group (LG) C1 and LG J (indicated by white boxes in Fig. 2) and there was a linkage gap in LG M.

Overall, the ratio of *Misuzudaizu* homozygous segments to *Moshidou Gong 503* homozygous segments in RHL1-156 was approximately 1.4:1, calculated as genetic distance. The heterozygous region of RHL1-156 was located on LG C2, where *FT1* was mapped. The regions of three other QTLs for flowering time were homozygous. Therefore, RHL1-156 was identified as a RHL that is heterozygous in the *FT1* region.

Variations in flowering time in an RHL-derived population

The next generation of this RHL had the genetic effects for flowering time fixed at all genetic regions except for those near the *FT1* locus. This enabled us to handle the *FT1* locus as a single Mendelian factor in a population consisting of 1,006 plants derived from RHL1-156. We observed a clear segregation into early- and late-flowering phenotypes (Fig. 3), which strongly suggests the presence of a QTL for flowering time within the approximately 17-cM-long heterozygous region between RFLP markers A063 and A538a. These two markers were homozygous in RHL1-156 and located above A635 and below Satt134 in LG C2, respectively (Fig. 1).

In the group of plants that was homozygous for the *FT1* allele of *Moshidou Gong 503*, the time to flowering was the shortest, with an average time to flowering of 41.9 days (range: 38–48 days; Table 1). In the group

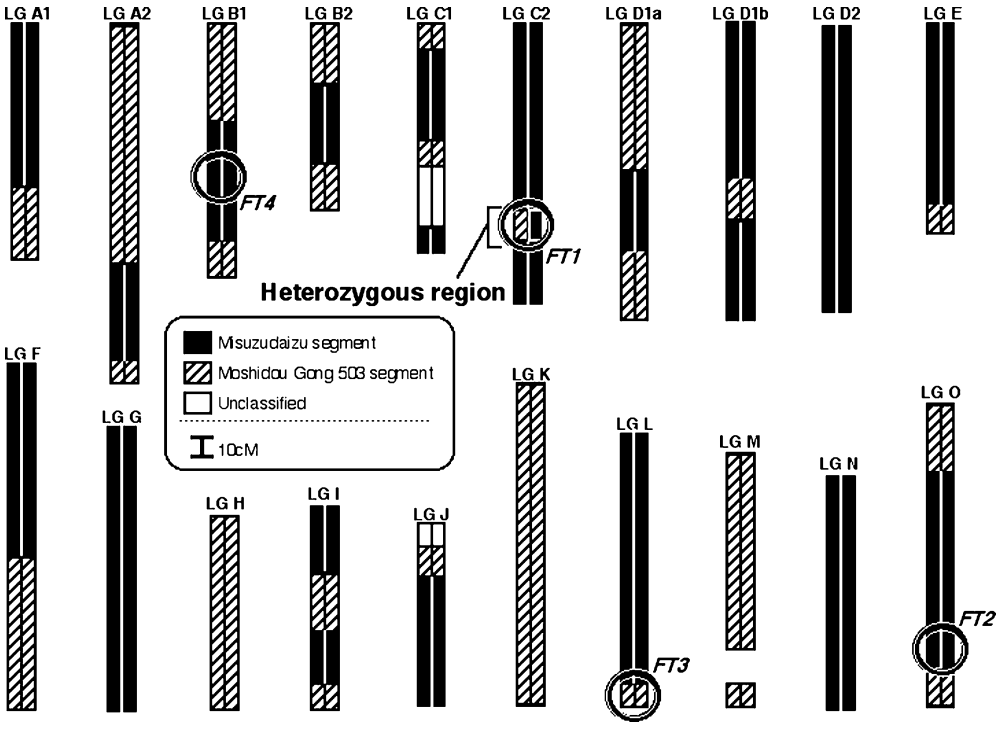

Fig. 2 Graphical genotype of the RHL1-156 line determined from 416 markers. *Solid bars* and *bars with diagonal lines* represent the *Misuzudaizu* segments and *Moshidou Gong 503* segments, respectively. *Open bars* represent unclassified segments. Putative location of each QTL is *circled*

that was heterozygous for the *FT1* locus of *Misuzudaizu* and *Moshidou Gong 503*, the flowering time was intermediate, with an average of 60.6 days (range: 51–68 days), while in the group that was homozygous for the *FT1* locus of *Misuzudaizu*, the flowering time was the longest, with an average of 67.9 days (range: 63–75 days). The time required for the heterozygous plants to flower was clearly longer than that required by homozygous plants of *Moshidou Gong 503* and slightly shorter than that required by homozygous plants of *Misuzudaizu*, indicating that the *Misuzudaizu* allele of *FT1* is semi-dominant over the *Moshidou Gong 503* allele. In addition, the variability in flowering time within the *Moshidou Gong 503* homozygous genotype was relatively high compared with the other two genotypes, based on the coefficient of variation (CV in Table 1).

From Table 1 shows that 234 plants had an early flowering time, 523 had an intermediate flowering time, and 249 had a late flowering time. These values were close to the ratio of 1:2:1 expected for single Mendelian segregation ($\chi^2 = 2.038$).

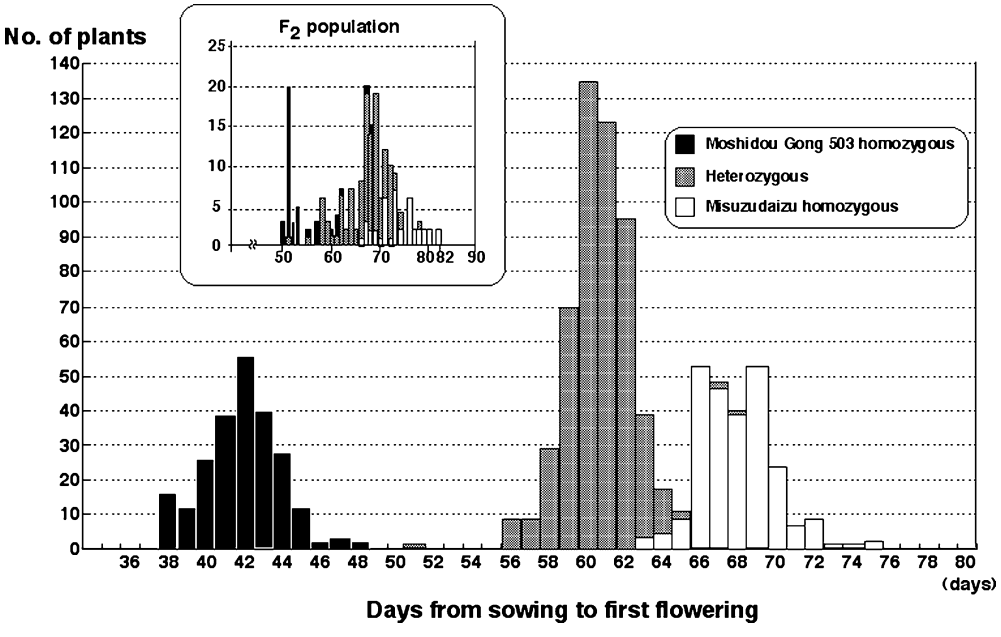

Fig. 3 Frequency distribution of flowering time of 1,006 individuals in a segregating population derived from RHL1-156. *Shading* indicates the genotypes of the *FT1* locus. *Insert* Frequency distribution of flowering time in F_2 population (Yamanaka et al. 2000) is shown for comparison

Table 1 Mean value and the number of plants in the RHL1-156-derived population

FT1 genotype	Number of plants	Range (days)	Mean ± standard deviation (days)	MS	Coefficient of variance
Moshidou Gong 503 homozygote	234	38–48	41.9 ± 1.9	3.712	4.597
Heterozygote	523	51–68	60.6 ± 1.7	2.958	2.840
Misuzudaizu homozygote	249	63–75	67.9 ± 2.0	3.990	2.944
Total	1,006	38–75			

Fine-mapping of *FT1*

Based on these 1,006 plants, we were able to construct a linkage map around the *FT1* locus and locate the *FT1* between Satt365 (BH126553) and GM169 (homolog of AY133612) as a single Mendelian factor (Fig. 4). The location of *FT1* was almost the same as that of the LOD peak in the QTL analysis of the F_2 population (Fig. 1). Comparison of the linkage map derived from 1,006 plants with the map from 210 F_2 plants showed a slight difference in the recombination frequencies. The most remarkable difference in the genetic distance was observed between Satt365 and GM169, where *FT1* was located. In the F_2 population, the distance between Satt365 and GM169 was 1.5 cM, and the flowering-time QTL was located at a distance of 0.4 cM from Satt365 (Fig. 1). In the RHL-derived population, however, the distance between Satt365 and GM169 was only 0.4 cM. Analysis with MAPMAKER did not reveal any difference in the location between Satt365 and *FT1*; however, one recombinant between these two loci was found among the 1,006 plants, indicating that *FT1* is located at a distance of approximately 0.1 cM from Satt365.

Discussion

For the fine-mapping of QTLs, it is necessary to carry out high-resolution linkage analysis using a large number of plants that segregate only around the QTL being investigated. However, the development of such populations for each QTL of interest is extremely laborious. In the investigation reported here, we succeeded in fine-mapping the soybean flowering-time QTL using an RHL derived from an RIL. This strategy has two main advantages over using an NIL developed by backcrossing. The first is that in developing the population for fine-mapping, only one line from an RIL population need be selected on the basis of its genotype; repeated backcrossings and selections based on DNA markers or phenotypes are not required. The second advantage is that the genomic composition of a RHL can be determined merely by checking the genotype data of the linkage map; it is not necessary to analyze the genotypes of any of the markers. We were successful in selecting an RHL (RHL1-156) from the RILs that was heterozygous for the *FT1* region. We were then able to determine the graphical genotype of RHL1-156 by using the genotypes of the single plant in the F_2 population and the RIL1-156 from which the RHL was derived. With respect to the genome composition of RHL1-156, the genotypes of 116 markers were homozygous, as determined in the plant of the F_2 population, while 291 of 300 markers, with the exception of nine markers around the *FT1* locus, were identified as being homozygous in the RIL. Based on these facts, we conclude that this RHL-based mapping strategy is a useful and simple protocol for the fine-mapping of QTLs and that it also facilitates the isolation of genes identified as QTLs. This method has only a few intrinsic disadvantages: (1) if the size of the RIL population is too small, it may be difficult to find the desired RHL; (2) it is impossible to detect interactions among QTLs by crossing each NIL due to the difference in the NIL background.

Due to the efficiency of this technique, we were able to perform fine-mapping of *FT1* QTL as a single gene. The position we determined was almost the same as that of the LOD peak found using 210 F_2 plants due to the large effect of *FT1* (Fig. 1). If, in contrast, a QTL having a small genetic effect is influenced strongly by other loci, or shows a relatively low heritability, the position obtained from the QTL analysis using an F_2 or RIL population might differ from that obtained by RHL-derived population. An RHL might then be more effective for the precise mapping of such a less-than-ideal QTL. Further studies should be carried out to demonstrate usefulness of RHL1-156.

While we successfully used the RHL1-156 line for fine-mapping *FT1*, other QTLs are also located around this region, such as loci for germination rate, seed hardness, water absorbance, seed weight, and leaflet shape (Yamanaka et al. 2001; Watanabe et al. 2004). Therefore, this population could also be used for the fine-mapping of these QTLs.

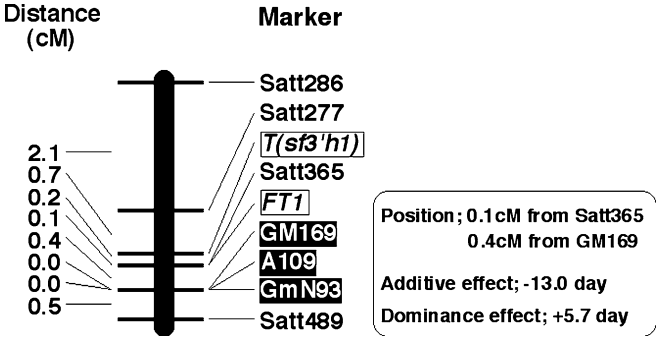

Fig. 4 Position and genetic effects of *FT1* determined using 1,006 plants

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