A major QTL, *qPDH1*, is commonly involved in shattering resistance of soybean cultivars

Tetsuya Yamada¹, Hideyuki Funatsuki*², Seiji Hagihara³, Shohei Fujita⁴, Yoshinori Tanaka⁵, Hiroyuki Tsuji⁶, Masao Ishimoto⁷, Kaien Fujino⁸ and Makita Hajika⁹

1) National Institute of Crop Science (NICS), 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan
2) National Agricultural Research Center for Hokkaido Region (NARCH), Hitusijigaoka, Sapporo, Hokkaido 062-8555, Japan
3) Tokachi Agricultural Experiment Station (TAES), Shinsei, Memuro, Hokkaido 082-0071, Japan
4) Hokkaido Central Agricultural Experiment Station (HCAES), Naganuma, Hokkaido 069-1395, Japan
5) Department of Crop Physiology, Graduate School of Agriculture, Hokkaido University, 9 Kita 9 Nishi, Sapporo, Hokkaido 060-8589, Japan

A major quantitative trait locus (QTL) controlling pod dehiscence (shattering) in soybean, designated *qPDH1*, has previously been identified using progeny of shattering-resistant cultivars derived from a Thai cultivar, SJ2. The QTL was located near a simple sequence repeat marker, Sat_366, on linkage group J. To determine whether shattering-resistance genes originating from different resources are located at *qPDH1* in general, we conducted genetic analysis using DNA markers for several populations. In an F₂ population derived from a cross between a shattering-susceptible cultivar, Toyomusume, and a shattering-resistant cultivar, Harosoy, a major QTL for pod dehiscence was identified in the region near *qPDH1*, which was confirmed in the progeny of F₄₅ populations. A major QTL was identified near *qPDH1* also in F₂ populations derived from crosses including Wasekogane and Kariyutaka as shattering-resistant parents. The heterozygous genotypes at the QTL showed high degrees of pod dehiscence, suggesting that shattering resistance behaves as a nearly recessive trait. In F₂ populations derived from crosses between shattering-resistant cultivars, heterozygous genotypes at the Sat_366 locus were shattering-resistant. These results suggest that shattering-resistant cultivars harbor recessive shattering-resistance allele(s) at *qPDH1* regardless of their origin and that molecular markers near *qPDH1* could be used for marker-assisted selection for shattering resistance in soybean.

Key Words: *Glycine max* (L.) Merr., pod dehiscence, MAS (marker-assisted selection), QTL, Genetic resources.

Introduction

Pod dehiscence (shattering) leads to a significant yield loss in soybean (*Glycine max* (L.) Merr.) production. Shattering resistance has been introduced into leading cultivars in some regions, including North America (Bailey et al. 1997), where soybean cultivation has been carried out on a large scale, while other regions still face the problem of pod dehiscence (Bhatnagar and Karmakar 1995, Jiang et al. 1991, Tiwari and Bhatnagar 1991, Tukamuhabwa et al. 2002). In Japan, shattering resistance has been conferred to several cultivars, including the leading cultivar in Hokkaido. However, few cultivars with shattering resistance are available in the southwestern part of Japan, and losses due to shattering have been reported to be up to 422 kg/ha (Shirota et al. 2001). A stable soybean production system requires shattering-resistant cultivars under the recent conditions of widespread use of combine harvesters and frequent occurrence of high temperatures after maturation of soybean.

To accelerate breeding of shattering-resistant cultivars that could be grown widely throughout Japan, the development of molecular markers for selection is needed. Recently, a major quantitative trait locus (QTL), designated *qPDH1*, was identified using recombinant inbred lines (RILs) derived from a cross between shattering-susceptible and -resistant cultivars of Hokkaido (Funatsuki et al. 2006). The shattering-resistant cultivar used was Hayahikari, the shattering-resistant nature of which is derived from a Thai cultivar, SJ2 (Yumoto et al. 2000). The QTL was found to be located between simple sequence repeat (SSR) markers, Sat_366 and Sat_093, on linkage group J (LG J, according to the study of Song et al. 2004), accounting for more than 50% of the total variance (Funatsuki et al. 2006, Funatsuki et al. 2008). In addition, the shattering-resistance allele from SJ2 has been demonstrated to be useful, regardless of genetic background and culture conditions (Funatsuki et al. 2008).

In addition to SJ2, two other sources of germplasm for shattering resistance have been used for soybean breeding in Hokkaido (Tsuchiya 1986). One of them is the introduction of accessions from North America, and the other is germplasm...
accessions derived from China. For example, an American shattering-resistant line, Clark-Dt2 (L62-1251), was used to develop Kariyutaka (Tanaka et al. 1993), and the shattering resistance of Wasekogane was derived from a Chinese cultivar, Zihua 4 (Japan Legume Foundation Association 1991). North American and Chinese accessions are used for breeding of Japanese soybean cultivars, typically for introduction of pest resistance (e.g., Harosoy from Canada, for resistance to soybean mosaic virus). Since marker-assisted selection (MAS) for pest resistance has been started in breeding programs (Ishimoto 2005), the identification of the markers for shattering resistance derived from these accessions would enable simultaneous selection for these traits, contributing to the improvement of breeding efficiency. A major QTL associated with shattering resistance of an American cultivar, Young, was identified near qPDH1 on LG J (Bailey et al. 1997). However, QTLs associated with shattering resistance of other cultivars have not been identified.

In the present study, we conducted QTL analysis for shattering resistance derived from genetic resources other than SJ2. The objectives of this study were to determine whether shattering-resistance genes of various origins are located at qPDH1 in common, and, if not, to identify new QTLs controlling pod dehiscence in soybean.

Materials and Methods

Plant materials

The cultivars used for crossing are listed in Table 1. An F2 population (n = 96) derived from a cross of Toyomusume (shattering-susceptible) × Harosoy (resistant) was used for QTL analysis scanning the entire genome. RILs of the same combination at the generations of F2 were used for confirmation of putative QTLs. F2 populations derived from crosses of Kariyutaka (resistant) × Sachiyutaka (susceptible) (n = 126) and Wasekogane (resistant) × Sachiyutaka (n = 164) were used for QTL analysis of LG J. F2 populations derived from crosses of Kariyutaka × Hayahikari (resistant) (n = 96) and Wasekogane × Yukihomare (resistant) (n = 158) were used for single-marker analysis with Sat_366. Hayahikari and Yukihomare carry the shattering-resistant allele from SJ2 at pPDH1.

Evaluation of shattering resistance

The degree of shattering resistance was evaluated by monitoring the percentage of dehiscent pods after heat treatment at 60°C for 1 or 3 h in circulation driers. For the F2 plants, 10 to 20 pods per plant were examined. For RILs, 10 plants per line were harvested and 10 pods per plant were heat-treated. The percentages of dehiscent pods were recorded for individual plants.

Data analysis

Percentages of dehisced pods were converted into arcsine-transformed values before performing one-way analysis of variance (ANOVA) using the GLM procedure of the SAS program (SAS Institute 1996).

Genotyping with SSR markers

DNA was isolated from leaves or seeds as described by Funatsuki et al. (2008). Polymerase chain reaction (PCR) for SSR markers was performed, and the products were

Table 1. Soybean cultivars used as shattering-resistant and -susceptible parents

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shattering resistance</th>
<th>Source of resistance</th>
<th>Allele at SSR marker loci</th>
<th>Location*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayahikari</td>
<td>Resistant</td>
<td>SJ2 (Thailand)</td>
<td>A</td>
<td>Hokkaido</td>
</tr>
<tr>
<td>Yukihomare</td>
<td>Resistant</td>
<td>SJ2</td>
<td>A</td>
<td>Hokkaido</td>
</tr>
<tr>
<td>Kariyutaka</td>
<td>Resistant</td>
<td>Clark-Dt2 (U.S.)†</td>
<td>B</td>
<td>Hokkaido</td>
</tr>
<tr>
<td>Wasekogane</td>
<td>Resistant</td>
<td>Zihua 4 (China)</td>
<td>B</td>
<td>Hokkaido</td>
</tr>
<tr>
<td>Harosoy</td>
<td>Resistant</td>
<td>Unknown</td>
<td>B</td>
<td>Canada</td>
</tr>
<tr>
<td>Toyomusume</td>
<td>Susceptible</td>
<td>—</td>
<td>B</td>
<td>Hokkaido</td>
</tr>
<tr>
<td>Sachiyutaka</td>
<td>Susceptible</td>
<td>—</td>
<td>B</td>
<td>Kyushu</td>
</tr>
</tbody>
</table>

*Location at which the cultivar was developed.
†Line name: L62-1251.
A major QTL associated with shattering resistance of soybean cultivars analyzed as described previously (Funatsuki et al. 2005, Ikeda et al. 2009).

Map construction

The parental cultivars were tested with SSR markers previously placed on integrated soybean genetic linkage maps (Yamanaka et al. 2001, Song et al. 2004). In the regions where markers were placed densely and polymorphic markers had previously been found, no more markers were screened. For the F<sub>2</sub> population derived from a cross between Toyomusume and Harosoy, 161 polymorphic markers were chosen to avoid redundancy in terms of map position. Ninety-six F<sub>2</sub> plants were genotyped with these SSR markers. MAPMAKER/EXP 3.0b (Lander et al. 1989) and MapManager QTX (Manly et al. 2001) were used to construct linkage maps as described by Ikeda et al. (2009). As for RILs, the heterozygous genotype at each marker locus was dropped from the data set according to the programs used. The analysis employed the default values including Kosambi and Haldane map functions for F<sub>2</sub> and RIL populations, respectively. The nomenclature of LGs was according to the study of Song et al. (2004), based on the common markers mapped. The maps of LG J for the other populations were constructed by the same procedure.

QTL analysis

QTL analysis was performed using the arcsine-transformed values of percentages of dehisced pods and the linkage maps constructed as described above. Single-marker regression analysis was performed using MapManager QTX. Composite interval mapping (CIM) was performed using QTL Cartographer ver. 2.5 (Wang et al. 2007). We ran CIM model 6 with a window size of 10 cM. Marker covariates for CIM were identified using a stepwise forward and backward regression (P = 0.1). The genome was scanned at 2-cM intervals. One thousand permutation tests were performed for the F<sub>2</sub> population derived from a cross of Toyomusume × Harosoy to establish empirical logarithm of the odds (LOD) thresholds at the 5% α level for experimentwise Type I error (Churchill and Doerge 1994).

Results

QTLs associated with shattering resistance of Harosoy

For the F<sub>2</sub> population derived from a cross of Toyomusume × Harosoy, the resulting genetic linkage map covered 2780 cM. Few or no polymorphic markers were found in some regions, resulting in the separation of several of 20 LGs constructed by Song et al. (2004). Consequently, the markers constituted 26 LGs and one unlinked marker.

QTL analysis of shattering resistance was conducted for the F<sub>2</sub> population using the linkage map constructed. Composite interval mapping at the empirical threshold value (LOD = 3.8) revealed the presence of two QTLs on LG J and LG A2 (Table 2). The QTL on LG J was located in the region near qPDH1 (Fig. 1A). The LOD score of this QTL was

---

**Fig. 1.** LOD score plot on LG J for the QTL associated with shattering resistance in four segregating populations. A, F<sub>2</sub> plants derived from a cross of Toyomusume × Harosoy. B, F<sub>2</sub> RILs derived from a cross of Toyomusume × Harosoy. C, F<sub>2</sub> plants derived from a cross of Kariyutaka × Sachiyutaka. D, F<sub>2</sub> plants derived from a cross of Wasekogane × Sachiyutaka. E, Consensus linkage map of SSR markers used for analyses based on the study of Song et al. (2004) with a modification in the positions of Satt620 and Satt621, which were calculated with the population used in Funatsuki et al. (2008).
To confirm the locations and the effects of the QTLs, QTL analysis was also conducted using the F4:5 RIL populations. A significant QTL with the LOD peak at Satt621 was detected (Fig. 1B) near qPDH1 (Fig. 1E). The LOD score was 9.7 and the QTL accounted for 34% of total variance (Table 2). No LOD peak was detected for the other minor QTL on LG A2.

Mapping of QTL for shattering resistance on LG J

Since the results described above suggested that the shattering resistance gene of Harosoy is located at qPDH1 as in the case of Hayahikari, two other F2 populations segregating for shattering resistance were used for composite interval mapping of QTL for shattering resistance on LG J. The marker Sat_366 was not used for these populations since no polymorphism was obtained (Table 1). In the population derived from a cross of Kariyutaka × Sachiyutaka, an LOD peak was detected between Satt620 and Sat_350 with a value of 50.1 (Fig. 1C and Table 2). This QTL explained 86% of total variance. In the population derived from a cross of Wasekogane × Sachiyutaka, an LOD peak was also found between Satt620 and Sat_350 with a value of 42.6 (Fig. 1D and Table 2).

Since the QTL analyses suggested that the shattering-resistant allele is recessive (Table 2), the percentages of dehiscent pods were compared among marker genotypes at Satt620 in these segregating populations to examine the dominance effect of these QTLs. The heterozygous genotypes showed nearly the same percentage of dehiscent pods as the homozygote for the Sachiyutaka genotype in both populations (Fig. 2A and 2B). These data suggested that the shattering-resistance alleles at the QTLs had a recessive effect.

Allelism test of shattering-resistance genes using SSR marker

To compare these shattering-resistance genes with that of SJ2 at qPDH1, the F2 populations derived from crosses between a shattering-resistant cultivar derived from SJ2 and a shattering-resistant cultivar derived from other resources were examined for genotype at Sat_366 and pod dehiscence percentage (Fig. 3). Since the shattering-resistance alleles at the QTLs on LG J were shown to be recessive, the heterozygous genotypes for this genomic region should be more susceptible to pod dehiscence than the homozygous ones, if the QTLs associated with the shattering resistance of Kariyutaka and Wasekogane differed from qPDH1. Indeed, the heterozygous genotypes exhibited percentages of dehiscent pods similar to those for the homozygous ones, which was confirmed by analysis of variance with arcsine-transformed values of pod dehiscence percentage indicating no significant effect of marker genotype. On the whole, the percentages

---

**Table 2. Summary of quantitative trait loci (QTLs) for shattering resistance**

<table>
<thead>
<tr>
<th>Parents</th>
<th>Generation</th>
<th>Position</th>
<th>LOD</th>
<th>r²</th>
<th>Additive effect</th>
<th>Dominance effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toyomusume (f) Harosoy (m)</td>
<td>F2</td>
<td>J</td>
<td>Satt621</td>
<td>10.2</td>
<td>0.31</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>Satt409</td>
<td>3.8</td>
<td>0.11</td>
<td>10.3</td>
</tr>
<tr>
<td>Sachiyutaka (m) Kariyutaka (f)</td>
<td>F4:5</td>
<td>J</td>
<td>Satt621</td>
<td>9.7</td>
<td>0.34</td>
<td>8.8</td>
</tr>
<tr>
<td>Sachiyutaka (m) Wasekogane (f)</td>
<td>F2</td>
<td>J</td>
<td>Sat_350</td>
<td>50.1</td>
<td>0.86</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F2</td>
<td>Satt620</td>
<td>42.6</td>
<td>0.65</td>
<td>22.3</td>
</tr>
</tbody>
</table>

a Linkage groups (LG) were designated according to Song et al. (2004).
b The marker closest to the QTL.
c Arcsine-transformed value. The additive effect indicates the effect of alleles from the shattering-susceptible parent.
d Arcsine-transformed value.
e f: female parent, m: male parent.
of dehiscent pods were relatively high, probably due to the prolonged period for drying outdoors prior to heat treatment and the low ambient temperature and humidity. However, considering that the shattering-susceptible cultivar Toyomusume dehisced 100% of pods with the shattering-resistant parental cultivars displaying 50–80% of pod dehiscence under the same conditions, all genotypes were considered to be shattering-resistant.

Discussion

There is considerable genetic diversity among soybean cultivars with regard to shattering resistance (Caviness 1965, Tsuchiya 1986, Helms 1994, Romkaew and Umezaki 2006). QTL analyses revealed the presence of one major QTL in each population derived from a cross between shattering-resistant and -susceptible cultivars in previous studies (Bailey et al. 1997, Funatsuki et al. 2006). In the present study, a major QTL was detected also in the segregating populations derived from a cross between Toyomusume (susceptible) and Harosoy (resistant). The linkage map of the F₂ population revealed that all genotypes, including the heterozygote, were shattering-resistant. Considering that the shattering resistance conferred by the QTL on LG J is a nearly recessive trait (Fig. 2), the shattering-resistance genes of various genetic resources are likely to be alleles at qPDH1. The SSR markers around qPDH1, such as Satt621, Satt620, and Sat_366, could be widely used for marker-assisted selection (MAS) for shattering resistance in soybean.

Although qPDH1 has a large effect, there are likely to be some minor QTLs for shattering resistance in soybean. Tsuchiya (1986) estimated up to three genes for shattering resistance. Bailey et al. (1997) identified several minor QTLs for shattering resistance on linkage groups other than LG J. We also identified a minor QTL in the F₂ population derived from a cross between Toyomusume and Harosoy. Since this minor QTL could not be confirmed in the advanced generations, it may be involved in shattering resistance, along with interactions with environmental conditions. Further research is needed to confirm the effects and the locations of minor QTLs.

In conclusion, we demonstrated that most shattering-resistant cultivars have a shattering-resistance gene with a large effect in the genomic region including qPDH1. The genes are likely to be alleles at qPDH1. The molecular markers around qPDH1 could be used for MAS in progeny derived from various cross-combinations of shattering-resistant and -susceptible parents. These molecular markers are especially useful for introduction of the trait by backcrossing, since shattering resistance conditioned by qPDH1 behaves as a nearly recessive trait.

Acknowledgments

The authors thank R. Narita, S. Furuhata, Y. Ogasawara, K. Yoshida, and R. Sugisawa for their technical assistance. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries (Research and Development Projects for Application in Promoting New Policy of Agriculture Forestry and Fisheries, No. 18038).
Literature Cited


