Introduction

Soybean mosaic virus (SMV), a member of the genus Potyvirus, is one of the most devastating diseases of soybean [Glycine max (L.) Merr.]. SMV is carried to some extent through seeds from infected soybean plants and transmitted by aphids in a non-persistent manner (Hashimoto and Nagasawa 1986). Plants infected with SMV show mosaic discoloration and deformation of leaves and seed motting, which reduce seed yield and degrade seed quality (Koshimizu and Iizuka 1963, Yamada et al. 2006). The virus is classified into six strains, A, A2, B, C, D, and E, on the basis of pathogenicity to a set of Japanese differential varieties (Nakano et al. 1982, Takahashi et al. 2000). SMV strains A and B are found in all parts of Japan, and strains C and D are mainly found in Kanto, Hokuriku, and the middle and southern parts of the Tohoku region (Hashimoto and Nagasawa 1986, Takahashi et al. 1980, Yagasaki et al. 2000). Thus, resistances to SMV strains C and D are indispensable characteristics in the breeding of cultivars for these areas. In Japan, cultivars suitable for late sowing after wheat harvesting are needed for rotations, including rice, wheat, and soybean cultivations. However, late planting sometimes lead to late maturation that delays harvesting and/or to a decrease of seed weight that degrades the quality of soybean food products such as boiled bean (Beatty et al. 1982, Ishida and Tange 1984). Therefore, a cultivar with large seed weight and early maturation is needed. The cultivar ‘Ohsuzu,’ which was developed by the National Agriculture and Food Research Organization (NARO): Tohoku Agricultural Research Center in 1998 (Tabuchi et al. 1999), possesses desirable agricultural characteristics including large seed, early maturation, and high adaptability to plant growth in all regions.

Transfer of the \textit{Rsv3} locus from ‘Harosoy’ for resistance to \textit{soybean mosaic virus} strains C and D in Japan

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Resistance to \textit{soybean mosaic virus} (SMV) is imperative for soybean (\textit{Glycine max} (L.) Merr.) production in the Tohoku region. Molecular markers for SMV resistance were previously reported for U.S. SMV strains, but they cannot be applied because of the differences in strain classification between Japan and the U.S. A U.S. variety ‘Harosoy’ has been used mainly as a donor of resistance to SMV strains C and D in a Japanese breeding program, resulting in resistant varieties such as ‘Fukuibuki.’ Because ‘Harosoy’ harbors the \textit{Rsv3} gene conferring resistance to the virulent SMV strain groups, G5 through G7, it appears that the \textit{Rsv3} gene confers resistance to strains C and D. In this study, we introduced resistance to the two strains from ‘Fukuibuki’ into a leading variety ‘Ohsuzu’ by recurrent backcrossing with marker-assisted selection. All lines selected with markers near \textit{Rsv3} showed resistance to the strains, suggesting that the \textit{Rsv3} locus is responsible for the resistance. Three years of trials showed that one of the breeding lines, ‘Tohoku 169,’ was equivalent to ‘Ohsuzu’ with respect to agricultural characteristics such as seed size, maturity date, and seed yield, except for the SMV resistance.

\textbf{Key Words:} \textit{Glycine max}, \textit{soybean mosaic virus}, SMV strains, \textit{Rsv3}, marker-assisted selection.
parts of the Tohoku region, the Hokuriku region, and northern parts of the Kanto region (Tabuchi et al. 1999), but its cultivation is limited because of its susceptibility to SMV strains C and D. Development of a new cultivar having resistance to strains C and D and favorable agricultural characteristics similar to those of ‘Ohsuzu’ has been requested. To introduce resistance to strains C and D into ‘Ohsuzu,’ one of the most efficient methods is to develop backcross lines using ‘Ohsuzu’ as a recurrent parent by marker-assisted selection. However, DNA markers for resistance genes to strains C and D have not been developed.

In Japan, ‘Harosoy’ (PI 548573) (PI, plant introduction number in the United States Department of Agriculture Agricultural Research Service National Plant Germplasm System) has been used as a donor of resistance to SMV strains C and D, and some resistant cultivars, e.g., ‘Suzuyutaka,’ ‘Dewamurasume,’ and ‘Fukuibuki,’ have been developed by NARO: Tohoku Agricultural Research Center (Hashimoto et al. 1984, Ishikawa et al. 1979, Shimada et al. 2004). Shigemori (1991) studied the mode of inheritance of SMV resistance by artificial inoculation tests in a greenhouse using F$_1$ and F$_2$ populations derived from three or five crosses between susceptible cultivars and the resistant cultivar ‘Suzuyutaka,’ whose resistances to SMV strains C and D originated in ‘Harosoy.’ It was concluded that each resistance to SMV strain C or D derived from ‘Harosoy’ was controlled by a single dominant gene and that the genes for resistance to strains C and D were strongly linked to each other, although it was unclear whether the single gene for resistance to SMV strain C was the same one as that to SMV strain D.

In the U.S., SMV has been classified into seven strains, G1, G2, G3, G4, G5, G6, and G7, on the basis of pathogenicity on a set of U.S. differential varieties, which differ from the Japanese varieties (Cho and Goodman 1979). Based on the genetic analysis of resistance to each strain, three independent single dominant resistance genes, Rsv$_1$, Rsv$_3$, and Rsv$_4$, have been identified and mapped on chromosomes 13, 14, and 2, respectively (Gore et al. 2002, Hayes et al. 2000, Hayes and Sajjadi Maroof 2000, Jeong and Saghai Maroof 2004, Saghai Maroof et al. 2010, Shi et al. 2008). Nine resistance alleles of Rsv$_1$ have been previously reported, and these Rsv$_1$ alleles confer resistance to strains G1–G3, although the reactions to strains G4–G7 differed depending on the alleles of Rsv$_1$ (Moon et al. 2009). In contrast, lines carrying a resistance allele of Rsv$_3$ showed resistance to strains G5–G7 and susceptibility to strains G1–G4 (Gunduz et al. 2001). The resistance alleles of Rsv$_1$ and Rsv$_3$ are strain-specific and associated with hypersensitive reaction. Hayes et al. (2004) and Suh et al. (2011) have identified nucleotide-binding leucine-rich repeat clusters as candidate genes of Rsv$_1$ and Rsv$_3$. Rsv$_4$ confers resistance to all strains from G1 to G7 and is suggested to reduce infection by restricting short- and long-distance movement of the virus (Gunduz et al. 2004). In addition, Gunduz et al. (2001) have shown, by investigating reactions to the SMV strains from G1 to G7, that ‘Harosoy’ carries susceptible alleles at the Rsv$_1$ and Rsv$_4$ loci and a resistance allele at the Rsv3 locus.

To exploit available information for introducing resistances to strains C and D into Japanese cultivars, relationships between the Japanese and U.S. strains of SMV should be clarified. Shigemori (1991) investigated the relationships by the artificial inoculation of U.S. differential varieties with the Japanese strains. Consequently, the Japanese strains were classified into three groups: one containing A and B, one containing C and D, and the other containing only E. Although strains A and B corresponded to strain G3, strains C, D, and E corresponded to no U.S. strains. Kanematsu and Nakano (2015) artificially inoculated the Japanese differential varieties with the U.S. strains. The U.S. strains were also classified into three groups: one containing G1 and G4; one containing G2, G3, G6, and G7; and the third containing only G5. Strains A and B corresponded to the group of G2, G3, G6, and G7 and the group of G1 and G4, respectively, and the pathogenicity of strain C was similar to that of G5, whereas strains D and E corresponded to no U.S. strains. The authors accordingly reported that it was impossible to clarify the complicated relationship between Japanese and U.S. strains using differential varieties. Thus, DNA markers for SMV resistance reported in the U.S. could not be used for soybean breeding in Japan.

Considering that ‘Harosoy’ has been used as a donor of resistance to SMV strains C and D in Japan and that these two resistances are controlled by a single dominant gene, the Rsv$_3$ allele derived from ‘Harosoy’ is expected to confer resistances to strains C and D. In the present study, to verify the effect of the Rsv$_3$ allele on the resistances to SMV strains C and D, the alleles of DNA markers covering a genomic region containing Rsv$_3$ were compared between ‘Harosoy’ and ‘Fukuibuki,’ whose resistances to SMV strains C and D are derived from ‘Harosoy.’ In addition, backcross lines were developed for introducing the Rsv$_3$ alleles derived from ‘Fukuibuki’ into ‘Ohsuzu’ (susceptible to SMV strains C and D) by marker-assisted selection to develop breeding lines with favorable agricultural characteristics similar to those of ‘Ohsuzu.’ The effect of the genomic regions near Rsv$_3$ on agricultural characteristics was evaluated with yield trials.

**Materials and Methods**

**Plant material**

‘Ohsuzu’ and ‘Fukuibuki’ were used as plant materials (Supplemental Fig. 1). ‘Ohsuzu’ and ‘Fukuibuki’ were registered as ‘Soybean Norin No. 109’ in 1998 and ‘Soybean Norin No. 122’ in 2002, respectively. ‘Ohsuzu,’ which is susceptible to strains C and D, is classified into medium-early maturity in the Tohoku region and has large seed size at approximately 350 mg per seed (Tabuchi et al. 1999). ‘Fukuibuki,’ which is resistant to strains C and D, is classified into medium-late maturity in the Tohoku region and has a medium large seed size at approximately 300 mg per seed.
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et al. (2010), are indicated as B1403, B1408, B1410, B1414, and B1416, respectively. Additionally, BC2F1 plants and a BC2F2 population were grown in 2004 and 2005, respectively, and the seeds of BC2F2:3 lines were obtained for inoculation tests. Differential varieties of SMV artificial inoculation tests and their resistances to SMV strains C and D were as follows: ‘Peking’ (JP28432) (JP, plant accession number of National Institute of Agrobiological Sciences Genebank), ‘Harosoy,’ ‘Dewamusume’ (resistant to the C and D strains), ‘Shiromame’ (JP27560) (resistant to the C strain, susceptible to the D strain), ‘Fukusennari’ (JP28295) (susceptible to the C strain, resistant to the D strain), ‘Ouu 3’ (JP27779), ‘Tokachinagaha’ (JP27439), ‘Nemashirazu’ (JP27696), and ‘Norin 4’ and ‘Tsurunotamago 1’ (JP27731) (susceptible to the C and D strains) (Hashimoto and Nagasawa 1987, Ishikawa et al. 1979, Takahashi et al. 1987).

Development of backcross lines
Backcross lines were developed from a cross between ‘Ohsuzu’ (susceptible to SMV strains C and D) as a recurrent parent and ‘Fukuibuki’ (resistant to SMV strains C and D) as a donor of the resistance allele of Rsv3 (Supplemental Fig. 1). ‘Ohsuzu’ and ‘Fukuibuki’ were crossed in 2001, and backcrosses to ‘Ohsuzu’ were repeated five times from 2002 to 2006. Following each backcross, DNA markers near the Rsv3 locus, namely Satt726 and M3SattM, were genotyped, and heterozygous plants were selected as pollen parents (Fig. 1). BC2F1 plants were grown in 2007, and then, plants homozygous for the ‘Fukuibuki’ allele were selected from a BC2F2 population in 2008 based on the alleles of DNA markers Satt726, BARCSOYSSR_14_1403, and M3SattM (Fig. 1).

DNA marker analysis
Marker alleles located near Rsv3 of ‘Ohsuzu,’ ‘Fukuibuki,’ and ancestors of ‘Fukuibuki’ were investigated. According to Suh et al. (2011), Rsv3 is located between A519F/R and M3Satt. Accordingly, simple sequence repeat (SSR) markers in the vicinity of A519F/R and M3Satt, namely Satt726, BARCSOYSSR_14_1403, BARCSOYSSR_14_1408, BARCSOYSSR_14_1410, BARCSOYSSR_14_1414, BARCSOYSSR_14_1416, M3Satt modified (hereafter, M3SattM), and Satt560 were used [Song et al. 2004, Song et al. 2010, Daizubase (http://daizu.dna.affrc.go.jp/)] (Fig. 1, Supplemental Table 1). The position of each DNA marker on a physical map was based on the soybean genome database Wm82.a1 (Schmutz et al. 2010). Sizing of polymerase chain reaction (PCR) fragments amplified with fluorescently-labeled SSR markers was performed with a 3730 Genetic Analyzer DNA sequencer [Applied Biosystems (now Life Technologies), CA, U.S.], according to Sayama et al. (2011).

Soybean mosaic virus artificial inoculation tests
Artificial inoculation tests with SMV were performed according to the method of Nagasawa et al. (1987) in a net house in a nursery. The breeding lines ‘Karikei 814’ and ‘Tohoku 169’ (‘Karikei 813’) were inoculated in 2011 and/or 2012, and the BC2F2:3 lines were inoculated in 2006. The SMV strains used in this study were originally isolated by Takahashi et al. (1987). SMV strains C and D correspond to SV-15 (MAFF 104065) (MAFF, microorganism accession number of National Institute of Agrobiological Sciences Genebank) and SV-70 (MAFF 104066), respectively. To obtain sufficient viruses to inoculate all lines and cultivars and to repopulate viruses, susceptible cultivar ‘Norin 4’ was inoculated. Frozen (–80°C) SMV-infected leaves were homogenized in phosphate buffer solution (40 mM potassium dihydrogen phosphate, 60 mM disodium hydrogen phosphate, pH 7.0) to prepare the inoculum. Carborundum of 600 mesh was dusted on the leaves of ‘Norin 4’ before inoculation,

Fig. 1. Schematic illustration of soybean chromosome 14 near the Rsv3 gene. The Rsv3 gene was reported to be located in the region between two markers, A519F/R and M3SattM (a double-headed arrow), by Suh et al. (2011). Another five markers, BARCSOYSSR_14_1403, 1408, 1410, 1414, and 1416 (Song et al. 2010), are indicated as B1403, B1408, B1410, B1414, and B1416, respectively.
Yield trials and evaluation of agricultural characteristics

‘Tohoku 169,’ which carries the ‘Harosoy’ allele of Rsv3 in an ‘Ohsuzu’ background, and ‘Ohsuzu’ were subjected to yield trials in a rotational paddy field and upland field from 2012 to 2014. Plots at each location were arranged in a randomized complete block design with three replications. In all trials, the seeds of each tested line or cultivar were sown at four seeds per hill, and seedlings were thinned to leave two plants per hill. Other growth conditions are shown in Supplemental Table 3.

We evaluated the following 11 agricultural characteristics according to the test guidelines of agricultural characteristics for the plant variety protection and seed acts of the Ministry of Agriculture, Forestry and Fisheries, Japan (http://www.hinsyu.maff.go.jp/info/sinsakijun/botanical_taxon.html): flowering time, seed-filling period, maturity, main stem length, lodging, number of pods, number of seeds per pod, seed yield, 100-seed weight, protein content, and oil content. Flowering time, maturity, and seed-filling period were defined as the number of days from sowing to flowering, from sowing to maturation, and from flowering to maturity, respectively. Lodging was visually scored from 0 (erect) to 5 (completely lodged). For evaluating the main stem length and number of pods, 10 hills (20 plants) of each plot were randomly selected and measured. The number of seeds per pod was investigated according to Kato et al. (2014) using five randomly selected plants in 10 hills. Seed yield was calculated by dividing the seed mass by the number of hills harvested in each plot. Measurement of 100-seed weight, protein content, and oil content were evaluated according to the method of Kato et al. (2014).

Statistical analyses were performed with the statistical package R 2.12.2 (http://www.r-project.org/). The statistical significance of differences was evaluated by analysis of variance (ANOVA). A P value <0.05 was considered statistically significant.

Results

Genotyping of DNA markers near Rsv3 in ‘Ohsuzu,’ ‘Fukuibuki’ and its ancestors

The DNA markers located near the Rsv3 locus in ‘Ohsuzu,’ ‘Fukuibuki,’ and ancestors of ‘Fukuibuki,’ namely ‘Harosoy,’ ‘Nemashirazu,’ ‘Tairadatezairai,’ and ‘Dewamusume,’ were genotyped (Table 1, Supplemental Fig. 1). The sizes of PCR products of all tested markers in ‘Dewamusume’ were identical to those of ‘Harosoy.’ The sizes of all markers in ‘Fukuibuki’ were also identical to those of ‘Harosoy,’ with the exception of Satt560 (Table 1). In contrast, the sizes of PCR products of all markers in ‘Nemashirazu’ and ‘Tairadatezairai’ were completely different from those of ‘Harosoy’ (Table 1). Based on the alleles of ‘Dewamusume’ and ‘Fukuibuki’ at BARCSOYSSR_14_1410, 1414, 1416 and M3SattM, which cover the genomic region of the Rsv3 locus, the Rsv3 allele of these cultivars was inferred to be derived from ‘Harosoy’ (Fig. 1, Table 1).

Introduction of Rsv3 into ‘Ohsuzu’ by backcrossing

‘Ohsuzu’ and ‘Fukuibuki’ were crossed in 2001, and five backcrosses to ‘Ohsuzu’ were made from 2002 through 2006. Six BC2F1 plants carrying heterozygous alleles in a genomic region containing Rsv3 were grown in 2007 and 218 plants homozygous for the ‘Harosoy’ allele of Rsv3 were selected from a BC2F2 population consisting of 891 plants by genotyping of DNA markers Satt726, BARCSOYSSR_14_1403, and M3SattM in 2008. Of these 218 plants, 59

Table 1. The sizes of PCR products of simple sequence repeat (SSR) markers near Rsv3 in eight tested cultivars or lines and their resistance to soybean mosaic virus (SMV) strains C and D

| Variety      | Satt726  | B1403b | B1408b | B1410b | B1414b | B1416b | M3SattM | Satt560 | Resistancea |
|--------------|----------|--------|--------|--------|--------|--------|---------|---------|-------------|
| Harosoy      | 278      | 128    | 262    | 93     | 160    | 158    | 224     | 289     | R           |
| Nemashirazu  | 309      | 107    | 284    | 138    | 151    | 156    | 230     | 292     | S           |
| Tairadatezairai | 306    | 140    | 338    | 132    | 163    | NDb    | 230     | 292     | S           |
| Ohsuzu       | 305      | 150    | 330    | 130    | 193    | NDb    | 230     | 298     | S           |
| Dewamusume   | HAc      | HA     | HA     | HA     | HA     | HA     | HA      | HA      | R           |
| Fukuibuki    | HA       | HA     | HA     | HA     | HA     | HA     | HA      | NE/TAd   | R           |
| Tohoku 169   | HA       | HA     | HA     | HA     | HA     | HA     | NE/TAd  | –       | –           |
| Kariket 814  | HA       | HA     | HA     | HA     | HA     | HA     | NE/TAd  | –       | –           |

a “R” and “S” indicate resistance and susceptibility, respectively, to SMV, following Ishikawa et al. (1979), Shimada et al. (2004), Tabuchi et al. (1999) and Takahashi et al. (1987).

b B1403, B1408, B1410, B1414, and B1416 indicate BARCSOYSSR_14_1403, 1408, 1410, 1414, and 1416, respectively (Song et al. 2010).

c Marker alleles derived from ‘Harosoy.’

d Marker alleles derived from ‘Nemashirazu’ or ‘Tairadatezairai.’
BC$_2$F$_2$ plants were selected again by the evaluation of seed qualities, and 59 BC$_3$F$_3$ lines were grown in 2009. Two elite breeding lines were selected from these 59 lines and named ‘Karakei 813’ and ‘Karakei 814.’ From the results of the preliminary yield trials for these two elite lines in 2010 and 2011, ‘Karakei 813’ was selected and renamed ‘Tohoku 169.’ In addition, the alleles in the genomic regions containing $Rsv3$ in ‘Tohoku 169’ (Karakei 813) and ‘Karakei 814’ were determined, confirming that alleles of all the SSR markers of these lines, except Satt560, matched the alleles of ‘Harosoy’ (Table 1). Additionally, BC$_2$F$_1$ plants were grown in 2004, and six BC$_2$F$_2$ plants homozygous for the ‘Harosoy’ allele of $Rsv3$ and four BC$_2$F$_2$ plants homozygous for the ‘Ohsuzu’ allele of $Rsv3$ were selected from a BC$_2$F$_2$ population using the DNA marker M3SattM, and the seeds of each BC$_3$F$_3$ line were collected.

**Artificial inoculation tests of SMV**

Artificial inoculation tests of the BC$_2$F$_2$-3 lines, which were developed for introducing the ‘Harosoy’ allele of $Rsv3$ into ‘Ohsuzu,’ were conducted in 2006. The results of inoculation tests using the differential varieties corresponded to the results of previous studies, although only half of the tested plants of ‘Norin 4’ showed symptoms with strain D (Table 2). Additionally, BC$_2$F$_2$ plants were homozygous for the ‘Harosoy’ allele of $Rsv3$ and four BC$_2$F$_2$ plants homozygous for the ‘Ohsuzu’ allele of $Rsv3$ were selected from a BC$_2$F$_2$ population using the DNA marker M3SattM, and the seeds of each BC$_3$F$_3$ line were collected.

### Table 2. Artificial inoculation tests with *soybean mosaic virus* (SMV) strains C and D

| Variety | SMV-C | SMV-D |
|---------|-------|-------|
|         | 2006  | 2011  | 2012 | 2006  | 2011  | 2012 |
| Peking  | 0/11$^a$ | 0/10 | 0/10 | R     | 0/11 | 0/10 | 0/10 | R     |
| Harosoy | 0/8   | 0/10 | 0/10 | R     | 0/10 | 0/10 | 0/10 | R     |
| Ozu 3   | 10/10 | 10/10 | 10/10 | S     | 11/11 | 10/10 | 10/10 | S     |
| Tokachinagahara | 10/10 | 10/10 | 10/10 | S     | 7/8   | 10/10 | 10/10 | S     |
| Nemashirazu | 12/12 | 10/10 | 10/10 | S     | 9/12 | 10/10 | 10/10 | S     |
| Fukusennari | 6/12 | 10/10 | 4/10 | S     | 0/10 | 0/10 | 0/10 | R     |
| Norin 4 | 11/12 | 10/10 | 10/10 | S     | 5/11 | 10/10 | 10/10 | S     |
| Tsurunotamago 1 | 5/6 | 10/10 | 9/9 | S     | 5/5 | 10/10 | 10/10 | S     |
| Shiromame | 0/11 | 0/10 | 0/10 | R     | 8/12 | 10/10 | 6/9 | S     |
| Dewamusume | 0/11 | 0/10 | 0/10 | R     | 0/12 | 0/10 | 0/10 | R     |
| BC2F3-49 (OH) | 8(12)/12$^c$ | – | – | – | 11(11)/11 | – | – | S     |
| BC2F3-56 (OH) | 8(12)/12 | – | – | – | 11(11)/12 | – | – | S     |
| BC2F3-57 (OH) | 8(9)/12 | – | – | – | 12(11)/12 | – | – | S     |
| BC2F3-71 (OH) | 9(10)/11 | – | – | – | 9(7)/10 | – | – | S     |
| BC2F3-43 (HA) | 0(0)/12 | – | – | R     | 0(0)/12 | – | – | R     |
| BC2F3-50 (HA) | 0(0)/12 | – | – | R     | 0(0)/12 | – | – | R     |
| BC2F3-54 (HA) | 0(0)/12 | – | – | R     | 0(0)/12 | – | – | R     |
| BC2F3-67 (HA) | 0(0)/12 | – | – | R     | 0(0)/12 | – | – | R     |
| BC2F3-68 (HA) | 0(0)/12 | – | – | R     | 0(0)/12 | – | – | R     |
| BC2F3-69 (HA) | 0(0)/12 | – | – | R     | 0(0)/12 | – | – | R     |
| Tohoku 169 | – | 0/10 | 0/10 | R | – | 0/10 | 0/10 | R     |
| Karakei 814 | – | 0/10 | – | R | – | 0/10 | – | R     |

$^a$ (OH) indicates that the allele of an SSR marker near $Rsv$, M3SattM, was the ‘Ohsuzu’ type, and (HA) indicates that the allele of M3SattM was the ‘Harosoy’ type.

$^b$ The number of infected plants judged by visual inspection/the number of all tested plants.

$^c$ The number of infected plants judged by visual inspection (judged by dot immuno-binding assay)/the number of all tested plants.

$^d$ R and S indicate resistance and susceptibility, respectively, to SMV.
containing \( Rsv3 \), and GMES5027 on chromosome 16. The alleles of the eight markers near the \( Rsv3 \) locus completely matched those of ‘Fukuibuki’ (Table 1, Fig. 2).

**Yield trial**

To compare the agricultural characteristics of ‘Ohsuzu’ and ‘Tohoku 169,’ ‘Ohsuzu’ and ‘Tohoku 169’ were subjected to yield trials in rotational paddy and upland fields from 2012 to 2014. Although there were significant differences in flowering time and main stem length between ‘Ohsuzu’ and ‘Tohoku 169’ (factor “variety” in ANOVA), differences in other characteristics such as maturity and 100-seed weight were not significant (Table 3).

Similarly, there were significant differences in all characteristics, except for seed-filling period, between the rotational paddy and upland fields (factor “field”) and there were significant differences in all the characteristics between cultivation years (factor “year”) (Table 3). These results showed that field and year affected almost all characteristics, whereas variety affected only flowering time and main stem length. The effects of interaction between field and year were significant for all characteristics, except for main stem length, number of pods, and number of seeds per pod (Table 3). In contrast, there was no significant effect of the interactions between field and variety or year and variety or of the interaction between all three factors (Table 3). These results indicated that the differences between ‘Tohoku 169’ and ‘Ohsuzu’ were consistent over different cultivation conditions and that the differences between the two cultivars with respect to agricultural characteristics were relatively small despite differences in cultivation conditions across years and fields.

**Discussion**

The cultivar ‘Harosoy’ was introduced into the soybean breeding program in Japan as the source of resistance to SMV strains C and D. Gunduz et al. (2001) showed that ‘Harosoy’ carries a resistance allele at the \( Rsv3 \) locus and susceptibility alleles at the \( Rsv1 \) and \( Rsv4 \) loci. Considering these facts, \( Rsv3 \) derived from ‘Harosoy’ has been suggested to confer resistance to SMV strains C and D. To determine
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The number of pods was calculated as the total from two plants per hill.

Flowering time, maturity, and seed-filling period are defined as the number of days from sowing to flowering, from sowing to maturation, and from flowering to maturity, respectively.

The number of pods was calculated as the total from two plants per hill.

whether resistant Japanese cultivars carry the genomic region containing Rsv3 derived from ‘Harosoy,’ we compared the alleles of DNA markers located near Rsv3 in resistant and susceptible cultivars. Resistant cultivars and ‘Harosoy’ carried the same alleles in the region containing Rsv3, whereas susceptible cultivars carried alleles that differed from the ‘Harosoy’ allele. These results indicate that Rsv3 derived from ‘Harosoy’ has been used as a gene for resistance to SMV strains C and D.

Lines and individual plants resistant to SMV strains C and D have been selected from a population by growing plants in fields severely infected with strains C and D or by inoculating plants with SMV using air-pressure guns (Hashimoto et al. 1984, Hashimoto and Nagasawa 1987, Ishikawa et al. 1979, Takahashi et al. 2003). However, temperature affects the severity of symptoms induced by SMV, impeding the selection of resistant plants (Shigemori 1991). It is difficult to inoculate all tested plants uniformly and to distinguish resistant and susceptible plants after inoculation without error. Takahashi et al. (2003), evaluating the efficiency of artificial air-inoculation tests, showed that 3% of tested susceptible plants escaped selection by artificial inoculation, depending on the cultivation environment. In the present study, artificial inoculation was performed in a net house, and some inoculated plants of susceptible varieties or lines showed no symptoms. This finding shows that evaluation by artificial spray inoculation in a net house or in the field is not perfect. We used marker-assisted selection with DNA markers near Rsv3 in backcross breeding to introduce a gene for resistance to strains C and D into the leading variety ‘Ohsuzu’ and developed ‘Tohoku 169’ and ‘Karikei 814.’ Our results showed that the DNA markers of Rsv3 can be used more accurately than evaluation by artificial inoculation to select plants with resistance to SMV strains C and D.

In introducing a resistance gene into breeding materials, the resistance gene itself should not cause undesirable changes in other agricultural characteristics. If the resistance is linked to undesirable agricultural characteristics, breeders have to break the linkages between the resistance gene and undesirable genes. One example is the ‘Peking’ allele of the Rhg4 gene, one of the major resistance genes to soybean cyst nematode (Heterodera glycines) (Matson and Williams 1965). Rhg4 is closely linked (0.35 cM) to the I locus, which controls seed coat color, and ‘Peking’ had a black seed coat (Matson and Williams 1965). When Rhg4 from ‘Peking’ was introduced, the seed coats of the recipient lines also became black, an undesirable characteristic. The use of Rhg4 derived from ‘Peking’ has been limited for this reason. Another example is rice pi21, a gene for durable resistance to rice blast (Magnaporthe oryzae) (Fukuoka et al. 2009). The location of the pi21 gene is near that of a gene that degrades eating quality. The eating quality of lines carrying pi21 in the elite cultivar background was equivalent to that of the elite cultivar only when a donor fragment did not contain the neighboring gene. To test the effects of fragments containing Rsv3 genes on agricultural characteristics, we performed yield trials for three years and compared ‘Ohsuzu’ and ‘Tohoku 169,’ which carried donor fragments in the ‘Ohsuzu’ background. We found that donor fragments affected only flowering time and main stem length and did not affect other characteristics, including

| Filed condition | Year | Variety | Flowering (day) | Seed-filling period (day) | Maturity (day) | Main stem length (cm) | Lodging | Number of pods | Number of seeds per pod | Seed yield (kg/ha) | 100-seed weight (g) | Protein content (%) | Oil content (%) |
|-----------------|------|---------|-----------------|--------------------------|---------------|----------------------|---------|---------------|------------------------|------------------|--------------------|-------------------|------------------|
| Upland field    | 2012 | Ohsuzu  | 59.7            | 74.0                     | 133.7         | 61.2                 | 0.0     | 89.5          | 1.99                   | 27.3             | 28.1               | 40.8              | 22.2             |
|                 | 2013 | Ohsuzu  | 56.7            | 74.3                     | 131.0         | 57.3                 | 1.0     | 89.9          | 1.94                   | 31.2             | 34.5               | 42.7              | 21.4             |
|                 | 2014 | Ohsuzu  | 56.0            | 72.7                     | 129.7         | 67.9                 | 2.3     | 76.6          | 2.01                   | 30.7             | 37.6               | 43.1              | 20.9             |
|                 | 2012 | Tohoku 169 | 62.7          | 71.7                     | 134.3         | 67.1                 | 0.0     | 97.0          | 1.99                   | 33.2             | 29.6               | 40.5              | 22.7             |
|                 | 2013 | Tohoku 169 | 58.7          | 73.0                     | 130.7         | 63.0                 | 1.0     | 94.4          | 1.97                   | 30.4             | 33.4               | 42.2              | 21.3             |
|                 | 2014 | Tohoku 169 | 58.0          | 71.3                     | 129.3         | 70.6                 | 2.3     | 72.9          | 2.03                   | 28.3             | 37.7               | 43.4              | 20.5             |

| Rotational paddy field | Year | Variety | Flowering (day) | Seed-filling period (day) | Maturity (day) | Main stem length (cm) | Lodging | Number of pods | Number of seeds per pod | Seed yield (kg/ha) | 100-seed weight (g) | Protein content (%) | Oil content (%) |
|------------------------|------|---------|-----------------|--------------------------|---------------|----------------------|---------|---------------|------------------------|------------------|--------------------|-------------------|------------------|
| 2012                   | Ohsuzu | 60.0   | 70.0            | 129.0                    | 62.7          | 0.0                   | 102.9   | 2.05          | 48.7                   | 35.7             | 43.5               | 21.7              | 21.7             |
| 2013                   | Ohsuzu | 49.7   | 72.3            | 122.0                    | 61.4          | 1.3                   | 98.8    | 2.02          | 41.4                   | 35.4             | 43.3              | 20.9              | 21.7             |
| 2014                   | Ohsuzu | 52.3   | 76.0            | 128.3                    | 69.9          | 1.3                   | 76.9    | 2.03          | 38.9                   | 39.8             | 46.2              | 19.2              | 19.6             |
| 2012                   | Tohoku 169 | 53.3   | 71.7            | 125.0                    | 72.8          | 1.7                   | 109.2   | 2.03          | 42.2                   | 35.9             | 42.7              | 21.1              | 21.1             |

Table 3. Agronomic characteristics of ‘Ohsuzu’ and ‘Tohoku 169’ by yield trials

| ANOVA                          | Field × Variety | Variety | Field × Year | Field | Year | Variety | Field × Year × Variety |
|-------------------------------|-----------------|---------|--------------|-------|------|---------|------------------------|
| ***                           | ns              | ns      | ***          | **    | **   | **      | ***                    |
| ***                           | ns              | ns      | ***          | ns    | ns   | ns      | ns                    | ns              | ns      | ns     | ns | ns |
| ***                           | ns              | ns      | ***          | ns    | ns   | ns      | ns                    | ns              | ns      | ns     | ns | ns |

a Flowering time, maturity, and seed-filling period are defined as the number of days from sowing to flowering, from sowing to maturation, and from flowering to maturity, respectively.

b Lodging was visually scored from 0 (no lodging) to 5 (completely lodged).

c The number of pods was calculated as the total from two plants per hill.
maturity and seed weight, and that seed yields of ‘Tohoku 169’ were equivalent to those of ‘Ohsuzu.’ It has been reported that late flowering elongates main stem length (Cober and Morrison 2010, Yamada et al. 2012), suggesting that the difference between these cultivars in main stem length is due to differences in flowering time. From the comparison of graphical genotypes between ‘Ohsuzu’ and ‘Tohoku 169,’ regions on chromosome 14 and 16 of ‘Tohoku 169’ originated in the Rsv3 donor, ‘Fukuibuki.’ We could not determine whether the introduced genomic region containing Rsv3 affected the duration from sowing to flowering, although major candidate genes and QTLs controlling flowering previously identified as E1, E2, E3, E4, E7, and E8 are not located on chromosomes 14 or 16 (Watanabe et al. 2012). Although changes in flowering time and main stem length have been reported to affect maturity and lodging, respectively (Wilcox and Sediyama 1981, Yamada et al. 2012), there were no significant differences in maturity or lodging between ‘Ohsuzu’ and ‘Tohoku 169.’ Thus, we showed that differences between ‘Ohsuzu’ and ‘Tohoku 169’ in agricultural characteristics were negligible and/or not significant. These results suggest that Rsv3 itself does not have undesirable pleiotropic effects on other agricultural characteristics and that Rsv3 is not linked to undesirable genes.

‘Ohsuzu’ has high adaptability in all parts of the Tohoku region, Hokuriku region, and northern parts of the Kanto region (Tabuchi et al. 1999); therefore, ‘Tohoku 169’ is expected to have equivalent adaptability. In these areas, ‘Ohsuzu,’ ‘Ryuho,’ (Nakamura et al. 1996) and ‘Tanrei’ (Mikoshiba et al. 1983) have been cultivated as early maturation varieties, but these varieties are susceptible to SMV strains C and D (Shigemori 1991). With the aim of displacing these varieties, we have been performing yield trial tests and evaluating the adaptability of ‘Tohoku 169’ in these areas for registration as a new cultivar.

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