ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2014) **12**(S1); S41–S44
doi:10.1017/S1479262114000227

Multiple genes confer resistance to soybean mosaic virus in the soybean cultivar Hwangkeum

Namhee Jeong and Soon-Chun Jeong*

Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Cheongwon, Republic of Korea

Abstract

Recombinant inbred lines (RILs) generated from a cross between a cultivated species and its wild progenitor species serve as important germplasm for introgressing valuable genes from a wild species to a cultivated species. During this breeding process, it is equally important to prevent the loss of agronomically important genes in the cultivated species. In an effort to establish an efficient selection system for the single *Rsv*1 gene conferring durable resistance to soybean mosaic virus (SMV) in the soybean cultivar Hwangkeum (also known as Suweon 97), which is a parent of a RIL population from Hwangkeum (cultivated soybean) × IT182932 (wild soybean), in the present study, we unexpectedly identified an additional necrosis-conditioning gene unmasked by a recombination in the middle of the *Rsv*1-containing nucleotide-binding leucine-rich repeat gene cluster region and the *Rsv*3 gene in Hwangkeum. Thus, Hwangkeum contains at least three SMV resistance genes consisting of two tightly linked genes at the *Rsv*1 locus and the *Rsv*3 locus. The results of this study provide additional important information for use of the Hwangkeum genome in soybean breeding programmes.

Keywords: Hwangkeum; resistance gene; soybean; soybean mosaic virus

Introduction

Soybean mosaic virus (SMV) is the most economically relevant virus in soybean [*Glycine max* (L.) Merr.] production. The symptoms of SMV infection are stunted growth, mosaic patterns on leaves, seed mottling, foliage discolouration, and necrosis (Cho and Goodman, 1982). Utilization of resistant cultivars is considered to be the most effective and environmentally friendly way to combat this disease. Efforts to find SMV resistance genes have resulted in the mapping of three resistance loci, *Rsv*1, *Rsv*3 and *Rsv*4, on different soybean chromosomes (Yu *et al*., 1994; Hayes *et al*., 2000; Jeong *et al*., 2002). Subsequent efforts to find new resistance genes in soybean lines conferring resistance to a broad spectrum of SMV strains have frequently revealed that combinations of the known genes or alleles rather than those of new gene(s) are the cause of the durable resistance (e.g. Li *et al*., 2010). These observations are further supported by the reconstruction of durable resistance through pyramiding of the respective *Rsv* genes (Saghai Maroof *et al*., 2008). Interestingly, among the soybean lines with high levels of resistance to SMV, 'Suweon 97', which was later registered as cultivar Hwangkeum (also translated as Hwang-Kum) in Korea (Yu *et al*., 2008), was found to contain a single dominant *Rsv*1 allele that is resistant to all SMV strains identified in the USA (Chen *et al*., 2002). In the present study, during the course of mapping the *Rsv*1 locus in a mapping population having Hwangkeum as a parent, we demonstrated that Hwangkeum contains more than two resistance genes at the classical *Rsv*1 locus as well as the *Rsv*3 locus.

* Corresponding author. E-mail: scjeong@kribb.re.kr

Materials and methods

SMV resistance genes were initially mapped in a RIL population generated from Hwangkeum (cultivated soybean) × IT182932 (wild soybean) (hereafter HI population), in which more than 1500 molecular markers have been mapped (Lee *et al.*, 2013). $F_{2:3}$ populations from a cross between 'Williams 82K', which does not carry any of the three known dominant *Rsv* genes (Viel *et al.*, 2009), and Hwangkeum (hereafter WH population) were used for validation.

SMV data were collected from 8–15 F_{12} plants from each of the HI lines by inoculating them with the SMV strain G1 or G7 in a greenhouse as described previously by Jeong *et al.* (2002). Hwangkeum, IT182932 and a set of reference soybean cultivars of known SMV strain response ('York', 'V94-5152', 'Essex', 'L29', 'PI507389' and 'PI96983') were inoculated to confirm the effectiveness of inoculation and to verify the identity and purity of the SMV strain used in the study. The presence of the *Rsv3* locus in Hwangkeum was validated by collecting G7 inoculation data from $F_{2:3}$ WH populations. A new marker InR1 (Table 1) and public makers described by Song *et al.* (2004), Suh *et al.* (2011) and Lee *et al.* (2013) were used for genotyping. The χ^2 test and linkage analysis were carried out as described by Jeong *et al.* (2002).

Results and discussion

We assayed SMV symptoms in the parental lines, reference lines and 95 RILs from the HI population using the SMV strain G1 or G7 (Fig. 1(a)). Most of the known *Rsv*1 alleles confer resistance to the strain G1 and to the mosaic or necrotic symptoms caused by the strain G7 (Chen *et al.*, 2002). When the strain G1 was inoculated, all RILs exhibited SMV disease reactions similar to those exhibited by either of the parental lines, IT182932 (mosaic) or Hwangkeum (extreme resistance), except one RIL, HI-55 (Fig. 1(a)). The aberrant HI-55 line exhibited lethal necrosis, a resistance reaction, which is similar to that conditioned by *Rsv1-n*, an *Rsv*1 allele, in PI507389 (Ma *et al.*, 2003). The segregation of the RILs for resistance to the strain G1 displayed a 1:1 ratio (resistant:mosaic = 49:46, $\chi^2 = 0.094$, $P = 0.758$), consistent with the results reported by Chen *et al.* (2002) that Hwangkeum contains a single dominant *Rsv*1 gene. With regard to molecular markers that were developed from the *Rsv*1-containing nucleotide-binding leucine-rich repeat (NB-LRR) gene cluster region (Collier and Moffett, 2009), the disease phenotypes cosegregate with Sca172b and Sca_172a and are separated by one recombination with the upper marker Sca172d and by one recombination with the lower marker InR1 (Fig. 1(b)). Marker genotyping of HI-55 revealed a recombination in the middle of the NB-LRR cluster (Fig. 1(b)), resulting in the phenotypic detection of the masked *Rsv1-n*-like gene (Tucker *et al.*, 2009). When the strain G7 was inoculated, 24 of the G1 strain-susceptible RILs exhibited extreme resistance. As *Rsv*3 conditions mosaic reaction to the strain G1 and confers resistance to the strain G7 (Jeong *et al.*, 2002), this result would be expected if the *rsv*1-carrying RILs are segregating at the *Rsv*3 locus. As expected, the segregation of the RILs for resistance to the strain G7 displayed a 3:1 ratio (resistant:mosaic, $\chi^2 = 0.171$, $P = 0.678$) and all the 24 RILs carry chromosomal segments from Hwangkeum at the *Rsv*3 locus. HI-55 contains a chromosomal segment from IT182932 at the *Rsv*3 locus, indicating that the severe necrosis is not related to *Rsv*3.

The presence of the *Rsv*3 gene in Hwangkeum was confirmed in the WH population. The markers located near *Rsv*1 and *Rsv*3 including Sca_172b and Sca_156b were used to select an F_2 line, which contained a chromosomal segment only from Williams 82K at the *Rsv*1 locus but segregated at the *Rsv*3 locus. When the disease reaction of 120 $F_{2:3}$ plants from the F_2 line was assayed following inoculation with the strain G7, the segregation of the $F_{2:3}$ plants for resistance to the strain G7 displayed a 3:1 ratio (resistant:mosaic, $\chi^2 = 0.000$, $P = 1.000$). The disease phenotypes cosegregate with Sca_156b

Table 1. Attributes of a simple sequence length polymorphism marker linked to the *Rsv*1 locus generated from a mapping database[a] of soybean genome resequencing data

Marker name	Attribute of polymorphism	Primer specificity and sequence (5′ → 3′)	Predicted product size (bp)
InR1	Insertion of 13 bp (CTTCTTTCCCCCT) at position 29218490 of Gm13 detected in IT182932 relative to the Williams 82 genome sequence[b]	Forward: ATGACAAGTCTCATGTAAGC; reverse: GTGGAGATATGGTGGTACA	153 (Hwangkeum) 166 (IT182932)

[a] Soya.re.kr; Chung *et al.* (2013), assessed on 24 November 2013. [b] Version Glyma1; www.phytozome.net, assessed on 24 November 2013.

https://doi.org/10.1017/S1479262114000227 Published online by Cambridge University Press

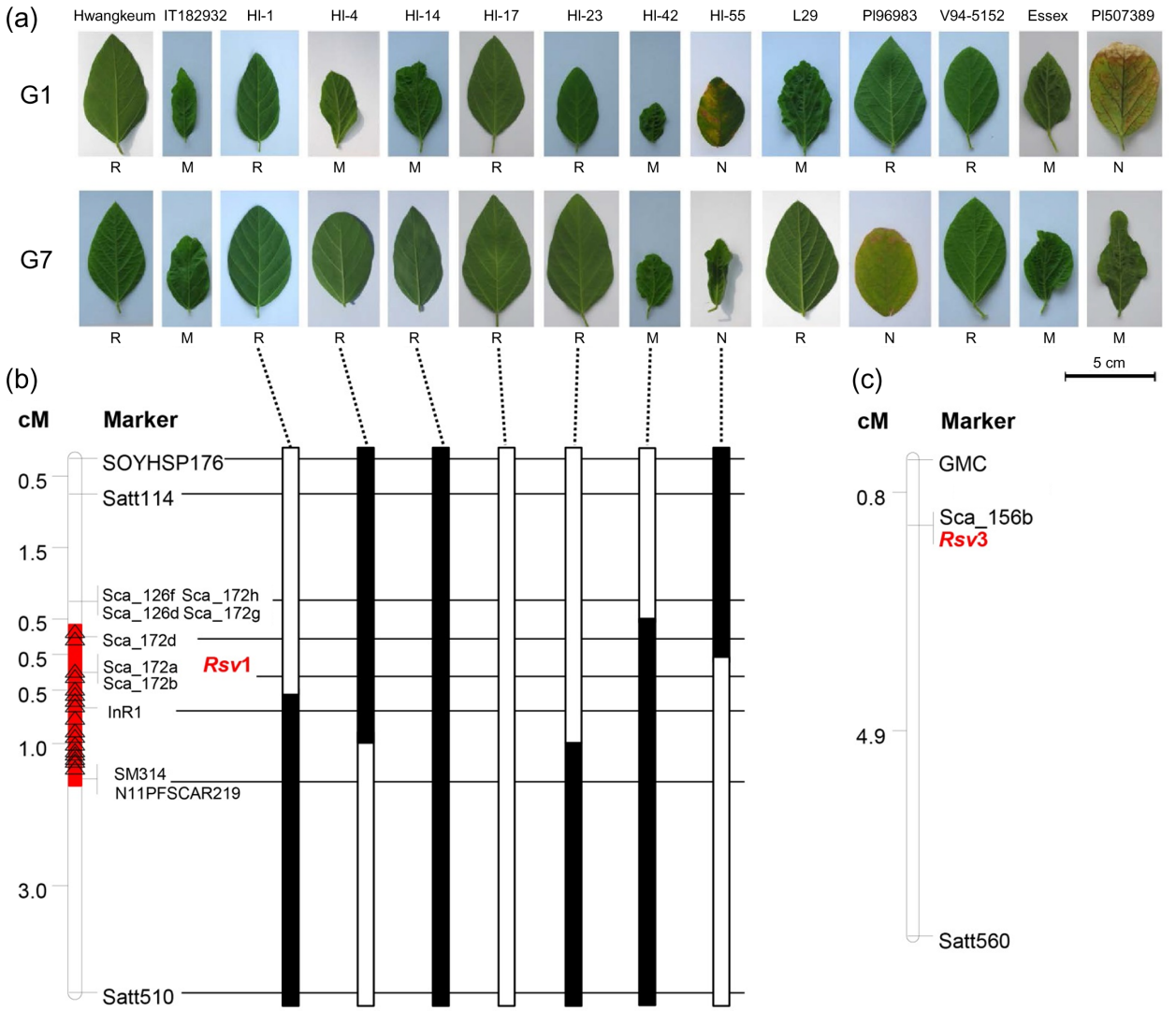

Fig. 1. Genetic analysis of soybean mosaic virus (SMV) resistance in Hwangkeum. (a) Disease reactions after inoculation of the SMV strain G1 (upper panel) or G7 (lower panel) in different soybean lines: parental lines (Hwangkeum and IT182932), recombinant inbred lines (RILs) (HI-1, HI-4, HI-14, HI-17, HI-23, HI-42 and HI-55) and other reference lines (L29, PI96983, V94-5152 (PI596752), Essex (PI548667) and PI507389). The representative RILs HI-1, HI-4, HI-23, HI-42 and HI-55 have a recombination in the vicinity of *Rsv*1. The HI-14-containing chromosomal segment from IT182832 in the vicinity of *rsv*1 is susceptible to the strain G1 and resistant to the strain G7, suggesting the presence of a resistance gene other than *Rsv*1 in Hwangkeum. The HI-17-containing chromosomal segment from Hwangkeum in the vicinity of *Rsv*1 is resistant to both strains G1 and G7. Photos were taken from young fully expanded leaves 4–5 weeks after inoculation. Mosaic (M), necrotic (N) and resistance (R) reactions are indicated on bottom of the photos for clarification. (b) Genetic and physical maps of the soybean chromosome (Chr) 13 in the vicinity of the SMV resistance gene *Rsv*1 in a RIL population from Hwangkeum × IT182932. Distances between markers are indicated in centimorgans (cM). The nucleotide-binding leucine-rich repeat (NB-LRR) gene cluster region on the basis of soybean Glyma1.1 annotation of the chromosome-based Glyma1 assembly (http://www.phytozome.net) is indicated by a red box on soybean Chr 13. Triangles represent the approximate locations of individual NB-LRR genes on the Glyma1 assembly. Graphical genotypes of the seven RILs whose SMV reactions are shown in panel (a) are indicated by white (Hwangkeum) and black (IT182932) bars. RILs are connected by dotted lines between panels. (c) Genetic map of the soybean Chr 14 in the vicinity of the SMV resistance gene *Rsv*3 in an $F_{2:3}$ population from Williams 82 × Hwangkeum. Distances between markers are indicated in cM.

(Fig. 1(c)), which is tightly linked to *Rsv*3 (Suh *et al.*, 2011). When the disease reaction of 19 $F_{2:3}$ plants from the F_2 line was assayed following inoculation with the strain G1, all the plants exhibited mosaic symptoms.

Herein, we report that, contrary to the report by Chen *et al.* (2002), Suweon 97 (cultivar Hwangkeum) contains at least two *Rsv*1 locus-residing genes as well as *Rsv*3. Chen *et al.* (2002) used sound experimental designs and reported

https://doi.org/10.1017/S1479262114000227 Published online by Cambridge University Press

what we observed in this study such as the valuable *Rsv*1 gene and its inseparable necrosis-conditioning *Rsv*1 gene. However, they acknowledged that 'The York × Suweon 97 cross exhibited a rather poor fit to the 3:1 segregation when inoculated with G6 because of a deficiency of susceptible plants' and that the 'Suweon 97 × Essex cross' exhibited 'a poor 3:1 fit in the field test because of a deficiency of susceptible plants'. In light of our results, these data should have been interpreted as 'an abundance of susceptible plants' exhibiting a 15:1 fit as expected from a digenic segregation.

In conclusion, our results support the presence of a valuable *Rsv*1 gene in Hwangkeum conferring durable resistance to diverse SMV strains, as emphasized by Viel *et al.* (2009). In addition, we found that Hwangkeum contains another *Rsv*1 gene conditioning necrosis caused by the strain G1 as well as *Rsv*3 conferring resistance to the strain G7. Our observation of an additional gene at the *Rsv*1 locus (Fig. 1(b)) is not unusual, as proposed by Gore *et al.* (2002) and further confirmed by Yang *et al.* (2013) with PI96983, suggesting that when breeding SMV-resistant cultivars, introgression of the *Rsv*1-containing NB-LRR cluster should be monitored carefully. Therefore, we have provided additional valuable information about Hwangkeum, which has already been widely used as a parent in Korean breeding programmes by virtue of clear golden and large, round-shaped seeds (Yu *et al.*, 2008; Yang *et al.*, 2010), as an important germplasm for breeding SMV-resistant cultivars.

Acknowledgements

This study was supported partly by a grant from the Next-Generation BioGreen 21 Program (PJ009091), by the Rural Development Administration, and by the KRIBB Research Initiative Program.

References

Chen P, Buss GR, Tolin SA, Gunduz I and Cicek M (2002) A valuable gene in Suweon 97 soybean for resistance to soybean mosaic virus. *Crop Science* 42: 333–337.

Cho EK and Goodman RM (1982) Evaluation of resistance in soybeans to soybean mosaic virus strains. *Crop Science* 22: 1133–1136.

Chung W-H, Jeong N, Kim J, Lee WK, Lee Y-G, Lee S-H, Yoon W, Kim J-H, Choi I-Y, Choi H-K, Moon J-K, Kim N and Jeong SC (2013) Population structure and domestication revealed by high-depth resequencing of Korean cultivated and wild soybean genomes. *DNA Research*. doi: 10.1093/dnares/dst047.

Collier SM and Moffett P (2009) NB-LRRs work a "bait and switch" on pathogens. *Trends in Plant Science* 14: 521–529.

Gore MA, Hayes AJ, Jeong SC, Yu YG, Buss GR and Saghai Maroof MA (2002) Mapping tightly linked genes controlling potyvirus infection at the *Rsv*1 and *Rpv*1 region in soybean. *Genome* 45: 592–599.

Hayes AJ, Ma GR, Buss GR and Saghai Maroof MA (2000) Molecular marker mapping of *Rsv4*, a gene conferring resistance to all known strains of soybean mosaic virus. *Crop Science* 40: 1434–1437.

Jeong SC, Kristipati S, Hayes AJ, Maughan PJ, Noffsinger SL, Gunduz I, Buss GR and Saghai Maroof MA (2002) Genetic and sequence analysis of markers tightly linked to the soybean mosaic virus resistance gene, *Rsv3*. *Crop Science* 42: 265–270.

Lee WK, Kim N, Kim J, Moon JK, Jeong N, Choi IY, Kim SC, Chung WH, Kim HS, Lee SH and Jeong SC (2013) Dynamic genetic features of chromosomes revealed by comparison of soybean genetic and sequence-based physical maps. *Theoretical and Applied Genetics* 126: 1103–1119.

Li D, Chen P, Alloatti J, Shi A and Chen YF (2010) Identification of new alleles for resistance to soybean mosaic virus in soybean. *Crop Science* 50: 649–655.

Ma G, Chen P, Buss GR and Tolin SA (2003) Genetic study of a lethal necrosis to soybean mosaic virus in PI507389 soybean. *Journal of Heredity* 94: 205–211.

Saghai Maroof MA, Jeong SC, Gunduz I, Tucker DM, Buss GR and Tolin SA (2008) Pyramiding of soybean mosaic virus resistance genes by marker-assisted selection. *Crop Science* 48: 517–526.

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Sphecht JE and Cregan PB (2004) A new integrated genetic linkage map of the soybean. *Theoretical and Applied Genetics* 109: 122–128.

Suh SJ, Bowman BC, Jeong N, Yang K, Kastl C, Tolin SA, Saghai Maroof MA and Jeong SC (2011) The *Rsv*3 locus conferring resistance to soybean mosaic virus is associated with a cluster of coiled-coil nucleotide-binding leucine-rich repeat genes. *The Plant Genome* 4: 55–64.

Tucker DM, Maroof MAS, Jeong SC, Buss GR and Tolin SA (2009) Validation and interaction of the soybean mosaic virus lethal necrosis allele, *Rsv1-n*, in PI 507389. *Crop Science* 49: 1277–1283.

Viel C, Ide C, Cui X, Wang A, Farsi M, Michelutti R and Strömvik M (2009) Isolation, partial sequencing, and phylogenetic analyses of soybean mosaic virus (SMV) in Ontario and Quebec. *Canadian Journal of Plant Pathology* 31: 108–113.

Yang K, Jeong N, Moon JK, Lee YH, Lee SH, Kim HM, Hwang CH, Back K, Palmer RG and Jeong SC (2010) Genetic analysis of genes controlling natural variation of seed coat and flower colors in soybean. *Journal of Heredity* 101: 757–768.

Yang Y, Zheng G, Han L, Dagang W, Yang X, Yuan Y, Huang S and Zhi H (2013) Genetic analysis and mapping of genes for resistance to multiple strains of soybean mosaic virus in a single resistant soybean accession PI 96983. *Theoretical and Applied Genetics* 126: 1783–1791.

Yu YG, Saghai Maroof MA, Buss GR, Maughan PJ and Tolin SA (1994) RFLP and micro satellite mapping of a gene for soybean mosaic virus resistance. *Phytopathology* 84: 60–64.

Yu YH, Yu H, Jeong J, Park H, Song D, Kim C, Kim S, Lee Y, Park J and Park K (2008) *A General Survey of Korean Legume Cultivars* (in Korean). Suwon: National Institute of Crop Science.