Identification and Characterization of the *SSB1* Locus Involved in Symptom Development by *Spring beauty latent virus* Infection in *Arabidopsis thaliana*

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The natural variation of *Arabidopsis thaliana* in response to a bromovirus, *Spring beauty latent virus* (SBLV), was examined. Of 63 *Arabidopsis* accessions tested, all were susceptible when inoculated with SBLV, although there was a large degree of variation in symptom development. Most accessions, including Columbia (Col-0), were symptomless or developed only mild symptoms, but four accessions, including S96, showed severe symptoms of SBLV infection. Genetic analysis suggested that the difference in the responses of Col-0 and S96 to SBLV was controlled by a single semi-dominant locus. We have designated this locus *SSB1* (symptom development by SBLV infection). By using genetic markers, *SSB1* was mapped to chromosome IV. The patterns of distribution and accumulation of SBLV in sensitive accessions were similar to those in the insensitive accessions. In addition, symptom development in S96 by SBLV infection was critically interrupted by the presence of the *NahG* gene, which encodes salicylic acid (SA) hydroxylase. These data suggest that symptom development in *A. thaliana* controlled by *SSB1* is independent of the efficiency of SBLV multiplication and is dependent on SA signaling.

Viral diseases of plants are established under the control of many plant and viral factors, including those required for viral multiplication and symptom induction. The development of symptoms is determined by interactions between these factors. To date, however, our understanding of such plant–virus interactions remains insufficient.

Arabidopsis thaliana has been used as a model plant in many studies, including the analysis of plant–microbe interactions (Eckardt 2001; Eckardt et al. 2001). This plant species grows in diverse environments and many accessions of *A. thaliana* have diverged significantly in their susceptibility to pathogens (Alonso-Blanco and Koornneef 2000; Kover and Schaal 2002). As for plant viruses, different responses in *Arabidopsis* accessions have been reported (Dardick et al. 2000; Lee et al. 1994, 1996; Leisner and Howell, 1992; Martín Martín et al. 1999; Park et al. 2002; Revers et al. 2003; Simon et al. 1992). If virus infection is restricted, viruses cannot induce systemic symptoms. For instance, *Beet curly top virus* (BCTV) Logan strain and *Turnip mosaic virus*, which induce systemic symptoms in the *A. thaliana* accession Col-0, do not induce visible symptoms in some other accessions because

their systemic spread is restricted (Lee et al. 1994; Martín Martín et al. 1999). A mechanism of conditioning virus infectivity in *A. thaliana* accessions has been well studied for *Tobacco etch virus* (TEV) infection, and the *RTM1* and *RTM2* genes controlling restriction of the long-distance movement of TEV have been isolated (Chisholm et al. 2000; Whitham et al. 2000). In other cases, viruses induce necrotic lesions known as the hypersensitive response (HR), which is a typical plant defense response restricted to initially infected regions (Dempsey et al. 1993; Takahashi et al. 1994). On the other hand, systemic infection of viruses does not necessarily induce symptoms. The grape strain of *Tobacco ringspot virus* (TRSV) systemically infects some *A. thaliana* accessions, including Col-0, without visible symptoms, which is termed ‘tolerance.’ TRSV induces severe symptoms in other accessions, including Estland. By using genetic analysis, the *TTR1* locus controlling tolerance to TRSV was identified on chromosome V (Lee et al. 1996). Similar observations have been reported using the BCTV CFH strain (Park et al. 2002). Thus, although relatively many studies have focused on resistance genes for virus multiplication in plants, poor information has been available about plant genes controlling symptom development.

Bromoviruses are a group of icosahedral plant viruses whose genome comprises positive-sense tripartite RNAs designated RNA1, RNA2, and RNA3 (Lane 1981). The bromovirus *Brome mosaic virus* (BMV) was the first RNA virus for which infectious in vitro transcripts were synthesized from cDNA clones of genomic RNAs (Ahlquist et al. 1984). The viral factors required for BMV multiplication have also been well characterized (Ahlquist 1999; Sullivan and Ahlquist 1997). In contrast, little is known about plant factors involved in bromovirus infection and symptom, partly because BMV shows low infectivity in *A. thaliana*. Recently, we have shown that another bromovirus, *Spring beauty latent virus* (SBLV), efficiently infects the *A. thaliana* accession Col-0 (Fujisaki et al. 2003a), suggesting that SBLV is a useful virus for analyzing plant–bromovirus interactions by the reverse and forward genetic approach.

As a first step toward the elucidation of SBLV–*A. thaliana* interactions, natural variation in response to SBLV infection was examined. SBLV asymptomatically infects Col-0 (insensitive) (Fujisaki et al. 2003a), whereas it induced severe symptoms in some other *A. thaliana* accessions, such as S96 and Ei-2 (sensitive). Infectivity of SBLV in the tested sensitive accessions was similar to that in the insensitive one. Plant factors controlling symptom production in SBLV infection were analyzed using a genetic approach.

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RESULTS

Multiple responses

of various *Arabidopsis* accessions to SBLV.

A total of 63 *Arabidopsis* accessions were inoculated with SBLV virions at 3 to 4 weeks after sowing, and symptoms were observed at 14 days postinoculation (dpi). SBLV systemically infects the accession Col-0 but does not induce visible symptoms in this accession (Fujisaki et al. 2003a). Similarly, most of the accessions tested were also symptomless or developed only mild symptoms at 14 dpi. However, four accessions (S96, Fr-2, Ei-2, and Abd-0) infected with SBLV exhibited severe symptoms (Table 1). Inoculated leaves of these four accessions exhibited yellowing and necrotic symptoms. In S96 and Fr-2, uninoculated rosette leaves also showed yellowing and curling, and most of these leaves died by 14 dpi (Table 1, Fig. 1A). In some S96 and Fr-2 plants, newly emerging leaves did not die, but showed severe curling at 14 dpi (Fig. 1B). In addition, S96 and Fr-2 plants infected with SBLV exhibited curling of stems and cauline leaves and produced very few seeds (data not shown). In the Ei-2 and Abd-0 accessions, yellowing and necrotic symptoms on uninoculated rosette leaves were mild, in contrast to those of S96 and Fr-2, although severe stunting of uninoculated rosette leaves of Ei-2 and Abd-0 was observed (Fig. 1A). Ei-2 and Abd-0 are late flowering compared with S96 and Fr-2, and no bolting tissues emerged at 14 dpi, even in mock-inoculated plants. However, when older plants of Ei-2 and Abd-0 (at 6 to 7 weeks after sowing) were inoculated, curling stems were observed at 14 dpi (data not shown).

Systemic infectivity of SBLV in the 63 accessions was examined. At least 10 plants were tested for each accession. Press blot analysis of SBLV infection at 14 dpi demonstrated that SBLV coat protein (CP) was detected in uninoculated upper tissues of all accessions (data not shown). The frequency of systemic infection with SBLV in S96, Fr-2, Ei-2, and Abd-0, in which severe symptoms were induced by SBLV infection was approximately 100, 91, 92, and 100%, respectively. Moreover, the frequency of systemic infection with SBLV in many other accessions, including Col-0, was similar to that in the accessions sensitive to SBLV (approximately 80 to 100%), whereas that in the Pla-0 accession was low (approximately 40%). Together, these data indicate that susceptibility to SBLV does not correlate with sensitivity to SBLV in *Arabidopsis* accessions.

Time-course of distribution and accumulation of SBLV in several *Arabidopsis* accessions.

To examine infectivity of SBLV in detail, four accessions, Col-0, Pla-0, S96, and Ei-2, were selected on the basis of their

different responses to SBLV infection and were used for time-course analysis of SBLV spread. Inoculated plants were harvested at 2, 4, and 7 dpi, and SBLV CP was detected by press blot analysis. SBLV infection in Col-0 was first detected as small dots at 2 dpi and had spread through the whole area of inoculated leaves and into some uninoculated leaves by 4 dpi. At 7 dpi, almost all plant tissues were infected with SBLV (Fig. 2). However, in the inoculated leaves of Pla-0, SBLV infection was observed as small dots even at 4 dpi, indicating that at least local spread of SBLV is delayed in Pla-0. On the other hand, the SBLV-sensitive accessions S96 and Ei-2 showed a distribution pattern of SBLV CP similar to that of Col-0 (Fig. 2).

We then checked the time-course accumulation of SBLV CP in Col-0, S96, and Ei-2 plants. The accumulation patterns of SBLV CP in inoculated leaves of Col-0 and Ei-2 were similar. Accumulation of SBLV CP in S96 was delayed to some degree at 1 and 2 dpi but reached similar levels in the three accessions at 3 dpi, at which timepoint symptoms were first observed (Fig. 3). Moreover, accumulation of SBLV CP in uninoculated rosette leaves was similar among the three accessions (Fig. 3). Therefore, we concluded that Col-0, S96, and Ei-2 show different sensitivities to SBLV infection because of a mechanism that is independent of the efficiency of SBLV multiplication in plants.

Genetic traits of symptom phenotypes.

To determine the genetic basis of the symptom phenotypes in SBLV infection, we crossed Col-0 and S96 plants. All F₁ plants from the reciprocal crosses between Col-0 and S96 developed symptoms intermediate to those of the two parental accessions (Table 2). Necrotic symptoms were observed on inoculated leaves of the F₁ plants and were like those of parental S96 at 10 dpi but were mild on uninoculated leaves. The curling of leaves and stems in uninoculated tissues was also mild at 14 dpi (Fig. 1B), and F₁ individuals produced seeds (data not shown). These results indicate that the symptom phenotype of S96 is conferred by a semidominant trait. The F₁ plants were self-pollinated, and the resulting F₂ plants were inoculated with SBLV to determine the segregation of the symptom phenotype of S96. In 212 F₂ individuals from the F₁ population (♀S96 × ♂Col-0) inoculated with SBLV, severe symptoms were observed in 48 plants, mild symptoms were observed in 110 plants, and 54 plants were symptomless (Table 2). This segregation ratio of severe symptoms/mild symptoms/no symptoms was 0.9:2:1 ($\chi^2 = 0.64$), which was similar to the expected ratio of 1:2:1. In an additional analysis using F₂ plants from the F₁ population (♀Col-0 × ♂S96), a similar segregation ratio was observed (Table 2). These results indicate that the symptom phenotype of S96 was conditioned by a single nuclear locus; this locus was designated *SSBI* (symptom development by SBLV infection).

Mapping of the *SSBI* locus.

The map position of the *SSBI* locus was determined by using the F₂ population from the F₁ plants (♀S96 × ♂Col-0). Because it was difficult to extract genomic DNA from the plants showing severe symptoms, we selected mapping lines that did not show any symptoms of SBLV infection (Col-0 phenotype). The genotype at PCR-based DNA markers covering the *A. thaliana* genome was then determined. Nine markers displayed polymorphisms between Col-0 and S96 (ADH, AG, DHS1, GPA1.1, LFY3, nga6, nga8, nga225, and nga280). Initial mapping using these markers showed that two markers, AG and DHS1, which are located on chromosome IV, linked to the Col-0 phenotype (recombination frequency of AG and DHS1 were 16.3 and 4.9%, respectively). The other markers showed no significant linkage with the Col-0 phenotype (re-

Table 1. *Arabidopsis* accessions phenotypes based on symptoms after Spring beauty latent virus (SBLV) infection

Symptom	<i>Arabidopsis</i> accession
No or mild symptoms	Ag-0, Ak-1, Ang-0, Bay-0, Be-0, Berkeley, Bla-2, Bs-1, Bscl-0, C24, Chi-0, Co, Col-0, Condara, Da, Db-0, Di-G, Dra-0, En-D, ENF, Er, EST, Fe-1, Fi-0, Ga-0, Gd-1, HI-0, Hodja-Obi-Garm, Jm-0, KI-0, Ler-0, Li-5, LIN, Litva, Mh-0, Nd-1, No-0, Nok-0, Nw-0, Ob-0, Old-1, Oy-1, Pa-1, Petergof, Pi-0, Pla-0, RLD-1, Rubeznoc-1, Sg-1, Shahdara, Sn, Sorbo, Tol-0, Tsu-1, Tu-0, Wc-1, Wil-1, Wt-1, Wu-0
Severe necrotic symptoms ^a	S96, Fr-2
Severe stunting symptoms ^b	Ei-2, Abd-0

^a S96 and Fr-2 infected with SBLV showed severe necrotic symptoms in uninoculated rosette leaves.

^b Ei-2 and Abd-0 infected with SBLV showed severe stunting in uninoculated rosette leaves.

combination frequency 45 to 65%). Further mapping was performed using 76 F_2 plants showing no symptoms. As shown in Figure 4, some markers on chromosome IV, especially CAT2, showed linkage with the *SSB1* locus. These data suggest that the *SSB1* locus is located within a region between markers ATMYB3R (map position 82.27 cM) and nga1107 (map position 104.73 cM) on chromosome IV.

To examine whether the symptoms induced in Ei-2 were also controlled by the *SSB1* locus, Ei-2 plants were crossed with Col-0 or S96. F_1 plants from crosses between Ei-2 and Col-0 and between Ei-2 and S96 were late flowering like Ei-2. When F_1 plants from the cross between Ei-2 and Col-0 were inoculated with SBLV at 4 weeks after sowing, mild stunting was observed at 14 dpi (Table 2), demonstrating that the symptoms

Fig. 1. *Arabidopsis* accessions showing symptoms induced by *Spring beauty latent virus* (SBLV) infection. **A**, Col-0, S96, and Ei-2 or **B**, F_1 plants from the cross between S96 and Col-0 were inoculated with SBLV virions and were photographed at 14 days postinoculation. In **A**, Col-0, S96, and Ei-2 plants inoculated with buffer (upper) or SBLV (lower) are shown from left to right. Bar = 20 mm.

of Ei-2 are also conferred by a semidominant trait like those in S96. However, all F₁ plants from the cross between Ei-2 and S96 showed severe stunting symptoms like those of the parental Ei-2 (Table 2), indicating that the *SSB1* locus of S96 functioned for development of severe symptoms in Ei-2. Furthermore, the symptom traits of Ei-2 showed linkage to a marker DHS1 that is located near the *SSB1* locus (recombination frequency 7.1%) in the mapping analysis using F₂ population (♀Col-0 × ♂Ei-2). These data suggest that allelic *SSB1* in Ei-2 is also involved in symptom development by SBLV infection.

Involvement of salicylic acid in necrotic symptoms of S96.

Salicylic acid (SA) is a key factor for plant defense responses to pathogens and is intimately associated with cell death (Overmyer et al. 2003). To examine whether SA is involved in symptom development in *A. thaliana* by SBLV infection, we tested the effect of the *NahG* gene encoding salicylate hydroxylase on necrotic symptoms induced in S96. We crossed S96 with either *NahG* transgenic plants (background: accession Nossen) or nontransgenic Nossen plants (No-0) and inoculated F₁ plants from these crosses with SBLV RNA,

Fig. 2. Time-course for *Spring beauty latent virus* (SBLV) spread in Col-0, Pla-0, S96, and Ei-2. Plants were inoculated with SBLV virion and pressed onto chromatography papers at 2, 4, and 7 days postinoculation (dpi). The distribution of SBLV coat protein was immunologically detected. Arrowheads indicate inoculated leaves. Because inoculated leaves of S96 and Ei-2 were dead and desiccated at 7 dpi, these leaves are not shown. Bar = 10 mm.

which was used as inoculum instead of SBLV virion to avoid detecting input CP in press blot assay. Parental No-0 and *NahG* plants showed no visible symptoms of SBLV infection on either inoculated or uninoculated leaves, in contrast to S96

(Table 1 and Fig. 5A). Symptoms induced in F₁ plants from the cross between S96 and No-0 were intermediate compared with those in the two parental accessions (data not shown), as were those of F₁ plants from the cross between S96 and Col-0,

Fig. 3. Time-course of spring beauty latent virus (SBLV) coat protein (CP) accumulation in Col-0, S96, and Ei-2 plants. Two expanded leaves were inoculated with SBLV transcripts at 3 to 4 weeks after sowing. Inoculated and uninoculated rosette leaves were harvested at 0, 1, 2, and 3 days postinoculation (dpi) and 2, 4, 6, and 8 dpi, respectively. The accumulation levels of SBLV CP were analyzed by Western blotting and then densitometrically quantified with the NIH Image program. The SBLV CP accumulations in inoculated (upper graph) and uninoculated (lower graph) rosette leaves of Col-0, S96, and Ei-2 are shown as closed, hatched, and open columns, respectively. Relative values for the accumulation of SBLV CP in leaves compared with those in inoculated leaves of Col-0 at 3 dpi (upper graph) or in uninoculated rosette leaves of Col-0 at 8 dpi (lower graph) are indicated. The mean values and standard deviations (vertical lines) were calculated from six to eight samples. Symptoms in S96 and Ei-2 were so severe after 3 dpi in inoculated leaves and after 8 dpi in uninoculated rosette leaves that accumulation of SBLV CP was not examined afterwards.

Fig. 4. Map position of *SSB1* on chromosome IV in *Arabidopsis thaliana*. The map positions of the markers (the Arabidopsis Information Resource) are indicated at left. The markers on chromosome IV that were used for linkage analysis are indicated on the right. The number of chromatids recombined between a marker and the *SSB1* locus with respect to the total number of chromatids examined is shown in parentheses.

and inoculated leaves of F₁ plants (S96 × No-0) had died by 10 dpi (Fig. 5A). When F₁ plants from the cross between S96 and *NahG* were inoculated with SBLV RNA, inoculated leaves of the F₁ plants (S96 × *NahG*) exhibited mild yellowing and small necrotic lesions at 10 dpi but, in contrast to F₁ plants (S96 × No-0), did not die (Fig. 5A). Even when symptoms were observed at 20 dpi, necrotic symptoms did not expand (data not shown). Press blot assays demonstrated that SBLV spread throughout the inoculated leaves (Fig. 5B). These data strongly suggest that SA has a significant involvement in symptom development in S96 by SBLV infection.

DISCUSSION

In this study, we examined the response of 63 *Arabidopsis* accessions against SBLV infection as a model for analyzing plant–bromovirus interactions. Recently, precise infectivity of BMV in *A. thaliana* was reported, and BMV was shown to systemically infect Col-0 under a certain plant growth condition (Dzianott and Bujarski 2004), which suggests that BMV can be used to identify plant factors governing its systemic infection. On the other hand, the accumulation level of BMV RNA in uninoculated leaves of Col-0 was at least 500-fold lower than that in barley (Dzianott and Bujarski 2004). In our data, the accumulation level of SBLV CP was approximately 800 µg per g of tissue in uninoculated rosette leaves of Col-0 at 14 dpi (F. Hagihara and K. Mise, *unpublished data*), which is fivefold lower than that of BMV CP in barley (Okinaka et al. 2001). Taken together, these data indicate that SBLV multiplication in Col-0 is more efficient than that of BMV. Therefore, SBLV may be more useful in some studies, such as the analysis of virus pathogenicity in plants, as reported in this article.

In most *Arabidopsis* accessions, including Col-0, SBLV infection does not induce visible symptoms, but severe symptoms are induced in some accessions, such as S96. Genetic analysis suggests that symptoms induced in S96 are conditioned by a single nuclear gene, *SSB1*. It has been reported that natural diversity in response to pathogens often occurs in plant genes involved in the recognition of plant pathogens, for example, *R* genes for gene-for-gene resistance (Cooley et al. 2000; Takahashi et al. 2002). Importantly, plant components functioning downstream of the plant defense responses are not diverse among accessions. Therefore, the *SSB1* gene product of S96 but not of Col-0 may be involved in the recognition of SBLV components and may activate plant responses to develop necrotic symptoms, as do *R* genes. Another possibility is that allelic *SSB1* in Col-0 is functional and confers tolerance to SBLV infection. In this model, recognition of SBLV in *A. thaliana* for symptom induction may be

Table 2. Genetic analysis of symptoms in response to *Spring beauty latent virus* (SBLV) infection

Cross	Symptoms ^a			χ^2 ^b	<i>P</i> ^c
	Severe	Mild	None		
F ₁ (♀ S96 × ♂ Col-0) ^d	0	21	0	–	–
F ₁ (♀ Col-0 × ♂ S96) ^d	0	18	0	–	–
F ₂ (selfed F ₁ [♀ S96 × ♂ Col-0]) ^e	48	110	54	0.64	0.73
F ₂ (selfed F ₁ [♀ Col-0 × ♂ S96]) ^e	24	50	20	0.72	0.70
F ₁ (♀ Col-0 × ♂ Ei-2) ^f	0	10	0	–	–
F ₁ (♀ S96 × ♂ Ei-2) ^g	11	0	0	–	–

^a Plants were inoculated with the SBLV virion and scored for symptoms at 14 dpi.

^b χ^2 values were calculated for an expected ratio of 1: 2: 1 for severe/mild/no symptoms in plants.

^c Probability (*P* value) was calculated using GraphPad Quickcalc online calculator.

^d F₁ plants from the reciprocal crosses between S96 (severe) and Col-0 (none) developed symptoms intermediate to those between the two parental accessions.

^e F₂ plants were obtained by allowing F₁ individuals to self-fertilize.

^f F₁ plants from crosses between Ei-2 (severe) and Col-0 (none) developed mild stunting symptoms.

^g F₁ plants from crosses between S96 and Ei-2 showed systemic stunting symptoms like parental Ei-2.

independent of *SSB1*, and *SSB1* may repress the activation of plant responses involved in symptom development, for example, by interfering with the SBLV recognition. In addition, we cannot rule out the possibility that the *SSB1* genes in both S96 and Col-0 are involved in normal development and maintenance of living cells in *A. thaliana*, and SBLV may specifically interfere with the function of *SSB1* in S96. Because the S96 phenotype is semidominant, we cannot postulate whether *SSB1* functions to develop symptoms in S96 or to confer tolerance in Col-0.

In this study, the *NahG* gene critically affected development of necrotic symptoms, suggesting that SA plays a crucial role in symptom production in S96. In the case of a bacterial pathogen, it has been shown that plant hormones such as SA are essential for the production of disease symptoms in *A. thaliana* (O'Donnell et al. 2003). Moreover, SA is associated with leaf senescence and HR formation in gene-for-gene resistance, which are regarded as programmed cell death (PCD) (Overmyer et al. 2003; Yoshida 2003). Considering these reports, SBLV in S96 may activate the SA signaling pathway

A

B

Fig. 5. Necrotic symptoms in inoculated leaves of F_1 plants from crosses between S96 and No-0 and between S96 and *NahG* transgenic plants. **A**, Plants were inoculated with spring beauty latent virus (SBLV) RNA and were photographed at 10 dpi. **B**, Then SBLV coat protein distribution in the leaves was checked immunologically by press blot analysis. Mock-inoculated (upper) and SBLV-inoculated (lower) leaves are shown in each panel. Inoculated leaves of parental S96, No-0, and *NahG* are also shown as controls. SBLV infection in inoculated leaves of S96 and an F_1 plant (S96 $\times$ No-0) was not detected by press blot assay, because the leaves were dead and desiccated. Bar = 10 mm.

and induce PCD. Although the *NahG* gene clearly interfered with symptom production in F₁ plants from the cross between S96 and *NahG* plants, the F₁ plants showed mild yellowing and small necrotic lesions, indicating that symptom development was incompletely inhibited in this generation. This may imply that other pathways independent of SA signaling are also involved in symptom development, although there remains a possibility of unexpected effects from using the F₁ generation, for example, heterozygosity of the *NahG* gene. Further analyses of the effects of mutation in the components involved in SA-dependent signaling and other pathways, such as jasmonic acid- and ethylene-dependent signaling, on symptom production will facilitate understanding the mechanisms of symptom development in the SBLV–S96 system.

Multiplication of SBLV in S96 was similar to that in Col-0, indicating that *SSB1* controls symptom development in *A. thaliana* but not SBLV infectivity. A previous study has demonstrated that TRSV systemically induced necrotic symptoms in Estland but does not induce visible symptoms in Col-0. In that study, Lee and associates (1996) reported that the *TTR1* locus, which controls responses to TRSV, does not have significant effects on virus multiplication. Similar responses to SBLV and TRSV in *A. thaliana* suggest that the *SSB1* and *TTR1* loci control responses to viruses via similar mechanisms although, because the *TTR1* locus is located on chromosome V, it is not allelic to *SSB1*. Identification of *TTR1* and *SSB1* genes will contribute to the elucidation of a mechanism that determines symptom development in plants by virus infection.

MATERIALS AND METHODS

Plants, viruses, and virus inoculation.

Seeds of *A. thaliana* accessions were obtained from the *Arabidopsis* Biological Resource Center, Ohio State University (Columbus, OH, U.S.A.). Seeds of *NahG* transgenic *A. thaliana* (Shah et al. 1999) were provided by H. Takahashi. Seeds were sown on rockwool, were treated at 4°C in the dark for 2 days, and then, were grown in a growth room at 25°C with 16 h of illumination per day in nutrient medium (Dhingra and Sinclair 1985). SBLV (ATCC PV-369, American Type Culture Collection) virion (0.5 µg/µl), virion RNA (0.5 µg/µl), and in vitro transcripts (0.2 µg/µl each of RNAs 1, 2, and 3) were prepared and inoculated into *A. thaliana* as described previously (Fujisaki et al. 2003a).

Detection of SBLV infection.

The press blot assay for detecting the distribution of SBLV CP in *A. thaliana* was performed as previously described (Fujisaki et al. 2003b; Takahashi et al. 2001). To analyze the accumulation of SBLV CP, infected leaves were ground in Laemmli's sample buffer (Laemmli 1970) and were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (samples from 0.4 mg of fresh weight tissues were loaded into each lane), followed by immunoblot analysis as described previously (Damayanti et al. 1999). In both assays, SBLV CP was detected using a rabbit anti-BMV antiserum (ATCC PVAS-178). The accumulation of SBLV CP was densitometrically quantified using the NIH Image program (National Institutes of Health, Bethesda, MD, U.S.A.).

Genetic analysis.

To analyze the genetic basis of sensitivity to SBLV, S96 plants were crossed with Col-0, Ei-2, No-0, or *NahG* plants. F₁ plants were self-fertilized to obtain F₂ seeds, and symptoms of F₁ and F₂ plants induced by SBLV infection were observed at 10 or 14 dpi. Infection with SBLV was confirmed by press blot analysis. In addition, we crossed Ei-2 with Col-0 (♀Col-0 ×

♂Ei-2). F₁ plants from these crosses were inoculated with SBLV as described above.

Mapping of the *SSB1* locus.

To map the *SSB1* locus, we used 14 simple sequence-length polymorphism (SSLP) markers (nga63, nga248, nga280, nga168, nga6, nga112, nga8, nga225, nga139, nga76, nga111, nga1139, nga1107, and DET1.2) and 17 cleaved amplified polymorphic sequence (CAPS) markers (NCC1, ADH, GPA1.1, GL1, AG, DHS1, ASA1, DFR1, LFY3, G4539, PG11, RPS2, PRHA, ATMYB3R, CAT2, UM596A, and CCR1). Information on these DNA markers was obtained from the *Arabidopsis* Information Resource database. Genomic DNA of *A. thaliana* was purified as described previously (Edwards et al. 1991). PCR was performed under the conditions described by Bell and Ecker (1994) for SSLP markers and those described by Konieczny and Ausubel (1993) for CAPS markers. Genotypes at these markers in S96 and Col-0 genomes were checked, and markers showing polymorphism between the two accessions were selected. F₂ plants from the F₁ population (♀S96 × ♂Col-0) were inoculated with SBLV, and plants showing no visible symptoms at 14 dpi were used for mapping. In initial mapping, we used nga63, nga280, nga6, nga112, nga8, nga225, nga139, NCC1, ADH, GPA1.1, AG, DHS1, and LFY3. Further mapping of the *SSB1* locus on chromosome IV was performed using nga1139, nga1107, ATMYB3R, and CAT2.

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