Chapter Two

Biological and Molecular Diversity of Naturally Occurring Resistance Breaking Strains of *Soybean mosaic virus*

ABSTRACT

Resistance to *Soybean mosaic virus* (SMV; Genus *Potyvirus*; Family *Potyviridae*) is controlled by single dominant genes at three distinct loci, *Rsv1*, *Rsv3* and *Rsv4*, in soybean (*Glycine max* [L.] Merr.). SMV isolates are classified into pathotypes G1 through G7 based on the differential reactions on resistant soybean cultivars. Most SMV isolated in the USA is of the G1 to G3 pathotypes. Since the cultivar Hutcheson carrying the $Rsv1^y$ allele for resistance to SMV G1 to G3 is widely used in the Mid-South region of the USA, SMV isolates that overcome that resistance are of particular interest for their biological and genomic diversity, as well as for their potential economic importance. Greenhouse experiments showed that SMV isolates collected between 1998 and 2002 have characteristics of the G5 and G6 pathotypes, and produce distinctive symptoms on Hutcheson. These resistance-breaking (RB) isolates systemically invaded cvs. Hutcheson and York, induced tip necrosis on Kwanggyo, and showed no symptoms on PI 96983 indicative of an extreme resistance (ER) response. These new isolates also induced a diversity of responses on Marshall and PI 507389 and on L29, an *Rsv3* carrying cultivar. Regions of the SMV genome encoding the coat protein of these RB isolates were PCR amplified and sequenced. Phylogenetic analysis of the coat protein showed that RB isolates of SMV are G6-like based on their nucleotide sequence. The coat protein is diverse at the amino and carboxy termini and highly conserved in the core region. The RB isolates were also G6-like based on the amino acid sequence of the coat protein. Amino acid substitutions were detected at 16 positions, indicating higher diversity than previously reported for SMV. All of the RB isolates retained the DAG triplet required for aphid transmission Emergence of SMV-G6 like isolates is a threat to resistance in soybean carrying the $Rsv1^y$ allele.

KEYWORDS: *Glycine max, Coat Protein, Emerging Viruses*

INTRODUCTION

Soybean mosaic virus (SMV) occurs worldwide and is one of the most important viruses in soybean (*Glycine max* [L.] Merr.) (Hill , 1999; Brunt et al., 2003). SMV is a member of the Genus *Potyvirus* and Family *Potyviridae*, the largest family of plant viruses (Shukla et al., 1994). Early infections of soybean can result in reduction of pod set, reduction in seed size and weight, increase in seed coat mottling, and decrease in seed quality. In contrast, late infections with SMV have a limited effect on yield and seed quality (Hill 1999; Hill et al., 1987).

Cho and Goodman (1979, 1982) used a set of resistant soybean cultivars [Davis, Kwanggyo, Marshall, Ogden, and Buffalo] to assign different SMV isolates to strain groups. The SMV isolates were classified as inducing systemic mosaic (S), necrosis (N), and symptomless or resistance (R). SMV isolates were classified, based on responses of this set of differential cultivars, into seven strain groups (G1 to G7) with the low numbered groups being least virulent (Table 1.1).

Resistance to SMV was first reported by Kiihl and Hartwig (1979) to be regulated by a single dominant gene in soybeans. The different cultivars used by Cho and Goodman for isolate classification have single SMV resistance genes that map to the same locus (Chen et al., 1991). Roane et al. (1986) suggested a gene for gene model for the SMV-soybean interactions. To date, genes for resistance to SMV have been identified in soybeans at three distinct loci *Rsv1*, *Rsv3,* and *Rsv4* (Gunduz et. al., 2004; Hayes and Saghai Maroof 2000; Hayes et al., 2000; Ma et al., 1995; Chen et al., 1993). *Rsv1* is a dominant gene identified from PI 96983 and originally named *Rsv*. The *Rsv1* locus was mapped to linkage group F in the soybean genome (Yu et al., 1994, 1996). The *Rsv1* locus conditions both necrotic and resistant reactions (Chen et al., 1994). Eight *Rsv1* alleles have been identified (Buss et al., 1989; Chen et al., 1991; Chen et al., 2001; Ma et al., 2003) that counter resistance to the lower numbered strains; however, plants carrying *Rsv1* display mosaic or necrotic reactions to the higher numbered strains (Chen et al., 1994, 2001).

The cultivar Hutcheson, which is homozygous for $Rsv1^y$, was released in 1988 (PVP 8800138; Buss 1988). It is derived from a cross between Essex and V68-1034, from York x PI71.506. Hutcheson is a high-yielding and widely-used cultivar in the Mid-South region

where maturity group V cultivars are grown (Ustun et al., 2001) and in high or low yielding environments. Less than 10 years after its release as a cultivar, we started detecting resistance-breaking (RB) isolates of SMV in natural infections in a virus nursery (Tolin, unpublished). The common SMV strains in natural infections in US soybean fields over 20 years ago were SMV-G1 and SMV-G2 (Cho and Goodman, 1979). In the same study, only 25% of all field isolates were found to be of the G5 or G6 strains and less than 2% were of the G7 type strain. SMV G4 was not found in field samples and was limited to seeds. Since Hutcheson is resistant to the most common SMV strains in the US and since the occurrence of SMV isolates that break its resistance happened relatively quickly, there is a significant threat to the durability of SMV resistance and the success of these widely used cultivars. The threat is that severe SMV strains have evolved to break the Hutcheson resistance, the most widely used source of resistance to SMV. In this study, we (i) determine the pathotype to which these RB field isolates belong using cultivars used by Cho and Goodman for strain determination; (ii) determine their biological diversity on additional SMV resistant soybean cultivars, and (iii) determine their molecular diversity with respect to the CP region of the genome.

Members of the *Potyviridae* are classified based on the nucletoide sequence of their genome (Shukla and Ward, 1988). Berger et al. (1997) reported that nucleotide or amino acid sequences could be used to differentiate closely related virus species, strains and isolates. Sequence comparisons of the 3' untranslated regions (UTR) and the coat protein (CP) were used to classify members of the family *Potyviridae*, including the different taxa, species and strains. Berger et al. (1997) also reported that members of the *Potyviridae* that infect the *Fabaceae* have 56% or more similarity at the amino acid level in the CP region. Bousalem et al. (2000) showed that variation at the CP could be used to classify isolates of *Yam mosaic virus* (YAM; Genus *Potyvirus;* Family *Potyviridae*) into nine groups based on the phylogenetic analysis of their respective CP sequences. Isolates of *Turnip mosaic virus* (TuMV; Genus *Potyvirus*; Family *Potyviridae*) have been classified based on their CP sequences (Walsh and Jenner, 2002). Even though isolated from small geographic regions, TuMV isolates have different host ranges, serological properties, single strand comformation polymorphism (SSCP) profiles, and induce different symptoms (Stavolone et al., 1998). Sequence analysis of the *Wheat streak mosaic virus* (WSMV; Genus *Tritimovirus*; Family *Potyviridae*) CP revealed

four regions of genetic diversity, which were used for classification of the different isolates of WSMV (Chenault et al., 1996).

The CP is variable at the amino and carboxy termini in potyviruses. Within the amino terminus of potyvirus CP, a conserved DAG triplet is essential for aphid transmission. Mutation in the *Tobacco vein mottling virus* (TVMV; Genus *Potyvirus*; Family *Potyviridae*) and *Tobacco etch virus* (TEV; Genus *Potyvirus;* Family *Potyviridae*) genomes that result in amino acid substitutions at the DAG motif affected the ability of the mutants to infect tobacco plants systemically (Lopez-Moya and Pirone 1998). Bateson et al. (2002) found that the amino terminus of the CP of *Papaya ring spot virus* (PRSV; Genus *Potyvirus;* Family *Potyviridae*) was the most variable in the PRSV genome. Different isolates of *Lettuce mosaic virus* (LMV; Genus *Potyvirus*; Family *Potyviridae*) were used for sequence comparison. Krause-Sakate et al. (2002) reported that the highest variation in the LMV genomes occurred at the carboxy terminus of the NIb and the amino terminus of the CP regions.

Jayaram et al. (1991, 1992) determined the complete nucleic acid sequence of strains G2 and G7 of SMV. These are the only full-length genome sequences of SMV that have been reported from the US. Coat protein (CP) sequences have also been determined for SMV-N (Eggenberger et al., 1989), SMV-VA (Gunyuzlu et al., 1987), and SMV G1 and G6 (Qusus, 1997). Studies by Dolja et al. (1994, 1995) showed that the different domains of the CP play distinct roles in cell-to-cell and long distance movement of the virus. Thus, the CP is an effective target for distinguishing among SMV isolates and may provide information on the evolution or source of these resistance-breaking isolates. Because the CP has been shown to be critical in mediating host-virus interaction and symptom development, such analyses could identify changes at the molecular level that are associated with changes in pathogenicity. This study will serve as the basis of subsequent studies involving site-specific alterations or SMV chimeras to experimentally establish key regions in the coat protein that are important for mediating virus-host interactions.

MATERIALS AND METHODS

Virus Isolation, Identification, and Maintenance

The source and designation of the SMV field isolates are summarized in Table 2.1. These isolates are a subset of SMV field isolates that were collected, and were selected because they overcome the resistance gene in Hutcheson (*Rsv1*^{y}). Hereafter, they are referred to as resistance-breaking (RB) isolates. The isolates designated S97, S98, and 2K were collected in 1997, 1998, and 2000, respectively. Cultures of each isolate were initiated by mechanical transmission to Lee 68 or Hutcheson and were maintained in the greenhouse. Leaves from symptomatic plants were sampled, brought to the greenhouse, and maintained on Hutcheson by transferring to seedlings every 2-3 weeks. Seedlings at the unifoliolate leaf stage were inoculated with a pestle using 1:10 inoculum (1 g tissue in 10 ml 0.01 M sodium phosphate buffer pH 7.0) prepared by homogenizing leaves using chilled mortars and pestles. Leaves were first dusted with 600 mesh carborundum (Buehler, Lake Bluff, IL), rub-inoculated with SMV, and rinsed with tap water. All isolates were identified as SMV serologically by tissue blot immunoassay using 0.45 µ NitroPure nitrocellulose transfer membranes (Osmonics®, Westborough, MA), 1:10,000 rabbit polyclonal antisera to SMV-G1 whole particles (Hunst and Tolin, 1982), 1:15:000 alkaline phosphatase conjugated goat anti-rabbit (Sigma®, Saint Louis, MO), and nitroblue tetrazolium salt/5 bromo-4-chloro-3-indolyl phosphate substrate (Zymed®, San Francsico, CA).

Virus Pathotype Determination

Pathotype determination was performed under greenhouse conditions using the cultivar series of Cho and Goodman (1979), as modified by Chen et al. (1991). Cultivars used included Lee 68 or Essex, Kwanggyo, Marshall, Ogden, York, and PI 96983. In addition, cultivars with other known *R* genes were used. These included Essex-*Rsv1* and Essex-*Rsv4* developed by G. R. Buss (Crop and Soil Environmental Sciences Dept., Virginia Polytechnic Institute and State University, Blacksburg VA), Hutcheson (Rsv1^{y}) (Buss et al., 1988), PI 507389 (*Rsv1*^{n}) (Ma et al., 2003) and L29 (*Rsv3*) (Bernard and Nelson, 1991). All mechanical inoculations were done as described earlier using 1:10 inoculum from the isolates maintained on Hutcheson in the greenhouse. Pathotype determination and comparative pathogenicity experiments were

conducted in duplicate using one pot (4-6 plants) of each cultivar for each isolate and type member of strain groups of SMV (G1, G5, G6 and G7). The strain group members were the same as ATCC accessions PV571, PV573, PV612, and PV613, respectively, submitted by S.A Tolin. Symptoms were observed and regularly recorded 6 to 28 days following inoculation.

RNA Extraction

Sequence analyses were performed on known strains and the field isolates of SMV that break the resistance of Hutcheson described in Table 2.1. Total RNA was extracted from leaves of plants approximately 2 weeks after inoculation with SMV, using the Qiagen® RNeasy Minikit with a silica-gel-based membrane, following the manufacturer's instructions (www.qiagen.com). Fresh leaf tissue (0.1 g) was homogenized in a microfuge tube using liquid nitrogen, and centrifuged through the QIAshredder at maximum speed in a benchtop centrifuge. Following ethanol addition, the sample was transferred to the RNeasy spin column membrane. Total RNA bound to the membrane was washed three times with buffer, then eluted in water by centrifugation (at maximum speed). RNA was kept frozen at –20 ^{o}C until use.

Generation of cDNA and PCR Amplification

RNA extracts were used to generate first strand cDNA by reverse transcription (RT) using the Promega® Reverse Transcription System (www.promega.com) with the *Avian myeloblastosis virus* reverse transcriptase enzyme. Oligo $(dT)_{15}$ was used as primer for first strand cDNA synthesis from polyadenylated RNAs. To 6 µl of RNA extracts, the following were added (according the manufacturer's instruction) to make the 20 µl total volume: 2µl RT-buffer, 3.75 µl water, 4 µl $MgCl_2$, 2 µl dNTP, 0.5 µl RNAsin inhibitor, 1 µl Oligo $(dT)_{15}$, 0.75 µl AMV-RT. Samples were incubated at 42^{o}C for 15 min, 95^{o}C for 5 min and 4^{o}C for 5 min. The resulting cDNAs were stored at -20^{o}C until further use.

PCR was used for amplification of the CP region of the SMV genome using the Promega® PCR Core Systems (www.promega.com) with *Taq* DNA Polymerase from *Thermus aquaticus*. A PTC-100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA) was used for both RT and PCR steps. Primers used for the CP amplification were described by Qusus (1997). The sequences of the upstream (P6) and downstream (P5) primers flanked the

coat protein coding region by 40 and 27 nucleotides respectively are given in Table 2.2. To each 6 µl of cDNA, the following were added to make a 50 µl total volume/reaction: 32.75 µl RNAse-free water, 5 µl 10X buffer, 1 µl dNTP, 1 µl primer P5 (50 pmol/µl), 1 µl primer P6 (50 pmol/µl), 3 µl $MgCl_2$, 0.25 µl Taq DNA Polymerase. Reaction mixtures were incubated at 95°C for 2 min, followed by 35 cycles of 1 min denaturation at 95°C, 2 min annealing at 50°C, and 2 min extension at 72°C; followed by a final extension at 72°C for 5 min. In order to characterize the amplified fragments, PCR samples (10 µl of PCR products and 5 µl loading dye) were separated by electrophoresis in 1% agarose in TAE buffer at 50 V, stained with ethidium bromide sheets (DNA InstaStain ™) and viewed with a UV light. Digitized images were recorded using an Alpha Imager (Alpha Innotech Co., San Leandro, CA).

PCR Product Cleanup and Sequencing

Bands of the expected size were purified using the Qiagen QIA PCR Purification Kit according to manufacturer's instructions (www.qiagen.com). DNA was re-suspended in RNAse-free water in the final step and sequenced at the DNA Core Laboratory, Virginia Bioinformatics Institute, CRC, Blacksburg, VA using Applied Biosystems Big Dye Terminator (v2.0) Chemistry.

Sequence Analyses

The resulting sequences were compared to those of the G1, G2, G5, G6 and G7 (accessions AH008451, S42280, AY294044, AF242845, and AY216010, respectively) pathotypes of SMV using the Blast Engine from the NCBI Server (www.ncbi.nlm.nih.gov). CP sequences were paired with each other and with G1, G2, G5, G6, and G7 using BlastN to determine percent identities of the nucleotide sequences. Deduced amino acid sequences were generated by transforming the nucleic acid sequences using the Bioinformatics.org public server (http://www.bioinformatics.org/. Phylogenetic analysis was performed on the nucleotide and amino acid sequences using the PHYLIP program (http://workbench.sdsc.edu) from Molecular Biology Workbench.

RESULTS

Differential Response to SMV Pathotypes and RB Isolates

Pathotypes of RB isolates of SMV were determined using a standard set of strain-differentiating soybean cultivars (Cho and Goodman, 1979; Chen et al., 1991). The results of these trials are presented in Table 2.3. As expected, all of the RB isolates invaded the susceptible Essex (*rsv*) causing typical mosaic and chlorosis; this susceptible reaction is designated S. The *Rsv1* allele in PI 96983 and Essex-*Rsv1* exhibited extreme resistance to all the RB isolates (no detectable symptoms) and to SMV-G1 through G6, hence the designation R. These cultivars displayed necrosis in response to SMV-G7, hence the N designation. Cutlivars Hutcheson and York, both carrying the $Rsv1^y$ allele, were susceptible to all the RB isolates. The RB isolates of SMV showed distinct symptoms (not shown) on inoculated leaves of Hutcheson that were reproducible in subsequent transfer of the isolate cultures in the greenhouse. These two cultivars were resistant to SMV-G1, G2 and G3, necrotic to SMV-G4, and susceptible to SMV-G5, G6, and G7. Kwanggyo was resistant to SMV-G1 to G4 and necrotic to all the other strains and to all the RB isolates. The tip necrosis response of Kwanggyo to all the RB isolates was similar to that caused by SMV-G5. Within 8-10 days after inoculation, upper non-inoculated leaves developed necrotic veins, followed by a spreading necrosis and death of all leaves and the apical meristem. Necrosis induced by SMV-G6 was not as extensive and developed more slowly. The RB isolates were thus most similar to SMV strains G5 and G6 in their interactions with various soybean hosts. In order to further differentiate among RB isolates and G5 and G6, we conducted detailed analyses to assess interactions with soybean cvs. Kwanggyo ($Rsv1^k$), Marshall ($Rsv1^m$), PI 507389 ($Rsv1^n$) and L29 (*Rsv3*).

The phenotypes of the Kwanggyo and Marshall responses induced by RB isolates of SMV were similar to those induced by SMV-G5 (Table 2.4). Kwanggyo showed that all RB isolates are SMV-G5-like except for 2K-13i and 2K-22, which induced a mild tip necrosis (Table 2.4). Symptoms induced by the RB isolates on Marshall were similar to those induced by SMV-G5 or G6 (Table 2.4). Marshall was expected to be resistant to SMV-G1 and G4, and necrotic to all the others (Cho and Goodman, 1979, 1982). Our results were not in agreement with Cho and Goodman (1979, 1982) with respect to the response of Marshall to SMV-G5

(Table 2.4). SMV-G5 induced a slow localized veinal necrosis versus the SMV-G6 that induced a faster localized and systemic veinal necrosis . Marshall showed that five RBs are SMV-G5-like, inducing slow local veinal necrosis. Marshall was resistant to the other five RB isolates, including 2K-13i and 2K-22. To further compare the biological diversity of the RB isolates, they were tested on PI 507389 and L29 with the *Rsv3* gene (Table 2.4) and Essex-*Rsv4* (Table 2.3). Essex-*Rsv4* was resistant to all strains and isolates of SMV tested. PI 507389 was susceptible to all the RB isolates and to SMV-G5 and G6. However, like SMV-G5, 2K-13i and S97-SBT-2 induced no necrosis whereas all others induced a transient necrosis on the inoculated unifoliolates and non-inoculated trifoliolate leaves. The line L29 carrying the *Rsv3* gene was resistant to the RB isolates 2K-22, S97-SBT-2, S98-51 and S98-52. However, L29 showed a hypersensitive response (HR) resulting in localized necrosis (LN) on the inoculated leaves in response to 2K-40 and 2K-44c (Figure 2.1A), and in response to 2K-13i, 2K-22, 2K-38 and 2K-39 showed localized and veinal necrosis on the inoculated unifoliolate leaves (Figure 2.1B) and systemic mosaic on trifoliolate leaves (Figure 2.1C) These reactions are unlike those produced on L29 by any of the other SMV strains. Use of these five cultivars provided interactions that effectively differentiated between the RB isolates and SMV-G5 and G6. We therefore targeted direct sequence of the CP regions of RB isolates to further differentiate their relatedness to each other and to SMV-G5 and G6.

Nucleotide and Amino Acid Sequence Analyses

Phylogenetic analysis was performed on the nucleotide sequences of CP determined from RB isolates of SMV. PCR amplification products from all the RB isolates and SMV-G1 and G7 with the primer pair P5 and P6 (Figure 2.2; Table 2.2) yielded products consistent with the predicted size of 921 bp. These products were sequenced using the same primer pair in order to generate the consensus sequence from the PCR population. All the RB isolates had nucleotide and amino acid sequence identities ranging from 94-95% and 96-98%, respectively, with the published sequences of SMV-G2 and G7 (Table 2.5). Those numbers were slightly lower for SMV-G1. Percentage similarity of nucleotides and amino acids between the RB and SMV-G5 and G6 (accessions AY294044 and AF242845) were greater than those with G7 or G1. Identity at the nucleotide level was 93-99% and was 97-99% at the amino acid level. The sequence from 2K-40 was not included because it showed only a small region of SMV

homology (240-270 bp) and aberrant non-related sequences for the rest of the fragment. New PCR amplifications and sequence analyses will be done for this isolate. Phylogenetic analysis showed all RB isolates are in the same branch (Figure 2.3) with SMV-G6 and are therefore most closely related to SMV-G6.

The deduced amino acid sequences of the CP were compared between the different strains and RB isolates of SMV (Table 2.6; Figure 2.1). The CP from all the SMV strains and RB isolates is composed of 265 amino acids, with serine at position 1 and glutamine at position 265, and a highly conserved DAG triplet (amino acid positions 10-12) sequence near the amino terminus. Except for 2K-38, percentage similarity for CP amino acid sequences between all the RB isolates was 96% or higher. Deduced amino acid sequences for the RB and SMV-G1 (PV 571) are shown in Figure 2.4. Alignment of the CP amino acid sequence from all the strains and RB isolates showed changes at 16 positions (shaded in Figure 2.4; positions 19, 21, 23, 26, 27, 29, 30, 37, 295, 228, 239, 241, 247, 250, 256, and 264). Amino acid substitutions in the CP among all strains and isolates of SMV were found mainly at the amino and carboxy termini of the CP leaving the core region conserved. Identical CP was found for 2K-24, 2K-39, and 2K-44c, two of which were isolated from Hutcheson but from different locations in the state of Virginia (Table 2.1).

Amino acid substitutions between all strains and isolates of SMV were found mainly at the amino and carboxy termini of the CP leaving the core region conserved except for substitution of valine to isoleucine at position 75 in G7, glycine to valine at position 134 for G1, lysine to glutamine at position 205 (G1 from PV 571, 2K-13, 2K-38, S98-51, S98-52), lysine to glutamine at position 228 in S97-SBT-2. Amino acid substitutions at the amino terminus occurred at positions 18 in G1, G2, G5, G7, G1-PV571 (serine instead of asparagine). 2K-38 had six additional amino acid substitutions at positions 21 (arginine for serine), 29 (arginine for isoleucine), 30 (isoleucine for lysine), 37 (isoleucine for lysine), 247 (proline for threonine), and 250 (asparagine for aspartic acid), the most of any of SMV strains and RB isolates. The amino acid substitutions at positions 30 and 37 (isoleucine for lysine) in 2K-38 are important since these change affect the charge and binding properties of the amino acid, and the could alter the structure of the capsid protein. Amino acid substitutions at the carboxy terminus were detected at positions 239 for S98-52 (histidine to asparagine), position 241 for 2K-38 and S97-SBT-2 (glutamic acid to glutamine), position 247 for 2K-13i and 2K-38

(threonine to proline), position 250 for 2K-38 (aspartic acid to asparagine), position 256 2K-13i and S98-52 (histidine to arginine), and position 264 where four of the sequences had a proline instead of glutamine.

DISCUSSION

Resistance-breaking isolates of SMV were collected from the resistant soybean cv. Hutcheson in natural infections. These RB isolates were tested on the differential soybean cultivars used by Cho and Goodman (1979, 1982) and Chen et al. (1991), all of which contain an allele at the *Rsv1* locus. Our results (Tables 2.3) showed that York and Hutcheson were susceptible to all the RB isolates with symptoms appearing about 1 week after inoculation. The only strains that induced a similar response on these cultivars were SMV strains G5, G6 and G7. PI 96983 was resistant to all the RB isolates with no necrosis. PI 96983 was also resistant to strains G1-G6 and necrotic to only SMV-G7. Therefore, the RB isolates cannot be G7 and may be either SMV-G5 or SMV-G6.

Kwanggyo differentiated between SMV-G5 and G6. It is resistant to SMV strains G1-G4 and necrotic to SMV-G5, G6, and G7. However strain G5 induced a fast tip necrosis (necrosis invading the apical meristem and eventually killing the plant) whereas with SMV-G6, the response was a slow necrosis. In addition, the necrosis was faster to appear and more severe, with SMV-G5 displaying a tip necrosis that eventually turns lethal. All of the RB isolates induced necrosis on Kwanggyo, but the extent of the response was variable permitting them to be grouped into two classes: (1) 2K-13i and 2K-22 causing mild tip necrosis similar to SMV-G6; (2) all the other RB causing fast tip necrosis more like SMV-G5.

To further differentiate among these strains, we used cultivars with additional SMV resistance genes. L29, an *Rsv3* carrying cultivar, showed a varied response to the RB isolates. It was resistant to both G5 and G6 and susceptible to four out of the ten RB isolates tested. Two of the six remaining RB induced a slow localized necrosis; the other four induced a fast localized and systemic necrosis with mosaic symptoms developing on the upper uninoculated leaves. It is worth noting here that L29 was completely susceptible to low numbered strains of SMV and resistant to high numbered strains. Although results from L29 showed a different response among SMV strains G5 and G6 and all the RB, it does biologically differentiate the

RB isolates into three classes. However we have not been able to draw a clear distinction among the RB isolates based on these additional cultivars, including PI 507389, and the *Rsv1*-containing cultivars used for the pathotype determination (Cho and Goodman 1979, 1982; Chen et al., 1991). Additional resistant soybean cultivars, all of which contain the *Rsv3* gene, are being investigated to further differentiate between the RB. However, biologically they all act more like G5 and G6 on soybean cultivars with *Rsv1* alleles (Table 2.3).

Coat protein sequences were used to further differentiate the RB isolates and investigate their phylogenetic relationships. The CP nucleotide sequences were highly similar among all the RBs and SMV-G6. Based on phylogenetic analysis of the CP nucleotide sequences, we suggest that all the RBs tested originated from SMV-G6 by natural selection in the field. This could well be attributed to an increasing usage of Hutcheson and its derivatives in soybean growing areas and in our virus nursery in particular. The similarity at the CP level is not surprising since the CP is a highly conserved region of the genome and all were serologically positive to antisera raised against SMV G1. Amino acid substitutions occurred at 16 positions in the CP region. This is a substantially higher number than was observed by Domier et al. (2003) for a different set of SMV isolates and strains of SMV. Their results showed 91 to 99% identity in the nucleotide sequence and 95 to 99% in the amino acid sequence. However no association, between geographic origin, including Asia and sequence identities or RFLP profiles, were detected (Domier et al., 2003).

Our results showed that except for 2K-38, the amino acid sequence of the CP is highly conserved within the RB isolates. This is expected for potyvirus coat proteins and for SMV in particular. Virus particle assembly and virus-vector interaction exert 'purifying selection' pressure (Domier et al., 2003) on the CP limiting the synonymous substitutions in the CP region. Although diverse in the biological responses they induce in legumes, potyviruses are closely related in terms of their genome identities (Berger et al., 1997). Phylogenetic analyses of members of the *Potyviridae* suggest that within each branch of the tree, there exist clusters of sequences that fit the quasispecies concept and that the natural diversity of the sequences is due to RNA-dependent RNA polymerase (RdRP) induced errors (Roossinck, 1997). Virus diversity has been reported to be a direct consequence of mutations accumulating due to these errors (Roossinck, 1997). Potyviruses have RNA genomes and replicate in a short time, and therefore have high population numbers and high mutation rates. Mutation or error rates are

estimated at 10^{-4} per nucleotide per generation. It is important to note, however, that the quasispecies are not plastic; they respond to environmental factors such as temperature (Harrison, 2002) and the consensus sequence around which the swarm of genome exists can vary. Interestingly, isolate 2K-38 showed the highest variability in the CP sequence compared to all the other RBs and strains tested, and induced the most unusual reactions on Hutcheson and Essex causing distinct symptoms including yellowing.

The CP were 100% identical at the amino acid level in 2K-24, 2K-39 and 2K-44c, isolated from Hutcheson at different locations in the state of Virginia (2K-24 and 2K-39 were also identical at the nucleotide sequence). This is likely due to the fact that all the RB were isolated from research plots and the same seed source was used in the various different fields or blocks. SMV is known to be seed transmitted (Hill, 1999). However, as shown in Table 2.4, the interactions of 2K44c on Marshall and L29 differed from 2K-24 and 2K-39 indicating that changes in other regions of the SMV genome are contributing to the specific responses these RB isolates induce on soybean.

Diversity in the viral genome is a result of one or more of the following: recombination, point mutation, or selection. Recombination events that occur between isolates or strains of the same virus, and between different virus species belonging to the same genus, have been considered a factor in virus genome diversity (Simon and Bujarski, 1994). Recombination between *Bean common mosaic virus* and *Bean common mosaic necrosis virus,* two Potyvirus species infecting the common bean (*Phaseolus vulgaris* L), has been reported (Silbernagel et al., 2001). Twenty-eight days after these two strains were inoculated on opposite primary leaves, recombinant forms were isolated that were different from each of the two parental forms and are phenotypically different (Silbernagel et al., 2001). Point mutations and recombination events in the TuMV were the causal factors in the diversity of the TuMV genomes (Walsh and Jenner, 2002).

Selection is a third factor that could influence genetic variation in virus genomes (McNeil et al., 1996; Garcia-Arenal et al., 2001). A balance between selection pressure and mutation errors due to replication is needed for diversity to be stable (Walsh and Jenner, 2002). This balance affects the biological fitness of the variants in terms of virus replication, virus-host interactions and virus-vector fitness. All these determine the fitness of the quasispecies in

the field (McNeil et al., 1996). In addition, the biological fitness of an RB strain is determined by the type and number of resistance genes the RB will break (Harrison, 2002).

Genes other than the CP have been used in comparing different strains and isolates of potyviruses. Hajimorad and Hill (2003) reported that no amino acid substitutions were found between pSMV-G7d, a mutant clone that evolved to evade the HR response mediated by *Rsv1*, and its progenitor pSMV-G7 in the cylindrical inclusion (CI), 6K, NIa-VPg and the 3'UTR. One substitution was detected in each of the helper component (HC-Pro), the P1 proteinase and the CP regions. Three AA substitutions were detected in the P3 proteinase region. Only seven amino acid substitutions caused the cloned mutant to evade detection by the *Rvs1* gene and change it from a compatible to an incompatible reaction. Similar work has been also done on other regions of the SMV genome. Kim et al. (2003) sequenced the CI regions of G7H, an isolate of SMV that developed to be severe on Suweon in 1999, and is believed to be prevalent in Korea at the present. G7H was compared to four other strains G5H, G5, G2 and G7 and percent amino acid similarity ranged from 95 to 99%. However there was no correlation between the amino acid identity and the response these strains induce in soybeans. Cultivated soybean is the only known host of SMV in the US, and genetic diversity of soybeans in the US is low. These factors are not considered favorable for enhancing genetic diversity in the virus population (Domier et al., 2003). The P1 region of SMV is reported to have higher variability that correlates to the geographic origins of different isolates of SMV (Domier et al., 2003). Continuing work in our laboratory will look at the diversity in the P1 gene from the RB isolates.

Harrison (2002) suggested that resistance-breaking isolates of a virus species should be compared to those of the non-RB phenotype to identify specific regions of their genome that are pathogenicity factors. However, all virus encoding genes can be potential factors in pathogenicity and pathogen-host interactions including *Avr-R* recognition and induction of resistance pathways described by Flor (1971) and Dangl and Jones (2001). Walsh and Jenner (2002) indicated that although a strain is able to break the resistance, it does not necessarily mean that the RB will result in successful infection. In most cases, RB strains are not economically important since they are not seed transmitted and thus limited to local outbreaks. However, when RBs are seed-borne, they would be considered a major threat (Krause-Sakate et al., 2002).

In conclusion, RB isolates that break the resistance of Hutcheson have emerged in the field. RB isolates have the biological characteristics of strains G5 and G6 on *Rsv1* soybean differentials, but are most closely related to G6 based on CP sequence. It is important to test the agronomic effects of these RB isolates of SMV since they break the resistance of a widely used cultivar in the Midsouth region of the US. This knowledge is essential in assessing the fitness of these RB strains in natural infections. It is equally important to further characterize these RB isolates at the genome level in order to understand their evolution and to design rapid, genome-based detection assays.

Table 2.1. Resistance-breaking field isolates of SMV: Nomenclature, location, and host.

Isolate	Year	Location of source	Cultivar
S97-SBT-2	1997	Sandhills, NC. (Variety trials)	Benning
S98-51	1998	Virus nursery, Blacksburg VA. (yield loss trials)	Hutcheson
S98-52	1998	Virus nursery, Blacksburg VA. (yield loss trials)	Hutcheson
2K-13i	2000	Breeding nursery, Blacksburg VA	PI556950
2K-22	2000	AREC, Warsaw, VA. (crossing and demonstration blocks)	PI 507389
2K-24	2000	AREC, Warsaw, VA. (yield trials)	RR-52 Hutcheson
2K-38	2000	Virus nursery, Blacksburg VA.(border row)	Hutcheson
2K-39	2000	Virus nursery, Blacksburg VA. (border row)	Hutcheson
2K-40	2000	Virus nursery, Blacksburg VA. (border row)	Hutcheson
2K-44c	2000	AREC, Warsaw, VA. (crossing and demonstration blocks)	Tambakura

Table 2.2. Synthetic oligonucleotide primers used in PCR amplifications of the coat protein genes of G1, G6, G7 and the RB isolates. Primers were synthesized based on the SMV-G2 sequences (Jayaram et al., 1992).

Primer	Length	Orientation	Position	Nucleotide sequence (5'-3')
P6	29	Forward	8472-8494	**CGTTCT**AGATATCTTGAAGTGCTGGATTT
P5	28	Reverse	9360-9379	**TGCTCTAG**ATAAAGCGACCCGAAATGAT

Bases in bold are not from SMV-G2 sequence and were designed to include nucleotides for *Xba* I restriction sites (Qusus, 1997).

Table 2.3. Pathotype determination of resistance-breaking (RB) field isolates of *Soybean mosaic virus.*

Results show differential response of soybean cultivars carrying one allele of the *Rsv1* gene or one allele of the *Rsv4* gene for resistance to SMV strain groups G1 through G7 (Cho and Goodman, 1979, Chen et al., 1991, Ma et al., 1995) and the RB isolates.

		SMV Strains G1-G7 and RB Isolates							
Cultivar	**Gene**	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**RB**
Essex	*rsv*	S	S	S	S	S	S	S	S
PI 96983	*Rsv1*	R	R	R	R	R	R	N_t	R
Essex-*Rsv1*	*Rsv1*	R	R	R	R	R	R	N_t	R
York	$Rsv1^y$	R	R	R	N_t	S	S	S	S
Hutcheson	$Rsv1^y$	R	R	R	N_t	S	S	S	S
Kwanggyo	$Rsv1^k$	R	R	R	R	N_t	N	N	N_t
Essex-*Rsv4*	*Rsv4*	R	R	R	R	R	R	R	R

R= resistant (no symptoms; no recoverable virus)

N= necrotic (local and systemic), N_t = tip necrosis (invading the apical meristem and eventually killing the plant)

S= susceptible (mosaic)

RB= resistance-breaking.

Table 2.4. Differential responses of Kwanggyo, Marshall, PI 507389, and L29 to SMV strains G5 and G6, and the resistance-breaking isolates of SMV.

Cultivar	Symptoms induced by SMV on four resistant cultivars[a] (inoculated / non-inoculated)											
	G5	G6	S97-SBT-2	2K-13i	2K-22	2K-24	2K-38	2K-39	2K-40	2K-44c	S98-51	S98-52
Kwanggyo (*Rsv1k*)	LN/FN_t	LN/SN	LN/FN_t	LN/MN_t	LN/MN_t	LN/FN_t	LN/FN_t	LN/FN_t	LN/FN_t	LN/FN_t	LN/FN_t	LN/FN_t
Marshall (*Rsv1m*)	LVN/SVN 2/5[b]	LVN/FVN 5/5	LVN/SVN 4/5	R/R 5/5	R/R 5/5	LVN/SVN 1/5	LVN/SVN 1/5	LVN/SVN 2/5	R/R 5/5	R/R 5/5	R/R 5/5	LVN/SVN 5/5
PI 507389 (*Rsv1-n*)	S/M	S/TN	S/M	S/M	S/TN	S/TN	S/TN	S/TN	S/TN	S/TN	S/TN	S/TN
L29 (*Rsv3*)	R/R	R/R	R/R	LN/NM	R/R	LN/NM	LN/NM	LN/M	LN/R	LN/R	R/R	R/R

[a]R= Resistant (no symptoms; no recoverable virus); M= mosaic; NM =necrotic mosaic.
LVN= local veinal necrosis (on the inoculated leaves); SVN = slow veinal necrosis; FVN = fast veinal necrosis; LN= localized necrosis; TN= transient necrosis; FN_t= fast tip necrosis; MN_t = mild tip necrosis.
[b]Numbers represent the number of plants showing the necrosis out of the total number of plants tested. Remaining plants were symptomless.

Table 2.5. Percentage identity of nucleotide sequences of the coat protein (CP) encoding region of SMV-G1, G5, G6 and G7 (GenBank accessions) and nine RB isolates. Percentage amino acid similarities are shown in parenthesis next to the nucleotide sequences.

Strains	G1[a]	G5[a]	G6[a]	G7[a]
G1[b]	96 (98)	95 (98)	93 (98)	93 (98)
G7[b*]	88	88	88	92
2K-13i	93 (98)	93 (97)	98 (98)	95 (97)
2K-22	94 (98)	94 (98)	99 (99)	95 (98)
2K-24	93 (98)	93 (99)	98 (99)	95 (98)
2K-38	92 (95)	92 (95)	96 (95)	93 (95)
2K-39	93 (98)	93 (99)	98 (99)	95 (98)
2K-44c	93 (98)	93 (99)	98 (99)	95 (98)
S98-51	93 (97)	94 (98)	98 (98)	95 (97)
S98-52	93 (96)	94 (97)	98 (97)	95 (96)
S97-SBT-2	93 (96)	94 (97)	98 (97)	94 (96)

[a] CP GenBank nucleotide sequences for SMV-G1, G2, G5, G6 and G7 are GenBank accessions AH008451, S42280, AY294044, AF242845, and AY216010, respectively.

[b] Sequence of SMV-G1 (PV 571) and SMV-G7 determined in our study.

(*) Sequence of SMV-G7 was not complete for the CP.

Table 2.6. Percent similarity in amino acid sequences among the RB isolates. Each pair of amino acid sequences was compared for percent identity using the Blast Engine (www.ncbi.nlm.nih.gov).

	2K-13i	**2K-22**	**2K-24**	**2K-38**	**2K-39**	**2K-44c**	**S98-51**	**S98-52**	**S97-SBT-2**
2K-13i	-	98	98	96	98	98	98	98	97
2K-22	98	-	99	95	99	99	98	97	97
2K-24	98	99	-	96	100	100	98	98	98
2K-38	96	95	96	-	96	96	95	95	95
2K-39	98	99	100	96	-	100	98	98	98
2K-44c	98	99	100	96	100	-	98	98	98
S98-51	98	98	98	95	98	98	-	98	98
S98-52	98	97	98	95	98	98	98	-	96
S97-SBT-2	97	97	98	95	98	98	98	96	-

A **B**

C

Figure 2.1. Symptoms on L29 soybean inoculated with resistance-breaking isolates 2K44c (A) and 2K-38 (B-C) 14 days post inoculation. A and B show necrotic lesions on inoculated leaves, C shows systemic mosaic on upper non-inoculated trifoliolate leaves

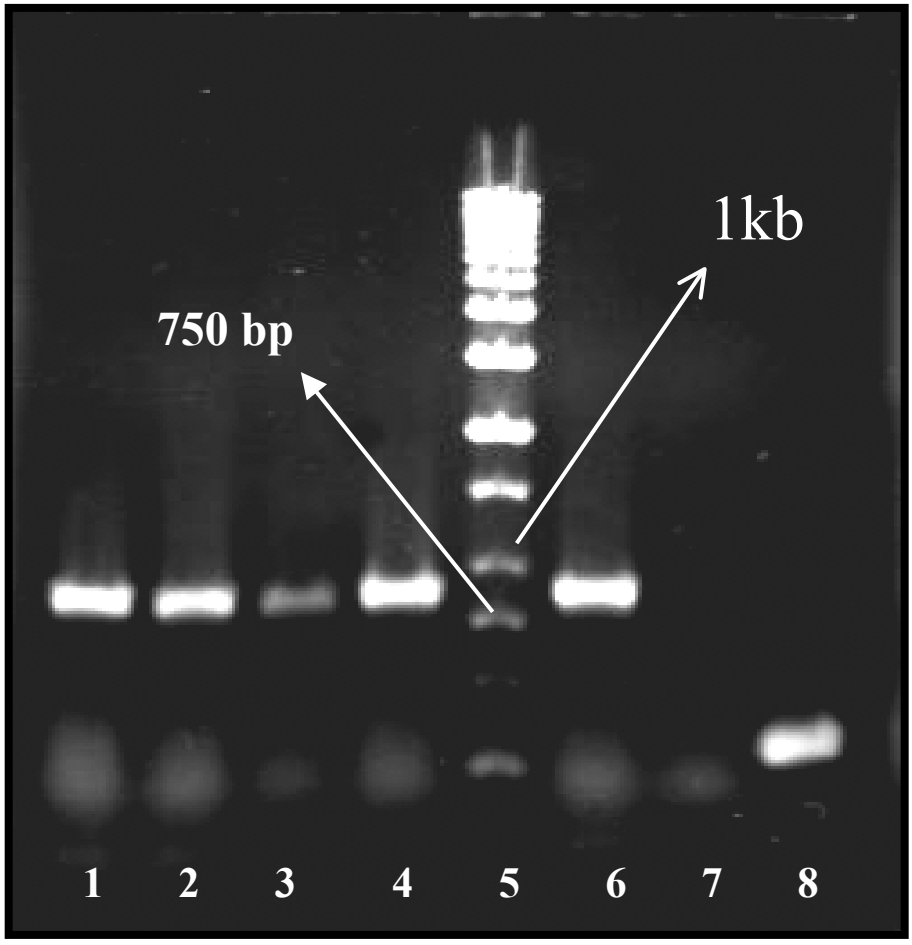

Figure 2.2. PCR amplification of SMV coat protein (CP) encoding sequences.

Agarose gel electrophoresis of PCR amplification products from the CP region of SMV-G1 (lane 1), G7 (lane 2), 2K-40 (lane 3), S98-51 (lane 4) and S98-52 (lane 6). 1Kb DNA marker (Promega®), healthy control, and 323 bp control reaction are present in lanes 5, 7, and 8, respectively.

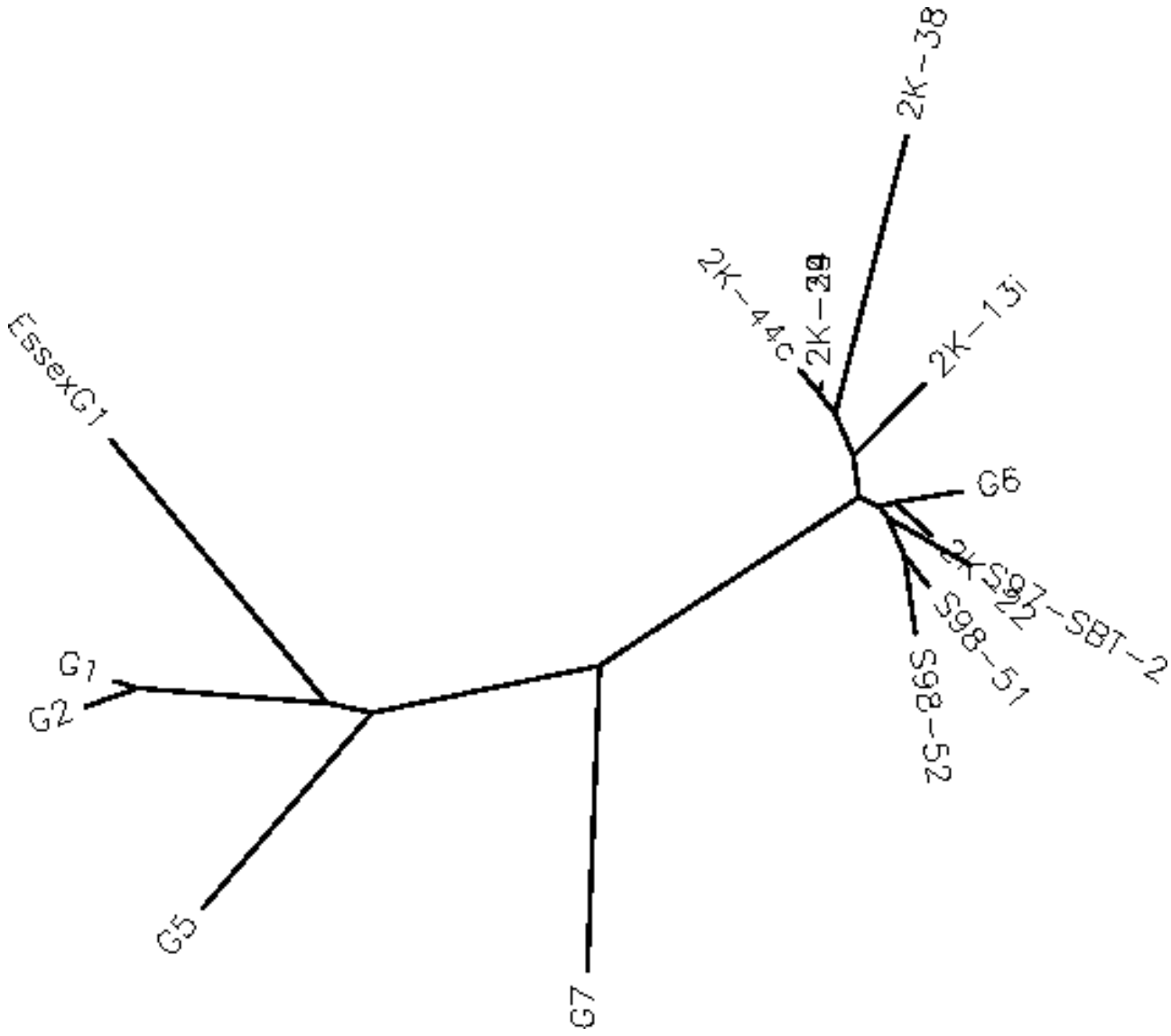

Figure 2.3. Phylogenetic analysis of nucleotide sequences encoding CP. Sequences are SMV-G1 and the resistance-breaking RB isolates from Essex and Hutcheson, respectively, with SMV G1, G2, G5, G6, and G7 from GenBank accessions AH008451, S42280, AY294044, AF242845, and AY216010, respectively. Unrooted tree generated by PHYLIP (http://workbench.sdsc.edu) using CLUSTAL W (1.81).

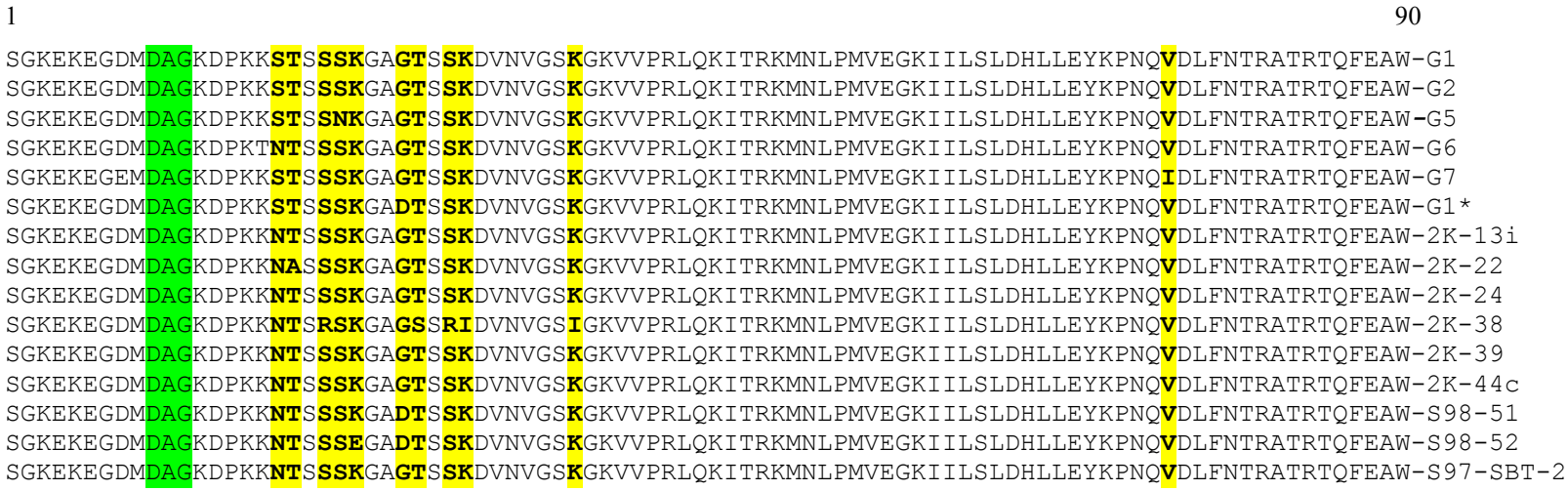

Figure 2.4. Multiple sequence alignment of deduced amino acid sequences from SMV coat protein. Sequences are SMV-G1 and the resistance-breaking RB isolates from Essex and Hutcheson, respectively, with SMV G1, G2, G5, G6, and G7 from GenBank accessions AH008451, S42280, AY294044, AF242845, and AY216010. Amino acids that differ among the sequences are highlighted in yellow. The conserved DAG triplet (10-12) is highlighted in green.

The coat protein includes 265 amino acids. Numbers indicate the position of the amino acid in the coat protein.

*= Sequence from SMV-G1 PV 571

Figure 2.4. Continued

REFERENCES

Bateson, M.F., Lines, R.E., Revill, P., Chaleeprom, W., Ha, C.V., Gibbs, A.J., and Dale, J.L. 2002. On the evolution and molecular epidemiology of the potyvirus Papaya ringspot virus. J. Gen. Virol. 83:2575-2585.

Berger, P.H., Wyatt, S.D., Shiel. P.J., Silbernagel, M.J., Druffel, K., and Mink, G.I. 1997. Phylogenetic analysis of the Potyviridae with emphasis on legume-infecting potyviruses. Arch. Virol. 142:1979-1999.

Bernard, R.L., and Nelson, R.L. 1991. USDA soybean genetic collection: isoline collection. Soybean Genet. Newsl. 18:27-57.

Bousalem, M., Douzery, E.J.P., and Fargette, D. 2000. High genetic diversity, distant phylogenetic relationships and intraspecies recombination events among natural populations of Yam mosaic virus: a contribution to understanding potyvirus evolution. J. Gen. Virol. 81:243-255.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) 2003. Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996. URL http://biology.anu.edu.au/Groups/MES/vide/

Buss, G.R., Camper, H.M., and Roane, C.W. 1988. Registration of 'Hutcheson' soybean. Crop Sci. 28:1024-1025.

Buss, G.R., Roane, C.W., Tolin, S.A., and Chen, P. 1989. Inheritance of resistance to soybean mosaic virus in two soybean cultivars. Crop Sci. 29:1439-1441.

Chen, P., Buss, G.R., and Tolin, S.A. 1993. Resistance to soybean mosaic virus conferred by two independent dominant genes in PI 486355. J. Hered. 84:25-28.

Chen, P., Buss, G.R., Gunduz, I., Roane, C.W., and Tolin, S.A. 2001. Inheritance and allelism tests of Raiden soybean for resistance to soybean mosaic virus. J. Hered. 92:51-55.

Chen, P., Buss, G.R., Roane, C.W., and Tolin, S.A. 1991. Allelism among genes for resistance to soybean mosaic virus in strain-differential soybean cultivars. Crop Sci. 31:305-309.

Chen, P., Buss, G.R., Roane, C.W., and Tolin, S.A. 1994. Inheritance in soybean of resistant and necrotic reactions to soybean mosaic virus strains. Crop Sci. 34:414-422.

Chenault, K.D., Hunger, R.M., and Sherwood, J.L. 1996. Comparison of the nucleotide sequence of the coat protein open reading frame of nine isolates of wheat streak mosaic rymovirus. Virus Genes 13:187-188.

Cho, E.K., and Goodman, R.M. 1979. Strains of soybean mosaic virus: classification based on virulence in resistant soybean cultivars. Phyopathology 69:467-470.

Cho, E.K., and Goodman, R.M. 1982. Evaluation of resistance in soybeans to soybean mosaic virus strains. Crop Sci. 22:1133-1136.

Dangl, J.L. and Jones, J.D.G. 2001. Plant pathogens and integrated defense responses to infection. Nature 411:826-833.

Dolja, V.V., Halderman, R., Robertson, N.L., Dougherty, W.G., and Carrington, J.C. 1994. Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. EMBO J. 13:1482-1491.

Dolja, V.V., Halderman-Cahill, R., Montgomery, A.E., Vandenbosch, K.A., and Carrington, J.C. 1995. Capsid protein determinants in cell-to-cell and long distance movement of tobacco etch potyvirus. Virology 206:1007-1016.

Domier, L.L., Latorre, I.J., Steinlage, T.A., McCoppin, N., and Hartman, G.L. 2003. Variability and transmission by *Aphis glycines* or North American and Asian *Soybean mosaic virus* isolates. Arch. Virol. 148:1925-1941.

Eggenberger, A.L., Stark, D.M., and Beachy, R.N. 1989. The nucleotide sequence of a soybean mosaic virus coat protein-coding region and its expression in *Escherichia coli*, *Agrobacterium tumefaciens* and tobacco callus. J. Gen. Virol. 70:1853-1860.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.

Garcia-Arenal, F., Fraile, A., and Malpica, J.M. 2001. Variability and genetic structure of plant virus populations. Annu. Rev. Phytopathol. 39:157-186.

Gunduz, I., Buss, g.R., Chen, P., and Tolin, S.A. 2004. Genetic and phenotypic analysis of Soybean mosaic virus resisance in PI 88788. Phytopathology (in press).

Gunyuzlu, P.L., Tolin, S.A., and Johnson, J.L. 1987. The nucleotide sequence of the 3' terminus of soybean mosaic virus. Phytopathology 77:1766. (Abstract).

Hajimorad, M.R., Eggenberger, A.L., and Hill, J.H. 2003. Evolution of Soybean mosaic virus-G7 molecularly cloned genome in *Rsv1*-genotype soybean results in emergence of a mutant capable of evading *Rsv1*-mediated recognition. Virology 314:497-509.

Harrison, B. 2002. Virus variation in relation to resistance-breaking in plants. Euphytica 124: 181-192.

Hayes, A.J., and Saghai Maroof, M.A. 2000. Targeted resistance gene mapping in soybean using modified AFLPs. Theor. Appl. Genet. 100:1279-1283.

Hayes, A.J., Ma, G., Buss, G.R., and Saghai Maroof, M.A. 2000. Molecular marker mapping of *Rsv4*, a gene conferring resistance to all known strains of soybean mosaic virus. Crop Sci. 40:1434-1437.

Hill, J. 1999. Soybean mosaic. Pp 70-71. In Hartman, G.L., Sinclair, J.B., and Rupe, J.C (Eds) Compendium of soybean diseases. 4th edition. APS Press. St Paul, MN.

Hill, J., Bailey, T.B., Benner, H.I., Tachibana, H., and Durand, D.P. 1987. Soybean mosaic virus: Effects of primary disease incidence on yield and seed quality. Plant Dis. 71:237-239.

Hunst, P.T., and Tolin, S.A. 1982. Isolation and comparison of two strains of soybean mosaic virus. Phytopathology 72:710-713.

Jayaram Ch., Hill, J.H., and Miller, W.A. 1991. Nucleotide sequences of the coat protein genes of two aphid-transmissible strains of soybean mosaic virus. J. Gen. Virol. 72:1001-1003.

Jayaram Ch., Hill, J.H., and Miller, W.A. 1992. Complete nucleotide sequences of two soybean mosaic virus strains differentiated by response of soybean containing the *Rsv* resistance gene. J. Gen. Virol. 73:2067-2077.

Kiihl, R.A.S., and Hartwing, E.E. 1979. Inheritance of reaction to soybean mosaic virus in soybeans. Crop. Sci. 19:372-375.

Kim, Y-H., Kim, O-S., Lee, B-C., Moon, J-K., and Lee, J-Y. 2003. G7H, a new soybean mosaic virus strain: its virulence and nucleotide sequence of CI gene. Plant Dis. 87:1372-1375.

Krause-Sakate, R., Le gall, O., Fakhfakh, H., Peypelut, M., Marrakchi, M., Varveri, C., Pavan, M.A., Souche, S., Lot, H., Zerbini, M., and Candresse, T. 2002. Molecular and biological characterization of Lettuce mosaic virus (LMV) isolates reveals a distinct and widespread type of resistance-breaking isolate: LMV-Most. Phytopathology 92:563-572.

Lopez-Moya, J.J., and Pirone, T.P. 1998. Charge changes near the N terminus of the coat protein of two potyviruses affect virus movement. J. Gen. Virol. 79:161-165.

Ma, G., Chen, P., Buss, G.R., and Tolin, S.A. 1995. Genetic characteristic of two genes for resistance to soybean mosaic virus in PI 486355 soybean. Theor. Appl. Genet. 91:907-914.

Ma, G., Chen, P., Buss, G.R., and Tolin, S.A. 2003. Genetic study of a lethal necrosis to soybean mosaic virus in PI 507389 soybean. J. Hered. 94:205-211.

McNeil, J.E., French, R., Hein, G.L., Baenziger, P.S., and Eskridge, K.M. 1996. Characterization of genetic variability among natural populations of wheat streak mosaic virus. Phytopathology 86:1222-1227.

Qusus, S. 1997. Molecular Studies on Soybean Mosaic Virus-Soybean Interations. PhD Dissertation. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Pp 162.

Roane, C.W., Tolin, S.A., and Buss, G.R. 1986. Application of the gene-for-gene hypothesis to soybean mosaic virus interactions. Soybean Genet. News. :13:136-139.

Roossinck, M.J. 1997. Mechanisms of plant virus evolution. Annu. Rev. Phytopathol. 35:191-209.

Shukla, D.D., Ward, C.W., and Brunt, A.A. 1994. *The Potyviridae*. CAB International, University Press, Cambridge, UK.

Silbernagel, M.J., Mink, G.I., Zhao, R-L., and Zheng, G-Y. 2001. Phenotypic recombination between bean common mosaic and bean common mosaic necrosis potyviruses in vivo. Arch. Virol. 146:1007-1020.

Simon, A.E., and Bujarski, J.J. 1994. RNA-RNA recombination and evolution in virus-infected plants. Ann. Rev. Phytopathol. 32:337-362.

Ustun, A., Allen, F.L., and English, B.C. 2001. Crop breeding, genetics and cytology. Crop Sci. 41:993-998.

Walsh, J.A., and Jenner, C.E. 2002. Turnip mosaic virus and the quest for durable resistance. Molec. Plant Pathol. 3:289-300.

Yu, Y.G., Saghai Maroof, M.A., and Buss, G.R. 1996. Divergence and allelomorphic relationship of a soybean virus resistance gene based on tightly linked DNA microsatellite and RFLP markers. Theor. Appl. Genet 92:64-69.

Yu, Y.G., Saghai Maroof, M.A., Buss, G.R., Maughan, P.J., and Tolin, S.A. 1994. RFLP and microsatellite mapping of a gene for soybean mosaic virus resistance. Phytopathology 84:60-64.