Chapter One

Literature Review

Characteristics of SMV and Potyviruses

Importance and Taxonomy

Soybean mosaic virus (SMV; Genus *Potyvirus*; Family *Potyviridae)*, the causal agent of soybean mosaic, is one of the most important viruses in soybean (*Glycine max* [L.] Merr.) and occurs worldwide (Brunt et al., 2000). SMV may cause significant yield losses reaching in some cases as high as 94% of the total yield. Infection of plants at an early stage results in reduction of pod set, reduction in seed size and weight, increase in seed coat mottling and decrease in seed quality. Late infection with SMV, however, has a limited effect on yield and seed quality (Hill 1999; Hill et al., 1987). The family *Potyviridae* has the largest number of known plant viruses (Barnett, 1991; Shukla et al., 1994). If potyviruses occur in complex with other unrelated viruses, the effects on yield quantity and quality are more severe. Plants infected by potyviruses are also more susceptible to fungal pathogens (Shukla et al., 1994).

SMV causes up to 40% yield loss when plants are infected at or before floral development (Hill, 1999). Similarly, 91% of the seeds have seedcoat mottling and higher levels of SMV infected plants result from mottled than non-mottled seeds. Seed transmission of SMV ranges from 0 to 68% but on average it is 10% and varies with the viral strains and the plant genotype and time of infection (Koning et al., 2002). For these reasons, the rate of seedcoat infection was suggested as an estimate of SMV incidence in the field. Necrosis, a hypersensitive type of response, can spread systemically into leaves and stems resulting in severely stunted plants with a typical tip necrosis invading the apical meristem and, no seed and eventually death (Buss et al., 1989).

SMV Strain Groups

The differential reactions of resistant soybean cultivars to various SMV isolates were used by Cho and Goodman (1979, 1982) to classify SMV into different strain groups. The reactions observed by them were: susceptible or systemic mosaic (S), necrosis (N), and symptomless or resistance (R). The strain groups were named G1 to G7 with the low numbered

groups being least virulent on a set of soybean cultivars used [Essex, Davis (York), Kwangyo, Marshall, Ogden, and Buffalo (PI 96983)] (Table 1.1).

Morphology and Genome Properties

Potyvirus particles are flexuous rods about 750 nm long and 11-15 nm in diameter, and consist of capsid proteins arranged in helical symmetry around a single-stranded monopartite positive sense RNA genome of approximately 10,000 bases. The RNA genome has a 3' poly A tail and a genome-linked virion protein, VPg, that is covalently bound to the 5'end. The viral RNA is translated into a polyprotein which is cleaved by proteases encoded by the viral genome, into at least nine mature proteins (Figure 1.1) (Jayaram et al., 1992). The potyvirus life cycle consists of entry into cells via wounds, uncoating, translation, polyprotein processing, genome replication, virus particle assembly, and movement to other cells, plant parts, and other plants (Shukla et al., 1994). During the life cycle of a potyvirus, the viral genome is translated into one protein and then processed into eight different gene products. The genome-linked protein (VPg) and the coat protein (CP) are the two only protein products of the viral genome that are present in the virion (Shukla et al., 1994).

A considerable amount is known about the genome of SMV and other potyviruses. Nucleic acid sequence is being used increasingly to analyze the different viruses and virus strains. Jayaram et al. (1992) mapped and determined the nucleic acid sequence of strains G2 and G7 of SMV (Figure 1). CP sequences of SMV G2 and G7 have been described by Jayaram et al. (1992), SMV-N by Eggenberger et al. (1989), SMV-VA by Gunyuzlu et al. (1987), and SMV G1 and G6 by Qusus (1997). Jayaram et al. (1992) observed that the most conserved regions of the potyvirus genome based on TEV, TVMV, PVY, PPV, SMV-G2, and SMV-G7 are the cylindrical inclusion protein gene (CI), the putative RNA-dependent-RNA polymerase gene (POL or NIb), and the coat protein gene (CP). The 21K and the 27K proteins in SMV are analogous to the TEV NIa protein and the genome associated protein VPg. VPg, NIa, P1, P3, and HC-Pro are the proteins responsible for the protein processing activity (Dougherty and Parks, 1991; Jayaram et al., 1992).

Sequencing of the CP gene from four strains of SMV confirmed it resides in the 3'-terminal 798 bases, followed by a un-translated region (UTR) of 259 bases excluding the poly A tail (Jayaram et al., 1992; Qusus, 1997). Within the N terminus of potyvirus CP, a conserved

DAG triplet is reported to be essential for aphid transmission. 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.

A GRD tripeptide at position 2120 to 2122 aa in the 21K protein (VPg) is conserved in a number of different potyvirus genomes (Jayaram et al., 1992). This GRD tripeptide is also conserved in SMV G2 but not in SMV G7, wherein lysine is substituted for an arginine residue at aa position 2121 resulting in a GKD tripeptide. This aa substitution interestingly correlates with the ability of strain G7 to overcome soybean *Rsv* gene of resistance to infection by SMV (Jayaram et al., 1992).

In their 1991 and 1992 studies, Jayaram et al. noted that the largest number of differences in the NA sequence between G2 and G7 strains of SMV occurred in the 5' region of the viral genome mainly at the P1, the P3, the CI and the HC-Pro regions of the viral genome. Omunyin et al. (1996) were able to differentiate SMV strains G2 and G7 in a mixed infection by using sequence-specific primers to the CI region. Kim et al. (1999) used restriction enzymes (*Eco*R1) to digest RT-PCR products of CI gene; they classified their SMV isolates from five strains (G5H, G5, G3, G6 and G7) into six different groups. Strains of the potyviruses *Papaya ringspot virus* (Bateson et al., 2002), and *Lettuce mosaic virus* (Krause-Sakate et al., 2002) have also been classified using restriction enzymes..

Functions of Potyvirus Genes

The potyviral genome includes the P1, HC-Pro, P3, 6K1, CI, 6K2, NIa, NIb and CP encoding genes from the amino to the carboxy terminus (Figure 1.1). The potyvirus proteins function in genome amplification and bind RNA except for P3, 6K1 and 6K2. Proteins encoded by genes at the amino terminal region function in virus movement; those at the carboxy terminal regions are involved in replication (Urcuqui-Inchima et al., 2001).

The P1 is the amino terminal gene and encodes a trypsin-like serine proteinase that function in autocleavage from the polyprotein. The P1 is not required for viral infectivity; it stimulates genome amplification and with HC-Pro acts as a pathogenicity enhancer by suppressing gene silencing (Urcuqui-Inchima et al., 2001).

Helper component proteinase (HC-Pro) is a multi component proteinase involved in aphid transmission of the virus, genome amplification, polyprotein processing and long-distance

transport of the virus in the host. Kasschau et al. (1997) hypothesized that the HC-Pro of TEV may function as an inhibitor of replication and long-distance movement-limiting host defense response. Wang and Maule (1995) reported that the potyvirus *Pea seed borne mosaic virus* suppressed the accumulation of a host mRNA at an advancing front of virus infection in immature pea embryos. Kasschau et al. (1997) reported that the central domain of HC-Pro of the TEV is functional in the viral long-distance movement in tobacco. HC-Pro of TEV is involved in the suppression of post-transcriptional gene silencing. HC-Pro mutant TEV strains are able to infect inoculated leaves of *Arabidopsis*, but are not capable of moving long distances (Whitham et al., 1999). Although SMV displays a similar phenotype on *Rsv4* soybeans (see Chapter 4), the potential role of HC-Pro in the SMV-soybean interactions has not yet been investigated.

The functions of the P3 gene product are not well known, but it is believed to function in viral pathogenicity as shown by mutation studies and is involved in inducing the wilting phenotype in plants (Urcuqui-Inchima et al., 2001).

The CI of potyviruses functions in mediating the cell-to-cell movement of the virus in host plants. Carrington et al. (1998) reported that amino acid substitutions in the N terminal region of the CI protein of TEV inhibited cell-to-cell movement of the virus in *Nicotiana tabacum* cv. Xanthi. However, these mutants were able to replicate to similar levels as wild type TEV in protoplasts. This means that the restriction of cell-to-cell movement is not due to inhibition or low levels of virus replication (Carrington et al., 1998). The CI of potyviruses localizes to special organized structures, pinwheel inclusion bodies, that attach to the plasmodesmata soon after virus infection, align with plasmodesmatal openings, and are associated with CP (Carrington et al., 1998). Hunst and Tolin (1983) showed that pinwheel-forming areas were present near the plasmodesmata early in soybean leaf cells infected with SMV.

The NIa is composed of both VPg and the carboxy terminal proteinase domain. The NIa localizes in inclusion bodies in the nucleus on infected cells. The VPg of many potyviruses is reported to be involved in virus movement (Nicolas et al., 1997; Schaad et al., 1997; Whitham et al., 1999). The VPg of TVMV and PVY may form complexes with host factors, either with or without other viral proteins, resulting in a protein complex that seems to be required for efficient cell–to-cell movement of the virus (Masuta et al., 1999). VPg of TEV was reported to be membrane associated and involved in viral replication (Restrepo-Hartwig and Carrington, 1994).

VPg is involved in systemic movement and, with 6K2, is involved in vascular movement (Urcuqui-Inchima et al., 2001). The NIb is the RNA-dependent RNA polymerase (RdRp) and is required for genome replication. This protein is involved in RNA binding activities (Urcuqui-Inchima et al., 2001).

Evidence for the importance of VPg in overcoming resistance to potyviruses has been reported for TVMV in the Burley tobacco cultivars TN86 (Nicolas et al., 1997) and *Pea seed-borne mosaic virus* (PSMV; Genus Potyvirus) in *Pisum sativum* (Keller et al., 1998). Findings from the TVMV studies have shown that changes within a cassette of four amino acids present within a six amino acid domain of the TVMV-S VPg region were responsible for breaking the virus resistance. These are Cys, Ser, Lys, and Ser at position 1928, 1929, 1932, and 1923 respectively. This was the first report that a determinant within the viral VPg cistron is involved in disease resistance breaking in plants. A single amino acid change could break the resistance as reported by Mastua et al. (1998) with PVY and the 'Virgin A Mutant' tobacco.

The CP gene is best characterized gene in potyviruses. It is divided into three domains: the amino terminus, the core region, and the carboxy terminus. Both the amino and carboxy termini are variable, exposed on the surface of the protein in virus particles, are cleaved by trypsin, and contain the major virus specific epitopes. The CP functions in virus encapsidation and amplification, virus movement both cell-to-cell and long distance, and aphid transmission (Urcuqui-Inchima et al., 2001).

Strain Diversity

Legume-infecting potyviruses are more diverse in biological properties than in nucleic acid sequence. One way to differentiate closely related viruses (related species, strains, or isolates) is to compare nucleic acid sequences, especially the 3'-end non-coding region (NCR) and CP gene sequences (Berger et al., 1997). The amino acid composition and sequence for the CP are characteristic of individual plant virus species. Very little sequence homology at the CP level is present between different plant virus genera. Except for the CP, many of the gene products of potyviruses share sequence homology with other plant virus genera and families. This makes classification of members of the potyvirus group based on CP possible (Shukla and Ward 1988; Berger et al., 1997).

Amino acid and nucleic acid analyses of the CP from potyviruses have been considered powerful tools not only for studying the taxonomy of the viruses belonging to this genus but also the strains (McKern et al., 1993). Phylogenetic analysis of potyvirus sequence data showed a bimodal distribution: members sharing 38-71% sequence identity are distinct viruses, and members at least 90% identity are strains of the same virus (Shukla and Ward, 1988). The data showed major differences in the length and sequence in the amino terminus of the CP, and a high sequence homology in the carboxy terminal regions of the CP. Sequence identity for the amino terminal 43 aa of two strains of *Passion fruit woodiness virus* (PWV; Genus *Potyvirus*) was only 53% whereas identity across the CP was 85-86% (Gough and Shukla, 1992).

Biologically distinct isolates of several viruses have also been distinguished by variability in their genome. Digestion of PCR amplified DNA from *Citrus tristeza virus* (CTV; Genus *Closterovirus*) CP with *Rsa*I and *Hin*f1 revealed sequence variation (Gillings et al., 1993). Hammond et al. (1999) reported the differentiation by PCR of biologically distinct *Prunus necrotic ringspot virus* (PNRSV; Genus *Ilarvirus*) isolates that are closely related. PCR primers specific for distinct virus isolates were designed. Eun et al. (2000) reported that real time reverse transcription (RT)-PCR was successful in differentiating two orchid viruses: *Cymbidium mosaic virus* (CymMV; Genus *Potexvirus*) and *Odontoglossum ringspot virus* (ORSV; Genus *Tobamovirus*). RT-PCR was also successful in detecting *Tomato spotted wilt virus* (TSWV; Genus *Tospovirus*) (Roberts et al., 2000), and differentiating two strains of *Potato virus Y* potyvirus (Walsh et al., 2001).

Phylogenetic analysis of viral genomes has been used to differentiate a wide range of other potyviruses. Berger et al. (1997) analyzed genome sequences of a number of legume potyviruses and constructed phylogenetic trees; the PILEUP program was used to align the sequences. PAUP, PHYLIP were used for the Fitch and Margoliash and parsimony analysis. Phylogenetic analysis was also used to differentiate other potyviruses including *Yam mosaic potyvirus* (Bousalem et al., 2000), *Wheat streak mosaic virus,* and *Oat necrotic mottle virus* (Rabenstein et al., 2002), *Papaya ringspot virus* (Bateson et al., 2002), and *Zucchini yellow mosaic virus* (Pfosser and Baumann 2002).

Genetics of Resistance of Soybean to SMV

Roane et al. (1986) suggested a gene for gene model for the SMV-soybean interactions. Inheritance studies of SMV resistance in soybeans were the basis for assigning the different *Rsv1*, *Rsv2*, and *Rsv3* gene symbols.

Rsv1 Gene

Resistance to SMV in soybeans has been detected in different cultivars with many of these not resistant to all of the SMV strains (Ma et al., 1995). The resistance was found to be regulated by a single dominant gene in most of the resistant lines, including the cultivars used by Cho and Goodman (Chen et al., 1991). This gene has been designated as R1 (*Rsv1*) (Kiihl and Hartwig 1979; Chen et al., 1991). Six alleles of *Rsv1* have been identified (Buss et al., 1989, Chen et al., 1991) that react differently to different strains of SMV. The *Rsv1* locus appears to condition both the necrotic and the resistant reactions (Chen et al., 1994). Hajimorad and Hill (2001) reported that the *Rsv1*-mediated resistance to SMV-N is HR-independent and is similar to extreme resistance *Potato virus X* (PVX) conditioned by the *Rx* gene.

Rsv, a single dominant gene, was identified in PI 96983 soybean, later named *Rsv1* (Kiihl and Hartwig 1979). Different alleles at the *Rsv* locus that have been identified are *Rsv1-t, Rsv1-y, Rsv1-k, Rsv1-m* from Ogden, York, Kwangyo, and Marshall, respectively (Table 1.1) (Chen et al., 1991). These alleles of *Rsv1* display different resistance reactions to SMV strain groups. The *Rsv1* alleles are usually resistant to the lower numbered strains and display mosaic or necrotic reactions to the higher numbered strains (Chen et al., 1991). Cultivars carrying an allele at the *Rsv1* locus, susceptible to lower virulence strains, show a necrotic reaction to more virulent strains of the virus. Similarly, cultivars showing a resistance reaction to lower virulence strains of SMV develop a necrotic reaction to more virulent SMV strains (Chen et al., 1994).

Rsv1 maps to linkage group F of the soybean genome and is linked to a cluster of resistance genes that includes genes conferring resistance to *Peanut mottle virus* (PMV; Genus *Potyvirus*) (*Rpv*), *Peanut stripe virus* (PStV; Genus *Potyvirus*), *Phytophthora (Rp3)*, and Javanese root-knot nematode. This mapping of resistance genes was done on populations from a cross of PI 96983 and Lee 68 through the use of RFLP and microsatellite molecular markers (Yu et al., 1994, 1996).

The Rsv3 and Rsv4 Genes

Molecular and genetic studies have revealed the presence of other genes for resistance to SMV in soybeans, which have been mapped to a region different from the *Rsv1* locus; these have been named *Rsv3* and *Rsv4* (Chen et al., 1993; Gunduz et al., 2001; Gunduz et al., 2002). The *Rsv2* symbol was assigned to an OX670 gene derived from Raiden (Buzzell and Tu, 1984), and is resistant to all strain groups G1-G7 (Gunduz et al., 2001). However, OX670 was found to have two genes, *Rsv1* derived from Raiden and *Rsv3* from Harosoy. Therefore the *Rsv2* designation was dropped (Gunduz et al., 2002). The *Rsv3* alleles result in different resistance reactions to SMV. The cultivar Columbia gives a necrotic reaction to SMV G1 (Buzzell and Tu 1989); the Harosoy *Rsv3* allele, however, is resistant to strains G5-G7 but susceptible to strains G1-G4 (Buss et al., 1999). Recent work shows that *Rsv3* maps to a chromosomal region of the linkage group B2 containing a cluster of disease resistance genes conferring resistance to soybean cyst nematodes and *Phytophthora sojae* (Jeong et al., 2002). Lines carrying *Rsv3* are susceptible to lower numbered strains, but are not affected by higher numbered strains. This strain specificity is different than that of *Rsv1*.

A third gene, distinct from *Rsv1* and *Rsv3* has been identified. Two independent resistance genes were reported from PI 486355 (Chen et al., 1993; Ma et al., 1995). One is at the *Rsv1* locus, the other is independent from *Rsv1* and *Rsv3* and termed *Rsv4*. *Rsv4* has been isolated from LR2, a selection of PI 486355 x Essex. A reselection from LR2 gave line V94-5152, which is registered as a germplasm (Buss et al., 1997). This *Rsv4* gene is completely dominant and confers resistance in both the homozygous and the heterozygous conditions and is non-necrotic (Ma et al., 1995). *Rsv4* has been mapped to linkage group MLG D1b (Hayes et al., 2000). Greenhouse experiments show that *Rsv4* is non-strain specific, non-necrotic and results in late susceptible reaction in the host (Ma et al., 1995; Gunduz et al., 2001, 2002).

Host Pathogen Interactions

Defense responses in plants include active mechanisms whereby resistance (*R*) genes in plants recognize a pathogen and initiate the cascades of defense pathways (Goldbach et al., 2003; Murphy et al., 2001; Jones, 1996). *R* genes encode pathogenicity targets, receptors that interact with ligands produced by the corresponding avirulence pathogen (*Avr*) gene and induce plant defense responses (Takken and Jossten, 2000). If an *R* gene or *Avr* gene are absent, a compatible

reaction occurs and the pathogen colonizes the host. Most of the *R* genes have an leucine-rich repeat (LRR) domain. The *N* gene for resistance to TMV from tobacco has Toll-interleukin-1 receptor/ nucleotide-binding site/ leucine-rich repeat (TIR-NBS-LRR) domains and these domains play an essential role in the induction of plant defense responses against TMV (Dinesh-Kumar et al., 2000).

Resistance Genes

Flor (1971) reported the classical gene-for-gene model for host-pathogen interactions using flax (*Linum usitatissimum*). He proposed that for this interaction to occur, one gene from the host (*R*) and another from the pathogen (*Avr*) should be complementary. *R* genes condition resistance to a broad range of pathogens, including bacteria, fungi, nematodes, and viruses, and have been cloned from monototyledons and dicotyledons (Takken and Jossten, 2000). Recognition of elicitors, *Avr* gene products, by *R* genes activates the host cascades of signal transduction pathways that include protein phosphorylation, reactive oxygen species, ion fluxes (Hammond-Kosack and Jones, 1997). Matching products of *R* and *Avr* genes are expected to localize and interact in the same cellular compartment, especially for obligate intracellular pathogens including viruses (Takken and Joosten, 2000). The 'guard' model for *R-Avr* interactions suggest that *R* proteins detect a complex of pathogen virulence factor (i.e elicitor) and pathogenicity target in the host. This model also suggest that Avr genes are both elicitors and virulence factors and are conserved in the pathogen (Takken and Joosten, 2000).

R genes share structural similarities and can be classified into five groups based on their structure (Hammond-Kosack and Jones, 1997; Richter and Ronald 2000). They are distinguished by the following domains: nucleotide-binding site (NBS), leucine-rich repeat (LRR), coiled-coil (CC), leucine zipper (LZ) and Toll and interleukin-1 domain (TIR) (Goldbach et al., 2003). Other classifications include the protein kinase (PK) and the transmembrane region (TM) (Takken and Joosten, 2000). *R* genes are therefore classified into LZ-NBS-LRR, NBS-LRR, TIR-NBS-LRR, LRR-TM-PK and LRR-TM. The LRR domains include sequence motif repeats and can be either intracellular or extracellular, act as receptors, and are involved in protein-protein interactions. Changes in the LRR domain can result in no HR induction; an example is LRR of RPS2 (Bent et al., 1994). The LZ and TIR also act as receptors but are believed to function in downstream signaling. Toll is a receptor protein and includes an extracellular

cytoplasmic LRR domain; TIR domains, on the other hand, have little homology to Toll but are believed to act similarly (Takken and Joosten, 2000).

Resistance to viruses is specific and branches away from resistance to fungi and bacteria downstream of the salicylic acid (SA) pathway. Signal transduction pathways induced by *R-Avr* interaction in the case of viruses include the induction of SA and activate multiple defense mechanisms including resistance to movement, both cell-to-cell and long distance, and virus replication (Hull, 2001). Murphy et al. (2001) reported that salicylhydroxamic acid (SHAM) was antagonistic to SA-induced resistance to TMV but not to PR-1 gene expression by SA. They suggested that defense pathways branched downstream of the SA into two pathways: one involving PR-1 proteins and another involving reactive oxygen species. The first is involved in resistance to fungi and bacteria; the latter in resistance to viruses (Murphy et al., 2001).

Host Resistance to Virus Through Activation of Defense Responses

The viral infection cycle includes several steps starting with the virus entering the cell, replication of the viral genome, movement of the virus from cell to cell, and spread of the virus into the vascular system (phloem) for long distance movement. Breaking the cycle at any of these steps could result in blocking the establishment of the viral infection in the plant (Masuta et al., 1999). Virus-host interactions are dictated the complex sets of genes in both the host and the virus; these determine the compatible and incompatible reactions. Compatible reactions permit such processes as genome replication and cell-to-cell and long-distance movement of the virus through the host vascular system. Incompatible reactions, on the other hand, may involve processes such as virus recognition and induction of the defense responses in the host, examples of which are extreme resistance, hypersensitive response, systemic acquired resistance (SAR), restriction of cell-to-cell and long distance virus movement, and homology dependent gene silencing (Whitham et al., 1999).

Hypersensitive Response and Extreme Resistance:

Typical host resistance through activation of defense responses includes the hypersensitive response (HR) whereby localized cell death takes place and the pathogen is restricted to the site of infection, and the extreme resistance (ER) whereby no symptoms are visible and no virus accumulation is detected. With HR, systemic acquired resistance (SAR) is triggered, salicylic acid (SA) and pathogenesis-related protein (PR) levels are increased, and

plants are more resistant to pathogens than those where SAR is not induced (Dinesh-Kumar et al., 2000). HR is not always conditioned by SA. Jasmonic acid (JA) and ethylene (ET) are triggered by necrotrophic pathogens (McDowell and Dangl, 2000) and are SA-independent. Local acquired resistance to pathogens triggers pathogen arrest at or near the site of infection. Both HR and ER are controlled by dominant genes and condition resistance through *R-avr* recognition. In the case of ER, no lesions are detected and the defense response is believed to be the result of high affinity between the receptor and the elicitor, and could be attributed to their presence at an early stage in infection. HR, however, is the result of lower affinity between the two or their delayed expression (Hajimorad and Hill, 2001)

Extreme resistance does not induce a HR or visible lesions on plants. The *Rx* gene in potato results in an extreme resistance to PVX with no HR, in response to inoculation with PVX (Bendahmane et al., 1999). Adams et al. (1986) suggested that such types of viral resistance are active and *R* genes act as suppressors of viral replication or promote viral RNA degradation. Lamb and Dixon (1997) argued that a HR is not an essential factor in disease resistance in plants. Rather, they suggested that cell death may deprive the biotrophic pathogens from vital substrates for growth or that the dying cells themselves may release biochemical signals that act as antimicrobials. Gilbert et al. (1998) generated transgenic potatoes carrying the PVX coat protein, which they showed to be the elicitor of the *Rx* gene. They showed that the *Rx*-mediated resistance acts by suppressing host cell viability. However, they suggested that a secondary HR or a latent HR might be involved in mediating this resistance response and plant cell death. Apoptosis or programmed cell death is one form of viral resistance in the host plants. Apoptosis may be both quantitatively and qualitatively assessed. It is characterized by nuclear DNA fragmentation into oligonucleosomal fragments of multiples of 200 bp (Ioannou and Chen, 1996).

Host Resistance to Virus Through Non Defense Responses

Not all resistance reactions between plant and pathogen involve induction of cell death and HR, or ER (Hull, 2001). For example, 65 % of viral resistance genes are not associated with HR or ER. Instead, the resistance to virus is attributed to restriction of virus replication and spread, and is the result of an incompatible interaction between host and viral factors (Hajimorad and Hill, 2001). For example, not all the *R* genes in soybean respond in a HR-like response to

infecting SMV. For example, initial observations have shown that *Rsv4* is not strain-specific and not necrotic, but displays a late susceptible response in heterozygotes.

Restriction to Virus Spread:

Two potyviral polypeptides are involved in viral movement; these are the CP and the helper component HC-Pro (Carrington et al., 1996). VPg may also play a role in the potyviral cell-to-cell movement (Revers et al., 1999). Nicolas et al. (1997) demonstrated the role of VPg in the cell-to-cell movement of *Tobacco vein mottling virus* (TVMV; Genus *Potyvirus*) in tobacco by conducting site directed mutagenesis and constructing chimeric TVMVs. They determined four amino acid changes were responsible for the resistance breaking phenotypes and the ability of the recombinant viruses to move in tobacco. Their studies strongly suggested that the VPg gene also determines viral resistance-breaking. The VPg interacts with host components to facilitate systemic virus movement (Nicolas et al., 1997). Moreno et al. (1999) reported that P1 and HC-Pro proteins of TVMV are involved in pathogenicity and enhance replication of a number of heterologous plant viruses.

To date, no movement proteins have been identified from potyviruses. In TEV, four viral proteins are needed, either directly or indirectly, for long-distance movement of the virus. These are the HC-Pro, CI, NIa, and CP. Therefore, these present a multi-target site for movement-restricting responses (Whitham et al., 1999). Cell to cell movement of many plant viruses not belonging to the potyvirus group is mediated by a viral-encoded protein, the movement protein (MP); in some cases the viral capsid protein CP may also be involved (Wang et al., 1998). MPs are believed to have a number of distinct functions in viral movement, including: (a) binding to RNA, (b) increasing the size exclusion limit (SEL) of plasmodesmata, and (c) trafficking of macromolecules through the plasmodesmata, thereby facilitating virus movement (Wang et al., 1998; Lee et al., 2000). It has been reported that viral MPs and endogenous plant proteins can form cargo complexes that interact with a cytoplasmic receptor or adapter sites in the plant host. However, little is known about plasmodesmata dilation and other factors that might be involved in signal recognition and trafficking. So, although much is known concerning the trafficking of macromolecules through plasmodesmata, little is known about the proteins comprising the supramolecular chaperon complex (Lucas, 1999). The receptor-docking complex that binds the cargo complex is yet to be characterized (Lee et al., 2000). Wang et al. (1998) showed that mutations in the MP of the *Red clover necrotic mosaic virus* (RCNMV; Genus *Dianthovirus*)

inhibited the movement of the virus from the bundle sheath or phloem parenchyma cells into the companion cell-sieve element complex in inoculated leaves. This study showed that the RCNMV MP is needed for the long distance movement of the virus in tobacco (*Nicotiana edwardsoni)* and cowpea (*Vigna unugiculata).*

Tracking Virus In Plants

Tracking virus movement and localization will allow better understanding of the soybean-SMV association at the cellular and sub-cellular levels. It may also show which genes or structures are involved in the virus-host interaction, viral movement, and subsequently resistance. Different methods have been used to track virus movement in plants in conjunction with studies to characterize movement proteins and resistance mechanisms. Tissue print hybridization with digoxigenin-labeled RNA probes has been useful in studying the movement of viruses through the vasculature of the plant (Mas and Pallas, 1995). In-situ hybridization is a similar detection method whereby fluorescent or digoxigenin-labeled nucleic acid probes are used to detect viral nucleic acid in plant tissues. Petioles and stem sections can be used and the hybridization may be detected using an epifluorescence or confocal microscope. Detection of the probe (gold, antibody) is also possible using either a light or electron microscope (Troxler et al., 1990).

Another approach to studying viral movement is the use of green fluorescent GFP or β-glucuronidase (GUS)-labeled infectious DNA clones of virus and monitoring virus movement by doing a time course analysis for detecting viral signals in the host tissue. Dolja et al. (1992) used an infectious clone of TEV containing an insertion of the bacterial GUS gene between the HC-Pro gene and the N-terminal 35 kDa protease gene. Time course analysis of GUS expression made possible the detection and visualization of virus activity in single mechanically-inoculated cells, in adjacent cells, and in phloem cells associated with systemic or long distance movement of the virus. This allowed the tracking of both cell-to-cell and long-distance movement of virus.

Wang et al. (1999) used a geminivirus*, Bean-dwarf mosaic virus* –green fluorescent protein reporter system (BDMV-GFP) to study the viral infection process in both compatible and incompatible virus-host systems. Viral distribution in host cells, and host-virus response were identified using confocal laser scanning microscopy, and epifluorescence microscopy, in addition to histochemical studies. Such tools are powerful in terms of identifying and visualizing the viral

determinants that act in the induction of resistance responses in the host, and in the viral life cycle itself including replication, movement and associations with the host vascular system.

Movement of SMV in soybeans has been studied to determine the possible host and viral factors that result in the different SMV-soybean responses. Leaf immunoprints with specific antibodies to SMV CP were used by Qusus (1997) to detect the virus in soybean leaves over a time course in order to investigate the relationship between the extent of resistance and movement in the host plant and to investigate the interactions between the different strain-cultivar combinations or genes for resistance. The presence of virus accumulation (detected by immunoassay) correlated to symptoms from the same leaves. Press blot immunoassay was also used by Mansky et al. (1991) for detecting SMV in inoculated leaves. Detection of CP in soybean leaves was used to determine resistance or susceptibility to SMV (Mansky et al., 1991).

Pathogen-Derived Resistance:

It is only recently that the different mechanisms of pathogen-derived resistance (PDR), a concept that was introduced by Sanford and Johnston (1985), are being understood (Baulcombe, 1996a). Such mechanisms include protein-mediated resistance whereby the expression of a protein interferes with the virus life cycle, i.e., the disassembly, and movement in the vascular system (Moreno et al., 1998). The first demonstration of PDR was reported in transgenic tobacco plants expressing the CP of TMV (Powel-Abel et al., 1986) wherein resistance was later correlated with the level of coat protein accumulation (Powel-Abel et al., 1990).

Homology-dependent gene silencing is a type of PDR based on RNA-mediated protection similar to post-transcriptional-gene-silencing (PTGS) (Moreno et al., 1998). It gives, however, a narrow range of resistance because it is sequence-dependent (Baulcombe, 1996b). Transgenic plants carrying a viral sequence show suppression of transgene mRNA accumulation and any viral RNA having a sequence similarity to the transgene, thereby, resulting in protection (English et al., 1996).

PTGS is an RNA-mediated silencing mechanism in natural defense against foreign or non-self sequences (Rovere et al., 2002). PTGS acts as a nucleotide sequence-specific defense mechanism targeting both cellular and viral mRNAs (Hamilton and Baulcombe, 1999). This type of RNA interference occurs in a wide range of organisms including animals, fungi, and plants (Llave et al., 2000). It has been associated with systemic spread of a silencing signal that directs RNA degradation in a sequence specific manner. Current research suggests that PTGS is

a naturally-occurring mechanism allowing plants to recognize and fight foreign nucleic acids (Voinnet et al., 1999; Baulcombe 1996). PTGS can occur as a localized silencing in one region of the plant. In such a case, a signal is released from the site of initiation and directs the systemic spread of silencing to other parts or tissue of the plant (Hamilton and Baulcombe, 1999). PTGS may occur as a result from a virus-induced RNA-mediated defense (Hamilton and Baulcombe, 1999). In some transgenic plant experiments, the virus was shown to be an inducer and a target of gene silencing (Ratcliff et al., 1999). Hamilton and Baulcombe (1999) reported that the short RNAs associated with PTGS occur in both the sense and antisense polarity and are homologous to the target of gene silencing. Hammond et al. (2000) reported that a component of PTGS resulted from a homology dependent, sequence specific nuclease, which incorporates these short RNAs as guides to target the PTGS.

In the potyviruses, the VPg, CP, P1, P3, NIa, NIb and the 6K2 portions of the genome have been studied for induction of RNA-mediated resistance. Lindbo et al. (1993) reported the first case of RNA-mediated resistance to plant viruses with the expression of untranslatable sequence of the CP gene of a potyvirus. Moreno et al. (1998) showed high levels of resistance in tobacco lines transformed with a translatable version of the P1 or P3 coding regions of TVMV. Two resistance phenotypes were reported: a high resistance, i.e., no viral symptoms, and a recovery (RC) phenotype where plants are initially infected and then escape the systemic infection, i.e., development of symptomless new leaves. A high inoculum level did not overcome the resistance. In all cases, a low level of transgene expression was observed with no detectable expression in the highly resistant lines (termed complete resistance by other researchers) and initially high levels of transgene expression in lines showing the RC or delayed resistance before virus inoculation. However, a different strain of TVMV (TVMV-S) was capable of breaking the resistance. Therefore, the mechanism of resistance in this case was believed to be acting in a gene-silencing manner since the TVMV-S strain differs from the wild type by six amino acids in the VPg region between positions 1927 and 1999, and since mRNA accumulation was prevented (Moreno et al., 1998).

It appears that most of the potyviral genome coding regions can induce RNA-mediated protection if expressed in transgenic plants (Moreno et al., 1998). The Vpg has been studied with PSMV, TEV, TVMV. It is believed that the gene acts in the PTGS type of resistance to plant viruses, and is sequence-specific (Nicolas et al., 1997; Keller et al., 1998; Swaney et al., 1995).

The P1 gene, which encodes a protease, was used in potatoes against PVY (Pehu et al., 1995) and showed a high degree of protection with a sequence-dependent protection. Another demonstration included the P1 and a part of the HC-Pro cistrons of the plum poxvirus (Tavert-Roudel et al., 1998).

Kasschau and Carrington (1998) reported that plants restrict TEV invasion by suppressing or silencing viral movement components. This mechanism could be mediated either by a signal generated at the initial site of infection that moves cell-to-cell ahead of the invading virus, therefore inducing silencing or suppression of the virus, or by a systemic signal that moves through the phloem and exits to the cells and initiates a similar response in emerging leaves. In many cases, plants carrying a viral transgene were resistant to that virus even when little or no protein was produced, suggesting a PTGS type of resistance (Waterhouse et al., 2001).

Rate reduction of disease is another form of resistance (Nutter et al., 1998). Steinlage et al. (2002) reported that both spatial and temporal spread of SMV in the field was reduced in soybeans transformed with the SMV CP. Management strategies that use several genes for resistance are likely to be beneficial in delaying virus invasion, may have the potential of limiting yield losses, and therefore increase the economic yield of the crop (Nutter, 1993).

Ability of Viruses to Overcoming R Genes

Viral Suppressors of Gene Silencing:

Viruses can suppress gene silencing and infect plants systemically. Plant viruses use an RNA-dependent RNA polymerase (RDRP) for their replication and have a dsRNA replicative intermediate. It is postulated that viruses that infect and invade plants do so by overcoming the plant's PTGS defense response (Waterhouse et al., 2001). Suppression of PTGS would permit the infecting virus to overcome resistance by blocking degradation of dsRNA, enabling cell-to-cell and long distance movement throughout the plant tissue. The HC-Pro of potyviruses has been shown to play a gene silencing role (Kasschau and Carrington, 1998). Expressing the HC-Pro sequence of a virus in plants using a transgene or a virus vector was enough to inhibit PTGS of a transgene. It was therefore proposed that the HC-Pro-PTGS suppressing activity accounts for the requirement for the HC-Pro in the virus replication and its long-distance movement (Llave et al., 2000).

A hypothesis that viruses are able to infect plants by avoiding or by suppressing the RNA-mediated defense has been investigated by Voinnet et al. (1999). HCPro of potyviruses and the 2b protein of CMV are suppressors of PTGS (Waterhouse et al., 2001). Interestingly, both act as pathogenicity determinants of their respective viruses. Brigneti et al. (1998) reported that HC-Pro of Potato virus Y (PVY) is a suppressor of PTGS. Transgenic *Nicotiana benthamiana* carrying the green fluorescence protein (GFP) transgene were infiltrated with an *Agrobacterium tumefaciens* carrying a binary Ti plasmid with the same GFP expression cassette used in transforming these plants. PTGS occurred and later was found to be systemic. When PVY was inoculated into these plants, the virus symptoms were present about two weeks after inoculation and were associated with green fluorescence, indicating a reversion of the PTGS of GFP.

Virus-induced gene silencing has been reported in plants that show a resistance response, similar to protection to subsequent infection by the same or closely related viruses. This reaction is believed to be de-novo through synthesis of complementary nucleic acid sequences to the target viral gene. Thus, plant viruses act similarly to animal viruses in initiating infection and invasion by suppressing host resistance (Kasschau and Carrington, 1998). A number of reports on viral genes suppressing PTGS strongly support such a hypothesis. Evidence for a role of the helper component protease (HC-Pro) of potyviruses in suppressing the gene silencing of transgenes or viral genes has been reported. HC-Pro has a wide range of functions including virus replication and accumulation, vascular and long distance movement, vector specificity, and enhancement of replication of unrelated RNA sequences (Pruss et al., 1997; Kasschau and Carrington, 1998).

Kasschau and Carrington (1998) reported that P1 protease gene of TEV enhanced the effect of the HC-Pro on virus amplification, therefore suggesting a possible cooperative function of these two genes. Corning et al. (1995) have previously suggested that HC-Pro facilitates long distance movement of TEV in plants. Surprisingly, virus genes can suppress the PTGS on endogenous plant genes or transgenes. An example of such a mechanism was reported by Andalakshmi et al. (2000). The HC-Pro of TEV suppressed the PTGS of a reporter gene (GFP). More interestingly, a plant gene encoding for a calmodulin-related protein that interacts with the TEV HC-Pro, was detected from tobacco plants using a yeast two-hybrid system. This protein was shown to function in suppressing PTGS in tobacco. (Andalakshmi et al., 2000).

Objectives

The objectives of this work are to:

a- Characterize the biological and genomic diversity of field isolates of SMV that break the resistance of Hutcheson.

b- Investigate the effect of the resistance-breaking field isolates of SMV on susceptible and resistant soybeans

c- Assess the effect of double infection with SMV and BPMV on soybeans

d- Characterize the mechanisms of resistance of *Rsv4*

e- Characterize the mechanisms of resistance of *Rsv3*

Table 1.1. Strain group classification of SMV on differential soybean cultivars containing the *Rsv1* alleles (based on Cho and Goodman, 1979, 1982; Chen et al., 1991).

		Reaction induced by strain groups of SMV on soybean cultivars						
Cultivar	Gene	G1	G2	G3	G4	G5	G6	G7
Essex	*rsv*	S[a]	S	S	S	S	S	S
York	$Rsv1^y$	R	R	R	N	S	S	S
Kwango	$Rsv1^k$	R	R	R	R	N	N	N
Marshall	$Rsv1^m$	R	N	N	R	R	N	N
Ogden	$Rsv1^t$	R	R	N	R	R	R	N
PI 96983	*Rsv1*	R	R	R	R	R	R	N

a: S= Susceptible, N= Necrotic, R= Resistant

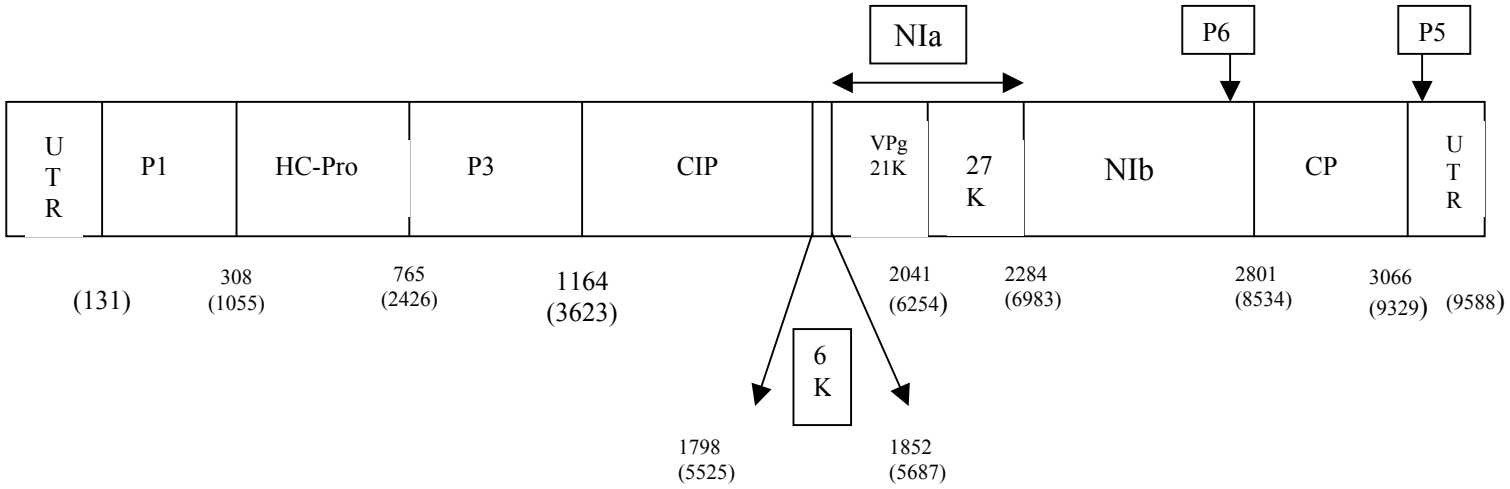

Figure 1.1: Proposed map of SMV genome and predicted protease cleavage sites of the polyprotein.

This map is based on the SMV G2 and SMV G7 sequsnces (Jayaram et al. 1992).

The different boxes refer to the different genes and the 5' and 3' untranslated regions (UTR).

The number below the genome refers to the position of the amino acid in the protein cleaved from the polyprotein, and the numbers in parenthesis refer to the nucleotide position of the cleavage site in the genome sequences of SMV G2 and G7.

CP is the coat protein; CI is the cylindrical inclusion protein;

HC-Pro is the helper component and protease; NIb is the RNA-dependent RNA polymerase;

P1, P3, and NIa are proteases; VPg is the viral protein-genome linked.

References

Adams, S.E., Jones, R.A.C., and Coutts, R.H.A. 1986. Expression of potato virus X resistance gene *Rx* in potato leaf protoplasts. J. Gen. Virol. 67:2341-2345.

Andalakshmi, R., Marathe, R., Ge, X., Herr Jr., J.M., Mau, C., Mallory, A., Pruss, G., Bowman, L., and Vance, V.B. 2000. A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. Science 290:142-145

Barnett, O.W. 1991. *Potyviridae*, a proposed family of plant viruses. Arch. Virol. 118:139-141.

Baulcombe, D.C. 1996a. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. Plant Cell 8:1833-1844.

Baulcombe, D.C. 1996b. RNA as a target and an initiator of post-transcriptional gene silencing in transgenic plants. Plant Molec. Biol. 32: 79-88.

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

Bendahmane, A., Kanyuka, K., and Baulcombe, D. 1999. The *Rx* gene from potato controls separate virus resistance and cell death responses. Plant Cell 11:781-791.

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. 142:1279-1999

Bent, A.F., Kundel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudt, J., Leung, J., and Staskawics, B.J. 1994. *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science 265:1757-1771.

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.

Brigneti, G., Voinnet, O., Li, W.X., Ji, L.H., Ding, S.W., and Baulcombe, D.C. 1998. Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. EMBO 17: 6739-6746.

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., Ma, G., Chen, P., and Tolin, S.A. 1997. Registration of V94-5152 soybean germplasm resistant to soybean mosaic potyvirus. Crop Sci. 37: 1987-1988.

Buss, G.R., Ma, G., Kristipati, S., Chen, P., and Tolin, S.A. 1999. a new allele at the *Rsv3* locus for resistance to soybean mosaic virus. p 490. In Kauffman (ed), Proc. World Soybean Res. Conf. VI. Chicago, IL. 4-7 Aug. 1999. Superior Printing, Champaign, IL.

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.

Buzzell, R.I., Tu, J.C. 1984. Inheritance of soybean resistance to soybean mosaic virus. J. Hered. 75:82

Buzzell, R.I., Tu, J.C. 1989. Inheritance of a soybean stem-tip necrosis reaction to soybean mosaic virus. J. Hered. 80:400-401.

Carrington, J.C., Jensen, P.E., and Schaad, M.C. 1998. Genetic evidence for an essential role for potyvirus CI protein in cell-to-cell movement. Plant J. 14:393-400.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., and Schaad, M.C. 1996. Cell-to-cell and long distance transport of viruses in plants. Plant Cell 8:1669-1681.

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., 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.

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.

Dinesh-Kumar, S.P., Tham, W.H., and Baker, B.J. 2000. Structure-function analysis of the tobacco mosaic virus resistance gene *N*. Proc. Natl. Acad. Sci. USA: 97:14789-14794.

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.

Dolja, V.V., McBride, H.J., and Carrington, J.C. 1992. Tagging of plant potyvirus replication and movement by insertion of β-glucuronidase into the viral polyprotein. Proc. Natl. Acad. Sci. USA 89:10208-10212.

Dougherty, W., and Parks, D. 1991. Post-translational processing of the tobacco etch virus 49-kDa small nuclear inclusion polyprotein: Identification of an internal cleavage site and delimitation of VPg and proteinase domains. Virology 183:449-456.

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.

English, J.J., Mueller, E., and Baulcombe, D.C. 1996. Suppression of virus accumulation in transgenic plants exhibiting silencing of nuclear genes. Plant Cell 8:787-797.

Eun, A.J.C, Seoh, M-L., and Wong, S-M. 2000. Simultaneous quantitation of two orchid viruses by the TaqMan real-time RT-PCR. J Virol. Meth. 87:151-160.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopathol. 9:65-73

Gilbert, J., Spillane, C., Kavanagh, T., and Baulcombe, D. 1998. Elicitation of *Rx*-mediated resistance to PVX in potato does not require new RNA synthesis and may involve a latent hypersensitive response. Molec. Plant Micro. Inter. 11:833-835.

Gillings, M., Broadbent, P., Indsto, J., and Lee, R. 1993. Characterisation of isolates and strains of citrus tristeza closterovirus using restriction analysis of the coat protein gene amplified by the polymerase chain reaction. J. Virol. Meth. 44:305-317.

Goldbach, R., Bucher, E., and Prins, M. 2003. Resistance mechanisms to plant viruses: an overview. Virus Research 92:207-212

Gough,K.H., and Shukla, D.D. 1992. Major sequence variations in the N-terminal region of the capsid protein of a severe strain of passionfruit woodiness potyvirus. Arch. Virol. 124: 389-396.

Gunduz, I., Buss, G.R., Chen, P., and Tolin, S.A. 2002. Characterization of SMV resistance genes in Tousan 140 and Hourei soybean. Crop Sci. 42: 90-95.

Gunduz, I., Buss, G.R., Ma, G., Chen, P., and Tolin, S.A. 2001. Genetic analysis of resistance to soybean mosaic virus in OX670 and Harosoy soybean. Crop Sci. 41: 1785-1791.

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

Hajimorad, M.R., and Hill, J.H. 2001. *Rsv1*-mediated resistance against Soybean mosaic virus-N is hypersensitive response-independent at inoculation site, but has the potential to initiate a hypersensitive response-like mechanisms. Molec. Plant Micr. Inter. 14:587-598.

Hamilton, A.J., and Baulcombe, D.C. 1999. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286:950-952.

Hammond-Kosack, K.E., and Jones, J.D.G. 1997. Plant disease resistance genes. Annu. Rev. Plant Physiol. Mol. Biol. 48:575-607.

Hammond, R.W., Crosslin, J.M., Pasini, R., Howell, W.E., and Mink, G.I. 1999. Differentiation of closely related but biologically distinct cherry isolates for prunus necrotic ringspot virus by polymerase chain reaction. J. Virol. Meth. 80:203-212.

Hammond, S.M., Bernstein, E., Beach, D., and Hannon, G.J. 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. Nature 404:293-296.

Hayes, A.J., and Saghai Marof, 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 Disease 71:237-239.

Hull, R. 2001. Mathew's Plant Virology. 4th ed. Academic Press, Orlando.

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

Hunst, P.L., and Tolin, S.A. 1983. Ultrastructural cytology of soybean infected with mild and severe strains of soybean mosaic virus. Phytopathology 73:615-619.

Ioannou, Y.A., and Chen, F.W. 1996. Quantification of DNA fragmentation in apoptosis. Nucleic Acid Res. 24:992-993.

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.

Jeong, S.C., Kristipati, S., Hayes, A.J., Maughan, P.J., Noffsinger, S.L., Gunduz, I., Buss, G.R., and Saghai Maroof, M.A. 2002. Genetic and sequence analysis of markers tightly linked to the soybean mosaic virus resistance gene, *Rsv3*. Crop Sci. 42:265-270.

Jones, J.D.G. 1996. Plant resistance genes: structure, function and evolution. Curr. Opin. Biotech. 7:155-160.

Kasschau, K.D., and Carrington, J.C. 1998. A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. Cell 95:461-470.

Kasschau, K.D., Cronin, S., and Carrington, J.C. 1997. Genome amplification and long-distance movement functions associated with the central domain of tobacco etch potyvirus helper component-proteinase. Virology 228:251-262.

Keller, K.E., Johansen I.E., Martin, R.R., and Hampton, R.O. 1998. Potyvirus genome-linked protein (VPg) determines pea seed-borne mosaic virus pathotype-specific virulence in *Pisum sativum*. Molec. Plant Micr. Inter. 11:124-130.

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., Roh,J-H., Kim, M-K., Im, D-J., Hur, I-B, and Lee, S-C. 1999. Detection of soybean mosaic virus using RT-PCR. Korean J. Crop Sci. 44:253-255

Koning, G., Tekrony, D.M., Ghabrial, S.A., and Pfeiffer, T.W. 2002. Seed physiology, production and technology. Crop Sci. 42:178-185.

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.

Lamb, C., and Dixon, R.A. 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Mol. Biol. 48:251-275.

Lee, J.Y., Yoo, B.C., and Lucas, W.J. 2000. Parallels between nuclear-pore and plasmodesmatal trafficking of information molecules. Planta 210:177-187.

Lin, N.S., Hsu, Y.H., and Hsu, H.T. 1990. Immunological detection of plant viruses and mycoplasmalike organisms by direct tissue blotting on nitrocellulose membranes. Phytopathology 80:824-828

Lindbo, J.A., Silva-Rosales, L., Proebsting, W.M., and Dougherty, W.G. 1993. Induction of a highly specific antiviral state in transgenic plants-implications for regulation of gene expression and virus resistance. Plant Cell 5:1749-1759.

Llave, C., Kassachau, K.D., and Carrington, J. 2000. Virus-encoded suppressor of posttranscriptional gene silencing targets a maintenance step in the silencing pathway. Proc. Natl. Acad. Sci. 97:13401-13406.

Lucas, W.J. 1999. Plasmodesmata and the cell-to-cell transport of proteins and nucleoprotein complexes. J. Exp. Bot. 50 SI:979-987.

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.

Mansky, L.M., Durand, D.P., and Hill, J.H. 1991. Effects of temperature on the maintenance of resistance to soybean mosaic virus in soybean. Phytopathology 81:535-538.

Mas, P., and Pallas, V. 1995. Non-isotopic tissue-printing hybridization: a new technique to study long-distance plant virus movement. J. Virol. Meth. 52:317-326.

Masuta, C., Nishimura, M., Morishita, H., and Hataya, T. 1999. A single amino acid change in viral genome-associated protein of potato virus Y correlates with resistance breaking in 'Virgin A Mutant' tobacco. Phytopathology 89:118-123.

McDowell, J.M, and Dangl, J.L. 2000. Signal transduction in the plant immune response. Trends Biochem. Sci. 25:79-82

McKern, N.M., Barnett, O.W., Whittaker, L.A., Mishra, A., Strike, P.M., Xiao, X.W., Ward, C.W., and Shukla, D.D. 1993. Sequence relationship among the coat proteins of strains of pea mosaic, white lupin mosaic and bean yellow moasaic potyviruses. Phytopathology 83:255-361.

Moreno M., Bernal, J.J., Jimenez, I., and Rodriguez-Cerezo, E. 1998. Resistance in plants transformed with the P1 or P3 gene of tobacco vein mottling potyvirus. J. Gen. Virol. 79:2819-2827.

Moreno, M., Brandwagt, B.F., Shaw, J.G., Rodriguez-Cerezo, E. 1999. Infectious virus in transgenic plants inoculated with a nonviable, P1-proteinase defective mutant of a potyvirus. Virology 257:322-329.

Murphy, A.M., Gilliland, A., Wong, C.E., West, J., Singh, D.P., and Carr, J.P. 2001. Signal transduction in resistance to plant viruses. Eur. J. Plant Pathol. 107:121-128.

Nicolas, O., Dunnington, S.W., Gotow, L., Pirone, T.P., and Hellmann, G.M. 1997. Variation in the VPg protein allow a potyvirus to overcome va gene resistance in tobacco. Virology 237:452-459.

Nutter, F.W. 1993. Quantification of components contributing to rate-reducing resistance in a plant virus pathosystem. Journal Paper No. J-14826 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa.

Nutter, F.W., Kuhn, C.W., and All, J.N. 1989. Models to estimate yield losses in bell pepper caused by tobacco etch virus epidemics. Phytopathology 79:1213

Omunyin, M.E., Hill, J.H., and Miller, W.A. 1996. Use of unique RNA sequence specific oligonucleotide primers for RT-PCR to detect and differentiate soybean mosaic virus strains. Plant Disease 80:1170-1174.

Pehu, T.M., Makivalkonen, T.K., Valkonen, J.P.T., Koivu, K.T., Lehto, K.M., and Pehu, E.P. 1995. Potato plants transformed with a potato virus Y P1 gene sequence are resistant to PVY. Amer. Potato J. 72:523-532.

Pfosser M.F., and Baumann, H. 2002. Phylogeny and geographical differentiation of Zucchini yellow mosaic virus isolates (Potyviridae) based on molecular analysis of the coat protein and part of the cytoplamsic inclusion protein genes. Arch. Virol. 147:1599-1610.

Powell-Abel, P., Nelson, R.S., De, B., Hoffman, N., Rogers, S.G., Fraley, R.T., and Beachy, R.N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.

Powell-Abel, P., Sanders, P.R., Tumer, N., Fraley, R.T. and Beachy, R.N. 1990. Protection against tobacco mosaic virus infection in transgenic plants requires accumulation of coat protein rather than coat protein RNA sequences. Virology 175:124-130.

Pruss, G., Ge, X.M., Carrington, J.C., and Vance, V.B. 1997. Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. Plant Cell 9: 859-868.

Qusus, S.1997. Molecular Studies on Soybean Mosaic Virus-Soybean Interactions. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 163p

Rabenstein, F., Seifers, D.L., Schubert, J., French, R., and Stenger, D.C. 2002. Phylogenetic relationships, strain diversity and biogeography of tritimoviruses. J. Gen. Virol. 83:895-906.

Ratcliff, F.G., MacFarlane, S.A., and Baulcombe, D.C. 1999. Gene silencing without DNA:RNA-mediated cross-protection between viruses. Plant Cell 11:1207-1215.

Restrepo-Hartwig, M., and Carrington, J.C. 1994. The tobacco etch potyvirus 6-kilodalton protein is membrane associated and involved in viral replication. J. Virol. 68:2388-2397.

Revers, F., Le Gall, O., Candresse, T., and Maule, A.J. 1999. New advances in understanding the molecular biology of plant/potyvirus interactions. Molec. Plant Micro. Inter. 12: 367-376.

Richter, T.E., and Ronald, P.C. 2000. The evolution of disease resistance genes. Plant Molec. Biol. 42:195-204.

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.

Roberts, C,A., Dietzgen, R.G., Heelan, L.A., and Maclean, D.J. 2000. Real-time RT-PCR fluorescnet detection of tomato spotted wilt virus. J Virol. Meth. 88:1-8.

Sanford, J.C., and Johnston, S.A. 1985. The concept of parasite-derived resistance-deriving resistance genes from the parasite's own genome. J. Theor. Biol. 113:395-405.

Schaad, M.C., Haldeman-Cahill, R., Cronin, S., and Carrington, J.C. 1996. Analysis of the VPg-proteinase (NIa) encoded by tobacco etch potyvirus: Effects of mutations on the subcellular transport, proteolytic processing, and genome amplification. J. Virol. 70:7039-7048.

Shukla, D.D., and Ward, C.W. 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J. Gen. Virol. 69: 2703-2710.

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

Steinlage, T.A., Hill, J.H., and Nutter, F.W. Jr. 2002. Temporal and spatial spread of soybean mosaic virus (SMV) in soybeans transformed with the coat protein gene of SMV. Phytopathology 92: 478-486.

Swaney, S., Powers, H., Goodwin, J., Rosales, L.S., and Dougherty, W.G. 1995. RNA-mediated resistance with nonstructural genes from the tobacco etch virus genome. Molec. Plant Micr. Inter. 8:1004-1011.

Takken, F.L.W., and Joosten, M.H.A.J. 2000. Plant resistance genes: their structure, function and evolution. Eur. J. Plant Pathol. 106:699-713.

Tavert-Roudet, G., Ravelonandro, M., Bachelier, J.C., and Dunez, J. 1998. Transgenic *Nicotiana benthamiana* plants containing the P1 gene of plum pox virus are resistant to virus challenge. Eur. J. Plant Pathol. 104:103-107.

Troxler, M., Pasamontes, L., Egger, D., Bienz, K. 1990. In situ hybridization for light and electron microscopy: a comparison of methods for the localization of viral RNA using biotinylated DNA and RNA probes. J. Virol. Meth. 30:1-14.

Urcuqui-Inchima, S., Haenni, A.L., and Bernardi, F. 2001. Potyvirus proteins: a wealth of functions. Virus Research 74:157-175.

Voinnet, O., Pinto, Y.M., and Baulcombe, D.C. 1999. Suppression of gene silencing: A general strategy used by diverse DNA and RNA viruses of plants. Proc. Natl. Acad. Sci. 96: 14147-14152.

Walsh, K., North, J., Barker, I., and Boonham, N. 2001. Detection of different strains of Potato virus Y and their mixed infections using competetive RT-PCR. J Virol. Meth 91:167-173

Wang, D., and Maule, A.J. 1995. Inhibition of host gene expression associated with plant virus replication. Science 267:229-231.

Wang, H.L., Giesman-Cookmeyer, D., Lommel, S.A., and Lucas, W.J. 1998. Mutations in viral movement alter systemic infection and identify an intercellular barrier to entry into the phloem long-distance transport system. Virology 245:75-89.

Wang, H.L., Sudarshana, M.R., Gilbertson, R.L., and Lucas, W.J.1999. Analysis of cell-to-cell long-distance movement of a bean dwarf mosaic Geminivirus-green fluorescent protein reporter in host and nonhost species: Identification of sites of resistance. Molec. Plant Micr. Inter. 12:345-355.

Waterhouse, P.M., Wang, M.B., and Lough, T. 2001. Gene silencing as an adaptive defence against viruses. Nature 411:834-842.

Whitham, S.A., Yamamoto, M.L., and Carrington, J.C. 1999. Selectable viruses and altered susceptibility mutants in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 96:772-777.

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.