# HIGH-RESOLUTION MAPPING OF THE REGION AROUND THE SOYBEAN VIRUS RESISTANCE GENES, *Rsv*1 AND *Rpv*1

By

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**High-Resolution Mapping of the Region around the Soybean**

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## ABSTRACT

*Soybean mosaic virus* (SMV) and *peanut mottle virus* (PMV) are potyviruses that can cause serious yield reductions in soybean [*Glycine max* (L.) Merr.]. Virus resistant soybean cultivars have been released with alleles at the *Rsv*1 and *Rpv*1 locus that confer resistance to SMV and PMV, respectively. A high-resolution map-based cloning approach was undertaken to isolate *Rsv*1 and *Rpv*1 from soybean, with hopes of providing insight into this host-pathogen relationship. A mapping population of 1,056 $F_2$ individuals was constructed from the cross of the resistant cultivar PI 96983 (*Rsv*1 and *Rpv*1) by the susceptible cultivar Lee 68 (*rsv*1 and *rpv*1). Ninety-one of the 1,056 $F_2$ individuals had a cross-over (recombination) in the chromosomal region between microsatellite, or simple sequence repeat (SSR) marker loci Hsp176 and Sat120, and these 91 recombinant lines (RLs) were selected for further genetic analysis. Genotypes of *Rsv*1 and *Rpv*1 for the 91 RLs were obtained by inoculating their $F_{2:3}$ progeny with SMV-G1 and PMV-P1, respectively. The 91 RLs also were used for mapping one random amplified polymorphic DNA (RAPD), five SSR, and 21 restriction fragment length polymorphism (RFLP) markers. Included in these RFLP markers were seven resistance gene candidate (RGC) and five resistance gene candidate flanking (RGCF) markers. RGC probes encode a protein with homology to previously cloned plant disease resistance genes, and RGCF probes are sequences obtained from the flanking regions of candidate disease resistance genes. The resultant high-resolution map consisted of 41 marker loci detected by 27 molecular markers. *Rsv*1 and *Rpv*1 cosegregated with one or more RFLP bands detected by RGCF probes: GG27-1a, 3gG2SP, and/or T3G. Analyses of the disease reaction and molecular marker data from seven RLs suggested that the map position of *Rsv*1 should be at a locus different from that designated by the linkage analysis software, Mapmaker 3.0. Compared to the other 89 RLs, a high percentage (>34%) of $F_3$ plants grown from four of these seven RLs gave a necrotic reaction when inoculated with SMV-G1. From this evidence, we believed that another locus independent of *Rsv*1 was involved in PI 96983's response to SMV-G1. The two loci conferring resistance to SMV-G1 were designated *Rsv*1a and *Rsv*1b.

**DEDICATIONS**

To my mother, Carmen,

whose love and guidance continues to shape my life.

She will live forever in my dreams.

To the appreciation of Dr. M. A. Saghai Maroof, for believing in me.

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# CHAPTER I.

# INTRODUCTION

In 2838 B.C., Chinese Emperor Sheng-Nung recorded the first written description of soybean [*Glycine max* (L.) Merr.] in the books Pen Ts'ao Kong Mu (Morse, 1950). Soybean was later domesticated by Northern Chinese farmers prior or during the Shang dynasty (ca. 1700-1100 B.C.). The culture of Eastern Asia utilized soybean for its medicinal properties and sustenance for several thousand years. In 1765, Samuel Bowen introduced soybeans from China into the North American colony of Georgia (USA). Dr. Benjamin Franklin Edwards introduced soybean into Illinois around 1851. The soybeans transported to Illinois were increased and dispersed into other surrounding states by 1854 (Hymowitz, 1989). Today, the largest commercial production of soybeans occurs in the U.S., followed by Brazil, China, and Argentina (Schmidt, 1989). From 1976 to 1984 the world production of soybean increased from 58.1 million to 89.9 million tons, and the total area of cultivated soybean expanded from 37.8 million to 52.1 million hectares (Singh and Rachie, 1987).

Soybean is the primary source of vegetable oil and protein in many areas of the world (Hymowitz, 1989). The high quality oil and protein are about 20 and 40%, respectively, of the bean (Singh, 1987). Manufacturers use oil from soybeans to produce cooking oil, salad oils, margarine, and shortening (Hymowitz, 1989). The oil is easily digested, low in saturated fats, and contains no cholesterol (Singh, 1987). The soybean meal, or cake remaining after the extraction of the oil, is often used to feed farm animals and household pets. Soybeans are used as a protein substitute for meat and fish because of its high level of essential amino acids (Hymowitz, 1989). Soybean also is used to produce milk, tofu, flour, and many snack foods (Singh, 1987).

Soybean diseases caused by bacteria, fungi, viruses, nematodes, and mycoplasmalike organisms have strengthened in severity and abundance, as the utilization of land for the cultivation of soybean has increased throughout the world (Sinclair and Backman, 1989). Soybeans are susceptible to at least 111 viruses or virus strains in experimental and natural environments, including 17 definite or possible members of the *potyvirus* genus (Demski et al., 1989). Among the *potyvirus* genus, the *soybean mosaic virus* (SMV) and the *peanut mottle virus* (PMV) are two economically important soybean viruses. SMV and PMV can inflict substantial losses in quality and yield of soybean (Shipe et al., 1979; Bays et al., 1986; Thottapilly and Rossel, 1987; Buss et al., 1989). SMV and PMV are obligate parasites that only can reproduce within living plant cells, and their genome consists of positive, single-stranded RNA (Demski et al., 1989; Shukla et al., 1994). These two *potyviruses* are transmitted to soybean plants through wounds created by aphids and mechanical inoculation. Soybean seed contaminated with SMV, and peanut seed infected by PMV allows these two viruses to persist as potential infectious agents of soybean throughout the world (Cho and Goodman, 1979; Bays et al., 1986; Thottapilly and Rossel, 1987; Buss et al., 1989). Symptoms resulting from SMV or PMV infection include stunting of plant growth, mosaic or mottling patterns on leaves, seed mottling, discoloration of foliage, and necrosis (Cho and Goodman, 1979; Bays et al., 1986).

**Background**

The use of virus resistant soybean cultivars is the most efficient and cost-effective method to limit crop damage resulting from SMV and PMV (Buss et al., 1989; Jacobsen and Backman, 1989). Plant breeders have reduced substantial economic losses for the grower by releasing soybean cultivars that dually possess resistance to SMV and PMV at the *Rsv*1 and *Rpv*1 loci, respectively (Smith, 1968; Buss et al., 1988). Cloning and characterizing *Rsv*1 and *Rpv*1 may provide further insight into the molecular genetic basis of these host-virus interactions.

The first step to cloning any of these virus resistance genes in soybean is the arduous task of constructing a highly saturated genetic linkage map in the vicinity of *Rsv*1 and *Rpv*1. Yu et al. (1994) developed a linkage map for the *Rsv*1 region using an $F_2$ population constructed from a cross between soybean line PI 96983 (*Rsv*1) and cultivar Lee 68 (*rsv*1). *Rsv*1 mapped to soybean molecular linkage group (MLG) F, as determined by the linkage of one SSR marker, four RFLP markers, and a gene for flower color, *w*1. *Rsv*1 is a member of a disease resistance gene cluster that consists of other specificities for bacterial, fungal, nematode and insect phytopathogens (Diers et al., 1992; Ashfield et al., 1996; Tamulonis et al., 1997a; Tamulonis et al., 1997b; Rector et al., 1999). *Rpv*1 also may map to the disease resistance gene cluster on MLG-F, since an inheritance study determined that *Rsv*1$^y$ and *Rpv*1 were linked (Roane et al., 1983). Continuing research has focused on saturating the *Rsv*1 chromosomal region with resistance gene candidate (RGC) markers that contain nucleotide sequences with partial homology to disease resistance genes with nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains. Yu et al.

(1996) developed a PCR approach to isolate resistance gene candidate (RGC) sequences in soybean using degenerate primers based on the conserved nucleotide-binding site (NBS) domains of disease resistance genes, *N* (tobacco) and *Rps*2 (*Arabidopsis*). The degenerate primers amplified eleven (*a-k*) different classes of RGC sequences. Two RGC sequences, NBS5 and NBS61, mapped to the disease resistance gene cluster on MLG-F of soybean. Hayes and Saghai Maroof (2000) generated four markers linked to *Rsv*1, taking advantage of the conserved NBS region of resistance genes and efficiency of AFLP.

**THE SPECIFIC OBJECTIVES OF THIS STUDY WERE:**

i) to determine the molecular linkage relationship between *Rsv*1 and *Rpv*1;

ii) to develop a high-resolution map using a population of 1056 $F_2$ individuals; by saturating the chromosomal region of *Rsv*1 and *Rpv*1 with molecular markers, particularly RGC markers and sequences obtained from the flanking regions of candidate disease resistance genes, or resistance gene candidate flanking (RGCF) markers.

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# CHAPTER II.

# LITERATURE REVIEW

**Peanut Mottle Virus**

*Peanut mottle virus* (PMV) was first recorded as a disease on peanut (*Arachis hypogaea* L.) in Georgia (Kuhn et al.,1965) and later observed naturally infecting various crop species in Africa (Bock, 1973), Australia (Behncken, 1970), Philippines (Reddy et al., 1985), India (Reddy et al., 1978), Europe (Schmidt and Schmelzer, 1966), and South America (Herold and Munz, 1969; Sreenivasulu and Demski, 1988). Multiple members of *Fabaceae* are natural hosts to PMV, as well as a limited number of species outside the family (Bock and Kuhn, 1975). In all, PMV is reported to infect 49 species in 23 genera of five families (Edwardson and Christie, 1991).

Kuhn (1965) demonstrated soybean [*Glycine max* (L.) Merr.] and 13 other species of *Fabaceae* to be a host of PMV under experimental circumstances. In 1971, a mild strain of PMV was detected to infect soybeans in Georgia under natural field conditions (Kuhn et al., 1972). PMV was reported to spread beyond Georgia into the soybean and peanut fields of Virginia and South Carolina (Demski and Kuhn, 1977; Shipe et al., 1979). Soybeans infected with PMV also have been observed in East Africa (Bock, 1973) and Australia (Behncken and McCarthy, 1973). Demski and Kuhn (1975) concluded that plant height and yield of susceptible field-grown soybean cultivars were reduced six and 20%, respectively, after mechanical inoculation with PMV. Demski and Kuhn (1977) encountered yield losses ranging from 12 to 28% on two Georgia-grown soybean varieties infected with PMV. A severe strain of PMV lowered the mean yield of 21 PMV-susceptible cultivars by 44% in a Virginia experiment (Shipe et al., 1979).

Peanut seeds contaminated with PMV serve as the primary inoculum source for the virus spreading throughout fields of susceptible soybean and peanut cultivars (Demski, 1975). Experimental data from different sources have demonstrated that PMV transmission occurs through infected peanut seeds (Kuhn, 1975; Sun and Herbert, 1972; Bock, 1973; Paguio and Kuhn, 1974a). Paguio and Kuhn (1974a) were unsuccessful in isolating PMV from other potential sources of inoculum such as sap from leaves of trees, shrubs, herbaceous plants, or vines within or proximal to infested peanut fields. Two research groups harvested seed from susceptible soybean cultivars and determined that PMV was not seed transmitted in soybean (Bock, 1973; Demski and Harris, 1974).

There is a high incidence of soybean infection occurring when they are planted near peanut fields or in a field that grew peanuts the previous year. The movement of disease between crop species results from aphids transmitting PMV in a nonpersistent manner from virus infected peanut plants to soybean plants (Demski, 1975). A variety of aphids, such as *Aphis craccivora* Koch, *Rhopalosiphum padi* (L.), *Myzus persicae* (Sulzer), *Aphis gossypii* Glover, and *Hyperomyzus lactucae* (L.) has demonstrated the ability to transmit PMV (Bock, 1973; Paguio and Kuhn, 1976; Sreenivasulu and Demski, 1988; Behncken, 1970). Highland et al. (1981) observed a higher incidence of peanuts infected with PMV than cowpea and soybean, which could result from *A. craccivora* and *M. persicae* composing 31% of the aphid population in peanuts compared to 17% in cowpeas and 14% in soybeans. Aphids appear to exhibit a greater propensity for peanut or infect peanut with more efficiency (Highland et al., 1981).

Substantial yield losses amongst soybeans from PMV are attributed to three main factors: (1) distance of virus movement in field, (2) initial level of inoculum, and (3) the stage of growth at which plants are infected (Demski, 1975). PMV is transmitted 48 m or more in a single movement, but the time necessary for infection decreases at shorter distances from the inoculum source. The infectivity rate of soybeans with PMV increases with decreasing distances from infected peanut plots (7% infection with 48 m separation and >80% infection with 1 m separation). There is a more accelerated dissemination of the virus in peanut than soybean, with reported cases of the virus spreading as far as 6.5 km in peanut (Demski, 1975). Tests conducted by Demski and Kuhn (1975) demonstrated that susceptible soybean plants have reduced incidences of systemic symptoms with increased maturity. Generally, soybean plants mechanically inoculated after 20 and 30 days of age have a low probability of developing systemic infections in the field or greenhouse.

In Georgia, the first mild mottle PMV isolate (PMV-M1) was isolated from infected peanuts exhibiting leaf mottling, upward curling of leaflets, and depression of interveinal tissue (Kuhn, 1965; Table 1). PMV-M1 was later isolated from naturally infected soybeans (Kuhn et al., 1972). Paguio and Kuhn (1973a) developed a purification scheme to isolate a second mild mottle PMV isolate (PVM-M2), which became prevalent in the fields of North Carolina and Georgia. PMV-M2 induced slight chlorotic mottling or spot patterns, while PMV-M1 only caused mottling in the infected leaves of peanut and soybean. Sun and Hebert (1972) designed a technique to purify a North Carolina isolate of peanut severe mosaic virus (PMV-S) from peanut leaves exhibiting chlorosis and necrosis. The authors observed that PMV-S caused

the leaves of susceptible soybean cultivars to become malformed and mottled. Paguio and Kuhn (1973b) described a necrosis isolate (PMV-N) and chlorotic line pattern isolate (PMV-CLP) obtained from peanut fields in Georgia. The PMV-N isolate caused infected peanut and soybean plants to become severely stunted and develop irregular necrotic spots on all new growth. The CLP variant initially induced vein clearing on developing leaflets of peanut and soybean plants followed by chlorosis with dark green spots. In the 1980's, Tolin and Ford (1983) developed a purification method for potyviruses that enabled high recovery and reduced aggregation of virus. The procedure was utilized to purify PMV isolates PMV-H and PMV-B. Susceptible soybean cultivars experienced a severe mosaic and line pattern in trifoliolate leaves as well as stunting after being inoculated with PMV-H, whereas PMV-B only caused mild symptoms in susceptible soybean cultivars.

Soybean genotypes display a variance of symptoms to different strains of PMV that only induce a mild mottle in peanut; therefore, a set of differential soybean varieties can accurately classify strains of PMV. A large number of soybean genotypes can be employed to further refine classification schemes of PMV strains (Bijaisoradat et al., 1988). Bays et al. (1986) classified twelve Virginia-PMV isolates by differentiating them into five strain groups (P1-P5) based on variable reactions of soybean cultivars, 'York', 'Lee 68', 'Virginia', and 'Cumberland'. In Virginia (USA), most PMV stains isolated from infected peanut or soybean fields tend to be members of the P1 strain group. The PMV M-2 strain described by Paguio and Kuhn (1973a) was classified into strain group P4 because of the symptoms observed in the four test cultivars. Bays et al. (1986) did not classify any other of the previously described Georgia and North Carolina PMV strains.

Bijaisoradat et al. (1988) characterized eight isolates of PMV based on disease reactions in peanut, bean (*Phaseolus vulgaris* L.), *Chenopodium amaranticolor*, and soybean. These differential hosts were screened with eight PMV isolates: arrowleaf (AR), chlorotic stunt (CS), desmodium (DE), India (IN), lima bean (LB), PMV-M1, PMV-N, and necrosis/chlorosis (NC). The disease symptoms caused by the PMV-M1, CS, PMV-N, and NC isolates on the four legume species were unique enough to be classified as strains of PMV. The other four isolates were variants of PMV that could not be considered separate strains, because they induced the same disease reaction symptoms as the mild strain on all hosts except soybean and *C. amaranticolor*.

Bijaisoradat et al. (1988) also observed that the reaction of eight soybean genotypes: 'Bragg', 'Buffalo', 'Davis', 'Dorman', 'Hardee', 'PI 96983', 'Virginia' and 'York' to the PMV isolates was more diverse than in other legume species. No soybean genotype was resistant to every inoculated isolate of PMV, but Davis, Dorman, Hardee, PI 96983, Virginia, and York were resistant (no virus detected in non-inoculated trifoliolate leaves) to one or more of the isolates. Bragg and Buffalo developed mottle symptoms for all eight isolates. Bays et al. (1986) determined that Buffalo was resistant (no symptoms, ELISA negative, and no virus recovered) to all five strain groups (P1-P5) found in Virginia, but the one India and seven Georgia isolates from the study were not utilized by the Virginia virologists.

**Soybean Mosaic Virus**

Clinton (1915) observed a disease causing chlorosis and crinkling of soybean leaves in Connecticut, USA. Gardner and Kendrick later described the disease as *soybean mosaic virus* (SMV) in 1921. SMV exists wherever soybeans are cultivated in the world due to its transmission through infected seeds (Kendrick and Gardner, 1924; Buss et al., 1989). Hill et al. (1980) conducted field cage tests to further understand the epiphytology of SMV. They determined that SMV spreads plant-to-plant by aphids from primary inoculum foci, and the primary inoculum consists of mosaic seedlings germinated from SMV-contaminated seeds. SMV is naturally transmitted in a nonpersistent manner by aphid species *Acyrthosiphon pisum*, *Aphis fabae*, *Myzus persicae*, *Rhopalosiphum maidis*, and experimentally by an additional 28 aphid species belonging to 14 different genera (Lucas and Hill, 1980; Shukla et al., 1994). SMV has been reported to infect 47 species of 27 genera in five families (Edwardson and Christie, 1991).

Many soybean cultivars infected with SMV exhibit transient systemic vein clearing, followed by a distortion mosaic in the younger leaves with dark green, later swelled areas along the primary veins and chlorosis amid the dark green regions (Bos, 1972). Cultivar dependent symptoms induced by SMV in soybean include mottling, shortening of petioles and internodes, bud blight, male sterility, flower abnormalities, reduced pubescence, etiolation of older leaves, necrosis, and seed coat mottling (Shukla et al., 1994). The symptoms of SMV become more severe at 18-20 $^{0}$C than at 27-30 $^{0}$C (Conover, 1948). Infected plants are slightly stunted and produce fewer pods that may be deformed, glabrous, and seedless

(Bos, 1972). Seeds harvested from SMV infected soybean plants tend to have increased levels of free amino acids and iodine (El-Amrety et al., 1985).

Soybean seeds contaminated with SMV are the primary source of virus inoculum (Hill et al., 1987). Gardner and Kendrick (1921) determined from tests conducted in the greenhouse that 13% of the seedlings germinated from SMV infected seed developed the disease. Primary leaves of soybean seedlings grown from SMV contaminated seeds showed mottling and downward curling. Subsequent leaves developed more severe symptoms than the initial leaves. Kendrick and Gardner (1924) continued studying the effects of SMV contaminated seeds. Fifteen U.S. grown varieties of soybean differed in their susceptibility and ability to transmit SMV through the seed. Conover (1948) harvested seeds from infected soybean plants and observed a seedling infection rate of 2 to 75% depending on the genotype of the cultivar. Koshimizu and Iizuka (1963) detected SMV in the embryo and seed coat of immature seeds from infected plants, and there was a rapid loss of the virus from seeds as they matured. The authors also discovered soybean plants infected with SMV after flowering did not produce contaminated seeds. Fett (1978) concluded from a study in Brazil that volunteer soybean plants from seeds left in the field after harvest were potential between-season survival sites for SMV. Goodman et al. (1979) screened 897 accessions using the "Illinois severe" isolate of SMV (SMV-Il-S) and identified 19 SMV-infected lines that did not seed transmit SMV.

Mottling is a common occurrence in soybean in which pigments of the hila are diffused in irregular patterns or streaked across the seed coat (Woodworth and Cole, 1924; Kennedy and Cooper, 1967). Mottling in soybean is attributed to the effects of environment, cultivar, and genotype (Hill et al., 1980). Kendrick and Gardner (1924) discovered both diseased and healthy plants produced mottled seeds, and mosaic seedlings developed from mottled and nonmottled seeds. Porto and Hagedorn (1975) observed the absence of virus seed transmission for a Brazilian isolate of SMV with one tested soybean cultivar that had a mottling rate of 91%. Hill et al. (1980) reported that mottled seeds were an imprecise indicator of SMV infected seeds and infection of the mother plant.

SMV lowered the yield of soybean by 30 to 75% in 1921 and 1922 Indiana test trials conducted by Kendrick and Gardner (1924). Ross (1968) reported 8-25% reductions in yield of soybeans inoculated as seedlings with SMV strains. Losses were dependent on the strain of SMV, location, and variety. Ross (1977) analyzed the effect of SMV naturally transmitted by aphids in field plantings of closely related resistant and susceptible soybean lines. Decline in seed yield of susceptible lines ranged from 20-35% compared to resistant controls. Dhingra and Chenulu (1980) studied the effects of SMV on seed yield in mechanical inoculations of greenhouse grown soybeans. The researchers calculated yield losses of 94%, 83%, 62% and 25% after inoculations at the cotyledonary stage, pre-bloom, bloom, and post-bloom stages, respectively. The results indicated that plants experience less reduction in yield as their age at the time of inoculation progressively increases.

Tu et al. (1970) reported that the number, size, and weight of nodules were reduced by SMV infection. The greatest reductions occurred when soybean plants were inoculated 2 weeks after sowing. The decrease of plant fresh weight in SMV-infected plants has been attributed to the alteration of the *Rhizobium*-soybean symbiotic relationship, causing a decrease in photosynthesis, nodulation, and increase in respiration. The authors provided evidence that nodules on SMV-infected plants were less effective than nodules on healthy plants. The total nitrogen was always higher in nodules of SMV-infected plants than in non-inoculated plants. Plants inoculated with SMV exhibited reduced susceptibility to *Rhizobium japonicum* infection and decreased leghemoglobin compared to virus-free soybeans. Dhingra and Chenulu (1980) evaluated the effects of SMV on nodulation in mechanically inoculated soybeans grown in the greenhouse. Soybeans infected at the pre-bloom stage of growth experienced a reduction in average nodulation and dry weight of nodules by 69% and 63%, respectively.

The behavior of two or more viruses in a solitary plant can be synergistic, additive, or cross protective (Demski et al., 1989). A synergistic effect occurs between SMV and bean pod mottle virus (BPMV) when infecting soybean plants together. This harmonious interaction results in poorer seed quality, greater reductions in seed size, and more seed mottling than plants singly inoculated with one of the two viruses (Quiniones et al., 1971). Ross (1969a) determined from experiments that susceptible soybean plants simultaneously inoculated with SMV and BPMV consistently produced less than plants singly inoculated at the same time with SMV or BPMV. Quiniones et al. (1971) evaluated the performance of three soybean varieties inoculated with SMV and/or BPMV. The authors observed an 18% loss in mean yield for three soybean varieties infected with SMV. Additionally, a 10% and 66% decline in mean yield was reported for the same three varieties inoculated with BPMV and both BPMV and SMV, respectively.

There was a 22% and 29% reduction in germination of seeds from plants infected with SMV and doubly inoculated with SMV and BPMV, respectively. SMV caused mottling on 92% of the seed harvested from SMV infected plants, and on 96% of seeds from plants inoculated with both SMV and BPMV. SMV was transmitted in 27% of the seed from SMV-infected plants, and in 39% of the seed harvested from plants inoculated with both SMV and BPMV.

Ross (1969b) used 24 soybean selections and seven SMV strains to discover that SMV isolates may vary in their virulence depending on the soybean genotype. Ross (1975) identified the blister strain of SMV (SMV-B), a novel variant of SMV-1, which resulted from a mutation of SMV-1 and/or selection pressure. SMV-B caused the terminal buds of some SMV-1 resistant soybean cultivars to become necrotic, along with severely distorted and dwarfed leaves having pronounced blisters. SMV-1 and SMV-B have many of the same physical and biological properties, but differ in their severity of disease reaction in certain cultivars. Cho et al. (1977) described a severe necrotic strain of SMV (SMV-N) that inflicted bud blight and drastic reduction of pod set in SMV resistant Korean and Japanese soybean cultivars. It is thought that the introduction of cultivars highly resistant to common SMV strains induced the evolution of SMV-N or the strain was undetected in the field until highly resistant cultivars were utilized.

Cho and Goodman (1979) classified 98 isolates of SMV from seed in the USDA soybean germplasm collections into seven strains based on symptomology (resistance, mosaic, or necrosis) of eight inoculated differential soybean cultivars. Twenty-seven, 24, 17, 3, 8, 17, and 2 isolates were categorized into strain groups SMV-G1, SMV-G2, SMV-G3, SMV-G4, SMV-G5, SMV-G6, and SMV-G7, respectively. There were marked differences in the degree of virulence exhibited by SMV strains and the disease response of the eight soybean cultivars to these strains. Cho and Goodman (1979) suggested that a wide range of SMV strains should be utilized to develop resistance in a soybean breeding program, because of virulent strains present in germplasm collections. The strain groups SMV-G3, SMV-G4, SMV-G5, SMV-G6, and SMV-G7 differ from any of the isolates analyzed by Ross (1969). The SMV-B belongs to SMV-G3; SMV-1 and SMV-I1-S are members of SMV-G2. The authors postulated that SMV-N would be classified in strain group SMV-G5.

Buzzell and Tu (1984) described SMV strain G7A, which caused symptoms different from SMV strains G1 through G7 in a cultivar with a single dominant SMV resistance gene, *Rsv*1, derived from 'PI 96983'. Lucas and Hill (1980) distinguished three Iowa isolates of SMV Ia SMV-0, Ia 12-18, and Ia 75-16-1 on the basis of host reaction and transmissibility by two aphid species. The three isolates were not classified with the system developed by Cho and Goodman (1979). In Virginia, Hunst and Tolin (1982) classified SMV-VA and SMV-OCM into the G1 and G3 strain groups, respectively. Lim (1985) conducted inoculations with an unclassified SMV isolate, C14.

Jayaram et al. (1992) sequenced the entire RNA genome of strains G2 and G7 of SMV. The 9,588 nucleotide long genome of G2 and G7 encoded a polyprotein of 3,066 amino acids, which was believed to be proteolyticly cleaved into nine mature proteins. Qusus (1997) cloned and sequenced the coat protein genes of SMV strains G1 and G6. The author compared the amino acid similarity and nucleotide identity of the coat protein genes from SMV strains G1, G2, G6, and G7. The results of the study suggested that the coat protein gene was not responsible for overcoming resistance mediated by a SMV resistance gene. Jain et al. (1992) used high-performance liquid chromatography (HPLC) to distinguish between SMV strains based on coat protein peptide profiles. Omunyin et al. (1996) performed a reverse transcription-polymerase chain reaction (RT-PCR) assay to detect and differentiate between SMV strains G2 and G7. The assay was specific and capable of detecting a specific strain of SMV in a mixed infection of a single soybean host plant.

**Molecular Markers Employed in Evaluation of Plant Genomes**

Classical plant breeders practiced their art by selecting superior individuals among the segregating progeny of a sexual cross based solely on a visible phenotype. Environmental effects on the measured trait and the complex inheritance of quantitative trait loci (QTL) can disrupt the accuracy of phenotypic selection (Rafalski et al., 1991). Isozymes were incorporated into early molecular genetic studies to follow the inheritance of traits. The use of isozymes was restricted in many species because they detected a low number of informative loci. Isozymes allowed researchers to develop fundamental principles applicable to present-day DNA-based molecular marker technology (Helentjaris, 1985). Genetic maps consisting of DNA-based molecular markers genetically linked to an important trait can be used for gene cloning and marker-assisted selection in plant and animal breeding programs (Williams et al., 1990). Molecular

markers allow breeders to examine wild germplasm, heterosis, pedigrees, and epistatic interactions. Breeders can fingerprint their products with markers to protect patent rights (Helentjaris, 1985). Restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR) or microsatellite, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and single nucleotide polymorphism (SNP) are DNA-based molecular markers utilized by researchers.

**Restriction Fragment Length Polymorphism (RFLP)**

RFLPs were originally used to follow the inheritance of disease loci in humans (Phillips et al., 1980). Recently, RFLPs have become an instrumental tool for the plant breeder and molecular geneticist. Any restriction fragment out of the possible million restriction fragments generated by genome digestion can possibly serve as a marker locus (Helentjaris, 1992). A change in the length of a restriction fragment generates a novel allele, however mutations at the protein level are often neutral (Burr et al., 1983). DNA is digested with different restriction endonucleases, separated on an agarose gel and transferred onto nylon or nitrocellulose membranes. Unique, low copy, sequences are cloned from nuclear, mitochondrial, or chloroplast genomes. The genomic or cDNA clones are radioactively or non-radioactively labeled, and hybridized to the membrane to detect homologous sequences in DNA. The differences in restriction fragment sizes recognized by the labeled probe between progeny of a population are defined as polymorphisms (Tanksley et al., 1988). RFLPs are typically inherited in a simple Mendelian codominant fashion, when linked to a trait segregating in a population. RFLP analysis requires a large amount of capital and time, which makes this assay impractical for some plant breeding facilities (Burr et al., 1983).

**Simple Sequence Repeat (SSR)**

SSRs are composed of short tandemly repeated core sequences, mostly two or three nucleotides in length, that are variable in repeat number. The repeated core sequences are flanked by conserved DNA sequences. The conserved sequences can be exploited to design complementary polymerase chain reaction (PCR) primers that are capable of amplifying the locus (Yu et al., 1994). The SSR is then separated on standard polyacrylamide or agarose gels to visualize the variation in the repeat region (Yu et al., 1994; Akkaya et al., 1995). The usefulness of SSRs is dependent on the abundance and frequencies of alleles. SSRs are single locus markers with many alleles, while RFLPs usually only detect a single locus with two alleles (Cregan et al., 1999). Saghai Maroof et al. (1994) determined that the number of alleles per locus ranged from 3 to 37 in a sample of 207 accessions of wild and cultivated barley. Maughan et al. (1995) detected 79 alleles in a sample of 94 accessions of wild (*Glycine soja*) and cultivated soybean (*Glycine max*) with five microsatellite primer pairs. Cregan et al. (1999) demonstrated that SSRs could be used to integrate existing linkage maps across mapping populations of soybean. Generally, an SSR is observed every 64.6 kb for monocots and every 21.2 kb for dicots, suggesting SSRs are evenly distributed in the plant genome (Wang et al., 1994). SSRs are inherited codominantly in a Mendelian manner and potentially highly polymorphic in crops that have a low-level of polymorphism with other molecular markers (Liu et al., 1996; Saghai Maroof et al., 1994; Weber, 1990). SSRs are important for linkage mapping projects, germplasm analysis, and uncovering phylogenetic relationships between species (Saghai Maroof et al., 1994; Liu et al., 1996). The primer sequence of SSRs can be widely dispersed throughout the world via publications and public databases (Saghai Maroof et al., 1994; Weber, 1990). GenBank and EMBL database sequences can be scanned to locate SSR-containing sequences for the crop species of choice (Akkaya et al., 1992; Saghai Maroof et al., 1994; Maughan et al., 1995; Liu et al. 1996). It is laborious and expensive to proceed from the initial construction of the genomic library to the actual PCR amplification of microsatellite alleles (Liu et al., 1996).

**Random Amplified Polymorphic DNA (RAPD)**

RAPD assays involve using short, random-sequence oligonucleotides as PCR-primers for the amplification of DNA without prior knowledge of target DNA sequence. The products of the RAPD assay are separated on an agarose gel and observed by staining with ethidium bromide. The fragments visible from staining are dependent on the primer sequence and the sequence of the DNA template. Primers differing by a single nucleotide amplify a different set of bands; therefore, a set of RAPD primers can be used to discover polymorphic bands at different loci randomly distributed throughout the genome. RAPDs

can be used to rapidly construct saturated genetic maps in various understudied organisms, since no knowledge of sequence is required (Williams et al., 1990; Rafalski et al., 1991). Nucleotide polymorphisms at one or both annealing sites results in no amplification, thus the RAPD assay amplifies only one polymorphic band per locus. RAPDs require less DNA, start-up time, labor, and money compared to RFLPs. Also, RAPDs do not require restriction enzymes, species-specific probes, or radioactivity (Rafalski et al., 1991; Williams et al., 1990). RAPDs are being used less by geneticists, because other PCR-based markers such as SSRs are more reproducible and codominant (Mudge et al., 1997).

**Amplified Fragment Length Polymorphism (AFLP)**

AFLP is a DNA fingerprinting technique developed by Vos et al (1995) based on the selective amplification of DNA fragments. The DNA fragments are generated from the digestion of total genomic DNA with two restriction endonucleases, followed by subsequent ligation of site-specific adapters to the restriction fragments. Primers complementary to the restriction sites and adapters are designed to achieve PCR-amplification of the restriction fragments. Primers have selective nucleotides attached to their 3' ends that extend into the restriction fragments, thus amplifying fragments with nucleotides flanking the restriction site complementary to the primer extension. The DNA fragments are observed on high-resolution, denaturing sequencing gels. AFLP, a high-throughput marker, is capable of coamplifying 50-100 restriction fragments in a single reaction. The number of fragments amplified is linearly correlated with the complexity of the host genome (Vos et al., 1995). Maughan et al. (1996) visualized 759 AFLP fragments with 15 primer pairs in a sample of 23 wild and cultivated soybean accessions. AFLP analysis allows thousands of independent genetic loci to be amplified in a minimal amount of time. Similar to RAPDs, no prior sequence knowledge is required to employ AFLPs in genome analysis. Heterozygotes cannot be differentiated from homozygotes, because AFLP markers have a dominant Mendelian segregation pattern. Insertion and deletion mutations in the internal amplified region of DNA fragments can produce codominant AFLP markers (Vos et al., 1995). An AFLP band can be purified from a polyacrylamide gel and used as a probe in RFLP analysis, which usually results in a codominant marker at the same locus (Hayes and Saghai Maroof, 2000).

**Single Nucleotide Polymorphism (SNP)**

SNPs are DNA sequence polymorphisms that occur commonly in plant and animal genomes, differing by as little as a single base pair (Alcala et al., 1997). SNPs usually exist as point mutations between alleles, with 2-3 SNPs occurring every 1 kb in humans (Newton et al., 1989; Gilles et al. 1999). SNPs have the potential to replace SSRs as the marker of choice. Large sets of SNPs can be developed near or in a gene of interest, while SSRs do not occur as frequently throughout the plant genome (Landegren et al., 1998). Grimm et al. (1999) postulated that SNPs occur at a frequency of 3.4/kb in the soybean genome, while an SSR in dicots is observed every 21.2 kb (Wang et al., 1994). SNPs are more stably inherited than SSRs, because the repeated sequences comprising SSRs sometimes suffer mutations that alter the size of the allele (Landegren et al., 1998). DNA chip technology and denaturing high-performance liquid chromatography (DHPLC) are expensive, high-throughput methods used by large laboratories to quickly identify and genotype SNPs (Hauser et al., 1998). SNPs can be detected with gel- or non-gel based methods. Some laborious gel-based procedures include RFLPs, single strand conformation polymorphism (SSCP) detection, and chemical or enzymatic mismatch modification assays (Nikiforov et al., 1994). Oligonucleotide ligation assays, allele-specific PCR, ligase chain reaction, 5' nuclease assay, and primer guided nucleotide incorporation assays are reasonably priced non-gel based assays (Nikiforov et al., 1994; Holloway et al., 1999).

**Near-Isogenic Lines and Bulk Segregant Analysis**

Molecular markers are powerful when utilized with near-isogenic lines (NILs) or bulk segregant analysis (BSA) (Young et al., 1988; Michelmore et al., 1991). Qualitative traits in plants regulated by single genes can be mapped using NILs (Young et al., 1988). The generation of NILs through backcrossing is achieved by crossing $F_1$ hybrid plants (Rr) containing a dominant allele, which are progeny of a cross between a recurrent (rr) and donor parent (RR), successively back to the recurrent parent (rr) for several generations. The genome of the NIL becomes almost identical to the recurrent parent after six or more

backcrosses, with the exception of the NIL retaining the dominant allele. Molecular markers that are polymorphic between the NIL and the recurrent parent have a high probability of being linked to the gene introgressed into the NIL. The putatively linked marker is mapped in a population segregating for both the gene of interest backcrossed into the NIL and the marker (Diers et al., 1992; Muehlbauer et al., 1989, 1991). Young et al. (1988) quickly identified tightly linked DNA markers to a gene of tomato that provides resistance to the tobacco mosaic virus, *Tm-2a*. Two tightly linked DNA markers were isolated by hybridizing sets of genomic clones to Southern blots comprising DNA from NILs with or without *Tm-2a*. Yu et al. (1994) confirmed close linkage between *Rsv*1 and three markers by marker analysis of NILs that carry alleles at the *Rsv*1 locus.

Michelmore et al. (1991) developed the BSA procedure for directly identifying markers linked to a gene or genomic region of interest. Two bulked DNA samples are created from individuals of a population segregating for a desired trait. Each bulk has individuals that are exactly alike for a trait or genomic region but variable at all unlinked loci. Polymorphisms between the two bulk samples are identified employing molecular markers. Michelmore et al. (1991) identified three RAPD markers in lettuce linked to a downy mildew resistance gene using BSA. Poulsen et al. (1995) constructed two separate DNA bulks of $F_2$ plants resistant or susceptible to barley leaf rust and identified a RAPD marker linked 12 centimorgans (cM) away from the leaf rust resistance gene, *Rph*Q. Bendahmane et al. (1997) utilized AFLPs and BSA to develop a high-resolution linkage map around the *Rx*1 locus in potato that confers extreme resistance to *potato virus x*.

BSA overcomes some problems associated with utilizing NILs to identify linked markers. NILs have problems with linkage drag and polymorphic markers between NILs are sometimes monomorphic in a segregating mapping population. NILs require years of backcrossing to develop, while BSA pools can be generated immediately after a segregating population has been developed (Michelmore et al., 1991).

**Control of Pathogens with Host Resistance Genes**

Plant resistance genes effectively control the spread of pathogens among plants. Plants have developed advanced resistance mechanisms to counteract deleterious pathogen attack, which coevolve with a corresponding pathogen (Tang et al., 1999). Disease resistance in plants is usually under the regulation of single dominant resistance genes (Flor, 1971). A single resistance gene in the plant recognizes signal molecules produced by the avirulence gene of the pathogen (Flor, 1971; Tang et al., 1996). This gene-for-gene interaction activates resistance gene products in response to pathogen elicitors (Tang et al., 1999). A susceptibility reaction occurs if the plant resistance gene products do not recognize the elicitors manufactured by the avirulence gene (Martin et al., 1993). The gene-for-gene concept has been exhibited only in plants with qualitative (monogenic and oligogenic) types of resistance to a particular pathogen (Agrios, 1988). The resistance gene products rapidly initiate a defense response that alters the cell physiology and metabolism of the plant. The defense response generally evokes a hypersensitive response that causes necrosis of infected plant cells. Hypersensitive reactions result in a stimulation of phytoalexin synthesis, temporary increase in hydrogen peroxide production, cell reinforcement, and activation of defense-related genes. The hypersensitive response confines invading pathogens to necrotic tissue, thus causing the isolated pathogens to eventually die (Tang et al., 1999). Antisense, ribozymes, and coat-protein-mediated protections are possible nontraditional techniques that can be used to combat pathogens, but extensive research will be required to integrate these techniques into plant breeding (Powell-Abel et al., 1986; Hull and Davies, 1992).

**PMV Resistance Genes in Soybean**

In 1973, Bock screened 21 cultivars and breeding lines of soybean with a PMV isolate from naturally infected soybean in East Africa, and found all were susceptible to the virus. Demski and Kuhn (1975) evaluated 70 soybean cultivars and breeding lines for resistance to PMV. A susceptible disease reaction was not observed for 14 cultivars. Resistant cultivars were not infected by two species of aphids in greenhouse experiments and maintained resistance in a field environment. The authors suggested that resistant plants have a high level of resistance to the establishment of viral infection, because the virus was isolated from only two of 674 resistant plants. Shipe at al. (1979a) screened 2,161 soybean plant introductions (Maturity Groups II, III, and IV) for PMV resistance by airbrush inoculating seedlings in the field. The authors identified 7, 16, and 122 resistant entries in Maturity Groups II, III, and IV, respectively.

Boerma and Kuhn (1976) studied the inheritance of PMV resistance in progeny of four crosses between resistant ('CNS' & 'Dorman') and susceptible soybean cultivars ('Ransom', 'Bragg', and 'Pickett'). Mechanical inoculation tests on $F_1$, $F_2$, and $F_3$ generations in the greenhouse determined that a single dominant gene, *Rpv*, governed resistance to PMV in CNS and Dorman. Shipe et al. (1979b) utilized populations segregating for PMV resistance to discover a single dominant gene that controlled resistance in 'Arksoy' (*Rpv*1) and a recessive resistance gene (*rpv*2) that conferred resistance in 'Peking.' Dorman and Arksoy most likely have the same single dominant gene for PMV resistance, since Dorman is derived from Arksoy.

Buss et al. (1985) determined the allelic relationship among the single dominant genes for PMV resistance in soybean cvs. Arksoy, Dorman, York, Shore, and CNS. Segregation was not observed in $F_3$ progenies of individual $F_2$ plants of crosses among Arksoy, Dorman, York, and Shore. The authors concluded that the four cultivars had resistance to PMV at *Rpv*1. $F_3$ progeny from crosses of CNS with Dorman, York, and Shore inoculated with PMV segregated in a pattern typical for two independent dominant genes. The data contradict the gene designation of *Rpv* assigned to CNS by Boerma and Kuhn (1976), since the gene for PMV resistance in CNS was at a locus different from Dorman. The first mentioned PMV resistance gene in CNS was later designated a gene symbol of *Rpv*3 (Bagade, 1998). Bagade (1998) determined the PMV resistance genes in PI 96983, 'Toano', 'Jizuka', and 'Suweon 97' were allelic to *Rpv*1. PI 486355 had two dominant genes for resistance to PMV, one gene allelic to *Rpv*1 and the other at a locus different from *Rpv*1 and *Rpv*3.

**SMV Resistance Genes in Soybean**

From 1955-1958, Koshimizu and Iizuka (1963) working in Japan tested 110 cultivars for resistance to SMV by inoculation tests in the greenhouse and field. Eighty-three soybean cultivars showed typical mosaic symptoms, but 27 cultivars exhibited resistance to the virus. Ross (1969b) screened 478 soybean selections from four different maturity groups in a North Carolina field. Only 24 selections were resistant to SMV-1.

Inheritance studies, conducted by Koshimizu and Iizuka (1963), provided evidence for the existence of a single dominant gene controlling SMV resistance in soybean. Kiihl and Hartwig (1979) determined a single dominant gene, *Rsv* (now *Rsv*1), regulated SMV resistance in PI 96983 (Table 2). In addition, the authors designated a partially dominant SMV resistance gene in 'Ogden' as $rsv^t$ (later $Rsv1^t$). Kwon and Oh (1980) determined a single recessive gene controlled resistance to SMV-N, but considered stem-tip necrosis a susceptible reaction (Buzzell and Tu, 1989). A single dominant gene in York controlled resistance to SMV-VA (G1), but a gene symbol was not designated (Roane et al., 1983). Buss et al. (1989) demonstrated that soybean cvs. Marshall and Kwanggyo carried single dominant resistance genes to SMV-G1 that were probably alleles at a shared locus. The alleles of Marshall and Kwanggyo exhibited differential symptoms to strains of SMV. PI 96983, Ogden, York, Marshall, and Kwanggyo were evaluated by Chen et al. (1991) to determine the allelic relationships among their single genes conditioning resistance to SMV-G1. All five cultivars had single dominant resistance genes that were alleles at the *Rsv*1 locus. York, Marshall, Ogden, and Kwanggyo were assigned the gene symbols $Rsv1^y$, $Rsv1^m$, $Rsv1^t$ and $Rsv1^k$, respectively.

There are other dominant resistance genes at loci different from *Rsv*1 that confer resistance to SMV. Buzzell and Tu (1984) discovered a second single dominant gene for SMV resistance at a locus different from *Rsv*1, and designated it *Rsv*2. *Rsv*2 provided resistance to SMV strains G1 through G7A in the breeding line OX670, a derivative of 'Raiden.' Researchers later determined that the gene for SMV resistance in Raiden was actually at the *Rsv*1 locus (Buss et al., 1995). Gunduz (2000) discovered that OX670 had an allele of *Rsv*1 from Raiden, and also an allele of *Rsv*3 probably derived from Harosoy. Buzzell and Tu (1989) determined a single dominant gene, *Rsv*3, controlled the stem-tip necrosis reaction to strains of SMV in OX686 (*Rsv*3 from 'Columbia'). Bowers et al. (1992) determined that Buffalo and the breeding line HLS have single, dominant non-allelic SMV resistance gene. The authors did not assign gene designations to the two different loci, because relationships to *Rsv*1, *Rsv*2, and *Rsv*3 were not elucidated. Results from marker analysis by Yu et al. (1994 and 1996) suggested that the *Rsv* gene in Buffalo was most likely at the *Rsv*1 locus. Lim (1985) reported that PI 486355 (SS74185) was resistant to the seven (G1-G7) strains of SMV and to an unclassified highly virulent SMV isolate, C14. The soybean line PI 483084 (Suweon 97) was susceptible to C14, but resistant to all seven strains of SMV. The author determined that

the SMV resistance gene in each of these lines were single dominant genes at different loci. Lim concluded that *Rsv*2 most likely mediates SMV resistance in PI 483084.

Chen et al. (1993) evaluated PI 486355 to determine the number of loci controlling resistance to SMV strains G1, G6, and G7. They discovered that a gene at the *Rsv*1 locus and a dominant gene at another unknown locus controlled resistance to SMV in PI 486355. Ma et al. (1995) separated the two dominant SMV genes from PI 486355 into separate breeding lines, LR1 and LR2. The researchers designated the allele at the *Rsv*1 locus in LR1 as *Rsv*1-s. *Rsv*1-s conferred resistance to strains G1-G4 and G7, but developed systemic necrosis with G5-G6. The other dominant gene in LR2 [later designated *Rsv*4 by Hayes et al., (2000)] conferred resistance to all known strains of SMV. *Rsv*4 produced no necrotic or hypersensitive type reaction to all strains of SMV (G1-G7). *Rsv*4 exhibited complete dominance in the heterozygous state, whereas a heterozygous genotype at the *Rsv*1 locus results in systemic necrosis from incomplete dominance (Chen et al., 1994).

**Mapping Disease Resistance Genes in Soybean**

In 1987, the soybean genetic map consisted of only 40 classical markers spanning 420 cM. The application of RFLPs, AFLPs, SSRs and other molecular markers in soybean genetic mapping has resulted in the expeditious construction of highly saturated maps (Diers et al., 1992). Shoemaker and Olson (1993) constructed a soybean linkage map from an interspecific *Glycine max* x *Glycine soja* cross that incorporated 365 RFLPs, 11 RAPDs, four isozymes, and three classical loci. Shoemaker and Specht (1995) made marginal progress assimilating molecular and classical marker maps using a set of anchoring RFLP loci that segregated in two soybean mapping populations. Akkaya et al. (1995) succeeded at integrating 40 SSR markers into an existing soybean linkage map. The SSRs were randomly distributed throughout the soybean genome with limited clustering of SSR loci. Keim et al. (1997) developed a high-density soybean genetic map, starting with 165 RFLP markers as anchors and accomplishing widespread saturation of the soybean genome with 650 AFLP markers. Cregan et al. (1999) constructed an integrated genetic linkage map with three populations that included classical markers with discernible phenotypic effects, isozyme markers, SSRs, RFLPs, RAPDs, and AFLPs, accounting for 1,423 unique loci among the three maps.

Research groups have succeeded in identifying the chromosomal location of qualitative disease resistance genes in soybean. Weisemann et al. (1992) used molecular and classical markers to map a soybean cyst nematode (SCN) resistance gene, *Rhg*4, to soybean molecular linkage group (MLG) -A2. Diers et al (1992) mapped *Rps*4 and *Rps*1 to MLG-G and -N, respectively. *Rps*4 and *Rps*1 confer resistance to root and stem rot, caused by races of *Phytophthora sojae* Kaufmann & Gerdemann. Hayes et al. (2000) mapped the SMV resistance gene *Rsv*4 to MLG-D1b using the combination of AFLP and BSA. *Rsv*4 confers resistance to all known strains of SMV in a manner different from typical SMV resistance genes. Similar to *RTM*1, which restricts long-distance transport of *tobacco etch virus* in *Arabidopsis*, a necrotic reaction is not produced in response to the entry of a pathogen (Chisholm et al., 1999).

Molecular markers have been used to study the quantitative inheritance of resistance to economically relevant pathogens. Concibido et al. (1996) utilized BSA, comparative genome mapping, and integrated mapping to saturate the region surrounding a major partial-resistance locus to SCN (*Heterodera glycines Ichinohe*) on MLG-G of soybean. The identified locus controlled over 50% of total variation for the quantitatively inherited trait. Mudge et al. (1997) developed two microsatellite markers that flanked the major partial-resistance locus for SCN mapped by Concibido et al. (1996). The PCR-based markers will facilitate selection of lines with SCN resistance without the need for laborious RFLPs. Hnetkovsky et al. (1996) mapped two QTLs for partial resistance to sudden death syndrome (SDS) caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Synd. & Hans., type A (FSA) on MLG-C and -N of soybean. The two QTLs cumulatively accounted for 34% of total phenotypic variability in mean disease incidence. Kim and Diers (2000) mapped significant QTLs for partial resistance to stem rot caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary. Interval mapping based on mean disease severity index values allowed the identification of a single QTL on MLG-K and -M, and at least two separate QTLs on MLG-C2.

**Disease Resistance Genes on MLG-J and -F of Soybean**

Resistance genes in plants appear to be conserved and clustered, thus allowing unequal crossing over to increase or decrease the size of the resistance gene cluster (Kanazin et al. 1996; Yu et al. 1996;

Crute and Pink, 1996). Gene conversion and/or intragenic recombination might generate novel loci by combining existing genes (Crute and Pink, 1996). A cluster of disease resistance genes exists on MLG-J of soybean. Diers et al. (1992) mapped *Rps*2 and *Rj*2 to MLG-J of soybean. *Rj*2 prevents normal nodulation with some strains of *Bradyrhizobium japonicum* (Kirchner) Buchanan. *Rps*2 governs resistance to phytophthora root and stem rot. Lohnes et al. (1993) evaluated the linkage among *Rj*2, *Rmd*, and *Rps*2. *Rmd* provides resistance to powdery mildew, caused by *Microsphaera diffusa* Cooke & Peck. The authors discovered that all three genes were linked in classical linkage group 19, and Polzin et al. (1994) integrated *Rmd*, *Rj*2, and *Rps*2 into MLG-J. Mian et al. (1999) mapped the *Rcs*3 gene of soybean in MLG-J. *Rcs*3 mediates resistance to frogeye leaf spot, which is a foliar disease caused by *Cercospora sojina* Hara. Kanazin et al. (1996) isolated conserved resistance gene analogs (RGAs), sequences with homology to the NBS of *N*, *Rps*2, and *L*6, from soybean with an approach similar to Yu et al. 1996. These authors mapped five different classes of RGAs to the resistance gene cluster in MLG-J.

Another disease resistance gene cluster resides on MLG-F of soybean. Diers et al. (1992) mapped a phytophthora root rot resistance gene, *Rps*3, with RFLPs to MLG-F. Ashfield et al. (1998) determined that the *Rpg*1 locus resides on MLG-F. *Rpg*1 confers resistance to races of *Pseudomonas syringae* pv. *glycinea* that express the avirulence genes *avr*B or *avrRpm*1. Tamulonis et al. (1997a) used RFLP markers to locate QTLs conferring resistance to Javanese root-knot nematode [*Meloidogyne javanica* (Treub) Chitwood] (Mj) in soybean. Two QTL alleles providing resistance to Mj were identified on MLG-F and -D1 of soybean. The data best fit an additive model, with marker B212 on MLG-F and A725-2 on MLG-D1 explaining 46% and 13%, respectively, of the variation in gall number. Rector et al. (1999) utilized RFLPs to determine the location of QTLs for antixenosis (nonpreference) resistance to corn earworm (*Helicoverpa zea* Boddie) in soybean plant introductions. A major QTL was identified on MLG-H and M, along with minor QTLs on MLG-C2, -D1, and -F. Tamulonis et al. (1997b) located QTLs to peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood] on the F- and E-MLG of soybean. A QTL each on MLG-F and -E accounted for 32% and 16%, respectively, of the variation in gall number. An additive to partially dominant model explained the gene action for the QTL on the MLG-F, whereas a dominance model explained the resistance conferred by the QTL on MLG-E of soybean. *Rsv*1 mapped to soybean MLG-F, as determined by the linkage of SSR marker, Hsp176, four RFLP markers, A186, K644, K2, and K390, and the gene for flower color, *w*1. Hsp176, A186, and K644 were closely linked to *Rsv*1, with genetic distances of 0.5, 1.5, and 2.1 cM, respectively (Yu et al., 1994).

Plant breeders have conducted inheritance studies involving potyvirus resistance genes in soybean. Roane et al. (1983) utilized a segregating population, York (*Rsv*1$^{y}$, *Rpv*1) x Lee 68 (*rsv*1$^{y}$, *rpv*1), to conclude that *Rsv*1$^{y}$ and *Rpv*1 were linked with $3.7 \pm 0.8$ % recombination. The segregation ratios for SMV and PMV reactions were indicative of two, single, dominant genes. Choi et al. (1989) observed a linkage relationship between two dominant genes providing resistance to SMV-G1 and peanut stripe virus (PStV-isolate PN) in AGS 129. The two dominant resistance genes were linked with $9 \pm 2.4$ % recombination as coupling phase. The chromosomal location of the dominant SMV gene in AGS 129 was later determined to be at *Rsv*1 (Buss, personal communication).

**Homology-based Cloning of *Rsv*1 from Soybean**

It is possible for new resistance genes to be rapidly isolated based on sequence homology, without the arduous methods of transposon tagging and map-based cloning (Yu et al., 1996). Yu et al. (1996) developed a PCR approach to isolate candidate disease resistance genes in soybean based on a conserved nucleotide-binding site (NBS) of disease resistance genes. The NBS region encodes three motifs involved in signal transduction, P-loop, kinase-2, and kinase-3a. They designed degenerate oligonucleotide primers based on the amino acid sequences of the P-loop and kinase-3a domains of the NBS region in tobacco *N* gene and *Arabidopsis Rps*2 gene. The amplified sequences from soybean were cloned and sequenced. All NBS clones encoded the three motifs of NBS and shared high sequence similarity to the *N* and *Rps*2 genes. The NBS clones were categorized into 11 classes based on sequence and hybridization data. Two NBS clones, *NBS61* and NBS5, mapped in the F-linkage group around *Rsv*1 and appear to be members of a clustered multigene family.

Hayes and Saghai Maroof (2000) developed a targeted mapping approach that took advantage of the conserved NBS region of disease resistance genes, sensitivity of bulk segregant analysis, and capability of AFLP. A degenerate primer corresponding to the P-loop motif encoded by the NBS region of cloned

resistance genes *N*, *L*6, and *Rps*2 was able to amplify multiple polymorphic bands between parents of a population segregating for *Rsv*1. Four dominant AFLP bands, R11 to R14, were cloned and used in RFLP analysis. R11, R12, R13, and R14 mapped within a 6 cM region that encompassed *Rsv*1. Sequence analysis revealed that all four clones possess the P-loop sequence analogous to the degenerate primer. R14 has an open reading frame sequence homologous to other cloned resistance genes, and mapped 0.4 cM away from *Rsv*1. These researchers are making significant strides in the cloning and characterization of the first virus resistance gene in soybean.

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Table 1. Isolates of PMV

| Isolate | Abbreviation | Reference |
|---|---|---|
| Mild-1 | PMV-M1 | Kuhn, 1965 |
| Mild-2 | PMV-M2 | Paguio and Kuhn, 1973a |
| Severe | PMV-S | Sun and Herbert, 1972 |
| Necrosis | PMV-N | Paguio and Kuhn, 1973b |
| Chlorotic line pattern | PMV-CLP | Paguio and Kuhn, 1973b |
| Holland, VA | PMV-H | Tolin and Ford, 1983 |
| Blacksburg, VA | PMV-B | Tolin and Ford, 1983 |
| Arrowleaf | AR | Bijaisoradat et al.,1988 |
| Chlorotic stunt | CS | Kuhn et al., 1984 |
| Desmodium | DE | Bijaisoradat et al.,1988 |
| India | IN | Rajeshwari et al., 1983 |
| Lima bean | LB | Bijaisoradat et al.,1988 |
| Necrosis/chlorosis | NC | Bijaisoradat et al.,1988 |

Table 2. Disease reaction of soybean genotypes to nine SMV strain groups

| Source | Resistance Gene | G1 | G2 | G3 | G 4 | G5 | G6 | G7 | G7A | C14 | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PI 96983 | *Rsv*1 | R | R | R | R | R | R | N | S | R | 1, 5 ,7,10 |
| Buffalo | *Rsv*1 | R | R | R | R | R | R | N | NA | R | 4, 7,10 |
| Ogden | *Rsv*1$^{t}$ | R | R | N | R | R | R | N | S | NA | 1, 5, 8 |
| Marshall | *Rsv*1$^{m}$ | R | N | N | R | R | N | N | S | NA | 1, 5, 8 |
| York | *Rsv*1$^{y}$ | R | R | R | N | S | S | S | S | NA | 1, 5, 7 |
| Kwanggyo | *Rsv*1$^{k}$ | R | R | R | R | N | N | N | N | NA | 1, 5, 7 |
| Raiden | *Rsv*1$^{r}$ | R | R | R | R | N | N | R | R | N | 1, 2,10 |
| OX670 | *Rsv*1,*Rsv*3 | R | R | R | R | R | R | R | R | NA | 9 |
| OX686 | *Rsv*3 | N | NA | NA | N | NA | NA | R | NA | NA | 3 |
| LR1 | *Rsv*1$^{s}$ | R | R | R | R | N | N | R | NA | NA | 11 |
| LR2 | *Rsv*4 | R | R | R | R | R | R | R | NA | NA | 11 |
| PI 483084 | *Rsv?* | R | R | R | R | R | R | R | R | N | 1, 6,10 |
| PI 486355 | *Rsv*1,*Rsv*4 | R | R | R | R | R | R | R | R | R | 1,10 |
| HLS | *Rsv*? | S | S | S | S | R | R | R | NA | NA | 4 |

R= resistant; S= susceptible; N= necrotic; NA= not available


1) Buss et al., 1994
2) Buss et al., 1995
3) Buzzell and Tu, 1989
4) Bowers et al., 1992
5) Chen et al., 1991
6) Chen et al., 1999
7) Cho and Goodman, 1979
8) Cho and Goodman, 1982
9) Gunduz, 2000
10) Lim, 1985
11) Ma et al., 1995

## CHAPTER III.

## High-Resolution Mapping of Candidate Disease Resistance Genes Flanking the Soybean Potyviral Resistance Genes, *Rsv*1 and *Rpv*1

**Abstract**

*Soybean mosaic virus* (SMV) and *peanut mottle virus* (PMV) are potyviruses that persist globally and can cause substantial economic loss. Plant breeders have limited crop damage for the grower by developing soybean [*Glycine max* (L.) Merr.] cultivars with alleles at the *Rsv*1 and *Rpv*1 loci that confer resistance to SMV and PMV, respectively. Isolating and characterizing *Rsv*1 and *Rpv*1 are necessary for a complete understanding of this host-virus relationship. An initial population of 243 $F_2$ individuals from the cross of the SMV and PMV resistant line PI 96983 (*Rsv*1 and *Rpv*1) by the susceptible cultivar Lee 68 (*rsv*1 and *rpv*1) was expanded to 1,056 $F_2$ individuals. The new population provided adequate recombinant individuals for high-resolution mapping in the interval between simple sequence repeat (SSR) marker loci Hsp176 and Sat120, and the capacity to determine the molecular linkage relationship between *Rsv*1 and *Rpv*1. Marker-based selection was used to construct a high-resolution genetic linkage map surrounding *Rsv*1 and *Rpv*1 on soybean molecular linkage group (MLG) F. In the original population, SSR markers 64-A8C and Sat120 mapped to one side of *Rsv*1 (0.2 cM and 3.7 cM, respectively) and Hsp176 to the other side (3.1 cM). These three SSR markers were scored in the 813 additional $F_2$ individuals to identify recombinant lines (RLs) with a cross-over (recombination) between Hsp176 and 64-A8C, or 64-A8C and Sat120. Ninety-one RLs were selected from the large mapping population. The genotype was inferred for the other 722 individuals that did not show recombination within the chromosomal region between Hsp176 and Sat120. Plants grown from remnant $F_{2:3}$ seeds of each of the 91 RLs were separately screened with the G1 strain of SMV and P1 strain of PMV to ascertain their genotype for *Rsv*1 and *Rpv*1, respectively. For the purpose of high-resolution mapping, $F_{2:3}$ DNA samples from each of the 91 RLs were analyzed with 27 DNA markers. These markers contained one randomly amplified polymorphic DNA (RAPD), five SSRs, and 21 restriction fragment length polymorphisms (RFLPs). The mapped RFLP markers included seven resistance gene candidates (RGCs) and five resistance gene candidate flanking (RGCF) markers. RGC probes encode a putative protein product with homology to resistance genes that contain a nucleotide-binding site (NBS) and leucine-rich repeat (LRR), while RGCF probes are sequences isolated from the flanking regions of candidate disease resistance genes. Cumulatively, the 27 molecular markers detected 41 marker loci that spanned 7.1 cM in the new population, and RFLP bands detected by RGCF markers cosegregated with *Rsv*1 and *Rpv*1. There were 19 RLs that had a recombination event in the chromosomal region between flanking disease resistance loci, *Rsv*1 and *Rpv*1. Analyses of the disease reaction and molecular marker data from seven of these 19 RLs suggested that *Rsv*1 should map to a locus different from that assigned by the linkage analysis software, Mapmaker 3.0. A percentage (8-61%) of plants grown from $F_{2:3}$ remnant seed of these seven RLs developed necrosis following inoculation with SMV-G1. From this data set, we postulated that another gene was involved in PI 96983's response to SMV-G1. The two SMV resistance genes were designated *Rsv*1a and *Rsv*1b.

## Introduction

Plants have developed advanced resistance mechanisms to defeat deleterious pathogen attack (Bent, 1996; Hinrichs-Berger et al., 1999). A single resistance gene in the plant recognizes signal molecules produced by the avirulence gene of the pathogen (Flor, 1971; Hammond-Kosack and Jones, 1996; Tang et al., 1996; Taraporewala and Culver, 1996). This gene-for-gene interaction activates resistance gene products that rapidly alter the cell physiology and metabolism of the plant (Hammond-Kosack and Jones, 1996; Tang et al., 1999). Commonly, a hypersensitive response is evoked that induces localized necrotic lesions at the site of pathogen entry. The necrotic tissue surrounds the invading pathogen and isolates it from the nutrient-rich living tissue, eventually causing the confined pathogen to die (Hammond-Kosack and Jones, 1996; Hinrichs-Berger et al., 1999; Tang et al., 1999). In addition, the hypersensitive response is accompanied by increased levels of phytoalexins, reactive oxygen species, and materials for cell wall reinforcement (Agrios, 1988; Hammond-Kosack and Jones, 1996; Tang et al., 1999).

Disease resistance genes in plants have been observed to occur in tightly linked clusters, and different loci in the clusters convey specific resistance to different phytopathogens (Bent, 1996; Crute and Pink, 1996). The cluster of resistance genes allows multiple specificities to be maintained in a single haplotype (Crute and Pink, 1996). In addition, tight linkage between cognate sequences of these complex loci enables the generation of novel specificities through the mechanisms of gene conversion, unequal crossing-over, and gene duplication, thus permitting coevolution with corresponding pathogen populations (Hulbert and Bennetzen, 1991; Hu and Hulbert, 1994; Richter et al., 1995; Bent, 1996; Crute and Pink, 1996; Michelmore and Meyers, 1998).

Cloned disease resistance genes from plant species conferring resistance to viral, bacterial, nematode, and fungal pathogens encode proteins with similar sequences and structural domains (Bent et al., 1994; Jones et al., 1994; Whitham et al., 1994; Grant et al., 1995; Lawrence et al, 1995; Song et al., 1995; Salmeron et al., 1996; Milligan et al., 1998). Many of these isolated resistance genes possess the N-terminal nucleotide-binding site (NBS)[1] and C-terminal leucine-rich repeat (LRR) domains (Bent et al., 1994; Mindrinos et al., 1994; Whitham et al., 1994; Grant et al., 1995; Lawrence et al, 1995; Salmeron et al., 1996; Milligan et al., 1998). The NBS domain is thought to be involved in the defense signal transduction pathway and consists of the conserved P-loop, kinase-2, and kinase-3a motifs (Traut, 1994; Bent, 1996; Baker et al., 1997). The LRR domain is variable in size among disease resistance genes and is believed to mediate protein-protein interactions (Bent, 1996). Researchers have amplified candidate disease resistance sequences from various plant species using degenerate PCR primers designed from the conserved NBS domain of cloned resistance genes. These NBS sequences have been shown to map near or cosegregate with resistance gene loci (Kanazin et al., 1996; Leister et al., 1996; Yu et al., 1996; Aarts et al., 1998; Collins et al., 1998; Shen et al., 1998; Speulman et al., 1998).

In soybean [*Glycine max* (L.) Merr.], *Rsv*1 and *Rpv*1 are commonly used virus resistance genes that counteract the pestilent nature of soybean mosaic virus (SMV) and peanut mottle virus (PMV), respectively. SMV, the most prevalent and important viral disease in soybean, has been reported to exist wherever soybeans are cultivated (Thottapilly and Rossel, 1987; Buss et al., 1989). The spread of PMV in the southeastern United States has been blamed for significant yield reduction in both soybean and peanut (Bays et al., 1986). Using virus resistant soybean cultivars is the most practical method of preventing an epiphytic disaster (Buss et al., 1989; Jacobsen and Backman, 1989).

Soybean cultivars with alleles at the *Rsv*1 locus can develop severe necrosis when inoculated with more virulent strains of SMV (Cho and Goodman, 1979; Chen et al., 1994). Generally, plants that show a necrotic reaction following infection with SMV have symptoms on both inoculated (necrotic local lesions and veinal necrosis) and noninoculated (necrotic lesions and veinal necrosis) leaves (Cho and Goodman, 1979), whereas in resistant plants the necrotic lesions indicative of the hypersensitive response are localized

[1] Abbreviations: NBS, nucleotide binding site; LRR, leucine rich repeat; SMV, soybean mosaic virus; PMV, peanut mottle virus; RFLP, restriction fragment length polymorphism; MLG, molecular linkage group; RGC, resistance gene candidate; TIR, toll-interleukin-1 cytoplasmic receptor; AFLP, amplified length polymorphism; BSA, bulk segregant analysis; RGCF, resistance gene candidate flanking; SSR, simple sequence repeat; NIL, near isogenic line; IDP, insertion deletion polymorphism; RAPD, random amplified polymorphic DNA; RL, recombinant line; NTN, non TIR NBS; HRF, *Hin*dIII restriction fragment; BAC, bacterial artificial chromosome.

to the area of pathogen entry (Chen et al., 1994; Hinrichs-Berger et al., 1999). Necrotic soybean plants become severely stunted and eventually die without producing any seed (Cho and Goodman, 1979; Chen et al., 1994). Chen et al. (1991) reported 98% of necrotic plants from varied crosses between resistant and susceptible soybean cultivars were heterozygous for *Rsv*1. Chen et al. (1994) discovered that the necrotic and resistant reactions of PI 96983 and four other cultivars to strains of SMV were attributed to alleles at the *Rsv*1 locus. Incomplete dominance of alleles at the *Rsv*1 locus, and dominance of necrotic alleles to resistant or susceptible alleles at the *Rsv*1 locus have been suggested as reasons for the necrotic reaction (Buss et al., 1989; Chen et al., 1994). Cloning and characterizing *Rsv*1 may provide further insight into the molecular genetic basis of the necrotic response elicited by more virulent SMV strains.

*Rsv*1 has been mapped to a gene cluster of molecular linkage group (MLG) F that consists of several disease resistance genes flanked by restriction fragment length polymorphism (RFLP) markers K644 and B212 (Yu et al., 1994). Tightly linked members of this disease resistance gene cluster include *Rps*3 and *Rpg*1 (Diers et al., 1992; Ashfield et al., 1996). Quantitative trait loci for resistance to the Javanese root-knot nematode, peanut root-knot nematode, and corn earworm are also present in this chromosomal region (Tamulonis, 1997a; Tamulonis et al., 1997b; Rector et al., 1999). Roane et al. (1983) used a segregating population, York (*Rsv*1$^{y}$, *Rpv*1) x Lee 68 (*rsv*1$^{y}$, *rpv*1), to determine that *Rsv*1$^{y}$ and *Rpv*1 were linked. This observation suggests that *Rpv*1 is a member of the disease resistance gene cluster on molecular linkage group (MLG)-F.

Yu et al. (1994) first initiated the development of a genetic linkage map for the *Rsv*1 region. A 107 individual $F_2$ population was constructed from a cross between soybean line PI 96983 *(Rsv1*) and cv. Lee 68 (*rsv*1). Five molecular markers mapped in this population were linked to *Rsv*1 on soybean MLG-F. Ensuing research has centered on identifying markers specific for the *Rsv*1 chromosomal region. Yu et al. (1996) developed an approach to isolate resistance gene candidate (RGC) sequences in soybean with homology to a class of disease resistance genes with NBS and LRR domains. Degenerate oligonucleotide primers based on the conserved NBS domains of *N* (tobacco) and *RPS*2 (*Arabidopsis*) amplified 11 (*a*-*k*) different classes or multigene families of RGCs. Two RGC clones, NBS5 and NBS61, mapped to the MLG-F of soybean and appear to be members of a disease resistance gene cluster. NBS5 and NBS61 were grouped into NBS classes *b* and *j*, respectively, according to sequence and RFLP hybridization information. NBS5 is highly homologous to the cloned *N* (tobacco) and *L*6 (flax) genes, which form a subclass of NBS-LRR disease resistance genes that contain a Toll-Interleukin-1 cytoplasmic receptor (TIR). On the other hand, NBS61 is most homologous to *I*2C (tomato), which is a member of a second subclass of NBS-LRR disease resistance genes that are without a TIR domain (Saghai Maroof, unpublished data). NBS5 was used as a probe to screen a cDNA library and two full-length genes, L20a and L33, were identified. L20a and L33 mapped to the MLG-F and MLG-E of soybean, respectively (Hayes et al., 2000). Kanazin et al. (1996) developed a similar degenerate primer approach to isolate RGC sequences from soybean, but none of their RGC clones mapped to MLG-F. Hayes and Saghai Maroof (2000) extended the degenerate primer approach and developed a targeted mapping strategy that exploited the conserved NBS region of typical NBS-LRR disease resistance genes, efficiency of amplified fragment length polymorphism (AFLP), and sensitivity of bulk segregant analysis (BSA). Four markers, R11 to R14, were identified and mapped in a 243 individual $F_2$ population segregating for *Rsv*1, constructed from a cross of PI 96983 x Lee 68. One of the four markers, R14, has an NBS sequence homologous to other cloned resistance genes and mapped 0.4 cM away from *Rsv*1.

In this study, an initial $F_2$ population of 243 individuals (Yu et al., 1996; Hayes and Saghai Maroof, 2000), constructed from a cross between resistant soybean line PI 96983 (*Rsv*1, *Rpv*1) and susceptible cv. Lee 68 (*rsv*1, *rpv*1), was expanded to 1,056 individuals. The newly constructed population was used to develop a high-resolution map around the *Rsv*1 and *Rpv*1 loci. An RGC probe (L61-95-B8) was used to identify a 22 kb lambda genomic clone (G27-1-1) containing a candidate disease resistance gene, from which several RFLP probes were generated. Two newly developed RFLP probes, GG27-1a and EG27-1b, are sequences acquired from regions flanking the candidate disease resistance gene contained in G27-1-1. These two resistance gene candidate flanking (RGCF) markers detected RFLP bands that mapped near or cosegregated with *Rsv*1 and *Rpv*1. GG27-1a was used to identify RGC clones from a lambda genomic library, and three additional RGCF probes were generated to map these clones. The objectives of this study were (i) to determine the molecular linkage relationship between *Rsv*1 and *Rpv*1, and (ii) to saturate the *Rsv*1 and *Rpv*1 chromosomal region with RGC and RGCF markers.

## Materials and Methods

Genetic Materials

A cross between PI 96983 (*Rsv*1, *Rpv*1) x Lee 68 (*rsv*1, *rpv*1) was made in the field. In the winter of 1996, six $F_1$ plants were grown in the greenhouse and were selfed to produce $F_2$ seeds. $F_2$ plants (813) were grown in Blacksburg and Warsaw, VA during 1997. Seeds from each $F_2$ plant were harvested to form $F_{2:3}$ lines.

The new $F_2$ (1056 individuals) mapping population was developed by combining the original $F_2$ (243 individuals) population from PI 96983 x Lee 68 (Yu et al., 1996) with the additional $F_2$ (813 individuals) population. Ninety-one of the additional 813 $F_2$ lines carry a chromosome that has undergone recombination within the region between flanking simple sequence repeat (SSR) marker loci, Hsp176 and Sat120. These recombinants were used for high-resolution mapping.

Williams 82, York, Buffalo, Ogden, Marshall, CNS, L81-4420, and PI 507389 were additionally used in RGCF hybridization experiments. L81-4420 is a near-isogenic line (NIL) of the cv. Williams carrying *Rsv*1 and *Rpv*1 derived from PI 96983.

Virus Screening

Progeny tests were done to determine the *Rsv*1 and *Rpv*1 genotype for each recombinant $F_2$ line. From each $F_{2:3}$ line, 15-20 10-day-old seedlings were inoculated with SMV-G1 (strain G1, VA isolate). An additional 15 to 20 10-day-old seedlings from each $F_{2:3}$ line were inoculated with PMV-P1 (Bays et al., 1986). The inoculation procedure was conducted as described by Hunst and Tolin (1982) and Shipe et al. (1979) for SMV-G1 and PMV-P1, respectively. The first reading of symptoms was recorded 7 days after inoculation. Reactions of individual plants to SMV-G1 and PMV-P1 were recorded as resistant (symptomless), susceptible (mosaic symptoms), or necrotic (necrosis). Three additional observations were made at 1-week intervals following the initial reading.

Based on the SMV reaction of the $F_{2:3}$ lines, each $F_2$ individual was categorized as homozygous resistant (*Rsv*1*Rsv*1), heterozygous (*Rsv*1*rsv*1), or homozygous susceptible (*rsv*1*rsv*1). Each $F_2$ individual was also categorized as homozygous resistant (*Rpv*1*Rpv*1), heterozygous (*Rpv*1*rpv*1), or homozygous susceptible (*rpv*1*rpv*1), according to the PMV reaction of the $F_{2:3}$ lines. SMV-G1 and PMV-P1 were propagated separately on the susceptible soybean cv. Lee 68 in the greenhouse. PI 96983, Lee 68, and a set of soybean differentials (York, Marshall, CNS, L29, and PI 507389) were inoculated to confirm the effectiveness of inoculation and to verify the identities of the SMV and PMV strains. Transfers of virus to host seedlings was performed every 2-3 weeks.

DNA Extraction Procedure

Two-week-old trifoliolate leaves from field-grown $F_2$ plants were cut as tissue samples for DNA extraction. An equivalent amount of plant tissue (0.3-0.5 g) was taken individually from each $F_2$ plant. In 1997, the tissue was placed on dry ice and transported from Warsaw and Blacksburg, VA to the laboratory where it was stored at -70 °C. DNA samples from the 813 additional $F_2$ lines were isolated according to the protocol described previously by Doyle and Doyle (1987), with slight modification. Leaf tissue (0.3-0.5 g fresh weight) was removed from the -70 °C freezer, and immediately placed into a mortar with 20 ml liquid nitrogen. The tissue was ground into a fine powder and 5-10 ml of extraction buffer [0.1 M Tris, pH 8.0/1.4 M NaCl/0.02 M EDTA/2% hexadecyltrimethyammonium bromide (CTAB)/1% β-mercaptoethanol] was added to the mortar for further grinding. All of the ground tissue was transferred to a 15 ml Falcon centrifuge tube and incubated for 2 h in a 60 °C water bath. After incubation, 10 ml chloroform/isoamyl alcohol 24:1 was added to each sample. The samples were mixed by manually inverting tubes, and then placing them horizontally on a shaker for 15 min. The samples were centrifuged at 3200 rpm at 4 °C for 15 min according to Saghai Maroof et al. (1984). The upper aqueous phase was transferred to a second tube with 10 µl (10 mg/ml) RNase A. After 30 min, 2/3 volume of isopropanol was added and mixed by inversion. A glass hook was used to transfer the precipitated pellet of DNA into a glass tube containing 20 ml of 76% ethanol/10 mM $NH_4OAc$. After incubating overnight, the DNA pellet was dried and then dissolved in 1 ml of 10 mM $NH_4OAc$/0.25 mM EDTA. The DNA concentration of each $F_2$ DNA sample was measured with a fluorometer. The quality of the extracted DNA was observed on a 1% agarose gel,

after staining with ethidium bromide. The $F_2$ DNA was subsequently analyzed with SSR markers: Hsp176, Sat120, and 64-A8C.

Two week-old trifoliolate leaf tissue was collected and bulked from 15-20 plants for each $F_{2:3}$ individual. The $F_{2:3}$ tissue was carried to the laboratory from the greenhouse in a similar fashion. DNA samples from parental and $F_{2:3}$ lines were extracted from powdered freeze-dried tissue with CTAB extraction buffer, adhering to the protocol described previously by Saghai Maroof et al. (1984). The $F_{2:3}$ DNA was subsequently analyzed with 27 molecular markers.

RFLP Markers

Generation of D3, GG27-1a, and EG27-1b

Resistance gene candidate (RGC) cDNA clone L61-95-B8 encodes a putative protein product with high sequence similarities to disease resistance genes having NBS and LRR domains. The cDNA clone was identified from an L81-4420 cDNA library using another RGC (NBS61) as a probe. L61-95-B8 was used as a probe to isolate a 22 kb genomic lambda clone (G27-1-1) that contains a candidate disease resistance gene from a Williams 82 lambda genomic library. G27-1a (7 kb) and G27-1b (15 kb) were generated from the digestion of the G27-1-1 insert with *Sst*I. Both fragments were subcloned into pBluescript K/S (-) II (Stratagene, La Jolla, CA). Dominate RFLP marker D3 was derived from G27-1b. Resistance gene candidate flanking (RGCF) probes GG27-1a and EG27-1b were generated from regions flanking the candidate disease resistance gene identified in G27-1-1. GG27-1a is a 1500 bp probe that was isolated by digesting the G27-1a insert with *Dde*I. EG27-1b is a 800 bp probe that was isolated by double digesting G27-1b with *Hin*fI and *Sst*I (Saghai Maroof, unpublished data).

Generation of RGCF probes: 3gG2SP, T3G, and 3T3G1

GG27-1a identified two highly similar RGC clones (3gG2 and 5gG3) from a genomic library made from leaf DNA of the Williams NIL, L81-4420. T3G and 3gG2SP both were generated from 3gG2, and flank a region of 3gG2 that has homology to previously cloned NBS-LRR type disease resistance genes. Analysis of the sequencing information from 3gG2 and 5gG3 determined that a 586 bp insertion deletion polymorphism (IDP) exists between these two RGC clones. A 586 bp insertion is present in 3gG2 and absent from 5gG3. The 586 bp IDP was amplified from 3gG2 to generate 3gG2SP. T3G (1.8 kb) was isolated by PCR amplification of 3gG2. The genomic clone T3G1 was identified from screening an L81-4420 genomic library using T3G. RGCF marker 3T3G1 (1.8 kb) was an isolated restriction fragment of *Sst*I-digested T3G1 (Saghai Maroof, unpublished data). Dr. Randy Shoemaker (ISU/USDA/ARS) kindly provided additional framework soybean RFLP clones.

RFLP Analysis

RFLP analysis was performed according to previously published procedures (Yu et al., 1994). In summary, 8 μg of parental and $F_{2:3}$ DNA was individually digested with each of seven restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Bgl*II, *Ase*I, *Bam*HI, and *Xba*I) for 16-18 h at 37 °C. The digestion was electrophoresed on 1% agarose gels with 1x TAE (Tris-acetate, EDTA) buffer at 70-95 mAmps for 12-18 h or 24-30 h, followed by DNA transfer to Hybond-N+ nylon membranes (Amersham, Piscataway, NJ) via Southern blotting with 0.4 N NaOH. Blots were hybridized overnight at 65 °C with 25 ng/blot of probe DNA, labeled with randomly primed [$\alpha$-$^{32}$P]dCTP (Ambion, Austin, TX). After hybridization, blots were washed twice for 5 min at room temperature with 2x SSC and 0.5x SDS. Subsequently, the blots were washed twice for 10 min at 65 °C with 0.5x SSC and 0.1x SDS. The blots were then exposed to Kodak (New Haven, CT) Xomat film with intensifying screens at –70 °C for 3-7 days.

SSR Analysis

The SSR procedures were as described by Yu et al. (1994). Briefly, a 20 μl PCR reaction contained 50 ng of parental, $F_2$, or $F_{2:3}$ genomic DNA, 1x reaction buffer (20 mM Tris, pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.1 μM of each primer, 1.0 U of *Taq* DNA polymerase (Gibco-BRL Life Technologies), 160 μM each of dGTP, dTTP, and dATP, 4 μM dCTP, and 1 μM [$\alpha$-$^{32}$P]dCTP. The reaction mixture was denatured at 94 °C for 3 min, followed by 32 cycles at 94 °C for 30 sec, 47 °C for 30 sec, and 68 °C for 1 min, with a final extension step of 68 °C for 7 min. After PCR 16 μl of stop solution

containing 98% deionized formamide, 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 10 mM EDTA (pH 8.0) was added to each sample. PCR products were denatured at 94 °C for 10 min. Five µl of each sample was loaded on a 7 M urea, 6% denaturing polyacrylamide gel at 1200-V constant power in 1x TBE (Tris- borate, EDTA) running buffer for 2-3 h, using a DNA sequencing unit (Model STS-45, IBI, New Haven, CT). Gels were covered with plastic wrap and exposed to Kodak (New Haven, CT) Xomat film for 1-1.5 h. Perry Cregan (USDA/ARS) and Roger Innes kindly provided additional primer sequences used in this study.

Randomly Amplified Polymorphic DNA (RAPD) Analysis

RAPD analysis was performed according to previously published procedures (Williams et al., 1990). PCR amplification was performed in a 25 µl reaction containing 25 ng of parental or $F_{2:3}$ genomic DNA, 0.2 mM primer (OPN O-11, Operon Technologies Inc., Alameda, CA), 200 µM each of dGTP, dTTP, dATP, and dCTP, 2.5 mM $MgCl_2$, 1x reaction buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), and 1.0 U of Taq DNA polymerase (Gibco-BRL Life Technologies). The reaction mixture was denatured at 94 °C for 3 min, followed by 42 cycles at 94 °C for 1 min, 37 °C for 1 min, and 72 °C for 2 min, with a final extension step of 72 °C for 3 min. PCR products were separated on 3% agarose gels, and then stained with ethidium bromide and visualized under ultraviolet light.

Linkage Analysis

The most probable order and map distances were ascertained by multiple linkage analysis with the computer program Mapmaker 3.0 at LOD=3.0 and a maximum Haldane distance of 50 cM (Lander et al., 1987; Lincoln and Lander, 1993).

## Results

Selection of lines with recombination between flanking SSRs

Marker-based selection was employed to identify informative lines with a cross-over (or recombination) in the chromosomal region of the disease resistance gene cluster on MLG-F. In the original population, SSR marker Hsp176 mapped to one side of *Rsv*1 (3.1 cM), and 64-A8C and Sat120 to the other side of *Rsv*1 (0.2 and 3.7 cM, respectively; Fig. 1). Hsp176 mapped above K644 (1.2 cM), and Sat120 below B212 (2.1 cM) in the original population (Saghai Maroof, unpublished data); therefore, these SSR markers flank the disease resistance gene cluster on MLG-F of soybean. Hsp176, 64-A8C, and Sat120 were scored in the 813 additional individuals. With these SSR markers we identified 91 recombinant lines (RLs) exhibiting a crossover between two of the three *Rsv*1 flanking markers. The genotype of each of the 722 non-RLs entire chromosomal interval between Hsp176 and Sat120 was scored identically as their three SSRs. For example, for a given $F_2$ individual, if Hsp176, 64-A8C, and Sat120 were heterozygous, then the entire chromosomal region between Hsp176 and Sat120 was inferred to be heterozygous.

Mapping framework markers in the 91 RLs

Plants grown from remnant $F_{2:3}$ seed of each of the 91 RLs were scored for both *Rsv*1 and *Rpv*1. Probes and primers that do not have (RFLP) or amplify (RAPD and SSR), respectively, nucleotide sequences specific for disease resistance genes were categorized as framework markers. $F_{2:3}$ DNA samples from each of the 91 RLs were used to map 15 framework markers from MLG-F, which were previously mapped in the original population. Nine RFLP, one RAPD, and five SSR framework markers were employed in the development of a skeletal linkage map, which was later saturated with RGC and RGCF markers. Hsp176, 64-A8C, and Sat120 were again mapped in the 91 RLs to verify the genotyping of the 91 selected $F_2$ recombinants. In the new expanded population of 1,056 individuals, SSR markers Hsp176 and 64-A8C mapped to one side of *Rsv*1 (2.9 and 0.5 cM, respectively), and Sat120 to the other side of *Rsv*1 (3.8 cM; Fig. 2). Hsp176 mapped 1.8 cM away from *Rpv*1, while 64-A8C mapped 0.6 cM away from *Rpv*1. K644 mapped 0.9 cM above *Rpv*1, and B212 1.0 cM below *Rsv*1. RAPD marker, $OPN\text{-}11_{1000}$ (Li et al., 1998), cosegregated with B212. Hayes and Saghai Maroof (2000) have previously described R11, R12, and R13. These three markers possess only the P-loop region associated with the NBS of NBS-LRR type resistance genes. R11 mapped closely to *Rsv*1, whereas both R12 and R13 mapped distantly from *Rsv*1 and *Rpv*1.

Saturation of framework map with RGC markers

RFLP probes NBS5 and NBS61 encompass nucleotide sequences with partial homology to previously cloned NBS-LRR type disease resistance genes (Yu et al., 1996). NBS5 is a class *b* NBS clone that has high homology to TIR-NBS-LRR disease resistance genes. NBS61 is a class *j* NBS clone that is most homologous to an NBS-LRR disease resistance gene, which lacks a TIR domain. R14 was isolated using an AFLP primer identical to one of two degenerate PCR primers originally used to amplify NBS61. R14 has the P-loop and kinase-2 motifs homologous to other NBS-LRR type resistance genes. Pairwise comparison of DNA sequences from R14 and class *j* member, NBS61, determined they are 75% similar, but R14 is only 28% similar to class *b* member, NBS5 (Hayes and Saghai Maroof, 2000). Additional non-TIR-NBS (NTN) members of soybean NBS class *j* family were isolated from PI 96983 via primers designed from the sequences of NBS61 and R14 (Jeong et al., in preparation). Three NTN clones: NTN1, NTN5, and NTN8 contain sequences homologous to NBS61, and collectively the three probes detected six loci that mapped within a 1.7 cM region around *Rsv*1 and *Rpv*1 (Fig. 2). R14 and NTN5 have identical sequence, and show the same RFLP banding pattern when hybridized to a parental Southern blot (Saghai Maroof, unpublished data). Z40a is a clone with candidate resistance gene sequences homologous to members of NBS class *j* family, and was identified from a Williams 82 (soybean cultivar without *Rsv*1 and *Rpv*1) cDNA library. All of these NBS class *b* and *j* markers mapped near *Rsv*1, but most notably the locus NTN1d cosegregated with *Rpv*1.

Mapping GG27-1a and EG27-1b

RFLP probes GG27-1a and EG27-1b were generated from a 22 kb lambda genomic clone. The 22 kb lambda genomic clone was identified from screening a Williams 82 library with a soybean cDNA clone (L61-95-B8), which has a nucleotide sequence with close homology to NBS61. Analysis of GG27-1a and EG27-1b determined they both are from regions flanking a candidate disease resistance gene (Saghai Maroof, unpublished data). The resistance gene candidate flanking (RGCF) probes GG27-1a and EG27-1b, detected seven and four polymorphic RFLP bands, respectively, that mapped within a 1.0 cM region around *Rsv*1 and *Rpv*1 (Fig. 2). The RFLP bands detected by the two probes were scored dominantly, because of the ambiguity in associating allelic bands. Most notably, GG27.1aA, GG27.1aB, and EG27.1bA cosegregated with *Rpv*1, and GG27.1aE and GG27.1aF mapped 0.1 cM from *Rsv*1.

Mapping RGCF markers related to 3gG2 and 5gG3

RGC clones 3gG2 and 5gG3 were identified from library screening with GG27-1a, and encode putative protein products with high sequence homology to NBS-LRR type disease resistance genes (Saghai Maroof, unpublished data). RGCF probes T3G and 3T3G1 were generated to assist in identifying map positions of the two RGC clones. T3G was amplified from a region flanking the putative protein product within 3gG2. 3T3G1 is a restriction fragment isolated from a digested genomic clone (T3G1). T3G1 was identified from a lambda genomic library using T3G as a probe. T3G and *Rsv*1 both cosegregated, and mapped 0.1 cM from 3T3G1.

The PI 96983 RFLP band, GG27.1aF, detected by GG27-1a corresponds to a portion of the unique but highly similar 3gG2 and 5gG3 RGC clones. 3gG2 and 5gG3 have two conserved *Hin*dIII restriction sites which, upon digestion, generate 4.8 kb *Hin*dIII restriction fragments (HRFs). GG27-1a hybridized to the 4.8 kb HRF from both 3gG2 and 5gG3. GG27-1a detected a 4.8 kb RFLP band in *Hin*dIII-digested PI 96983 genomic DNA that was scored as GG27.1aF. Therefore, GG27.1aF was actually two co-migrating 4.8 kb HRFs representative of the RGCs, 5gG3 and 3gG2. An approach was developed to map 3gG2 and 5gG3 separately. A 586 bp insertion that is present in 3gG2 is not present in 5gG3. The 586 bp IDP was selectively amplified from 3gG2 to generate the RGCF probe, 3gG2SP. 3gG2SP selectively hybridized only to 3gG2-related sequences, because the probe detected only HRFs with the 586 bp IDP. 3gG2SP detected only a subset of the four RFLP bands detected by GG27-1a in PI 96983, when hybridized to *Hin*dIII-digested PI 96983 genomic DNA (Saghai Maroof, unpublished data). 3gG2SP hybridized two HRFs that were detected in PI 96983 and Lee 68, when GG27-1a was used as an RFLP probe, 3gG2SPa (GG27.1aC) and 3gG2SPb (GG27.1aD). 3gG2SP detected the 4.8 kb HRF specific to 3gG2, while the other 5gG3-related co-migrating 4.8 kb HRF was not hybridized because it lacked the 586 bp IDP. The 3gG2 specific 4.8 kb RFLP band was scored as 3gG2SPc and cosegregated with *Rsv*1. RL 943 was the only individual with RFLP band GG27.1aF without the presence of 3gG2SPc. The three 3gG2-related bands detected by 3gG2SP were scored dominantly, because of the ambiguity in associating allelic bands. RFLP

bands, GG27.1aG, GG27.1aE, GG27.1aB, and GG27.1aA are apparently 5gG3 related because 3gG2SP did not detect them.

Evaluation of conflicting SMV-G1 disease reaction and molecular marker data

The 91 RLs were screened with SMV-G1 and PMV-P1 to determine their genotype for *Rsv*1 and *Rpv*1, respectively. The computer program Mapmaker was used to calculate linkage relationships between *Rsv*1, *Rpv*1, and markers from MLG-F. The map position of *Rsv*1 in the new population contradicted its map position in the original population (Figs. 1 and 2). In the original population, *Rsv*1 mapped above cosegregating markers 64-A8C and php2385 (0.2 cM), and below K644 (1.9 cM); therefore, *Rsv*1 should map between these flanking marker loci in the new population, but instead it mapped below 64-A8C and php2385 (0.5 and 0.1, respectively). Nineteen RLs with crossovers between *Rsv*1 and *Rpv*1 were most informative for calculating the map position of these two disease resistance loci (Fig. 3). Among the 19 RLs, Mapmaker designated the *Rsv*1 genotype of RLs 128, 248, 647, 938, 943, 957, and 982 as candidate errors, since the placement of *Rsv*1 below php2385 conflicted with the genotypes of their marker data (Table 1). The disease reaction and marker data from these seven RLs suggested that *Rsv*1 should be placed above 64-A8C and php2385, similar to the placement of *Rsv*1 in the original population. Assuming that the *Rsv*1 locus is a single dominant gene and maps 0.1 cM below php2385, RLs 647, 938, 943, and 982 would be homozygous susceptible at the *Rsv*1 locus (based on markers), but instead are heterozygous for SMV-G1 resistance (Fig. 3; Table 2). Compared to other RLs, a high percentage (>35%) of plants grown from remnant $F_{2:3}$ seed of RLs 647, 938, 943, and 982 developed necrotic symptoms after inoculation with SMV-G1 (Fig. 4; Table 2), and these four RLs are segregating for SMV-G1 resistance. In addition, RLs 128, 248, and 957 would be heterozygous at the *Rsv*1 locus (based on markers), but are homozygous resistant to SMV-G1. Necrotic plants were not observed in the progeny from these three RLs.

Analysis of SMV and PMV differential cultivars and breeding lines with GG27-1a and 3gG2SP

PI 96983 and York have resistance to PMV controlled by different alleles at the *Rpv*1 locus (Roane et al., 1983; Bagade, 1998), while Ogden, Marshall, PI 507.389, Williams 82 and Lee 68 are susceptible to PMV (Table 4). CNS has resistance to PMV mediated by *Rpv*3. The chromosomal location of the PMV resistance gene in Buffalo is unknown. L81-4420, a NIL of Williams, carries *Rpv*1 derived from PI 96983. These nine cultivars and one NIL were evaluated with RGCF probe, GG27-1a (Fig. 5). The RFLP bands, GG27.1aB (1.5 kb) and GG27.1aA (1.2 kb) were detected in PI 96983 and Lee 68 by GG27-1a, respectively, and both cosegregated with *Rpv*1. GG27-1a also hybridized to a 1.5 kb HRF in L81-4420, York, Buffalo, and CNS, and to a 1.2 kb HRF in Williams 82 and PI 507.389. The 1.5 kb HRF exclusively was present in cultivars that have resistance to PMV, and the 1.2 kb HRF solely was seen in cultivars that are susceptible to PMV. Neither Ogden nor Marshall had the 1.5 or 1.2 kb HRF, but apparently both possess a susceptibility allele different from other PMV susceptible cultivars. CNS has resistance to PMV at the *Rpv*3 locus, but had the same 1.5 kb HRF as cultivars with *Rpv*1. The RFLP band, GG27.1aG, only was detected in cultivars that have resistance to PMV, with the exception of York.

PI 96983, York, Buffalo, Ogden, and Marshall are resistant to various SMV strains conferred by different alleles at the *Rsv*1 locus (Table 4 and 5), while CNS, Williams 82, and Lee 68 are susceptible to all strains of SMV. PI 507.389 carries an allele of *Rsv*1, and becomes necrotic when infected with SMV-G1. L81-4420 has resistance to SMV at the *Rsv*1 locus derived form PI 96983. RGCF probe, 3gG2SP, was used to evaluate these nine cultivars and one NIL (Fig. 6). The RFLP band, 3gG2SPc (4.8 kb), was detected in PI 96983 by 3gG2SP, and cosegregated with *Rsv*1. A 4.8 kb HRF was hybridized in L81-4420, Buffalo, Ogden, and Marshall by 3gG2SP. The 4.8 kb HRF only was present in cultivars with resistance to SMV. In addition, 3gG2SP detected the RFLP bands 3gG2SPa (2.5 kb) in PI 96983, and 3gG2SPb (2.7 kb) in Lee 68. Additionally, GG27-1a detected a 2.7 kb HRF in Williams 82, Buffalo, Ogden, and PI 507.389. A 2.5 kb HRF was hybridized by GG27-1a in all cultivars, with the exception of Lee 68, York, and PI 507.389. The 2.7 kb HRF detected in PI 96983 probably is not associated with SMV resistance, because it also was present in SMV susceptible cultivars, Williams 82 and CNS.

## Discussion

Mapping of *Rsv*1 and *Rpv*1

The *Rpv*1 locus mapped to a chromosomal region flanked by RFLP markers K644 and B212 of soybean MLG-F that consists of a cluster of previously described disease resistance genes, conferring resistance to bacterial, viral, fungal, nematode, and insect phytopathogens (Diers et al., 1992; Yu et al.,

1994; Ashfield et al., 1996; Tamulonis et al., 1997a; Tamulonis et al., 1997b; Rector et al., 1999). Past resistance-gene-mapping studies involving members of this cluster have only scored a lone qualitative or quantitative resistance trait. In this study, we have mapped two virus resistance genes in a single mapping population of 1,056 $F_2$ individuals, and determined *Rsv*1 and *Rpv*1 have a linkage of 1.1 cM. This research not only confirmed the linkage relationship of *Rsv*1 and *Rpv*1 observed in a previous inheritance study (Roane et al., 1983), but also provided the linkage relationship of these two resistance genes with many RGC and RGCF markers.

Utility of a large mapping population

The map position of *Rsv*1 was placed between flanking SSR marker loci Hsp176, 64-A8C, and Sat120. These markers identified 91 individuals with a recombination between SSR marker loci Hsp176 and Sat120 from the additional 813 individuals. This marker-based selection method allowed the successful construction of a high-resolution map with 27 molecular markers that map no further than 4.9 cM from either *Rsv*1 or *Rpv*1. Previous researchers have used a similar marker-based selection approach to construct a high-resolution map with selected recombinant individuals from a large segregating population (Meksem et al., 1995; Bendahmane et al., 1997). Additionally, the large mapping population increased the probability of obtaining a few individuals in which recombination had occurred near *Rsv*1 and *Rpv*1. In the original population, three individuals possessed a crossover in the chromosomal region between *Rsv*1 and *Rpv*1, while the additional 813 individuals provided 16 more individuals with a recombination between *Rsv*1 and *Rpv*1.

The high-resolution map saturated with RGC and RGCF markers is a valuable asset for cloning *Rsv*1, *Rpv*1, and other multigene family members. A high-resolution map has been utilized in map-based cloning strategies to successfully clone disease resistance genes from plants (Martin et al., 1993; Bent et al., 1994; Mindrinos et al., 1994). Martin et al. (1993) selected an RFLP marker from a high-resolution linkage map that cosegregated with the *Pto* locus on chromosome 5 of tomato, and used the RFLP marker to identify a YAC clone containing the *Pto* gene. In this study, the high-resolution map developed from the large population helped to determine the map position of RGC clones, 3gG2 and 5gG3. In addition, the generation of RGCF probes from 3gG2 further refined the resolution of the map, and ruled out the possibility of 5gG3 being *Rsv*1.

The tight linkage among members of the resistance gene cluster on MLG-F impedes the ability to separate individual genes for map-based cloning purposes. Constructing a high-resolution map from the expanded population allowed previously cosegregating marker loci detected by class *j* NBS probes to be separated from their tightly linked family members. In the expanded population, NTN1b and NTN1c both cosegregated and mapped 0.2 cM away from NBS61, but the three marker loci cosegregated in the original population. NTN8 and NTN1a cosegregated in the original population, but mapped 0.4 cM away from each other in the expanded population. The six unique loci detected by class *j* clones appear to be members of the NBS61 gene family, considering their shared close distances to one another and high sequence similarities. In addition, each clone hybridized to multiple bands on a parental RFLP Southern blot. R14 (NTN5) and NBS5 cosegregated in both populations, which indicates there is a tight linkage between NTN and TIR-NBS sequences on MLG-F of soybean. The clustering of NTN and TIR-NBS sequences has also been observed to exist on chromosome five of *Arabidopsis* (Meyers et al., 1999).

Evidence of a new SMV resistance gene

In 1979, Kiihl and Hartwig discovered that an allele at a single locus, *Rsv*1, mediated resistance to SMV in PI 96983. Yu et al. (1994) mapped molecular markers in a $F_2$ population (PI 96983 x Lee 68) segregating for SMV resistance at the *Rsv*1 locus, and the data indicated a single, dominant gene controlled SMV resistance in PI 96983. The population used to map the *Rsv*1 locus by Yu et al. (1994, 1996) and Hayes and Saghai Maroof (2000) was not large enough to rule out the possibility of other tightly linked genes providing SMV resistance. Taken together, the marker and disease reaction data from this study indicated that disease resistance to SMV-G1 in PI 96983 was conferred by two closely linked loci. First, mapping *Rsv*1 and *Rpv*1 in the large population has allowed the designation of a new map position for *Rsv*1, and identification of seven RLs that had a *Rsv*1 genotype not in concordance with their molecular marker data. Second, a high percentage (>34%) of necrotic plants was observed among plants grown from remnant $F_{2:3}$ seed of four of these seven RLs, thus suggesting a gene other than *Rsv*1 contributed to their necrotic response. An attempt was made to map the other SMV resistance gene (hereafter designated as *Rsv*1b) using Mapmaker. *Rsv*1 (hereafter designated as *Rsv*1a) in the heterozygous state prohibited the

genotype of *Rsv*1b to be elucidated based on the disease reaction data for RLs 613, 840, 937, and 1044, because *Rsv*1a masked the action of *Rsv*1b by conferring SMV resistance. These four RLs were assigned a *Rsv*1b genotype of C (B-Lee 68 susceptible allele or H-heterozygous) for the purpose of mapping *Rsv*1b with Mapmaker (Table 6). RLs 647, 938, 943, and 982 had a homozygous susceptible genotype for *Rsv*1a based on the position of recombination inferred from the marker data. *Rsv*1b was heterozygous for these four lines, because the SMV-G1 disease reaction data from RLs 647, 938, 943, and 982 indicated they were segregating for SMV resistance. The *Rsv*1b genotype for each of the other RLs (except 1044) was the same as their *Rsv*1a genotype. The *Rsv*1b locus mapped 0.1 cM below *Rpv*1, and 1.0 cM above *Rsv*1a.

The results of a study conducted by Chen et al. (1991) suggested that soybean individuals heterozygous at the *Rsv*1 locus developed necrosis from SMV infection due to incomplete dominance of the SMV resistance gene. It is possible that the existence of necrotic plants from six RLs was attributed to the SMV resistance response evoked solely by either *Rsv*1a or *Rsv*1b in the heterozygous state. RLs 647, 938, 943, and 982 were heterozygous for *Rsv*1b and homozygous susceptible for *Rsv*1a. RLs 709 and 896 were heterozygous for *Rsv*1a and homozygous susceptible for *Rsv*1b. The percentage of necrotic plants from RLs 647, 938, 943, and 982 was greater than 34%, but 12% or less for RLs 709 and 896. *Rsv*1a maybe functionally stronger than *Rsv*1b, as indicated by the lower number of necrotic plants from RLs 709 and 896. It appears that *Rsv*1a and *Rsv*1b exhibited incomplete dominance when they were heterozygous, thus allowing necrosis and virus to spread throughout the infected plant.

The *Rsv*1b genotype of RL 1044 was either heterozygous or homozygous susceptible, because *Rsv*1a masked the action of *Rsv*1b. The hypothetical recombination breakpoint of RL 1044 was identical to RLs 613, 840, and 937, but SMV-G1 only caused necrosis in plants grown from RL 1044. Therefore, it is possible that *Rsv*1b was homozygous susceptible in RL 1044, while heterozygous in RLs 613, 840, and 937. There was a higher percentage of necrotic plants from RL 1044, compared to RLs 709 and 896, which also were heterozygous for *Rsv*1a and homozygous susceptible for *Rsv*1b. Most likely the higher number of necrotic plants from RL 1044 was due to variation in sampling or another unidentified resistance gene contributed to the necrotic response in RL 1044. There were either one or two necrotic $F_{2:3}$ plants observed from RLs 640, 685, 723, and 758, and *Rsv*1a and *Rsv*1b had a heterozygous genotype in these four RLs (data not shown). Therefore, these necrotic plants could have resulted from the SMV resistance response exclusively mediated by *Rsv*1a or *Rsv*1b in the heterozygous state. Necrosis was not seen among plants grown from each of 34 other RLs heterozygous for both *Rsv*1a and *Rsv*1b. It seems unusual that only 11 RLs had plants with necrosis, but Chen et al. (1991) also observed the frequency of necrotic plants induced by SMV-G1 among crosses with PI 96983 to be lower, compared to crosses with other cultivars. This phenomenon might be explained by the paucity of individuals with *Rsv*1a heterozygous and *Rsv*1b homozygous susceptible, or vice versa. The necrotic plants induced by PMV-P1 from RL 943 may have resulted from an environmental effect, or an unusual mutation in RL 943 that causes a high level of necrosis when inoculated with SMV-G1 or PMV-P1. One necrotic plant infected with PMV-P1 was observed among progeny from each of eight different RLs that were segregating or homozygous resistant to PMV-P1 (data not shown).

It seems logical to develop homozygous lines for each of these eight unusual RLs. Crossing a line homozygous resistant for *Rsv*1b or *Rsv*1a and homozygous susceptible for *Rsv*1a or *Rsv*1b with Lee 68 could provide insight as to why necrosis was observed in these lines. Additionally, screening lines homozygous resistant for either *Rsv*1a or *Rsv*1b with different strains of SMV will give insight into which locus provides the specific resistance response.

Are there more members of the *Rsv*1 complex waiting to be discovered? The existence of linked, functionally identical genes has been observed in other plant species. The complex *Rp*1 locus of maize consists of multiple linked *Rp* genes that confer resistance to different races of the rust fungus *Puccinia sorghi* (Saxena and Hooker, 1968; Hulbert, 1997). At least ten downy mildew resistance genes have been observed at the *Dm*3 locus of lettuce (Meyers et al., 1998). The 19 RLs with recombination between *Rpv*1 and *Rsv*1a should be screened with various strains of SMV to detect other specificities. The five SMV-G1 resistant cultivars analyzed with GG27-1a and 3gG2SP have different disease reactions to strains G2-G7 of SMV (Table 5). All five of these cultivars have alleles at the *Rsv*1 locus. The variation in disease reaction to SMV strains G2-G7 among these five cultivars may be due to the presence or lack of other *Rsv*1 multigene family members, as indicated by the variation of the RFLP patterns detected by RGCFs, GG27-1a and 3gG2SP. Analysis of the nine cultivars and L81-4420 with GG27-1a and 3gG2SP strongly indicates that the two HRF, designated as, 3gG2SPc and GG27.1aB, are regions flanking the *Rsv*1a and *Rpv*1 loci, respectively. GG27-1a and 3gG2SP should be used to screen a soybean bacterial artificial chromosome

(BAC) library, and then construct a physical map with the selected clones. Subsequent studies would be to transform full-length RGC clones into susceptible soybean cultivars. High-resolution and physical mapping of select BAC clones will allow in-depth investigation of multigene regions on MLG-F.

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Table 1. Molecular markers and disease reaction data from 19 of 1,056 $F_2$ individuals with a recombination between *Rsv*1 and *Rpv*1 from a cross between PI 96983 (*Rsv*1 and *Rpv*1) and Lee 68 (*rsv*1 and *rpv*1).

| | | Marker Genotypes | | | |
|---|---|---|---|---|---|
| **$F_{2:3}$ no.** | **K644** | **64-A8C** | **php2385** | ***Rsv*1** | **Candidate error [a]** |
| 128 | A[+] | H | H | A | Yes |
| 161 | H[++] | A | A | A | No |
| 241 | H | A | A | A | No |
| 248 | A | A | H | A | Yes |
| 613 | B[+++] | H | H | H | No |
| 647 | H | H | B | H | Yes |
| 709 | B | B | H | H | No |
| 758 | A | H | H | H | No |
| 800 | H | H | A | A | No |
| 840 | B | H | H | H | No |
| 867 | H | A | A | A | No |
| 896 | B | B | H | H | No |
| 937 | B | H | H | H | No |
| 938 | H | B | B | H | Yes |
| 943 | H | H | B | H | Yes |
| 957 | A | A | H | A | Yes |
| 966 | H | A | A | A | No |
| 982 | H | B | B | H | Yes |
| 1044 | B | H | H | H | No |

[+]A, PI 96983 allele – Homozygous Genotypes
[++]H, Heterozygous Genotypes
[+++]B, Lee 68 allele – Homozygous Genotypes

a. Mapmaker designated the *Rsv*1 genotype of 128, 248, 647, 938, 943, 957, and 982 as candidate errors, since their *Rsv*1 genotype did not agree with the marker data. Mapmaker placed *Rsv*1 0.1 cM below php2385, but the *Rsv*1 genotype of the seven RLs suggests it is between *Rpv*1 and 64-A8C.

Table 2. SMV disease reaction data from $F_{2:3}$ families derived from $F_2$ individuals that exhibited recombination between *Rsv*1 and *Rpv*1 from a cross between PI 96983 (*Rsv*1 and *Rpv*1) and Lee 68 (*rsv*1 and *rpv*1).

| | No. of plants | | | | | | |
|---|---|---|---|---|---|---|---|
| **$F_{2:3}$ no.** | $R^+$ | **N[b]** | **S** | **Total** | **% N** | **Segregation of $F_{2:3}$ families for SMV resistance** | **No. times screened** |
| 128[a] | 18 | 0 | 0 | 18 | 0 | All R | 1 |
| 161 | 19 | 0 | 0 | 19 | 0 | All R | 1 |
| 241 | 20 | 0 | 0 | 20 | 0 | All R | 1 |
| 248 | 12 | 0 | 0 | 12 | 0 | All R | 1 |
| 613 | 16 | 0 | 3 | 19 | 0 | 3(R+N):1S | 1 |
| 647 | 7 | 9 | 10 | 26 | 35 | 3(R+N):1S | 2 |
| 709 | 30 | 3 | 4 | 37 | 8 | 3(R+N):1S | 2 |
| 758 | 13 | 1 | 5 | 19 | 5 | 3(R+N):1S | 1 |
| 800 | 43 | 0 | 0 | 43 | 0 | All R | 3 |
| 840 | 22 | 0 | 5 | 27 | 0 | 3(R+N):1S | 2 |
| 867 | 35 | 0 | 0 | 35 | 0 | All R | 2 |
| 896 | 24 | 4 | 5 | 33 | 12 | 3(R+N):1S | 2 |
| 937 | 25 | 0 | 9 | 34 | 0 | 3(R+N):1S | 2 |
| 938 | 5 | 7 | 4 | 16 | 44 | 3(R+N):1S | 1 |
| 943 | 4 | 22 | 10 | 36 | 61 | 3(R+N):1S | 2 |
| 957 | 13 | 0 | 0 | 13 | 0 | All R | 1 |
| 966 | 30 | 0 | 0 | 30 | 0 | All R | 2 |
| 982 | 5 | 10 | 7 | 22 | 46 | 3(R+N):1S | 2 |
| 1044 | 13 | 9 | 8 | 30 | 30 | 3(R+N):1S | 2 |

+ R, resistant (symptomless); N, necrotic; S, susceptible (mosaic symptoms)

%N = (no. N plants/no. Total plants)

a. From previous work by Yu et al. (1996).

b. Plants were scored necrotic if necrosis spread to non-inoculated leaves.

Table 3. PMV disease reaction data from $F_{2:3}$ families derived from $F_2$ individuals that exhibited recombination between *Rsv*1 and *Rpv*1 from a cross between PI 96983 (*Rsv*1 and *Rpv*1) and Lee 68 (*rsv*1 and *rpv*1).

| | No. of plants | | | | | | |
|---|---|---|---|---|---|---|---|
| **$F_{2:3}$ no.** | $R^+$ | **N** | **S** | **Total** | **% N** | **Segregation of $F_{2:3}$ families for PMV resistance** | **No. times screened** |
| 128[a] | -[++] | - | - | - | - | All R | - |
| 161 | - | - | - | - | - | 3(R+N):1S | - |
| 241 | - | - | - | - | - | 3(R+N):1S | - |
| 248 | 13 | 0 | 0 | 13 | 0 | All R | 1 |
| 613 | 0 | 0 | 33 | 33 | 0 | All S | 2 |
| 647 | 11 | 0 | 5 | 16 | 0 | 3(R+N):1S | 1 |
| 709 | 0 | 0 | 20 | 20 | 0 | All S | 1 |
| 758 | 31 | 0 | 0 | 31 | 0 | All R | 3 |
| 800 | 12 | 0 | 3 | 15 | 0 | 3(R+N):1S | 1 |
| 840 | 0 | 0 | 18 | 0 | 0 | All S | 1 |
| 867 | 20 | 0 | 5 | 25 | 0 | 3(R+N):1S | 2 |
| 896 | 0 | 0 | 17 | 17 | 0 | All S | 1 |
| 937 | 0 | 0 | 37 | 37 | 0 | All S | 2 |
| 938 | 5 | 0 | 1 | 6 | 0 | 3(R+N):1S | 1 |
| 943 | 10 | 4 | 4 | 18 | 22 | 3(R+N):1S | 1 |
| 957 | 15 | 0 | 0 | 15 | 0 | All R | 1 |
| 966 | 19 | 0 | 10 | 29 | 0 | 3(R+N):1S | 2 |
| 982 | 25 | 0 | 7 | 22 | 0 | 3(R+N):1S | 2 |
| 1044 | 0 | 0 | 34 | 0 | 0 | All S | 2 |

+ R, resistant (symptomless); N, necrotic; S, susceptible (mosaic symptoms)

++ -, not tested

%N = (no N plants/no Total plants)

a. *Rpv*1 genotypes for $F_2$ individuals 128, 161, and 241 were inferred from their molecular marker data.

b. Plants were scored necrotic if necrosis spread to non-inoculated leaves.

Table 4. Differential reactions of soybean cultivars to inoculation with SMV and PMV[a].

| Cultivars Or NIL | SMV-G1 | PMV-P1 |
|---|---|---|
| PI 96983 | $R^{+}$ | R |
| L81-4420 | R | R |
| York | R | R |
| Buffalo | R | R |
| Ogden | R | S |
| Marshall | R | S |
| CNS | $S^{++}$ | R |
| PI 507389 | $N^{+++}$ | S |
| Williams 82 | S | S |
| Lee 68 | S | S |

\+ R, resistant (symptomless)
++ S, susceptible (mosaic)
+++ N, necrotic

a. Buss, unpublished data

Table 5. Disease reaction of differential soybean cultivars to SMV strains[a].

| Cultivar | SMV Resistance Gene | SMV Strain Group | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G7A |
| PI 96983 | *Rsv*1 | $R^{+}$ | R | R | R | R | R | N | S |
| Buffalo | *Rsv*1 | R | R | R | R | R | R | N | $-^{++++}$ |
| Ogden | $Rsv1^{t}$ | R | R | N | R | R | R | N | S |
| Marshall | $Rsv1^{m}$ | R | N | N | R | R | N | N | S |
| York | $Rsv1^{y}$ | R | R | R | N | S | S | S | S |
| PI 507389 | $Rsv1^{n}$ | $N^{++}$ | N | $S^{+++}$ | S | N | N | S | - |

\+ R, resistant (symptomless)
++ N, necrotic
+++ S, susceptible (mosaic)
++++ -, not tested

a. Buss et al., 1989; Buss et al., 1994

Table 6. Genotypes of *Rsv*1a, *Rsv*1b, and *Rpv*1 for the 19 of 1,056 RLs with recombination between *Rsv*1 and *Rpv*1 from a cross between PI 96983 (*Rsv*1 and *Rpv*1) and Lee 68 (*Rsv*1 and *Rpv*1).

| **$F_{2:3}$ no.** | ***Rsv*1a** | ***Rsv*1b** | ***Rpv*1** |
|---|---|---|---|
| 128 | H[+] | A | A |
| 161 | A[++] | A | H |
| 241 | A | A | H |
| 248 | H | A | A |
| 613 | H | C[++++] | B |
| 647 | B[+++] | H | H |
| 709 | H | B | B |
| 758 | H | H | A |
| 800 | A | H | H |
| 840 | H | C | B |
| 867 | A | A | H |
| 896 | H | B | B |
| 937 | H | C | B |
| 938 | B | B | H |
| 957 | H | A | A |
| 966 | A | A | H |
| 982 | B | B | H |
| 1044 | H | C | B |

\+ H, Heterozygous Genotypes: *Rsv*1a/*rsv*1a, *Rsv*1b/*rsv*1b, or *Rpv*1/*rpv*1
++ A, PI 96983 allele - Homozygous Resistant Genotypes: *Rsv*1a/*Rsv*1a, *Rsv*1b/*Rsv*1b, or *Rpv*1/*Rpv*1
+++ B, Lee 68 allele - Homozygous Susceptible Genotypes: *rsv*1a/*rsv*1a, *rsv*1b/*rsv*1b, or *rpv*1/*rpv*1
++++ C, Lee 68 allele or Heterozygous: *Rsv*1b/*rsv*1b, or *rsv*1b/*rsv*1b

a. *Rpv*1 genotypes for lines 128, 161, and 241 were inferred from their molecular marker data.

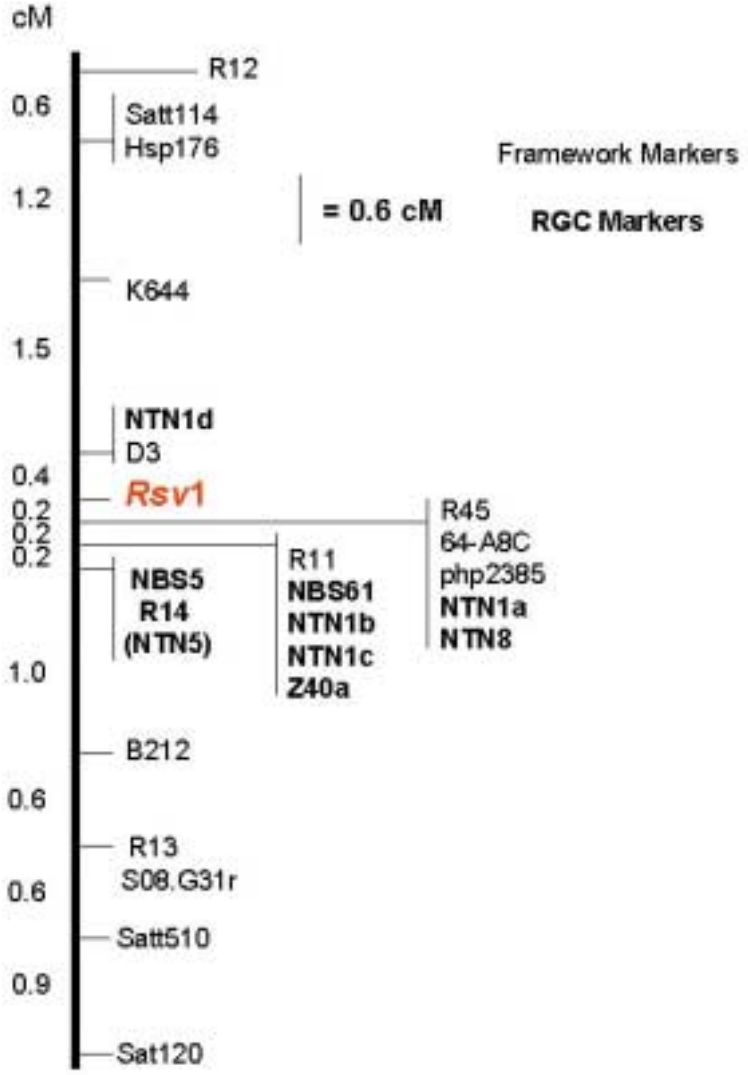


Figure 1. Genetic linkage map of the region around *Rsv*1 on soybean MLG-F based on framework and RGC markers. The map was constructed using the original 243 individual population. Map distances are given in centimorgans (cM).

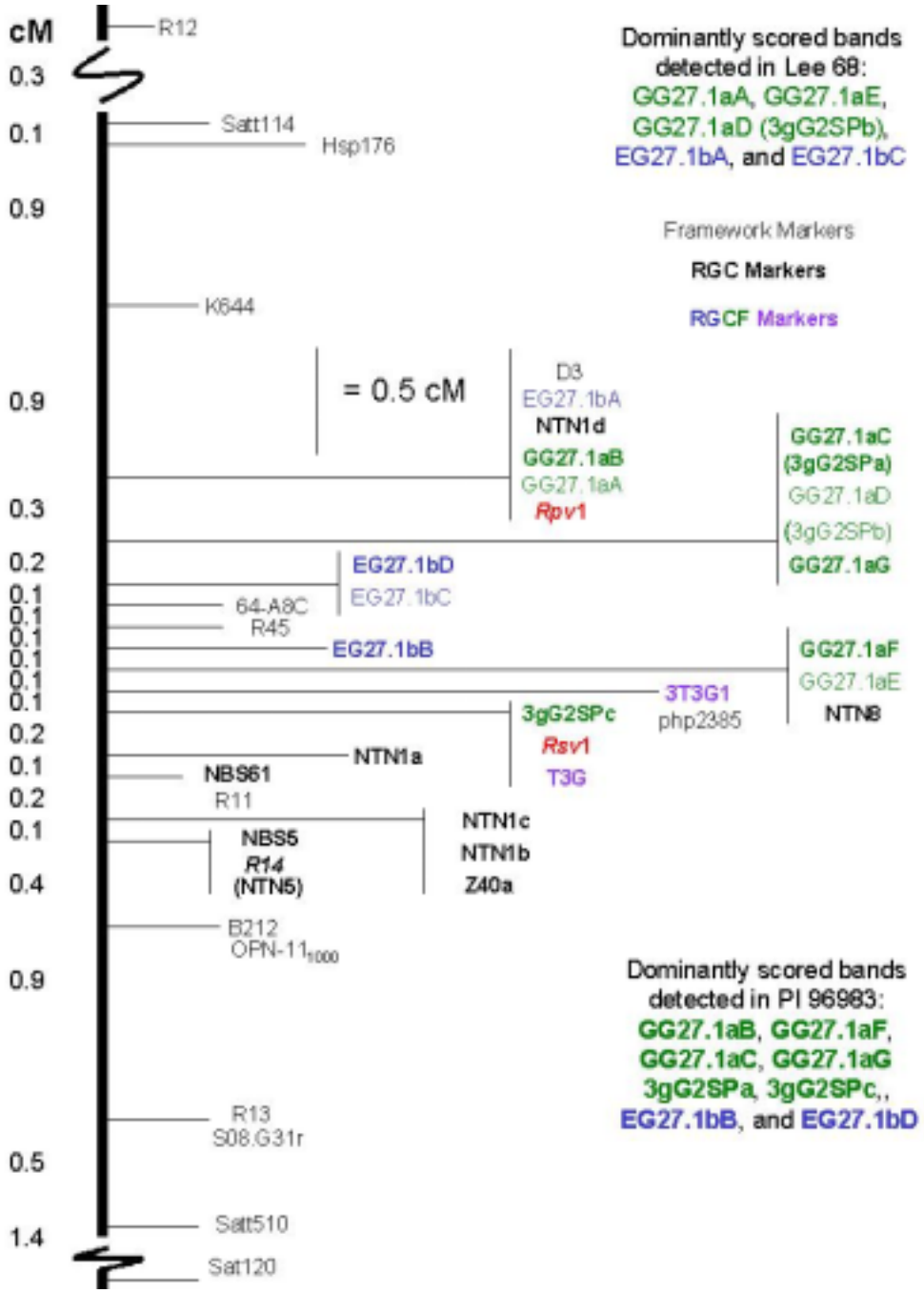


Figure 2. High-resolution map of the region around the *Rsv*1 and *Rpv*1 loci on soybean MLG-F based on framework, RGC, and RGCF markers. Map distances are given in centimorgans (cM).

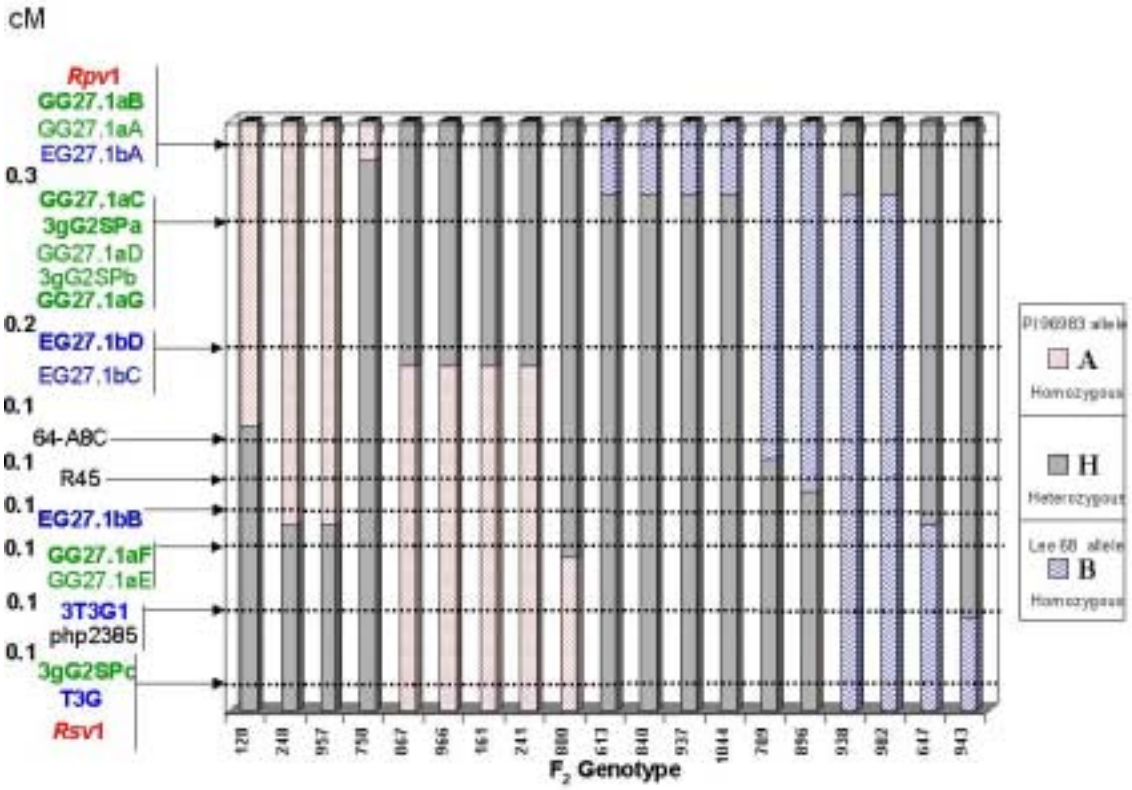


Figure 3. Genotype representation of 19 of 1,056 $F_2$ individuals with recombination between *Rsv*1 and *Rpv*1 from a cross between PI96983 (*Rsv*1 and *Rpv*1) and Lee 68 (*rsv*1 and *rpv*1)[a]. The vertical bars represent the chromosomal region between *Rsv*1 and *Rpv*1 for each recombinant line (RL). The point of color transition for each vertical bar represents the hypothetical location (based on marker genotypes) at which a recombination transpired. A vertical line after a group of markers indicates they cosegregated. The arrow, to the right of the marker(s), points to a dotted line that intersects the vertical bar at the location of the marker(s); furthermore, the coloration of the vertical bar at the point of intersection indicates the genotype of the marker for each $F_2$ individual.

a. Molecular marker data for $F_2$ individuals with recombination between *Rsv*1 and *Rpv*1 (RLs) was obtained by analyzing bulked DNA from 15-20 $F_{2:3}$ plants that were derived from each $F_2$ individual.


Figure 4. Necrotic symptoms on non-inoculated leaves of RL 982 (PI 96983 x Lee 68) two weeks after inoculation of unifoliolate leaves with SMV-G1. Lesions of trifoliolate leaves are a characteristic symptom of necrosis.

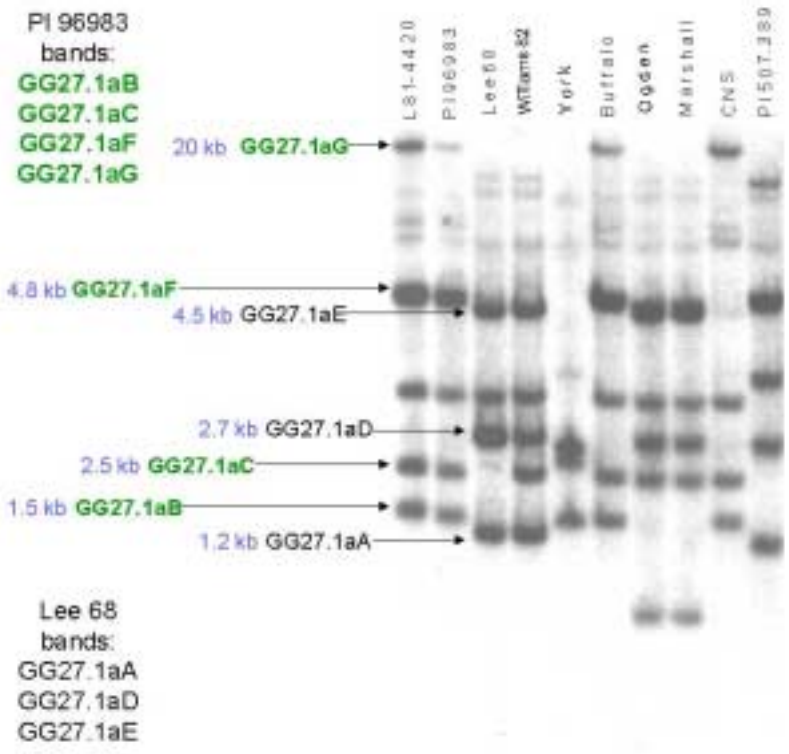


Figure 5. Southern blot analysis of nine cultivars and one NIL of Williams using the RGCF probe GG27-1a. Genomic DNA was digested with *Hin*dIII. The size and designation of the restriction fragments detected by GG27-1a in PI 96983 and Lee 68 are indicated on the left. L81-4420 (Williams NIL, *Rsv*1 and *Rpv*1 derived from PI 96983) and PI 96983 are assumed to be identical for the *Rsv*1 and *Rpv*1 region.

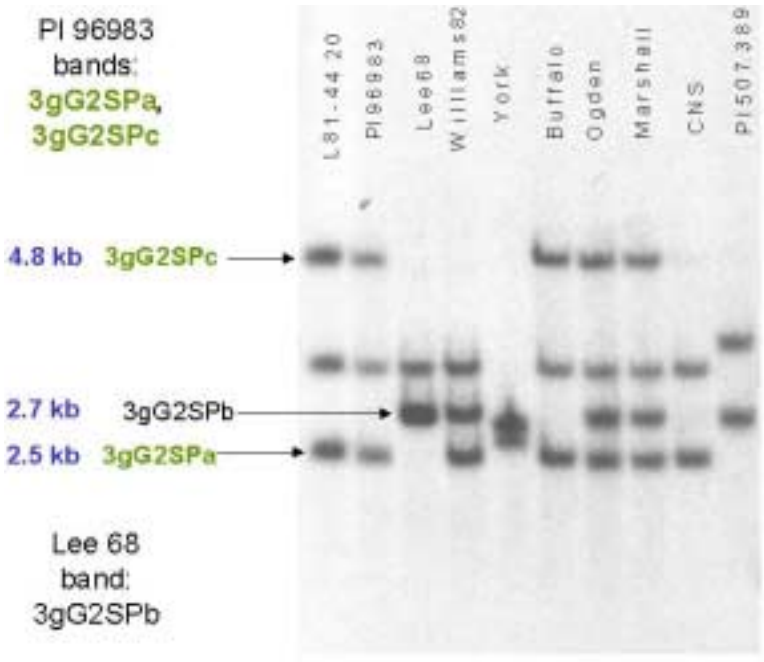


Figure 6. Southern blot analysis of nine cultivars and one NIL of Williams using the RGCF probe 3gG2SP. Genomic DNA was digested with *Hin*dIII. The size and designation of the restriction fragments detected by 3gG2SP in PI 96983 and Lee 68 are indicated on the left. L81-4420 (Williams NIL, *Rsv*1 and *Rpv*1 derived from PI 96983) and PI 96983 are assumed to be identical for the *Rsv*1 and *Rpv*1 region. 3gG2SPa and 3gG2SPb are the identical RFLP bands designated as GG27.1aC and GG27.1aD, respectively.

# Chapter IV

## SUMMARY AND FUTURE PROSPECTS

*Soybean mosaic virus* (SMV) and *peanut mottle virus* (PMV) are potyviruses that can cause substantial reductions in soybean yield worldwide. The utilization of resistant soybean [*Glycine max* (L.) Merr.] cultivars by the grower is the most efficient and economical practice to limit yield losses. Breeders have developed soybean cultivars that possess resistance to SMV and PMV conferred by *Rsv*1 and *Rpv*1, respectively. Cloning and characterizing *Rsv*1 and *Rpv*1 will help provide further insight into this host-virus interaction.

A new population of 1,056 $F_2$ individuals from the cross of the SMV and PMV resistant line PI 96983 (*Rsv*1 and *Rpv*1) by the susceptible cultivar Lee 68 (*rsv*1 and *rpv*1) was constructed. A marker-based selection approach was used to identify 91 individuals from the new population that had a recombination in the interval between SSR marker loci Hsp176 and Sat120. The individuals were used to construct a high-resolution map in the *Rsv*1 and *Rpv*1 region of soybean molecular linkage group (MLG)-F. Genotypes of *Rsv*1 and *Rpv*1 were determined by inoculating $F_{2:3}$ progeny with SMV-G1 and PMV-P1, respectively. $F_{2:3}$ DNA samples from each of the 91 RLs were analyzed with seven resistance gene candidate (RGC), five resistance gene candidate flanking (RGCF) markers, and 15 other molecular markers. Analysis of the data revealed that four different RGCF markers detected RFLP bands that cosegregated with *Rsv*1 or *Rpv*1. Analyses of the disease reaction and molecular marker data suggested that another SMV resistance gene, *Rsv*1b, was present in PI 96983's response to SMV-G1. The two closely linked SMV resistance genes were designated *Rsv*1a and *Rsv*1b. The SMV resistance response provided exclusively by *Rsv*1a or *Rsv*1b in the heterozygous state may allow SMV-G1 to induce necrosis in a percentage of plants grown from remnant $F_{2:3}$ seed of six of the 91 RLs.

Researchers have successfully cloned disease resistance genes from tomato, rice, and *Arabidopsis* using map-based cloning (Martin et al., 1993; Bent et al., 1994; Mindrinos et al., 1994; Yoshimura et al., 1998). The RGCF markers cosegregating with *Rsv*1 and *Rpv*1 should be used to screen a bacterial artificial chromosome (BAC) library, and then construct a physical map with the selected clones. A BAC clone confirmed to span the *Rsv*1 or *Rpv*1 locus can be used to screen a cDNA library made from a resistant soybean line. An isolated cDNA clone that encodes a putative protein product with homology to cloned disease resistance genes and cosegregates with either *Rsv*1 or *Rpv*1 should be utilized in complementation studies. The *N* gene of tobacco was transformed into susceptible tomato plants, and effectively confers resistance to the tobacco mosaic virus in these transgenic tomato plants (Whitham et al., 1996). In a related study, *Rsv*1 and *Rpv*1 could be transformed into other susceptible species of *Fabaceae*, enabling host-resistance without the incompatibility problems associated with cross-species breeding. This is a logical progression in SMV and PMV research, since SMV and PMV cause susceptible symptoms in 37 and 38 species of *Fabaceae*, respectively (Edwardson and Christie, 1991).

The disease resistance gene cluster on MLG-F includes the potyviral resistance genes, *Rsv*1a, *Rsv*1b, *Rpv*1, and possibly a resistance gene for peanut stripe virus (Choi et al., 1989; Buss, unpublished data). The potyviral resistance genes on MLG-F may be members of the NBS61 multigene family, since RFLP bands detected by RGC markers homologous to NBS61 map near or cosegregate with *Rsv*1a, *Rsv*1b. and *Rpv*1. Cloning and characterizing members of the NBS61 gene family may provide insight into the necrotic response evoked by more virulent strains of SMV.

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## VITA

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