GENETIC ANALYSIS OF SOYBEAN MOSAIC VIRUS RESISTANCE IN SOYBEAN

by

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ABSTRACT

This research was conducted to analyze the genetics of soybean mosaic virus (SMV) resistance in soybean [Glycine max (L.) Merr.] and to determine allelic relationships of SMV resistance genes and their interactions with SMV strain groups.

In the first part of this study, the inheritance of SMV resistance in OX670 and 'Harosoy' was studied to determine the source and identity of the resistance gene/genes in OX670. Other researchers reported that OX670 possesses a single gene at a locus independent of Rsv1 and assigned the gene symbol Rsv2. Rsv2 was presumably derived from the cultivar 'Raiden'. However, later work showed that Raiden contains a single resistance gene at the Rsv1 locus, raising the possibility that the resistance gene in OX670 was not from Raiden. Harosoy and its derivatives make up much of the remaining pedigree of OX670. Results from crosses of OX670 with susceptible cultivars indicate that it contains two independent genes for SMV resistance. One is allelic to the Rsv1 locus, expresses resistance to SMV-G1 and G7 and is derived from Raiden. The other is allelic to the Rsv3 locus, expresses resistance to SMV-G7 but susceptibility to SMV-G1 and is derived from Harosoy. Therefore the Rsv2 locus does not appear to exist in OX670 or its ancestors. The presence of Rsv1 and Rsv3 makes OX670 resistant to all SMV strains from G1 through G7.

The second study was conducted to investigate the inheritance and allelomorphic relationships of resistance gene(s) in 'Tousan 140' and 'Hourei', which were reported to carry single independent resistance genes when inoculated with the Japanese SMV strain C. Both of these lines exhibit resistance to strains SMV-G1 through G7. This inheritance study shows that Tousan 140 and Hourei each possess two resistance genes. One of the genes in Hourei confers resistance to SMV-G1 and G7 strains; the other gene confers susceptibility to SMV-G1 but resistance to SMV-G7. Allelism tests indicate that one of the genes in both Hourei and Tousan 140 is allelic to Rsv1, and the other is allelic to Rsv3. The two genes in Tousan 140 were separated into individual lines, R1 and R2. R1, most probably containing Rsv1, exhibited resistance to SMV-G1 through G3 but was susceptible to SMV-G5 through G7. Line R2, most likely possesses Rsv3 gene, was susceptible to SMV-G1 through G3 but resistant to SMV-G5 through G7. Therefore, presence of these two genes makes Tousan 140 resistant to SMV-G1 through G7.

The objective of the third study was to investigate inheritance and allelomorphic relationships of SMV resistance in PI88788. PI88788 exhibits resistance to SMV-G1 through G7. Genetic analysis of our data reveals that SMV resistance in PI88788 is conferred by a single gene at a locus tentatively labeled 'Rsv4'. Expression of this gene in the homozygous state decreased accumulation rate and prevented vascular movement of SMV. In the heterozygous state vascular movement of the SMV was delayed but not prevented.

To my wife Nazan and my daughter Erin Ilge Gunduz

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Literature Review

Soybean mosaic (SM) disease was first reported by Clinton in 1916, but the nature of the disease was first described in detail by Gardner and Kendrick in 1921. They reported that SM diseased plants were stunted, had mosaic dark green areas on leaflets and the leaflets were misshapen. It was also demonstrated that the causal agent of SM disease could be transmitted through seed and by mechanical inoculation (Gardner and Kendrick, 1921). Now it is known that soybean mosaic disease, caused by soybean mosaic virus (SMV: potyvirus, potyviridae) is one of the most common diseases of soybean (Glycine max L.) (Buss et al., 1989a). The disease can cause significant yield reduction (Ross, 1983; Hill et al., 1987 and Ren et al., 1997b), change chemical composition of the seed, reduce seedling viability (El-Amretz et al., 1987b), seedling vigor and cause seed coat mottling (Kwon and Oh, 1983). Depending on genotype and SMV strain interaction, yield reductions of 25% (Ren et al., 1997b) and 60% (Cho et al., 1977) have been reported.

SMV Properties, Structure and Genome

SMV is a member of the potyvirus group (Bos, 1972), which is the largest genus of plant viruses with 180 possible members causing significant losses in a wide range of crop plants. SMV belongs to the family Potyviridae and the genus Potyvirus (Fauquet and Mayo, 1999).

SMV is a flexuous particle about 750 nm long (Bos, 1972). The thermal inactivation point is usually 10 min at 55-60 ^{o}C. The longevity of the virus in vitro is 14 to 15 days in plant sap stored at 4 ^{o}C (Galvez, 1963), and the dilution end point is usually around 10^{-3} to 10^{-5}. SMV is most stable at pH 6 in expressed sap and loses infectivity at pH levels below 4 and above 9.

SMV has a single-stranded positive sense RNA genome of approximately 10 kb. Complete nucleotide sequences of SMV strains G2 and G7 have been reported (Jayaram et al., 1992). The viral RNA is polyadenylated at the 3` end and has a virus-encoded genome-linked protein (VPg) covalently linked to the 5` end. The RNA encodes a single polyprotein that is proteolytically cleaved by self-encoded protease into functional proteins. The only structural protein is the coat protein. Other identified functions

include the aphid transmission helper-component protease, which also has gene silencing suppressor activity, the cylindrical inclusion protein, which assists virus movement, the combined VPg-protease protein (NIa; nuclear inclusion a), and the RNA replicase (NIb; nuclear inclusion b) (Jayaram et al., 1992; Revers et al., 1999).

The estimated molecular weight of the coat protein is 28,300 daltons according to Hill and Benner (1980a) and 32,150 daltons according to Soong and Milbrath (1980). Single stranded RNA, which is 5.3% of the virus particle, has a molecular weight of 3.02 x 10^6 daltons (Hill and Benner, 1980b).

Host Range

SMV is known to naturally infect only soybean and its wild relatives (Bos, 1972; Sinclair, 1982). However, it has been shown that SMV is transmissible to about 30 plant species by mechanical inoculation (Bos, 1972). It causes local lesions in the genus Chenopodium, (C. quinoa and C. album) and in species of the Leguminosae (Galvez, 1963). SMV has been isolated from naturally infected Vicia faba showing mild symptoms of yellow mottle in China (Anonymous, 1985) and from Cassia occidentalis in Nigeria (Thottappilly, 1985).

Transmission of SMV

SMV is seed-borne and can be transmitted via aphids, and mechanical inoculation.

Seed transmission: SMV infected soybean seed is the major source of primary virus inoculum (Hill and Benner, 1980b). Thus, SMV transmission through seeds plays an important role in the epidemiology of the virus (Maury, 1985). Seed transmission rate can vary depending on the SMV strain, the soybean genotype, and the time of infection. Ross (1968) reported that seed transmission of two SMV isolates in a given cultivar was different. While the percentage of seed transmission for isolate SMV-1 was 11% in Lee, it was only 5.8% for SMV-2. SMV-1 transmitted through seed at a rate of 1.3% in Hill, while SMV-2 was not seed transmitted in the same cultivar. Moreover, some soybean

plant introductions and cultivars have been found to be resistant to SMV seed transmission (Bowers and Goodman, 1982), and can be used to control SMV disease by preventing its spread (Irwin and Goodman, 1981). Bowers and Goodman (1979) reported that when the plant was inoculated before flowering, 18% transmission was observed. The transmission rate dropped to 11% for inoculation during the R1 (flowering) stage and then to 4% for later inoculations. Infection after flowering results in lower rates of seed transmission (Iizuka, 1973; Irwin and Goodman, 1981). Ren et al. (1997b) also reported between the incidence level of SMV infection and inoculation stage. At 100% incidence of infection, the seed transmission percentage averaged 10% for plants that were inoculated prior to flowering compared with 3.5% for plants inoculated after flowering. As the incidence of SMV infection increased by 1%, the percentage of seed transmission increased by 0.11%, 0.08%, and 0.04 % when the infection occurred at growth stage V_2, R_1, and R_3, respectively.

Aphid transmission: Aphids are the only known insect vectors. SMV is transmitted by over 30 aphid species in a non-persistent manner (Koshimizu and Iizuka, 1963; Irwin and Goodman, 1981; Maury, 1985). Among those, only A. glycines, A. craccivora, and Aulacorthum solani are known to colonize soybean in Asia and parts of Africa (Irwin and Schultz, 1981). In South America, young soybeans are occasionally colonized by A. craccivora and A. gossypii (Irwin and Goodman, 1981). Aphids have not been reported to colonize soybean to any extent elsewhere (Irwin and Schultz, 1981).

SMV transmission via aphids is dependent on SMV strains and aphid species. Takahashi et al. (1980) found that some strains of SMV were hardly aphid transmissible. A non-transmissible isolate of SMV strain G5 was transmitted via aphid when plants were also infected with an aphid-transmissible SMV strain (Cho, 1981). The non-transmissible SMV-G5 strain probably does not contain the helper-component, which is essential for aphid transmission. When plants are infected with both non-transmissible and transmissible virus, non-transmissible virus binds with the helper component of the transmissible virus, thereby enabling aphid transmission of a usually non-transmissible virus (Matthews, 1991).

Mechanical transmission: SMV is readily transmitted mechanically (Bos, 1972), which is important for genetic studies and breeding purposes in obtaining 100% infection (Buss et al. 1985). Simple hand inoculation by rubbing a pestle or sponge dipped into inoculum on leaves dusted with abrasive is adequate and efficient to inoculate a small number of plants (Cho and Goodman, 1979; Buss et al., 1985). An artist's airbrush with air pressure of 4.2 to 5.6 kg cm^2 is the most efficient method for inoculating many plants in the field (Kiihl and Hartwig, 1979; Bowers and Goodman, 1982; Roane et al., 1983; Buss et al., 1989b).

Maintenance of pure cultures of different virus strains is essential for genetic studies (Buss et al., 1985). SMV can be maintained by continuos passage in susceptible plants. Chen et al. (1988) reported that SMV also can be maintained on soybean callus induced from infected leaf explants as long as the callus is alive. For long-term maintenance, SMV inoculum can be stored by desiccation of infected tissue over calcium chloride at 4 C or by preparing a liquid nitrogen powder of infected leaves and stored at -20 C (Roane et al. 1983). Infectivity of SMV can be maintained indefinitely, by simply freezing infected leaf tissue at -70 C (Ma, 1995).

SMV Strains

Conover (1948) was the first to recognize that soybean mosaic disease is caused by more than one strain of SMV. Since then, different isolates of SMV have been reported. These SMV isolates have been classified into different groups in Japan (Takahashi et al., 1963, 1980), the United States (Ross 1969, 1975; Cho and Goodman, 1979, 1982a), Taiwan (Han and Murayama, 1970), Korea (Cho et al., 1977), Brazil (Almeida, 1981) and China (Pu et al., 1982; Xu et al., 1983; Chen et al., 1986).

In the United States, Cho and Goodman (1979) classified 98 SMV isolates, which were derived from seeds in the USDA soybean germplasm collections, into seven strain groups, G1 through G7, based on symptoms induced on a set of soybean differential cultivars. The reaction of the eight soybean differentials, which include cultivars Clark, Rampage (the latter two are SMV universal susceptibles), Buffalo, Davis, Kwanggyo, Marshall, Ogden and York, to seven SMV strains was classified as mosaic, necrotic, or

symptomless. All of the SMV isolates tested caused infection and typical mosaic symptoms in cultivars Clark and Rampage. Table 1 summarizes the Cho and Goodman (1979, 1982a) system. In addition to Cho and Goodman's seven SMV strain groups, Buzzell and Tu (1984) reported a distinct isolate labeled G7A that caused mosaic symptoms in a PI96983 derivative (L78-379) but did not produce symptoms on the cultivar 'Raiden'.

Eight isolates from China were designated Sa through Sh (Pu et al., 1982; Chen et al., 1986). In Japan 102 SMV isolates were classified into five strain groups, A, B, C, D, and E based on symptoms induced on four soybean cultivars including Harosoy, Shiromame, Ou 13 and Tokachinagaha (Takahashi et al., 1980). When the soybean differential cultivars used by Cho and Goodman (1979; 1982a) were inoculated with Japanese strains A, B, C, D and E, the differentials exhibited identical reactions to Japanese A, B and SMV-G3 strains. Similarly, the differentials exhibited identical reaction to Japanese E and SMV-G5 strains (Shigemori, 1988) indicating that these strains might be similar or the same (Table 1).

Xu et al. (1986) classified 23 SMV isolates from China into 13 groups when they used the soybean differential cultivars from Cho and Goodman (1979, 1982a). Only six of 23 isolates, including isolates 24, Sb, 13, 14, 16, and 28, did not belong to any of the original G1-G7 SMV groups reported by Cho and Goodman (1979; 1982a).

Disease Symptoms of SMV on Soybean

SMV-host interactions result in four distinct reactions, which include resistant, susceptible, late susceptible and necrotic (Cho and Goodman, 1979,1982a; Ma, 1995). A host plant is considered fully susceptible to a virus if the virus can successfully complete replication, cell-to-cell movement and long distance movement. Similarly a host plant is resistant if the host can block one of these three processes (Carrington and Whitham, 1998). Disease symptoms can be seen in different parts of the plants including, roots, stems, petioles, leaflets, and seeds (Gardner and Kendrick, 1921; Koshimizu and Iizuka, 1963; Bos, 1972; Cho et al., 1977; Sinclair, 1982; Tu and Buzzell, 1987; Bowers et al., 1992).

Susceptible (mosaic): Generally, mosaic symptoms appear as yellowish vein-clearing along the small, branching veins of the first trifoliolate leaves 7 to 10 days after mechanical inoculation of unifoliolate leaves. These symptoms are transitory. Typical rugosity usually does not appear until the third trifoliolate leaf. More severe symptoms develop on subsequent leaves, which eventually show dark green enations along the main veins. Leaf margins frequently curve down at the side (Sinclair, 1982). The youngest and most rapidly growing leaves show the most severe symptoms. Plants infected early in the season in the field are severely stunted, with shortened petioles and internodes, and may mature later than non-infected plants (Ross, 1969; Cho and Goodman, 1979; Sinclair, 1982); however, infected plants of certain soybean genotypes mature earlier (Sinclair, 1982).

Late Susceptible: Late susceptible symptoms were first observed in the segregating populations of Columbia (R) x Lee 68 (S) (Buss, unpublished data). Late susceptible plants express resistant reactions to virus for approximately 20 days after inoculation, but then susceptible symptoms appear as large mosaic islands on the first trifoliolate leaves. Apparently, the host delays viral replication or movement of virus due to partial dominance of the resistance gene and, therefore, this reaction can be considered a type of resistance.

Necrosis: The necrotic reaction is a hypersensitive reaction of plants to pathogens, including viruses. The necrotic reaction, which can be classified into two subgroups, is often observed on soybean cultivars that have resistance genes (Cho and Goodman, 1982a: Buss et al., 1989a).

1) Local necrosis: The hypersensitive reaction of the plant is limited to initially infected cells on the inoculated leaves. The formation of such localized necrotic spots, caused by host cell death in the vicinity of pathogen-infected cells, suggests that virus proliferation is restricted to the initial infected cell (Greenberg, 1997). Therefore, it is considered a resistant reaction (Fraser, 1990; Matthews, 1991).

2) Stem tip (systemic) necrosis: In this case the hypersensitive reaction is not restricted to the initial infection site and can be systemic. The symptoms associated with stem tip necrosis include a brown discoloration of leaf veins, yellowing of the leaves, confined systemic necrotic lesions on leaves, stunting of the plants, browning of petioles,

stem or stem-tips, bud blight, and defoliation, usually leading to plant death (Cho et al., 1977; Sinclair, 1982). Soybean cultivars that show systemic necrotic symptoms usually have necrotic lesions and/or veinal necrosis on inoculated leaves (Cho and Goodman, 1979; Pu et al., 1982).

Development of the mosaic and stem tip necrosis symptoms may be temperature dependent (Conover, 1948; Walters, 1963; Cho and Goodman, 1979; Tu and Buzzell, 1987). Tu and Buzzell (1987) reported that the development of stem-tip necrosis in the soybean line OX686 was temperature-dependent. The majority of inoculated plants developed stem-tip necrosis at 20 and 24 ^{o}C, but developed typical mosaic symptoms at 28 and 30 ^{o}C in the growth chamber. Temperature, however, did not affect mosaic symptom development in 'Amsoy 71' soybean. Cho and Goodman (1979) observed that necrosis was more conspicuous on plants held in a growth chamber at 30 ^{o}C than on those at 24 ^{o}C. No qualitative differences in reactions were noted at the higher temperature. Cho et al. (1977) also observed that temperatures above 25 ^{o}C favored early development of necrotic symptoms.

Effects of SMV on Soybean Yield and Seed Quality

Infected plants often have reduced foliage, poor pod set, reduced nodulation and may exhibit increased susceptibility to other pathogens (Sinclair, 1982). Therefore, the virus has significant economic impact on soybean production, causing considerable yield loss (Ross, 1983; El -Amretz et al., 1987a; Hill et al., 1987). However, the amount of yield loss depends on the growth stage at which infection occurs, frequency of infected plants, degree of susceptibility, virulence of the virus strain, environmental conditions and population density of the vector (Ross, 1987). Ren et al. (1997a) reported that SMV infection at an early stage of soybean plant development resulted in significant yields loss. If SMV infection occurred at or before the R1 growth stage, yield loss ranged from 3 to 22% depending on the incidence level of SMV infection.

Genetic resistance is the most effective, convenient and inexpensive control method and is especially applicable to SMV in soybean (Buss et al., 1989a). Introgression of resistance genes into adapted cultivars decreases yield reduction in the presence of

SMV. Ross (1977) used closely related SMV resistant and susceptible sibling lines (27 pairs) that were developed by back-crossing SMV resistance from PI96983 or PI170893 into the cvs, Semmes, Dare, Pickett 71, Lee 68 and Ransom. Some rows in the field were inoculated artificially to serve as inoculum source for aphid transmission. Susceptible lines in two of four pairs of a Dare backcross family showed no significant yield reduction (5.2%). Yield reduction in other backcross families averaged from 20 to 35 %. Buss et al. (1989c) used SMV resistant isolines of soybean cultivars Williams and Essex to assess yield loss caused by natural field infestation of SMV in Virginia and Illinois during 1987 and 1988. A significant average yield advantage for the resistant isolines of 11% was observed in the Virginia test in 1988. Slightly larger, but non-significant, differences were observed in two other tests. Ren et al. (1997b) using SMV resistant and susceptible genotype pairs of Essex and Williams with Rsv1 gene, reported that SMV resistance provided a yield benefit in double crop but not in early planting. In 18 resistant/susceptible genotype pairs (ten selected from F4 derived lines of 'A3935 x 'Hutcheson', six selected from F4 derived lines of 'A4393' x Hutcheson, Williams and its resistant line L78-379 and Essex and its resistant isoline V85-5344), the resistant genotypes overall had 12% higher yield than the susceptible genotypes in late plantings. Only border rows were inoculated with SMV-G2 to provide the virus source to be transmitted by aphids. The reason SMV resistance benefit was more noticeable in the double crop system (late-planting) is that a high aphid population is present at an early stage of growth in this system. During late July and August when aphid populations are high, double cropped soybean plants are either still in the vegetative growth stage or at R1 (Fehr and Caviness, 1977) while full-season (conventional tillage system) soybean plants are near the mid-reproductive growth stage (R3/R4), which is less vulnerable to SMV infection (Ren et al., 1997a).

In addition to reducing yield, SMV infection has a negative effect on seed quality. SMV decreases seed quality by causing seed coat discoloration (mottling), which is unfavorable in soyfood processing (Quiniones et al., 1971). Ren et al. (1997a) reported that infection at or before flowering increased the percentage of seed coat mottling. Percentage of seed coat mottling was linearly related to the incidence of SMV infection occurring prior to flowering. However, if the soybean plant is infected with SMV after

flowering, the level of incidence did not increase seed coat mottling. SMV reduces seed viability and germinability (El-Amretz et al., 1987b), and seedling vigor (Kwon and Oh, 1983). SMV also alters chemical composition of the seed (El-Amretz et al., 1987b). The percentage of oil and protein in the seed may be slightly altered by virus infection (Demski and Jellum, 1975).

SMV may have a greater negative effect on quality and yield when soybean is co-infected with other viruses. Ross (1968) and Quiniones et al. (1971) reported that seed mottling was more severe when plants were infected with both SMV and bean pod mottle virus (BPMV) than when they were infected with SMV or BPMV alone. Moreover, yield reductions of 66% (Quiniones et al., 1971) and 80% (Ross, 1968) were reported when co-infection of SMV and BPMV occurred. Yield reduction was only 18% and 10% when soybeans were infected individually with SMV and BPMV, respectively (Quiniones et al., 1971).

Inheritance of Soybean Resistance to SMV

To date, many studies have been conducted to elucidate the inheritance of resistance to SMV in soybean. In most cases, resistance to SMV is controlled by a single dominant or incompletely dominant gene (Kiihl and Hartwig, 1979; Roane et al., 1983; Buzzell and Tu, 1984; Shigemori, 1988; Buss et al., 1989a; Chen et al., 1991; Bowers et al., 1992; Ma et al., 1995; Wang et al., 1998). In 1963, Koshimizu and Iizuka conducted inheritance studies using 13 resistant x susceptible crosses. The F_1 plants from 11 crosses were resistant and the F_2 populations segregated in a pattern of 3R : 1S. In the other two crosses, the F_1 plants were susceptible and the F_2 populations segregated to fit a 7 resistant : 9 susceptible ratio. In the latter case, they conclude that two complementary genes controlled resistance to SMV.

Kiihl and Hartwig (1979), investigated the inheritance of SMV resistance in eight cultivars using SMV-1 and SMV-1-B isolates, which were later assigned by Cho and Goodman (1982a) to strain group SMV-G2 and G3, respectively. Segregation patterns in F_2, F_3 and BC_1F_1 populations indicated that a single gene governs resistance to SMV in PI96983 and Ogden. They noted that while PI96983 was resistant to SMV-1-B, Ogden

gave a necrotic reaction. The resistance gene in PI96983 was dominant to the gene conferring a necrotic reaction in Ogden. The genes in PI96983 and Ogden were allelic and assigned the symbols Rsv and rsv-t, respectively. Kiihl and Hartwig (1979) observed a necrotic reaction in plants of some F_1 and F_2 populations from resistant x susceptible crosses and obtained a good fit to a 3 resistant : 1 susceptible ratio when plants with the necrotic reaction were classified as resistant. Such necrotic reactions were interpreted as being a response to heavy inoculum dosage. However, Cho and Goodman (1982b) reported that the necrotic reaction did not appear to be a response to inoculum dosage. In fact, soybean plants can become necrotic when inoculated with a given strain, whether the plants are mechanically inoculated with sap inoculum or inoculated with a much smaller dose provided by aphids (Bowers et al., 1992).

There has been disagreement among researchers in terms of the classification of necrotic plants as either susceptible or resistant; however, results from some genetic studies suggested that necrotic plants should be classified as resistant when evaluating segregating populations (Chen, 1989; Buss et al., 1989a; Bowers et al., 1992; Chen et al., 1994; Ma et al., 1995). Buss et al. (1989a) reported that the majority of necrotic plants occurred in segregating $F_{2:3}$ families of resistant x susceptible crosses and proposed that the necrotic reaction was associated with heterozygosity of resistance alleles. Chen et al. (1994) reported that alleles that confer resistance in the homozygous state often exhibit necrotic reaction when combined with a susceptible allele in heterozygous plants. Although plants with necrotic reaction have been classified as resistant for genetic analysis in most studies, Kwon and Oh (1980), Buzzell and Tu (1984), Lim (1985) and Zhang et al. (1989) classified necrotic plants as susceptible for genetic analysis.

The necrotic reaction observed in progeny of resistant x susceptible segregating populations can be used to determine dominance of resistance genes. For example, necrotic reactions observed in the segregating population of Lee 68 x Ogden indicated that the resistance gene in Ogden was partially dominant (Kiihl and Hartwig, 1979). However, the necrotic reaction was not observed when PI96983 was used as the resistant parent, indicating that the resistance gene in PI96983 is completely dominant. Bowers et al. (1992) reported that progeny from crosses of the resistant line HLS with susceptible lines never produced necrotic plants when inoculated with any SMV strain, indicating

that the resistance allele from HLS is completely dominant. However, necrotic plants were observed in certain environments in the F_2 populations of Buffalo x susceptible genotypes.

A low frequency of necrotic plants also has been observed in F_2 populations from resistant x resistant crosses between parents with alleles at the Rsv1 locus (Chen et al. 1991, 1994; Ma, 1995).

The necrotic reaction generally results when soybean cultivars possessing resistance to SMV strains SMV-G1, G2, G3, G4 and G5 are infected by SMV-G6 and G7 strains (Cho et al., 1977; Cho and Goodman, 1979, 1982b; Chen et al., 1994). However, lower numbered SMV strains have the capacity to incite necrosis in an appropriate host. PI507389 gives a necrotic reaction to SMV-G1 but susceptible reaction to G7. Ma et al. (1994) and Ma (1995) conducted a study to investigate inheritance of lethal necrosis in PI507389, a large seeded germplasm accession from Japan. PI507389 was found to give a quick lethal necrosis response to SMV-G1, G2, G5, and G6 and a susceptible response to G3, G4 and G7. Data from F_1, F_2 and $F_{2:3}$ populations derived from PI507389 x Lee 68 , PI507389 x PI96983, PI507389 x York, and PI507389 x Marshall indicate that a lethal necrosis reaction was expressed by progeny that were homozygous for an allele at the Rsv1 locus. This allele is recessive to the resistance alleles in PI96983, York and Marshall. Gene symbol Rsv1-n for the allele at the Rsv1 locus conferring lethal necrosis in PI507389 was proposed. Dominance of the necrotic reaction to susceptibility has been reported by other researchers as well (Kiihl and Hartwig , 1979; Tu and Buzzell, 1987; Shigemori, 1988; Chen et al., 1994). Kwon and Oh (1980) reported that a single recessive gene conferred resistance in some of the Korean cultivars. Their results were based on F_2 populations of resistant x ”susceptible” cultivars Kwanggyo and Tachisuzunari, which exhibited necrosis to a severe SMV-N strain. However necrotic plants were classified as susceptible in this study. If they had classified necrotic as resistant they would have concluded that necrosis is dominant to resistance.

Chen et al. (1991) conducted allelism studies among the differential cultivars PI96983, Ogden, York, Marshall, and Kwanggyo which are all resistant to SMV but react differently to strains SMV-G1 through SMV-G7. They found that each cultivar has a single dominant gene conditioning resistance to SMV. They concluded that the

resistance genes in these cultivars are alleles at Rsv1 locus and assigned the gene symbols Rsv1-y, Rsv1-m and Rsv1-k for the resistance alleles in York, Marshall and Kwanggyo, respectively, and changed the symbol for the resistance gene in Ogden from Rsv1-t to Rsv1-t because of its apparent dominance to alleles for susceptibility.

Buzzell and Tu (1984) reported that OX670, a breeding line, possesses a single dominant gene, which is independent of the resistance gene Rsv1 in L78-379. L78-379 is a resistant selection from Williams(6) x PI96983. OX670 was derived as a SMV resistant selection from OX615 x OX613. OX615 is a SMV resistant line derived from Harcor x OX315, and OX613 is a susceptible line derived from Harcor x a Harosoy isoline. OX315 is a resistant selection from Harcor x Raiden. The F_2 population from OX670(R) x L78-379 (S) inoculated with SMV-G7 segregated to fit a 3R : 1S ratio indicating that OX670 possesses a single dominant gene (Buzzell and Tu, 1984). However, L78-379 contains Rsv1 from PI96983 (Bernard et al., 1991) and is necrotic to SMV G7 (Chen et al., 1991). Buzzell and Tu (1984) did not distinguish necrotic plants from susceptible plants and apparently they classified all necrotics including L78-379, as susceptible. Therefore, they might have observed 3R:1N instead of 3R:1'S'. When SMV-G6 was used, the OX670 (R) x L78-379 (R) F_2 population segregated to fit a 15R:1S ratio, indicating that the gene in OX670 is independent of the Rsv1 locus. Again, it appears likely that the S plants were actually N. Based on their interpretation of the data, the gene symbol Rsv2 was assigned to the resistance gene in OX670, which presumably was derived from Raiden. They also noted that this new gene confers resistance to all SMV strains tested including SMV-G1 through G7 and isolate SMV-G7A. However, a later study revealed that Raiden exhibits resistance to SMV-G1, G2, G3, G4 and G7, and a necrotic reaction to G5 and G6 (Buss et al., 1995). Buss et al. (1995) conducted a study to investigate the inheritance of resistance in Raiden and L88-8431, a Williams isoline with SMV resistance derived from Raiden using SMV-G1 and G7. When inoculated with SMV-G1 the F_2 population of Raiden x Lee 68 and L88-8431 x Lee 68 segregated to fit a single gene ratio of 3(R+N):1S. F_2 and $F_{2:3}$ populations derived from R x R crosses of Raiden with resistant cultivars possessing Rsv1 alleles, did not segregate for susceptible progeny indicating that the resistance gene from Raiden is

allelic to Rsv1. These contradictory results suggest that the resistance gene in OX670 was not derived from Raiden.

Tu and Buzzell (1987) reported that OX686 exhibits stem tip necrosis (STN) to SMV-G1 and G4. OX686 is a true breeding line for STN and was derived from an F_2 plant of Columbia x Harosoy. Harosoy, is susceptible to SMV G1 and G4. They also reported that the development of STN in OX686 was temperature dependent. At 20 and 24 ^{o}C the majority of the inoculated leaves developed STN, while at 28 and 32 ^{o}C nearly all inoculated plants developed typical mosaic symptoms.

Buzzell and Tu (1989) studied the inheritance of the STN gene in OX686. The $F_{2:3}$ progeny of OX686 (STN) x Harosoy (S) segregated 1 all STN : 2 segregating for STN and NN (nonnecrotic) : 1 all NN, indicating that STN was controlled by a single dominant gene when inoculated with SMV-G4. $F_{2:3}$ progeny from crosses OX686(STN) x L78-379 (Rsv1) and OX686 (STN) x OX670 (Rsv2) segregated for two genes when inoculated with SMV-G1 and G4. This indicated that the STN gene is independent of both the Rsv1 and Rsv2 loci and, therefore, was assigned the gene symbol Rsv3 (Buzzell and Tu, 1989).

Bowers et al. (1992) reported that 'Buffalo' and HLS, a late maturing selection from the cultivar 'Hardee', have single dominant genes at independent loci for SMV resistance. Ma (1995) and Buss et al. (1999) reported that the resistance gene in L29, a resistant selection from Williams (6) x Hardee, is allelic to Rsv3. They also reported that L29 shows a susceptible reaction to SMV-G1 through G4 and a resistant reaction to SMV-G5 through G7.

Lim (1985) reported that resistance in PI483084 (Suweon 97), PI96983 and PI486355 was conferred by single dominant gene at independent loci. Each of the F_2 populations from crosses among these three lines segregated to fit a ratio of 15 resistant : 1 susceptible. However, Chen et al. (1993) found that PI486355, which is resistant to SMV-G1 through G7, possesses two independent resistance genes, and one of these genes likely was at the Rsv1 locus. Later Ma et al. (1995) separated these two genes in two lines, LR1 and LR2 derived from Essex x PI486355. Inheritance studies confirmed that each of these lines possesses a single dominant resistance gene. Allelism studies, indicated that the R1 gene was allelic to the Rsv1 locus and expression of the gene was

dosage dependent, with the homozygotes conferring resistance but the heterozygotes showing systemic necrosis to SMV-G7. They assigned gene symbol Rsv1-s, which is the only reported Rsv1 allele with resistance to SMV-G7. The gene in LR2 confers resistance to strains SMV-G1 through G7 and exhibits complete dominance. It is also epistatic to the gene in LR1. The gene in LR2 is independent of Rsv1 and Rsv3 and therefore, was tentatively called Rsv4. LR2 has been developed into a homozygous line, V94-5152, a subline selection from PI486355 x Essex and registered as germplasm (Buss et al., 1997).

Inheritance of resistance in Peking and PI556949, which are resistant to G1 through G7 was studied by Buss et al. (unpublished data). Both lines were crossed with Lee 68 and with each other. They were also crossed with soybean lines possessing Rsv1, Rsv3 and Rsv4 to test allelism. Genetic analysis of F_2 populations and $F_{2:3}$ progeny indicated that the same partially dominant single gene at Rsv4 locus governs the resistance in Peking and PI556949 (Buss unpublished data). Late susceptible plants observed in the segregating populations of Peking x Lee 68 and PI556949 x Lee 68 were combined with resistant plants as a single class for chi-square test because of their association with heterozygotes (Buss unpublished data).

Shigemori (1988) found that the cultivars Tousan-140 and Hourei both possess a single gene conferring resistance to the C strain of SMV in Japan. The resistance gene in Tousan 140 was shown to be independent of the resistance gene in Hourei. A single gene confers systemic necrosis in Tousan 122 is dominant to the allele for mosaic reaction to the C strain of SMV in Japan. The gene in Tousan 122 conferring necrosis to the C strain was proposed to be at a locus independent of the resistance gene in Tousan 140 and the resistance gene in Hourei. Segregation of each of the F_2 populations from crosses of Tousan 122 with Tousan 140, and Hourei conformed to a ratio of 15 (resistant+necrotic):1 mosaic. These results indicated that Tousan 122, Tousan 140 and Hourei each possess an independent SMV resistance gene. The resistance gene in Tousan 140 and Hourei confer resistance to Japanese SMV strain A, B, C, D and systemic necrosis to strain E. The resistance gene in Tousan 122 caused systemic necrosis to Japanese A, B, C, D and a resistant reaction to strain E.

Wang et al. (1998) studied the inheritance of SMV resistance in four soybean cultivars, including, Da bai ma, Ke feng No.1, Feng shou huang and Xu dou No.1 from China. They found that each of the Chinese cultivars had a single dominant gene for resistance to SMV-G1. They concluded that the resistance gene in Ke feng No.1 (PI 556949) is not at the Rsv1 locus reported for PI486355 (Ma et al., 1995), since the F_2 populations of Ke feng No.1 x PI 96983 (R, Rsv1) and Ke feng No.1 x PI 486355 did not segregate for susceptible progeny. They also reported that the resistance gene carried by Da bai ma (PI 556948) is not at the Rsv1 locus. The resistance gene in Xu dou No.1 (PI 556950) is not at the Rsv1 locus or non-Rsv1 locus of PI 486355, since the F_2 populations of PI96983 x Xu dou No.1 and Xu dou No.1 x PI486355 gave digenic and trigenic segregation ratios, respectively.

The Rsv1 gene from PI96983 maps to linkage group F (Yu et al., 1995) and the Rsv4 gene from PI486355 maps to linkage group D1b (Hayes et al., 2000). Closely linked molecular markers to these genes also were reported.

Interactions between SMV Resistance Genes and SMV Strains

The same resistance gene, or closely linked genes, likely confers resistance to different strains of SMV in a given soybean genotype. Chen et al. (1994) indicated that the resistant and necrotic reactions of a given soybean genotype to different SMV strains are controlled by the same gene. Buss et al. (1989c) found that an SMV resistant isoline of the susceptible cultivar Essex retained the same reactions to SMV strains G1 and G7 as did PI96983, the original source of resistance after 11 generations of backcrossing. There was selection only for resistance to SMV-G1 during five generations of backcrossing to Essex. These results indicate that a single gene or closely linked genes, rather than distantly linked or independent genes, probably conferred resistance to different SMV strains in PI96983. Similarly, L78-379, an SMV resistant isoline of the susceptible cv Williams, developed by six generations of backcrossing with PI96983 as the resistant donor parent (Bernard et al. 1991), gave the same reactions to SMV strains G1 through G7 as PI96983 (Chen et al., 1991).

Ma (1995) conducted a study to investigate whether the same gene or separate genes control reactions to SMV strains G1 and G7. Both LR1 and LR2 are resistant to SMV-G1 and G7. LR1 possesses a resistance gene at the Rsv1 locus, while LR2 has an non-Rsv1 gene. Two SMV resistant soybean lines, LR1 and LR2, were crossed with the susceptible cv Lee 68. Their data based on 200 $F_{2:3}$ progeny from LR1 x Lee 68 and 262 $F_{2:3}$ progeny from LR2 x Lee 68 confirmed that resistance to SMV-G1 and G7 was conferred by a single gene in both lines, since no recombinants were found for reactions to SMV-G1 and SMV-G7 in either cross. In the $F_{2:3}$ progeny of LR1 x Lee 68 necrotic plants were classified as resistant since a portion of heterozygotes expressed systemic necrosis when inoculated with G1; all heterozygotes expressed necrosis when inoculated with G7.

In linkage tests for resistance to different SMV strains, Gai et al. (1989), reported that separate single genes conditioned resistance to each of the four local SMV strains, Sa, Sc, Sg, and Sh, isolated in China. Those four genes, A, C, G, and H, were at different loci but linked in the order G-H-A-C with recombination frequencies estimated as 25-28%, 24% and 13-16%, respectively.

The reactions of resistance genes at the Rsv1, Rsv3 and Rsv4 loci to SMV strains G1 to G7 are summarized in Table 2. In general, alleles at the Rsv1 locus show resistance to the lower numbered strains such as SMV-G1 to G4 and susceptibility or necrosis to higher numbered strains such as SMV-G5 to G7. The gene in Suweon 97 is an exception since it is resistant to all SMV strains (Chen et al., 1999). They are incompletely dominant since F_1 plants of R x S crosses frequently produce a necrotic reaction to SMV-G1. Roane et al. (1986) proposed that SMV-G1 must lack a virulence gene since G1 does not produce symptoms on cultivars carrying resistance genes at the Rsv1 locus. At that time all cultivars that were resistant to more virulent strains were also resistant to SMV-G1. Thus it was proposed that SMV-G1 should be ideal for genetic studies because it should detect any resistance gene. However, it has since been discovered that some genes, such as Rsv3 are completely susceptible to G1 but are resistant to higher numbered strains.

Genes at the Rsv3 locus generally are completely dominant and show susceptibility to lower numbered strains, but resistance to higher numbered strains as

seen in L29. Bowers et al. (1992) reported that the resistance gene derived from the cultivar Hardee, which was later found to be at the Rsv3 locus (Ma, 1995), confers resistance to strains, SMV-G5 through G7, but susceptibility to G1 through G4.

Alleles at the Rsv4 locus confer resistance to SMV-G1 through G7 (Ma et al., 1995; Ma, 1995), although some exhibit late susceptibility in the heterozygous state (Buss unpublished data, 1999).

SMV resistance in soybean generally is controlled by single gene. Reliance on a single gene will result in genetic uniformity and potential vulnerability. Cultivars that were resistant to common SMV strains in Korea exhibited necrotic reaction against a new strain called SMV-N (Cho et al., 1977). This situation caused great concern among plant breeders and farmers alike. Thus, plant breeders need to increase the diversity of SMV resistance genes in order to develop cultivars with durable resistance to multiple strains of SMV.

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Table 1. Reaction of a set of soybean cultivars to US, Japanese, and Chinese SMV strains.

SMV Strains[#]	Soybean cultivars						
	Williams	Clark	York Rsv1^{y}	Marshall Rsv1^{m}	Ogden Rsv1^{t}	Kwanggyo Rsv1^{k}	PI96983 Rsv1
G1	-	S†	R	R	R	R	R
G2	-	S	R	N	R	R	R
G3	-	S	R	N	N	R	R
G4	-	S	N	R	R	R	R
G5	-	S	S	R	R	N	R
G6	-	S	S	N	R	N	R
G7	-	S	S	N	N	N	N
Japanese strains[#]							
A	-	S	R	N	N	R	R
B	-	S	R	N	N	R	R
C	-	S	S	N	N	S	N
D	-	S	S	N	N	S	N
E	-	S	S	R	R	N	R
Chinese isolates[#]							
4,6,8,30,Sd,Sf (G1)	S	-	R	R	R	R	R
18-1(G2)	S	-	R	N	R	R	R
11,21,23 (G2a)	S	-	R	S	R	R	R
17,Sc (G3)	S	-	R	N	N	R	R
20 (G3a)	S	-	R	S	S	R	R
7(G4a)	S	-	S	R	R	R	R
15(G5)	S	-	S	R	R	N	R
25(G6a)	S	-	N	S	R	N	R
26(G7b)	S	-	S	S	S	N	S
24(G8)	S	-	S	S	R	N	S
Sb(G9)	S	-	S	S	S	R	S
13, 14, 16(G10)	S	-	S	R	R	N	R
28 (G11)	S	-	S	S	S	S	S

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

[#] Data for US strains obtained from Cho and Goodman (1979, 1982 a); Data for Japanese strains obtained from Shigemori (1988); Data for Chinese isolates taken from Xu et al. (1986).

Table 2. Reaction of some soybean genotypes to seven SMV strain groups.

Source	Resistance Gene	SMV Strains G1	G2	G3	G4	G5	G6	G7	Reference
PI96983	Rsv1	R†	R	R	R	R	R	N	Chen et al., 1991
Ogden	Rsv1-t	R	R	N	R	R	R	N	Chen et al., 1991
York	Rsv1-y	R	R	R	N	M	M	M	Chen et al., 1991
Marshal	Rsv1-m	R	N	N	R	R	N	R	Chen et al., 1991
Kwanggyo	Rsv1-k	R	R	R	R	N	N	N	Chen et al., 1991
PI507389	Rsv1-n	N	N	S	S	N	N	S	Ma, 1995
LR1[#]	Rsv1-s	R	R	R	R	N	N	R	Ma et al., 1995
Raiden	Rsv1-r	R	R	R	R	N	N	R	Buss et al., 1995
Suweon 97	Rsv1-?	R	R	R	R	R	R	R	Chen et al., 1999
OX686[#]	Rsv3	N	NA	NA	N	NA	NA	R	Buzzell and Tu, 1987
L29[#]	Rsv3-h	S	S	S	S	R	R	R	Buss et al., 1999
LR2[#]	Rsv4	R	R	R	R	R	R	R	Ma et al., 1995
Peking	Rsv4-p	R	R	R	R	R	R	R	Buss, unpublished

† R, resistant symptomless; N, necrotic; S, susceptible (mosaic); NA, not available

[#] LR1, derived from PI486355; LR2, derived from PI486355; OX686, derived from Columbia ;

L29, [Williams (6) x Hardee], a Williams isoline with SMV resistance derived from Hardee.

CHAPTER I

Genetic Analysis of Resistance to Soybean mosaic virus in OX670 and Harosoy Soybean

Abstract

Soybean mosaic virus (SMV) resistance in OX670 previously was postulated to be controlled by a gene derived from the cultivar 'Raiden' and designated as Rsv2. Subsequently, it was shown that Raiden has only a single resistance gene at the Rsv1 locus, suggesting that the resistance gene in OX670 was not from Raiden. Except for Raiden, 'Harosoy' is the predominant parent in the ancestry of OX670. Therefore, the inheritance of SMV resistance in OX670 and Harosoy was studied to determine the source and identity of the resistance gene/genes in OX670. OX670 and Harosoy were crossed with the SMV susceptible cultivar 'Lee 68' to study the inheritance of resistance. OX670 and Harosoy were also crossed with the resistant cultivars PI96983, L29 and V94-5152, possessing the genes Rsv1, Rsv3, and Rsv4, respectively, to elucidate the allelomorphic relationships between the genes in OX670, Harosoy and previously reported genes. Our results indicate that Harosoy, which is resistant to SMV-G5 through G7 and susceptible to SMV-G1 through G4, possesses a single partially dominant SMV resistance gene at the Rsv3 locus. Inheritance studies indicate that OX670, which is resistant to SMV-G1 through G7, possesses two independent dominant genes for SMV resistance. One is allelic to the Rsv1 locus, and probably derived from Raiden, while the other is allelic to the Rsv3 locus, and probably derived from Harosoy. The Rsv1 gene confers resistance to SMV-G1 through G4 and G7 while the Rsv3 gene confers resistance to only SMV-G5 through G7. The combination of Rsv1 and Rsv3 in OX670 confers resistance to SMV-G1 through G7. Therefore, the previously proposed Rsv2 locus does not appear to exist in OX670 or its ancestors.

Key words: Glycine max, incomplete dominance, disease resistance

Introduction

Soybean mosaic virus (SMV) is one of the most common diseases of soybean [Glycine max (L.) Merr.] (Buss et al., 1989). Since it is seed-borne, it is commonly observed wherever soybean is grown. The disease can cause significant yield reduction (Ross, 1983; Hill et al., 1987 and Ren et al., 1997), change chemical composition of the seed (El-Amretz et al., 1987), cause seed coat mottling (Kwon and Oh, 1983) and reduce seedling viability (El-Amretz et al., 1987) and seedling vigor (Kwon and Oh, 1983). Ren et al. (1997) reported up to 25% yield reductions in the USA and 60% yield reductions were reported by Cho et al. (1977) in Korea resulting from SMV.

SMV is a member of the genus Potyvirus, the largest genus of plant viruses. Numerous SMV isolates have been classified into different pathogenic strain groups in the USA, China and Japan by different investigators. Cho and Goodman (1979, 1982) classified 98 isolates of SMV into seven groups, G1 through G7, based on the differential reactions of a set of soybean cultivars.

SMV can cause three distinct reactions in soybean: mosaic (susceptible-S), systemic necrotic (N) and symptomless (resistant-R). Buzzell and Tu, (1989) suggested that necrosis is a hypersensitive form of resistance. The necrotic reaction often has a more severe effect on the plant than the typical mosaic reaction since death or severe stunting of the plant usually results in little or no seed production (Buss et al., 1989). Depending on the SMV strain, a homozygous soybean genotype can exhibit the necrotic reaction as seen in PI96983 to SMV-G7 or the necrotic reaction can be observed in heterozygotes of segregating populations from resistant x susceptible crosses. There has been disagreement on whether necrotic plants should be classified as susceptible or resistant. The results from most genetic studies suggest that necrotic plants should be included in the resistant class when evaluating segregating populations since necrosis is always associated with resistant genotypes (Shigemori, 1988; Chen et al., 1991, 1993, 1994; Bowers et al., 1992; Ma et al., 1995).

A number of studies have been conducted to elucidate the inheritance of resistance to SMV in soybean. Kiihl and Hartwig (1979) reported a single dominant resistance gene in PI96983, designated Rsv (now Rsv1) and a resistance gene in ‘Ogden’ designated as rsv-t, which was later re-designated as Rsv1-t (Chen et al., 1991). The

single resistance genes in 'York', 'Marshall' and 'Kwanggyo' were found to be alleles at the Rsv1 locus and were assigned the gene symbols, Rsv1-y, Rsv1-m and Rsv1-k, respectively (Chen et al., 1991). These five Rsv1 alleles confer differential reaction to SMV strains SMV-G1 through G7. One of the resistance genes in PI486355 is also an allele at the Rsv1 locus. This allele is resistant to SMV-G1, G2, G3, G4 and G7 but expresses necrosis to SMV-G5 and G6. Due to its unique reaction, the gene symbol Rsv1-s was assigned to this gene in PI486355 (Ma et al., 1995). Ma et al. (1994) reported that the necrotic gene in PI507389, an accession from Japan, is an allele at the Rsv1 locus. That produces lethal necrotic response to SMV-G1, G2, G5 and G6 and a susceptible reaction to SMV-G3, G4 and G7. The gene symbol Rsv1-n was proposed for this gene in PI507389. Wang et al. (1998) reported that PI458507, a germplasm accession from China, has a single dominant gene at the Rsv1 locus. The Rsv1 locus has been mapped to linkage group F and closely linked molecular markers have been reported (Yu et al., 1994).

Buzzell and Tu (1989) found that stem-tip necrosis was controlled by a single gene in 'Columbia' which was dominant to the mosaic reaction. This gene was designated as Rsv3. Bowers et al. (1992) reported that 'Buffalo' and HLS, a late maturing selection from 'Hardee', have single dominant genes at different loci for SMV resistance but their allelism to other reported genes was not determined. Ma (1995) and Buss et al. (1999) reported that the resistance gene in L29, a resistant selection from Williams (6) x Hardee, is an allele at the Rsv3 locus.

Chen et al. (1993) reported that two independent dominant genes in PI486355 conferred SMV resistance, and one of the genes appeared to be at the Rsv1 locus. Later, Ma et al. (1995) separated these two genes into two different lines, LR1 and LR2, and studied the inheritance and allelomorphic relationships of these two genes. The gene in LR1 is allelic to the Rsv1 locus, and the gene in LR2 is independent of Rsv1 and Rsv3 and was tentatively labeled Rsv4. A re-selection of LR2, V94-5152, was released as germplasm (Buss et al., 1997). While the SMV resistance gene in LR1 confers resistance to SMV-G1 through G4 and G7, a systemic necrotic response is observed against SMV-G5 and G6. The SMV resistance gene in LR2 confers resistance to SMV-

G1 through G7 and has been mapped to the D1b linkage group and associated with closely linked molecular markers (Hayes et al., 2000)

Buzzell and Tu (1984), based on the segregation pattern obtained in an F_2 population of OX670(R) x L78-379 (S), reported that OX670 contained a single dominant gene presumably from Raiden. The F_2 population from OX670(R) x L78-379 (S) inoculated with SMV-G7 segregated 3R : 1S, indicating that OX670 possesses a single dominant gene. The F_2 population of the same cross OX670 (R) x L78-379 (R, Rsv1) inoculated with the SMV-G6 strain fit a 15R:1S segregation ratio indicating that the gene in OX670 is independent of the Rsv1 locus. Therefore, the gene symbol Rsv2 was assigned to the resistance gene in OX670. However, it was later shown that Raiden possesses a single SMV resistance gene at the Rsv1 locus (Buss et al., 1995; Wang et al., 1998). These contradictory results suggested that the Rsv2, reported in OX670 was not from Raiden. A pedigree analysis of OX670 reveals that, except for Raiden, Harosoy or its derivatives were the primary ancestors of OX670 (Figure 1). Harosoy was postulated to be susceptible based on its reaction to SMV-G1 (Buzzell and Tu, 1984). Raiden however, is resistant to SMV-G1 through G4 and G7 and necrotic to SMV-G5 and G6 (Buss et al., 1995). On the other hand OX670 exhibits resistant reactions to SMV-G1 through G7 (Buzzell and Tu, 1984). Thus, either the resistance gene in OX670 is not from Raiden or there could be another resistant gene in OX670, which exhibits a resistant reaction to SMV-G5 and G6 strains and makes OX670 resistant to SMV-G1 through G7. Therefore, the objectives of this study were to determine (i) the reaction of OX670 and Harosoy to SMV-G1 through G7 strains; (ii) the inheritance of SMV resistance in Harosoy and OX670 and; (iii) the allelomorphic relationship of resistance genes in these cultivars with previously described resistance genes.

Materials and Methods

OX670 and Harosoy were crossed with the SMV-susceptible cultivar, Lee 68 to study the inheritance of resistance. They were also crossed with resistant lines PI96983, L29 and V94-5152 which contain Rsv1, Rsv3 and Rsv4, respectively, to study the allelomorphic relationships between the resistance genes in OX670, Harosoy and previously identified genes. OX670 was crossed with L88-8431, a Williams isoline with SMV resistance gene Rsv1 derived from Raiden (Bernard et al., 1991) and Harosoy, a potential contributor of the non-Rsv1 gene in OX670. Crosses were made and F_1 plants were grown either in the greenhouse or in the field at Blacksburg, Virginia. F_2 plants were grown in the field without virus inoculation either at Blacksburg or Warsaw, and individual plants were harvested for $F_{2:3}$ progeny. Crosses were distinguished from selfs in the F_1 and F_2 generations using leaf shape, flower color, pubescence color, and maturity as morphological markers.

The F_2 populations and $F_{2:3}$ progeny were tested with SMV-G1 in the greenhouse and field. SMV-G6 and G7 inoculations of genetic populations were conducted only in the greenhouse at Blacksburg. An average of 200 F_2 plants and 20 $F_{2:3}$ plants from each of 50 $F_{2:3}$ families were inoculated. In addition, an average of 15 seeds from each of the parents were planted and inoculated along with segregating populations. Individual plant reactions were examined about 10, 20, 30, and 40 d after inoculation and classified into three distinct groups: resistant (R) (symptomless or local necrotic lesions on inoculated leaves), necrotic (N) stem tip necrosis (that usually kills plants), and susceptible (S) (mosaic) (Figures 1 and 2).

All parents used in this study were tested with SMV-G1 through G7 in the greenhouse to compare their differential reactions. Three strains, SMV-G1, SMV-G6 and SMV-G7 were used to screen F_1 plants, F_2 populations and $F_{2:3}$ families. The isolate of SMV-G1 was originally isolated from 'Lee' soybean in Virginia (Hunst and Tolin, 1982) and is analogous to the SMV-G1 of Cho and Goodman (1979, 1982). Strains SMV-G2 through G7 were originally obtained from Dr. R.M. Goodman in 1984, at the University of Illinois. Cultures of SMV-G1 and SMV-G7 have been deposited as PV-571 and PV613, respectively, in the American Type Culture Collection (10801 University

Boulevard, Manassas, Virginia 20110-2209, USA). The SMV-G1 through G4 cultures were maintained by continuous passage in Lee 68, while SMV-G5 through G7 were maintained on 'York'.

For the greenhouse tests, inocula were prepared from infected trifoliolate leaf tissue homogenized in 0.01 M sodium phosphate buffer solution, pH 7.0, at an approximate rate of 1 g infected tissue per 10 ml buffer. Unifoliolate leaves were inoculated before trifoliolate leaves emerged, approximately 10 d after planting. A small amount of 600-mesh carborundum was dusted on the leaves to be inoculated. Inoculum was applied by rubbing both primary leaves of each plant with a pestle dipped in the inoculum. Inoculated leaves were rinsed with tap water. The differential cultivars were also included in each set of inoculations to confirm the identity and purity of the strain used. A daylength of approximately 14 h was maintained by using both fluorescent and incandescent supplemental lighting during winter months. Greenhouse temperatures were maintained at 24 to 30 ^{o}C during daylight hours and 15 to 20 ^{o}C at night.

Only SMV-G1 strain was used in field inoculations. The procedure used for field inoculation is described in Roane et al. (1983). Randomly selected susceptible and necrotic plants were tested for confirming infection with SMV in the field by dot-blotting immunoassays (Srinivasan and Tolin, 1992).

Results and Discussion

Inheritance and Allelomorphic Relationship of Resistance Gene/Genes in Harosoy

Buzzell and Tu (1984) considered Harosoy to be a susceptible cultivar, probably based on its reaction to SMV-G1. Our results indicate that Harosoy is also susceptible to SMV-G2 through G4, but is resistant to SMV-G5 through G7 (Table 1). F_1 plants of Harosoy (R) x Lee 68 (S) exhibited a lethal necrotic reaction to SMV-G7 at 15 to 20 d after inoculation, indicating that the resistance gene in Harosoy was incompletely dominant (Table 2). The F_2 population of Harosoy (R) x Lee 68 (S) segregated to fit a 1R:2N:1S single gene ratio when the population was inoculated with SMV-G7. Thus, it appears that a single resistance gene is segregating, with the homozygous dominant and

heterozygous genotype being expressed as resistant and necrotic, respectively. The same population was completely susceptible to SMV-G1 strain, verifying the susceptibility of Harosoy to that strain. $F_{2:3}$ families of the same cross segregate to fit a 1 (all R) : 2 [3(R+N):1S] : 1 (all S) genotypic ratio, which verified that Harosoy indeed has a single gene for resistance to SMV-G7 (Table 3).

No susceptible plants were observed in the F_2 populations and $F_{2:3}$ families of Harosoy x L29 when inoculated with SMV-G7 (Tables 2 and 3) indicating that the gene in Harosoy is allelic to the gene at the Rsv3 locus. The F_2 population of L78-379 (N) x Harosoy (R) inoculated with SMV-G7 segregated to fit a digenic ratio of 12R:3N:1S (Table 2). The $F_{2:3}$ families of the same cross segregated 4(all R) : 2(3R:1S) : 2(3R:1N) : 2(3N:1S) : 4(12R:3N:1S) : 1(all N) : 1(all S) (Table 4), indicating that the SMV resistance gene in Harosoy is independent of the SMV resistance gene, Rsv1, in L78-379.

Segregation in the F_2 population of Harosoy (R) x V94-5152 (R) conformed to a digenic ratio when tested with G7 (Table 2). This result was conformed by the observed 7 (all R) : 4[3(R+N):1S] : 4[15(R+N):1S] : 1 (all S) segregation pattern in the $F_{2:3}$ families (Table 5), and verifies that the single dominant gene in Harosoy is independent of the Rsv4 locus of V94-5152.

Inheritance of resistance to SMV strains G1 and G7 in OX67O

OX670 exhibits resistant reactions to SMV-G1 through G7 (Table 1). The F_2 population from OX670 (R) x Lee 68 (S) segregated to fit the single gene ratio of 3(R+N) : 1S when inoculated with SMV-G1 (Table 6). However, a fit to the digenic ratio of 15(R+N):1S was observed when SMV-G7 was used (Table 6). These results indicate that OX670 has two genes; one is resistant to SMV-G1 and G7, and the other gene is also resistant to SMV-G7 but susceptible to SMV-G1. The overall segregation of the $F_{2:3}$ families from OX670 (R) x Lee 68 (S) conformed to the 1(all R): 2 [3(R+N):1S] :1(all S) ratio expected with SMV-G1 (Table 7) and fit a 7(all R): 4[15(R+N):1S]:4[(3R+N):1S]:1(all S) ratio when SMV-G7 was used (Table 8). This study verifies the presence of two genes in OX670 for SMV resistance.

F_1 plants from OX670 x Lee 68 were symptomless when inoculated with SMV-G7, and necrotic when inoculated with SMV-G1 (Table 6). Therefore, one of the genes in OX670 confers a necrotic reaction to SMV-G1 in the heterozygous condition. However, the frequency of necrotic plants was well below that expected for heterozygotes in the F_2 population and $F_{2:3}$ families (1% of the total plants) (Table 6). Such a low number of necrotic plants might be result of environmental factors. While F1 plants and F2 populations were planted and inoculated at different time of the year in the greenhouse, F2:3 families were planted and inoculated in the field. In addition no homogenous necrotic $F_{2:3}$ lines were observed, indicating that the necrotic reaction was not expressed by a homozygous genotype. Because of the reaction of the F_1 plants to SMV-G1, necrotic (heterozygous resistant) plants were grouped in the resistant class for genetic tests. The association between heterozygosity and necrosis was reported by other investigators, and necrotic plants were combined with the resistant plants for genetic analysis (Kiihl and Hartwig 1979; Shigemori, 1988; Buss et al., 1989; Bowers et al., 1992; Chen et al., 1991, 1994; Ma et al., 1995). Although many investigators have reported the association, still it is not clear why all heterozygous plants in F_2 and $F_{2:3}$ populations do not show the necrotic reaction.

Allelic Relationship of the Resistance Genes in OX670 with Rsv1

No susceptible plants were found in the F_2 and $F_{2:3}$ populations of the cross between PI96983 (Rsv1) and OX670, when inoculated with SMV-G1, G6, and G7 (Tables 6, 9, and 10). The lack of susceptible plants indicates that one of the genes in OX670 is an allele at the Rsv1 locus.

Necrotic plants were observed in the F_2 of PI96983 (R) x OX670 (R) subjected to SMV-G1 in a 15(R):1(N) ratio (Table 6). Of seventy $F_{2:3}$ families, eight segregated for the necrotic reaction (Table 7). However, each family had only one or two necrotic plants and there were no homogeneous necrotic $F_{2:3}$ families, suggesting that the necrotic plants observed in this population did not appear to represent genetic segregation. Necrotic plants previously have been reported in R(Rsv1) x R (Rsv1) crosses and were assumed to not be the result of genetic segregation (Chen et al., 1994; Ma, 1995).

The F_2 population from PI96983 (N) x OX670 (R) inoculated with SMV-G7 segregated to fit a 13(R) : 3 (N) ratio (Table 6), and $F_{2:3}$ families segregated 7 (all R) : 2 (3R:1N) : 2(3N:1R) : 4(15R:1N) : 1 (all N) (Table 10), indicating digenic segregation. In contrast, Buzzell and Tu (1984) obtained a 3(R):1"S" monogenic ratio from the F_2 population of L78-379("S") x OX670 (R) using SMV-G7. L78-379 contains Rsv1 from PI96983 and is necrotic to G7, which Buzzell and Tu (1984) classified as susceptible, hence the "S" designation here. The 3 : 1 ratio is statistically close to the 13(R):3(N), that we obtained from the F_2 of PI96983(N/"S") x OX670 (R) (Table 6). Thus it seems likely that most, if not all, of the susceptible plants reported by Buzzell and Tu were actually necrotic. In fact, no susceptible plants were found in the segregating populations of PI96983 x OX670 inoculated with SMV-G7 in the present study (Tables 6 and 10).

Segregation of the F_2 population of PI96983 (R) x OX670 (R) inoculated with SMV-G6 fit a 15(R):1(N) ratio (Table 6), which supported digenic segregation. $F_{2:3}$ families of the same cross, segregated to fit a 7(all R) : 4(3R:1N) : 4(15R:1N) : (1 all N) genotypic ratio (Table 9). Obviously, one of the SMV resistance genes in OX670 produces a necrotic reaction to SMV-G6 in the homozygous condition since homogeneous necrotic $F_{2:3}$ families were observed. The pedigree of OX670 (Figure 1) shows that Raiden, Harosoy, or Harcor are possible sources of SMV resistance genes in OX670. Harosoy is resistant to SMV-G6 and possesses an SMV resistance gene at the Rsv3 locus (Gunduz et al., 1999). Raiden, however, is necrotic to SMV-G6 and contains an SMV resistance gene at the Rsv1 locus (Buss et al., 1995). Thus, it is logical to assume that the Rsv1 gene in OX670, derived from Raiden, was the source of the necrotic reaction observed in the segregating populations of PI96983 (R) x OX670 (R) since the Rsv1 allele in PI96983 is resistant to SMV-G6. Buzzell and Tu (1984) obtained a 15R : 1"S" ratio from F_2 populations of L78-379 (R) [Williams(6) x PI96983] x OX670 (R) inoculated with SMV-G6 and concluded that the resistance gene in OX670 is independent of Rsv1. The F2 segregation patterns in Buzzell and Tu (1984) and the present study are the same; however, Buzzell and Tu classified necrotic plants as susceptible resulting in a different interpretation.

No susceptible plants, as well as no necrotic plants of consequence, were found in the F_2 population and $F_{2:3}$ families of L88-8431(R, Rsv1) x OX670 crosses when SMV-

G7 was used (Tables 6 and 7), verifying that one of the genes in OX670 is an allele at the Rsv1 locus and is derived from Raiden. L88-8431 is a Williams BC_5 isoline with SMV resistance derived from Raiden (Bernard et al., 1991). The two necrotic plants observed in the F_2 (Table 6), probably were either mechanical mixtures of seeds or the result of viral contaminations. A low percentage of necrotic plants in RxR crosses involving resistance alleles at the Rsv1 locus was also observed in a previous study (Chen et al., 1991).

Genetic analysis of crosses between OX670 and parents containing Rsv1 clearly demonstrated that one of the genes in OX670 is allelic to Rsv1. It is most likely derived from Raiden, because Raiden is the only parent in the pedigree of OX670 that possesses Rsv1 based on reaction to SMV-G1. Moreover, Raiden and the Rsv1 allele in OX670 exhibit identical reactions to SMV-G1, G6, and G7. Both exhibit a resistant reaction to SMV-G1 and G7 (Tables 1, 6, 7, and 8) and a necrotic reaction to SMV-G6 (Tables 1, 6, and 9). The genetic model explaining these results is presented in Table 13.

Allelic Relationship of the Resistance Genes in OX670 with Rsv3 and Rsv4

No susceptible plants were observed in the F_2 populations of L29 (R, Rsv3) x OX670 (R) or OX670 (R) x Harosoy (R, Rsv3) when inoculated with SMV-G7 in the greenhouse (Table 6). Nor were any susceptible plants found in the $F_{2:3}$ families from the same crosses (Table 7), which confirms that one of the genes in OX670 is an allele at the Rsv3 locus. These data and the pedigree information strongly suggest that the Rsv3 gene in OX670 was derived from Harosoy. Harcor is a descendent of Harosoy and exhibits the same reaction to G1 and G7. Apparently, Harcor inherited the gene from Harosoy (Figure 1). The combination of the Harosoy and Raiden genes likely occurred when Raiden was crossed with Harcor (Figure 1), since OX315 is resistant to SMV-G1 through G7 (Buzzell and Tu, 1984). The genes from the two sources complement each other, since Harosoy is resistant only to SMV-G5, G6 and G7 and Raiden is necrotic to SMV-G5 and G6 and resistance to the other strains (Table 1).

The F_2 population from OX670 (R) x V94-5152 (R, Rsv4) segregated to fit a digenic ratio of 15 (R+N) : 1 (S) when inoculated with SMV-G1 (Table 6). When the F_2

population of the same cross was inoculated with SMV-G7, a 63 (R+N) : 1 (S) trigenic ratio was obtained. The observed segregation pattern among $F_{2:3}$ families from the same cross with SMV-G1 fit a 7 (All R) : 4[3(R+N):1S] : 4[15(R+N):1S] : 1 (all S) genotypic ratio expected for two genes (Table 11). When tested with SMV-G7, families segregating 63(R+N):1S were combined with the homozygous resistant class because the number of plants tested for each $F_{2:3}$ family were insufficient to distinguish between the two classes. Therefore, a 45 [All R + 63(R+N):1S] : 12 [15(R+N):1S]: 6 [3(R+N) :1S] : 1 (all S) trigenic ratio was tested against the actual $F_{2:3}$ segregation with SMV-G7 (Table 12). The good fit indicated that neither of the genes in OX670 is allelic to the gene at the Rsv4 locus.

In general, the disagreement between the conclusion of Buzzell and Tu (1984) and that of current study regarding genes governing SMV resistance in OX670 can be attributed to the difference in classification of the necrotic plants, including the necrotic reaction of L78-379. Also, Buzzell and Tu (1984) apparently were not aware that Harosoy is resistant to SMV-G5, G6, and G7. Now it is known that OX670 also carries a resistance gene from Harosoy, which explains why our PI96983 x OX670 F_2 and $F_{2:3}$ (Table 6 and 9) and L78-379 x OX670 F_2 population of the Buzzell and Tu (1984) all exhibited digenic segregation patterns for the necrotic reaction. If we assume that all plants classified as "susceptible" by Buzzell and Tu (1984) were actually necrotic, all observations fit well with the genetic model in Table 13. When inoculated with SMV-G6, only the $Rsv1^{r}Rsv1^{r}rsv3rsv3$ genotype shows the necrotic reaction. Gene Rsv1r is derived from Raiden, which exhibits the necrotic reaction to SMV-G6 (Buss et al., 1995). When SMV-G6 was used, $F_{2:3}$ families of L78-379 x OX670 segregated to fit the expected genotypic ratio 7(allR) : 4(15R:1N) : 4(3R:1N) : 1(all N) (Table 9). None of the $F_{2:3}$ families segregated to fit a 3N:1R ratio, indicating that the resistant reaction of Rsv1 in PI96983 is dominant to the necrotic reaction of the resistance gene Rsv1r in Raiden when SMV-G6 is used. According to our genetic model Rsv1Rsv1rsv3rsv3 and $Rsv1Rsv1^{r}$ rsv3rsv3 genotypes give the necrotic reaction against SMV-G7. In fact, some $F_{2:3}$ families segregated 3N:1R when SMV-G7 was used (Table 10), indicating that the necrotic reaction of Rsv1 is dominant to the resistant reaction conferred by Rsv1r. Thus,

it appears that the resistance allele in PI96983 is dominant to the resistance allele in Raiden, regardless of the SMV strain used.

Results of this study clearly indicate that OX670 possesses two genes for resistance to SMV. One of the genes in OX670, derived from Raiden, is allelic to Rsv1, and the other, derived from Harosoy, is allelic to Rsv3. Therefore, the previously designated Rsv2 locus does not appear to exist in OX670 or its ancestors. Typical of alleles at the Rsv1 locus, the Rsv1 allele from Raiden in OX670 is incompletely dominant, as evidenced by the necrotic reaction of heterozygotes when inoculated with SMV-G1 (Tables 1and 6). In addition, the Rsv1 allele from Raiden is not resistant to all strains of SMV. The Rsv3 allele from Harosoy was also partially dominant when inoculated with SMV-G7, and is resistant only to SMV-G5 to G7 (Tables 1 and 2). The presence of Rsv1 and Rsv3 in OX670 confers resistance to SMV-G1 through G7, combining the resistances of both genes.

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Figure 1. Mosaic symptoms of soybean mosaic virus (SMV) on ‘Lee 68’ [17 days after inoculation (dai)with SMV-G1]

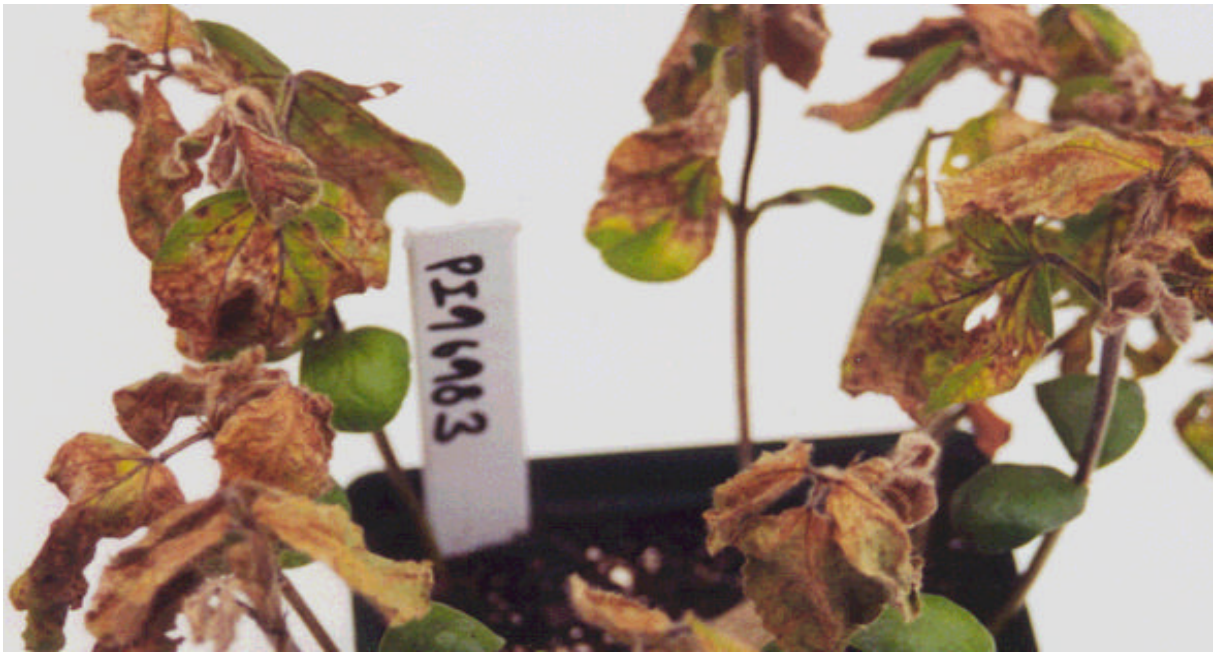

Figure 2. Necrotic reaction on PI96983, when inoculated with SMV-G7 in the greenhouse (12 dai).

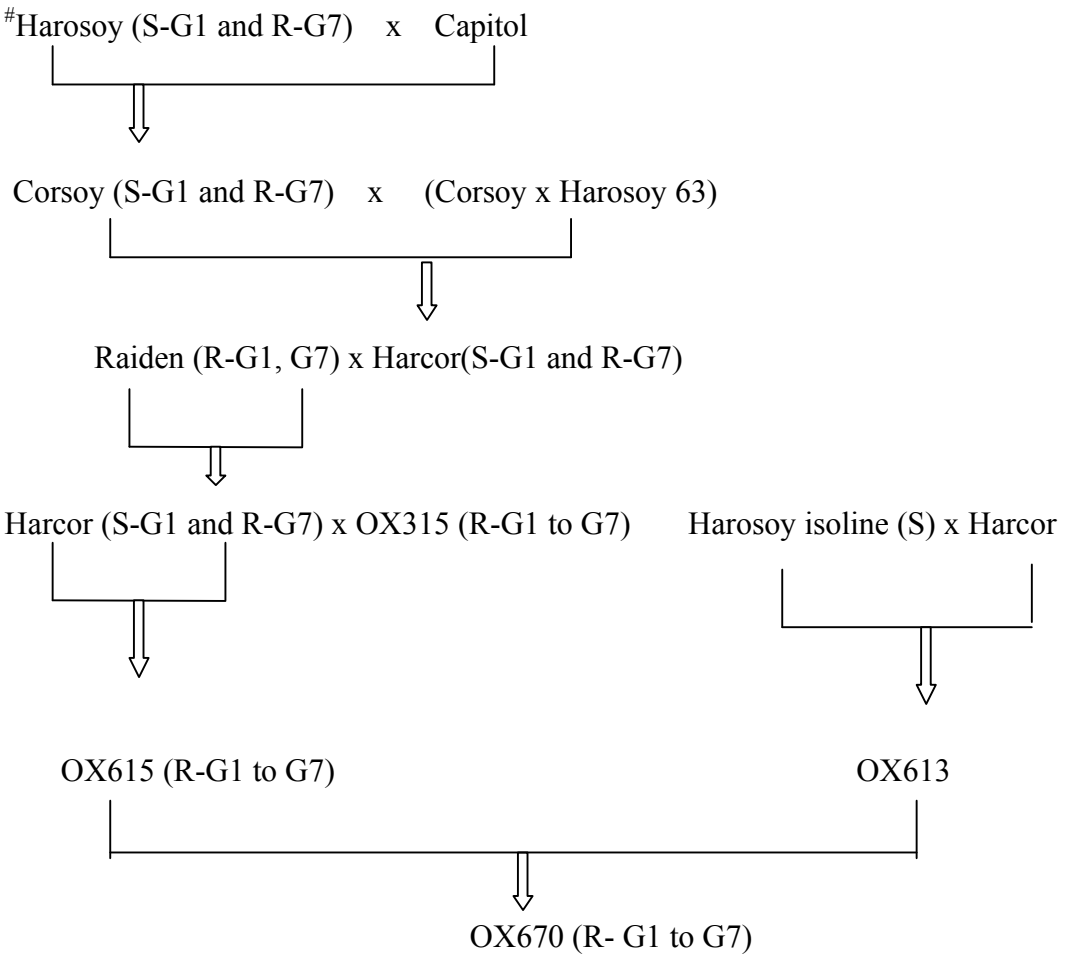

Figure 1. The Pedigree of the soybean line OX670 and its ancestral reactions to SMV strains

R, resistant; S, susceptible

Pedigree information obtained from Buzzell and Tu (1984) and National Plant Germplasm System web page http:// www.ars-grin.gov/npgs/

Table 1. Disease reaction of a set of soybean differential genotypes and cultivars, to seven SMV strain groups.

	SMV strains						
Parents	G1	G2	G3	G4	G5	G6	G7
OX670	R†	R	R	R	R	R	R
Raiden	R	R	R	R	N	N	R
L88-8431	R	NA	R	NA	N	N	R
Harosoy	S	S	S	S	R	R	R
Harcor	S	NA	NA	NA	NA	NA	R
‡L29(Rsv3)	S	S	S	S	R	R	R
‡PI96983(Rsv1)	R	R	R	R	R	R	N
‡L78-379(Rsv1)	R	R	R	R	R	R	N
‡V94-5152(Rsv4)	R	R	R	R	R	R	R

† R, resistant (symptomless); N, necrotic; S, susceptible (mosaic); NA, not available

‡ L29 (Buss et al., 1999); L78-379 (Buzzell and Tu, 1984 and Bernard et al., 1991); L88-8431 (Buss unpublished data); L29 (Buss et al., 1999); PI96983 (Chen et al., 1991); V94-5152 (Buss et al., 1998)

Table 2. Reaction of F_1 plants and F_2 populations from crosses between Harosoy and susceptible or resistant parents, when inoculated with SMV-G1 and G7 in the greenhouse.

Cross and Generation	SMV	Number of plants			Expected		
	Strain	R†	N	S	ratio	χ^2	P
Harosoy x Lee 68, F_1	G7	0	3	0			
Harosoy x Lee 68, F_2	G7	92	142	76	1R:2N:1S	3.42	0.18
Harosoy x Lee 68, F_2 allelism	G1	0	0	120			
L78-379 (Rsv1)x Harosoy, F_2	G7	174	39	18	12R:3N:1S	1.31	0.52
Harosoy x L29(Rsv3), F_2	G7	153	0	0			
Harosoy x L29 (Rsv3), F_2	G1	0	0	127			
Harosoy x V94-5152 (Rsv4), F_2	G7	130	26	12	15(R+N):1S	0.12	0.73

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 3. Segregation of $F_{2:3}$ families when inoculated with SMV-G7 in the greenhouse and G1 in the field.

Crosses	SMV Strain	Number of families			Expected Ratio	χ^2	P
		All R†	H	All S			
Lee 68(S)x Harosoy(R)	G7	20	27	23	1all(R+N) 2[3(R+N):1S] 1 all S	3.8	0.15
Lee 68(S) x Harosoy(S)	G1#	0	0	20			
Harosoy(R) x L29 (R,Rsv3)	G7	44	0	0			
Harosoy(S) x L29(S)	G1#	0	0	20			

†R: resistant (symptomless); H, segregating for resistant, susceptible and necrotic reaction; S, susceptible.
Data obtained from field

Table 4. Segregation of $F_{2:3}$ families from L78-379 (Rsv1) x Harosoy when inoculated with SMV-G7 in the greenhouse.

		Number of families			
Reaction†	Frequency	Observed	Expected	χ^2	P
All R	4	10	11.3	0.140	
3R : 1S	2	5	5.6	0.064	
3R : 1N	2	5	5.6	0.064	
3N : 1S	2	9	5.6	2.025	
12R : 3N : 1S	4	9	11.3	0.450	
All N	1	2	2.8	0.229	
All S	1	5	2.8	0.777	
Total	16	45	45	3.749	0.71

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 5. Segregation of $F_{2:3}$ families from Harosoy x V94-5152 (Rsv4) when inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families		χ^2	P
		Observed	Expected		
All R	7	18	18.8	0.03	
3(R+N) : 1S	4	13	10.8	0.44	
15(R+N) : 1S	4	11	10.8	0.003	
All S	1	1	2.7	1.07	
Total	16	43	43	1.54	
$\chi^2_{(7:4:4:1)}$				1.54	0.65

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 6. Reactions of F_1 plants and F_2 populations from crosses of OX670 with Lee 68 and known sources of Rsv1, Rsv3 nad Rsv4 when inoculated with SMV-G1, G6 and G7.

Cross and Generation	SMV Strain	Number of plants			Expected ratio	χ^2	P
		R†	N	S			
OX670(R) x Lee 68(S) F_1	G1	0	5	0			
OX670(R) x Lee 68(S) F_2	G1	72	1	21	3(R+N):1S	0.35	0.55
OX670(R) x Lee 68(S) F_1	G7	4	0	0			
OX670(R) x Lee 68(S) F_2	G7	360	48	22	15(R+N):1S	0.94	0.33
Allelism							
PI96983 (R,Rsv1)xOX670(R) F_2	G1	124	10	0	15R:1N	0.33	0.56
PI96983(R,Rsv1)xOX670(R) F_2	G6	117	11	0	15R:1N	1.23	0.27
PI96983(R,Rsv1)xOX670(R) F_2	G7	313	71	0	13R:3N	0.01	0.90
L29 (R,Rsv3)x OX670(R) F_2	G7	303	0	0			
OX670(R) x Harosoy (R,Rsv3) F_2	G7	224	0	0			
L88-8431(R,Rsv1) x OX670(R) F_2	G7	136	2	0			
OX670(R) x V94-5152(R,Rsv4) F_2	G1	120	4	11	15(R+N):1S	0.83	0.36
OX670(R) x V94-5152(R,Rsv4) F_2	G7	419	20	5	63(R+N):1S	0.55	0.46

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible (mosaic)

Table 7. Segregation of $F_{2:3}$ families derived from crosses of OX670 with susceptible and resistant soybean genotypes inoculated with SMV-G1 and G7.

Crosses	SMV Strain	Number of families			Expected ratio	χ^2	P
		All R†	H	All S			
OX670(R) x Lee 68(S)[#]	G1	22	53	16	1all R :2 [(3R+N):1S]: 1 all S	3.26	0.20
L29 (Rsv3)x OX670 (R)	G7	65	0	0			
OX670(R) x Harosoy (R,Rsv3)	G7	50	0	0			
PI96983 (R,Rsv1) x OX670 (R)[#]	G1	70[##]	0	0			
L88-8431(R,Rsv1) x OX670 (R)	G7	42	0	0			

†R: resistant (symptomless); H, segregating for resistance, necrotic and susceptible; S, susceptible ; N, necrotic.
[#] Data obtained from field
[##] One or two necrotic plants observed in 8 $F_{2:3}$ families

Table 8. Segregation of $F_{2:3}$ families from OX670 x Lee 68 inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families			
		Observed	Expected	χ^2	P
All R	7	26	24.9	0.04	
3(R+N):1S	4	15	14.3	0.03	
15(R+N) : 1S	4	13	14.3	0.11	
All S	1	3	3.6	0.08	
Total	16	57	57	0.28	
$\chi^2_{(7:4:4:1)}$				0.28	0.96

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 9. Segregation of $F_{2:3}$ families from PI96983 x OX670 inoculated with SMV-G6 in the greenhouse.

Reaction†	Frequency	Number of families			
		Observed	Expected	χ^2	P
All R	7	23	19.75		
3R:1N	4	11	11		
15R : 1N	4	7	11		
All N	1	3	2.75		
Total	16	44	44		
$\chi^2_{(7:4:4:1)}$				2.01	0.57

† R, resistant (symptomless); N, stem tip necrosis

Table 10. Segregation of $F_{2:3}$ families from PI96983 x OX670 inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families		$\chi^2_{7:2:2:4:1}$	P
		Observed	Expected		
All R	7	14	18.4		
3R:1N	2	6	5.3		
3N:1R	2	9	5.3		
13R : 3N	4	10	10.5		
All N	1	3	2.6		
Total	16	42	44	3.902	0.42

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 11. Segregation of $F_{2:3}$ families from OX670 x V94-5152 inoculated with SMV-G1 in the greenhouse.

Reaction†	Frequency	Number of families			
		Observed	Expected	$\chi^2_{7:4:4:1}$	p
All R	7	27	25		
3R:1S	4	15	14.3		
15(R+N) : 1S	4	10	14.3		
All S	1	5	3.6		
Total	16	57	57	2.06	0.56

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 12. Segregation of $F_{2:3}$ families from OX670 x V94-5152 inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families			
		Observed	Expected	$\chi^2_{45:12:6:1}$	P
All R + 63(R+N):1S	45	56	59.8		
15(R+N) : 1S	12	18	15.9		
3R:1S	6	10	7.9		
All S	1	1	1.3		
Total	64	85	85	1.1	0.77

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 13. A proposed genetic model for the R x R and R x N crosses between OX670 and PI96983[##].

F_2 genotype	Frequency	F_2 reaction to SMV-G6	$F_{2:3}$ reaction to SMV-G6	F_2 reaction to SMV-G7	$F_{2:3}$ reaction to SMVG7
$R_1R_1R_3R_3$[#]	1	R†	All R	R	All R
$R_1R_1R_3r_3$	2	R	All R	R	3R:1N
$R_1R_1r_3r_3$	1	R	All R	N	All N
$R_1R_1^rR_3R_3$	2	R	All R	R	All R
$R_1R_1^rR_3r_3$	4	R	15R:1N	R	15R:1N
$R_1R_1^rr_3r_3$	2	R	3R:1N	N	3N:1R
$R_1^rR_1^rR_3R_3$	1	R	All R	R	All R
$R_1^rR_1^rR_3r_3$	2	R	3R:1N	R	All R
$R_1^rR_1^rr_3r_3$	1	N	All N	R	All R
Total	16	15R:1N	7R:8H:1N	13R:3N	7R:8H:1N

† R, resistant (symptomless); N, systemic necrosis; S, susceptible (mosaic);
H, segregating for necrotic reaction

[#]R_1, Rsv1 gene from PI96983; R_1^r, Rsv1 gene from Raiden; R_3, Rsv3 gene in OX670

[##] PI96983 is resistant to SMV-G6 and produce a necrotic reaction to SMV-G7.

CHAPTER II

Characterization of SMV Resistance Genes in Tousan 140 and Hourei Soybean

Abstract

Tousan 140 and Hourei are two large-seeded soybean (Glycine max Merill.) accessions from Japan. It has been reported that these two accessions possess single gene at different loci for soybean mosaic virus (SMV) resistance to Japanese strain SMV C. The objective of this study was to determine i) the reaction of these two accessions to SMV-G1 through G7, ii) the inheritance and allelomorphic relationship of resistance genes in Tousan 140 and Hourei using SMV-G1 and G7 strains. Tousan 140 and Hourei were crossed with SMV susceptible cultivar Lee 68 to study the inheritance of resistance. They were also crossed with PI96983 and L78-379, possessing Rsv1, L29 and Harosoy, possessing Rsv3, and with V94-5152 possessing the Rsv4 gene to elucidate the allelomorphic relationships among the genes in Tousan 140, Hourei, and previously reported genes. Tousan 140 and Hourei were also crossed with each other to determine the allelic relationship of the SMV resistance genes in these cultivars.

Inheritance and allelism studies indicated that Tousan 140 possesses two SMV resistance genes. These two genes were separated in two F2:3 lines. One of the genes an allele of Rsv1 expresses resistance to SMV-G1 through G3 and susceptibility to SMV-G5 through G7 while the other one, an allele of Rsv3, expresses resistance to SMV-G5 through G7 and susceptibility to SMV-G1 through G3. Their presence in Tousan 140 makes it resistant to strains SMV-G1 through G7.

Hourei also is resistant to SMV-G1 through G7 and possesses two SMV resistance genes, which are also alleles of Rsv1 and Rsv3. One, probably the Rsv1 allele, expresses resistance to SMV-G1 and G7 and the other, probably the Rsv3 allele, expresses resistance to SMV-G7 but is susceptible to G1.

Key words: Glycine max, soybean mosaic virus, incomplete dominance, disease resistance, hypersensitive response, gene pyramiding, allelism

Introduction

Interaction between strains of soybean mosaic virus (SMV) and soybean genotypes results in four distinct reactions including resistant (symptomless), systemic necrotic, susceptible (mosaic) (Cho and Goodman, 1979, 1982), and late susceptible (early resistance) (Buss unpublished data).

Conover (1948) first recognized that soybean mosaic disease is caused by more than one strain of SMV. Since then, different isolates of SMV strains have been reported. These SMV isolates have been classified into different groups in Japan (Takahashi et al., 1963, 1980), the United States (Cho and Goodman, 1979, 1982), Taiwan (Han and Murayama, 1970), Korea (Cho et al., 1977), Brazil (Almeida, 1981), and China (Pu et al., 1982; Xu et al., 1983; Chen et al., 1986). In the United States, Cho and Goodman (1979) classified 98 SMV isolates into seven strain groups, G1 through G7, based on symptoms induced on a set of soybean differential cultivars. In Japan, 102 SMV isolates were classified into five strains, A,B,C,D and E, based on symptoms induced on four soybean cultivars, Harosoy, Shiromame, Ou 13, and Tokachinagaha (Takahashi et al. 1980). Eight isolates from China were designated Sa through Sh (Pu et al.,1982; Chen et al., 1986).

Many investigators have studied the inheritance of SMV resistance in soybean to determine the nature and relationship of resistance genes. To date, three genes conferring resistance to SMV, Rsv1, Rsv2 and Rsv3, have been identified. Eight alleles have been identified at the Rsv1 locus. Kiihl and Hartwig (1979) identified a single dominant gene for SMV resistance in PI96983 and designated it Rsv (later renamed Rsv1). Also a resistance gene in Ogden was designated as rsv-t but later was changed to Rsv1-t by Chen et al. (1991). The single resistance genes in 'York', 'Marshall' and 'Kwanggyo' were found to be alleles at the Rsv1 locus and were assigned the gene symbols, Rsv1-y, Rsv1-m and Rsv1-k, respectively (Chen et al., 1991). These five Rsv1 alleles confer differential reaction to SMV strains G1 through G7. Ma et al. (1994) and Ma (1995) reported that PI507389 exhibits a necrotic reaction to G1 and a susceptible reaction to G7, which is controlled by a single gene at the Rsv1 locus that is recessive to the resistance alleles in

PI96983, York, and Marshall. One of the genes in PI486355 was also found to be at the Rsv1 locus and was designated Rsv1-s (Chen et al., 1993; Ma et al., 1995). The SMV resistance gene in Suweon 97, exhibits resistance to SMV-G1 through G7. This gene is also located at the Rsv1 locus (Chen et al., 1999). General behavior of resistance genes at the Rsv1 locus can be summarized as follows:

1. Generally, alleles at the Rsv1 locus do not confer resistance to all SMV strains (G1 to G7), and exhibit necrotic or susceptible reaction to one or more of the strains. The gene in Suweon 97 is an exception since it is resistant to all strains.
2. Resistance alleles at the Rsv1 locus are incompletely dominant, as evidenced by necrotic reaction of F_1 plants of R (Rsv1) x susceptible crosses.
3. Resistance alleles at the Rsv1 locus generally exhibit resistant reactions to the "less virulent" strains (G1, G2, G3, G4), and necrotic or susceptible reactions to more virulent strains (G5 through G7). Exceptions include the Rsv1-r allele in Raiden (Buss et al., 1995) and the Rsv1-s allele derived from PI486355 (Ma et a., 1995) which exhibit resistant reaction to SMV-G7.

The necrotic reaction generally is observed in progeny of resistant (Rsv1) x susceptible crosses that are heterozygous for Rsv1. Although there has been disagreement whether necrotic plants should be classified as susceptible or resistant, results from some genetic studies indicate that only a portion of the heterozygous plants exhibit the necrotic reaction. Therefore, necrotic plants usually are included in the resistant class when evaluating segregating populations (Chen et al., 1994). The same association and conclusion have been obtained by other researchers (Kiihl and Hartwig, 1979; Shigemori, 1988; Chen et al., 1991, 1993; Bowers et al., 1992; Ma et al., 1995; Ma and Buss, 1995). Necrotic plants also were observed in the segregating populations of resistant (Rsv1) x resistant (Rsv1) crosses (Chen et al., 1991; Ma, 1995).

Two resistance alleles at the Rsv3 locus have been identified. Buzzell and Tu (1989) reported that the gene derived from 'Columbia' conferring necrotic reaction is independent of the Rsv1 and Rsv2 loci, and assigned it the Rsv3 gene symbol. Bowers et al. (1992) found that 'Buffalo' and 'HLS', a late maturing selection from the 'Hardee', have single dominant genes at different loci for SMV resistance but allelism tests with

reported loci were not conducted. Ma (1995) and Buss et al. (1999) reported that a resistance gene in L29, which is a resistant selection from 'Williams' (6) x Hardee, is allelic to Rsv3. 'Harosoy' possesses a single dominant gene at the Rsv3 locus that exhibits the same reaction to G1 and G7 as the gene in L29. Therefore, Harosoy and L29 may possess the same alleles at the Rsv3 locus. Generally, the resistance alleles at this locus exhibit resistance to SMV-G5, G6 and G7, but are susceptible or necrotic to SMV-G1 through G4.

Ma et al. (1995) reported that the single gene conferring SMV resistance in the line LR2, derived from PI486355, is independent of the Rsv1 and Rsv3 loci and therefore tentatively was designated Rsv4. LR2, an F_2 derived line from PI486355 x 'Essex', was reselected and developed into a homozygous breeding line, V94-5152, which was released as germplasm (Buss et al., 1997). Rsv4 confers resistance to SMV-G1 through G7. Interestingly, no local or systemic necrotic reaction has been observed with the Rsv4 gene (Ma et al., 1995; Buss personal communication).

Many of the SMV resistance genes studied to date are at the Rsv1 locus, and the majority of them exhibit resistance to lower-numbered strains of SMV and susceptibility or necrotic reaction to higher-numbered strains. On the other hand, alleles at the Rsv3 locus show a susceptible reaction to lower-numbered strains and a resistant reaction to higher-numbered strains. Complementary resistance conferred by these two loci can be combined to develop genotypes possessing resistance to SMV-G1 through G7. The Rsv4 locus exhibits resistance to all SMV strain groups reported by Cho and Goodman (1979, 1982). Although, some alleles possess relatively broad resistance, reliance on a single gene will result in genetic uniformity and potential vulnerability. Cultivars resistant to common SMV strains in Korea exhibited extreme necrosis when a new strain, SMV-N, arose (Cho et al. 1977). This situation caused great concern among soybean breeders and farmers alike. Thus, soybean breeders need to increase the diversity of SMV resistance genes used in order to develop cultivars with more durable resistance to SMV.

Shigemori (1988) found that the cultivars, Tousan 140, Hourei, and Suzuyataka each possesses a single gene conferring resistance to the C strain of SMV in Japan. The resistance gene in Tousan 140 was shown to be independent of the resistance gene in Hourei and Suzuyataka, which appeared to be allelic. Segregation of the F_2 populations

from resistant x resistant crosses of Tousan 140 and Hourei fit a ratio of 15 (resistant+necrotic) : 1 mosaic. An allelism test was not conducted to determine the relationships between these genes and previously reported loci. Therefore, the objectives of this study were to determine: i) the reaction of Tousan 140 and Hourei to SMV-G1 through G7 strains; ii) the inheritance of SMV resistance in Tousan 140 and Hourei and; iii) the allelomorphic relationship of resistance gene/genes in these accessions with previously known resistance genes using SMV strains grouped by Cho and Goodman (1979, 1982).

Materials and Methods

Tousan 140 and Hourei were crossed to the SMV susceptible cv Lee 68 to develop genetic population for determining the number of resistance genes in these accessions. To investigate the allelomorphic relationship between resistance genes in Tousan 140 and Hourei and previously identified genes, these accessions were crossed to resistant lines PI96983, L29 and V94-5152 possessing known genes Rsv1, Rsv3 and Rsv4, respectively. Crosses were made either in the greenhouse or in the field at Blacksburg, Virginia. F_1 plants were grown in the greenhouse or in the field at Blacksburg. F_2 plants were grown in the field without virus inoculation either at Blacksburg or Warsaw, and individual plants were harvested to obtain $F_{2:3}$ families. Crosses were distinguished from selfs in F_1 and F_2 generations using leaf shape, flower color, pubescence color and maturity as morphological markers.

The F_2 populations and $F_{2:3}$ families were tested with SMV-G1 in the greenhouse and field and with G7 only in the greenhouse at Blacksburg, Virginia. An average of 200 F_2 plants per population and 20 $F_{2:3}$ plants from each of 50 $F_{2:3}$ families were inoculated. Individual plant reactions in F_2 populations and $F_{2:3}$ families were examined about 10, 20, 30 and 40 d after inoculation and classified into three distinct groups: resistant (R) (symptomless or local necrotic lesions on inoculated leaves), necrotic (N) (necrosis develops as stem tip necrosis and may kill plants 10-15 days after inoculation), and susceptible (S) (mosaic). Systemic necrotic plants were observed in F_2 populations, and $F_{2:3}$ families and were combined with resistant plants for genetic analysis.

All parents used in this study were tested with SMV-G1 through G7 in the greenhouse to compare their differential reactions. Two strains, SMV-G1 and SMV-G7, were used to screen F_1 plants, F_2 populations, and $F_{2:3}$ families. The strain SMV-G1 was originally isolated from 'Lee' soybean in Virginia and is analogous to the SMV-G1 of Cho and Goodman (1979, 1982) with respect to reactions induced on the soybean differentials (Hunst and Tolin, 1982). Strains SMV-G2 through G7 were originally obtained from Dr. R. M. Goodman in 1984, at the University of Illinois. Cultures of SMV-G1 and SMV-G7 have been deposited as PV-571 and PV-613, respectively, in the American Type Culture Collection (10801 University Boulevard, Manassas, Virginia 20110-2209 USA).

The SMV-G1 through G4 cultures were maintained by continuous passage in Lee 68, while SMV-G5 through G7 were maintained in York. For the greenhouse tests, inocula were prepared from infected trifoliolate leaf tissue homogenized in 0.01 M sodium phosphate buffer solution, pH 7.0, at an approximate rate of 1 g infected tissue per 10 ml buffer. Unifoliolate leaves were inoculated before triofoliolate leaves emerged, approximately 10 d after planting. A small amount of 600 mesh carborundum was dusted on the leaves to be inoculated. Inoculum was applied by rubbing both unifoliolate leaves of each plant with a pestle dipped in the inoculum. Inoculated leaves were rinsed with tap water. The differential cultivars were also included in each test to confirm the identity and purity of the strain used. A daylength of approximately 14 h was maintained using both fluorescent and incandescent supplemental lighting during winter months. Greenhouse temperatures were maintained at 24-30 ^{o}C during daylight hours and 15 to 20 ^{o}C at night.

Only SMV-G1 strain was used for field inoculation. The procedure used for the field inoculation has been previously described by Roane et al. (1983). Susceptible and necrotic plants in the segregating populations were tested by dot-blotting immunoassays (Srinivasan and Tolin, 1992) for confirming infection of SMV in the field.

Results and Discussion

Reaction of Tousan 140 to SMV-G1 through G7

Tousan 140 was resistant to all SMV strains, G1 through G7 (Table 1). However, small necrotic spots were observed on the inoculated leaves of Tousan 140 when inoculated with SMV-G1 and G2 strains. SMV resistance to G1 and G2 strains in this accession occurs as a hypersensitive response resulting in localized necrosis. Virus replication and movement is inhibited by rapid tissue death around the virus-invaded area. Occasional systemic necrotic plants (5 to 10%) also were observed when Tousan 140 was tested with SMV-G1.

Inheritance of resistance to SMV strains G1 and G7 in Tousan 140

F_1 plants from Tousan 140 x Lee 68 exhibited stem tip necrosis when inoculated with SMV-G1 (Table 2), indicating partial dominance of the resistance gene with the dominant homozygotes expressing resistance (symptomless reaction) while the heterozygotes show systemic necrosis to SMV-G1. On the other hand, F_1 plants from the same cross were completely resistant to the SMV-G7 strain (Table 2).

Segregation in three F_2 populations from the crosses between Tousan 140 and the SMV-G1 susceptible cultivars Lee 68, L29, and Harosoy was consistent with a 3(R+N) : 1S ratio and was homogeneous among populations when inoculated with SMV-G1 (Table 2). Likewise, monogenic 3(R+N):1S inheritance of resistance was observed for the F_2 population of Tousan 140 x Lee 68 inoculated with the SMV-G7 strain (Table 2).

$F_{2:3}$ families of Tousan 140 x Lee 68 segregated to fit a 1 (all R) : 2 [3(R+N):1S]: 1(all S) when inoculated separately with SMV-G1 and G7. (Table 3). Interestingly, no $F_{2:3}$ families had all systemic necrotic plants, indicating that systemic necrosis was not the expression of any homozygous genotypes in this cross. Necrosis was associated with heterozygosity, as evidenced by the reaction of F_1 plants of Tousan 140 x Lee 68 to SMV-G1 (Table. 2). Therefore, necrotic plants in the F_2 and $F_{2:3}$ populations were combined with resistant plants for genetic analysis, as done in many previous studies

(Shigemori, 1988; Buss et al., 1989; Chen et al., 1991, 1994; Bowers et al., 1992; Kiihl and Hartwig, 1979; Ma et al., 1995).

When the reactions of the same $F_{2:3}$ families of Tousan 140 x Lee 68 to SMV-G1 versus G7 were examined, the joint segregation data were consistent with a 1:2:1:2:4:2:1:2:1 genotypic ratio (Table 4). These results indicated that two independent genes control the resistance in Tousan 140. One of the genes expresses the resistant reaction to SMV-G1 and is susceptible to SMV-G7, while the other gene is susceptible to SMV-G1 and resistant to SMV-G7.

Three $F_{2:3}$ lines derived from Tousan 140 x Lee 68 were postulated to possess only one of the two resistance genes identified in Tousan 140 because they were homogeneously resistant to SMV-G1 but susceptible to SMV-G7 and were temporarily designated R1. Two other $F_{2:3}$ lines of Tousan 140 x Lee 68 were homogeneously susceptible to G1 and resistant to SMV-G7, indicating that these lines possess the other resistance gene, temporarily called R2. Seeds of these $F_{2:3}$ lines were planted and inoculated separately with SMV-G1, G2, G3, G5, G6, and G7 to detect their differential reactions to SMV strains. No segregation for reaction to any of the SMV strains was observed in these $F_{2:3}$ lines. R1 Line exhibited susceptible reactions to SMV-G5, G6, and G7, but were resistant to SMV-G1, G2, and G3 (Table 1). In contrast R2 line exhibited a resistant reaction to SMV-G5, G6, G7 but were susceptible to SMV-G1, G2, and G3. Thus, the SMV resistance gene in R1 shows a resistance pattern similar to Rsv1 alleles (Kiihl and Hartwig, 1979; Chen et al., 1991; 1994), while the SMV resistance gene in R2 exhibits a reaction pattern similar to Rsv3 (Ma, 1995; Buss et al., 1999). Regardless of their identity, it is clear that the resistances of the two genes combine to make Tousan 140 resistant to each of the strains SMV-G1 through G7.

Allelic relationship of the resistance genes in Tousan 140 with Rsv1, Rsv3 and Rsv4

A few necrotic plants were observed in the F_2 and $F_{2:3}$ populations of Tousan 140 (R) x PI96983 (R, Rsv1), but no susceptible plants were observed when SMV-G1 was used (Table 5 and 3). This indicated that one of the resistance genes in Tousan 140 is at the Rsv1 locus. Ten percent of the Tousan 140 parental plants included in the test

developed systemic necrosis with SMV-G1, which could explain the source of necrotic plants in this segregating population. Necrotic plants have been observed among progeny of R (Rsv1) x R (Rsv1) crosses and were classified as resistant for genetic analysis by other researchers (Chen et al., 1994; Ma, 1995).

There were no susceptible plants in the F_2 and $F_{2:3}$ populations from Tousan 140 x L29 when inoculated with SMV-G7, indicating that the other resistance gene in Tousan 140 is allelic to Rsv3 (Tables 3 and 5) .

The F_2 population from Tousan 140 (R) x V94-5152 (R, Rsv4) segregated to give a satisfactory fit to a digenic ratio of 15 (R+N) : 1 (S) when inoculated with SMV-G1 (Table 5). $F_{2:3}$ populations of the same cross conformed to a 7 (all R) : 4 [15 (R+N) : 1S]: 4 [3 (R+N) : 1 S]: 1 (all S) genotypic ratio (Table 6) when inoculated with G1, indicating that the gene (Rsv1) conferring resistance to SMV-G1 in Tousan 140 is independent of Rsv4.

Reaction of Hourei to SMV-G1 through G7

Hourei is resistant to SMV-G1 through G7 (Table 1). However when inoculated with SMV-G1 strain, local necrotic lesions on inoculated leaves and occasional systemic necrotic plants were observed. Ma (1995) reported that the Rsv1-s allele derived from PI486355 also confers local necrosis on inoculated leaves, and occasionally develops systemic necrosis.

Inheritance of resistance to SMV strains G1 and G7 in Hourei

F_1 plants from Hourei x Lee 68 expressed necrosis when inoculated with SMV-G1 (Table 7), which suggests that the resistance gene in Hourei is incompletely dominant and necrosis was associated with heterozygosity in this population. On the other hand, when SMV-G7 was used, F_1 plants of Hourei x Lee 68 produced necrotic lesions only on the inoculated leaves but beyond that the plants remained symptomless and resistant.

When inoculated with SMV-G1, three F_2 populations from crosses between Hourei and the SMV-G1-susceptible cultivars Lee 68, L29, and Harosoy segregated to fit

a 3(R+N) : 1S ratio and were homogeneous (Table 7). When SMV-G7 was used, segregation in F_2 populations of Hourei x Lee 68 conformed to the digenic ratio of 15(R+N):1S (Table 7). These results indicated that Hourei possesses two resistance genes. One gene expresses resistant reaction to G1 and G7, while the other gene expresses susceptibility to SMV-G1 and resistance to SMV-G7.

The overall segregation of the $F_{2:3}$ families from Hourei (R) x Lee 68 (S) fit a genotypic ratio of 1(all R): 2 [3(R+N):1S] :1(all S) when the population was inoculated with SMV-G1 (Table 8). However, the same $F_{2:3}$ families segregated with a 7(all R): 4[15(R+N):1S]:4[(3R+N):1S]:1(all S) genotypic ratio, when SMV-G7 was used as inoculum (Table 9). These results verified the F_2 population data and confirmed that Hourei has two genes. These two genes in Hourei act in a complimentary manner in that together they confer resistance to SMV-G1 through G7.

Necrotic plants observed in the segregating populations of Hourei x Lee 68 were associated with heterozygosity, as the F_1 plants exhibited a necrotic reaction when SMV-G1 was used. Therefore, necrotic plants in F_2 and $F_{2:3}$ populations were combined with resistant plants for chi-square analysis. Other investigators reported the same association, and necrotic plants were combined with resistant plants in their genetic tests (Kiihl and Hartwig, 1979; Shigemori, 1988; Buss et al., 1989; Bowers et al., 1992; Chen et al., 1991, 1994: Ma et al., 1995).

When SMV-G7 was used, Hourei x Lee 68 F_1 plants produced local necrotic lesions on inoculated leaves but no systemic necrosis was observed (Table 7). However, systemic necrotic plants were observed in the F_2 population and $F_{2:3}$ families of Hourei x Lee 68 and there was no $F_{2:3}$ family which was completely necrotic. Thus, the systemic necrotic reaction observed in F_2 and $F_{2:3}$ populations most likely occurred due to environmental changes since F_1 plants, F_2 populations and $F_{2:3}$ families were grown in different time of the year in the greenhouse.

Allelic relationship of the resistance gene in Hourei with Rsv1, Rsv3, and Rsv4

There were no susceptible plants in the F_2 populations or $F_{2:3}$ families derived from L78-379 x Hourei inoculated with G1 (Tables 8 and 10) and L29 x Hourei

inoculated with SMV-G7 (Tables 8 and 10). This indicated that one of the genes in Hourei is allelic to Rsv1, and the other is an allele at the Rsv3 locus.

The F_2 population from V94-5152 x Hourei segregated to fit a digenic segregation ratio of 15 (R+N):1 (S) when inoculated with SMV-G1 (Table 10). Segregation among $F_{2:3}$ families of V94-5152 x Hourei conformed to a 7 (all R) : 4 [15 (R+N) : 1 S] : 4[3(R+N) : 1 S] : 1(all S) genotypic ratio and were homogeneous in the field and greenhouse tests (Table 11).

When SMV-G1 was used, there were no susceptible plants observed in F_2 populations of Hourei x Tousan 140, Tousan 140 x Hourei (Table 10) or in $F_{2:3}$ families of Tousan 140 x Hourei (Table 8). This result corroborates the individual allelism studies of Hourei and Tousan 140, which indicated that both cultivars possess alleles at the Rsv1 and Rsv3 loci. However, 25% of the Hourei x Tousan 140 F_2 population expressed a systemic necrotic reaction (Table 10), and there were some necrotic plants in 42 of the 50 $F_{2:3}$ families (Table 8). None of the $F_{2:3}$ families had all systemic necrotic plants, which indicated that systemic necrosis was not the expression of any homozygous genotype in this population. Less likely, the number of $F_{2:3}$ families might not have been large enough to detect any completely necrotic families. In fact, Hourei and Tousan 140 both exhibited local necrotic lesions on the inoculated leaves. Occasionally, systemic necrotic plants were observed on Tousan 140 and Hourei plants inoculated with SMV-G1 strain in greenhouse and field tests. Frequency of necrotic plants observed in the F_2 and $F_{2:3}$ populations of Tousan 140 x Hourei was approximately equal to frequency of necrotic plants observed in both parents. Ma (1995) reported that line LR1, which possesses a gene at Rsv1 and exhibits local necrosis on inoculated leaves, was usually resistant but occasionally developed systemic necrosis in the greenhouse at a high temperature when inoculated with SMV-G7. Ali (1950) reported that bean (Phaseolus vulgaris L.) exhibited a resistant reaction (local necrosis on inoculated or infected leaves) to common bean mosaic virus in the field, but developed top necrosis (systemic necrosis) in the greenhouse at high temperatures.

When SMV-G7 was used, a few plants exhibited local necrotic lesions on inoculated leaves in F_2 populations of Tousan 140 x Hourei, but no susceptible plants were observed (Table 10). This confirms that genes conferring resistance to G7 in Tousan

140 and Hourei are allelic. However, Shigemori (1988) reported that Tousan 140 and Hourei possess a single dominant gene at different loci conferring resistance to Japanese C strain. A difference in SMV strains used in the two studies could explain the contradictory results obtained. Probably only one of the two SMV resistance genes in Tousan 140 and Hourei exhibit resistance to Japanese C strain (either Rsv1 in Tousan 140 and Rsv3 in Hourei or vice versa). Therefore, the Japanese C strain did not permit the identification of a second gene in each cultivar due to its virulence for these genes in the study by Shigemori (1988). Also, Hourei and Tousan 140 must possess different alleles at both Rsv1 and Rsv3, since the opposite loci conferred resistance to the Japanese C strain in the two parents. In fact results of our study indicate that the Rsv1 allele in Tousan 140 express resistance to SMV-G1 and susceptibility to SMV-G7 (Tables 1, 2 and 3), while the Rsv1 allele in Hourei confers a resistant reaction to SMV-G1 and G7 (Tables 7, 8 and 9).

The Rsv1 allele in Tousan 140 appears to be different from other alleles at the Rsv1 locus. The Rsv1 allele in Tousan 140 exhibits local necrotic lesions on inoculated leaves to SMV-G2 strain, while none of the other alleles at Rsv1 exhibit such a reaction (Table 1). The Rsv3 alleles in Tousan 140 and Hourei exhibit a reaction similar to other alleles at the Rsv3 locus, such as susceptible reaction to SMV-G1 and resistant reaction to the most virulent strain, SMV-G7 as defined by Rsv1.

Results of this study clearly show that Tousan 140 and Hourei each possess two SMV resistance genes and each cultivar has a gene at the Rsv1 and Rsv3 loci. The presence of Rsv1 and Rsv3 genes in each of these accessions makes them resistant to SMV strains G1 through G7 as opposed to Rsv4, in which a single resistance gene confers resistance to SMV-G1 through G7 (Ma, 1995). Broader resistance conferred by two resistance genes at different loci theoretically is more durable than single-gene resistance, which could result in genetic uniformity and potential vulnerability. Pyramiding of genes at the Rsv1, Rsv3 and Rsv4 loci in different combinations should provide effective and more durable resistance.

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Table 1. Disease reaction of soybean cultivar and breeding lines to seven SMV strain groups.

	SMV strains						
Parents	G1	G2	G3	G4	G5	G6	G7
Tousan 140[#]	LN/N †	LN	R	NA	R	R	R
Hourei[#]	LN/N	R	R	NA	R	R	R
R1[##]	R	LN	R	NA	S	S	S
R2[##]	S	S	S	NA	R	R	R
[‡]PI96983(Rsv1)	R	R	R	R	R	R	N
[‡]L78-379 (Rsv1)	R	R	R	R	R	R	N
[‡]L29(Rsv3)	S	S	S	S	R	R	R
[‡]V94-5152 (Rsv4)	R	R	R	R	R	R	R

† R, resistant (symptomless); LN, Local necrosis on inoculated leaves; N, systemic necrosis; S, susceptible (mosaic)

[#] Occasionally produce systemic necrotic plants

[##] $F_{2:3}$ progeny derived from Tousan x Lee 68 possessing a single SMV resistance gene

[‡] PI96983 (Chen et al., 1991); L78-379 (Buzzell and Tu, 1984; Bernard et al., 1991); L29 (Buss et al., 1999); V94-5152 (Buss et al., 1997)

Table 2. Reactions of F_1 plants and F_2 populations from Tousan 140 x SMV-G1-susceptible genotypes.

Cross and Generation	SMV Strain	Number of plants R†	N	S	Total	Expected Ratio	χ^2	df	P
Tousan 140 x Lee 68 F_2	G1	124	16	54	194	3(R+N):1S	0.83	1	0.36
Tousan 140 x L29 F_2	G1	103	16	47	166	3(R+N):1S	0.97	1	0.32
Tousan 140 x Harosoy F_2	G1	123	30	52	205	3(R+N):1S	0.01	1	0.92
Total							1.81	3	
Pooled		350	62	153	565	3(R+N):1S	1.30	1	0.25
Heterogeneity							0.50	2	0.78
Tousan 140 x Lee 68 F_2	G7	79	6	21	106	3(R+N):1S	1.52	1	0.22
Tousan 140 x Lee 68 F_1	G1	0	4	0					
Tousan 140 x Lee 68 F_1	G7	3	0	0					

† R, resistant (symptomless); N, systemic necrosis; S, susceptible (mosaic)

Table 3. Segregation of $F_{2:3}$ families from crosses of Tousan 140 x Lee 68, Tousan 140 x L29 (Rsv3) and Tousan 140 x PI 96983 (Rsv1) inoculated with SMV-G1 and G7 strains in the greenhouse.

Cross	SMV Strain	Number of families			Expected ratio	χ^2	P
		All R†	H	All S			
Tousan 140 x Lee 68	G1	8	24	10	1(all R): 2[3(R+N):1S]: 1(all S)	1.04	0.59
Tousan 140 x Lee 68	G7	10	23	14	1(all R): 2[3(R+N):1S]: 1(all S)	0.70	0.70
Tousan 140 x L29	G7	64	0	0			
Tousan 140 x PI96983	G1	67	0	0			

† R, resistant; H, segregating for susceptible reaction; S, susceptible

Table 4. Segregation of $F_{2:3}$ families from Tousan 140 x Lee 68 inoculated with SMV-G1 and G7 in the greenhouse.

Reaction of $F_{2:3}$ families			Number of families			
SMV-G1	SMV-G7	Frequency	Observed	Expected	$\chi^2_{1:2:1:2:4:2:1:2:1}$	P
All R	All R	1	4	3.19		
All R	3(R+N):1S	2	2	6.38		
All R	All S	1	3	3.19		
3(R+N):1S	All R	2	4	6.38		
3(R+N):1S	3(R+N):1S	4	16	12.75		
3(R+N):1S	All S	2	8	6.38		
All S	All R	1	2	3.19		
All S	3(R+N):1S	2	8	6.38		
All S	All S	1	4	3.19		
Total		16	51	51	6.39	0.60

† R, resistant (symptomless); N, Stem tip necrosis; S, susceptible

Table 5. Segregation of F_2 populations from crosses between Tousan 140 and R parents inoculated with SMV-G1 and G7 in the greenhouse.

Cross and parents	SMV Strain	Number of plants			Expected ratio	χ^2	P
		R†	N	S			
Tousan 140 x PI96983 (Rsv1)	G1	238	8	0			
Tousan 140 x L29 (Rsv3)	G7	197	17	0			
Tousan 140 x V94-5152 (Rsv4)	G1	153	85	22	15(R+N):1S	2.17	0.14

†R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 6. Segregation of $F_{2:3}$ progenies from Tousan 140 x V94-5152 (Rsv4) inoculated with SMV-G1 in the greenhouse.

Reaction†	Frequency	Number of families		$\chi^2_{7:4:4:1}$	P
		Observed	Expected		
All R	7	17	22.31		
3(R+N) : 1S	4	16	12.75		
15(R+N) : 1S	4	12	12.75		
All S	1	6	3.19		
Total	16	51	51	4.59	0.20

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

Table 7. Reactions of F_1 plants and F_2 populations from Hourei x SMV-G1-susceptible parent crosses inoculated with SMV-G1 and G7.

Cross and	SMV	Number of plants				Expected			
Generation	Strain	R†	N	S	Total	Ratio	χ^2	df	P
Hourei x Lee 68 F_2	G1	62	5	29	96	3(R+N):1S	1.388	1	0.23
L29 x Hourei F_2	G1	93	7	43	143	3(R+N):1S	1.960	1	0.16
Hourei x Harosoy F_2	G1	110	8	39	157	3(R+N):1S	0.002	1	0.96
Total		265	20	111	400		3.35	3	
Pooled						3(R+N):1S	1.96	1	0.16
Heterogeneity							1.39	2	0.50
Hourei x Lee 68 F_2	G7	165	24	16	205	15(R+N):1S	0.846	1	0.36
Hourei x Lee 68 F_1	G1	0	3	0					
Hourei x Lee 68 F_1	G7	4[#]	0	0					

† R, resistant (symptomless); S, susceptible (mosaic); N, stem tip necrosis
[#] Local necrotic lesions on inoculated leaves

Table 8. Reaction of $F_{2:3}$ families from the crosses of Hourei x Lee 68, L29 x Hourei, L78-379 x Hourei and Hourei x Tousan 140 inoculated with SMV-G1 and G7 in the greenhouse.

Cross	SMV Strain	Number of families			Expected Ratio	χ^2	P
		All R†	H	All S			
Hourei x Lee 68	G1	10	26	11	1(all R):2[3(R+N):1S]:1(all S)	0.57	0.75
L29 (Rsv3) x Hourei[#]	G7	52	0	0			
Hourei x L78-379 (Rsv1)	G1	68	0	0			
Hourei x Tousan 140[##]	G1	50	0	0			

† R, resistant; H, segregating ; S, susceptible; N, systemic necrosis
[#] Four $F_{2:3}$ lines have 1 or 2 necrotic plants
[##] Systemic necrotic plants observed in 42 $F_{2:3}$ progenies

Table 9. Segregation of $F_{2:3}$ families from Hourei x Lee 68 when inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families		$\chi^2_{7:4:4:1}$	P
		Observed	Expected		
All R	7	19	19.25		
3(R+N+LS) : 1S	4	12	11		
15(R+N+LS) : 1S	4	11	11		
All S	1	2	2.75		
Total	16	44	44	0.298	0.96

† R, resistant (symptomless); N, Stem tip necrosis; S, susceptible; LS, late susceptible

Table 10. Reaction of F_2 populations from crosses between Hourei and R parents inoculated with SMV-G1 and G7 in the greenhouse.

Cross and parents	SMV Strain	Number of plants			Expected Ratio	χ^2	P
		R†	N	S			
L78-379(Rsv1) x Hourei	G1	287	0	0			
L29 (Rsv3)x Hourei	G7	188	9	0			
V94-5152 (Rsv4) x Hourei	G1	237	50	25	15(R+N):1S	1.65	0.19
Hourei x Tousan 140	G1	73	23	0			
Tousan 140 x Hourei	G1	72	20	0			
Tousan 140 x Hourei	G7	132[#]	0	0			

†R, resistant (symptomless); N, stem tip necrosis; S, susceptible
[#] Five plants exhibit local necrotic reaction on the inoculated leaves.

Table 11. Segregation of $F_{2:3}$ families from V94-5152 (Rsv4) x Hourei inoculated with SMV-G1 in the greenhouse and field.

	Number of families							
Location	All R	3(R+N):1S	15(R+N):1S	All S	Total	$\chi^2_{(7:4:4:1)}$	df[#]	P
Greenhouse	23	8	11	2	44	1.75	3	0.62
Field	11	7	4	3	25	2.06	3	0.52
Total	34	15	15	5	69	3.97	6	
Pooled						1.17	3	0.76
Heterogeneity						2.80	3	0.42

† R, resistant (symptomless); N, stem tip necrosis; S, susceptible

[#] Degree of freedom

CHAPTER III

Inheritance of Soybean mosaic virus Resistance in PI88788 Soybean

Abstract

The inheritance of resistance to soybean mosaic virus (SMV) in PI88788 soybean [Glycine max (L.) Merr.], a germplasm accession from China, was studied. PI88788 was crossed with susceptible cvs Essex and Lee 68 to determine the inheritance of resistance. Crosses with the resistant cultivars PI96983, L29 and V94-5152, possessing the Rsv1, Rsv3 and Rsv4 genes, respectively, were analyzed to elucidate allelomorphic relationships with previously reported genes. PI88788 is resistant to SMV-G1 through G7. However, SMV was detected in inoculated leaves but no vascular movement of the virus was observed. R x S crosses exhibited monogenic segregations, but, since F_1 plants show late susceptible symptoms when inoculated with SMV-G1, the gene is considered partially dominant. However, the gene is completely dominant when inoculated with SMV-G7. Analysis of F_2 data revealed that the resistance gene in PI88788 is independent of the Rsv1 and Rsv3 loci, but is allelic to the Rsv4 locus. While none of the reported resistance genes at the Rsv1 and Rsv3 loci confer resistance to all SMV strains, the resistance gene in PI88788 confers resistance to SMV-G1 through G7. Therefore, it has potential value for use in breeding broad resistance to SMV.

Key words: Glycine max, incomplete dominance, late susceptibility, broad resistance

Introduction

Depending on the virus strain and soybean genotypes, SMV induces four distinct reactions in soybean: resistant (symptomless), necrosis, susceptible (mosaic) (Cho and Goodman, 1979; 1982) and late susceptible (early resistance) (Buss unpublished data). Late susceptible plants show large mosaic spots three weeks after inoculation, while typical susceptible plants show veinal clearing and mosaic symptoms 7 to 10 d after inoculation (Buss, unpublished data).

Ninety-eight isolates of SMV were classified into seven groups, G1 through G7, based on symptoms produced on a set of soybean differential cultivars in the US (Cho and Goodman, 1979; 1982). Resistance sources to different virus strains also have been reported. Kiihl and Hartwig (1979) reported a single dominant resistance gene in PI96983, designated Rsv (later renamed Rsv1) and a resistance gene in 'Ogden' was designated as rsv-t, which later was changed to Rsv1-t (Chen et al., 1991). The single resistance genes in 'York', 'Marshall', and 'Kwanggyo' were found to be alleles at the Rsv1 locus and were assigned the gene symbols, Rsv1-y, Rsv1-m and Rsv1-k, respectively (Chen et al., 1991). These five Rsv1 alleles confer differential reaction to SMV strains G1 through G7. Ma et al. (1994) reported that lethal necrosis in PI 507389 was the expression of a homozygous allele at the Rsv1 locus and was recessive to the resistance alleles in PI96983, York, and Marshall. One of the genes in PI486355 was also found to be at the Rsv1 locus and was designated Rsv1-s. The Rsv1 gene has been mapped to linkage group F and closely linked molecular markers have been reported (Yu et al., 1994).

Buzzell and Tu (1984) reported that OX670 possesses a single gene, presumably derived from Raiden, at a locus independent of Rsv1. The gene symbol Rsv2 was assigned. However, it has been shown that Raiden contains a single resistance gene at the Rsv1 locus (Buss et al., 1995). Later studies showed that OX670 possesses two resistance genes to SMV. One, derived from Raiden, is allelic to Rsv1 and the other, derived from Harosoy, is allelic to Rsv3. Therefore, the Rsv2 locus does not appear to be in OX670 or its ancestors (Gunduz et al., 1999).

Buzzell and Tu (1989) reported that the gene derived from 'Columbia' conferring necrotic reaction is independent of Rsv1 and Rsv2 and assigned the Rsv3 gene symbol. Bowers et al. (1992) found that 'Buffalo' and 'HLS', a late maturing selection from the cultivar 'Hardee', have single dominant genes at different loci for SMV resistance but did not conduct allelism tests with reported loci. Ma (1995) and Buss et al. (1999) reported that the resistance gene in L29, a resistant selection from Williams (6) x Hardee, is allelic to Rsv3.

Chen et al. (1993) reported that two independent genes in PI486355 governed SMV resistance and one of the resistance genes appeared to be at the Rsv1 locus. These two genes were separated in two different lines, LR1 and LR2, and the inheritance and allelomorphic relationship of these two genes were studied. They found that the gene in LR1 is allelic to the Rsv1 locus but the gene in LR2 is independent from Rsv1 and Rsv3 (Ma et al., 1995; Ma, 1995). LR2 a subline selection from PI486355 x 'Essex' has been developed into a homozygous breeding line, V94-5152 (Rsv4), and registered as germplasm (Buss et al., 1997). While the gene in LR1 confers resistance to G1 through G4 and G7 and a systemic necrotic response to SMV-G5 and G6, the gene in LR2 confers resistance to SMV-G1 through G7. It has also been determined that Peking and PI556949 each possess a single gene at the Rsv4 locus. The resistance gene in Peking and PI556949 were considered partially dominant since some heterozygotes showed late susceptibility when inoculated with G1 (Buss unpublished data). The Rsv4 gene has been mapped to linkage group D1b and closely linked molecular markers have been identified (Hayes et al., 2000).

PI88788, an early soybean introduction from China, has been widely used as a source of cyst nematode resistance. Two dominant genes and one recessive gene condition resistance to soybean cyst nematode (SCN) in PI88788 (Rao-Arelli et al., 1988). It is also resistant to SMV, but the inheritance of resistance to SMV in PI88788 has not been studied. Therefore, the objective of this study was to determine: (i) the reaction of PI88788 to SMV-G1 through G7 strains, (ii) the inheritance of SMV resistance in PI88788, and (iii) the allelomorphic relationship of the resistance gene/genes in PI88788 with previously identified resistance genes.

Materials and Methods

PI88788 was crossed with SMV susceptible cvs Lee 68 and Essex to study the inheritance of resistance. PI88788 was also crossed with 'Peking' and resistant cultivars PI96983, L29, and V94-5152 which contain Rsv1, Rsv3, and Rsv4, respectively, to investigate allelomorphic relationships with previously identified genes. 'Peking' is a plant introduction from China, which possesses a single SMV resistance gene at Rsv4 (Buss, unpublished data). Crosses were made either in the greenhouse or in the field at Blacksburg, Virginia. F_1 plants were grown in the greenhouse or in the field at Blacksburg. F_2 plants were grown in the field without virus inoculation either at Blacksburg or Warsaw and plants were harvested to obtain $F_{2:3}$ families. Crosses were distinguished from selfs in the F_1 and F_2 generation using leaf shape, seed color, flower color, pubescence color and maturity as markers.

The F_2 populations and $F_{2:3}$ families were tested with SMV-G1 in the greenhouse and field and with G7 in the greenhouse at Blacksburg, Virginia. An average of 200 F_2 plants per population and 20 $F_{2:3}$ plants from each of 50 $F_{2:3}$ families were inoculated. Individual plant reactions were examined about 10, 20, 30, and 40 d after inoculation and classified into four distinct groups: resistant (R) (symptomless or with local necrotic lesions on inoculated leaves); necrotic (N) (necrosis developed as stem tip necrosis and killed plants 10 to 15 d after inoculation); susceptible (S) (mosaic); and late-susceptible (LS) (plant shows early resistance but develops mosaic symptoms 2 to 3 wk after inoculation). Systemic necrotic plants and late susceptible plants that were observed in F_2 populations and $F_{2:3}$ progenies were combined with resistant plants for testing fits to standard Mendelian ratios.

To study the genetics of late susceptibility, 200 F_2 plants from PI88788 x Essex and PI88788 x Lee 68 were planted in the virus nursery at Blacksburg, Virginia, and inoculated with SMV-G1. Susceptible, late susceptible, and resistant plants were tagged and harvested to obtain $F_{2:3}$ families. $F_{2:3}$ families were planted in the greenhouse and inoculated with SMV-G1.

SMV-G1 through G7 were used to test PI88788 but only two strains, SMV-G1 and SMV-G7, were used to screen F_1 plants, F_2 populations, and $F_{2:3}$ families. The strain SMV-G1 was originally isolated from 'Lee' soybean in Virginia and is analogous to the SMV-G1 of Cho and Goodman (1979, 1982) with respect to reactions induced on the soybean differentials (Hunst and Tolin, 1982). Cultures of type isolates of SMV-G2 through G7 were originally obtained from Dr. R.M. Goodman in 1984, at the University of Illinois. Cultures of SMV-G1 and SMV-G7 have been deposited as PV-571 and PV613 respectively, in the American Type Culture Collection (10801 University Boulevard, Manassas, Virginia 20110-2209 USA). The SMV-G1 through G4 cultures were maintained by continuous passage in Lee 68, while SMV-G5 through G7 were maintained in York.

For the greenhouse tests, inocula were prepared from infected trifoliolate leaf tissue homogenized in 0.01 M sodium phosphate buffer solution, pH 7.0, at an approximate rate of 1 g infected tissue per 10 ml buffer. Primary leaves were inoculated before trifoliolates emerged, approximately 10 d after planting. A small amount of 600 mesh carborundum was dusted on the leaves to be inoculated. Inoculum was applied by rubbing both primary leaves of each plant with a pestle dipped in the inoculum. Inoculated leaves were rinsed with tap water. The differential cultivars were also included in each set of inoculations to confirm the identity and purity of the virus culture used. A daylength of approximately 14 h was maintained by using both fluorescent and incandescent supplemental lighting during winter months. Greenhouse temperatures were maintained at 24 to 30 ^{o}C during daylight hours and 15 to 20 ^{o}C at night.

Only SMV-G1 was used in field inoculations. The procedure used for field inoculation has been previously described by Roane et al. (1983). Random susceptible and necrotic plants were tested by dot-blotting immunoassays (Srinivasan and Tolin, 1992) for detecting possible infection of alien viruses in the field. In addition, leaf tissue prints of inoculated leaves from PI88788 were taken to detect virus replication and invasion on the inoculated leaves. The leaf tissue print procedure was described by Gera (1994).

Results and Discussion

Reaction of PI88788 to SMV-G1 through G7

PI88788 exhibited no visible symptoms after inoculation with SMV-G1 through G7 (Table 1). However, SMV was detected in leaf tissue prints of leaves inoculated with SMV-G1 and G7 (Figures 4 and 5). The area invaded by virus enlarged in increments at 11, 19, 23 and 42 days after inoculation (dai) with SMV-G1 (Figure 4). No visual virus symptoms or SMV were detected on the upper leaves in the later growth stages, suggesting that the resistance gene in PI88788 decreased the invasion rate and prevented long distance movement. A plant host is considered resistant if it can block long distance movement of the virus (Carrington and Whitham, 1998).

Inheritance of resistance to SMV strains G1 and G7 in PI88788

F_1 plants of PI88788 (R) x Lee 68 (S) and PI88788 (R) x Essex (S) expressed the late susceptible reaction when inoculated with SMV-G1, indicating that the resistance gene in PI88788 is incompletely dominant (Tables 2 and 3). Essex and Lee 68 exhibited typical veinal clearing in first trifoliolate leaves 7 days after inoculation (dai), late susceptible symptoms were first observed on PI88788 x Essex F_1 plants at 19 dai but not on F_1 plants of PI88788 x Lee 68 until 35 dai. This difference in timing of symptom appearance was not observed when F_1 plants of the same cross were tested in the greenhouse in winter. While normal susceptible plants develop veinal clearing 7-10 days after inoculation, late susceptible plants remain symptomless approximately 20 days after inoculation (Figure 1). Then, late susceptible symptoms first appear on the lower leaves as transitory mosaic islands and then move to the upper leaves (Figure 3). At that time normal susceptible leaves are misshapen and puckered (Figure 2). These observations indicate that the resistance gene in the heterozygous state delays cell-to-cell and vascular movement of the virus but does not totally prevent replication and cell-to-cell movement.

The F_2 populations of PI88788 x Essex and PI88788 x Lee 68 segregated to fit a 3 (R) : 1 (S) based on the initial reaction to SMV-G1 at 7 to 10 dai. However, at 20 dai,

late susceptible plants were observed in the F_2 populations from PI88788 x Essex and PI88788 x Lee 68. The number of plants exhibiting late susceptible reaction increased in the third, (30 dai) and fourth (40 dai) readings. Segregation pattern based on the last reading of the PI88788 (R) x Essex (S) F_2 population was consistent with a 1(R):2(LS):1(S) ratio, indicating monogenic inheritance with incomplete dominance (Table 2). However, the F_2 population of PI88788 x Essex planted and inoculated in the field did not segregate in a 1R:2LS:1S ratio (Table 2). Late susceptible plants were observed, but their frequency was well below the number of expected heterozygotes. It is not clear whether this difference occurred as a result of environmental differences or inoculation procedure. In the greenhouse, both unifoliolate leaves were inoculated by rubbing them with a pestle dipped in inoculum; whereas, an airbrush gun, which is more destructive to plant tissue, was used to inoculate unifoliolate leaves in the field. Thus, a larger area of the unifoliolate leaves was likely exposed to SMV with pestle and mortar inoculation in the greenhouse. When late susceptible plants were combined with resistant plants, the F_2 segregation pattern of the Essex x PI88788 population tested in the field fit a single gene ratio (Table 2).

The segregation pattern observed in the F_2 population of PI88788 (R) x Lee 68 (S) did not fit the expected 1(R):2(LS):1(S) ratio in either the greenhouse or field (Table 3). Late susceptible plants were observed, but their frequency was well below the number of expected heterozygotes. Obviously all heterozygotes did not express symptoms. When late susceptible plants were combined with resistant plants for genetic analysis, the F_2 population fit a 3(R+LS):1S ratio when inoculated with SMV-G1 (Table 3).

Seeds from eight randomly selected, late susceptible F_2 plants from PI88788 x Essex, grown in the field, were planted in the greenhouse and inoculated with SMV-G1. Segregation in 8 late susceptible $F_{2:3}$ families was consistent and homogeneous with a 3R:1S nine dai and 1R:2LS:1S fortytwo dai (Table 4). This result suggests that the late susceptible reaction is associated with heterozygosity (Table 4).

F_1 plants of PI88788 x Lee 68 and PI88788 x Essex inoculated with SMV-G7, remained symptomless. F_2 populations of the same crosses segregated in a pattern with a

3 R : 1S ratio (Tables 2 and 3). $F_{2:3}$ families of PI88788 x Lee 68 segregated in a 1 (all R) : 2 [3 (R+LS):1S] : 1 (all S) genotypic ratio when inoculated with SMV-G1 (Table 5). The $F_{2:3}$ population of PI88788 x Essex likewise segregated to fit a 1(all R):2(segregating):1(all S) genotypic ratio when either SMV-G1 or G7 was used (Table 5). All lines of the latter cross exhibited the same reaction to both strains, indicating that the same gene controls the resistant reaction to both SMV strains. No late susceptible plants were observed in the F_1 plants, F_2 populations, and $F_{2:3}$ families of PI88788 x Essex when inoculated with G7 (Tables 2 and 5). These results demonstrate that the SMV resistance gene in PI88788 is completely dominant when inoculated with SMV-G7.

Allelic Relationship of the resistance gene in PI88788 with Rsv1, Rsv3 and Rsv4

The F_2 population from PI88788 (R) x PI96983 (R, Rsv1) segregated to fit a digenic ratio of 15 (R+LS+N) : 1 (S) when inoculated with SMV-G1 (Table 6). $F_{2:3}$ populations of the same cross conformed to a 7 (all R) : 4[(15 R+LS+N) : 1S)]:4[(3 (R+N+LS) : 1 S]:1 (all S) genotypic ratio indicating that the resistance gene in PI88788 is independent of Rsv1 (Table 7).

Segregation in the F_2 population from L29 (R, Rsv3) x PI88788 (R) was consistent with a digenic ratio of 15 (R):1 (S) when inoculated with SMV-G7 (Table 6). $F_{2:3}$ families of the same cross fit a 7 (all R) : 4(15 R : 1 S): 4(3R : 1S):1 (all S) genotypic ratio indicating that the resistance gene in PI88788 is independent of Rsv3 (Table 8).

There were no susceptible plants identified in the F_2 and $F_{2:3}$ populations from V94-5152 (R, Rsv4) x PI88788 (R) , PI88788 (R) x Peking (R, Rsv4), and Peking (R, Rsv4) x PI88788 (R) when inoculated with SMV-G1,which indicated that the resistance gene in PI88788 is allelic to Rsv4 (Tables 6 and 5).

The resistance allele in PI88788 is exhibits a similar reaction to the Rsv4 allele in Peking but was different from the other allele detected in V94-5152. Both V94-5152 and PI88788 are resistant to SMV-G1 through G7, but the reaction of these alleles to G1 is not identical. The Rsv4 allele in V94-5152 decreases the invasion rate of SMV-G1 much more than does the resistance allele in PI88788 as evidenced by the comparison of

invaded area by virus in the inoculated leaves of V94-5152 and PI88788 (Figure 4). The reverse situation occurs when SMV-G7 is used. Furthermore the Rsv4 allele in V94-5152 is completely dominant (Ma, 1995) while the allele in PI88788 is partially dominant with SMV-G1.

The SMV resistance gene in PI88788 is completely dominant with SMV-G7. However it is incompletely dominant when inoculated with SMV-G1 since F_1 plants of PI88788 x Lee 68 and PI88788 x Essex produced the late susceptible reaction. This implies that resistance to SMV-G1 is expressed more effectively in the homozygous (R/R) than heterozygous (R/r) state of the SMV resistance gene. Incomplete dominance is expressed as systemic necrosis with heterozygotes of Rsv1 (Kiihl and Hartwig, 1979; Chen et al., 1994; Ma, 1995) and Rsv3 (Gunduz et al., 1999). Local necrosis has been observed with other virus resistance genes in different crops (Fraser, 1990). However, local necrosis and systemic necrosis can be observed when a homozygous resistance gene at the Rsv1 locus interacts with specific SMV strains. For example, the Rsv1 from PI96983 produces systemic necrosis to SMV-G7 (Chen et al., 1991) and Rsv1-s from PI486355 produces a local necrotic reaction when inoculated with SMV-G1 (Ma, 1995). Likewise, Rsv3 derived from Columbia exhibits a necrotic reaction to SMV-G1 (Buzzell and Tu, 1989; Buss unpublished data). However, no allele has been reported at the Rsv4 locus that produces local or systemic necrosis or mosaic reaction to any SMV strain tested (Ma, 1995; Gunduz et al., 1999; Buss unpublished data). In addition, results of the present study show that the resistance gene at the Rsv4 locus acts by decreasing the virus accumulation and preventing long distance movement (Figures 4 and 5).

In general, plant resistance to a virus occurs by restricting any one of three steps including viral replication, cell-to-cell movement, and vascular movement, which are essential in order for a virus to complete its mission (Carrington and Whitham, 1998). Recent research demonstrated that post transcriptonal gene silencing, which is a homology-dependent mechanism and functions in trans to suppress identical or closely related sequences (Carrington and Whitham, 1998), is a potential defense mechanism against viruses that could restrict virus accumulation in infected cells and hence delay virus movement (Pruss et al., 1997). Therefore, resistance is not complete. We may speculate that resistance genes at the Rsv4 locus silence the viral genes, which results in

reduction of virus accumulation, thus preventing long distance movement of virus. Mutation studies on tobacco etch virus (TEV) showed that a TEV mutant with defects in helper component proteinase (HC-Pro) has decreased virus accumulation and long distance movement is prevented (Kasschau et al., 1997). SMV is also a member of the potyvirus group (Fauquet and Mayo, 1999) and has the genetic information to express HC-Pro component, which is necessary for vascular movement (Revers et al., 1999). Thus, we can speculate that the resistance gene at the Rsv4 locus might particularly silence the section of the virus, which codes for the HC-Pro component. Silencing of the virus gene may also vary depending upon the homozygous or heterozygous state of resistance genes. Theoretically, homozygous resistance genes should produce more complementary RNA for silencing viral genes than heterozygotes. Therefore, Rsv4 at the heterozygous state may decrease virus accumulation and delay long distance movement but can not totally prevent the spread of virus. If we accept this as true, the source of late susceptible plants in the segregating populations of PI88788 (R, Rsv4) x 'Essex' (S) (Table 2) can be explained easily. In fact, in the present study it has been shown that late susceptibility is associated with the heterozygous condition of resistance genes (Tables, 2, 3 and 4).

Plant resistance genes are postulated to encode receptors that, upon binding to their cognate Avr gene products, elicit defense responses (Keen and Dawson, 1992) for back-up system. Recent molecular cloning and characterization of several resistance genes support the elicitor/receptor model. The N gene in tobacco, which confers resistance to tobacco mosaic virus (TMV), produces the N protein, which is bound to the TMV derived elicitor. N protein has nucleotide binding site (NBS), leucine-rich repeat (LRR) domains which function as receptors for virus elicitors (Ericson et al., 1999). Resistance genes that encode an NBS-containing sequence are prevalent in plants. The majority of the plant disease resistance genes cloned to date, including at least 14 genes from six plant species, also encode a predicted NBS region attached to a C-terminal LRR of variable lengths (Baker et al., 1997). These domains function as a receptor. The elicitor-bound receptor then activates the hypersensitive response (HR), in which plant cells in the immediate vicinity of the pathogen undergo programmed cell death as part of the overall defense response (Bent, 1996). However, it can be postulated that this

recognition has some threshold level (Fraser, 1990) and depends on the homozygous (R/R) or heterozygous (R/r) state of resistance genes. It can be speculated that the resistance gene in the homozygous state has twice as many cellular receptors as the heterozygous state; therefore, a low concentration of virus is adequate to initiate the HR. In contrast, a resistance gene in the heterozygous state activates the HR only under a high concentration of virus. This allows the virus to complete its long distance movement throughout the entire plant. Since plant cells in the vicinity of the pathogen undergo programmed cell death, systemic necrosis can be observed.

The necrotic response is common when resistance is governed by alleles at the Rsv1 and Rsv3 loci. This implies that Rsv1 and Rsv3 may express receptor domains such as NBS to bind viral elicitors to activate a hypersensitive response. In fact, Yu et al. (1996) amplified and cloned NBS sequences from soybean by using degenerate primers, based on two conserved motifs of the NBS amino acid sequences of the N gene from tobacco and RPS2 gene from Arabidopsis. NBS-containing genes have been located on the 'F' linkage group in the vicinity of the Rsv1, Rpv, and Rps3 genes for resistance to SMV, peanut mottle virus (PMV), and Phytophthora root rot, respectively. These results provide strong evidence that alleles at the Rsv1 locus may code for proteins, which act as binding sites for elicitors of HR. Thus, resistance to SMV at Rsv1 and Rsv3 occurs in two steps: 1) reducing or blocking replication, or synthesis of virus components that are essential for disease induction and 2) if it can not prevent the replication or synthesis of virus components then it activates the hypersensitive response resulting in localized necrosis.

When Rsv1 and Rsv3 govern resistance SMV has an extremely low probability of overcoming replication inhibitors, a movement barrier, and the hypersensitive response since the resistance mechanism takes place in two steps. However, theoretically the HR increases the chance that unrecognizable-mutated viruses can survive and overcome resistance as it does not allow surviving of recognizable virus. Perhaps because of this reason, all of the alleles at Rsv1 and Rsv3 loci do not exhibit the resistant reaction to all SMV strains (Table 2 in literature review section).

In contrast, genes at the Rsv4 locus exhibit resistance to SMV-G1 through G7. Interestingly, all alleles discovered at the Rsv4 locus exhibit resistance to all SMV strains

without exception. Also, no hypersensitive responses were detected with the alleles at the Rsv4 locus, indicating that this gene may not code for a receptor, which binds to elicitors to activate a hypersensitive response or its receptor is not recognizable by the SMV strains. The expression of this gene is likely to exert less selection pressure on a virus and decreases the probability that a mutant virus can overcome resistance, since it allows virus replication and does not initiate HR. Therefore, deployment of cultivars possessing Rsv4 genes may contribute to broad, effective, and long-term resistance since the Rsv4 gene allows replication but prevents long distance movement of virus and thus decreases initial inoculum. Nutter et al. (1998) reported that SMV disease can be successfully managed by reducing the initial inoculum to levels approaching zero, reducing the temporal virus progress, and reducing the time span that host pathogen populations interact. However, SMV resistant cultivars have not been durable (Cho et al., 1977; Irwin and Goodman, 1981) because resistance is conferred by a single gene, which can be overcome easily by the pathogen. Thus, for SMV and related viruses, host resistance that reduces the initial inoculum and rate of within-plant and plant-to-plant spread may provide the greater benefits in terms of efficacy and durability (Padget et al., 1990; Bar-Zur and Salomon, 1995; Nutter et al., 1998). Expression of the Rsv4 gene not only reduces the initial virus inoculum (SMV G1 and G7) but also suppresses virus progress in the plant, thus, it seems to be a good candidate for preventing possible epidemics of SMV.

Conclusions

PI88788 possesses a single resistance gene at a locus, tentatively labeled 'Rsv4'. This gene exhibits resistance by blocking vascular movement of the virus (Figure 1) and is resistant to SMV strains G1 through G7. The gene expresses incomplete dominance when plants are inoculated with SMV-G1 but complete dominance when plants are inoculated with SMV-G7, as evidenced by the reaction of F_1 plants of PI88788 x Essex and PI88788 x Lee 68 (Tables 2,3 and 4).

Deployment of cultivars carrying Rsv4 genes may prevent an epidemic of virus in the field for two reasons: 1) it confers broad resistance to SMV strains and 2) it allows

some virus replication but decreases the rate of virus accumulation and prevents long distance movement, thus reducing the probability that more virulent strains can evolve.

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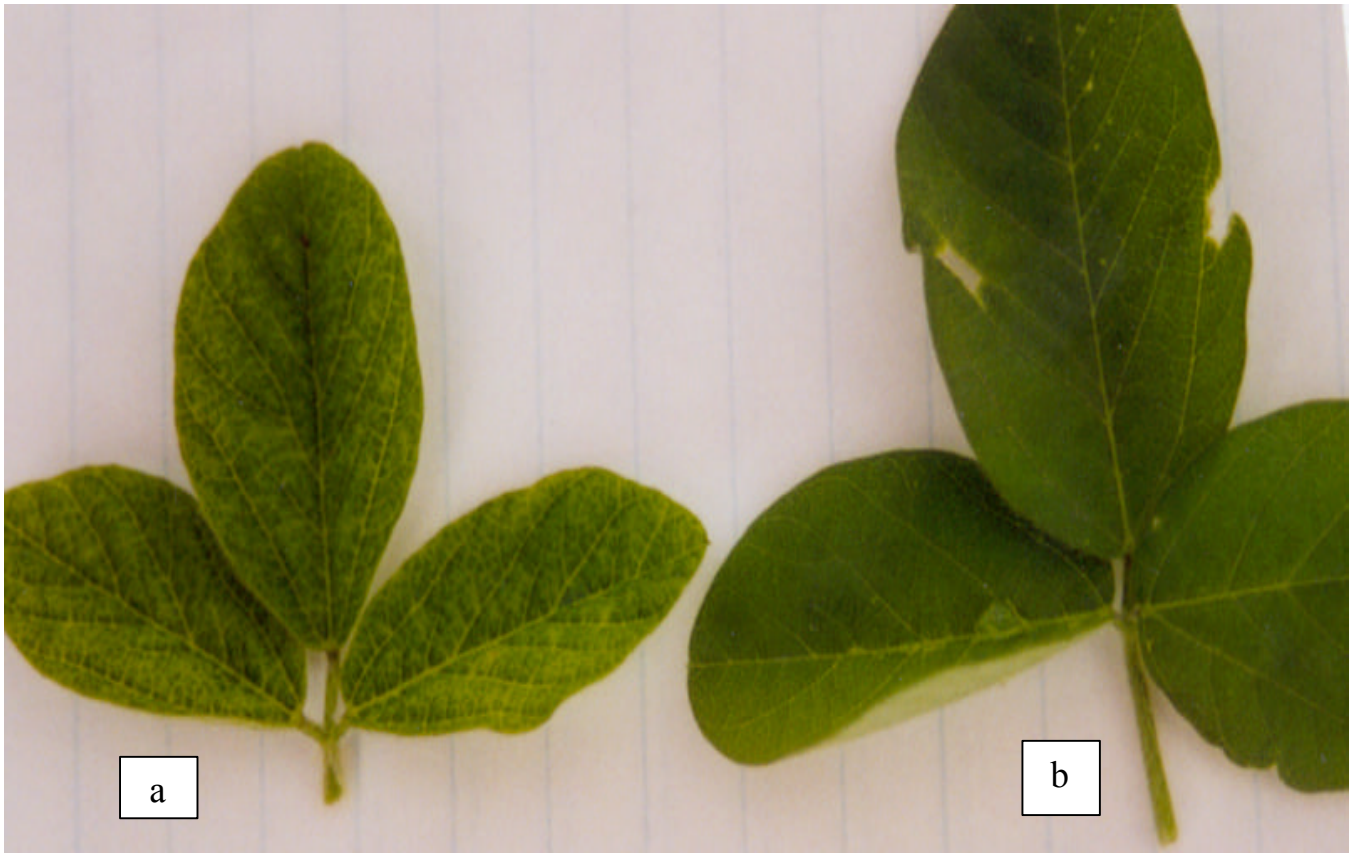
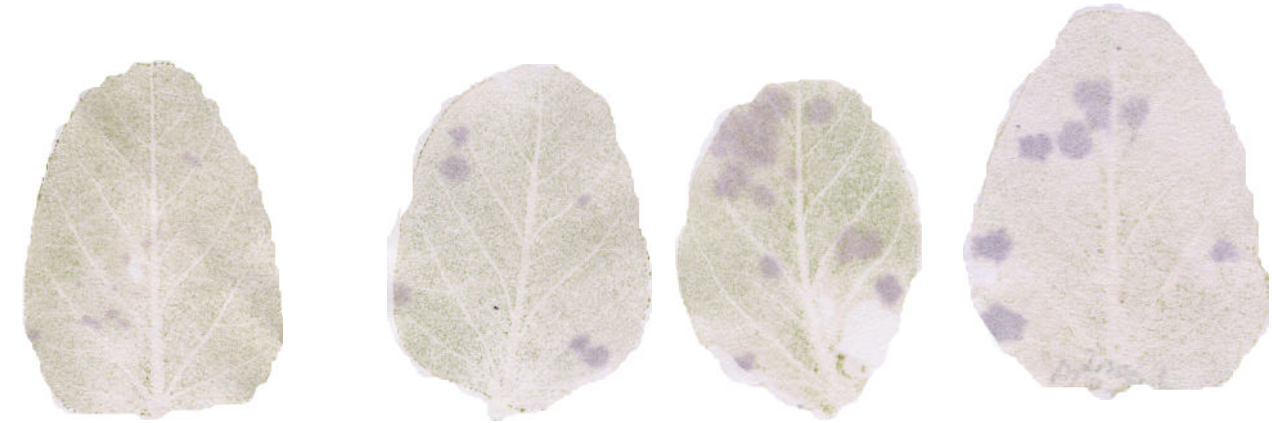


Figure 1. First trifoliolate leaves taken from susceptible (a) and resistant (b) plant 7 days after inoculation in the greenhouse.

Figure 2. Mosaic symptoms of soybean mosaic virus (SMV) on ‘Lee 68’ [17 days after inoculation (dai)with SMV-G1]

Figure 3. Late susceptible symptoms on F_1 plants of V94-5152 x Essex 32 dai with SMV-G1 in the greenhouse.



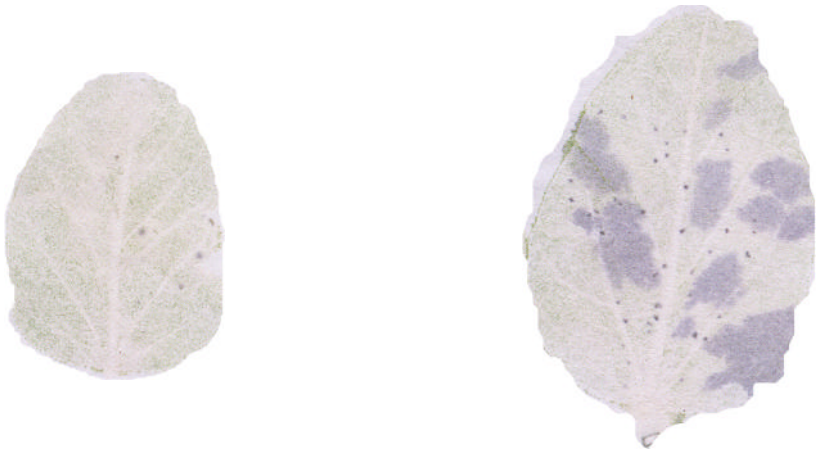
a b c d

e f g

Figure 4. Leaf tissue prints from G1 inoculated leaves of PI88788, V94-5152, and the susceptible cultivar Essex .

a) PI88788 11 days after inoculation (dai); b) PI88788 19 dai; c) PI88788 23 dai; d) PI88788 42 dai; e) Essex 6 dai; f) V94-5152 19 dai; g) Peking 23 dai.

Virus invaded areas appear as dark purple color.



a b

Figure 5. Leaf prints taken 35 days after inoculation with G7

a) PI88788 ; b) V94-5152

Dark purple area indicates SMV presence

Table 1. Reaction of parental genotypes to seven SMV strain groups.

Parents	SMV strains						
	G1	G2	G3	G4	G5	G6	G7
PI88788	R†	R	R	R	R	R	R
Peking	R	R	R	R	R	R	R
V94-5152 (Rsv4)[#]	R	R	R	R	R	R	R
L29(Rsv3)[#]	S	S	S	S	R	R	R
PI96983(Rsv1)[#]	R	R	R	R	R	R	N

† R, resistant (symptomless); N, necrotic; S, susceptible (mosaic)
[#] V94-5152 (Bus et al, 1997); PI96983 (Cho and Goodman, 1982); L29, (Buss et al., 1999)

Table 2. Reactions of F_1 plants and F_2 populations from PI88788 x Essex cross when inoculated with SMV-G1 and G7.

Cross and generation	SMV Strain	Number of plants			Expected Ratio	χ^2	P
		R†	LS	S			
PI88788 x Essex F_1	G1	0	10	0			
PI88788 x Essex F_2	G1	86	151	67	1R:2LS:1S	2.38	0.30
PI88788 x Essex F_2[#]	G1	41	24	16	3(R+LS):1S	1.18	0.27
PI88788 x Essex F_1	G7	4	0	0			
PI88788 x Essex F_2	G7	210	0	56	3R:1S	2.21	0.14

† R, resistant (symptomless); S, susceptible (mosaic); LS, late susceptible
[#] Data taken from field

Table 3. Reactions of F_1plants and F_2 populations from PI88788 x Lee 68 cross when inoculated with SMV-G1 and G7.

Cross and generation	SMV Strain	Number of plants			Expected ratio	χ^2	(P)
		R†	LS	S			
PI88788 x Lee 68 F_1	G1	0	7	0			
PI88788 x Lee 68 F_2	G1	90	45	35	3(R+LS):1S	2.57	0.11
PI88788 x Lee 68 F_2[#]	G1	55	16	22	3(R+LS):1S	0.08	0.76
PI88788	G1	14	0	0			
Lee 68	G1	0	0	14			
PI88788 x Lee 68 F_1	G7	4	0	0			
PI88788 x Lee 68 F_2	G7	156	0	45	3R:1S	0.77	0.39
PI88788	G7	12	0	0			
Lee 68	G7	0	0	11			

† R, resistant (symptomless); S, susceptible (mosaic); LS, late susceptible
[#] Data taken from field

Table 4. Reaction of $F_{2:3}$ families from late susceptible F_2 plants of PI88788 X Essex when inoculated with SMV-G1in the greenhouse.

$F_{2:3}$ family	Total plants	9 dai					41 dai					
		R	S	$\chi^2_{3:1}$	df[#]	P	R	LS	S	$\chi^2_{1:2:1}$	df[#]	P
1	15	14	1	2.68	1	0.10	4	10	1	2.86	2	0.24
2	11	7	4	0.75	1	0.39	0	7	4	3.73	2	0.15
3	14	10	4	0.09	1	0.77	3	7	4	0.14	2	0.93
4	17	14	3	0.49	1	0.48	4	10	3	0.64	2	0.73
5	16	13	3	0.33	1	0.57	2	11	3	2.37	2	0.31
6	15	11	4	0.02	1	0.89	2	9	4	1.13	2	0.57
7	16	10	6	1.33	1	0.25	6	4	6	4.00	2	0.14
8	13	9	4	0.23	1	0.63	4	5	4	0.69	2	0.71
Total	117			5.920	8					15.65	16	
Pooled		88	29	0.003	1	0.96	25	63	29	0.96	2	0.62
Heterogeneity				5.917	7	0.55				14.6	14	0.41

† R, resistant (symptomless); LS, Late susceptible; S, susceptible

[#] Degree of freedom

Table 5. Segregation of $F_{2:3}$ families derived from crosses of PI88788 x Essex, PI88788 x Lee 68, PI88788 x V945152 (Rsv4) and PI88788 x Peking (Rsv4) when inoculated with SMV-G1 and G7 in the greenhouse.

Cross and parents	SMV Strain	Number of families			Expected ratio	χ^2	P
		All R†	H	All S			
PI88788 x Essex $F_{2:3}$	G1	11	24	7	1(all R):2[3(R+LS):1S]:1(all S)	1.62	0.45
PI88788 x Essex $F_{2:3}$	G7	11	24	7	1(all R):2(3R:1S):1(all S)	1.62	0.45
PI88788 x Lee 68 $F_{2:3}$	G1	8	17	10	1(all R):2[3(R+LS):1S]:1(all S)	0.25	0.88
PI88788 x V94-5152 (Rsv4)	G1	46	0	0			
PI88788 x Peking (Rsv4)	G1	50	0	0			

† R, resistant; H, segregating ; S, susceptible

Table 6. Segregation of F_2 populations from crosses between PI88788 and R parents, when inoculated with SMV-G1 and G7 in the greenhouse .

Cross and parents	SMV Strain	Number of plants				Expected ratio	χ^2	P
		R†	N	LS	S			
PI88788 x PI96983 (Rsv1)	G1	262	4	36	26	15(R+N+LS):1S	1.57	0.21
L29 (Rsv3) x PI88788	G7	272	0	0	25	15R:1S	2.38	0.12
V94-5152 (Rsv4) x PI88788	G1	325	0	0	0			
Peking (Rsv4) x PI88788	G1	220	0	0	0			
PI88788 x Peking (Rsv4)	G1	198	0	0	0			

†R, resistant (symptomless); N, stem tip necrosis; S, susceptible; LS, late susceptible

Table 7. Segregation of $F_{2:3}$ families derived from PI88788 x PI96983 cross when inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families			
		Observed	Expected	$\chi^2_{7:4:4:1}$	P
All R	7	28	22.75		
3(R+N+LS) : 1S	4	12	13		
15(R+N+LS) : 1S	4	9	13		
All S	1	3	3.25		
Total	16	52	35	2.54	0.47

† R, resistant (symptomless); N, Stem tip necrosis; S, susceptible; LS, late susceptible

Table 8. Segregation of $F_{2:3}$ families derived from L29 x PI88788 when inoculated with SMV-G7 in the greenhouse.

Reaction†	Frequency	Number of families		$\chi^2_{7:4:4:1}$	P
		Observed	Expected		
All R	7	23	20.562		
3(R) : 1S	4	12	11.75		
15(R) : 1S	4	8	11.75		
All S	1	4	2.938		
Total	16	47	47	0.876	0.83

† R, resistant (symptomless); LS, Late susceptible; S, susceptible

CHAPTER IV

SUMMARY AND FUTURE RESEARCH

Reliance on single disease resistance gene can lead genetic uniformity and vulnerability. Deployment of multiple resistance genes either in separate cultivars or stacked in single cultivar should reduce genetic vulnerability. Therefore, plant breeders need to increase diversity of the SMV resistance genes to develop effective and durable resistance. The present study was done to identify new sources of SMV resistance genes. The major conclusions of this study can be summarized as follows:

The previously designated Rsv2 locus does not appear to exist in OX670 or its ancestors. This conclusion was obtained from results of inheritance studies of Harosoy and OX670. Genetic analysis of our data indicated that SMV resistance in Harosoy, which is resistant to SMV-G5 through G7 and susceptible to SMV G1 through G7, is governed by a partially dominant single gene at Rsv3 locus. OX670 which, is resistant to SMV-G1 through G7, possesses two SMV resistance genes. One of the gene in OX670 derived from Raiden and express necrotic reaction to SMV-G5 and G6 and resistance other strains another gene derived from Harosoy express resistant reaction to SMV-G5, G6, and G7. Therefore, complementary action of these two genes makes OX670 resistant to SMV-G1 through G7. Thus resistance in OX670 is not governed by a single gene at Rsv2 locus as proposed by Buzzell and Tu, (1984) but two genes at previously identified loci confer resistance to SMV-G1 through G7 in OX670.

Tousan 140 is resistant to SMV-G1 through G7. The SMV resistance in Tousan 140 is governed by two genes, one is allelic to Rsv1 while another is allelic to Rsv3. These two genes separated in two $F_{2:3}$ lines, which were temporarily labeled R1 and R2. R1 line possesses SMV resistance gene at Rsv1 locus is resistant to SMV-G1, G2, and G3 and susceptible to SMV-G5 through G7. R2 line however, exhibited resistant reaction to SMV-G5 through G7 and susceptible reaction to SMV-G1 through G3 possesses resistance gene at Rsv3 locus. Thus presence of these two genes makes Tousan 140 resistant to SMV-G1 through G7.

Hourei is resistant to SMV-G1 through G7, possesses two SMV resistance genes while one express resistance reaction to SMV-G1 and G7 the other expressed resistant reaction to SMV-G7 but susceptible reaction to G1. One of the genes in Houre is allelic to Rsv1, while another one is allelic to Rsv3.

The main conclusion we can obtain from these two plant introductions is that, combining Rsv1 and Rsv3 gene may create broad resistance to SMV.

PI88788 exhibited resistance reaction to SMV-G1 through G7. However presence of SMV was detected in the inoculated leaves of PI88788. In the inoculated leaves virus invasion rate occurred at a slow rate and no vascular movement of the virus were observed. Genetic analysis of our data reveals that SMV resistance in PI88788 is conferred by a single gene at a locus tentatively labeled 'Rsv4'. The gene expressed incomplete dominance when plants inoculated with SMV-G1 but complete dominance when plants inoculated with SMV-G7. From these results we can suggest that deployment of cultivars possessing Rsv4 genes may prevent an epidemic of virus in the field for two reasons: 1) It confers resistance to SMV-G1 through G7, and 2) It allows virus replication but decreases the rate of virus accumulation and prevents long distance movement. Therefore, expression of Rsv4 in PI88788 should not exert high selection pressure on the virus, and thus may decrease the probability for a mutated virus to overcome resistance.

Future Research

1- Resistance genes in Tousan 140, Hourei and PI88788 can be tested with new resistance breaking SMV isolates (if any).
2- Developing isogenic lines of Rsv4 and deployment of the isogenic line with the Rsv4 may prevent possible epidemic of SMV.
3- Pyramiding SMV resistance genes Rsv1, Rsv3, and Rsv4, may create durable and broad resistance.
4- To continue to search new sources of the SMV resistance genes.

VITA

Irfan Gunduz was born on August 27, 1966 in Mus, Turkey. After graduating from Antalya Caglayan high school, he was admitted to Cumhuriyet University, College of Agriculture in 1983. Mr. Gunduz received a B.S. degree with a major in Agronomy in 1987. He completed his one year mandatory military service. For a short time, he worked as a government official at Internal Revenue Service of Turkey. Then he joined to The Scientific and Technical Research Council of Turkey (TUBITAK) as assistant research scientist. In 1993, he was accepted as a teaching and research assistant to Akdeniz University, Turkey, where he received a M.S. degree in Forage crops in 1995. In 1996, he was admitted to the graduate program in the Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University to pursue his Ph.D. study. He is a member of the Crop Science Society of America and American Association for the Advancement of Science. He is married to Nazan Gunduz and has a two and half year old daughter, Erin Ilge Gunduz.

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Irfan Gunduz