GENETICS OF REACTIONS TO SOYBEAN MOSAIC VIRUS IN SOYBEAN

by

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"Disease resistance is the most feasible and often the only practical way of controlling many plant diseases"

Dr. Arthur L. Hooker, Professor of
Plant Pathology and Agronomy,
University of Illinois

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Chapter I

INTRODUCTION

Soybean mosaic (also known as soybean crinkle), caused by various strains and isolates of soybean mosaic virus (SMV), is one of the most prevalent viral diseases of soybean [Glycine max (L.) Merr.] in the world. SMV has been found wherever soybean is cultivated and is considered one of the more serious threats to soybean production in some areas (Sinclair, 1982).

SMV, a potyvirus, is a member of the potato virus Y group. Various SMV strains and isolates have been found in soybean germplasm throughout the world which differ in pathogenicity and symptomatology on soybean. A variety of symptoms ranging from mild mosaic to severe necrosis caused by SMV isolates have been observed in various soybean cultivars (Bos, 1970; Buzzell and Tu, 1984; Conover, 1948; Cho and Goodman, 1979, 1982; Cho et al., 1977; Lim, 1985; Ross, 1969, 1975; Sinclair, 1982; Takahashi et al., 1980; Tu and Buzzell, 1987).

In general, the virus causes systemic foliar enation, distortion, and severe stunting of infected plants. The infected plants often have reduced foliage, very poor pod set, reduced nodulation, and may exhibit increased susceptibility to other pathogens (Sinclair, 1982; Tu et al., 1970). Ultimately, the virus has significant economic impact on soybean production, causing considerable yield loss (El-Amretz et al., 1987a; Hill et al., 1987; Kwon and Oh, 1983; Ross, 1983); altering chemical composition of the seed (El-Amretz et al., 1987b; Gupta and

Joshi, 1976; Tu and Ford, 1970b); causing undesirable seed coat mottling (Dunleavy et al., 1970; Iwai et al., 1985; Kennedy and Cooper, 1967; Kwon and Oh, 1983; Ross, 1968; Tu, 1975); reducing seed viability and germinability (El-Amretz et al., 1987a), and seedling vigor (Kwon and Oh, 1983).

The primary factor affecting the world-wide distribution of SMV is that it is seedborne (Bowers and Goodman, 1979, 1982). The virus is also transmitted readily by aphids in a nonpersistent manner. At least 31 aphid species have been reported to transmit SMV (Sinclair, 1982). In addition, the alternate hosts (primarily species of Leguminosae and weeds) such as Phaseolus lathyroides L., Cassia occidentalis L., Amaranthus spp., and Setaria spp. also play some role in the epidemiology of SMV. At this time, no practical viricide treatments are available for chemical control of SMV. Although some control of the incidence of SMV can be accomplished by management practices such as sowing seeds from SMV-free fields, roguing out infected plants when first found, spraying chemicals to control the aphid vectors, eradicating the alternate hosts to minimize the inoculum reservoirs, only the use of virus-free seed has been effective to some extent. Using genetic resistance appears to be the most effective and inexpensive method and is especially applicable to control of SMV in soybean (Buss et al., 1985, 1989).

The basic requirements for a soybean breeding program in which SMV resistance is an objective are sources of resistance and methodology for combining resistance with commercially acceptable plant types. Once the resistant soybean types are identified, the choice of appropriate

parents and the inheritance of resistance play major roles in how they are used in a breeding program. If the resistance from a given source is simply inherited, any of the conventional breeding approaches can be used to incorporate the resistance gene into adapted germplasm. Backcrossing would be the simplest and most effective method if the resistance source is very unadapted. If the resistant parent is reasonably adapted, simple crosses followed by selection for resistance and adaptation could be used.

When multiple resistance genes at different loci are available, gene pyramiding can be used to incorporate as many of the genes as needed into a single line or cultivar, provided that appropriate tests can be made for the presence of each gene. When the resistance genes are alleles at a single locus, however, it would be impossible to incorporate two or more resistance alleles in a single line or variety unless a molecular manipulation technique is available and applicable. Alternatively, utilizing the multiple alleles could be accomplished, with great effort, by developing multi-isoline cultivars, mixtures or blends of cultivars with different resistance alleles (Buss et al., 1989; Ross, 1983). Obviously, the individual values of a resistant soybean genotype cannot be assessed in a breeding program, nor can an appropriate breeding method be selected until the inheritance of SMV resistance is better understood.

A unique system of soybean genotype x SMV strain interactions has been established (Cho and Goodman, 1979), in which SMV strains are differentiated by resistant, mosaic, or necrotic reactions on a group of soybean cultivars. Little is known about the genetic relationships of

resistance genes in this specific set of differential cultivars, although inheritance studies have been conducted using several of them. Knowledge of the inheritance of SMV reactions in Cho and Goodman's differential hosts will lead to a better understanding of soybean x SMV interactions.

The broad objective of this research was to explore the genetic basis of the differential reactions of a number of representative soybean types to the established SMV strains. The specific objectives of this research were: 1) to determine the inheritance of resistance in a member of each of Cho and Goodman's differential cultivar groups, 2) to examine and establish the allelomorphic relationships among the resistance genes from different sources, and 3) to identify new sources of resistance in soybean germplasm.

Chapter II

LITERATURE REVIEW

2.1 Aspects of soybean mosaic virus

History: Soybean mosaic virus (SMV) is probably the most common virus of soybean known today. SMV is distributed throughout soybean growing areas by seedborne infection and within fields by aphid vectors (Dunleavy, 1973; Sinclair, 1982). The virus is believed to have been introduced to the United States with the first soybean plant introduction from the Orient (Sinclair, 1982). The disease of soybean mosaic was first described by Clinton in 1915. It was not until 1921, however, that Gardner and Kendrick established the viral nature of the mosaic disease. These same investigators in 1924 established that the virus was transmitted through the seed of the cultivated soybean. Later, in 1940, Heinze and Kohler showed SMV was also transmitted by aphids (Dunleavy, 1973; Walters, 1963).

Properties: SMV is a member of the potyvirus group of plant viruses (Bos 1972; Sinclair, 1982). It is a flexuous rod ranging in length from 300-900 nm. The longevity *in vitro* is 2-5 days, thermal inactivation point is 55-70^{o}C, and the dilution end point is between 10^{-3} and 10^{-5}. SMV is most stable at pH 6-7 and loses infectivity at pH levels below 4 and above 9. It moves both upward and downward in plants and can be detected in all parts of systemically infected plants. Multiplication and movement occur most rapidly at 26^{o}C and must occur for systemic infection to take place. In systemically infected plants, higher virus

content is correlated with more severe symptoms (Ford et al., 1989; Sinclair, 1982). Little or no virus is detectable in plants exhibiting severe necrosis (Tolin, personal communication).

Transmission: SMV has three modes of transmission: mechanical, seed, and aphid (Bos, 1972). Mechanical transmission has been accomplished with sap extracted from infected leaves. The sap was rubbed on leaf surfaces previously dusted with an abrasive, using cotton swabs, gauze or pestles (Galvez, 1963; Ross, 1969, 1970). Several species of aphids transmit the virus efficiently in a nonpersistent manner and bring about local movement of SMV. The most efficient transmission vector reported is Myzus persicae. Aphids become viruliferous by feeding on infected stems or leaves. At least 31 aphid species have been reported to transmit SMV (Sinclair, 1982; Ford et al., 1989). Oils have been used to prevent the spread of SMV by aphids. Joshi and Gupta (1974) tested 7 oils and found that a weekly spraying with 1% coconut oil emulsion for 5 weeks had some effect on control of SMV spread by aphids. Reifman (1974) recommended the use of systemic aphicides for control of the vectors and, in turn, control of SMV.

Seed transmission is probably the most important method of widespread distribution of SMV (Demski and Harris, 1974; Hill et al., 1980; Porto and Hegedorn, 1975). Seed coat mottling (bleeding hilum), a discoloration of the seed coat, is a characteristic of seed produced on SMV-infected plants (Cooper, 1966; Dunleavy, 1973; Hill et al., 1980; Ross, 1970; Tu, 1975b). The mottling is caused by the accumulation of flavenoid compounds, such as anthocyanins, in the seed coat (Ross, 1970). Ross (1970) also found that seed mottling was significantly

increased on susceptible cultivars when plants were exposed to 20°C or were infected with both SMV and bean pod mottle virus (BPMV). However, seeds exhibiting over 40% mottling produced no more infected seedlings than seeds rated at 0% mottling. Thus, he concluded that the percentage of virus transmission cannot be predicted from the amount of seed coat mottling. Hill et al. (1980) concurred with this finding and stated that seed mottling is not a reliable indicator of SMV infection of plants.

Seed transmission of SMV occurs at relatively low frequencies ranging from 1 to 18% (Ross, 1963). Bowers and Goodman (1979) observed a much lower (less than 1%) incidence of seed transmission in seeds from SMV inoculated plants. The low rate of seed transmission was thought to be due to inactivation of the virus during seed maturation, especially the drying process (Bowers and Goodman, 1979). SMV-infected seeds produce diseased seedlings or fail to germinate (Sinclair, 1982). Infected seedlings tend to be spindly and the primary leaves are rugose and curled downward. These leaves often become chlorotic prematurely and trifoliolate leaves are rugose and stunted (Ross, 1970; Sinclair, 1982).

Host range: SMV is known to naturally infect only soybean and its wild relatives (Sinclair, 1982). Bos (1972) showed it to be transmissible to about 30 plant species. Most of the host species belong to the Leguminosae. Three Leguminosae members, *Phaseolus lathyroides* L., *Cassia occidentalis* L., and *Sesbania exaltata* (Raf.) Cory. are the major systemic hosts. *Amaranthus* spp., *Setaria* spp., *Physalis virginiana* Mill., *P. longifolia* Nutt., and *Solanum carolinense* L. are prevalent

weed species that have been suggested as possible non-legume hosts for SMV (Hill et al., 1980).

2.2 Disease symptoms of SMV on soybeans

Mosaic: Various workers have reported symptoms ranging from mild mosaic to severe necrosis (Cho et al., 1977; Cho and Goodman, 1979; Han and Murayama, 1970; Kwon and Oh, 1980; Ross, 1969; Sinclair, 1982; Takahashi et al., 1980). Generally, the first symptom of infection on soybean plants after mechanical inoculation of the primary leaves is the appearance of yellowish vein-clearing along the small, branching veins of the first trifoliolate leaflets. This symptom is transitory and occurs only in the first trifoliolate leaflets. Typical rugosity generally appears on the third trifoliolate leaf. Increasingly more severe symptoms develop on subsequent leaves, which eventually show dark green enations along the main veins. Enations may be scattered or aligned on either side of the veins. Leaflets may become prematurely chlorotic among enations near the margins. Leaf margins frequently curve down at the side. The youngest and most rapidly growing leaves show the most severe symptoms. Plants infected early in the season are more severely stunted, with shortened petioles and internodes, and may mature later than uninfected plants.

Necrosis: Some SMV strains can produce necrosis on certain cultivars. The symptoms associated with necrosis include a brown discoloration of leaf veins, yellowing of the leaves, defined 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; Cho and Goodman, 1979, 1982; Sinclair, 1982). The necrosis is often observed on soybeans that have resistance genes or alleles (Buss et al., 1989; Cho and Goodman, 1982).

Temperature effect: Development of symptoms in SMV-infected plants is reported to be temperature-dependent (Cho and Goodman, 1979; Sinclair, 1982; Tu and Buzzell, 1987). The rugosity of trifoliolate leaflets tends to increase in severity on successive leaves when plants are grown at 20 to 25 ^{o}C (Conover, 1948; Dunleavy,1973; Sinclair, 1982). Low temperatures (18.5^{o}C) lengthen the time between inoculation and symptom development to 14 days as compared to 4 days at 29.5^{o}C (Dunleavy, 1973, Sinclair, 1982). The development of stem-tip necrosis was also shown to be temperature dependent (Tu and Buzzell, 1987). The majority of the inoculated plants developed necrosis at 20 and 24^{o}C, but developed typical mosaic symptoms at 28 and 32^{o}C.

Development of different symptoms also depends largely upon the combination of soybean genotypes and SMV strains (Cho and Goodman, 1979, 1982; Ford et al., 1989)

2.3 SMV strains

Soybean mosaic disease was first recognized to be caused by more than one strain of SMV by Conover (1948). Since then various SMV isolates obtained from soybean germplasm have been found to differ in pathogenicity and symptomatology on soybean (Cho et al., 1977; Cho and Goodman, 1979, 1982; Ross, 1969, 1970; Takahashi et al., 1980).

Cho and Goodman (1979) first attempted to classify a large number of diverse SMV isolates using differences in symptoms of soybean geno-

types. They developed a classification system for SMV strains based on their virulence on 8 soybean cultivars, and assigned 98 isolates of SMV obtained from seeds of USDA soybean germplasm collections to 7 groups (G1-G7). The reactions of the 8 soybean differentials to 7 SMV strains were classified as mosaic, necrotic, or symptomless. The differential cultivars included Clark and Rampage (both SMV-susceptible), Buffalo, Davis, Kwanggyo, Marshall, Ogden, and York (all SMV-resistant). All SMV strains tested caused infection and typical mosaic symptoms in cultivars Clark and Rampage. Strain G1 did not infect any of the 6 resistant cultivars. Strain G2 caused local and systemic necrosis in Marshall but did not infect other resistant cultivars. Strains G3 and G4 caused local and systemic necrosis in both Ogden and Marshall; strain G4 also infected Davis and York, causing either local and systemic necrosis or mosaic symptoms. Strains G5, G6, and G7 all caused mosaic symptoms in Davis and York; strain G5 also caused necrosis in Kwanggyo. Strain G6 caused necrosis in both Kwanggyo and Marshall, and strain G7, which infected all cultivars tested, caused necrosis in Marshall, Ogden, Kwanggyo, and Buffalo. Later, Cho and Goodman (1982) found that PI 96983 has the same reactions as Buffalo to the 7 SMV strains. Their results (1979, 1982) confirmed that virulent SMV strains cause severe necrosis in soybeans possessing resistance to less virulent strains of the same virus. Based on their results, Cho and Goodman suggested that a range of SMV strains, differing in virulence, should be used in breeding programs for SMV-resistance in soybean.

In addition to Cho and Goodman's 7 SMV strain groups, Buzzell and Tu (1984) reported a distinct isolate labeled G7A (originally from

Goodman) that caused mosaic symptoms in a PI 96983 derivative (L78-379) but did not produce symptoms on the cultivar 'Raiden'. Lim (1985) reported an isolate named C14 and found that the pathogenicity of C14 differed from that of any of the 7 SMV strain groups described by Cho and Goodman. C14 caused necrotic symptoms on Suweon 97 (PI 483084) and no symptoms on PI 96983 and PI 486355. In 1977, Cho et al. described a necrotic strain of SMV, designated SMV-N, which severely affected soybean cultivars carrying resistance genes for the common strain of SMV. The cultivar Kwanggyo, which was widely grown in Korea, was most severely affected. The incidence of necrosis was as high as 86% in the field and yields were considerably reduced. Another necrotic strain was reported in Canada causing stem-tip necrosis on cultivar Columbia and its derivatives and was identified as similar to the ATTC type strain of SMV. The necrosis was found to be a hypersensitive, temperature-dependent reaction (Tu and Buzzell, 1987). Takahashi et al. (1980) reported 5 SMV strains (A-E) in Japan differing in symptomatology on soybean. Strains A-C induced a mosaic and strains D and E caused crinkling, yellowing and tip necrosis. Four isolates from China, designated Sa, Sc, Sg, and Sh, recently proved more pathogenic based on disease severity than any previously studied (Ford et al., 1989; Gai et al., 1989). The relationships of SMV-N, A-E, Sa, Sc, Sg, and Sh to G1-G7 have not been determined. SMV strains recognized in the U.S. and differentiating cultivars are summarized in Table 1.

Table 1. Reaction of differential soybean cultivars to identified SMV strains

Cultivar	Reaction to SMV strains[1]									
	G1	G2	G3	G4	G5	G6	G7	G7A[2]	C14	Ref.[3]
Clark/Rampage	M	M	M	M	M	M	M	M	-	a
Lee 68/Essex	M	M	M	M	M	M	M	M	-	b
York/Davis	R	R	R	N	M	M	M	M	-	a
Marshall	R	N	N	R	R	N	N	M	-	a
Ogden	R	R	N	R	R	R	N	M	-	a
Kwanggyo	R	R	R	R	N	N	N	N	-	a
Buffalo/PI96983	R	R	R	R	R	R	N	M	-	a,c
Suweon 97	R	R	R	R	R	R	R	R	N	d
PI 486355	R	R	R	R	R	R	R	R	R	d

[1] M = mosaic, R = symptomless, N = necrotic, - = not tested.

[2] Reactions of cultivars other than Suweon 97 and PI 486355 are based on the present investigations.

[3] a = Cho and Goodman (1979), b = Chen et al. (1988), c = Buzzell and Tu (1984), d = Lim (1985)

2.4 Maintenance of SMV strains

It is important to maintain pure cultures of the virus and to maintain the purity during production of inoculum (Buss et al., 1985, 1989). Since SMV can only replicate in living cells of the host, it is often maintained continuously by periodically transferring the virus to young susceptible plants (Buss et al., 1985, 1989). The method has a constant requirement for space to propagate stock plants and time to make inoculations every few weeks. Additional disadvantages of the method include the potential for contamination of cultures by other viruses or other SMV strains, genetic modifications of the virus through multiple passage through the host, and possible loss of strains through accidental death of plants.

A preferred method for maintenance of SMV is by preservation of virus-infected tissue *in vitro*. However, the longevity of viable SMV *in vitro* varies, depending on the methods used. Lim (1985) described a method in which the infected leaves were freeze-dried in small vials that were sealed and placed in test tubes containing calcium sulfate. Sealed tubes were stored at -5°C. Long-term storage of inoculum was achieved by desiccation of infected tissue over calcium chloride and storage at 4°C or by preparing a liquid nitrogen powder of infected leaves and storing at -20° (Roane et al., 1983). Occasionally, however, these *in vitro* preservation techniques may be unreliable (Buss et al., 1989). Decrease of virus infectivity or even complete loss of the virus by the above *in vitro* techniques, and contamination of SMV strains by *in vivo* methods were frequently observed (Tolin, personal communication). Chen et al. (1988) established a tissue culture system to main-

tain SMV in callus culture grown on defined synthetic media in vitro. With this approach, SMV has been maintained viable for more than two years with no change of the virus properties or decrease of infectivity. The method is inexpensive, simple and greatly reduces the risk of contamination and strain loss.

2.5 Inoculum preparation

Small quantities of inoculum for greenhouse studies are usually prepared by grinding infected leaves with a chilled mortar and pestles or in a Waring blender in 0.01 M sodium phosphate buffer, pH 7.0 (Bowers and Goodman, 1982; Buss et al., 1985, 1989; Cho and Goodman, 1979, 1982; Hunst and Tolin, 1982; Lim, 1985; Roane et al., 1983) Potassium phosphate (0.01 M) and sodium citrate (0.05 M) may also be used as a buffer solution (Cho and Goodman; 1979; Roane et al., 1983). Sometimes the buffer concentration can be increased up to 0.05M (Bowers and Goodman, 1982). The ratio of the amount of buffer (ml) to the weight of infected tissue (g) can vary from 3:1 to 10:1 (v/w) (Cho and Goodman, 1979; Tolin, personal communication). A small amount (5-10g/l) of 22 um (600 mesh) carborundum is often added to the inoculum suspension as an abrasive that helps to wound cells and provide an entry point for the virus (Bowers and Goodman, 1982; Buss et al., 1985, 1989; Cho and Goodman, 1979). The inoculum may be squeezed through several layers of cheesecloth plus one layer of Miracloth to remove plant debris (Bowers and Goodman, 1982; Cho and Goodman, 1982, Lim, 1985).

Large quantities of SMV inoculum for field use can be obtained by homogenizing freshly harvested infected leaves in a blender using 2-3

ml of 0.05 M sodium citrate buffer solution per gram of tissue. The preparation is usually strained through 4 thicknesses of cheesecloth, additional buffer is then added to make 10 ml per g tissue and 0.05% (w/v) carborundum powder is added to the suspension (Roane et al., 1983).

2.6 Inoculation techniques

Successful screening under natural infestations is dependent on having a reliable source of inoculum as well as aphids to transmit the virus and these conditions do not always occur when needed. It is rare that a field will be uniformly infected with only a single virus or that infection would occur within a short time span. Thus, artificial inoculation is an important aspect of breeding for virus resistance (Buss et al., 1985, 1989).

Different methods of inoculation may be used, depending on the number of plants to be inoculated and the desired accuracy of the results. To inoculate up to a few hundred plants, a simple hand inoculation method is adequate and efficient (Buss et al., 1985, 1989; Cho and Goodman, 1979). The procedure involves rubbing a leaf of each plant with a pestle dipped into the inoculum (Buss et al., 1985; Cho and Goodman, 1979; Hunst and Tolin, 1982) or with a cotton tipped applicator that had been dipped into the inoculum (Cho and Goodman, 1982; Lim, 1985). Inoculated leaves are often washed with a spray of tap water (Buss et al., 1985; Cho and Goodman, 1979). The hand method produces a high proportion of infected plants and is suggested for most greenhouse work and small field studies.

If large numbers of plants are to be inoculated, some degree of mechanization of the inoculation process is desirable (Buss et al., 1989). A hand-pushed inoculator was developed by Ross (1978) to inoculate rows in the field. It is essentially a set of inoculum-soaked pads mounted on wheels. The pads rub the plants on both sides as the device is pushed down rows of plants. When it is more critical that every plant be inoculated, as in a genetic study, an artist's airbrush is the most effective method for inoculation of large numbers of plants (Buss et al., 1989). The inoculum is applied to the plants with the airbrush that is supplied with air pressure of 4.2-5.6 kg/cm^2 (Bowers and Goodman, 1982; Kiihl and Hartwig, 1979; Roane et al., 1983). In some cases, double or multiple inoculations can be performed to increase the disease pressure (Roane et al., 1983; Quiniones, 1971).

2.7 Examination of plant reactions and detection of SMV

Disease rating is generally made at least 10 days after inoculation when the inoculated plants begin to show symptoms of infection (Cho and Goodman, 1979). Symptom development may be observed at 7-10 day intervals for at least a month following inoculation since certain genotypes may show very late response to the virus inoculation (Buss and Tolin, personal communication). Notes on symptom development may be taken daily for specific purposes (Cho and Goodman, 1979). Individual plant reactions should be rechecked at least twice for accurate classification.

Inoculated plants are usually classified as resistant (symptomless), necrotic, or susceptible (mosaic) (Chen et al., 1988; Cho and

Goodman, 1979; Kiihl and Hartwig, 1979; Lim, 1985). The necrotic reaction often has a more severe effect on the plant than the typical mosaic reaction since death or severe stunting of the plants usually results, with little or no seed production. Thus, it might be regarded as a form of extreme susceptibility (Buss et al., 1989). However, the results from most genetic studies indicate that necrotic plants should be included with the resistant class when evaluating segregating populations. There are four cases in the SMV literature in which the necrotic reaction was regarded as resistant (Buss et al., 1989, Chen et al., 1988; Kiihl and Hartwig, 1979; Tu and Buzzell, 1987), one case in which necrotic plants were included with the susceptible class (Lim, 1985), and one case in which the classification of necrosis was not stated (Buzzell and Tu, 1984). Kwon and Oh (1982) treated necrotic plants as a separate class but called them susceptibles. Chen et al. (1988) used ELISA and infectivity assay to test the presence of SMV in necrotic plants and found that little or no virus was detectable and that the leaf sap from the necrotic plants did not induce symptoms on susceptible hosts, thus suggesting that the necrotic plants should be rated as resistant.

Buss et al. (1989) pointed out that an immunological test or host plant test should be used to test for the presence of SMV in a plant if the specific plant reaction classification is critical to the experiment. SMV can be detected serologically using an Ouchterlony double diffusion test or physically by electron microscopy (Hunst and Tolin, 1982). The presence and the content of SMV in a plant can be tested by enzyme-linked immunosorbent assay (ELISA) (Bowers and Goodman, 1982;

Cho and Goodman, 1979; Lister, 1978; Moore et al., 1982) and by Top Crop bean indexing techniques (Bowers and Goodman, 1982; Cho and Goodman, 1979, 1982; Lim, 1985; Milbrath and Soong, 1976) or by infectivity assay on differential soybean types (Chen et al., 1988).

2.8 Inheritance of SMV resistance

Knowledge of the inheritance of resistance and the genetics of host-pathogen interactions are essential to an effective breeding program for SMV resistance (Buss et al., 1989). Inheritance of reaction to SMV has been investigated by various workers using different soybean genotypes and SMV strains.

Koshimizu and Iizuka (1963) conducted inheritance studies using resistant and susceptible plants in Japan. In two crosses, the F_1 was resistant and the F_2 population segregated in a ratio of 3 resistant to 1 susceptible. In another cross, they considered the F_1 to be susceptible and the F_2 to segregate in a ratio of 7 resistant to 9 susceptible, and concluded that the SMV resistance was controlled by two complementary genes.

Kiihl and Hartwig (1979) conducted an inheritance study using 8 SMV-resistant and 3 susceptible cultivars and lines. The resistant types had resistance tracing to either PI 96983 or Ogden. They inoculated F_1 plants, BCF_1, F_2, and F_3 populations with SMV-1 and SMV-1-B isolates which were later assigned by Cho and Goodman (1982) to strain groups G2 and G3, respectively. They found that F_1 plants from crosses having PI 96983 as the resistant parent showed no symptoms and that F_1 plants with the Ogden source of resistance exhibited necrosis. F_2 popu-

lations segregated 3 resistant : 1 susceptible with necrotic plants being included in the resistant class. F_3 progenies from symptomless F_2 plants gave a good fit to 1 resistant : 2 segregating. The backcrossed progenies segregated with a good fit to 1:1 ratio for resistant and susceptible plants. They concluded that a pair of dominant alleles condition resistance to SMV. They further detected 2 different types of resistance, using graft techniques to determine the reaction of heterozygous scions, and found that the highest level of resistance in PI 96983 gave protection against SMV-G2 and G3, the lesser level of resistance in Ogden gave protection against SMV only in homozygous condition. They proposed that the highest level of resistance be symbolized Rsv, the lesser level of resistance as rsv^t, and the susceptible allele as rsv. In their allelomorphic series, Rsv is dominant to rsv^t and rsv, and rsv^t is dominant to rsv.

Bowers (1980) provided evidence for a new locus involved in resistance to certain SMV strains. A late-maturing selection from 'Hardee' was resistant to SMV strains G5, G6, and G7 and this resistance was conditioned by a dominant allele at a locus distinct from that found in Buffalo.

Kwon and Oh (1980) investigated the inheritance of resistance to a necrotic strain SMV-N isolated in Korea, which caused severe necrosis on cultivars having resistance genes for the common SMV strain. They crossed five resistant lines with a necrotic-reacting cultivar Kwanggyo and found that all the F_2 progenies segregated in a ratio of 3 susceptible (refers to the necrotic reaction) to 1 resistant plants. Thus, they concluded that the resistance to SMV-N was conferred by a single

recessive gene. This gene was not tested with any of the strains established by Cho and Goodman (1979).

Roane et al. (1983) screened F_3 progenies from the cross of York x Lee 68 separately with two viruses, SMV and peanut mottle virus (PMV). They found that reaction to SMV-G1 in York is conditioned by one gene with resistance dominant. This gene was demonstrated to be independent of pubescence color, but is closely linked with the single dominant gene for resistance to PMV.

Buzzell and Tu (1984) found a line carrying SMV resistance derived from Raiden (PI 360844) had a single dominant gene conditioning resistance to strains G7 and G7A. When this line was crossed to a line containing Rsv and the cross was tested with G6, segregation ratios observed in F_2 indicated that there were two independent genes conferring the resistance. The Raiden gene was then designated Rsv_2.

Lim (1985) reported that crosses of PI 96983, Suweon 97 (PI 483084), and PI 486355 with a susceptible cultivar all segregated monogenically when F_2 populations were inoculated with strains G2, G7, or C14. The segregation ratio of 3R:1S was obtained when necrotic plants were included in the susceptible class. F_2 plants derived from all the possible crosses involving the three resistant parents segregated in a 15 resistant : 1 susceptible ratio. They concluded that resistance in each of the three lines was conferred by a different dominant gene. Gene symbols were not assigned to the independent loci in PI 486355 and Suweon 97 since the allelism tests were not done to determine their relationships with the Rsv_2 gene in Raiden. However, Lim assumed that the Suweon 97 gene was identical to the Raiden gene since both culti-

vars reacted very similarly to strains G1-G7 and C14.

Buss et al. (1987, 1989a) showed that resistant cultivars Marshall and Kwanggyo each carried one dominant gene for resistance to SMV-G1 and that these single genes were allelic at a common locus. However, the allelic relationships of these two genes with other recognized genes were not established.

Tu and Buzzell (1987) reported that the necrotic reaction exhibited by the cultivar Columbia and its derivatives was a hypersensitive and temperature dependent reaction, and was controlled by a single dominant gene. The necrotic reaction was found to be dominant to the susceptible (mosaic) reaction. The SMV strain used in their study was identified as similar to the ATTC type strain of SMV, based on the particle morphology and differential host series.

Gai et al. (1989) investigated the inheritance of soybean to 4 local strains of SMV isolated in China using 9 resistant and 4 susceptible cultivars. Their data from F_2 plants, F_3 families, and testcross plants indicated that resistance to each of the 4 strains was conditioned by separate dominant genes labeled A, C, G, and H, respectively. All the 4 loci were shown to be in one linkage group with the order of G-H-A-C. The map units were estimated as 25-28, 24, and 13-16, respectively. The four loci were independent of T (pubescence color) in linkage group 1, W1 (hypocotyl color) in linkage group 8, and Ln (leaflet shape) probably in linkage group 4. However, their results were not very conclusive and their genetic interpretation was obscure.

2.9 Summary

Soybean mosaic virus, a potyvirus, is worldwide in distribution. Various strains and isolates differing in pathogenicity and symptomatology have been found since the virus was recognized in the early 1900s. SMV is seedborne, and transmitted both mechanically and by aphids. The virus is most stable at pH 6-7, and multiplies most rapidly in soybean leaves at 26°C. SMV has a narrow host range, mostly restricted to species of Leguminosae.

SMV has significant deleterious effects on soybean, inducing symptoms ranging from mild mosaic to severe necrosis, causing drastic yield loss, reducing seed quality, viability, germinability, and seedling vigor.

The response of soybean to SMV infection depends on cultivar, virus strain, and environmental conditions. Several strains with different virulence have been differentiated by resistant, mosaic, or necrotic reactions on a set of soybean cultivars with different reaction-conditioning genes or alleles. SMV strains can be maintained in living plants, desiccated tissue, or callus culture.

SMV inoculum is usually prepared by grinding infected leaves in 0.01 M sodium phosphate buffer at pH 7, in presence of carborundum or citrate as abrasive. Inoculations can be made by a pestle, cotton-tipped applicator, a hand-pushed inoculator, or an artist's airbrush.

SMV can be detected serologically using Ouchterlony double diffusion tests or ELISA, and biologically using Top Crop indexing or infectivity assay on differential soybean genotypes.

The inheritance of resistance has been studied in 7 cultivars, and

gene symbols have been assigned for 2 loci. Most of the genetic studies revealed that SMV resistance is conditioned by single dominant genes or alleles. However, exceptions were also reported, such as two complementary genes, single recessive gene, two duplicate genes, and four linked genes.

Chapter III

MATERIALS AND METHODS

3.1 Growth of F_1 and F_2 plants for seed production

Crosses among the selected parents were made in the greenhouse at Blacksburg or in the field at the Eastern Virginia Agricultural Experiment Station, Warsaw, Virginia. Seeds from each cross and seeds from both parental plants were harvested separately. Up to six seeds from each cross were space-planted in the field free of SMV at Warsaw. Progeny of the parent plants were also grown along with the F_1 plants for cross verification and for seed increase. Hypocotyl color, flower color, color of pubescence, and pod color were used as genetic markers to identify true F_1 plants. Mature seeds from each F_1 plant were threshed and stored separately in coin envelopes. Color of seedcoat and hilum color were also used as genetic markers to verify true crosses post harvest. Plants identified as arising from self-pollinated seeds were discarded.

For production of F_3 seeds, more than 100 F_2 plants from each F_1 family were grown in the field at Warsaw or at Blacksburg with no SMV inoculation. Notes on segregation of indicator traits mentioned above were taken for each F_2 population for true-cross identification. Plant height and maturity were also used as verification markers. Individual F_2 plants were harvested and threshed separately.

It is essential when testing for goodness-of-fit to a specific genetic ratio that the sample size (number of plants grown) is large

enough to avoid chance omission of a genotype and to distinguish between different segregation ratios, i. e., 15 resistant : 1 susceptible vs. all resistant. In general, sample size is sufficiently large if one can expect 3 or 4 plants in the least frequent class. In this case, the F_3 ratio with the lowest expected frequency of susceptible plants was 15R:1S. Thus 50-60 seeds per F_2 family should give satisfactory results. Eleven plants in an F_3 row would ensure a 95% chance of obtaining one susceptible plant if the expected ratio is 3R:1S (Snedecor, 1977).

3.2 Propagation of the virus and inoculation procedures

The SMV strains used throughout the study were obtained from Dr. Sue A. Tolin, Department of Plant Pathology, Physiology, and Weed Science, VPI&SU. SMV-G1 was described by Hunst and Tolin (1982). All other strains were those of Cho and Goodman (1979). The virus cultures were initially maintained in Lee 68 or York soybean grown in the greenhouse, and then transferred to soybean callus cultures *in vitro* (Chen et al., 1988). When a strain was needed for test, the virus was taken from callus culture and increased by propagation in greenhouse-grown plants of Lee 68 or York.

For the greenhouse studies, inoculum of each strain was prepared by homogenizing trifoliolate leaves showing typical mosaic symptoms 2-3 weeks after inoculation of the stock plants. The leaves were ground with a mortar and pestle in 0.01 M sodium phosphate buffer solution, pH 7.0, at an approximate rate of 1 g infected tissue per 10 ml buffer. A pestle was dipped in the inoculum and rubbed onto both unifoliolate

leaves of each seedling approximately 2 weeks after planting when trifoliolate leaves had not yet emerged. Leaflets were pre-dusted lightly with 600-mesh carborundum powder. The inoculum dosage was about 100 um per leaflet.

For the field experiment, large quantities of SMV inoculum were prepared from freshly harvested leaves 2-3 weeks following inoculation. Leaves were ground with a Waring blender in 2-3 ml of 0.05 M sodium citrate buffer solution per g of tissue. This preparation was strained through 4 thicknesses of cheesecloth, additional buffer was added to make 10 ml per g tissue and 0.5% (w/v) carborundum powder was added. The inoculum was kept on ice immediately following preparation and was used in the field within 3 hours.

Field inoculations were made by spraying inoculum onto leaves with an artist's airbrush (Bowers and Goodman, 1982; Cho and Goodman, 1982; Kiihl and Hartwig, 1979; Roane, et al., 1983), at an air pressure of 60-80 psi, supplied by a gasoline-powered compressor. Plants at the V1-V3 stage (Fehr and Caviness, 1977) were inoculated on the youngest fully expanded leaf or leaflet. About 0.2 ml of inoculum was dispensed onto the lower surface of one leaflet of each plant by spraying for about one second from a distance of 1-2 cm. The inoculum was frequently agitated to keep the carborundum in suspension.

The virus strains were checked for their biological integrity periodically by inoculating differential cultivars in the greenhouse. This precaution was taken to detect any strain contamination or genetic changes in the virus strains.

3.3 Greenhouse evaluation of F_2 populations

F_2 seeds from a single F_1 plant were planted in a metal flat with dimensions of 8 x 35 x 50 cm filled with a greenhouse soil mixture (approximately 75% soil, 15% sand, and 10% peat). In each flat, approximately 60-75 F_2 seeds were planted in three rows (20-25 seeds/row). Also included in each flat was one row of Lee 68, the susceptible check cultivar, and one row of each parental cultivar. The parent row provided a sample of resistant or necrotic reactions for comparison. F_2 populations from a cross were tested at least twice using seeds from either the same or different F_1 plants. One pot with 6-10 plants of each additional differential cultivar was also included in each test for verifying the strain identity. Plants were maintained under natural daylength from late spring to early fall. By using artificial lighting, a constant daylength of 14 hours was provided during winter months. Greenhouse temperatures ranged from 24 to 30°C during daylight hours and from 15 to 20°C at night.

Before inoculation, each flat was carefully examined and any seedling that appeared abnormal in any way was rogued. All plants within a flat or pot were examined for SMV symptoms at 7-10 day intervals for at least a month after inoculation. Specific notes on type of symptoms and plant counts were taken on each scoring date. Individual plant reactions were divided into three categories: symptomless, systemic mosaic, or systemic necrosis. The necrotic class included plants exhibiting stem-tip necrosis, stem necrosis, systemic necrotic lesions, systemic veinal necrosis, or any combinations among these necrotic symptoms. The necrotic plants were removed from F_2 populations and recorded when they

were observed. The necrotic plants were included in the resistant class in X^2 tests for goodness of fit to expected ratios.

3.4 Field evaluation of F_3 progenies

Approximately 40 seeds from each of about 100 F_2 plants of each cross were planted in rows 0.9 m long with 0.9 m between rows and tiers. Lee 68 and York were planted every 50 rows across the field as susceptible and resistant checks, respectively. The parents of each cross were also included as checks in the section of the field where their F_3 progeny rows occurred. Only strain G1 was used for field inoculations because it is the prevalent strain in the field in Virginia.

Counts of susceptible, necrotic, and total plants were obtained for each F_3 progeny row one month after inoculation and rechecked approximately a month later. For genetic analysis, the necrotic plants were classified as resistant. The F_3 rows were classified as homogeneous resistant, segregating (either 3R:1S or 15R:1S), or homogeneous susceptible based on plant counts. X^2 tests were used to classify rows when the appropriate class was not obvious upon inspection.

In F_3 rows containing only one or two symptomatic (often questionable susceptible or necrotic) plants, leaf samples were taken to test for the presence of SMV using ELISA (Lister, 1978). Antiserum against SMV-G1, made in 1980, was used in the tests (Hunst and Tolin, 1982). The antigen extracts were prepared by grinding leaf samples with a Tekmar homogenizer in phosphate-buffered saline solution at 1:5 w/v at pH 7.4; 2% polyvinylpyrollidone and 0.05% Tween-20 were added as anti-

oxidant and surfactant, respectively. Absorbance at 405 nm was measured with a Bio-Tek EL 307 EIA Reader 2 hours after the addition of substrate. Identity of the virus from the 1986 nursery was checked biologically on indicator hosts in the greenhouse.

3.5 Proposed genetic models

Single gene segregation: In the case of single gene inheritance, F_1 plants from a resistant x susceptible cross should be all resistant if resistance is completely dominant. R x S F_2 populations should segregate 3R:1S. In the F_3 generation, 1/2 of the F_2-derived lines should segregate 3R:1S, and the remainder should be equally divided between homogeneous resistant and susceptible.

Two gene segregation: In the case of two independent dominant genes conditioning resistance, all F_1 plants should be resistant. F_2 populations should segregate 15R:1S. F_3 progenies will exhibit a ratio of 7 (all R) : 4 (3R:1S) : 4 (15R:1S) : 1 (all S). Linkage between the genes would increase the frequency of totally resistant F_3 progenies, as well as those segregating in a ratio similar to the F_2. The frequency of both totally susceptible and 3R:1S F_3 progenies would decrease correspondingly. However, as long as some recombination occurred, some progenies segregating 3R:1S should be found.

Allelism and linkage: To determine whether two resistant cultivars have allelic genes, it is necessary to cross them and genetically analyze the F_2 and F_3 progenies. If the resistance in each parent is controlled by two different genes at separate loci, an F_2 phenotypic ratio of 15R:1S is expected and a 7:4:4:1 genotypic ratio is expected from F_3

progeny tests. Absence of segregation for susceptible plants would indicate that the parents possess either identical genes for resistance or different alleles at the same locus. Close linkage of separate resistance genes could also produce an apparent lack of segregation and could be detected only with extremely large populations.

Chapter IV

Genetics of Resistance to Soybean Mosaic Virus Type Strain G1 in Five Differential Soybean Cultivars[1]

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Abstract

Five differential soybean cultivars [Glycine max (L.) Merr.] identified as resistant to soybean mosaic virus (SMV) were studied to determine the mode of inheritance of reaction to the type strain SMV-G1. Each cultivar has been previously reported to have a single dominant gene conditioning SMV resistance. These cultivars were crossed in all possible combinations with each other to determine the allelomorphic relationships of the resistance genes. Field-grown F_3 populations from 10 crosses were inoculated with virus strain SMV-G1. Greenhouse studies were also conducted with 17 F_2 populations. A single dominant gene with incomplete dominance was found to condition resistance to SMV-G1 in each of the resistant cultivars 'PI 96983', 'Ogden', 'York', 'Marshall', and 'Kwanggyo'. The lack of segregation for susceptibility in F_2 and F_3 progenies from the resistant x resistant crosses indicates that the resistance genes in these cultivars are probably alleles at a common locus. Gene symbols, $\underline{Rsv}^y$, $\underline{Rsv}^m$, and $\underline{Rsv}^k$ are proposed for the resistance alleles in York, Marshall, and Kwanggyo, respectively. Data from the experiment also furnished evidence that the necrotic reaction to SMV-G1 inoculation is highly associated with plants heterozygous for the resistance gene in segregating populations.

Additional index words: Glycine max, ELISA, allelism, necrosis.

Introduction

Soybean mosaic virus (SMV) causes significant yield losses and reduction of seed quality in soybean [Glycine max (L.) Merr.]. Various SMV isolates obtained from soybean germplasm have been classified into 7 (G1 - G7) strain groups (Cho and Goodman, 1979) based on the differential reactions (resistant, necrotic, or susceptible) of a group of soybean cultivars. The common strain G1 is the least virulent strain and does not infect any resistant cultivars. Roane et al. (1986b) postulated that the G1 strain lacks a virulence gene, and caused no symptoms on cultivars carrying resistance genes; they suggested that G1 should be used in all genetic studies to detect any resistance gene.

Various sources of SMV resistance have been identified in soybean (Cho and Goodman, 1982; Lim, 1985; Bowers and Goodman, 1982; Kwon and Oh, 1980). The genetics of resistance to SMV has been reviewed by Buss et al. (1985, 1989b). The method of utilizing resistance to SMV in a breeding program depends largely on the number of genes conditioning the resistance. Breeding for SMV-resistance will be simplified when the resistance is controlled by a single gene. If more than 1 gene is available, lines with multigenic resistance could be developed, provided tests can be made for the presence of each gene. The individual values of a resistant soybean type in a breeding program cannot be assessed until the inheritance of resistance is better understood.

Several genes conferring resistance to SMV have been reported and some have been assigned gene symbols. Kiihl and Hartwig (1979) detected different reactions in resistance to SMV-1 and SMV-1B isolates which

were later assigned by Cho and Goodman (1982) to strain groups G2 and G3. The high level of resistance in PI 96983, giving complete protection against SMV-G2 and G3, was controlled by a single dominant gene designated Rsv. The resistance gene in Ogden gave protection against SMV-G2 only in the homozygous condition, but produced necrosis in heterozygotes. Plants homozygous for the Ogden gene were necrotic after inoculation with SMV-G3. The Ogden gene was found to be allelic to Rsv, but given a recessive label, rsv^t, because of its lesser degree of resistance compared to Rsv. Buzzell and Tu (1984) found the single dominant resistance gene in cultivar Raiden to be at a different locus and labeled it Rsv_2. Lim (1985) reported that PI483084 and PI 486355 each had single dominant genes for resistance which were at a locus other than Rsv. Gene symbols were not assigned since allelism tests with Rsv_2 had not been conducted. Roane et al. (1983) demonstrated that a single dominant gene conditions resistance to SMV-G1 in cultivar York. However, no gene symbol was assigned for this gene since allelism tests had not been conducted against any other symbolized genes. Buss et al. (1989a) also reported that resistant cultivars Marshall and Kwanggyo each had a single dominant gene for resistance to SMV-G1 and that these single genes were allelic at a common locus. Their allelomorphic relationships with other reported genes have not been investigated.

Our objectives in this study were: 1) to confirm the inheritance of SMV resistance in PI 96983, Ogden, York, Marshall, and Kwanggyo, each of which is a member of a different strain differential group, and 2) to establish the genetic relationships among the resistance genes.

Materials and Methods

Five soybean cultivars identified as resistant (R) to SMV-G1 were used as parents. They were PI 96983, Ogden, York, Marshall, and Kwanggyo. 'Lee 68' and 'Essex' were used as susceptible (S) parents. Crosses were made either in the field at the Eastern Virginia Agricultural Experiment Station at Warsaw or in the greenhouse at Blacksburg. F_1 plants were grown in the field free of SMV at Warsaw. F_2 populations were grown in the field without SMV inoculation either at Warsaw or at Blacksburg and plants were harvested individually. Crosses were distinguished from selfs and outcrosses in F_1 and F_2 generations using appropriate genetic markers.

A Virginia SMV isolate previously designated SMV-VA, which was classified into Cho and Goodman's strain group G1 (Hunst and Tolin, 1982), was used in this study. The virus strain (referred to as SMV-G1 hereafter) was maintained in the greenhouse by regular passage in Lee 68 soybean. The strain identity was checked periodically by inoculating plants of the differential cultivars (Cho and Goodman, 1979) in the greenhouse.

Seven F_2 populations from R x S crosses and 10 populations from R x R crosses were screened with SMV-G1 in the greenhouse during the winter. The photoperiod was adjusted to 14 hours a day and the temperature was maintained at 21° to 26°C. Each of the F_2 populations was grown in a 8 x 35 x 50 cm metal flat. In each flat, 3 rows of 20-25 F_2 seeds were planted along with a row of each parent and a row of Lee 68 as a susceptible check. A pot (6 - 10 plants) of each differential cultivar

was also included in each test for strain verification. Inoculum was prepared by grinding SMV-G1 infected Lee 68 leaves in 0.01 M sodium phosphate buffer (approx. 10 ml per g leaf tissue) at pH 7 with a chilled mortar and pestle. Inoculations were made by gently rubbing the inoculum with a pestle onto both expanded unifoliolate leaves of each seedling (approx. 2 weeks after planting) which had been lightly dusted with 600-mesh carborundum powder. The inoculated plants were examined for symptoms of infection 2 to 3 weeks after inoculation and were rechecked approximately 2 weeks later.

F_3 progenies were tested with SMV-G1 in the field at Blacksburg from 1985 through 1987. Approximately 40 seeds from each of about 100 F_2 plants of each cross were planted in rows 0.9 m long with 0.9 m between rows and tiers. Lee 68 and York were planted every 50 rows across the field as susceptible and resistant checks, respectively. The parents of each cross were also included as checks in the section of the field where its F_3 progeny rows occurred. Large quantities of SMV-G1 inoculum for field use were prepared according to Roane et al. (1983) from freshly harvested Lee 68 leaves 2-3 wks following inoculation. The field inoculations were made by applying inoculum onto leaves of 3-wk-old plants with an artist's airbrush (Roane et al., 1983).

Plants in each F_3 row were classified 3 wks and 6 wks after inoculation for SMV-G1 reaction. Individual plant reactions in F_2 and F_3 populations were classified into 3 categories: no symptoms (R), systemic mottling (S), and systemic necrosis (N). The systemically necrotic plants were rated resistant as Kiihl and Hartwig (1979) proposed. The F_3 rows were classified as homogeneous resistant, segregat-

ing, or homogeneous susceptible based on plant counts. Chi-square tests were made on F_2 and F_3 data for goodness-of-fit to the expected segregation ratios. X^2 tests were also used to determine whether comparable F_2 or F_3 populations were homogeneous.

Leaf samples were taken from symptomatic plants in F_3 rows containing 1 or 2 susceptible or necrotic plants for enzyme-linked immunosorbent assay (ELISA) to detect the presence of SMV. The ELISA tests followed closely the procedures described by Lister (1978). Antiserum against SMV-VA/G1, made in 1980, was used in the tests (Hunst and Tolin, 1982). The antigen extracts were prepared by grinding the leaf samples with a Tekmar homogenizer in phosphate-buffered saline solution at 1:5 w/v at pH 7.4, containing 2% polyvinylpyrollidone and 0.05% Tween-20. Absorbance at 405nm was measured with Bio-Tek EL 307 EIA Reader after 2 hours of incubation. Negative results from ELISA tests were assumed to indicate the absence of SMV. Leaf samples were also taken from representative plants of each reaction class in 1986 for infectivity assay on a group of SMV-differential cultivars for virus identification.

Results and Discussion

Resistant parents grown either in the greenhouse or in the field and inoculated with SMV-G1 showed no symptoms of virus infection. Inoculated susceptible cultivars, Lee 68 and Essex, developed typical mosaic symptoms 2 weeks after inoculation, indicating that both mortar-pestle and airbrush techniques were effective.

The reactions of F_2 plants from R x S crosses and their parents to SMV-G1 are presented in Table 1. The data from all 7 crosses showed satisfactory fits to the 3R:1S segregation ratio, indicating that PI 96983, Ogden, York, Marshall, and Kwanggyo each possesses a single dominant gene conferring resistance to SMV-G1. The combined data show that the 7 crosses are homogeneous and provide an acceptable fit to the 3R:1S ratio. The results agree with the previous reports of a single dominant gene for resistance in York (to SMV-G1) (Roane et al., 1983), PI 96983 and Ogden (to SMV-G2 and G3) (Kiihl and Hartwig, 1979), and Marshall and Kwanggyo (to SMV-G1) (Buss, et al., 1989a, Roane, et al., 1986a). The similar genetic behavior of the reciprocal crosses of Ogden and Essex indicated no apparent cytoplasmic effect involved in the expression of SMV-G1 reaction.

In the segregating F_2 populations from the R x S crosses, approximately 30% of the plants in the resistant class were necrotic (Table 1). These necrotic F_2 plants were assumed to be heterozygous for the resistance gene. Kiihl and Hartwig (1979) found that the progeny of necrotic F_2 plants segregated for SMV reaction, which is an indication of heterozygosity for the resistance gene in the necrotic plants. We

were unable to test the progenies of the necrotic F_2 plants because they produced no seeds. However, in our tests of F_2-derived F_3 lines from the R x S crosses, the great majority (98%) of the necrotic plants occurred in segregating rows (data not shown), supporting the assumption that the necrosis is often expressed in plants that are heterozygous for the resistance gene. The association of necrotic reaction with heterozygosity for the resistance gene suggests an incomplete dominance.

None of the 10 R x R crosses produced any susceptible segregates in their F_2 populations (Table 2). The populations tested were not sufficiently large to rule out the possibility that the single dominant genes in these cultivars were closely linked rather than alleles at a common locus, but that possibility seems remote. In any of the R x R F_2 populations, one would expect either no plants with mosaic type reaction if the resistance genes are alleles or some plants with mosaic symptoms if genes are nonallelic. If resistance genes are completely independent, 1/16 of the population should be plants with mosaic type reaction. The complete lack of susceptible plants provides strong evidence that the resistance genes in PI 96983, Ogden, York, Marshall, and Kwanggyo are alleles at a common locus. The few necrotic plants observed in the F_2 did not appear to represent genetic segregation since occasional necrotic plants were also observed in Kwanggyo. although the possibility of a close linkage among the genes cannot be excluded by the population size investigated, it seems relatively unlikely that two or more genes controlling the same trait would be so closely linked.

No apparent segregating pattern was observed in F_2-derived F_3 progenies from the R x R crosses (Table 3) although occasional susceptible plants were observed. Most of the symptomatic (either susceptible or necrotic) plants detected in 1985 and 1987 gave negative results in the ELISA test, indicating probable infections by alien pathogens other than SMV. ELISA testing was not available in 1986, but it seems safe to assume that the observed symptomatic plants did not represent genetic segregation for susceptibility. Of greater concern are the single rows in York x Marshall, PI 96983 x York, and York x Ogden which segregated 3R: S. They do not appear frequently enough to fit any simple genetic models. A possible explanation is that our inoculum was contaminated with more virulent SMV strains that could cause necrotic or susceptible symptoms on plants having resistance genes (Cho and Goodman, 1979, 198), but that does not appear to be the case since a contaminant would not be expected to concentrate in 1 or 2 rows. The most likely explanation is that the 3R:1S F_3 rows resulted from natural outcrosses to susceptible plants in the F_1 generation and the single positive plants in some rows resulted from outcrosses in the F_2 generation. This level of outcrossing (less than 1%) is in the range normally observed in soybean (Carlson and Lersten, 1987). Also the possibility of mechanical mixture of a few seeds from susceptible plants cannot be ruled out.

The identity of the SMV strain was confirmed by infectivity assay on plants of Lee 68, Ogden, York, and Marshall in the greenhouse (Table 4). Leaf samples from symptomatic plants in segregating rows, homogeneous susceptible rows and susceptible check rows resulted in symptoms

of infection on Lee 68 but not on Ogden, York or Marshall. Because no other SMV strain gives a negative reaction on all three resistant cultivars, the presence of the G1 strain was confirmed. Samples from healthy or necrotic plants did not cause infection on any of the cultivars. In addition, all the necrotic plants detected in F_3 lines which were tested by ELISA showed negative results (Table 3). Both these findings suggest that the virus had not multiplied in the necrotic plants and provide additional evidence that they should be classified as resistant rather than susceptible.

The results of this study and previous investigations indicate that the 5 resistant cultivars each have a single dominant gene for resistance to SMV-G1 and that these genes are alleles at the Rsv locus. We propose that the symbols Rsv^y, Rsv^m, and Rsv^k be assigned to the resistance genes in York, Marshall, and Kwanggyo, respectively. We would also suggest that the symbol for the Ogden gene be changed to Rsv^t, because it is clearly dominant to rsv and the dominance relationships among Rsv, Rsv^t, Rsv^y, Rsv^m, and Rsv^k are as yet undetermined.

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List of Tables

Table 1. Segregation and X^2 tests for reaction to SMV-G1 in F_2 populations from resistant x susceptible crosses grown in the greenhouse.

Cross and parents	Class & frequency[1]				X^2	P
	Resistant			Susc.	(3:1)	(df=1)
	R	N	Total			
York x Lee 68	100	45	145	43	0.454	.50-.75
York	32	0	32	0		
Lee 68	0	0	0	66		
Ogden x Lee 68	58	34	92	26	0.554	.30-.50
Ogden	30	0	30	0		
Lee 68	0	0	0	32		
Ogden x Essex	52	46	98	29	0.318	.50-.75
Ogden	20	0	20	0		
Essex	0	0	0	19		
Essex x Ogden	59	74	133	36	1.233	.25-.50
Essex	0	0	0	28		
Ogden	24	0	24	0		
Marshall x Lee 68	61	56	117	38	0.019	.75-.90
Marshall	24	0	24	0		
Lee 68	0	0	0	56		
PI 96983 x Lee 68	158	5	163	49	0.403	.50-.75
PI 96983	40	0	40	0		
Lee 68	0	0	0	95		
Kwanggyo x Lee 68	57	117	174	52	0.478	.30-.50
Kwanggyo	41	2	43	0		
Lee 68	0	0	0	95		
F_2 total (df=7)	545	377	922	273	3.459	
F_2 pooled (df=1)					2.959	.05-.10
Heterogeneity (df=6)					0.500	>.995

[1] No. of F_2 plants, R = symptomless, N = systemic necrosis, and Susc. = systemic mosaic.

Table 2. Reaction of F_2 populations from crosses among resistant parents to inoculation with SMV-G1 in the greenhouse.

Cross and parents	No. of plants[1] R	N	S
PI 96983 x Ogden	112	5	0
PI 96983	19	0	0
Ogden	19	0	0
PI 96983 x Marshall	119	3	0
PI 96983	19	0	0
Marshall	22	0	0
York x PI 96983	118	4	0
York	23	0	0
PI 96983	22	0	0
PI 96983 x Kwanggyo	121	9	0
PI 96983	22	0	0
Kwanggyo	19	2	0
York x Ogden	94	5	0
York	24	0	0
Ogden	30	0	0
York x Marshall	66	17	0
York	18	0	0
Marshall	16	0	0
York x Kwanggyo	79	19	0
York	15	0	0
Kwanggyo	12	2	0
Ogden x Marshall	124	5	0
Ogden	19	0	0
Marshall	13	0	0
Ogden x Kwanggyo	81	5	0
Ogden	16	0	0
Kwanggyo	19	4	0
Kwanggyo x Marshall	140	15	0
Kwanggyo	11	1	0
Marshall	12	0	0

[1] R = resistant, N = stem-tip necrosis or systemic necrotic lesions, and S = systemic mosaic.

Table 3. Seedling reaction to inoculation with SMV-G1 in the field of F_3 lines from crosses among resistant cultivars.

Crosses	No. of F_3 rows			No. of plants[#]	
	Total	Homo-R	Seg.[+]	S	N
1985 nursery:					
Kwanggyo x Marshall	82	80	2	12(0)	11(0)
1986 nursery:					
York x Marshall	94	80	14[++]	21	2
Kwanggyo x Marshall	56	54	2	1	1
York x Kwanggyo	32	29	3	3	1
PI 96983 x Ogden	61	53	8	12	0
PI 96983 x York	80	55	25[++]	34	2
PI 96983 x Marshall	142	140	2	4	0
1987 nursery:					
PI 96983 x Kwanggyo	41	40	1	1(0)	0
York x Ogden	158	151	7[++]	11(5)	3(0)
Ogden x Marshall	131	122	9	10(0)	9(0)
Ogden x Kwanggyo	114	104	10	14(0)	13(0)
York x Kwanggyo	71	52	19	32(4)	23(0)
Kwanggyo x Marshall	71	68	3	4(0)	9(0)
Total	1133			165	74

[+] all rows had 1 or 2 susceptible plants, except as noted.

[++] only 1 row fits 3:1 segregation; all others had 1 or 2 susceptible plants.

[#] No. of susceptible (S) or necrotic (N) plants observed in segregating F_3 progeny rows, also represents no. of samples taken from either susceptible S) plants or necrotic(N) plants for ELISA tests, numbers in parentheses are plants giving a positive reaction in ELISA for SMV.

Table 4. Infectivity assay on greenhouse-grown plants for SMV-G1 of leaf samples taken from F_3 progeny and check rows in the 1986 field nursery.

Samples from	No. of samples	Reaction on differential cultivars in greenhouse			
		Lee 68	Ogden	York	Marshall
S Lee 68 plants	3	12/13[+]	0/18	0/9	0/17
S plants in seg. rows	3	12/14	0/20	0/19	0/9
Necrotic plants	2	0/11	0/15	0/9	0/9
Healthy plants	3	0/10	0/20	0/16	0/16
S plants in susc. rows	3	15/15	0/19	0/15	0/11

[+] Number of infected plants/total no. of plants inoculated.

Chapter V

Reaction and Genetic Segregation of SMV Resistance Genes in Soybean after Inoculation with Virulent SMV Strains[1]

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Abstract

Five SMV differential soybean cultivars, 'PI 96983', 'Ogden', 'York', 'Marshall', and 'Kwanggyo', were studied to determine the inheritance of their resistant or necrotic reaction to virulent strains (G4-G7 and G7A) of SMV. Each of the five parents was crossed reciprocally with a susceptible cultivar ('Essex', or 'Lee 68') to determine the number of genes for resistance or necrosis. The five SMV-resistant parents were also crossed among each other to test the allelism of the genes conditioning the resistant or necrotic reaction. F_2 plants from all resistant x susceptible crosses segregated in a 3 resistant to 1 susceptible ratio. All the F_2 populations from necrotic x susceptible crosses segregated in a 3:1 ratio with necrosis dominant to susceptibility. The results indicate that the resistant and necrotic reaction to virulent SMV strains were governed by the same genes. There is no cytoplasmic effect involved in expression of SMV reaction. The resistant x necrotic crosses consistently produced more necrotic plants than resistant plants in F_2 populations, indicating that heterozygous plants frequently exhibit necrotic reaction. The absence of susceptible segregants in F_2 populations of necrotic x necrotic and resistant x resistant crosses and the lack of segregation in susceptible x susceptible crosses indicates that the single dominant genes carried in the 5 resistant parents are alleles at a common locus.

Introduction

Strains of soybean mosaic virus (SMV), differing in pathogenicity and symptomatology on soybean [Glycine max (L.) Merr.] have been found worldwide in soybean germplasm since 1948 (Conover, 1948; Ross, 1969; Cho et al., 1977; Cho and Goodman, 1979, 1982; Takahashi et al., 1980). Cho and Goodman (1979) established a classification system for SMV strains based on their virulence on resistant soybean cultivars and assigned a number of SMV isolates from USDA germplasm collections to 7 strain groups (G1-G7) by the differential reactions of 8 soybean cultivars. Mosaic, necrotic, or symptomless reactions have been observed in soybean cultivars infected with SMV. The necrotic reaction was observed to be the reaction of soybeans possessing resistance to less virulent SMV strains when inoculated with a more virulent strain of the same virus (Cho et al., 1977; Cho and Goodman, 1979, 1982). The necrosis caused by virulent strains of SMV is considered a serious problem for soybean production because this reaction often has a more severe effect on the plant than the typical mosaic reaction. The necrotic reaction occurs both on the inoculated leaf and on subsequent leaves and stems. The infected plants are usually severely stunted and eventually die with little or no seed production (Buss et al., 1989b). The seriousness of necrosis in soybean has emphasized the need for determining the genetic basis of the necrotic reaction.

The inheritance of resistance (symptomless reaction) to SMV in soybean cultivars has been studied by various investigators. Kiihl and Hartwig (1979) used SMV-G2 and G3 to detect 2 allelic genes, symbol-

ized Rsv and rsv^t, in resistant cultivars PI 96983 and Ogden, respectively. The rsv^t gene was demonstrated to be recessive to Rsv, but dominant to susceptibility (rsv). Buzzell and Tu (1984) identified a gene at a different locus, labeled Rsv_2, for resistance to SMV-G7 and G7A in cultivar Raiden. Roane et al. (1983) reported that resistance in York to SMV-G1 was controlled by a single dominant gene, but did not assign a gene symbol. Buss et al. (1989a) found single allelic dominant genes conferring resistance to SMV-G1 in Marshall and Kwanggyo. Lim (1985) reported that Suweon 97 and PI 486355 carry single dominant genes at different loci from each other. He concluded that these two genes were not at the Rsv locus, but he did not test for allelism with the Rsv_2 locus.

The information on genetics of necrotic reaction, however, is limited. Kwon and Oh (1980) reported that the resistance to a necrotic strain SMV-N isolated in Korea was governed by a single recessive gene. Tu and Buzzell (1987) found that the stem-tip necrosis on cultivar Columbia and its derivatives was a hypersensitive, temperature-dependent reaction and was controlled by a single dominant gene. It is not known whether the resistant and necrotic reactions to SMV are conferred by the same gene or different ones. The objective of this study was to further characterize the inheritance of resistant and necrotic reaction to virulent SMV strains in 5 soybean cultivars that have been reported to have single dominant genes for resistance.

Materials and Methods

Five soybean cultivars (PI 96983, Ogden, York, Marshall, and Kwanggyo) previously identified as resistant to SMV-G1 and giving various combinations of resistant (R), necrotic (N) and susceptible (S) reactions to virulent strains of SMV (G4-G7 and G7A) were used as parents. They were crossed with cultivars Essex and Lee 68 which are susceptible to all SMV strains to determine the mode of inheritance of resistance. The 5 resistant parents were also crossed among each other to test the allelic relationships of the genes conditioning the resistant or necrotic reactions. All crosses were made in the greenhouse at Blacksburg. F_1 plants for producing F_2 populations were grown (space-planted) without SMV inoculation in the field at Warsaw, Virginia, and were verified for true crosses using appropriate genetic markers. F_2 populations were screened with selected SMV strains in isolated greenhouses. Each of the F_2 populations was grown in 8 x 35 x 50 cm metal flats containing a greenhouse soil mix. In each flat, 5 rows of F_2 seeds (20-25 seeds/row) were planted. Two pots (6-10 plants/pot) of each parent of a cross and 1 pot of each additional differential cultivar were included in the test for verification of strain identity. Crosses were labeled as R x R , R x N, R x S, N x N, N x S, and S x S, depending on reactions of the parents to the strain used for inoculation.

Strains of SMV used in the experiment were G4, G5, G6, G7, and G7A. The SMV strains were maintained in soybean callus cultures (Chen et al. 1988) and increased for producing inocula by propagation in York or Lee

68 soybean plants grown in the greenhouse. Inoculum of each strain was prepared by grinding infected leaves in 0.01 M sodium phosphate buffer (approx. 10 ml per g leaf tissue) at pH 7.0 with a chilled mortar and pestle. Inoculations were made by rubbing the inoculum with a pestle onto both unifoliolate leaves (75% expanded) of each seedling about 2 weeks after planting. Leaves were pre-dusted lightly with 600-mesh carborundum powder.

The inoculated plants were examined for symptoms of infection at 7-10 day intervals for a month after inoculation. Individual plants of each F_2 population were classified as R, N, or S. The N class included plants showing stem-tip necrosis, stem necrosis, systemic necrotic lesions, systemic veinal necrosis, or combinations among these necrotic symptoms. The N plants were counted and removed from F_2 populations. Remaining plants were classified as R or S. N plants in R x S and N x S crosses were grouped in the R class for X^2 tests. Chi-square tests were used to determine the goodness-of-fit of observed F_2 segregations to expected genetic ratios. A X^2 test for heterogeneity was also used to determine whether different F_2 populations displayed similar genetic behavior.

Results

The distribution of F_2 plants for reaction to SMV from eight PI 96983 x susceptible crosses is presented along with the parents in Table 1. All 5 R x S crosses segregated in a ratio of 3R:1S when the necrotic segregates were grouped in the resistant class. The combined data for the 5 populations showed an acceptable fit to 3R:1S and good homogeneity. The 3 N x S crosses also gave good fits to a 3N:1S ratio. In the cross of PI 96983 x Essex inoculated with G7, 8 F_2 plants showed no symptoms of infection and were combined with the N class in the X^2 test because all of the Essex plants were S and 1 of the PI 96983 plants was symptomless. The combined X^2 for the 3 F_2 populations showed good homogeneity. The similar reactions of crosses with PI 96983 as male or female indicated no cytoplasmic effects on the expression of SMV reaction.

Table 2 summarizes the segregation in Ogden x susceptible crosses when inoculated with SMV. The 5 F_2 populations from R x S crosses all segregated in a ratio of 3R:1S and were homogeneous. Each F_2 contained about 1/2 necrotic plants and they were combined in the resistant class. The data from each N x S cross provided a good fit to a ratio of 3N:1S. The combined F_2 data showed good homogeneity. Three R plants were obtained in each of the crosses of York x Ogden and Ogden x Lee 68 when inoculated with G7. They were included in the N class for X^2 tests since 2 out of 145 Ogden plants also showed R reaction upon inoculation. Crosses with Ogden as both male and female parent showed similar reactions.

The 5 F_2 populations from Marshall x susceptible crosses all produced 3(R+N):1S ratios (Table 3) when inoculated with 3 SMV strains. The X^2 values for testing goodness of fit to an expected ratio of 3:1 within each cross, and the homogeneity among the 5 F_2 populations were acceptable. In the 4 crosses with Marshall as necrotic parent, more symptomless (R) plants were found in both F_2 populations and Marshall inoculated with G6 than those inoculated with G7. However, the overall F_2 populations contained approximately 1/4 susceptible segregates that would be expected for segregation of a single gene. The reactions of crosses made in opposite directions were in good agreement.

The F_2 progenies of the Kwanggyo x susceptible crosses all segregated in a ratio of 3(R+N):1S, and the 7 populations were homogeneous in reaction to inoculation with the 4 SMV strains (Table 4). Monogenic segregations were also observed in F_2 populations from crosses between Kwanggyo and other parents giving susceptible reaction to SMV-G7A (Table 5).

Table 6 shows the results from F_2 populations of R x N crosses inoculated with G4, G5, and G6. No segregation for susceptibility was observed in any of the 9 crosses. Each cross produced more plants with necrotic than resistant reaction. In the X^2 tests for goodness of fit to a ratio of 3N:1R, 5 of 9 populations fit the expected ratio and 4 populations did not fit due to the deficiency of necrotic plants.

No susceptible segregates were obtained in F_2 progenies from the 7 crosses among the 4 necrotic parents inoculated with strains G6 or G7 (Table 7). The R plants obtained in the 4 crosses involving Kwanggyo do not appear to represent genetic segregation since Kwanggyo also pro-

duced a number of symptomless plants. Also given in Table 8 is the reaction of populations from R x R and S x S crosses, and there was no apparent segregation observed.

Discussion

The results indicate that single dominant, nuclear-inherited genes condition the resistant and necrotic reaction to virulent strains of SMV. The genes in the different parents appear to be alleles at the same locus.

Single dominant genes for SMV resistance were identified in PI 96983 and Ogden by Kiihl and Hartwig (1979) using SMV-G2 and G3 which cause no symptoms on PI 96983 and Ogden. In this study, we used SMV-G7 strain which produces severe stem tip necrosis on both PI 96983 and Ogden. All the F_2 populations segregated 3N:1S, indicating that both PI 96983 and Ogden have single dominant genes for necrotic reaction to SMV-G7. We postulate that the single dominant genes in PI 96983 and Ogden for necrosis to SMV-G7 are the same genes (Rsv and rsv^t) for resistance to SMV-G2 and G3 reported by Kiihl and Hartwig (1979) and to G5 and G6 (Tables 1 and 2). The monogenic segregation for reaction to SMV-G5 and G6 in R x S crosses having PI 96983 and Ogden as resistant parents (Tables 1 and 2) furnished additional evidence for the single dominant genes in PI 96983 and Ogden, and for the assumption that the reactions of PI 96983 and Ogden to G2, G3, and G7 are controlled by Rsv, and rsv^t, respectively. The differential reactions of PI 96983 and Ogden (necrotic to G7, resistant to G2, G3, G5, and G6) are probably because the Rsv and rsv^t genes are expressed or functioned differently when interacting with different SMV strains.

The monogenic segregations of both R x S and N x S crosses indicate the resistant and necrotic reactions are controlled by the same gene.

If the resistant and necrotic reactions are due to two separate genes, then F_2 populations from both R x S and N x S crosses should contain 1/16 susceptible plants because only homozygous recessive segregates would show mosaic symptoms. Our results appeared to exclude the 2 gene possibility.

Of 71 F_2 plants from York x Lee 68 inoculated with SMV-G4, 55 plants exhibited necrotic reaction and 16 plants were susceptible. The observed segregation fit a 3N:1S ratio. The result indicated that York possesses a single gene for necrosis to SMV-G4. This gene is assumed to be the same gene ($\underline{Rsv}^y$ that conditions the resistance to SMV-G1 (Roane, et al., 1983). The York gene can be defeated by virulent strains G5, G6, G7, and G7A and produce mosaic symptoms.

In a previous study of inheritance of resistance to the type strain G1 of SMV (Buss et al., 1989a), Marshall and Kwanggyo were shown to carry single dominant genes for resistance. Symbols $\underline{Rsv}^m$ and $\underline{Rsv}^k$ have been proposed in a previous report (Chapter IV) for the dominant genes in Marshall and Kwanggyo, respectively. In the present study, using virulent SMV strains, monohybrid ratios were obtained in all F_2 populations from the crosses involving Marshall and Kwanggyo (Tables 3, 4, and 5). The data provide clear evidence that the necrotic reactions of Marshall and Kwanggyo are controlled by single dominant genes that are the same genes for resistant reaction to SMV-G1.

It is obvious from the data (Tables 4 and 5) that Kwanggyo and F_2 populations inoculated with G7 or G7A frequently produced more symptomless plants than those inoculated with G5 or G6. However, the proportion of susceptible segregates in each of the F_2 populations was about

1/4, as expected from simple inheritance.

The R x N crosses with Marshall and Kwanggyo as necrotic parents did not exhibit consistent 3N:1R segregation for reaction to G5 and G6. However, Marshall and Kwanggyo do not always give clear-cut reactions (Table 6). The mixed reactions of these two parents probably explain the deviations from the expected ratio in the F_2 populations. More necrotic plants than resistant plants in F_2 appear to indicate that the heterozygous segregates in R x N crosses tend to exhibit necrosis rather than resistance. This association of heterozygosity and necrosis has also been reported in other studies (Kiihl and Hartwig, 1979; Buss et al., 1989a, 1989b).

In the crosses among the 4 necrotic parents, no segregation for susceptibility was observed. This indicates that the single dominant genes in the 4 necrotic parents probably reside at a common locus. Extremely large populations would have to be screened to distinguish allelism from close linkage. However, it seems very unlikely that 4 separate genes in distinct genetic backgrounds from different sources are closely linked. Therefore, we conclude that the genes in PI 96983, Ogden, Marshall, and Kwanggyo are alleles at the Rsv locus. There are variations in SMV-reactions among these cultivars with genes at a common locus but it has not been determined whether these differences are due to the action of different alleles, to modifying genes, or to variations in the total genetic background in which the SMV-conditioning genes are acting upon interaction with different SMV strains.

Our finding that only one pair of dominant genes conditions the necrotic reaction in each of the 4 cultivars agrees with the report by

Tu and Buzzell (1987), although different parents and SMV strains were used. They observed a 3N:1S segregation in a N x S cross. In our experiment, similar results were obtained in all N x S crosses. However, Kwon and Oh (1980) reported that F_2 progenies of R x N crosses segregated in a 3:1 ratio of necrotic to resistant plants, and thus concluded that resistance was conferred by a single recessive gene. In our study, most of the F_2 populations from R x N crosses segregated 3N:1R, which agrees with the results reported by Kwon and Oh (1980). However, their conclusion that resistance was conditioned by a recessive gene was based on the assumption of complete dominance. If necrosis is a reaction of the homozygous recessive and heterozygous plants, the 3N:1R ratio is expected when resistance is incomplete dominant. After all, the facts that 3/4 necrotic plants were always present in F_2 populations from N x S crosses and that about 1/3 N plants were obtained from R x S crosses indicate that necrosis is associated with heterozygosity.

Our classification of SMV-inoculated plants into resistant, necrotic, and susceptible classes conforms with the system devised for identification of SMV strains by Cho and Goodman (1979). The occasional R plants found in N x S F_2 populations were combined into the N class since the necrosis was regarded as a hypersensitive (a form of resistant) reaction (Tu and Buzzell, 1987). It has also been shown that the necrosis occurs almost exclusively on plants possessing SMV-resistance genes (Cho and Goodman, 1979, 1982; Buss et al., 1989b). In most of the genetic studies, R and N plants were combined to obtain good fits to Mendelian ratios (Buss et al., 1989b). The R plants unable to develop expected necrosis suggest that the expression of necrosis is somewhat

affected by environmental factors such as temperature (Tu and Buzzell, 1987). More R plants were obtained in crosses involving Kwanggyo inoculated with any of the virulent SMV strains. This is probably because Kwanggyo does not always give a consistent necrotic reaction to SMV.

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List of Tables

Table 1. Segregation and X^2 tests for SMV reaction in F_2 populations from PI 96983 x susceptible crosses.

Cross and parents	SMV strain	No. of plants[1]			Chi-square 3:1	
		R	N	S	X^2	P
PI 96983 x York (RxS)	G5	100	10	25	3.025	.05-.10
PI 96983		12	0	0		
York		0	0	16		
York x PI 96983 (SxR)	G6	52	16	14	2.748	.05-.10
York		0	0	10		
PI 96983		14	0	0		
PI 96983 x York (RxS)	G6	15	9	8	0.000	1.0
PI 96983		8	0	0		
York		0	0	15		
PI 96983 x Essex (RxS)	G6	12	20	15	1.199	.25-.50
PI 96983		8	0	0		
Essex		0	0	16		
PI 96983 x Lee 68 (RxS)	G6	56	51	36	0.002	.95-.98
PI 96983		33	0	0		
Lee 68		0	0	70		
F_2 total (df=5)		235	106	98	6.974	
F_2 pooled (df=1)					1.677	.10-.25
Heterogeneity (df=4)					5.307	.25-.50
York x PI 96983 (SxN)	G7	0	131	48	0.315	.50-.75
York		0	0	25		
PI 96983		0	49	0		
PI 96983 x Lee 68 (NxS)	G7	0	104	28	1.010	.25-.50
PI 96983		0	36	0		
Lee 68		0	0	50		
PI 96983 x Essex (NxS)	G7	8	80	34	0.536	.25-.50
PI 96983		1	23	0		
Essex		0	0	23		
F_2 total (df=3)		8	325	110	1.861	
F_2 pooled (df=1)					0.038	.75-.90
Heterogeneity (df=2)					1.823	.25-.50

[1] R = symptomless, N = stem-tip necrosis, S = mosaic symptoms.

Table 2. Segregation and X^2 tests for SMV reaction in F_2 populations from Ogden x susceptible crosses.

Cross and parents	SMV strain	No. of plants[1] R	N	S	Chi-square 3:1 ratio Value	Probability
Ogden x Lee 68 (RxS)	G5	19	43	25	0.648	.25-.50
Ogden		16	0	0		
Lee 68		0	0	15		
Ogden x Lee 68 (RxS)	G6	33	108	39	1.067	.25-.50
Ogden		56	0	0		
Lee 68		0	0	58		
York x Ogden (SxR)	G5	24	51	22	0.278	.50-.75
York		0	0	16		
Ogden		21	0	0		
York x Ogden (SxR)	G6	39	99	38	1.091	.25-.50
York		0	0	32		
Ogden		50	1	0		
Essex x Ogden (SxR)	G6	52	108	47	0.581	.25-.50
Essex		0	0	63		
Ogden		31	0	0		
F_2 total (df=5)		167	409	171	3.665	
F_2 pooled (df=1)					1.771	.10-.25
Heterogeneity (df=4)					1.894	.10-.25
York x Ogden (SxN)	G7	3	212	73	0.019	.75-.90
York		0	0	64		
Ogden		1	64	0		
Ogden x Lee 68 (NxS)	G7	3	102	38	0.189	.50-.75
Ogden		1	48	0		
Lee 68		0	0	75		
Essex x Ogden (SxN)	G7	0	126	55	2.801	.05-.10
Essex		0	0	26		
Ogden		0	31	0		
F_2 total (df=3)		6	440	166	3.009	
F_2 pooled (df=1)					1.473	.10-.25
Heterogeneity (df=2)					1.536	.25-.50

[1] R = symptomless, N = stem-tip necrosis, S = mosaic.

Table 3. Segregation and X^2 tests for SMV reaction in F_2 populations from Marshall x susceptible crosses.

Cross and parents	SMV strain	No. of plants[1]			Chi-square 3:1 ratio	
		R	N	S	Value	Probability
York x Marshall (SxR)	G5	33	77	42	0.561	.25-.50
York		0	0	43		
Marshall		18	0	0		
York x Marshall (SxN)	G6	68	133	69	0.044	.50-.75
York		0	0	75		
Marshall		26	29	0		
York x Marshall (SxN)	G7	9	210	62	1.292	.25-.50
York		0	0	89		
Marshall		2	83	0		
Marshall x Lee 68 (NxS)	G6	28	53	21	1.059	.25-.50
Marshall		31	41	0		
Lee 68		0	0	105		
Marshall x Lee 68 (NxS)	G7	12	123	39	0.621	.25-.50
Marshall		3	65	0		
Lee 68		0	0	106		
F_2 total (df=5)		150	596	229	3.577	
F_2 pooled (df=1)					1.190	.25-.50
Heterogeneity (df=4)					2.387	.50-.75

[1] R = symptomless, N = stem-tip necrosis, stem necrosis, systemic necrotic lesions, or systemic veinal necrosis, S = mosaic symptoms.

Table 4. Segregation and X^2 tests for SMV reaction in F_2 populations from Kwanggyo x susceptible crosses

Cross and parents	SMV strain	No. of plants[1] R	N	S	Chi-square 3:1 ratio Value	Probability
York x Kwanggyo (SxN)	G5	18	69	30	0.026	.75-.90
York		0	0	31		
Kwanggyo		1	17	0		
York x Kwanggyo (SxN)	G6	7	145	49	0.042	.50-.75
York		0	0	50		
Kwanggyo		5	26	0		
York x Kwanggyo (SxN)	G7	31	189	70	0.115	.50-.75
York		0	0	34		
Kwanggyo		34	31	0		
York x Kwanggyo (SxN)	G7A	31	66	35	0.162	.50-.75
York		0	0	37		
Kwanggyo		11	28	0		
Kwanggyo x Lee 68 (NxS)	G5	0	98	31	0.065	.75-.90
Kwanggyo		0	9	0		
Lee 68		0	0	35		
Kwanggyo x Lee 68 (NxS)	G6	15	171	50	1.831	.10-.25
Kwanggyo		7	50	0		
Lee 68		0	0	74		
Kwanggyo x Lee 68 (NxS)	G7	37	123	62	1.015	.25-.50
Kwanggyo		26	14	0		
Lee 68		0	0	48		
Kwanggyo x Lee 68 (NxS)	G7A	0	139	45	0.029	.75-.70
Kwanggyo		3	18	0		
Lee 68		0	0	25		
F_2 total (df=8)		139	1000	372	2.285	
F_2 pooled (df=1)					0.117	.50-.75
Heterogeneity (df=7)					2.168	.90-.95

[1] R = symptomless, N = stem-tip necrosis, systemic necrotic lesions, systemic veinal necrosis, or stem necrosis, S = mosaic.

Table 5. Segregation and X^2 tests for reaction to SMV-G7A in F_2 populations from crosses of Kwanggyo with necrotic parents giving susceptible reaction to strain G7A.

Cross and parents	No. of plants[1]			Chi-square 3:1 ratio	
	R	N	S	Value	Probability
Kwanggyo x PI 96983 (NxS)	0	86	25	0.282	.50-.75
Kwanggyo	0	7	0		
PI 96983	0	0	18		
PI 96983 x Kwanggyo (SxN)	11	140	56	0.465	.25-.50
PI 96983	0	0	40		
Kwanggyo	8	31	0		
Ogden x Kwanggyo (SxN)	29	94	38	0.168	.50-.75
Ogden	0	0	75		
Kwanggyo	11	56	0		
Kwanggyo x Marshall (NxS)	28	75	45	2.306	.10-.25
Kwanggyo	8	34	0		
Marshall	0	0	42		
F_2 total (df=4)	68	395	164	3.221	
F_2 pooled (df=1)				0.447	.50-.75
Heterogeneity (df=3)				2.774	.25-.50

[1] R = symptomless, N = stem-tip and/or stem necrosis, S = mosaic.

Table 6. Segregation of reactions of F_2 populations from resistant x necrotic crosses

Cross and parents	SMV strain	No. of plants[1] R	N	S	X^2(3:1)	P
PI 96983 x Kwanggyo (RxN)	G6	80	146	0	13.452	<.001
PI 96983		53	0	0		
Kwanggyo		7	32	0		
PI 96983 x Marshall (RxN)	G6	34	40	0	17.315	<.001
PI 96983		18	0	0		
Marshall		8	11	0		
Kwanggyo x PI 96983 (NxR)	G5	35	69	0	4.154	.03-.05
Kwanggyo		0	17	0		
PI 96983		21	0	0		
Ogden x Kwanggyo (RxN)	G6	167	291	0	32.069	<.001
Ogden		129	3	0		
Kwanggyo		12	80	0		
Kwanggyo x Ogden (NxR)	G6	31	77	0	0.790	.25-.50
Kwanggyo		0	21	0		
Ogden		23	0	0		
Ogden x Marshall (RxN)	G6	168	425	0	3.508	.05-.10
Ogden		123	6	0		
Marshall		52	77	0		
Kwanggyo x Marshall (NxR)	G5	34	115	0	0.378	.50-.75
Kwanggyo		3	25	0		
Marshall		22	0	0		
Kwanggyo x Ogden (NxR)	G5	38	79	0	3.490	.05-.10
Kwanggyo		0	17	0		
Ogden		19	0	0		
PI 96983 x York (RxN)	G4	40	83	0	3.710	.05-.10
PI 96983		21	0	0		
York		0	18	0		

[1] R = symptomless, N = systemic necrosis, S = mosaic.

Table 7. Reaction of F_2 populations from crosses among necrotic parents to inoculations with selected strains of SMV.

Cross and parents	SMV strain	No. of plants[1]		
		R	N	S
Kwanggyo x Marshall	G6	28	137	0
Kwanggyo		4	29	0
Marshall		24	10	0
Kwanggyo x Marshall	G7	12	62	0
Kwanggyo		14	31	0
Marshall		1	61	0
PI 96983 x Ogden	G7	0	83	0
PI 96983		0	18	0
Ogden		0	14	0
PI 96983 x Marshall	G7	0	80	0
PI 96983		0	18	0
Marshall		0	17	0
PI 96983 x Kwanggyo	G7	46	128	0
PI 96983		0	42	0
Kwanggyo		22	24	0
Ogden x Marshall	G7	0	183	0
Ogden		0	57	0
Marshall		1	51	0
Ogden x Kwanggyo	G7	9	80	0
Ogden		0	25	0
Kwanggyo		22	24	0

[1] R = symptomless, N = stem-tip necrosis, systemic necrotic lesions, systemic veinal necrosis, or stem necrosis, S = mosaic symptoms.

Table 8. Reaction of F_2 populations from the crosses among the five differential cultivars to inoculation with selected SMV strains.

Cross and parents	SMV strains	No. of plants[1]		
		R	N	S
Ogden x Marshall (RxR)	G5	17	3	0
Ogden		16	2	0
Marshall		6	0	0
PI 96983 x Ogden (RxR)	G6	125	1	0
PI 96983		12	0	0
Ogden		19	0	0
York x Lee 68 (SxS)	G5	0	0	26
York		0	0	9
Lee 68		0	0	7
York x Lee 68 (SxS)	G7	0	0	167
York		0	0	32
Lee 68		0	0	54
Marshall x Lee 68 (SxS)	G7A	0	0	36
Marshall		0	0	14
Lee 68		0	0	20
PI 96983 x Ogden (SxS)	G7A	0	0	114
PI 96983		0	0	8
Ogden		0	0	18
Ogden x Marshall (SxS)	G7A	0	0	399
Ogden		1	0	103
Marshall		0	0	79
York x Ogden (SxS)	G7A	0	0	224
York		0	0	36
Ogden		2	0	67
York x Marshall (SxS)	G7A	0	0	169
York		0	0	22
Marshall		0	0	40
PI 96983 x Marshall (SxS)	G7A	0	0	85
PI 96983		0	0	15
Marshall		0	0	19
York x Lee 68 (SxS)	G7A	0	0	160
York		0	0	14
Lee 68		0	0	24
Ogden x Lee 68 (SxS)	G7A	0	0	32
Ogden		0	0	20
Lee 68		0	0	24
York x PI 96983 (SxS)	G7A	0	0	94
York		0	0	14
PI 96983		0	0	21

[1] R = resistant, N = necrotic, S = susceptible.

Chapter VI

Identification of Genes in PI 486355 and Suweon 97 for Resistance to Soybean Mosaic Virus and Their Allelic Relationships with the Rsv Locus.[1]

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Abstract

A series of genetic studies was conducted to determine the number of gene loci conditioning reactions to soybean mosaic virus (SMV) in 'PI 486355' and 'Suweon 97' cultivars and to establish the allelomorphic relationships among their genes for SMV resistance and other reported genes for SMV resistance. F_2 populations and F_3 lines from resistant x susceptible, and resistant x resistant crosses were inoculated in the greenhouse or in the field. Strains SMV-G1, G5, G6, G7, or G7A whichever were used, as appropriate, to observe genetic segregations. The F_2 plants from the susceptible cultivars Lee 68 and Essex crossed with PI 486355 or Suweon 97 segregated in a 15 resistant : 1 susceptible ratio. The F_3 data from PI 486355 x Lee 68 exhibited 7 homogeneous resistant : 4 segregating 3R:1S : 4 segregating 15R:1S : 1 homogeneous susceptible. The results indicate that PI 486355 and Suweon 97 each have two independent dominant genes conditioning resistance to SMV. The F_2 populations derived from resistant x resistant crosses of PI 486355 or Suweon 97 with PI 96983, York, Ogden, Marshall, and Kwanggyo did not segregate for susceptibility, suggesting that PI 486355 and Suweon 97 each have at least one allele (one of the two resistance genes) at the Rsv locus. Dihybrid ratios were observed in crosses of PI 486355 and Suweon 97 with York, Marshall, and Ogden, when inoculated with SMV strains that induce mosaic reactions on York, Marshall, and Ogden. This suggests that the alleles that are at the Rsv locus in PI 486355 and Suweon 97 are not the alleles Rsv^y, Rsv^m or Rsv^t.

Introduction

Cho and Goodman (1979, 1982) described a set of 7 strains of soybean mosaic virus (SMV) that could be differentiated by resistant, mosaic or necrotic reactions on 6 cultivar groups having resistance to the common strain of SMV. The differential cultivars chosen to represent those strain groups included 'Suweon 97', 'Buffalo', 'Kwanggyo', 'Ogden', 'Marshall', and 'Davis'. Most of these SMV-resistance sources were found to be resistant to some, but not all 7 strains. Cho and Goodman did not conduct inheritance tests to locate genes for resistance to SMV.

Inheritance of SMV resistance has been studied by several investigators using various strains or isolates and different soybean types. A review of the available literature reveals that resistance to some mosaic-inducing strains is conditioned by single dominant genes (Bowers, 1980; Buss et al., 1985, 1989a; Buzzell and Tu, 1984; Kiihl and Hartwig, 1979; Koshimizu and Iizuka, 1963; Lim, 1985; Roane et al., 1983), whereas resistance to a necrosis-inducing strains has been reported to be controlled by a single recessive gene (Kwon and Oh, 1980). In addition, two complementary genes (Koshimizu and Iizuka, 1963) and four linked genes (Gai et al., 1989) for SMV resistance have been reported. Tu and Buzzell also showed that stem-tip necrosis on soybean was a hypersensitive and temperature-dependent reaction, and was conferred by a single dominant gene.

Three gene symbols have been assigned to previously recognized genes. The Rsv gene locus was first identified in PI 96983 for resis-

tance to SMV-G2 and G3 and was shown to provide a high level of resistance to SMV (Kiihl and Hartwig, 1979). Ogden was demonstrated to carry a gene designated $\underline{rsv}^t$ at the Rsv locus. The $\underline{rsv}^t$ gene gave protection against SMV only in homozygous condition and was recessive to Rsv. Both these genes were dominant to rsv in susceptible cultivars (Kiihl and Hartwig, 1979). $\underline{Rsv}_2$ was found in a breeding line 'OX670', a derivative of resistant cultivar 'Raiden', for resistance to SMV-G7 and G7A, and was shown to be independent of Rsv (Buzzell and Tu, 1984).

Suweon 97 and 'PI 486355' are resistant to all 7 identified SMV strains (Cho and Goodman, 1982; Lim, 1982, 1985) and the resistance was reported to be governed by a single dominant gene in each cultivar (Lim, 1985) and they were at independent loci. No gene symbols were proposed for these two genes since allelism tests had not been conducted against $\underline{Rsv}_2$. The three allelic, single, dominant genes, $\underline{Rsv}^y$, $\underline{Rsv}^m$, and $\underline{Rsv}^k$, in York, Marshall, and Kwanggyo (Chen et al., in a previous report, Chapter IV), have not been tested for allelism with the genes in Suweon 97 and PI 486355 (Lim, 1985).

Our experiments described here were conducted to further characterize the inheritance of SMV resistance in PI 486355 and Suweon 97 and to determine the genetic relationships between their resistance genes and those previously reported.

Material and Methods

The SMV resistant cultivars used in this study were PI 486355, Suweon 97, PI 96983, Ogden, York, Marshall, and Kwanggyo. PI 486355 and Suweon 97 were crossed with SMV-susceptible cultivars Essex or Lee 68. Advanced progenies from these crosses were tested with different SMV strains to determine the inheritance of resistance. PI 486355 and Suweon 97 were also crossed with PI 96983, Ogden, York, Marshall, and Kwanggyo to determine the genetic relationships of their genes with Rsv, Rsv^t, Rsv^y, Rsv^m, and Rsv^k, respectively.

The methods used in this study have been described previously (in Chapters IV and V) except that the F_3 lines derived from the cross of PI 486355 x Lee 68 were tested with SMV-G1 in the greenhouse, and that several F_2 populations from different crosses were screened with SMV-G1 under field conditions. In the greenhouse tests of F_3 progenies, each of the 53 F_3 lines was planted in a single row in a metal flat. Five rows were planted per flat and approximately 20 seeds were planted in each row. Two pots of each parent were included as checks and one pot each of PI 96983, York, Ogden, Marshall, and Kwanggyo was also included as checks on virus strain identity. In the field tests, each F_2 population from a cross was planted in 3 rows (approx. 40-45 seeds/row). Also included in the field tests were single rows of parental and susceptible check cultivars.

In presenting results of inoculations of segregating progenies, the original cross is classified as resistant x resistant if both parents are resistant to the SMV strain used for inoculation, while the same

cross is referred to as resistant x susceptible if one of the parents is susceptible to the strain used for inoculation.

Plant were classified as resistant (R), nerotic (N), or susceptible (S). N plants were combined with R plants for use in Chi-square tests.

Results and Discussion

The reactions of 9 F_2 populations from PI 486355 x susceptible crosses and the parents are given in Table 1. The F_2 plants from PI 486355 x Lee 68 segregated with a satisfactory fit to a ratio of 15 resistant (R) to 1 susceptible (S) when inoculated with SMV-G1 in the field and in the greenhouse. This cross also gave a good fit to a 15R:1S segregation ratio upon inoculation with SMV-G6 and G7 (Table 1). When the 53 F_3 lines derived from PI 486355 x Lee 68 were tested with G1, 27 progeny rows showed homogeneous resistant reactions, 12 and 11 rows segregated 3R:1S and 15R:1S, respectively, and 3 rows were homogeneous susceptible. These observations provide an excellent fit to 7:4:4:1 ratio which would be expected from a dihybrid segregation (X^2=1.155 with 3 df, P=.75-.95) for duplicate dominant genes. An excellent fit to a 15R:1S F_2 ratio was also obtained when the homozygous resistant and segregating rows were combined and compared to the homozygous susceptible rows (X^2=.031 with 1 df, P=.50-.75). All of the resistant checks were 100% resistant and the susceptible checks were 95% infected in the field. The F_2 data from York x PI 486355 inoculated with G5, G6, or G7 and PI 486355 x Marshall and PI 486355 x Ogden inoculated with G7A provided an acceptible fit to 15R:1S ratio. When data for the 9 F_2 populations were combined, a good fit to the 15R:1S was obtained, and the populations were homogeneous.

Table 2 summarizes the segregation of SMV reactions observed in resistant x susceptible crosses having Suweon 97 as the resistant parent. All six cross and strain combinations segregated into 15R:1S

ratios, indicating that two dominant genes were segregating in each cross x SMV strain combination. The X^2 values for goodness of fit for each population and for homogeneity among them were very acceptable.

In the 4 F_2 populations from the cross of PI 486355 x Suweon 97, no susceptible plants were detected with inoculations of G1, G6, G7, or G7A (Table 3), indicating that at least one of the two genes in each parent are allelic. If none of the PI 486355 or Suweon 97 genes were at the Rsv locus, the F_2's should have segregated 63R:1S. While some of the populations were somewhat small to have a high probability of at least one susceptible plant, there should have been one or two susceptible plants in most F_2's. Assuming that one of the genes in PI 486355 and Suweon 97 are alleles at the Rsv locus, it would appear that they are not the alleles Rsv^y, Rsv^m, or Rsv^t, because only 15R:1S ratios were observed in all the R x S crosses with York, Marshall, and Ogden (Tables 1 and 2). If PI 486355 or Suweon 97 did contain one of those alleles, these crosses should have segregated 3R:1S. Unfortunately, similar conclusions regarding the Rsv^k allele cannot be made, because no strains are available to which Kwanggyo gives a susceptible reaction. Since non strains are available to differentiate the reactions of the Rsv alleles in PI 48355 and Suweon 97, it cannot be determined whether or not there alleles are identical.

In Tables 4 and 5 are the results from the F_2 populations of resistant x resistant crosses involving PI 486355 and Suweon 97 with the other 5 resistant differentials which carry alleles at the Rsv locus, respectively, when inoculated with SMV-G1 or G6 in the greenhouse and in the field. No susceptible segregates were observed in any of the

crosses, providing strong evidence that one of the resistance genes in PI 486355 is an allele at the Rsv locus. The susceptible checks had 100% infected plants in the greenhouse, and 95% infected plants in the field.

In our experiment the F_2 populations from resistant x susceptible crosses involving PI 486355 or Suweon 97 always produced some necrotic plants. These necrotic plants were considered heterozygotes and combined with the resistant class, as other workers did. Any other classification would not provide a good fit to either monohybrid or dihybrid genetic ratios. The presence of a number of necrotics in the F_2 populations indicates that at least one of the genes in each parent exhibits incomplete dominance.

The F_2 populations derived from crosses of PI 486355 and Suweon 97 with PI 96983, Ogden, York, Marshall, and Kwanggyo had a few plants with necrotic symptoms but no susceptible segregates. The necrotic plants were assumed not to represent genetic segregation because a number of necrotic plants were also observed in inoculated plants of the parental cultivars.

It is clear from the data presented that PI 486355 and Suweon 97 both have two dominant genes for resistance to SMV. This is in apparent disagreement with Lim's (1985) conclusion that each had only one gene for resistance. However, we used strain G1, which should detect all resistance genes present, whereas Lim tested his crosses only with strains which apparently were virulent on one of the genes and thus obscured its presence. Very likely it was the gene which our data show to be at the Rsv locus. This would fit with Lim's conclusion that the

one gene he detected was not at the Rsv locus.

We were unable to detect any segregation in crosses between PI 486355 and Suweon 97, as Lim (1985) did. Again, this discrepancy can probably be explained by the fact that the strains we used were not able to overcome the Rsv alleles that appear to be present in both parents. Using the strains G1-G7A, it would not be possible to determine the genetic relationships of the non-Rsv genes in PI 486355 and Suweon 97 without first separating them from the Rsv alleles.

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List of Tables

Table 1. Segregation and X^2 tests for SMV reactions in F_2 populations from PI 486355 x susceptible crosses in the field and greenhouse.

Cross and parents	SMV strain	No. of plants			Chi-square 15:1 ratio	
		R	N	S	Value	Probability
PI 485355 x Lee 68	G1	164	14	10	0.278	.50-.75
PI 486355		37	0	0		
Lee 68		0	0	69		
PI 486355 x Lee 68*	G1	97	2	8	0.275	.50-.75
PI 486355		11	0	0		
Lee 68		4	0	50		
PI 486355 x Lee 68	G6	134	34	17	2.728	.05-.10
PI 486355		35	0	0		
Lee 68		0	0	43		
PI 486355 x Lee 68	G7	79	10	5	0.137	.50-.75
PI 486355		5	0	0		
Lee 68		0	0	12		
York x PI 486355	G5	98	50	10	0.002	.75-.90
York		0	0	16		
PI 486355		14	0	0		
York x PI 486355	G6	93	51	14	1.838	.10-.25
York		0	0	40		
PI 486355		42	0	0		
York x PI 486355	G7	374	35	32	0.762	.25-.50
York		0	0	63		
PI 486355		77	0	0		
PI 486355 x Marshall	G7A	127	18	14	1.771	.10-.25
PI 486355		23	0	0		
Marshall		0	2	23		
PI 486355 x Ogden	G7A	124	8	9	.0004	.95
PI 486355		26	0	0		
Ogden		0	0	27		
F_2 total (df=9)		1290	222	119	7.791	
F_2 pooled (df=1)					3.046	.05-.10
Heterogeneity (df=8)					4.745	.75-.90

* inoculated in the field.

Table 2. Segregation and X^2 tests for SMV reaction in F_2 populations from Suweon 97 x susceptible crosses

Cross and parents	SMV strain	No. of plants			Chi-square 15:1 ratio	
		R	N	S	Values	Probability
Suweon 97 x Lee 68	G1	149	18	11	0.002	.95-.98
Suweon 97		40	0	0		
Lee 68		0	0	42		
York x Suweon 97	G6	29	15	5	1.307	.10-.25
York		0	0	9		
Suweon 97		6	0	0		
Suweon 97 x York	G6	23	57	9	2.356	.10-.25
Suweon 97		8	0	0		
York		0	0	13		
Suweon 97 x York	G7	100	48	10	0.002	.95-.98
Suweon 97		11	1	0		
York		0	0	28		
Suweon97 x Essex	G7	47	22	5	0.032	.50-.75
Suweon		13	1	0		
Essex		0	0	12		
Suweon 97 x Marshall	G7A	108	22	9	0.012	.90-.95
Suweon 97		30	0	0		
Marshall		0	1	32		
F_2 total (df=6)		456	182	49	3.710	
F_2 pooled (df=1)					0.913	.25-.50
Heterogeneity (df=5)					2.797	.50-.75

Table 3. Reactions of F_2 populations from PI 486355 x Suweon 97 when inoculated with selected SMV strains.

Cross and parent	SMV strain	No. of plants		
		R	N	S
Suweon 97 x PI 486355	G1	59	2	0
Suweon 97		9	0	0
PI 486355		4	0	0
PI 486355 x Suweon 97	G6	71	12	0
PI 486355		11	0	0
Suweon 97		8	0	0
PI 486355 x Suweon 97	G7	71	1	0
PI 486355		25	0	0
Suweon 97		20	1	0
PI 486355 x Suweon 97	G7A	164	10	0
PI 486355		63	0	0
Suweon 97		65	3	0

Table 4. Reactions of F_2 populations from the crosses of PI 486355 x other resistant cultivars when inoculated with SMV in the greenhouse and in the field.

Cross and parents	SMV strain	No. of plants		
		R	N	S
Greenhouse tests:				
PI 486355 x Ogden	G1	94	1	0
PI 486355		20	0	0
Ogden		18	0	0
PI 486355 x Kwanggyo	G1	109	3	0
PI 496355		20	0	0
Kwanggyo		25	1	0
PI 486355 x Marshall	G1	107	1	0
PI 486355		20	0	0
Marshall		25	0	0
York x PI 486355	G1	60	6	0
York		20	0	0
PI 486355		19	0	0
PI 486355 x Ogden	G6	86	6	0
PI 486355		6	0	0
Ogden		9	0	0
PI 486355 x Marshall	G6	85	17	0
PI 486355		5	0	0
Marshall		7	0	0
Field tests:				
Ogden x PI 486355	G1	123	0	0
Ogden		20	0	0
PI 486355		11	0	0
York x PI 486355	G1	120	0	0
York		32	0	0
PI 486355		11	0	0
Marshall x PI 486355	G1	113	0	0
Marshall		31	0	0
PI 486355		11	0	0
Kwanggyo x PI 486355	G1	105	0	0
Kwanggyo		4	0	0
PI 486355		11	0	0

Table 5. Reactions of F_2 populations from crosses of Suweon 97 x other resistant cultivars when inoculated with SMV in the greenhouse and in the field.

Cross and parents	SMV strain	No. of plants		
		R	N	S
Greenhouse tests:				
Suweon 97 x PI 96983	G1	99	8	0
Suweon 97		23	1	0
PI 96983		22	0	0
Suweon 97 x York	G1	90	2	0
Suweon 97		19	3	0
York		25	0	0
Marshall x Suweon 97	G1	102	1	0
Marshall		20	1	0
Suweon 97		19	3	0
Kwanggyo x Suweon 97	G1	100	12	0
Kwanggyo		19	3	0
Suweon 97		19	3	0
PI 96983 x Suweon 97	G6	90	9	0
PI 96983		4	0	0
Suweon 97		8	0	0
Suweon 97 x PI 96983	G6	87	10	0
Suweon 97		18	2	0
PI 96983		14	1	0
Field tests:				
York x Suweon 97	G1	91	0	0
York		32	0	0
Suweon 97		20	0	0

Chapter VII

SUMMARY AND CONCLUSIONS

A series of genetic studies were conducted to: 1) determine the mode of inheritance of reactions to soybean mosaic virus (SMV) in nine soybean cultivars exhibiting differential SMV reactions, 2) identify different genes and/or alleles conditioning resistant, necrotic, and susceptible reactions to six SMV strains, and 3) establish the genetic relationships among genes for resistance or necrosis.

The soybean cultivars used as parents for various crosses include 'PI 486355', 'Suweon 97', 'PI 96983', 'York', 'Ogden', Marshall', 'Kwanggyo', 'Essex', and 'Lee 68'. The SMV strains used in this study were G1, G4, G5, G6, G7, and G7A. PI 486355 and Suweon 97 are resistant to all the strains. PI 96983 and Ogden are resistant to G1 and G4-G6, necrotic to G7, and susceptible to G7A. York is resistant to G1, necrotic to G4, and susceptible to G5-G7A. Marshall is resistant to G1, G4 and G5, necrotic to G6 and G7, and susceptible to G7A. Kwanggyo is resistant to G1 and G4, and necrotic to G5-G7A. Essex and Lee 68 are susceptible to all six strains.

Crosses among the seven resistant (R) and two susceptible (S) cultivars were made and the F_2 and F_3 generations were evaluated in the greenhouse and field for their reactions to SMV. Field inoculations were made using an artist's airbrush device. A mortar-pestle technique was used for greenhouse inoculation.

The genetic model proposed for determining the number of gene loci

conditioning SMV resistance in individual cultivars had two likely possibilities: 1) if F_2 populations from an R x S cross segregate 3R:1S, or F_3 lines exhibit a ratio of 1 homogeneous R : 2 segregating (3R:1S) : 1 homogeneous S, then a single dominant gene for resistance is indicated; 2) if segregation from an R x S cross exhibits a 15R:1S F_2 ratio or an F_3 ratio of 7(all R) : 4(3R:1S) : 4(15R:1S) : 1 (all S), the results indicate segregation for two independent dominant genes for resistance. To determine the relationships among genes from different resistance sources, a model with 3 alternatives from R x R crosses was proposed: (a) if all F_2 plants and F_3 lines are R, allelic genes are indicated; (b) a 15R:1S F_2 ratio and a 7:4:4:1 F_3 ratio indicate two different genes; (c) deviations from 15:1 and from 7:4:4:1 ratios in F_2 and F_3 generations could indicate the presence of linkage. Appropriate X^2 tests were made on F_2 and F_3 data for goodness of fit to the proposed ratios.

All F_2 populations from R x S crosses involving PI 96983, Ogden, York, Marshall, and Kwanggyo segregated 3R:1S when inoculated with virus strain G1. F_3 progenies from PI 96983 x Lee 68, Marshall x Essex, Marshall x Lee 68, and Kwanggyo x Lee 68 segregated into phenotypic classes consistent with 1(all R) : 2(3R:1S) : 1(all S). The results indicate that resistance to SMV-G1 in each of the five cultivars is monogenically controlled, which agrees with previously published reports (Kiihl and Hertwig, 1979; Roane, et al., 1983; Buss, et al., 1989a). A large proportion (nearly 1/2) of the F_2 plants from R x S crosses exhibited necrosis and 98% of the necrotic (N) plants from F_3 populations were observed in segregating F_2-derived rows, suggesting an

association of necrotic reaction with plants heterozygous for the resistance gene and an incomplete dominance of the resistance gene. All F_2 plants from crosses among these five resistant cultivars were resistant to G1 inoculation and no segregation for reaction was evident in F_3 progenies. It is concluded that the resistance genes in PI 96983, Ogden, York, Marshall, and Kwanggyo are alleles at a common locus.

Gene symbols Rsv and rsv^t have been assigned to PI 96983 and Ogden. It is proposed that symbols Rsv^y, Rsv^m, and Rsv^k be assigned to the resistance genes in York, Marshall, and Kwanggyo, respectively. It is suggested that the symbol for the Ogden gene be changed to Rsv^t because of its dominance to rsv.

F_2 populations from R x S crosses having PI 96983 or Ogden as resistant parents showed a 3R:1S segregation ratio when inoculated with SMV-G5 and G6. With G7 inoculation, to which PI 96983 and Ogden are necrotic, the F_2 plants segregated into a ratio of 3N:1S. The F_2 of York x Lee 68 (NxS) also produced 3N:1S plants when inoculated with G4. The F_2 populations from Marshall x susceptible crosses segregated 3R:1S for reaction to G5 and 3N:1S for reaction to G6 and G7. All the Kwanggyo x susceptible crosses segregated 3N:1S when inoculated with strains G5-G7A. These results appeared to indicate that the necrotic reactions to virulent strains in PI 96983, Ogden, York, Marshall, and Kwanggyo, are conferred by the same genes that condition resistance to SMV-G1. The absence of segregation for reaction to virulent SMV strains in, R x R, N x N, and S x S crosses among PI 96983, Ogden, York, Marshall, and Kwanggyo provided additional evidence for allelism of the genes in them, which was established by G1 inoculation. When the F_2 populations

from R x N crosses were tested with the virulent strains to which PI 96983 and Ogden are resistant, but Marshall, Kwanggyo and York are necrotic, 3N:1R ratios were observed, indicating that the heterozygous plants frequently showed necrotic reaction rather than resistant reaction. The facts that 3/4 of the F_2 plants from N x S crosses were necrotic and about 1/2 of the F_2 plants in R x S crosses were necrotic strongly support this conclusion.

The F_2 plants from PI 486355 x susceptible and Suweon 97 x susceptible crosses, when inoculated with strains G1 and G5-G7A, segregated with good fits to a ratio of 15R:1S. A ratio of 7 (all R) : 4(3R:1S) : 4(15R:1S) : 1(all S) was also obtained for F_2-derived F_3 progenies from PI 486355 x Lee 68. The data furnished clear evidence that PI 486355 and Suweon 97 each have two independent genes for resistance to SMV. The cross between PI 486355 and Suweon 97 did not produce any susceptible segregates in the F_2 population, when inoculated with G1, G6, G7, or G7A, suggesting that at least one of the genes in each of these two cultivars are allelic. When the two independent genes in PI 486355 and Suweon 97 were tested for allelism with the genes in PI 96983, Ogden, York, Marshall, and Kwanggyo, all F_2 plants from the crosses among them were resistant to SMV-G1. Therefore, it is concluded that the seven resistant cultivars each have one allele at the Rsv locus. The R x S crosses with York, Marshall, and Ogden as susceptible and with PI 486355 and Suweon 97 as resistant parents consistently segregated 15R:1S when inoculated with strains virulent on Ogden, York, and Marshall, indicating that the alleles which are at the Rsv locus in PI 486355 and Suweon 97 are not the alleles Rsv^y, Rsv^m, or Rsv^t but they

are resistant to the virulent strains.

As a basis for further research on a gene-for-gene system for soybean-SMV interactions in future, the cumulative genetic research on reactions of soybean to SMV is summarized in Table 1.

Table 1. Summary of SMV strains, differentiating soybean cultivars, and genetics of their interactions.

Cultivars	Resistance Genes	SMV strains, host gene(s), and reactions								
		G1	G2	G3	G4	G5	G6	G7	G7A	C14
PI 486355	Rsv^p Rsv?	R[a]	R	R	R	R	R	R	R	R
		2[b]	-	-	-	2	2	2	2	(1)
Suweon 97	Rsv^s Rsv?	R	R	R	R	R	R	R	R	N
		2	(1)	-	-	2	2	2(1)	2	0
Raiden	Rsv$_2$	R	R	R	R	R	R	R	R	N
		-	-	-	-	-	-	(1)	(1)	-
PI 96983	Rsv	R	R	R	R	R	R	N	S	R
		1	(1)	(1)	1	1	1	1	0	-
Ogden	Rsv^t	R	R	N	R	R	R	N	S	-
		1	(1)	(1)	-	1	1	1	0	-
York	Rsv^y	R	R	R	N	S	S	S	S	-
		1	-	1	1	0	0	0	0	-
Marshall	Rsv^m	R	N	N	R	R	N	N	S	-
		1	-	-	-	1	1	1	0	-
Kwanggyo	Rsv^k	R	R	R	R	N	N	N	N	-
		1	-	-	-	1	1	1	1	-

[a] R = no symptom, N = systemic necrosis, S = systemic mottling, - = no report.

[b] Number of genes in a host conditioning the reaction to a strain group. Numbers in parentheses are numbers of genes identified by other workers.

Chapter VIII

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GENETICS OF REACTIONS TO SOYBEAN MOSAIC VIRUS IN SOYBEAN

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(ABSTRACT)

The genetic interactions among 9 soybean [Glycine max (L.) Merr.] cultivars and 6 strains of soybean mosaic virus (SMV) were investigated. The objectives were to identify genes and/or alleles conditioning resistant and necrotic reactions to SMV and to determine the genetic relationships among resistance genes from cultivars exhibiting differential responses to the SMV strains.

Seven SMV-resistant (R) cultivars ('PI 486355', 'Suweon 97', 'PI 96983', 'Ogden', 'York', 'Marshall', and 'Kwanggyo') were crossed in all combinations among each other and with susceptible (S) cultivars 'Essex' and 'Lee 68'. F_2 populations and F_2-derived F_3 lines were inoculated in field with the SMV type strain G1 and in the greenhouse with the virulent strains G4, G5, G6, G7, and G7A.

All F_2 populations from R x S and necrotic (N) x S crosses having PI 96983, Ogden, York, Marshall, and Kwanggyo as either resistant or necrotic parents segregated 3R:1S and 3N:1S, respectively. F_2-derived F_3 progenies from R x S crosses exhibited an F_2 genotypic ratio of 1 homogeneous R : 2 segregating (3R:1S) : 1 homogeneous S. The results indicate that each of these five resistant parents has a single, dominant or partially dominant gene conditioning the resistant and necrotic reactions to SMV. No segregation for SMV reaction was evident in F_2 and F_3 generations from R x R, N x N, and S x S crosses among the

five differential cultivars, indicating that the resistance genes in the five cultivars are alleles at a common locus. The alleles in PI 96983 and Ogden were previously labeled Rsv and rsv^t, respectively. Gene symbols, Rsv^y, Rsv^m, and Rsv^k are proposed for the resistance genes in York, Marshall, and Kwanggyo, respectively. It is also proposed that the gene symbol rsv^t be changed to Rsv^t to more accurately reflect its genetic relationship to the susceptible allele.

The R x S crosses with PI 486355 and Suweon 97 as resistant parents segregated 15R:1S in the F_2 and 7 (all R) : 4 (3R:1S) : 4 (15R:1S) : 1 (all S) in the F_3, indicating that each has two independent genes for resistance to SMV. The F_2 plants of PI 486355 x Suweon 97 showed no segregation for SMV reaction, suggesting that they have at least one gene in common. The crosses among all 7 resistant parents produced no susceptible segregates when inoculated with strain G1. . It is concluded that the 7 resistant cultivars each have a gene or allele at the Rsv locus.

Data from the experiments furnished conclusive evidence that the necrotic reaction in segregating populations is highly associated with plants that are heterozygous for the resistance gene.

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