Introduction

Soybean (*Glycine max* L. Merrill) is one of the most important crops of the world including U.S.A. The world acreage during 1990-91 was 55,018,000 hectares and the production during the corresponding year was 104,525,000 tons out of which the United states accounts for 22,866,000 hectares and 52,302,000 metric tons ('91 Soya Bluebook). Major uses of soybean are as a source of protein and oil though it is also used in the manufacturing of some industrial products.

Like in any other crop, soybean is prone to yield reductions caused by a number of diseases and insect-pests. Soybeans are known to be susceptible to at least 111 viruses or viral strains and 33 of them are known to occur naturally in soybeans and are believed to have some economic significance (Sinclair and Backman, 1989). The most prominent virus affecting soybeans throughout the world is soybean mosaic virus (SMV). Another virus of significance in southeastern parts of the United States is peanut mottle virus (PMV). PMV has also been reported on peanuts from many parts of the world such as Australia (Behncken, 1970), East Africa (Bock, 1973), Japan (Inouye, 1969) and West Malaysia (Ting et al., 1972). In the U.S.A. it was reported for the first time on peanuts by Kuhn (1965).

Peanut mottle virus is a significant virus in those parts of the country where peanuts and soybeans are grown in close proximity. This type of cropping pattern is mostly seen in the southeastern parts of this country and a few other countries. In these areas, peanuts can act as the primary source of inoculum in the spread of the virus by aphids. As the virus is seed-borne in peanut (Demski and Kuhn, 1977), it can survive from season to season to infect other peanut and soybean plants.

To date only three resistant genes against peanut mottle virus have been identified. Dominant genes were identified in York at the *Rpv*1 locus (Boerma and Kuhn, 1976) and in CNS at a different locus (Buss et al., 1985). A gene symbol for the CNS resistance gene has not yet been assigned. Peking was identified as possessing a single recessive gene at the *rpv*2 locus (Shipe, 1979).

One of the most effective ways to control viral diseases is through the development of

resistant cultivars (Buss et al., 1989). The basic step in the development of resistant cultivars is identifying sources of resistance and understanding the mode of inheritance. In crop improvement programs, it is always desirable to have a broad base of resistant sources as it will be helpful in broadening the genetic base of the available germplasm. This imparts genetic stability against the ever changing viral strains because multiple gene resistance cannot be easily and quickly overcome.

PI 486355 was selected for study because it has two independent dominant genes for resistance against soybean mosaic virus (Chen et al., 1993). It was thought that these genes might also confer resistance to PMV. Most of the soybean cultivars which are susceptible to SMV are also susceptible to PMV. PI 398593 is an exception as it is susceptible to SMV but resistant to PMV. Because of this peculiar reaction it was thought that it might likely have a resistance gene different than those already reported. Other resistant cultivars tested for allelism in this study include Jizuka, Toano, Raiden, Kwanggyo and PI 96983. Most of these accessions are from foreign countries except Toano which is from the U.S.A. Accessions PI 486355, PI 398593, PI 96983 and Kwanggyo are from Korea, while Jizuka and Raiden are from Japan. Hence, it was thought that they might possess different resistance genes than those already reported. If they are different, they could be valuable sources of germplasm for breeding for disease resistance, and reducing genetic uniformity.

This research was conducted with the main objective of identifying new resistance genes and describing relations with known genes against peanut mottle virus.

Specific objectives were:

1. Assign a gene symbol to the gene identified in CNS.
2. Determine the inheritance of resistance in PI 486355 and PI 398593.
3. Test the allelic relationships of the resistance genes in PI 486355 and PI 398593 compared to those reported in York, Peking and CNS.
4. Study the mode of inheritance of resistance and allelism in PI 96983, Kwanggyo, Jizuka, Suweon 97 and Raiden.

Literature Review

A disease caused by peanut mottle virus (PMV), a potyvirus, was first described in the U.S.A. by Kuhn in 1965 on peanuts. In 1966 it was observed on peanuts in southeastern Queensland (Behncken, 1970). Bock (1973) identified the same virus in East Africa both on peanuts and soybean, on the basis of particle morphology, serology, host range and reaction, transmission and physical properties. Kuhn et al. (1972) for the first time reported the natural occurrence of this virus on soybean in Georgia. Other southeastern states including Virginia (Tolin et al., 1974) have subsequently described its occurrence (Demski and Kuhn, 1977). It has also been reported from Japan (Inouye,1969), West Malaysia (Ting et al., 1972), Venezuela (Herold and Munz, 1969), and Bulgaria (Schmidt and Schmelzer, 1966). In the U.S.A. it is most prevalent in the southeast (Tolin and Ford, 1983) where soybeans and peanuts are grown in close proximity (Tolin and Roane, 1975).

General description of the virus

The following description has been taken from Bock and Kuhn (1975), and Demski and Kuhn (1989) except where other references are cited. Peanut mottle virus is a RNA-containing virus belonging to the Potyvirus genus in the Potyviridae, and contains 740-750nm long flexous, filamentous particles. It is more closely related to pea seed-borne mosaic virus than to other legume infecting potyviruses, including soybean mosaic virus (SMV) and peanut stripe virus (PStV) viruses which are members of the bean common mosaic subgroup (Berger et al., 1997).

Host range: PMV occurs in nature in several important legume crops such as *Glycine max, Arachis hypogaea, Lupinus albus, L.angustifolius, Phaseolus vulgaris, Pisum sativum.* It has been islolated from a few weed species viz. *Cassia obtusifolia, C. leptocarpa, C. occidentalis* and *Desmodium canum*. Other plant species that can be infected with PMV include *Calapogonium mucunoides, Canavaia ensiformis, Chenopodium amaranticolor, Nicotiana benthamiana, Sesamum indicum, Trifolium hybridum, Vigna cylindrica.*

Propagation species: Cultures are best maintained in *Cassia occidentalis* or peanut, although soybean

can be used.

Transmission: Aphids serve as non-persistent vectors and seed transmission occurs at a rate of 0.02-2.0% in peanuts but probably not in other species. The virus is mechanically transmitted by inoculation of the sap which is facilitated by the use of phosphate buffer of 0.01-0.05M concentration at a pH of 8.0 (Demski and Kuhn, 1977) or by spraying the inoculum onto leaves with an artist's air brush (Jones and Tolin, 1972).

Stability in sap: American isolates have a thermal inactivation point between 60-64°C, whereas that for East African and Australian isolates is 55-59°C. The dilution end point of all the isolates is 10^{-3} to 10^{-4}. Infectivity can be retained for two days at 20°C whereas frozen leaves can retain live virus for at least 12 weeks at -12°C.

Disease symptoms: The first symptoms of PMV on soybean are small, enlarging chlorotic patches followed by dark green islands on young leaves. Later on yellow patches, line patterns or ring patterns may occur on third and fourth leaves formed after infection. A mosaic pattern is produced on the older leaves. Infected leaves show downward curling. Apart from these leaf symptoms, infected plants show a decrease in plant height, lower number of pods per plant and reduction in the 100 seed weight (Demski and Kuhn, 1977 ; Roane and Tolin, 1974).

Host traits affected by PMV infection

Demski and Kuhn (1977) conducted a field experiment with artificial inoculation to determine the effect of PMV on two soybean cultivars Hampton 266A and Jackson. Yield reductions of 5-28% were reported in these tests conducted from 1972-75. An average of 6 and 5% reductions in the plant height and 100 seed weight, respectively, were observed. There was no effect on maturity or lodging. They also reported an increase in protein content and decrease in oil content from the seeds of PMV inoculated plants compared to the non-infected seeds. Roane et al. (1978) in their experiment on the effect of a severe strain of PMV on twenty-five adapted cultivars found a 44% reduction in the mean yield for twenty-one susceptible cultivars.

In a greenhouse experiment carried out using three susceptible cultivars (Laredo, Ransom and

Lee 68) and two resistant cultivars (Davis and CNS), it was observed that PMV caused a significant reduction in plant height , root and shoot weight in 'Laredo' and 'Ransom' and number of pods in Laredo but the differences were not significant in the case of 'Lee 68', 'Davis' and 'CNS' (Demski and Kuhn, 1977).

Strains of Peanut mottle virus

Bays et al. (1986) studied the reactions of selected soybean cultivars and plant introductions to peanut mottle virus and were able to classify twelve isolates of the virus form Virginia into five strain groups (P1-P5) based on the reaction they caused on York, Lee 68, 'Virginia', CNS and 'Cumberland' . Ten of the twelve strains infected Lee 68, Cumberland and Virginia but symptom severity differences permitted separation into three groups. One isolate infected only Lee 68 and Cumberland and one strain only Lee 68 and York.

Inheritance of resistance to PMV in soybean

Boerma and Kuhn (1976) studied the inheritance of resistance to PMV using the following crosses: 'Dorman' (R) x Ransom (S), Ransom x Dorman, CNS (R) x 'Bragg' (S) and 'Pickett' (S) x Dorman (R). All the F_1s were found to be resistant. In the F_2 generation, plants segregated in a ratio of 3R:1S indicating that the resistance is controlled by a single dominant gene which tentatively was designated as *Rpv*. Single gene control was confirmed by screening the F_3 population derived from resistant F_2 plants. The allelic relationships of the genes in CNS and Dorman were not studied. Reciprocal crosses involving Dorman and Ransom gave similar results, so it was concluded that there is no maternal effect.

Shipe (1979) reported a single, recessive gene conferring resistance to PMV in Peking at the *rpv*2 locus. F_1s of the cross Peking x PI 229315 (susceptible) showed a susceptible reaction and the F_2 population segregated in a ratio of 1R:3S indicating the recessive nature of the resistance gene. It was further confirmed when the $F_{2:3}$ lines of the same cross segregated in a ratio of 1R:2(1R:3S):1S.

A second dominant gene for resistance to PMV was identified by Buss et al. (1985). In their study, crosses were made among the resistant cultivars Arksoy, Dorman, York, Shore and CNS. No segregation was observed between the crosses involving these cultivars except when CNS was a parent. Arksoy, Dorman, York and Shore were concluded to contain the same gene for resistance which was earlier designated as *Rpv*. $F_{2:3}$ lines of the crosses of CNS with Dorman, York and Shore segregated as expected in a ratio of 7R : 4(15R:1S) : 4(3R:1S) : 1S. This study concluded that CNS has a different dominant gene from the gene in York, Dorman, Arksoy and Shore but a gene symbol was not assigned because allelism with *rpv2* was not tested. The results of Bays et al. (1986) also indicate a genetic difference between CNS and other resistant cultivars. They found that CNS was resistant to strain group P5 while Arksoy, Dorman, Shore and York exhibited mild mottling. Strain groups P1 through P4 produced no symptoms on any of the five cultivars.

Reaction of soybean to other viruses:

Warwick and Demski (1988) studied 121 genotypes from the International soybean program collection for their reaction to PStV. Reactions varied from necrotic local lesions followed by systemic necrosis, chlorotic local lesions followed by systemic mottle, systemic mosaic, systemic mild mottle in the first trifoliolate leaves to completely resistant genotypes. As PMV, PStV is also seed borne in peanuts and is the source of virus for infecting soybeans. But, it is not found to be seed transmitted in soybean in contrary to the behavior of SMV. Resistance to SMV has been reported in PI 486355, PI 96983, York, Peking, Jizuka, Toano, Raiden, Kwanggyo, Suweon 97, 'Ogden' whereas Lee 68, Essex, PI 398593 are susceptible (Yu, 1994).

Materials and Methods

Virus maintenance and inoculation procedures

The PMV strain used in this study, V89-3B9 was obtained from Dr. Sue A. Tolin, Dept. of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg. It was isolated from peanut in eastern Virginia. Pure culture was selected through nine single lesion isolations on Top crop bean (*Phaseolus vulgaris)* alternating with passage in Lee 68 and selection for typical PMV symptoms. Biological properties of V89-3B9 are similar to that of P1 strain group discussed by Bays et al. (1986). The virus was maintained in the greenhouse on a susceptible soybean cultivar Lee 68 or peanut (*Arachis hypogaea)* by mechanical inoculation. It was also maintained *in vitro* in the form of callus culture from explants of Lee 68 and Essex soybean cultivars and tobacco (Bagade et al., 1993).

For greenhouse inoculations, leaves showing typical symptoms of the disease were ground using a pre-cooled mortar and pestle with 0.01M sodium phosphate buffer at pH 8.0. For every gram of infected leaf tissue about 10 ml of buffer were added to prepare the inoculum. The pestle was dipped into the inoculum and rubbed onto the upper surface of both unifoliolate leaves which were previously dusted with 600 mesh carborundum powder. The seedlings were inoculated 10 to 14 days after planting and before the emergence of trifoliolate leaves. All the greenhouse inoculations were performed during winter and early spring. Field inoculations were done during summer.

For field inoculations, inoculum was increased in the greenhouse on 'Lee 68' plants. Leaves showing typical symptoms of the disease were harvested 2-3 weeks after inoculation. The field inoculum was prepared by grinding the infected leaves in a blender with 0.05M sodium phosphate buffer, pH 8.0, and straining through four layers of cheese cloth. About 10 ml of buffer was used for a gram of infected tissue. To facilitate entry of the virus into plant tissue, 0.5% carborundum and 0.1% sodium sulfite was added to the inoculation buffer to increase the virus stability. The inoculum was kept on ice until used.

Field inoculations were made by spraying about 0.2 ml of the inoculum onto the lower surface of a leaflet from a distance of 1-2 cm with an artist's air brush (Roane et al., 1983) at an air pressure of 4.2-5.6 kg/cm^2 (60-80 psi) supplied by a gasoline powered portable air compressor. Plants at the V1-V3 stages (Fehr and Caviness, 1977) were inoculated on the youngest fully expanded leaf or leaflet.

Tissue Immunoblot Assay

The tissue immunoblot assay method was used to test for the presence of PMV in plant tissue in cases where positive identification of PMV in symptomatic plants was critical for genetic interpretation.

Lin et al. (1990) described an immunological method for the detection of plant viruses and mycoplasmalike organisms by direct tissue blotting on nitrocellulose membranes. This method was modified successfully by Srinivasan and Tolin (1992) for the detection of three viruses in clover. In practice, a leaf with suspicious symptoms is torn and rubbed onto a nitrocellulose membrane which is then immersed in 5% Triton X-100 and agitated for 10-15 minutes to remove excess green color. Then the membrane is rinsed in 1x 0.02M K_2HPO_4 + 0.15M NaCl + 0.05% Tween-20, pH 7.4 KPS-T buffer for three minutes, and then treated 30 minutes with blocking solution consisting of 5% non-fat dry milk and 0.5% bovine serum albumin (BSA). Then the membrane is transferred to rabbit whole serum PMV-specific antibody (Tolin and Ford, 1983) and rinsed thrice in 1x KPS-T buffer for 10, 5 and 5 minutes each. The membrane is incubated in secondary antibody (goat anti-rabbit/alkaline phosphatase, Sigma Chemical (St. Louis, MO), rinsed again with 1x KPS-T buffer as before and incubated in freshly prepared nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indoyl-phosphate (BCIP), obtained from Zymed Laboratories Inc. (So. San Francisco, CA). Blue or purple color on the filter paper indicates a positive reaction for PMV.

Inheritance of resistance and tests of allelism

PI 486355 was crossed with PMV susceptible cultivars Lee 68 and Essex to determine the number of resistance genes and their mode of inheritance. To test for allelism with reported genes, PI 486355 was crossed with York, having a resistance gene at the *Rpv*1 locus, and CNS which has a dominant resistance gene at a locus other than *Rpv*1.

To determine the mode of inheritance and also to test the allelic relationships with known

resistance genes, PI 398593 was crossed with cultivars Lee 68, York, CNS and Peking.

PI 96983, Kwanggyo, Toano and Jizuka (all R) were crossed with Lee 68; Suweon 97(R) was crossed with Essex(S); and Raiden(R) with Ogden(S) to study the inheritance of resistance in these cultivars against PMV. PI 96983, Toano, Jizuka and Suweon 97 were crossed with York and their F_2 populations were studied with an objective of determining their allelism with *Rpv*1.

Both F_2 populations and $F_{2:3}$ lines of the cross Peking x CNS were studied to determine whether or not the CNS gene is allelic to the Peking gene, *rpv*2.

Growth of F_1 and F_2 plants

Crosses were made both in the greenhouse at Blacksburg and in the field at the Eastern Virginia Agricultural Research and Extension Center, Warsaw, VA. Seeds from each cross and parent plant were harvested separately and the F_1 plants were grown for seed production either in the greenhouse at Blacksburg or space planted in the field at Warsaw. No virus symptoms were observed on them. This is done to ensure that no virus is being seed transmitted. Plants of each parental line were also grown along with the F_1 plants to determine whether each F_1 plant was a true cross by using morphological markers such as flower color, pubescence color, pod color and seed coat color. F_2 seeds were hand-harvested from each F_1 to avoid mechanical mixtures. Seeds from each F_1 plant were kept separate. $F_{2:3}$ lines were produced by planting F_2 seeds at Warsaw. Segregation for the marker traits such as flower color, pubescence color, pod color and seed coat color were observed to check the authenticity of the cross. Individual F_2 plants were threshed separately.

The F_2 populations and $F_{2:3}$ lines were evaluated in the greenhouse or field depending on the availability of space. In the greenhouse, F_2 populations for inoculations were planted in 15 cm diameter pots with 15 seeds per pot. In all the crosses at least 100 F_2 plants were inoculated. In the field, 30 seeds were planted in a 1 m long row. Data were collected only from $F_{2:3}$ rows which had a minimum of ten plants.

Collection and Analysis of data

Notes on segregation for resistance and susceptibility were taken and the data were analyzed using X^2 test. All inoculated plants were classified as resistant or susceptible based on typical symptom development. In the greenhouse, plants were scored 2-3 weeks after inoculation. In the field the first scoring was done about 5 weeks after inoculation and a second scoring was done about 8-9 weeks after inoculation. $F_{2:3}$ lines were classified as homogeneous susceptible, homogeneous resistant or segregating in a particular ratio. X^2 test of $F_{2:3}$ segregation data were performed for the most likely genetic ratios. Rows were classified as belonging to the category with the lowest X^2 value. A X^2 test for homogeneity was conducted among rows classified as segregating in a particular ratio within each cross.

Results and Discussion

Allelism test for the resistance gene in CNS

Results from inoculating the F_2 population of cross Peking x CNS with PMV are shown in Table 1A. Plants were inoculated in the greenhouse during spring 1994. The data fit a 13R:3S ratio, which is expected when one dominant and one recessive gene at different loci are segregating. These data suggest that the resistance genes in Peking and CNS are non-allelic to each other. It also corroborates the recessive nature of the resistance gene in Peking as reported by Shipe (1979).

These F_2 results were further corroborated by $F_{2:3}$ data involving the same cross (Table 1B) inoculated in the greenhouse during winter 1995. $F_{2:3}$ lines were categorized as: homogeneous resistant, segregating as either 13R:3S (this category also includes 3R:1S, as it is very difficult to distinguish these two categories in a population size of 30 or less plants per row) or 1R:3S, and homogeneous susceptible. Homogeneous resistant rows were 47, total number of segregants were 49 and 3 rows were homogeneous susceptible. These segregations were tested against a ratio of 7(Homo.R) : 6(13R:3S + 3R:1S) : 2(1R:3S) : 1(Homo.S) and were found to fit satisfactorily with a P-value of 0.25-0.5. This ratio is expected when one dominant and one recessive gene segregate at different loci. Hence, it can be said that the resistant genes of Peking and CNS are at different loci. These data are in agreement with the F_2 data. These results clearly establish the non-allelic nature of Peking and CNS resistance genes.

Hence, from this and previous studies (Boerma and Kuhn, 1976) it can be concluded that York and CNS each has one dominant gene for resistance against peanut mottle virus; whereas, Peking has one recessive gene. All of these genes are non-allelic to each other. So it can be confidently said that CNS has a dominant resistant gene which is non-allelic to the resistance genes of York and Peking. Since all the allelism tests are complete, the gene symbol of *Rpv*3 can be assigned.

Inheritance of resistance in PI 486355

Three hundred and forty five F_2 plants from two different F_1 plants of the cross PI 486355 x Lee 68 were inoculated in the greenhouse during spring 1994. Results of this inoculation are shown in Table 2A. No susceptible individuals were found among the PI 486355 plants and Lee 68 showed 100% susceptibility. The F_2 data showed a good fit to a 15R:1S ratio suggesting that PI 486355 has two independent, dominant genes for resistance against peanut mottle virus.

Fifty five $F_{2:3}$ lines from PI 486355 x Essex were inoculated in the field during 1993 and forty lines were inoculated in the greenhouse during spring 1994. Each line could be classified into one of the following four categories: homogeneous resistant, segregating in either 15R:1S or 3R:1S ratios, or homogeneous susceptible.

PI 486355 was completely resistant and both of the susceptible checks, Lee 68 and Essex, were 100% susceptible in the greenhouse, whereas Lee 68 showed 99% susceptible reaction in the field during 1993. If PI 486355 possesses two independent, dominant genes then the $F_{2:3}$ lines should segregate as 7 Homo.R: 4 (15R:1S): 4 (3R:1S): 1 Homo.S. All the segregating rows in each ratio class were tested for homogeneity. Both 15R:1S and 3R:1S pooled data showed an excellent fit to their respective ratios (Table 2B) and the segregating rows of each class were homogeneous. When the data were analyzed against a 7:4:4:1 ratio, they provided a very good fit, again indicating the presence of two independent, dominant genes in PI 486355 for resistance to PMV, as was hypothesized from the F_2 data.

Test of allelism of PI 486355 resistance genes

To test for the identity of the resistance genes in PI 486355, it was crossed with cultivars York and CNS which have dominant resistance genes at *Rpv*1 and *Rpv*3 loci, respectively.

F_2 plants of the cross PI 486355 x York were inoculated in the greenhouse during spring 1994. All 254 inoculated plants were found to be resistant (Table 3A). Both of the resistant parents showed complete resistance and the susceptible check was 100% susceptible. If neither of the PI 486355 genes are at the *Rpv*1 locus, then this cross should have segregated in a ratio of 63R:1S.

Complete absence of segregants strongly indicates that at least one of the resistance genes is at the *Rpv*1 locus of York. All F_2 seeds of the cross PI 486355 x CNS were used to produce $F_{2:3}$ lines, so none were available for screening F_2s.

$F_{2:3}$ lines of the crosses PI 486355 x York and PI 486355 x CNS were inoculated with PMV in the field in 1993 and 1995 respectively and classified into four categories: homogeneous resistant (this category also includes rows that would segregate as 63R:1S but could not be identified due to the small sample number of 30 plants per row); segregating either 15R:1S or 3R:1S, and homogeneous susceptible.

Out of the 167 $F_{2:3}$ rows of the cross PI 486355 x York, only two rows contained symptomatic plants (Table 3B). The remaining 165 rows were completely resistant.

The symptomatic plants observed in this cross do not appear to be the result of genetic segregation. One of the two apparently segregating rows had only one symptomatic plant and the other row had three. Intensity of the purple color developed from blot testing of the single symptomatic plant was not obviously different from the PMV-negative check, York. This suggests that the symptoms were caused by a virus other than PMV. Three symptomatic plants from the other row gave very positive immunoblot results. It is likely that this row resulted either from an outcross that occurred on the F_1 plant or from mechanical mixtures while threshing individual F_2 plants. If neither of the PI 486355 genes were allelic to *Rpv*1, a ratio of 45R : 12 (15R:1S) : 6 (3R:1S) : 1S would be expected. Complete lack of totally susceptible rows, along with the very low number of heterogeneous rows, is strong evidence that no genetic segregation for reaction to PMV occurred in this cross.

$F_{2:3}$ lines of the cross PI 486355 x CNS were inoculated in the field during 1995 and some were repeated in the greenhouse during early 1996. At the time of the first scoring, 8 weeks after inoculation, not many plants were showing symptoms indicating susceptibility. Second scoring was done 10 weeks after inoculation. Even by the time of second scoring many plants remained symptomless. During an additional scoring (apart from routine second scoring), which was done 15 weeks after inoculation most of the rows started showing susceptible plants. All rows showing

susceptible plants were selected and plants from remnant seed from those rows were screened in the greenhouse. Resistant rows were also repeated in the greenhouse where seed was available. The number of resistant rows in the field was 225 compared with 167 from the greenhouse studies. There were no homogeneous susceptible rows in the field, but one line was found to be completely susceptible in the greenhouse inoculations. Following greenhouse studies all the segregants were subjected to a X^2 test of homogeneity. The results indicated that the rows within each category were homogeneous, but the overall fit to the 15R:1S and 3R:1S ratios were not good. It might be due to the fact that not all susceptible plants showed symptoms. Complete lack of any susceptible row is an indication that all susceptible plants did not show symptoms. Both the resistant parents were completely resistant and the susceptible check, Lee 68, showed 100% susceptible plants in the greenhouse and 95% in the field. These results are shown in Table 3B. When the data were tested against 45(Homo.R.+63R:1S) : 12(15R:1S) : 6 (3R:1S) : 1S ratio which is expected if three independent, dominant genes are segregating, a good fit was obtained. This indicates that both the resistant genes in PI 486355 are at loci other than *Rpv*3.

From this data it can be said that PI 486355 has two independent, dominant genes for resistance against PMV. One of the genes is allelic to the York gene (*Rpv*1 locus) and the second gene is at a locus separate from *Rpv*3, the CNS resistance gene locus. Since the PI 486355 x Peking cross was not studied, the allelism tests are incomplete and a gene symbol cannot be assigned to the newly identified gene in PI 486355 until this cross is studied.

Inheritance of resistance in PI 398593.

The F_2 population of the cross PI 398593 x Lee 68, was used to determine the inheritance of resistance. Crosses of PI 398593 with other resistant parents possessing identified genes (York, CNS and Peking) were studied for the allelism test. All these crosses were inoculated in the greenhouse during spring 1994.

Results from inoculating the F_2 populations along with the reactions of the parents are shown in Table 4. The PI 398593 x Lee 68 cross showed a good fit to 1R:3S ratio suggesting a recessive

nature of the resistant gene. When PI 398593 was crossed with York and CNS which are known to contain single dominant genes, the F_2 populations segregated in a ratio of 13R:3S. These fit the hypotheses that PI 398593 possesses a single recessive gene at a locus different than *Rpv1* and *Rpv3*. But, the data obtained from the cross PI 398593 x Peking did not fit that model. If PI 398593 has a single recessive gene, then this cross should have segregated as 7R:9S. A very high X^2 value (98.9) was obtained when tested against the 7R:9S ratio. Nevertheless, it is clear that the gene or genes in PI 398593 are not allelic to those in Peking, York or CNS.

$F_{2:3}$ lines of the cross York x PI 398593 (Table 5A) were inoculated and scored in the field during 1993. A good fit was obtained when the data were fitted to a 7R : 6(13R:3S + 3R:1S) : 2(3S:1R) : 1S ratio expected from the segregation of one dominant and one recessive gene for resistance. These data indicate that the resistant genes in these two cultivars are non-allelic to each other. $F_{2:3}$ lines of the crosses PI 398593 x CNS and Peking x PI 398593 were studied during 1995 in the field. These results (Table 5B and C) did not support the findings from F_2 data in regard to resistance in PI 398593. Segregation patterns of these crosses did not fit the expected ratios. In these two crosses the number of susceptible rows were much less than expected if PI 398593 possesses a single recessive gene for resistance.

In addition, PI 398593 itself started behaving in an unexpected manner since 1994 in the field. A few symptomatic plants were noticed in the field during 1994 which were found to be clearly positive for PMV with the tissue immunoblot test. Seeds were harvested from single plants (both symptomatic and resistant) from the field during 1994 and were tested in the greenhouse during spring 1995 and also during summer 1995 in the field. Results of these inoculations are shown in Table 6. In the greenhouse all single plant progenies irrespective of whether they had been derived from resistant or symptomatic plants showed resistant reactions. It could be possible that the susceptibility was not expressed in the greenhouse environment. It could be possible that the resistance is temperature sensitive. From available data, the genetic nature of resistance in PI 398593 is unexplainable. In the 1995 field nursery, a number of lines remained completely resistant. However, a small number of susceptible plants were observed in seven out of a total of 24 rows. Five of these

rows had only one susceptible plant whereas two rows had three plants each. Presence of PMV in these susceptible plants was confirmed by immunoblot assay. Two of the seven rows were derived from resistant plants in the 1994 nursery. So there is no clear association between reaction of the plants in 1994 and their progeny in 1995. If the susceptible plants observed in the 1995 nursery were due to mechanical seed mixtures, then a few susceptible plants should have also been observed in greenhouse studies since the sample size in both cases was the same and the seeds were from the same source. Complete absence of susceptible plants in the greenhouse tends to indicate that the susceptible plants observed in the field during summer 1995 were not due to mixtures. Also, the observed numbers of resistant and susceptible plants in the mixed rows do not suggest any simple genetic segregation ratios. This observation tends to rule out the possibility of outcrossing. The presence of susceptible plants only in the field and the lack of agreement between 1994 and 1995 suggests that a virus might be present in the field that gives a positive test for PMV, but is virulent on the PI 398593 gene. If it is a new PMV strain, it is not clear how it survives from one season to the next since no known reservoir hosts are present in the area. Another possible explanation is that PI 398593 resistance gene is not stably expressed in some environments or genetic backgrounds.

Since the data show that PI 398593 possesses a gene or genes that are non-allelic to all the previously reported resistance genes, it is worth further investigation. Before making any crosses with this PI, it should be tested for uniformity of PMV reaction both in the field and greenhouse. After screening the progenies of the individual plants in the field during 1994 and 1995 and also in the greenhouse during 1995, nine lines were found to be resistant in all the cases. These should be tested for at least one more season before using them as parents in making crosses for further studies. If they were found to be non-uniform again, then only the progenies showing resistance should be selected. This process needs to be repeated until the selected plants and their progeny show uniform reaction to PMV. If uniformity cannot be obtained, then genetic segregation can be ruled out as the source of variation. Also, seeds should be harvested individually from all parent plants used in crossing. These seeds can be used to check the parental reactions at a later stage, if needed.

Genetics of PMV resistance of six soybean cultivars

The mode of inheritance of resistance was studied in PI 96983, Kwanggyo, Toano and Jizuka using F_2 populations. Resistant parents were crossed with a susceptible cultivar, Lee 68, for this purpose. F_2 populations of crosses PI 96983 x Lee 68 and kwanggyo x Lee 68 were inoculated in the field during 1993. Toano x Lee 68 was inoculated in the greenhouse during winter 1994 and Jizuka x Lee 68 in the field during 1994 Results from inoculating F_2 populations from each of these crosses are shown in Table 7 (A, B and C). All of the populations fit a 3R:1S ratio indicating that resistance is monogenic and dominant. In the cross PI 96983 x Lee 68, ten plants showed necrotic reaction. These necrotic plants were uniformly distributed in all six rows in which F_2s from this cross were grown, but were not found in any other crosses, indicating that necrosis is probably related to the resistance gene in PI 96983. The necrotic F_2 plants are probably heterozygous for the resistance gene. This assumption could not be tested as the necrotic plants did not produce any seed. If the necrotic plants are heterozygous for the resistance gene, then theoretically 50% of the F_2 population could be necrotic. But in this case, only about 5.5% of the F_2 population (10 out of 181 plants) showed a necrotic reaction. Necrotic plants were also observed in 43 of the 110 segregating $F_{2:3}$ rows from the same cross (Table 8B). Presence of necrotic plants only in the segregating $F_{2:3}$ rows supports the assumption of the heterozygous nature of necrotic plants. Kiihl and Hartwig (1979) found that the progeny of F_2 necrotic plants segregated when inoculated with SMV, indicating the heterozygous nature of F_2 necrotic plants. Chen et al. (1991) from their study of various soybean cultivars inoculated with SMV-G1 reported that the expression of necrotic symptoms is under the influence of environment and the expression varies among the resistant genes. They also reported a very low frequency of necrotic plants in crosses with PI 96983. Chen et al. (1994) studied several crosses using various soybean cultivars, wherein they reported that Resistant x Susceptible crosses segregated in a ratio of 1resistant : 2necrotic : 1susceptible. This is a clear evidence of the heterozygous nature of the necrotic plants. They had classified the necrotic plants as resistant for calculating genetic ratios. Similarly, in this study also necrotic plants were classified as resistant plants.

$F_{2:3}$ lines were used to determine the mode of inheritance in Raiden, Suweon 97 and PI 96983 (Table 8A and B). Progeny of the Raiden x Ogden and Suweon 97 x Essex crosses were inoculated in the field during 1993 whereas, those of the PI 96983 x Lee 68 cross were inoculated in the field during the following year. All three resistant x susceptible crosses gave a good fit to 1(Homo.R) : 2 (3R:1S) : 1 (Homo.S) indicating that all these cultivars possess a single dominant gene for resistance. X^2 test of homogeneity was run on the segregating rows of all these crosses. P-values for Raiden x Ogden, Suweon 97 x Essex and PI 96983 x Lee 68 were > 0.995, 0.05-0.1 and > 0.995, respectively. This indicates that all the crosses are segregating uniformly.

Since all of the cultivars have single gene for resistance to PMV, studies were conducted to determine allelism with known loci. To achieve this objective PI 96983, Toano, Jizuka and Suweon 97 were crossed with York which is known to contain the *Rpv*1 locus. PI 96983 x York was inoculated in the greenhouse during spring 1993. Crosses of York with Toano, Jizuka, and Suweon 97 were inoculated in the field during 1994. Complete lack of segregation (Table 9A and B) among the F_2 populations of these crosses indicates that the resistance gene in each of these cultivars is at *Rpv*1 locus. Even though the York x Suweon 97 cross had a fairly small sample size (Table 9B), the probability of finding at least one susceptible plant out of 58, when a 15R:1S ratio is expected, is greater than 95%.

It appears that the same gene or very tightly-linked genes may govern resistance to both SMV and PMV in the cultivars studied here. PI 96983 has a resistance gene at the *Rsv*1 locus for resistance to SMV (Kiihl and Hartwig, 1979) and at the *Rpv*1 locus for resistance to PMV. Toano apparently has the same PMV and SMV resistance as York, based on pedigree analysis and the results of this study. For PMV resistance Suweon 97 has one dominant resistance gene at the *Rpv*1 locus (Table 9 B) but its SMV resistance has not been studied. Kwanggyo has a resistance gene at *Rsv*1 locus (Chen et al., 1991) and has a single dominant gene for PMV resistance. Raiden has a single, dominant gene for resistance to PMV (Table 8A) and its SMV resistance gene is reported to be at *Rsv*1 locus (Ma and Buss, 1995). Jizuka possess a single dominant resistance gene at *Rpv*1 locus for resistance to PMV but no data on SMV genetics is available about this cultivar. If *Rsv1* and *Rpv1* were

independently segregating genes, it would seem improbable that both would be found together in all diverse cultivars studied here. Roane et al. (1983) reported that *Rsv1* and *Rpv1* are linked with about 3.8% crossing-over. The data now available would indicate the association between the two genes might even be closer than that. But 'Marshall' and Ogden are resistant to SMV and susceptible to PMV; whereas PI 398593 and V73-178 are found to be resistant to PMV and susceptible to SMV.

Summary

From this study it can be concluded that CNS has a resistance gene at a locus other than that of Peking. Since all allelism tests are complete its resistance gene can be assigned a gene symbol of *Rpv3*. PI 486355 possesses two independent dominant genes for resistance against PMV. One of the genes is at *Rpv1* locus and the second one is non-allelic to *Rpv3*. Since it has not been crossed with Peking, allelism tests are incomplete and a gene symbol cannot be assigned at this stage. Even though nothing conclusive can be said about the genetic nature of PI 398593, it seems to be non-allelic to all known resistance genes. PI 96983, Kwanggyo, Jizuka, Suweon 97, Toano, and Raiden were found to possess a single dominant gene for PMV resistance . The gene in PI 96983, Jizuka, Suweon 97, and Toano are allelic to *Rpv1*. PI 96983, Toano, Kwanggyo, and Raiden are also known to have different alleles of the same resistance gene at *Rsv1* against SMV (Chen, 1994). From these observations and also from the study of Roane et al. (1983), SMV and PMV resistance genes are either very closely linked or it may even be possible that the same gene may be governing resistance to both the viruses even though these two viruses are not closely related to each other based on phylogenetic analyses (Berger et al., 1997). They also suggest that peanut mottle virus is more closely related to pea seed borne mosaic virus than to other legume-infecting potyviruses and SMV is in a cluster of its own based on the analysis of coat protein and non-coding region. However, Marshall and Ogden have alleles of *Rsv1* confirming resistance to SMV but are susceptible to PMV (Bays et al., 1986).