Korean J. Crop Sci. 46(1): 17~21(2001)

Evaluation in Korean Soybean Cultivars of Resistance to Soybean Mosaic Virus

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ABSTRACT : Soybean mosaic virus (SMV) resistance of Korean recommended soybeans was evaluated naturally and by mechanical inoculation in Suwon. Based on the differential reaction of forty-four soybean genotypes tested to nine different SMV strains, soybeans were classified into twenty-four groups. Myeongjunamulkong and Ilpumgeomjeongkong showed a high degree of resistance to nine SMV strains, having no symptom. The other cultivars produced various reactions according to inoculation of each SMV strain: symptomless, mosaic or systemic necrosis. Only five cultivars such as Kwangankong, Eunhakong, Tawonkong, Namhaekong, Sobaegnamulkong were totally susceptible to every strain. There was variation in disease incidence. Soybeans, having the highest levels of resistance to G5H and G7H in the greenhouse, showed the lowest levels of SMV incidence in the field of Suwon. Myeongjunamulkong, Ilpumgeomjeongkong, Soyangkong, Pungsannamulkong, Sodamkong, Jangmikong, Geomjeongkong2, Pureunkong, Sinpaldalkong2, Duyoukong, and Geumgangkong were fairly resistant to SMV. And SMV incidence of Taekwangkong, Saealkong and Baegunkong was over 45% with symptom of bud necrosis. And soybeans, highly resistant to SMV in the field and the greenhouse, were mainly derived from Jangyeobkong and Hwangkeumkong resistant to G1-G7.

Keywords **:** soybean mosaic virus, Korean recommended soybean, mosaic, necrosis, disease incidence.

Soybean mosaic virus (SMV) is one of the most prevalent diseases in soybeans [*Glycine max* (L.) Merr.] of Korea and causes significant reduction in yield and quality (Kim *et al.*, 1997). Pathogenic variation among SMV isolates has been found worldwide in soybean cultivars (Cho *et al.*, 1979; Takahashi *et al.*, 1980; Kim *et al.*, 1999).

Soybean responses caused by SMV are symptomless or resistant, necrosis, and mosaic or susceptible depending on combination of soybean genotype and virus strain. Necrosis caused by more virulent strains of SMV is becoming a serious problem for soybean production because it can be much more lethal to the plant than a typical mosaic symptom.

Development of soybean varieties resistant to SMV is a major desirable strategy for managing this disease. Koshimizu & Iizuka (1963) evaluated 110 soybean cultivars in field or greenhouse condition, and rated 27 of these cultivars as immune. Ross (1969) screened 478 soybean lines for resistance to a SMV-1 strain and found 24 lines to be free of symptoms. In Brazil, Lima (1974) found 38 breeding lines resistant to five SMV isolates. Cho & Goodman (1982) reported five breeding lines developed in Korea (Suwon86, Suwon94, Suwon95, Suwon97 and Suwon106) were immune to seven SMV strains, G1 to G7. Soybean line SS74185 (PI486.355) collected in Korea, was reported to be resistant to seven (G1-G7) strains and C14 isolate by Lim (1985).

Studies on the genetic basis of resistance and susceptibility of soybean to SMV resulted in a better understanding of interaction between host and virus (Chen *et al.*, 1991; 1994). Gene for gene model was also proposed for SMV X soybean interactions (Roane *et al.*, 1986). In a majority of cultivars, a single dominant gene, *Rsv*1, confers both resistant and necrotic responses. Qusus (1997) revealed that responses of SMV strains to soybean cultivars were four types of reactions : 1) susceptible, showing unrestricted replication and spread; 2) immune, where no virus was detected; 3) systemic spread, showing unrestricted replication but limited spread along the veins; and 4) restricted replication and spread, where infection was restricted to few foci along veins. Results of this study indicated that *Rsv*1-mediated resistance is a multicomponent type of resistance that involves both inhibition of virus replication as well as cell-to-cell movement.

Recently, a new strain of SMV, G7H was identified from the plants infected with SMV (Kim *et al.*, 1999). It induced mosaic and necrotic symptoms on the inoculated and upper leaves of Korean recommended soybeans resistant to G5H and G7 strains. Infection with G7H was up to 36%, and its

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necrosis became a severe problem especially in the northern area of South Korea such as Suwon.

This study was carried out to evaluate Korean recommended soybean cultivars of resistance to SMV and find a source of valuable resistant gene for the future soybean breeding.

MATERIALS AND METHODS

Soybean plants

Evaluation of the SMV resistance of soybeans was conducted naturally and by mechanical inoculation at National Crop Experiment Station (NCES) of Suwon. Forty-four Korean recommended soybean cultivars were used for a virulence test (Table 1). Phenotypic reactions to nine SMV strains in the greenhouse of NCES were evaluated mechanically from May through September of 1999 and 2000. Each of soybean entries was planted in 1/5000a pots. Nine pots (3 plants/pot) were prepared for each cultivar, and inoculated with each of nine SMV strains respectively. Reactions of the plants to SMV were checked for two months after showing SMV symptoms. Evaluation of the field resistance of thirty-nine soybeans to SMV was performed at the station in 1999. Disease incidence was evaluated with naked eyes.

SMV strains

Strains of SMV used in the experiment were G1, G3, G4, G5, G6, G7, G7a, G5H, and G7H. PV-571 (SMV-G1/VA), PV-616 (SMV-G3), PV-572 (SMV-G4/VA), PV-612 (SMV-G6), PV-613 (SMV-G7), and PV-614 (SMV-G7a) obtained from American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland 20852, USA). SB29 (G5), SB15 (G5H) and SB26 (G7H) were selected from seed-borne isolates deposited in Dept. of Upland, NCES. Each strain was

Table 1. Phenotypic classification of forty-four Korean recommended soybean cultivars based on reactions to nine soybean mosaic virus strains.

Groups	Reactions to SMV strains†									Soybean cultivars
	G1	G3	G4	G5	G6	G7	G7a	G5H	G7H	
A	-	-	-	-	-	-	-	-	-	Myeongjunamulkong, Ilpumgeomjeongkong
B	-	-	-	-	-	-	-	-	ST	Soyangkong
C	-	-	-	-	-	-	-	-	N	Pungsannamulkong, Jangsukong, Sodamkong, Jangmikong, Muhankong, Jinpumkong, Jinpumkong2
D	-	-	-	-	-	-	-	N,-	N	Mallikong, Alchankong, Jangyeobkong, Hwangkeumkong, Daewonkong, Dajangkong, Pokwangkong
E	-	-	N	-	-	-	M	-	-	Geomjeongkong2
F	-	-	N	-	-	N	M	-	-	Pureunkong
G	-	-	N	-	-	N	-	N, -	N	Geomjeongkong1
H	-	-	N	-	-	N	M	-	N,-	Sinpaldalkong2
I	-	-	-	N	N	-	-	N	N	Taekwangkong, Saealkong
J	M	-	-	M, -	M, -	-	-	-	M	Paldokong
K	M	M	M	-	-	-	-	-	M	Duyoukong, Geumgangkong
L	-	-	N, -	N	N	-	-	N	N	Baegunkong
M	-	-	N	M	M	M	-	M	M	Seonheukkong, Keunolkong, Hwaeomputkong
N	N	-	N	M	M	M	-	-	M	Hannamkong
O	M	M	M	-	N,-	-	-	M	M	Songhagkong
P	M	M	M	M	M	-	-	M	M	Bukwangkong
Q	M	-	N	M	M	M	-	M	M	Seokryangputkong
R	M	-	M	M	M	M	-	M	M	Danbaegkong
S	M	-	-	M	M	M	M	M	M	Iksannamulkong
T	M	M	M	N, M	M	N, M	-	M	M	Samnamkong
U	M	M	M	M	M	M	-	M	M	Danweonkong, Danyeobkong
V	N	M	M	M	M	M	M	M	M	Kwangankong
W	N	M	N, M	M	M	M	M	M	M	Eunhakong
X	M	M	M	M	M	M	M	M	M	Tawonkong, Namhaekong, Sobaegnamulkong

†Symbols for symptoms: –(symptomless); M(mosaic); N(necrosis); ST(stunting).

confirmed by inoculation on soybean differentials of Cho and Goodman (1982) and Korean soybeans such as Hwangkeumkong, Daewonkong, Doyoukong, Myeongjumanulkong (Kim *et al.*, 1999). Rampage (susceptible to G1, G3, G4, G5, G6, G7, G5H and G7H) and Buffalo (susceptible to G7a) were used as the maintenance hosts.

Inoculation procedures

Inoculum of each SMV strain was prepared by homogenizing leaves of the hosts, showing mosaic symptoms approximately 20 days after inoculation, with 0.01M sodium phosphate buffer (pH 7.0). Inoculations were made on fully expanded primary leaves pre-dusted with Carborundum powder, and the leaves were rinsed immediately with tapwater.

Distribution of SMV strains

To find distribution of SMV strains in the field of NCES, reverse transcription-polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) analysis was used the same as that did previously (Kim *et al.*, 2000). Total RNA was isolated from SMV-infected leaves. RT-PCR was performed with specific primers amplifying 1385-bp fragments from 4176 to 5560 nucleotides, which were based on the sequence of SMV-G2 (NCBI Accession No. S42280). Thermocycling was programmed as follows; DNA synthesis of 1 cycle at 48°C for 45 min, AMV RT inactivation and RNA/cDNA/primer denaturation of 1 cycle at 94°C for 2 min; followed by 40 cycles for template denaturation at 94°C for 30 sec, primer annealing at 60°C for 1 min, extension at 68°C for 2 min; followed by 1 cycle for final extension at 68°C for 7 min. The products were precipitated and analyzed in a 3% Nusieve 3 : 1 agarose gel (FMC) after cleavage with *Eco*RI and *Rsa*I at 37°C for 3 hours.

RESULTS

Reactions of forty-four Korean recommended soybean cultivars to nine SMV strains were given in Table 1. Korean strains G5H and G7H causing necrosis in Hwangkeumkong, were designated previously (Kim *et al.*, 1999). Various host reactions were produced and grouped into twenty-four subgroups on the basis of their symptoms when inoculated with each of strains. Out of 44 soybeans, only two cultivars of Group A, Myeongjunamulkong and Ilpumgeomjeongkong, were showed a high degree of resistance to all SMV strains including G7H which was reported to be the most prevalent strain in Korea (Kim *et al.*, 1999). Every inoculated primary leaf and upper leaf of them was symptomless. Group E and F were also immune to G7H but produced necrosis and mosaics by other strains. The above four soybeans can be used as resistance sources to G7H strain. Eight cultivars of Group B and C had stunting and systemic necrosis respectively by inoculation of G7H, while they were resistant to the other strains. Group D showed the same phenotypic responses with Group C except necrotic symptoms caused by G5H. Group V, S, and X were susceptible to G5H and G7H of the virulent Korean strains as well as the previous G1 to G7, G7a. Soybeans of Group T and U were also susceptible to SMV strains, but G7a strain could induce no reaction. In fact, mosaic by G7a was limited only in nine cultivars. G1 strain showed unpredited necrotic sympoms in three soybeans of Group N, V and W.

The incidence of SMV disease in the soybean field of NCES was investigated with naked eyes and classified into three groups according to their symptoms: symptomless,

Table 2. Soybean mosaic virus (SMV) disease incidence in soybeans under natural infection in the field of Suwon.

Symptoms	SMV incidence[†]	Recommended soybean cultivars
Symptomless	-	Myeongjunamulkong, Ilpumgeomjeongkong
Necrosis (%)	1~5	Pungsannamulkong, Sodamkong, Jangmikong, Geomjeongkong2, Sinpaldalkong2
	6~15	Jangsukong, Alchankong, Hwangkeumkong, Dajangkong
	16~30	Jinpumkong, Jinpumkong2, Jangyeobkong, Daewonkong, Pokwangkong, Geomjeongkong1
	31~45	Muhankong, Mallikong
	>45	Taekwangkong, Saealkong, Baegunkong
Mosaic (1~9)[‡]	1~3	Soyangkong, Pureunkong, Duyoukong, Geumgangkong
	4~5	Keunolkong, Hwaeomputkong, Seokryangputkong, Samnamkong, Danyeobkong, Kwangankong, Tawonkong, Namhaekong, Sobaegnamulkong
	6~9	Bukwangkong, Danbaegkong, Danweonkong, Eunhakong

[†]Investigation was conducted with naked eyes in 1999
[‡]Degree of severity was numbered from 1(mild) to 9(severe).

mosaic, and necrosis (Table 2). Degrees of mosaic and necrosis were due to its severity and the number of necrotic bud, respectively. Group A of Table 1, under the natural infection, was also evaluated to be highly resistant to SMV, showing symptomless. Among the other 37 cultivars tested, twenty soybeans produced bud necrosis and seventeen had mild or severe mosaic symptoms. SMV incidence of Group C and D soybeans was relatively low, except that Muhankong and Mallikong were grouped into 31~45%. Taekwangkong, Saealkong and Baegunkong of Group I and L showed high levels of incidence with bud necrosis, 54.5%, 51.0% and 50.8%, respectively (data not shown). Variety of severity in mosaics was observed; Mosaic of Group A, F, and K soybeans was mild, and they were highly resistant to SMV in the cultivated areas. Group P, R, U, and W soybeans had very severe mosaic symptoms, and those of Group V and X, susceptible to SMV, showed moderate field resistance.

To find the most prevalent SMV strain in the field of NCES in Suwon, fifty-two leaves showing mosaic symptoms were collected during 1999 and 2000 (Table 3). Even

Table 3. Distribution of soybean mosaic virus strains in the field of Suwon.

Years	No. of SMV strains†						Total isolates tested
	G2	G3	G5	G5H	G7H	Others	
1999	7	1	-	2	22	5	37
2000	4	-	1	-	8	2	15
%	21.2	1.9	1.9	3.8	57.7	13.5	100

†SMV strains were classified using RT-PCR/RFLP during 1999 and 2000.

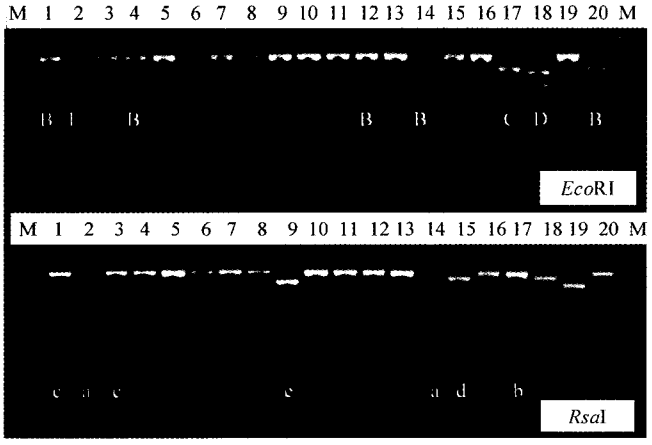

Fig. 1. DNA profiles of various SMV-infected soybeans in Suwon after digestion with restriction enzymes. M: 1kb ladder, combination (B, c) : G7H, combination (F, a) : G3, combination (C, b) : G2, the other combinations: unidentified strains.

though all SMV strains were not classified with combination of DNA profiles using RT-PCR/RFLP, we could distinguish some major strains from unidentified ones (Fig. 1, Kim *et al.*, 2000). G7H and G2 were characterized with a very wide distribution, taking up 57.7% and 21.2%, respectively.

DISCUSSION

Development of soybean varieties, resistant to SMV, has been one of the most desirable strategies for managing this disease. In this study, we evaluated the resistance of forty-four Korean soybeans to SMV, which was based on their phenotypic reactions after each inoculation of nine SMV strains and the disease incidence. The results offered a diversity of SMV-resistant cultivars for soybean breeders. Seventeen of Group A, B, C and D soybeans were resistant to the general SMV strains of Cho and Goodman (1982) and Group A, E and F soybeans were to G7H, the most prevalent Korean strain (Table 1). Thirty-nine cultivars except Group V, W and X were resistant to one or more SMV strains. We hereby presumed that various phenotypic reactions resulted from successive change of genetic backgrounds and sink of resistance genes.

Table 4 shows origins of several SMV-resistant soybean cultivars. Their resistance to SMV seemed to be affected by Jangyeobkong (Suwon86), Hwangkeumkong (Suwon97) or PI96983. Myeongjunamulkong and Ilpumgeomjeongkong reacted similarly to the nine strains but their genetic backgrounds were derived from Jangyeobkong and Hwangkeumkong of Group D, respectively. Sodamkong of Group C became more resistant to G5H than Jangyeobkong, a soybean of its genetic background; it did not cause necrosis by inoculation of G5H. It is considered that resistance of Sodamkong was conferred by other cultivars as well as Jangyeobkong. Contrarily, although Geomjeongkong2 was originated from both Jangyeobkong and Hwangkeumkong

Table 4. Origin of Korean soybean cultivars resistant to soybean mosaic virus.

Soybean cultivars	Origin
Myeongjunamulkong	Jangyeob × Baegun
Ilpumgeomjeongkong	SLSB87-3 × YS588 (Hwangkeum/SLSB-45)
Sodamkong	SNUA78010 (Jangyeob/LC103) × Dongsan127
Geomjeongkong 2	SS83021 (Jangyeob/LC7865) × SS83033 (L78-434/SS82053)
Pureunkong	Cheonsaeknamul × L78-379 (Willams /PI96983)
Sinpaldalkong 2	Deogyou (SS74185/Jangyeob) × Paldal (Elt/SS74185)

(origin of SS82053), it caused necrosis by G4. Sinpaldalkong2 was from SS74185 of having two resistance genes (Lim, 1985) and Jangyeobkong, and Pureunkong was from L78-379, susceptible to G7 but resistant to G1-G6 (Buzzell & Tu, 1984), they all had necrotic disease after each inoculation of G4 and G7. Group E, F, and H soybeans, however, showed the same reactions with Group A after inoculation of the virulent G5H and G7H strains; their resistance was donated from other origin cultivars. Their incidence in the soybean field was very low (Table 2). In conclusion, soybeans such as Group A, E, F, and H may be used as valuable sources of soybean breeding, possessing multiple resistance genes.

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