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Studies on Identification and Classification of Soybean Virus Diseases in Korea*

I. Preliminary Studies on a Soybean Virus Disease

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한국 大豆 바이러스의 分類, 同定에 關한 硏究

Ⅰ. 一種의 大豆 바이러스에 관한 硏究

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Abstract

Leading soybean cultivars such as Kwanggyo, Yugu No. 3, Dongbugtae, Gangrim, and Eundaedu were heavily diseased by a virus in Korea.

The disease was most severe in the northern provinces where soybean mosaic virus also occurrs, but the disease has also been observed in other provinces where soybean diseases are less prevalent.

The disease symptoms were similar to bud blight caused by tobacco ringspot virus; but this was not confirmed in inoculation tests on indicator plants and serological experiments.

There were some differences in varietal susceptibility to the disease, with symptom variation depending on the soybean cultivar and source of inoculum. Disease symptoms on infected soybean plants were mottling and necrosis. The present results, therefore, indicate some strains of SMV or a mixture of legume viruses may or may not be responsible for the disease.

Introduction

A severe soybean virus disease was found in Korea in both 1974 and 1975. Although the disease had previously been observed by workers of the Crop Experiment Station, O.R.D., Suweon, Korea, it did not attract any attention until 1974(K. Y. Park, personal communication).

The soybeans in Gangweon, and Gyeonggi provinces were heavily diseased, especially at the Crop Experiment Station field in Suweon. Soybeans in other provinces were not affected as severely, although the disease was observed in other provinces, including Jeju island where the cultivation of soybean has been recently initiated.

The disease was generally severe on the recommended or leading soybean cultivars such as Kwanggyo, Dongbugtae and Yugu No. 3. This disease was prevalent in fields where soybean mosaic virus(SMV) was very severe and also in fields planted to cultivars

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resistant to SMV(5).

The disease symptoms on soybean plants in the field varied considerably depending on cultivar and probably infection time. The initial shock symptoms, such as bud curving and necrosis, were similar those caused by tobacco ringspot virus (1, 6, 12).

This research was conducted to determine the causal virus of this soybean disease by using indicator plants and serological tests.

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Materials and Methods

Indicator plants for detection of tobacco ringspot virus were used in sap inoculation tests because the symptoms on diseased soybean plants were similar to those of bud blight. Indicator plants included: *Nicotiana tabacum* Samsun, White Burley, Bright Yellow; *Nicotiana glutinosa*, *Chenopodium amaranticolor*, *Phaseolus vulgaris*, *Cucumis sativus* and *Glycine max* var. Buseog, Sipseungjangyeop, and Chungbugbaig.

As a result of a field survey in July in 1975 in Suweon, however, some differences from the descrip tion of typical bud blight symptomatology were observ ed: e.g., dark streaks on stems and petioles, and l ocal or systemic lesions on soybean leaves(12). Therefore, the differential susceptibility of different soybean cultivars was investigated(Table1). Generally speaking, soybean cultivars resistant to mosaic were susceptible.

Table 1. Varietal reaction to the soybean disease in the field[1]

Cultivar	No. of plants observed	No. of plants diseased(%)	Reaction to SMV(%)[2]
Miyagysirome	25	6(24.0)	?
Eundaedu	53	8(15.1)	?
Yugu No.3	53	7(13.2)	R(15.3)
Gangrim	60	5(8.3)	R(28.2)
Dongbugtae	63	4(6.3)	R(12.1)
Kwanggyo	76	3(3.9)	R(0)
Hill	52	0	S(53.1)
Igamgwan	31	0	S(100)
Bongeui	47	0	S(53.4)
Geumgangdaerip	29	0	S(79.8)
Buseog	31	0	S(100)
Haman	57	0	S(53.4)

1 : The disease was observed on July 30.
2 : Chung et al (1972).

(5). Since the soybean cultivars Kwanggyo and Eundaedu were found to be the most susceptible to this disease, cultivars Buseog and Chungbugbaig were replaced with these cultivars for inoculation tests.

Inoculation tests:

Soybean buds and leaves showed necrotic symptom were used as inoculum sources. However, in a previous field survey, a few plants of cultivar Kwanggyo were found with mosaic symptoms on the newly dewly developed leaves and with bud necrosis. Samples of these plants were collected from the field and used as an inoculum source in screenhouse tests.

The diseased materials were macerats dusted in 0.01 MPO_4 buffer (pH 7.0) and indicator plants dusted with carborandum (600 mesh), were inoculated by rubbing leaves with the virus suspension. The inoculated plants were rinsed with tap water and kept on the greenhouse bench. The development of the sy mptoms on the plants was investigated daily for one

month.

Microprecipitin tests:

Serological investigations were not carried out intensively or extensively. The serological microprecipitin test for SMV and TRSV was carried out by first coating the bottom of a clean Petri dish with a red wax pencil. The fresh diseased samples weighed from 0.5~1.2 g. Each sample was mixed with four times its weight of buffer solution and then ground in a mortar and pestle.

The juice was centrifuged at low speed. Partiallycleaned sap was serially diluted 2^n in saline solution. The dilution of both SMV and TRSV antisera was 1/4.

A small drop of the appropriate dilution of a sample and of the antiserum were added to each grid square in the Petri dish. The Petri dishes were flooded with liquid paraffin and incubated at approximately 36°C. After 30 minutes, two hours, and 24~28 hours incubation they were observed through a dark field microscope(2).

Agar diffusion tests:

In the agar diffusion test, 0.6% Ionagar medium was autoclaved at 15 pound pressure for 10 minutes. Before the media cooled, sodium azide was added to make a 0.25% solution. Ten ml of the medium was poured into a flat-bottom Petri dish. After solidifying a well pattern was cut using a pattern tool and the agar was removed by suction.

The 1/4 dilution of each antiserum was placed in the center well, and the sample dilutions were placed in the peripheral wells. The dishes were kept at room temperature and results were recorded daily for 10 days(2). The antisera used for SMV and TR SV were purchased from the American Type Culture Collection in 1974.

Results

Soybean plants that were probably infected at an early stage were stunted, the leaves became dark green, buds curved downward and became necrotic as in bud blight, and dark streak-like lesions appeared on stems and petioles in the field (Fig. 1-A and B).

However, soybean plants infected at a late stage showed considerable variation in symptoms including systemic brown lesions which ranged from 2 mm to 4 mm on leaves and a reddish-brown necrosis of veinlets without stunting of plants in the field (Fig. 1-C).

These symptoms, especially bud necrosis on soybean, resembled those of bud blight caused by tobacco ringspot virus. Therefore, Chung et al. investigated the possiblity that the virus might have been introduced into Korea. They failed to detect tobacco ringspot virus by inoculation of indicator plants, but they did find SMV-like particles on material prepared by the dipping method in electron microscopy(4).

Attempts to isolate bacteria or fungi from lesions of soybean leaves and stems were unsuccessful. When the diseased buds and leaves of soybean were used as a source of inoculm, none of the indicator plants, including soybean cultivars such as Buseog and Chungbugbaig, reacted.

As indicated above, varietal susceptibity to the disease was investigated in the field. Results are given in Table 2. In addition, a few plants of Kwanggyo cultivar, which is highly resistant to SMV, showed mosaic on the newly developed leaves with bud and vein necrosis on the old leaves (5). This, however, was not a common symptom in the field.

Because there was no reaction in the indicator plants for TRSV(e.g., tobacco) and mosaic symptoms were observed on the Kwanggyo soybean cultivar, another strain of SMV was suggested to be involved in the soybean disease which resulted in a hypersensitive or resistant reaction on soybean cultivars resistant to ordinary strain of SMV (3, 5, 8).

In one trial, 7 different soybean cultivars including Kwanggyo, Gyeongdu, Sipseungjangyeop, Buseog, York, Hood and Harsoy were inoculated with inoculum from diseased leaves showed both mosaic and necrosis, to investigate varietal reactions. Unexpectedly, Kwanggyo was the only cultivar to show the same bud necrosis symptom of the disease, but without the mosaic symptom. At the same time two other soybean cultivars, Haman and Gyeongdu with what appeared to be a mild discoloration of the disease, were collected from the field and used as an inoculum. The inoculated same cultivars produced the same mild symptoms.

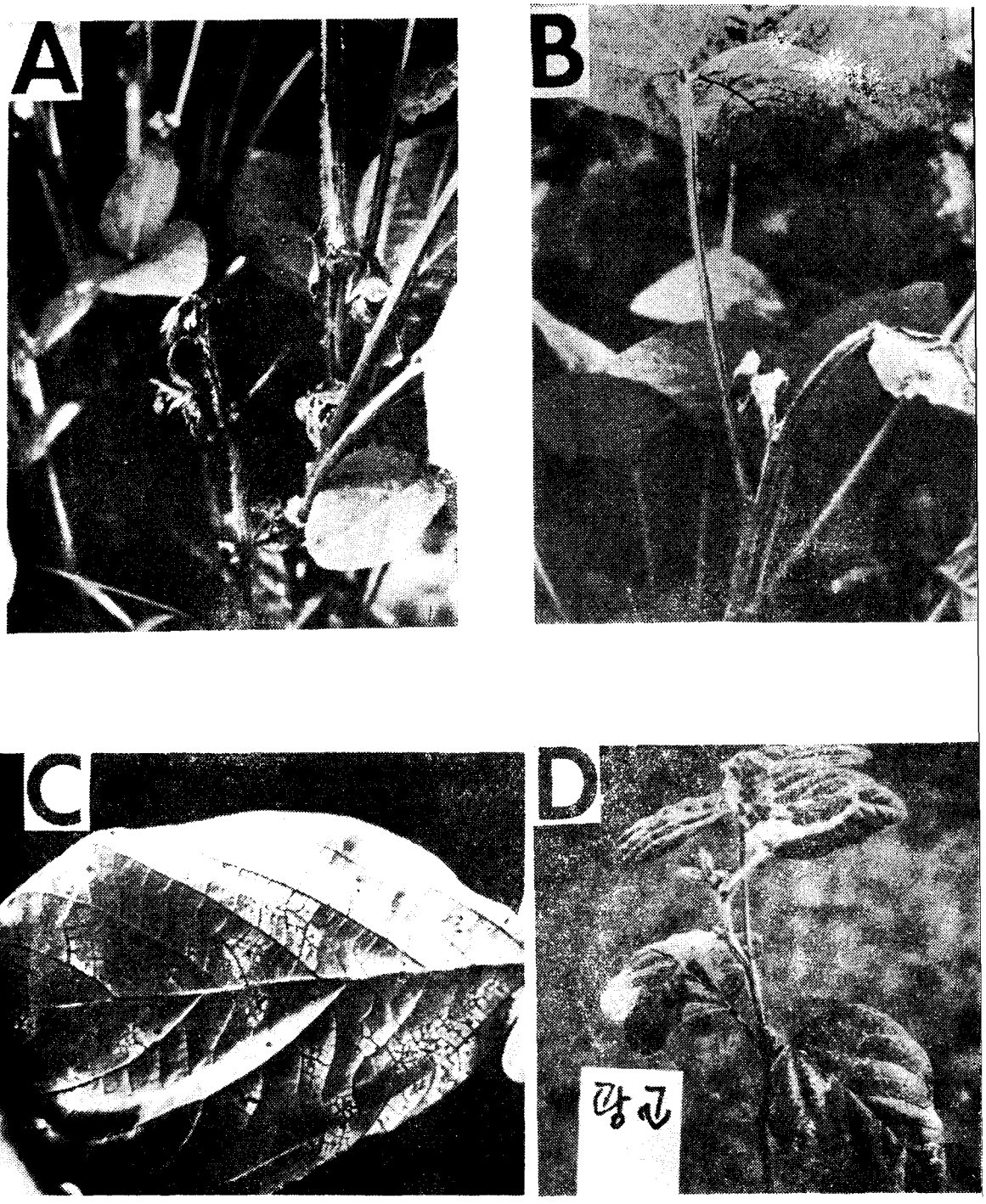

Fig. 1 The symptoms on soybean cultivar Kwanggyo diseased by an undetermined virus. (A) Bud necrosis (B) Discoloration of stems and petioles (C) Systemic lesions on leaves (D) Symptoms on soybean seedling by sap inoculation

Several plants of the Kwanggyo cultivar with the well-opened leaves were inoculated with the same source plant used above in order to multiply the inoculum source. Well-defined local lesions were produced within four days in the screenhouse in August. Later, the veinlets of leaves became progressively discolored. As the plants grew, the newly developed upper leaves produced a mottling comparable to the mosaic symptom but without bud necrosis or darkening of the stems (Fig. 1-D).

Using mottled leaves, several cultivars(Kwanggyo, Eundaedu, Ou No.3. Miyagisirome, Haman, Wellman,

Table 2. *Varietal reaction to the soybean virus disease in the field*[1]

Cultivar	No. of plants observed	No. of plants diseased[2]	Percentage diseased
Kwanggyo	15	8	61.5
Eundaedu	18	11	57.9
Gogesijiro	15	6	37.5
Suweon 66	13	4	36.4
Nemasiraz	12	4	33.3
Suhae 20	25	8	33.3
Miyakisirome	12	4	28.6
Shin 3	26	6	26.1
Nongrim 3	14	3	20.0
Iwatae 2	16	3	15.0
Suweon 27	19	3	14.3
Nongrim 2	14	3	20.0
Suweon 49	14	2	12.5
Granger	18	2	9.5
Suweon 67	14	1	8.3
Suweon 54	13	1	6.3
S.R.F.	8	1	6.3
Suweon 51	19	1	5.3
Dajissnari	24	1	4.6
Suweon 68	17	1	4.6
Eunbaek	6	1	16.7
70 other cultivars		0	

1 : Soybeans were planted on July 4 and 5
2 : The soybean virus disease was observed on Aug. 8.

Table 3. Reactions of indicator plants to the severe soybean virus disease

Indicator plants	Reaction
Glyiine max; Kwanggyo, Eundaedu	Mottling or necrosis on upper leaves, and local lesions and vein necrosis on the inoculated soybean leaves
Vigna sinensis	Occasional local lesions
Chenopodium amaranticolor	Occasional local lesions
Nicotiana tabacum Samsun	—
〃 〃 White Burley	—
〃 〃 Bright Yellow	—

Nicotiana glutinosa	—
Vicia faba	—
Cassia occidentalis	Vein-clearing, mosaic

— : no reaction

Extra, Sipseungjangyeop, Bougeui, Hong Kong, Southern prolific, Hood, Kwangdu and Wilson) were inoculated to find the variation of symptom expression. The variation observed could be grouped into

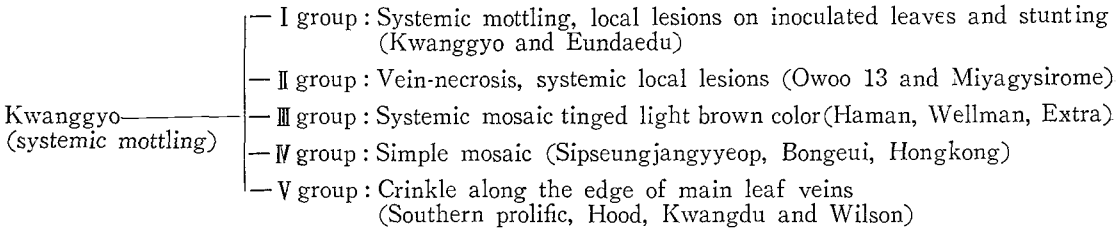

Fig. 2. Varietal variation in symptom expression of the disease using leaves showing mottling symptom on cultivar Kwanggy as inoculum.

5 distinct categories (Fig. 2).

Chlorotic local lesions were sometimes formed on *Chenopodium amarantocolor* but in other tests there was no resaction. Many reddish specks on the inoculated leaves of *Vigna sinensis* were produced, however, these results were not consistent in all inoculation tests (Table 3).

To compare the reaction of different indicator plants, soybean, cowpea and *Chenopodium amaranticolor* were inoculated separately using two different inoculum sources; leaves showed systemic mottling or typical necrosis. The symptom expression in soybeans was somewhat different, but both types of inoculum produced similar local lesions on cowpea and no local lesions on *Chenopodium amaranticolor* (Table 4). It, therefore, appears that the same virus

Table 4. Differences in symptom expression using two different types of diseased soybean leaves as an inoculum source on indicator plants

Test plant	Virus source	
	Leaves showing systemic mottling	Leaves showing typical necrosis
	Symptom expression	
Glycine max; Kwanggyo Eundaedu	Stunting, vein-necrosis on inoculated leaves, systemic mottling with lesions. Local lesions on the inoculated leaves in 10 days followed by a systemic infection.	Local and systemic lesions with darkening of the petiole and stems. Some plants showed mosaic on the upper leaves but this soon disappeared and local lesions appeared on the inoculated leaves in 14 days.
Vigna sinensis	Local lesions on inoculated leaves.	Local lesions on inoculated leaves.
Chenopodium amaranticolor	No local lesions.	No local lesions.

may be present in both types of inoculum.

Although negative reults were consistently obtained for TRSV in screenhouse and glasshouse tests, the results may have been influenced by unfavorable environmental conditions at the time of testing. These tests will be repeated next year.

Using either mottled or necrotic leaves, no positive reactions were observed in microprecipitin tests with

SMV and TRSV antisera. No precipitin band was observed in the agar diffusion test with TRSV antiserum.

Discussion

The most important finding is that the leading soybean cultivar, Kwanggyo, is susceptible to this severe soybean disease, and shows mottling symptoms when inoculated with homogenates from infected plants with necrotic symptoms or mosaic symptoms.

The variation in symptom expression depending on soybean cultivars are common in the field as demonstrated in this experiment (Fig. 2). Thus, the present data strongly suggest that the virus causing a mottling on Kwanggyo was a soybean mosaic virus, because no indicator plants except soybeans responded clearly. It is possible this virus is not the causal virus in this severe soybean disease observed in the field. More extensive serological microprecipitin tests should help to confirm the identity of the virus. However, the necrotic type symptoms in soybean may be responsible for some strains of SMV because some strains of SMV can cause top and vein necrosis(8).

If the variation in symptom expression is due to a mixture of viruses, some legume viruses might be considered as the eausal agents of the undetermined severe soybean virus disease. These include; soybean mosaic virus (SMV), alfalfa mosaic virus (AMV), tobacco ringspot virus (TRSV), bean yellow mosaic virus(BYMV), cowpea chlorotic mottle virus(CCMV) and bean pod mottle virus (BPMV) (1, 3, 6, 7, 9, 10, 11, 12) reported by Han and Murayama(8).

SMV generally produces mosaic symptoms in soybeans, but sometimes can produce local lesions on inoculated leaves in some soybean cultivars (11). Some strains of SMV were reported to produce local lesions in soybeans and produce chlorotic local lesionso n *Cheno-podium amaranticolor* (8). Our results appear to be quite similar to those caused by the T-38 starin of SMV reported by Han and Murayama(8).

AMV produces necrotic specks, rings and vein necrosis on inoculated soybean leaves with systemic symptoms developing later in the plants. AMV also causes a top necrosis and darkening of the stems in soybeans (9). The variation of symptom expression in soybean cultivars in our tests also resembles those caused by AMV, but the results of the host reaction tests did not verify this.

TRSV causes bud blight in soybeans (12). The symptomatology of this disease suggested that TRSV might be involved. However, the differences in soybean varietal susceptibility to the disease, negative results in serological microprecipitin and agar diffusion tests with TRSV antiserum, and lack of positive reactions on indicator plants such as tobacco and *Nicotiana glutinosa* indicate that is not the causal virus.

TSV also causes bud blight in soybeans(6, 7). The severe soybean virus disease is difficult to transmit mechanically, as is TSV, but the results of host range tests show that TSV is not the causal agent.

BYMV produces a yellow mottling or sometimes an indefinite yellow band along the major veins with rusty necrotic ringspots on leaves. It does not cause stunting of the infected plants, or a systemic infection with mosaic type symptoms. It has a larger host range than SMV, and can easily be differentiated from SMV (6, 9).

Mixtures of some viruses can cause top and vein necrosis (6, 10). In view of the complexity of legume viruses, additional extensive research work must be conducted to determine the causal virus of this severe soybean virus disease.

抄　錄

光敎, 東北太, 강림, 陸羽3號, 은대두등과 같은 大豆奬勵品種이 바이러스에 依하여 심하게 罹病되었다. 이 病은 主로 모자익病의 發生이 많은 江原·京畿地方에서 發病이 심하였으나 모자익病이 심하지 않은 全南 등 南部地方에서도 發病되고 있다.

病徵으로 보아 tobacco ringspot virus에 依한 大豆의 被害와 類似한 것으로 보였으나 指標植物檢定과 血淸檢定에 依하여 調査한 結果 모두 否定的이었으며 大豆品種에 따른 罹病程度의 相異, 品種과 接種源에 依한 病徵의 變異가 많았다.

罹病株에서 分離되는 病徵型은 Mottling과 necrosis 였으며 지금까지의 硏究結果 이 大豆病害는 모자익바이러스(SMV)의 계통 내지는 tobacco ringspot virus 이외의 豆類바이러스의 複各感染에 依한 것으로 생각할 수 있으나 SMV의 계통에 依한 被害일 可能性이 더욱 有力視되고 있다.

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