STUDIES ON SOYBEAN MOSAIC VIRUS

I. Separation of Virus Strains by Differential Hosts

You Hsin HAN* and Daiki MURAYAMA

(Laboratory of Plant Pathology, Faculty of Agriculture,
Hokkaido University, Sapporo, Japan)

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Introduction

Soybean mosaic is one of the most serious diseases of the soybean [*Glycine max* (L.) MERR.]. Many investigators have noted its potential danger. The disease was first reported in the United States by Clinton in 1916, who described the symptoms of the disease. Since then reports concerning mosaic disease of soybean have been published by a number of workers. GARDNER (1921) reported the nature of the virus. Later, in 1940, HEINZE and KÖHLER demonstrated the properties of the causal virus. Mechanical transmission of soybean mosaic virus (SMV) from infected plants has been successful (GARDNER and KENDRICK, 1921; HEINZE and KÖHLER, 1940; Conover, 1948). The possibility of transmission of SMV through seeds was reported by KENDRICK and GARDNER in 1924. It has been demonstrated that several species of aphids are capable of transmitting SMV, and it is probable that they are responsible for most of the secondary spread of the disease in the field (KENDRICK and GARDNER, 1921; HEINZE and KÖHLER, 1940; CONOVER, 1948; DE VASCONCELOS, 1964). SMV, a thread-like virus particle belonging to potato virus Y group (BRANDES and WETTER, 1959; BRANDES und BERCKS, 1965) is differentiated from other viruses in the same group by its narrow host range as reported by GALVEZ (1963), CONOVER (1948) and QUANTZ (1961). In an investigation, various varieties of soybean were tested for susceptibility to SMV (KOSHIMIZU and IIZUKA, 1963). TAKAHASHI and IIZUKA (1968) have reported five strains of SMV found in Japan. Differentiation among SMV strains has been recognized on the basis of symptoms induced or, the ability or inability to infect certain species or varieties of plants. ROSS (1969) also reported SMV isolates varied significantly in their pathogenicity to soybean selections. Host reactions depended on the soybean genotype and

* Present address: Taiwan Provincial Chung-Hsing University, Taichung, Taiwan, Republic of China.

SMV strains. No report on strains of SMV has been published in Taiwan. In 1968, some isolates of SMV obtained from Taiwan were tested in this laboratory. To understand the occurrence of SMV strains in this island, an attempt was made to determine whether the different strains of SMV had become established in Taiwan and to ascertain whether the strains of this virus are similar to those found in Japan, and also to attempt to distinguish this apparently new strains from those already recognized on the basis of reactions on the differential hosts. In this paper we will give data about mechanical inoculation of plants particularly the soybean varieties with the SMV. The results of the present investigation are reported here.

Materials and Methods

SMV isolates SV-10, SV-18, SV-15, SV-70 and SV-127 as designated as strian A, B, C, D, and E, respectively were kindly supplied by Mr. IIZUKA, Tohoku Agricultural Experiment Station, Japan. They were used as standard isolates in this investigation. Some of the original sources of SMV were naturally infected soybean plants growing in soybean areas of Taiwan. The virus isolated from individual infected plant collected from the field in Taiwan was maintained in plants of soybean variety, Chung-hsing No 1 by inoculating young soybean plants in the primary leaf stage by means of mechanical inoculation. Leaf samples were sent by airplane from Taiwan to this laboratory, where transmission was done immediately. All the subsequent sources of the virus used in the experimental work were maintained by the sub-inoculation from soybean plants in the greenhouse. Soybean variety, Tokachi-nagaha was used throughout the experiment both as source of the virus and as test plants. All the plants used in the experiment were grown in five-inch clay pots containing sandy loam. Leaf development of the plants was in the seedling stage where the first trifoliate leaf had unfolded during inoculation. Because of the success in obtaining consistent transmission of the virus by mechanical inoculation method, the inoculum source consisted of expressed sap from leaves infected with SMV. Crude plant juice inocula were prepared by homogenizing freshly harvested infected leaves in a mortar and pestle with 0.01 M phosphate buffer (pH 7.0) and squeezing the liquid through two layers of cheesecloth. Usually 10 ml of buffer solution was used per one gram of infected leaves from the source plant. Carborundum (400 mesh) was used as an abrasive in virus inoculation (RAWLINS and TOMPKINS, 1934). A piece of absorbed cotton ball held in a sterilized forceps was dipped in the virus inoculum that had just been extracted from plants showing typical soybean mosaic symptoms three weeks after inoculation. The virus-soaked

cotton ball was then rubbed slightly over the upper surface of the test plants leaves. Five plants of soybean rubbed with distilled water served as control. Several varieties of soybean were inoculated in the greenhouse with the virus isolates both transported from Taiwan and those isolates obtained in Japan to attempt to distinguish the new strain from those already recognized in Japan (TAKAHASHI et al., 1963; TSUDA and TAKAHASHI, 1968). Therefore typical strains of SMV found in Japan were used as a check to characterize the virulence of the virus to the test plants. To avoid the contamination and mistakes in records, careful examinations of the test seedlings that were free from transmitted virus were made. The greenhouse was fumigated and spread periodically with insecticide in order to kill any insect that existed in the greenhouse. So the greenhouse was free from the insect during the experiment. Other plants in the greenhouse, not intentionally inoculated to the test virus, were symptom- and virus-free throughout the experiment. Precautions were also taken to prevent accidental spread of the virus inoculum onto uninoculated plants. The test plants before and after inoculation were placed on wooden benches and were kept separately.

Experimental Results

In this investigation, ten varieties of soybean and two speceis of *Chenopodium* (*C. album* L. and *C. amaranticolor* COSTE et REYN.) were used for their reactions to each strains of SMV. Soybean varieties were selected on the basis of known susceptibility to the type isolates found in Japan as mentioned above. The reactions on each test plants were compared. Each variety was recorded as infected or not infected. Plant that initially failed to infect or develop symptoms were retested twice. The inoculated plants were indexed on soybean variety, Tokachinagaha in order to establish the presence or absence of the virus. This recovery tests were made in any case where there is any doubt.

Symptoms were first evident 6–7 days after inoculation. The young expanding leaf at the top of the plant showed vein-clearing. In general, all the strains of the virus produced typical mosaic symptoms in soybeans and the virus was readily recovered from infected plants. Results of inoculation showed that the plant species and their varieties tested were resistance, susceptible or highly susceptible based on different virulence of the strains. The strains induced similar reactions on some varieties. Some varieties of soybean tested, Norin No. 2, Peking, Ōu No. 3, and Enshiken which gave differential reactions to each strains of SMV and four varieties of soybean such as Kariha-takidani No. 28, Norin No. 4, Chung-hsing No. 1 and Chung-hsing

TABLE 1. Reactions of differential hosts to soybean mosaic virus

Virus isolates	T-109		T-121		T-1		T-41	
Test plants	L	S	L	S	L	S	L	S
Soybean								
Peking	—	M	—	SL	—	M	—	SL
Harosoy	VN	M	VN	M	VN	M	VN	M
Ōu No. 3	—	—	NS	SL	NS	M	NS	ST
Enshiken	—	—	NS	—	NS	—	NS	M
Norin No. 2	NS	—	NS	—	NS	SL	NS	SL
Tokachinagaha	VN	M	VN	M	VN	M(ST)	VN	M(ST)
Howkeye	NS	M	NS	M	NS	M	NS	M
Chung-hsing No. 2	NS	M	NS	M	NS	M	NS	M
Kariha-takidani No. 28	VN	M	VN	M	VN	M(ST)	VN	M(ST)
Norin No. 4	VN	M	VN	M	VN	M(ST)	VN	M(ST)
Bean								
Kurodanekinugasa	NS	SL	NS	SL	NS	SL	NS	SL
Chenopodium album	CL	—	CL	—	CL	—	CL	—
Chenopodium amaranticolor	—	—	—	—	—	—	—	—

T=SMV isolates obtained from Taiwan, SV=SMV isolates obtained in Japan. L=tomless, ST=Stunting, NS=Necrotic spot, TN=Top necrosis, CL=Chlorotic local variety Tokachinagaha.

No. 2 did not. They were susceptible to all strains of the virus. Although Tokachinagaha failed to differentiate the mild strains, however, it served to differentiate severe strains from mild strains. The plants readily infected with the Japanese isolates also were susceptible to Taiwanese isolates of the virus. However, somewhat differences in host reactions by some of Taiwanese isolates were noted. The strains induced similar reactions on a number of soybean varieties, however, there were distinct differences in the effects of these strains on other plants and soybean varieties in regard to susceptibility; local lesion, mosaic, stunting and top necrosis. The results indicate that six strains of SMV found in Taiwan may be identified or isolated. The host range of this six strains are quite similar to Japanese strains as reported by TSUDA and TAKAHASHI (1968), but not identical. In consideration of the grade of virulence two groups of the isolates were divided. SMV isolates, T-109 and T-121 are mild strains, whereas isolates, T-38 and T-9 are the severe ones. Foliage symptoms induced by mild strains include vein-clearing

from mechanical inoculation in the greenhouse conditions

T-9		T-38		SV-10		SV-18		SV-15		SV-70		SV-127	
L	S	L	S	L	S	L	S	L	S	L	S	L	S
—	SL	—	M	—	SL	—	M	—	(M)	—	(M)	—	M
VN	M	VN	M	VN	M	VN	M	VN	M	VN	M	VN	M
NS	ST	NS	ST-TN	—	M	NS	SL	NS	M	NS	ST	NS	ST-TN
NS	TN	NS	ST-TN	NS	M	NS	SL	NS	M	NS	ST	NS	ST-TN
NS	M-TN	NS	ST-TN	NS	SL(M)	NS	SL	NS	M	NS	ST	NS	ST-TN
VN	M	VN	M(ST)	VN	M	VN	M	VN	M	VN	(ST)	VN	(ST)
NS	M	NS	M	NS	—	NS	M	NS	—	NS	—	NS	(ST)
NS	M	NS	M	NS	M	NS	M	NS	M	NS	M	NS	M
VN	M(ST)	VN	M(ST)	VN	M	VN	M	VN	M	VN	M(ST)	VN	M(ST)
VN	M(ST)	VN	M(ST)	VN	M	VN	M	VN	M	VN	M(ST)	VN	M(ST)
NS	TN	NS	(TN)	NS	SL	NS	SL	NS	SL	NS	SL	NS	(TN)
CL	—	CL	—	CL	—	CL	—	CL	—	CL	—	CL	—
—	—	—	CL	—	—	—	—	—	—	—	—	—	—

Local infection, S=Systemic infection. VN=Vein necrosis, M=Mosaic, SL=Symplesion, ( )=Mild symptom,—=infection as judged by the back inoculation to soybean

and mosaic. However, the severe strains reduce leaf size and produce stunting and necrosis in upper parts of infected plants. Among these symptoms particularly the stunting is more obvious in soybean varieties, Ōu No. 3, Enshiken and Norin No. 2. Isolate T-38 induces a symptom in soybean varieties that very similar, if not identical, to type strain E. It forms chlorotic local lesions on the inoculated leaves of *Chenopodium amaranticolor*, but type strain E and other strains do not. On the other hand, soybeans infected with severe strains show faint mottling, and both growth and fruiting of infected plants are eventially greatly reduced than those infected with mild strains. A distinctive difference is observed in soybean variety, Norin No. 2, where the local lesions induced by T-9 and T-38 are definitely larger than those resulting from infection with other isolates inoculated at the same time. In comparison with the symptoms produced by severe strains, it indicates that T-9 and strain D, and also T-38 and strain E are most closely related SMV strains, respectively. Some isolates failed to induce symptoms in soybean

variety, Norin No. 2. However, the virus was recovered from the infected plants 14 days after inoculation. On bean variety, Tsurunashikintoki, the symptoms induced by isolate T-9 consisted of chlorotic local lesions and vein necrosis on the inoculated leaves, and the systemically infected leaflets with vein-banding. Serious vein-necrotic lesions were not usually found on the infected leaves inoculated by other isolates. Attempts to perpetuate the virus responsible for these symptoms by further transfers to soybean were successful.

Discussion and Conclusion

Data obtained from this investigation which may be concluded that six strains of SMV may be separated by their differences of host reactions. TAKAHASHI et al. (1963), TSUDA and TAKAHASHI (1968), and ROSS (1969) found that different isolates of SMV showed a considerable variability in host range. This finding was confirmed in the present investigation. Visual comparison of the symptoms of the Japanese type strains and the strains collected in Taiwan among inoculated plants revealed that symptoms induced by all strains on any given susceptible plants were so similar that they could not accurately distinguished by using one variety of soybean. The reactions of inoculated plants produced by some Taiwanese strains were not sufficiently different from those incited by the Japanese strains to know the distinction by observation. Some of the isolates failed to produce any symptoms on test plants, they do not imply immunity of these plants or soybean varieties to the strain in question, but it seems probable that if not immune they are highly resistant to infection. In comparison of the varieties used, Ōu No. 3, Enshiken, Norin No. 2, and others are apparently, more suitable for separation of the virus strains than some of the others. The reactions of *Chenopodium album* to the infection indicated that this host might not be suitable for differentiating these isolates that were apparently strains of the SMV. However, *C. amaranticolor* could be used as the indicator of severe strains. This investigation suggests that the reactions of the test plants to each isolates of SMV may be due to the differences of SMV strains. The variations of the virulence may be due to subtle differences in the virus itself which limits or accelerates the ability to multiply within given hosts. The severe strains obviously have a genetic constitution which overcomes the resistance of soybean variety to systemic infection. On the other hand, the genetic aspects have been considered by COUCH (1955) who found that the genotype of the host is the major factor in determining the virus and host relationship.

The resistance of soybean to SMV is controlled by a single dominant

gene (KOSHIMIZU and IIZUKA, 1963). Soybean mosaic resistance in soybean is adequate for the breeding of resistant varieties. The breeding programs designed to develop SMV-resistant soybean varieties should evaluate hybrid progeny against a wide range of SMV isolates. The parent variety used for hybridization should have a wide range in the resistance of SMV isolates.

Information obtained from this study was important to breeders in breeding disease resistance in commercial varieties. Additional studies, such as serological tests, would be necessary to establish a more precise relationship among SMV strains.

Summary

Sevearl isolates of SMV collected from Taiwan were tested and compared with the typical isolates obtained in Japan. Six strains of SMV were found from soybeans in Taiwan and designated as SMV-T1, SMV-T2, SMV-T3, SMV-T4, SMV-T5, and SMV-T6. The host range of these six strains are quite similar, but not identical, to Japanese strains. Transmission studies with SMV isolates indicated considerable variability in symptom expression in soybean and certain specfic differences in host range. The isolates can be divided into two groups of the isolates, differing in virulence. Foliage symptoms induced by mild strains including vein-clearing and mosaic. Severe strains reduce leaf size and produce stunting and necrosis in upper parts of infected plants.

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Explanation of plates

PLATE I

A. Primary leaf of soybean variety, Tokachinagaha inoculated with SMV (T-109), showing vein necrosis.
B. Primary leaf of soybean variety, Norin No. 2 inoculated with SMV (T-9), showing necrotic local lesions and vein necrosis.
C. Primary leaf of bean variety, Tsurunashi-kintoki inoculated SMV (T-9), showing necrotic lesions and vein necrosis.

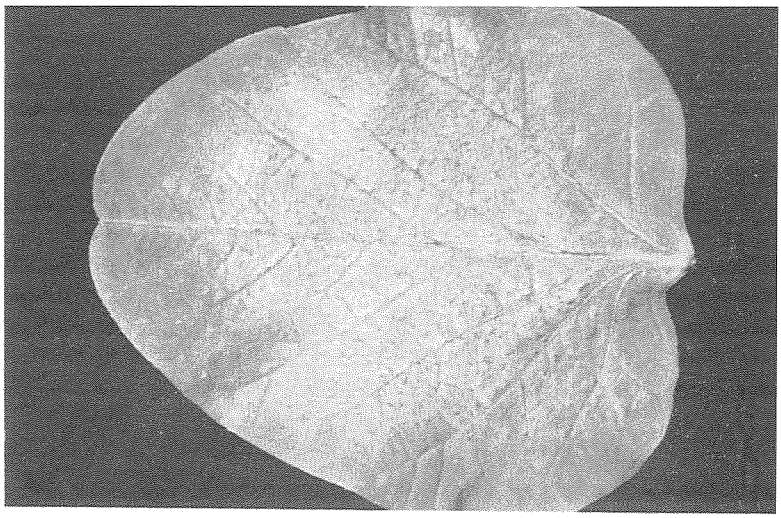
PLATE II

A. Soybean variety, Norin No. 2 infected with SMV (SV-15), showing a mild mottling.
B. Soybean variety, Ōu No. 3 infected with SMV (T-9), showing stunting.
C. Soybean variety, Norin No. 2 infected with SMV (SV-127), showing stunting and death of the plant.

PLATE III

A. Soybean variety, Norin No. 2 infected with SMV (T-38), showing local lesions and stunting.
B. Soybean variety, Norin No. 2 infected with SMV (T-38), showing necrotic lesions and vein necrosis on systemic ally infected leaf.
C. Bean variety, Kurodane-kinugasa infected with SMV (T-9), showing top necrosis and death of the infected plants.

A



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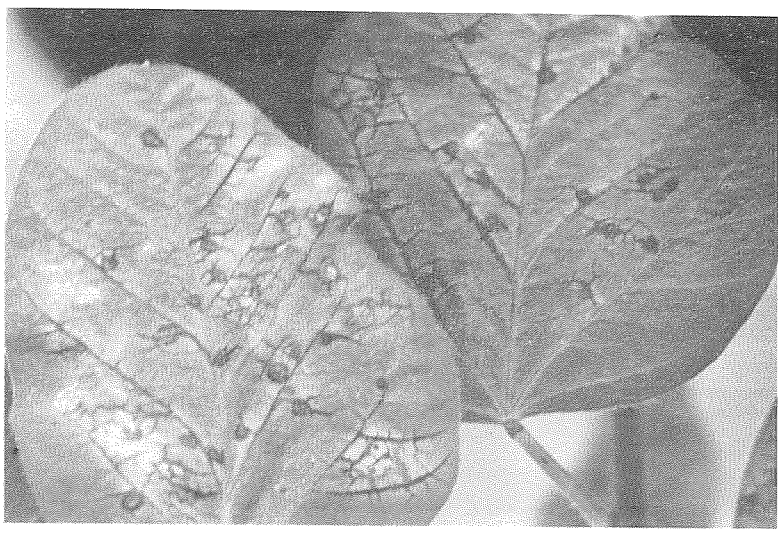

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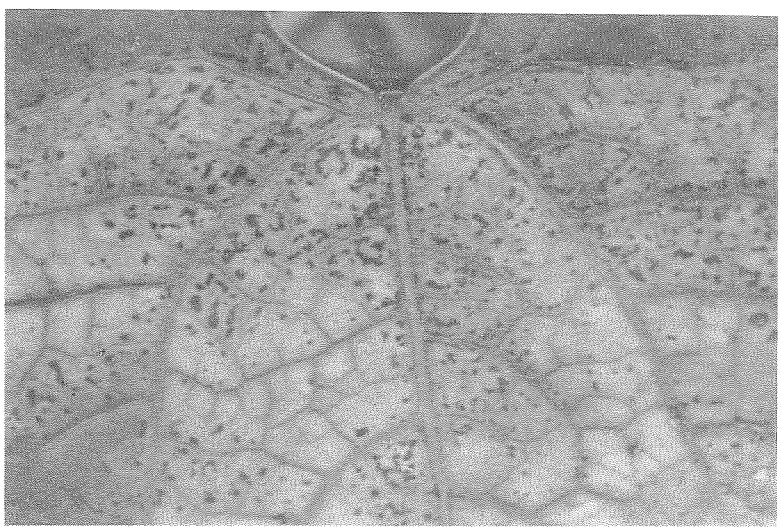

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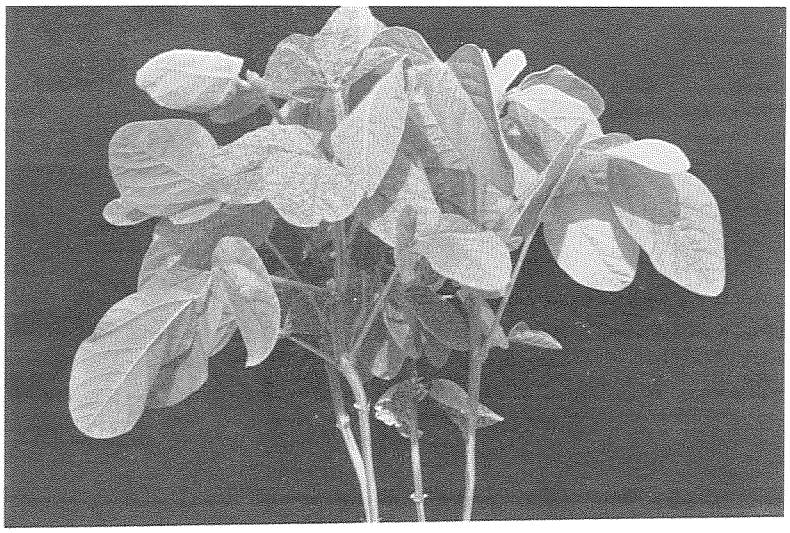

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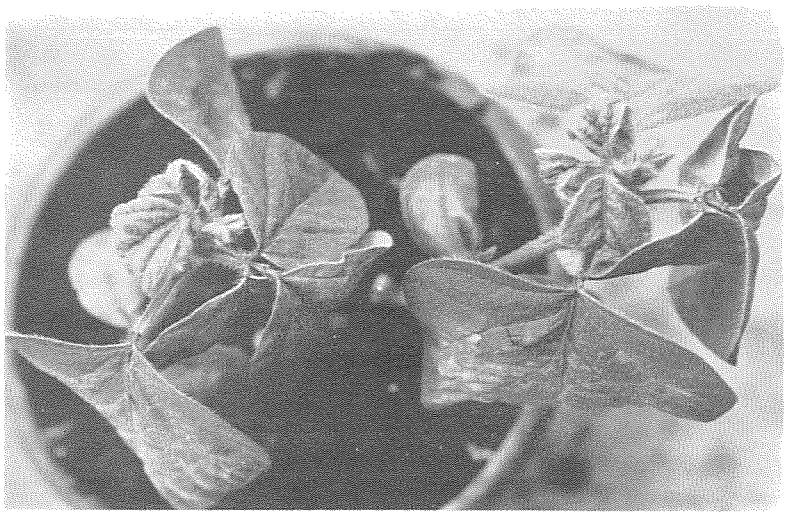

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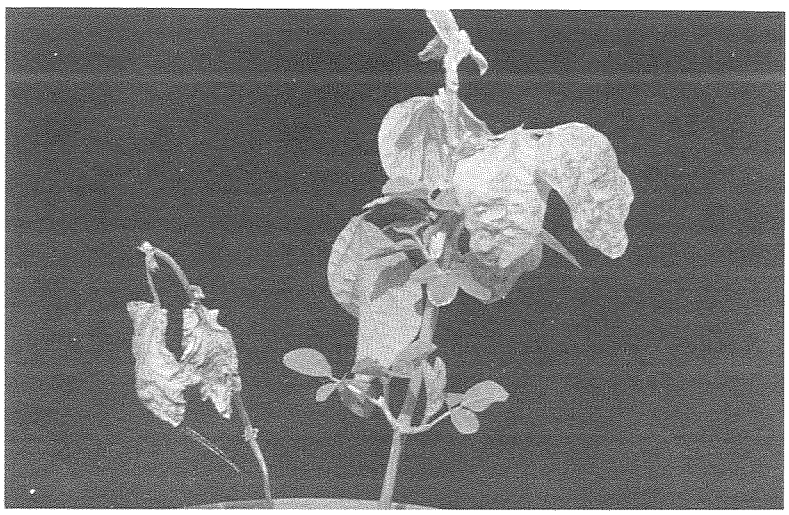

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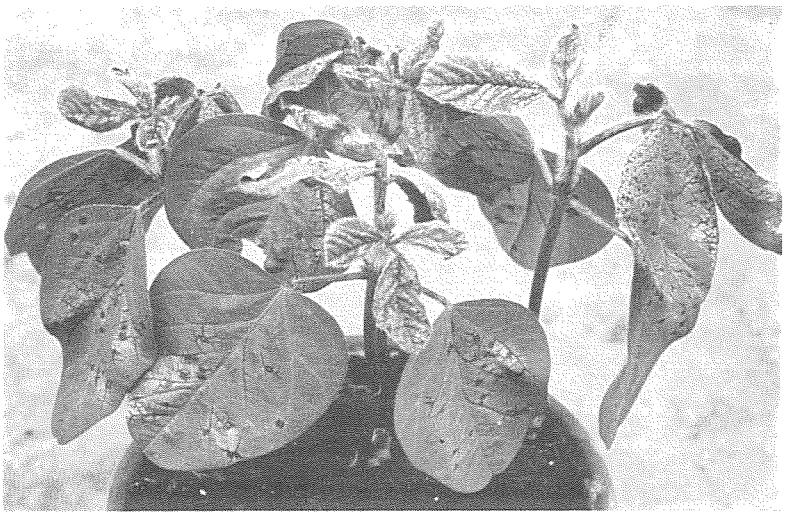

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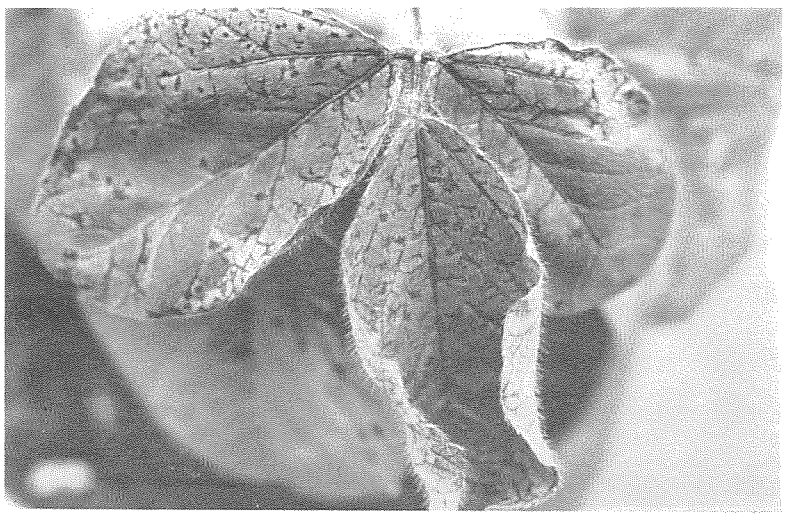

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