

CLASSIFICATION OF STRAINS OF SOYBEAN MOSAIC VIRUS AND SEED TRANSMISSIBILITY

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SUMMARY - Five isolates of soybean mosaic virus (SMV) were collected from soybean fields in the Cerrados, and five variants were derived from these isolates by passage through some resistant varieties. They were divided into 5 strain groups (Groups I-V) based on the pathogenicity to six soybean varieties. Also, the 130 soybean varieties and/or lines tested were divided into 6 varietal groups on the basis of the responses to the 5 virus strain groups. All the isolates obtained from soybean fields belonged to Group I, and showed higher rates of seed transmission compared with the other strain groups. All the SMV strains were transmitted through soybean seeds, and the rates varied with the virus strains, soybean varieties and infection period.

Key words: soybean mosaic virus, virus strain, seed transmission.

Introduction

The disease caused by soybean mosaic virus (SMV) is one of the most important viral diseases of soybeans, *Glycine max* (L) Merr., worldwide. Yield reductions of up to 35% have been recorded in SMV-susceptible varieties (Ross, 1977). The disease is transmitted by many species of aphids and through seeds from infected soybean plants (Bos, 1972). In Brazil also, the disease is one of the most important viral diseases of soybeans, and the identification of the pathogen, distribution of the disease, strains of the virus, etc., have been reported (Almeida, 1978; Almeida, 1981; Anjos, 1985).

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The worldwide distribution of SMV is primarily a consequence of its seed-borne nature. Seed transmission properties of SMV have been reported. It is generally recognized that the virus is transmitted in variable proportion to the seeds produced by virus-infected plants, depending on the soybean varieties and growth stage at which the plants become infected. Minimal or no transmission occurs if a plant is infected after the flowering stage (Iizuka, 1973; Bowers, 1979).

Pathogenic variation among SMV isolated and various types of soybean reactions have been reported. SMV isolates which were collected from infected soybean seeds, were classified into 7 strain Groups(G1-G7) in USA (Cho and Goodman, 1979). Many SMV isolates obtained from infected soybean plants were classified into 5 strains(A-E) in Japan (Takahashi et al., 1980), and ten isolates were classified into 3 strains (Ms1-Ms3) in Brazil (Almeida, 1981). All of the strains were classified based on the reactions of several soybean differentials. Since the strains of SMV may differ in their effects on soybeans, breeding programs for mosaic-resistant varieties should include studies on pathogenic variation among virus isolates, and determine whether SMV resistance in soybean is adequate for the breeding of resistant soybean varieties.

Our studies were undertaken to classify the isolates of SMV occurring in the Cerrados, and to analyse the virulence of these isolates to soybean varieties and/or lines. Also, the present studies aimed at determining whether soybean varieties, SMV isolates and/or selected conditions are the factors responsible for the low level or absence of seed transmission of SMV in soybeans.

Materials and Methods

Source of SMV isolates and differentiation of SMV strains: two isolates (Cp1 and Cp2) were obtained from soybean plants affected by the disease in a field of CPAC(DF) on January 1988, two isolates(Mt1 and Mt2) were collected from field-grown soybean in Lucas do Rio Verde(MT) on February 1988, and one isolate was collected from a field in Barreiras(BA) in March 1989. Seeds of 99 soybean varieties and/or lines were provided from CNPSoja and CPAC, 31 soybean varieties and/or lines (19 originating from Japan, seven from USA, four from Korea and one from Indonesia) were

introduced from Japan (Importação No. 167/89, CENARGEN). The reactions of the soybean differentials used for differentiating the SMV strains were reported previously, and six varieties; "Doko", "York", "FT-4", "Kwanggyo", "Campos Gerais" and "Buffalo", were also used. Plants for use as differentials were obtained by sowing eight-ten seeds in plastic pots. Tested varieties and/or lines are presented in the results.

Inoculation procedures: Mechanical inoculations were performed by rubbing carborundum-dusted leaves with a cotton pad dipped in sap prepared in 0.1M phosphate buffer, pH 7.2, the plants were observed for at least three-four weeks for symptom development, then they were back-inoculated to susceptible soybean varieties to determine the presence of the virus. All the tested plants were maintained in a greenhouse at CPAC.

Seed-transmission tests: Fifteen soybean varieties were planted in an isolated field on 17 November 1988, at CPAC, and two isolates were inoculated on primary leaves of soybean plants on 2 December, 1988. Two varieties, "Doko" and "FT-Estrela", were inoculated with 7 SMV isolates and/or variants on 2 December, 1988, respectively. Moreover, two varieties were inoculated with a virus isolate at 5 growth stages; 2 Dec., 22 Dec., 1988, 11 Jan., 31 Jan. and 20 Feb., 1989. Inoculated plants were inspected several times for the development of symptoms during the growing period. When the plants matured, ten plants from each entry were harvested and dried. Thereafter, the SMV-infected seeds were planted in a greenhouse for inspection of virus transmission through seed. The number of seedlings which emerged and the number of SMV-infected seedlings based on the symptoms were counted. When there was no obvious infection of the seedlings, they were inoculated onto a susceptible soybean variety.

Results

1. Classification of SMV strains and screening of resistant varieties

Classification of SMV strains: Five isolates collected from soybean plants in the fields were classified by the methods reported previously. All the isolates belonged to Group 1 based on the method of Cho and Goodman (1879). Also, all the isolate belonged to strain B according to the method of Takahashi et al. (1980). No isolates belonged to any of the strains reported by Almeida (1981).

Isolation of SMV variants and classification of strains: A few plants among some resistant varieties were occasionally infected systemically with these isolates, when several susceptible and resistant varieties were inoculated with 5 SMV isolates, respectively. Mosaic or necrotic symptoms were observed on the resistant varieties. Viruses were isolated from infected resistant varieties, and the varieties were reinoculated with the viruses. Thereafter, ten SMV variants which had infected some resistant varieties, were obtained. Five SMV variants out of the ten variants were selected based on the reactions to some resistant varieties. These virus variants were classified again by the methods reported previously. As a result, one variant was assigned to Group 4, while four other variants did not belong to any of the 7 Groups reported by Cho and Goodman (1979). One variant was assigned to Strain B, and another variant to Strain E, but three other variants did not belong to any of the 5 strains reported by Takahashi et al. (1980). Two variants belonged to Ms-1, but three other variants did not belong to any of the 3 strains reported by Almeida (1983).

Screening of resistant varieties and/or lines, and classification of SMV isolates and variants based on virulence: One hundred and thirty soybean varieties and/or lines were inoculated with 10 SMV isolates or variants, and the reactions of these varieties and/or lines to the SMV isolates or variants were observed. Only three varieties were found to be resistant to the 10 SMV isolates or variants. Seventy eight out of 130 varieties and/or lines were susceptible to all the SMV isolates or variants. Other varieties and/or lines were resistant or susceptible to some SMV isolates or variants. Based on these results, the 10 SMV isolates or variants used were classified into 5 strain Groups according to the differences in the virulence among 6 soybean varietal Groups. The results are shown in Table 1.

TABLE 1 - Reaction of soybean varieties and/or lines to SMV strains isolated from the Cerrados.

Varietal group	Typical variety	SMV strain				
		Group I	Group II	Group III	Group IV	Group V
A	Doko	M	M	M	M	M
B	York	0	0	M	N	M _s st
C	FT-4	0	0	0	N	M _s st
D	Kwanggyo	0	0	0	0	M _s st
E	Campos Gerais	0	M _s st	0	0	0
F	Buffalo	0	0	0	0	0

Remarks: M; mosaic, N; necrosis, st; stunting, O; no infection.

SMV strain Group I consisted of five isolates (Cp1, Cp2, Mt1, Mt2 and Ba1) collected from fields, Group II consisted of one variant(Mt2- 11) derived from isolate Mt2, Group III consisted of two variants(Cp2G and Mg1G), Group IV consisted of one variant(Cp2D) and Group V consisted of one variant(Mg2-12).

Reactions of the soybean differentials to the 5 strain Groups of SMV were as follows: Varietal Group A included susceptible varieties and/or lines to the 5 SMV strain Groups(10 isolates and variants), and consisted of 78 varieties and/or lines as follows; Arisoy, Bossier, BR 1, BR 3, BR 5, BR 6, BR 10, BR 13, BR 80-6989, BR 80-6123, BR 81-1072, BR 81-186, CEP 10, CEP 71-16, Cheio Kee, Coker 136, Coker 156, Cristalina, Delman, Doko, D69-442, FT-1, FT-2, FT-6, FT-7, FT-9, FT- 11, FT-12, FT-13, FT-17, FT 79-4401, FT 80-1992, FT 80-2161, FT 80- 2341, FT-Estrela, Hardee, IAC-2, IAC-6, IAC-12, IND 2006, IND 80-1007, OC 79-136, OCEPAR 2-Iap, OCEPAR 3-Primavera, OCEPAR 4-Iguaçu, OCEPAR 5-Piquiri, Paraná, PEL 8201, Perry, Santa'ana, Santa Rosa, Sertaneja, Soc 81-183, Tropical, UFV-1, UFV-4, UFV-5, UFV-6, UFV-7, UFV-9, UFV- 10, Vicoja, EMGOPA 301, EMGOPA 302, EMGOPA 303, Hakuto, Harosoy, Iwate-Wasekurome, Karibatakiya 28, Kitamishiro, Kurokawaseiou, Shiro-Tsurunoko, Tanyou, Nyonya, Rampage and Jangbeagkong.

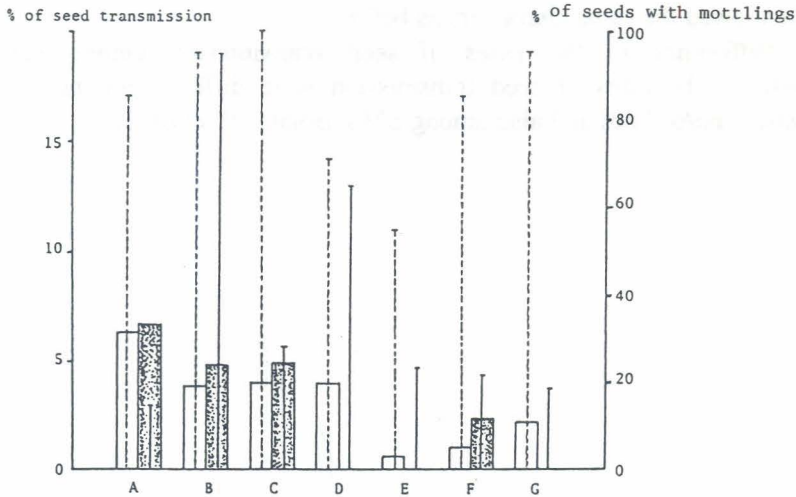
Varietal Group B which was resistant to SMV strain Groups I and II, consisted of 14 varieties and/or lines as follows: BR 81-8407, D71- 9951, FT-14, FT-15, Soc 81-210, Torsoy, Paranaiba, York, Akisen-nari, Dewamusume, Kyushyu 99, Ou 3, Tozankei E648 and Hwanggeumkong.

Varietal Group C which was resistant to SMV Groups I, II and III, consisted of only FT-4 and Akiyoshi. Varietal Group D was resistant to SMV Groups I, II, III and IV, and consisted of Ou 13, Toshiken and Kwanggyo. Varietal Group E was resistant to SMV Groups I, II, IV and V, and consisted of 21 varieties and or lines, as follows: Bienville, BR-4, BR-12, BR 80-826, BR 81-2291, Campos Gerais, CEP 12, CEP 82-52, CO 60-239, Dortchsoy, FT-16, FT 79-1901, Hampton, IVAI, Lancer, Tracy, Uniao, Marshall, Ogden, Hakubo 1 and Shiroshima 10.

Varietal Group F was resistant to all the 5 virus Groups, and consisted of Buffalo, Peking and L78-434.

2. Seed Transmission Tests and Occurrence of Seed Mottlings

Seed transmission of SMV strains and occurrence of seed mottlings:
The rates of seed transmission differed among the SMV isolates and/or variants (Picture 1).



Remarks: SMV isolates; A. Cp1(I), B. Cp2(I), C. Cp2D(IV), D. Mt1(I), E. Mt1G(III), F. Mt2-11(II), G. Mt2-12(V).



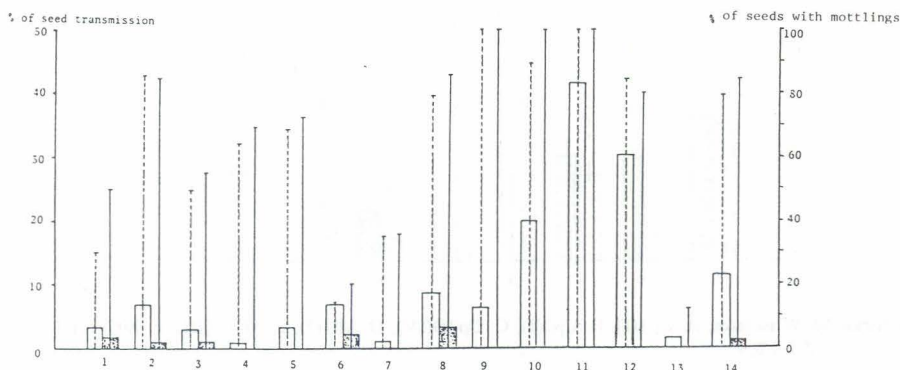
-  Seed transmission in variety "Doko", • • Seed mottlings caused by Cp1.
 Seed transmission in variety "FT-Estrela", • • Seed mottlings caused by Mt2-11.

FIG. 1 - Differences in seed transmission among SMV isolates and/or variants.

Three isolates belonging to the SMV Group I were transmitted through seeds at a rate of about 4-6%, while other isolates or variants were transmitted at a rate of less than 2.1% in the variety "Doko". Infected variety "FT-Estrela" with 2 SMV isolates belonging to Group I and a variant of Group IV, showed a seed transmission rate of about 5-7%. Three isolates or variants belonging to SMV Group I, III and V were not transmitted through seeds from infected "FT- Estrela".

SMV-infected plants exhibited black or brown mottlings on the surface of the seeds, and the rates of mottlings on the seeds varied with the SMV isolates and variants. In general, the rate of mottlings on the seeds produced by infected "FT-Estrela" in two isolates belonging to Group I, was higher than that of the plants infected with the five other isolates or variants. It was confirmed that the SMV isolates and variants transmitted through soybean seeds showed the same characters as before.

Difference in the rates of seed transmission among soybean varieties: The rates of seed transmission were different among soybean varieties and/or lines and also among SMV isolates (Picture 2).



Remarks: Varieties and/or lines; 1. Cristalina, 2. Doko, 3. EMGOPA 301, 4. EMGOPA 302, 5. FT-11, 6. FT-Estrela, 7. Parana, 8. Santa Rosa, 9. Iwate-Wasekurome, 10. Karibatakiya 28, 11. Kitamishiro, 12. Shiro-Tsurunobo, 13. Tanyou, 14. Nyonya.

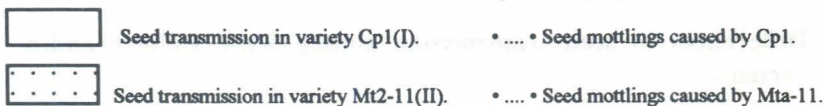


FIG. 2 - Differences in seed transmission among soybean varieties.

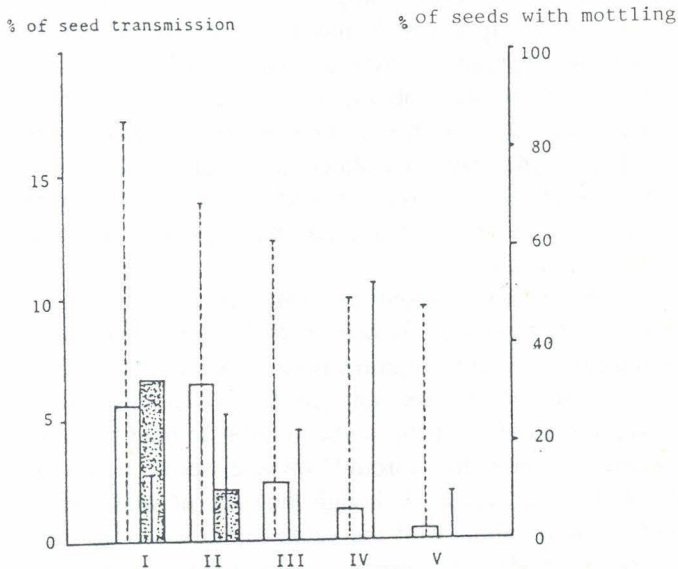
The rates of seed transmission in three varieties originating from Japan, "Karibatakiya 28", "Kitamishiro" and "Shiro-Tsurunoko", were 20-41.7% when they were infected with isolates Cp1(Group I), and 0.6- 8.8% in the varieties and/or lines from Brazil infected with isolate Cp1. The rate of seed

transmission was less than 3.1% after infection with a variant Mt2-11, and eight varieties, including the Japanese ones, did not show any seed transmission. In general, the varieties "Cristalina", "Doko", "EMGOPA 301", "FT-Estrela" and "Santa Rosa" showed higher rates of seed transmission, than the varieties "EMGOPA 302", "FT-11" and "Paraná".

Rates of mottlings on seeds were higher in the Japanese varieties than in the Brazilian varieties.

Relationship between seed transmission and period of inoculation:

The rates of seed transmission were correlated with the stage of plant growth when the infection occurred, and decreased as the time of infection approached the flowering period (Picture 3).



Remarks: Date of inoculation.

I. 02/12/88, II. 22/12/88, II. 11/01/89, IV. 31/01/89, V. 20/02/89.

- Seed transmission in variety "Doko"
- Seed transmission in variety "FT-Estrela"
- Seed mottlings caused by Cp1.
- Seed mottlings caused by Mt2-11.

FIG. 3 - Relationship between seed transmission and period of inoculation.

The rates of seed transmission decreased gradually with late inoculation in the variety "Doko". The seeds, from the variety "FT- Estrela" inoculated 55 days after planting, showed mottlings, but no seed transmission was observed.

Discussion and Comments

SMV isolates and/or variants obtained from the Cerrados were divided into 5 virus Groups, and all the five isolates collected from soybean fields directly belonged to Group I. All the isolates belonging to Group I showed higher rates of seed transmission compared with the SMV variants derived from the isolates of Group I, which may be the reason why SMV belonging to Group I was widespread in soybean fields. SMV variants belonging to Groups II, III, IV and V, were obtained from isolates belonging to Group I. This phenomenon may account for the fact that the virulence of SMV readily differed from that of the original isolates, and that in the areas where resistant varieties to SMV were cultivated next to infected susceptible varieties with SMV, the virulence of the strain may have changed and resistant varieties could become infected.

Presently the disease caused by SMV is not a serious problem in soybean fields in the Cerrados. However, the disease is occurring in many fields of agricultural research organizations, and also the vector (aphid) of the disease was observed in some soybean fields. Therefore, it is likely that the disease may spread all over the soybean fields in the Cerrados. Moreover, since the disease is transmitted through soybean seeds, it can be distributed wherever soybean is cultivated. Although the rates of seed transmission were not very high, if the population of the vector(aphid) were to increase in susceptible varieties to SMV, the disease could spread rapidly in the soybean fields.

Methods of control of the disease are as follows: (1) never use soybean seeds with mottlings, and (2) cultivate resistant varieties to the disease. It may be difficult to control the disease by the use of resistant varieties. However when resistant varieties are infected with some SMV strains, the strains show very low rates of seed transmission or even seed transmission is not observed. Moreover, breeding for superior resistant varieties to the disease is a future objective.

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References

- ALMEIDA, A.M.R.; MIRANDA, L.C. Ocorrência do vírus do mosaico comum da soja no estado do Paraná e sua transmissibilidade pelas sementes. *Fitopatologia Bras.* 4:293-297, 1979.
- ALMEIDA, A.M.R. Levantamento do vírus do mosaico comum e queima do broto em soja, nas diversas regiões do estado do Paraná. *Fitopatologia Bras.* 5:125-128, 1980.
- ALMEIDA, A.M.R. Identificação de estirpes do vírus mosaico comum da soja no estado do Paraná. *Fitopatologia Bras.* 6:131-136, 1981.
- ANJOS, J.R.N.; LIN, M.T.; KITAJIMA, E.W. Caracterização de um isolado do vírus do mosaico da soja. *Fitopatologia Bras.* 10:137-151, 1985.

- BOS, L. **Soybean mosaic virus**. C.M.I./A.A.B. Descriptions of Plant Viruses. N° 93, 1972.
- BOWERS, JR.; GOODMAN, R.M. Soybean mosaic virus: Infection of soybean seed parts and seed transmission. **Phytopathology** 69:569-572, 1979.
- CHO, E.K.; GOODMAN, R.M. Strains of soybean mosaic virus: Classification based on virulence in resistant soybean cultivars. **Phytopathology** 69:467-470, 1979.
- IIZUKA, N. Seed transmission of viruses in soybeans. **Tohoku Nat. Agric.Expt.Stn. Bull.** 46:131-141, 1973.
- ROSS, J.P. Effect of aphid-transmitted soybean mosaic virus on yields of closely related resistant and susceptible soybean lines. **Crop Sci.** 17:869-872, 1977.
- TAKAHASHI, K.; TANAKA, T.; IIDA, W.; TSUDA, Y. Studies on virus diseases and causal viruses of soybean in Japan. **Tohoku Nat.Agric. Expt. Stn. Bull.** 62:1-130, 1980.