Application of Cross-Protection to the Control of Black Soybean Mosaic Disease

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ABSTRACT

Kosaka, Y., and Fukunishi, T. 1994. Application of cross-protection to the control of black soybean mosaic disease. Plant Dis. 78:339-341.

In 1990 and 1991, approximately 20,000 and 78,000 seedlings, respectively, of black soybean (*Glycine max* (L.) Merr. cv. Shin Tambaguro) were transplanted 1–3 days after protective inoculation with an attenuated isolate of soybean mosaic virus (SMV), Aa15-M2, in seed farms in Kyoto Prefecture, Japan. In the plants that received the protective inoculation, virulent strains of SMV were effectively suppressed. Seeds virtually free of SMV were produced in 1990 (971 kg/ha), and free of SMV in 1991 (4,740 kg/3.9 ha). Black soybean seedlings grown from these seeds were widely transplanted in growers' fields in 1991 (45 ha) and 1992 (50 ha) in Wachi, Kyoto. The incidence of virulent strains of SMV in late July (preflowering stage, about 40 days after transplanting) was found to be 1.3% in 1991 and 0.8% in 1992, in contrast to the rates of over 60% in 1989 and 1990. The use of seeds from Aa15-M2 inoculated plants was also found to be effective for the control of SMV in other areas during 1992.

Mosaic disease caused by soybean mosaic virus (SMV) is commonly found in soybean (*Glycine max* (L.) Merr.) in Japan (10). The first step in the control of this seedborne and aphid-borne potyvirus (1) is the widespread use of SMV-free seeds or seedlings to minimize the primary inoculum source that is spread by aphids (5–7). However, it is difficult to obtain SMV-free seeds from field-grown soybean plants under high disease pressure, to implement this initial control measure. Previously, we reported that attenuated isolates of SMV obtained by cold treatment may be able to control mosaic disease on black soybean cv. Shin Tambaguro with a black seed coat (4). To produce SMV-free seeds, we determined the effectiveness of the use of an attenuated isolate of SMV to protect black soybean plants from the seed-transmitted mosaic disease caused by SMV.

MATERIALS AND METHODS

Cross-protection strategy. To evaluate the effectiveness of the cross-protection strategy, black soybean seedlings inoculated with the attenuated SMV isolate Aa15-M2 were grown on the seed farms in Wachi, the main black soybean-producing area in Kyoto Prefecture, in 1990 and 1991. The efficiently aphid-transmissible and poorly seed-transmissible isolate Aa15-M2 has been described (4). Aa15-M2 was propagated in soybean cv. Shin Tambaguro in a temperature-controlled greenhouse (23–30 C) at the Kyoto Agricultural Research Institute in Kameoka, Kyoto, during the spring months of 1990 and 1991. The first to fourth trifoliolate leaves of infected plants were harvested 17–20 days after inoculation and were stored at −40 C for 1–2 wk or 4 C for 2–3 days prior to the protective inoculation. All of the seedlings for protective inoculation originated from seeds produced by plants that had been maintained in a screenhouse to avoid viral infection by aphids. These seedlings were grown in 105-cell paper pots in a mixture of sand and vermiculite (8 L), placed in the plastic houses of the Wachi Farmers Cooperative (JA-Wachicho). Inoculum was prepared by homogenizing Aa15-M2 infected soybean leaves in 10 volumes (milliliters per gram) of 0.1 M sodium phosphate buffer (pH 7.2) in a blender, then filtering the mixture through cheesecloth. At the fully expanded primary leaf stage (11–15 days old), the primary leaves were rubbed with cotton puffs dipped in inoculum containing 3% (w/v) Carborundum. One puff inoculated 10–15 seedlings. The seedlings were transplanted in early June, 1–3 days after the protective inoculation. In 1990, approximately 20,000 seedlings were planted in 10 fields that were massed in one block (1.0 ha). In 1991, approximately 78,000 seedlings were planted in 35 fields in two blocks (3.9 ha). The fields ranged in size from 0.03 to 0.2 ha. Because the seedlings inoculated with Aa15-M2 were not fully protected from subsequent infection for a period of at least 6 days after inoculation (4), transplanting was carried out about 1 wk earlier than the local standard transplanting date to avoid infection with virulent SMV from aphids in the commercial fields. Protective inoculations were performed through cooperation with local growers, except for the preparation of leaf extracts. Plants in each field were maintained according to local standard practices for black soybean production. Insecticides (organophosphates) were applied four to five times from transplanting to harvest to control aphids, soybean pod worm and borer, and bugs.

Disease survey. The progression of black soybean mosaic disease was surveyed in the seed farms or growers' fields that were 0.1–0.2 ha during 1989–1992 (Fig. 1). Plants (100–311) from a randomly selected three to five rows in the centers of the fields were observed from July to August for symptoms of SMV (mosaic, leaf roll, and rugose) and for symptoms associated with infection by other viruses. Leaf samples collected from plants with distinct symptoms were tested to identify viruses by the double-sandwich method (2) or the nonprecoated indirect method (9) of the enzyme-linked immunosorbent assay (ELISA) with antiserum against SMV-C (12), peanut stunt virus (PSV) (11), soybean dwarf virus (SDV) (3), or cucumber mosaic virus (Institute of Japan Plant Protection Association). To determine the transmission rates of SMV through seeds produced from unprotected (1988 and 1989) or cross-protected (1990 and 1991) fields, the seeds obtained in 1988, 1989, 1990, and 1991 were divided into

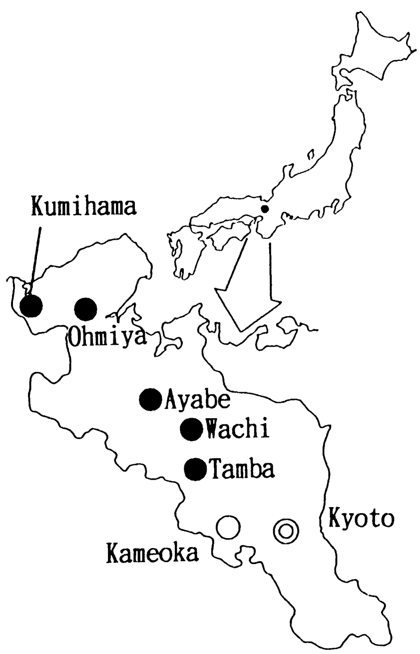

Fig. 1. Map of black soybean-producing areas (●) surveyed in Kyoto Prefecture.

Accepted for publication 9 December 1993.

13, 14, 7, and 7 lots, respectively. Each seed lot consisted of seeds produced in 1–5 fields. The seed samples (200–450 seeds per lot) were sown in flats containing sand and vermiculite and maintained in a greenhouse. The plants were grown to the two trifoliolate leaves stage and observed for symptoms. All of the abnormal plants and a few randomly selected plants without symptoms were assayed by ELISA for the presence of SMV. The seed-transmission rates were arcsine-transformed prior to analysis of variance, and means were tested according to the least significant difference (LSD) test.

RESULTS

Effectiveness of cross-protection strategy. On the seed farm during 1989 (survey of 430 plants in two fields), the average incidence of virulent SMV was 58.4% on 21 July and 78.9% 10 days later (preflowering stage). However, the mean percentage of diseased plants in fields planted with Aa15-M2 infected plants in 1990 (450 plants in three of 10 fields) was 0.3% on 24 July and remained low when surveyed on 4 and 17 August. In 1991 (4,200 plants in 35 fields), only 0.5% of the plants in fields planted with Aa15-M2 infected plants had severe symptoms characteristic of virulent SMV on 29 July (Fig. 2). The diseased plants were rogued 1 wk after the survey. Seed yields on the seed farm in 1989, 1990, and 1991 were 952, 971, and 1,215 kg/ha, respectively. The mean rates of seed transmission of SMV in seed samples from unprotected fields in 1988 and 1989 were 7.12 and 3.63%, respectively. On the other hand, SMV seed transmissions from cross-protected fields in 1990 and 1991 were only 0.07 and 0%, respectively (LSD 0.05 = 0.2%) (Table 1). No transmission of the attenuated isolate of SMV was observed in these seeds. In Wachi during 1990, black soybean plants were grown under high disease pressure (Fig. 3). To determine the transmission rate of SMV through seeds produced from unprotected plants in this year, 600 seeds were sampled at random from the commercial field (surveyed field shown in Fig. 3). The seed-transmission rate was 2.69% (significantly different [$P < 0.001$] from 0.07% of the cross-protected fields in 1990 and not significantly different from 3.63% of the unprotected fields in 1989, based on the chi-square test of independence).

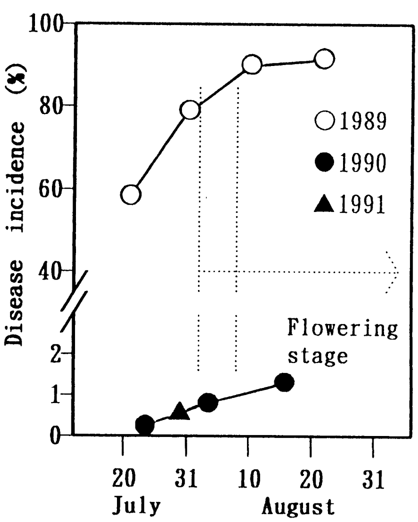

Fig. 2. Incidence of the mosaic disease caused by soybean mosaic virus in unprotected (1989) and cross-protected (1990 and 1991) fields of black soybean. Seedlings were transplanted in early June.

Effect of use of SMV-free seeds in growers' fields. The incidence of mosaic disease due to SMV-infection was surveyed in a field located in the center of the black soybean–growing area (about 50 ha) of Wachi during the period 1989–1992. Seeds produced on the seed farm (see Table 1) were widely supplied to this area (21–25 kg/ha) in the next year. Seedlings (10–15 days old) were grown from the seeds supplied and were transplanted in the fields by local growers in early to mid-June. In 1989, the mosaic disease rapidly progressed after transplanting, and almost all the plants showed severe symptoms on 10 August (early flowering stage) (Fig. 3). In 1990, eight seed lots with lower transmission rates of SMV (mean rate 2.6%) were selected out of 14 seed lots that had been obtained the previous year. The percentage of plants with severe symptoms exceeded 60% in late July (preflowering stage) and reached a level of over 80% in mid-August (Fig. 3). For control of this disease, seedlings grown from seeds produced in cross-protected fields were transplanted over about 45 ha in 1991 and almost every field in 1992. Consequently, the disease incidence in late July was 1.3% in 1991 and 0.8% in 1992. In both years, however, the rate of diseased plants increased rapidly later in the growing season (Fig. 3). The average seed yields in 1989, 1990, 1991, and 1992 in Wachi were 714, 561, 724, and 794 kg/ha, respectively (data from Wachi Agricultural Statistics).

To evaluate the usefulness of SMV-free seeds, seeds produced from Aa15-M2 inoculated plants in 1991 were supplied to other areas in 1992. Seedlings grown from these seeds were transplanted by local growers in mid-June. Disease incidence was surveyed in randomly selected fields during the preflowering to early flowering stages (late July to early August). Each field was located within a black soybean–growing area geographically separated from the other fields by mountains. The incidence of SMV was significantly lower ($P < 0.001$, by chi-square test of independence) in the fields where seedlings from SMV-free seeds were grown (0–14.8%), compared to commercial seed areas (35.9–59.7%). Although PSV and SDV were found in most of the fields, the incidence was very low except for one field (Table 2).

Table 1. Transmission rates of soybean mosaic virus (SMV) in seed samples from cross-protected and unprotected fields[a]

Year	SMV transmission rates (%)
1988	7.12[b]
1989	3.63
1990	0.07
1991	0
LSD ($P = 0.05$)	0.2

[a]The fields were located in Wachi, Kyoto. Cross-protected plants were planted in 1990 and 1991.

[b]Actual values are shown; statistical analyses were done with arcsine-transformed data.

DISCUSSION

This appears to be the first use of cross-protection with a mild isolate of SMV, Aa15-M2, to control the mosaic disease caused by SMV on soybean. This isolate provided effective protection against field spread and seed infection by virulent SMV. In the fields where seedlings from seeds of Aa15-M2 inoculated plants were grown, the occurrence of SMV was low before the flowering stage; however, a rapid increase in the number of diseased plants was observed later in the growing season. SMV plant infection during the late growth stages (flowering to post-flowering) was shown to reduce soybean yield by very little (5,8). In addition, early natural infection by SMV was found to reduce seed production by 10–25% in black soybean (5) and by 20–40% in other

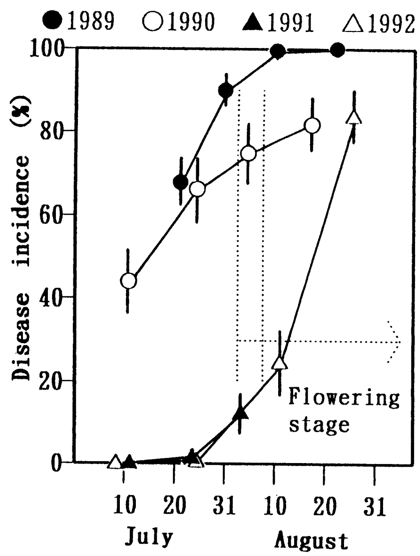

Fig. 3. Progression of black soybean mosaic disease caused by soybean mosaic virus (SMV) in a grower's field during 1989–1992. The surveys of SMV incidence were conducted in a field situated in the center of the black soybean–producing area of Wachi, Kyoto. The transplanting dates were early to mid-June. Seedlings grown from seeds produced in cross-protected fields were widely transplanted in this area in 1991 and 1992. Vertical lines represent the 95% confidence levels.

Table 2. Incidence of viral disease in black soybean-producing areas of Kyoto Prefecture in 1992[a]

Seed source	Location	Date	Plants surveyed (no.)	Incidence of viruses (%)[b]		
				SMV	PSV or PSV + SMV	SDV or unknown
Aa15-M2 inoculated plants	Sakahara, Wachi	28 Jul	300	0	0.7	0
	Nakayama, Wachi	28 Jul.	300	2.3	0.3	1.3
	Obata, Wachi	28 Jul.	200	0	2.5	1.0
	Niwa, Ayabe	4 Aug.	199	3.5	1.5	0.5
	Boguchi, Ayabe	4 Aug.	204	0.5	2.5	0.5
	Shinada, Kumihama	6 Aug.	311	14.8	5.8*	0
Commercial seeds	Tomita, Tamba	30 Jul.	210	49.0	4.3*	1.9
	Kamiyata, Ayabe	4 Aug.	221	42.5	20.8*	4.1
	Sano, Kumihama	6 Aug.	270	35.9	3.7*	0.4
	Kawabe, Ohmiya	6 Aug.	211	59.7	4.7*	2.4

[a] Field surveys were conducted from 28 July to 6 August in 1992. The surveyed fields were located within black soybean-growing areas geographically separated from each other by mountains.
[b] SMV, soybean mosaic virus; PSV, peanut stunt virus; SDV, soybean dwarf virus; PSV + SMV, a mixed infection with PSV and SMV; unknown, severe stunting by unknown virus or causal agent. Values with an asterisk include those with mixed infections.

soybean cultivar (8). Therefore, a control strategy that delays SMV plant infection would be helpful in soybean production. After the application of our strategy in growers' fields, the annual seed yield tended to increase. This may be a result of reduced early SMV infection. More research is needed to determine the impact of our strategy on seed yield, because yield response is strongly dependent on the weather and field conditions.

It is important to determine why the mosaic disease appeared despite the use of SMV-free seeds. This could be due to the presence of virulent SMV at an extremely low percentage in numerous seeds obtained from the Aa15-M2 inoculated plants; infection of SMV-free plants by the spread of SMV from the only weed host, tsurumame (*Glycine soja* Siebold & Zucc.) (5); spread from diseased homegrown soybean plants within an area and/or from diseased plants in other areas where SMV-free seeds were not supplied; or intensification of aphid flights (flights peak in mid to late August) from SMV source plants. These factors should be substantiated by further experiments.

The control strategy of using SMV-free seed from plants inoculated with the Aa15-M2 isolate was found to be effective against chronic disease caused by seedborne and aphid-borne SMV. If an attenuated isolate, Aa15-M2, with aphid transmissibility could be improved into an efficient seed-transmissible isolate, the control of black soybean mosaic disease may be more effective due to the natural spread of the attenuated isolate.

ACKNOWLEDGMENTS

We thank T. Tsuchizaki for supplying antisera of SMV and PSV, and Y. Mikoshiba for helping us in ELISA for SDV. We also thank the Wachi Farmers' Cooperative for field work.

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