Control of Russet Crack Disease in Sweetpotato Plants Using a Protective Mild Strain of *Sweet potato feathery mottle virus*

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ABSTRACT

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A mild isolate (designated as 10-O) of *Sweet potato feathery mottle virus* (SPFMV) was obtained from *Ipomoea batatas*. This isolate rarely caused skin discoloration and did not cause russet crack disease on the storage roots of *I. batatas* that are typical of infection with the severe strain of SPFMV (SPFMV-S). The yield of the 10-O-infected *I. batatas* ranged from 92 to 105% of the yield of healthy *I. batatas*. The coat protein gene of 10-O encodes 315 amino acids and has sequence identities of 91.1% at the nucleotide level and 95.6% at the amino acid level with the corresponding region of SPFMV-S. When *I. batatas* cuttings were inoculated with 10-O and later challenged with SPFMV-S, russet crack symptoms were much reduced and SPFMV-S was not detected in the challenged plants. These results indicate that 10-O can be an effective biological control agent against russet crack disease.

Additional keywords: cross-protection

Sweetpotato (*Ipomoea batatas* L.) is a major field crop in Japan. In 2005, approximately 1 million tons of sweetpotato were harvested, ranking Japan sixth in the world in sweetpotato production (Food and Agriculture Organization corporate statistical database [FAOSTAT] Classic). In the southern part of Japan, some varieties of sweetpotato are commonly cultivated for use in the manufacture of distilled spirits.

Some strains of *Sweet potato feathery mottle virus* (SPFMV), a member of the genus *Potyvirus* (25), cause russet crack and skin discoloration of the storage roots of *I. batatas*. Several strains of SPFMV have been distinguished on the basis of symptom differences, amino acid sequence identities, and serological relationships (1,2). In Japan, three strains have been described, SPFMV-O (26), SPFMV-T (24), and SPFMV-S (25). SPFMV-S causes russet crack and severe skin discoloration on the storage roots. Although SPFMV-O is serologically related to SPFMV-S, it induces only mild skin discoloration (26).

SPFMV-T is asymptomatic on the roots and is serologically related to SPFMV-S and SPFMV-O by complement fixation test, but is not detected by SSEM using protein A gold and protein A sandwich enzyme-linked immunosorbent assay (ELISA) using antisera made against SPFMV-S or SPFMV-O (24). SPFMV-S and SPFMV-O are transmitted by the green peach aphid (*Myzus persicae*) (25,26), although the primary vector of SPFMV-T in the field is unknown.

In Oita Prefecture, Japan, SPFMV is estimated to infect 10 to 40% of the sweetpotato crop in any given year, resulting in a 20 to 50% reduction in yield and quality (13). To maintain the best quality of skin color, sweetpotato is often planted using virus-free cuttings, and then cuttings from those plants are used to plant the subsequent years' crops. Usually the incidence and disease severity of SPFMV-S is low in the first crop due to limited transmission of the virus by aphids; however, cuttings from these plants often produce roots with typical symptoms of russet crack and severe skin discoloration. Not all farmers purchase virus-free cuttings each year due to the expense, so virus incidence continues to increase in subsequent years' crops. Other strategies for virus disease control are lacking for sweetpotato.

Cross-protection is a phenomenon in which systemic infection of a plant with one strain of a virus, generally an attenuated or less virulent strain, prohibits or

restricts secondary infection by a related, more virulent viral strain (6,12,17). Cross-protection has been used as a management strategy for several viruses, including *Papaya ringspot virus* (7), *Citrus tristeza virus* (4), *Cucumber mosaic virus* (22), *Zucchini yellow mosaic virus* (9,10), *Japanese yam mosaic virus* (5), and *Bean yellow mosaic virus* (23) in many different crops, including those propagated by vegetative reproduction (7). The idea that culling out roots with russet crack symptoms has resulted in selection of strains of SPFMV that confer cross-protection was suggested by Campbell et al. (3); however, mild isolates of SPFMV that can protect *I. batatas* from severe disease have not been characterized.

In this study, an isolate of SPFMV from *I. batatas* roots that induced mild or undetectable disease was identified and characterized, and shown to be effective at protecting plants from subsequent infection with a virulent isolate.

MATERIALS AND METHODS

Growing conditions for plants. SPFMV is very difficult to transmit mechanically to *I. batatas*, and graft-inoculation is the most effective means of transmission. On the other hand, *I. nil* at the cotyledonary stage can be readily infected with SPFMV by mechanical transmission, and these *I. nil* seedlings were then used to graft-inoculate large numbers of *I. batatas* plants that could be planted in the field. To challenge the *I. batatas* plants infected with mild isolate(s), SPFMV-S-infected *I. nil* seedlings were grafted onto the sweetpotato plants.

To mechanically inoculate *I. nil* seedlings, sap from symptomatic leaves of *I. nil* plants previously infected with SPFMV was triturated in 100 volumes of 0.1 M potassium phosphate buffer (pH 7.0) rubbed onto the cotyledons of healthy *I. nil* seedlings. These seedlings were grown for 2 weeks, by which time they developed typical symptoms on upper leaves, and then grafted onto *I. batatas* ('Tosabeni') cuttings at the three-open-leaf stage. These were grown in a growth chamber at 25°C in 16 h of daylight for 2 weeks, the SPFMV-infected *I. nil* scion was removed from the *I. batatas* cuttings, and these cuttings were planted in the field. Daugh-

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*The e-Xtra logo stands for "electronic extra" and indicates that Figure 1 appears in color online.

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ter storage roots produced on these plants were used for further experiments.

Screening for mild isolates. Approximately 50,000 storage roots of *I. batatas* were harvested over 5 years from four fields in Oita Prefecture, Japan. A total of 249 storage roots showing mild skin discoloration were selected to search for mild isolates of SPFMV. SPFMV infection of the roots was first confirmed by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using Isogen (Nippon Gene, Japan) according to the manufacture's protocol. RT-PCR was performed using a GeneAmp RNA PCR kit (Applied Biosystems, Foster city, CA) under the following conditions: (i) RT, 20 min at 42°C and 6 min at 99°C; (ii) PCR, 40 cycles of 95°C for 40 s, 53°C for 1.5 min, and extension at 72°C for 2 min, final extension at 72°C for 10 min. Nucleotide primers 5'-CACTTCAGTGACGTTGCTGA-3' and 5'-GGCTCGATCACGAACAAAA-3' were designed from the coat protein (CP) gene and the 3' untranslated region, respectively, of SPFMV-S and -O, and amplify a 545-bp DNA fragment (14–16). RT-PCR products were fractionated on a 2% agarose gel and visualized under UV light after staining with ethidium bromide (50 µg/ml).

Storage roots with mild symptoms and confirmed to be infected with SPFMV were planted in a greenhouse, and cuttings were taken from the shoots when they reached the three-open-leaf stage. These cuttings were subsequently challenged with SPFMV-S and grown in the field. These experiments were conducted each year from 1995 to 1999, and daughter storage roots produced on these challenged plants that continued to show mild symptoms were used for further experiments.

Separation and selection of mild isolates. To obtain pure isolates of mild SPFMV, the *I. batatas* daughter storage roots were planted in a greenhouse, and infected cuttings at the three-fully-open-leaf stage were transplanted and grown in the greenhouse. Healthy *I. nil* seedlings at the cotyledonary stage were grafted onto the infected plants, and upper leaves from these scions were selected when symptoms developed and used to mechanically inoculate *Chenopodium amaranticolor* and *C. quinoa* plants in an attempt to obtain local lesion isolates.

RT-PCR and restriction fragment length polymorphism (RFLP) analyses. Two assays were used to discriminate between SPFMV isolates. The nucleotide primers 5'-GACAACACACTTATGGTTGT-3' and 5'-CGCGCAAGACTCATATCA GT-3' were designed from the N1b and the CP cistrons, respectively, of SPFMV-S and SPFMV-O (8,14,15). They amplify a 1,343-bp DNA fragment. RT was carried out by incubation at 42°C for 20 min, followed by incubation at 99°C for 6 min. PCR of the cDNA was carried out by incu-

bation of 30 cycles of 40 s at 95°C, 1.5 min at 53°C, and 2 min at 72°C, with a final extension for 7 min at 72°C. The amplified products of each isolate were digested with restriction enzymes *Eco*RI and *Bam*HI following the manufactures protocol and fractionated on 2% agarose gel.

A second assay used three oligonucleotide primers: 490-RT (5'-GATTTAGGTGACACTTAGTTTTTTTTTTTTTTTGTG-3'), 490-5' (5'-ATGAGTCTTGCGCGATATGC-3'), and 490-3' (5'-GATTTAGGTGACACTATAG-3') designed from the CP gene and the 3' untranslated region (14,18,19). RT was carried out with primer 490-RT by incubation at 42°C for 20 min, followed by incubation at 99°C for 6 min. PCR of the cDNA was carried out with the two primers, 490-5' and 490-3', by incubation at 95°C for 1 min, followed by 35 cycles of 15 s at 94°C, 30 s at 45°C, and 1 min at 72°C, with a final extension for 7 min at 72°C. The amplified products from each isolate were digested with restriction enzyme *Alu*I and fractionated on 2% agarose gel.

Partial nucleotide sequence of a mild isolate selected. The 3'-terminal 1781 nt encoding bp fragment containing the N1b gene, the CP gene, and the 3' untranslated region of a mild isolate referred to as 10-O (selected because this isolate produced milder symptoms on upper leaves of *I. nil* than the other isolates and showed good protection on sweetpotatoes against SPFMV-S; see results for detail) was amplified by RT-PCR as described by Mori et al. (15). The amplified fragment was cloned into the plasmid vector pBluescript SKII+ (Stratagene, Torrey Pines Road, La Jolla, CA) and sequenced by the dideoxynucleotide chain termination method by using ABI373A (Applied Biosystems, Foster City, CA).

Host range and pathogenicity of the 10-O SPFMV isolate. The mild isolate 10-O was mechanically inoculated to *I. batatas* (cv. Tosabeni, Koukei-14, Tamaotome, and Beniazuma), *I. nil*, *I. setosa*, *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana benthamiana*, *N. glutinosa*, *N. tabacum* 'Xanthi-nc', *Vigna sesquipedalis*, *Solanum lycopersicum*, *S. melongena*, *Capsicum annuum*, *Petunia hybrida*, *Cucumis sativus*, *C. melo*, *Raphanus sativus*, and *Brassica oleracea* var. *capitata*. *I. batatas* was also graft-inoculated by 10-O-infected *I. nil* seedlings. Three separate plants were inoculated for each species. Inoculated leaves and upper leaves from the inoculated plants were sampled when they showed apparent symptoms, and the virus infection was examined by RT-PCR. Those that did not show any symptoms were similarly examined 21 days after inoculation.

Cross-protection tests of 10-O against SPFMV-S. *I. batatas* plants were infected with SPFMV 10-O using graft-inoculation as described above. Infection with 10-O

was confirmed by RT-PCR followed by *Alu*I digestion, and the cuttings were multiplied in a greenhouse equipped with 1-mm mesh netting in order to protect them from subsequent aphid-borne virus infection. The 10-O-infected *I. batatas* ('Tosabeni') were challenge-inoculated with SPFMV-S using graft-inoculation as described above and grown each year from 2000 to 2003 in the field. 10-O-infected and virus-free *I. batatas* cuttings were used as controls. After harvest, storage roots were evaluated for symptoms, number, and fresh weight. Symptom severity for each storage root was scored by the formula described in Table 1. Every year, leaves were sampled from 10 SPFMV-S-inoculated cuttings and 10-O-infected cuttings at approximately 30 days after transplanting, and infection by SPFMV-S was determined by RT-PCR followed by *Alu*I digestion.

Transmission by aphids. *Myzus persicae* reared on *I. batatas* ('Tosabeni') in a growth chamber were starved for 2 h and placed on 10-O-infected *I. nil* for 10 min. Ten aphids were transferred to each of nine healthy *I. batatas*. The aphids were then killed by insecticide after 24 h, and the plants were grown in the growth chamber for 2 months. 10-O infection was examined by RT-PCR-RFLP (see above). As a control, SPFMV-S was compared by the same method.

RESULTS

Isolation of a mild isolate of SPFMV.

Four of the 249 SPFMV-infected *I. batatas* plants produced storage roots with mild symptoms. Plant 95-10 was chosen as the source of virus for further experiments because its daughter storage roots did not show any russet crack symptoms and showed only slight skin discoloration. Amplification using the N1b and CP primers described above yielded 1,463- and 1,337-bp fragments in addition to the expected 1,343-bp fragment. Digestion of these three amplicons together by *Eco*RI yielded two fragments of 715 and 628 bp in addition to the undigested 1,463, 1,343, and 1,337 amplicons. Digestion by *Bam*HI yielded four fragments of 1,044, 717, 620, and 419 bp in addition to the 1,343-bp undigested amplicon. These results suggested that the plant was infected by multiple genetically distinct isolates of SPFMV and that further selection for a mild SPFMV isolate was required.

Although mechanical inoculation of the two *Chenopodium* spp. did result in local lesion formation, mechanical inoculation from *Chenopodium* spp. back to healthy *I. nil* for increase and maintenance of the isolates was unsuccessful. As an alternative, mild SPFMV isolates were separated using a dilution based method. Sap from symptomatic leaves of *I. nil* scions were triturated in 1,000 volumes of 0.1 M potassium phosphate buffer (pH 7.0) and used

for mechanical inoculation of healthy *I. nil* cotyledons. After 6 serial passages in *I. nil*, the isolates were examined by RT-PCR coupled with RFLP (RT-PCR-RFLP) analysis and compared to each other and the viruses in the original daughter roots.

Three distinct isolates were identified by RFLP analysis in different plants following serial passage. Following *Bam*HI digestion, one isolate yielded a fragment of approximately 1,300 bp, another generated two fragments of approximately 1,000 and 400 bp, and the third generated two fragments of approximately 700 and 600 bp. *Eco*RI digestion only separated two isolates, one that yielded a fragment of approximately 1,300 bp, another generated a fragment of approximately 1,400 bp. The isolate that yielded the 1,300-bp fragment after digestion with both enzymes induced milder symptoms on upper leaves of *I. nil* than the other two isolates and was selected for further use and designated as SPFMV 10-O.

Host range and pathogenicity. Although 10-O was not mechanically transmissible to *I. batatas*, it was transmitted by grafting with 10-O-infected *I. nil* seedlings. No symptoms were observed on the leaves of 10-O-infected *I. batatas*, in contrast to symptoms of chlorotic spots with purple rings observed on the leaves of SPFMV-S-infected plants (25). 10-O-inoculated *I. batatas* grew similarly to virus-free plants, and storage roots were asymptomatic, or in the sweetpotato cultivars Tosabeni and Koukei-14 there was a slight mild storage root skin discoloration (Fig. 1). Russet crack did not appear on any of the tested cultivars. This was in contrast to the russet crack, skin discoloration, and deformity observed in SPFMV-S infected storage roots (Fig. 1).

The yield of fresh storage roots of the 10-O-infected *I. batatas* was not significantly different from that of storage roots from noninfected *I. batatas* (Table 1). The weight of storage roots from plants infected with 10-O ranged from 137.5 to 237.4 g, and the number of roots per stock ranged from 2.7 to 3.9. These values were not significantly different from those obtained from the noninoculated controls (Student's *t* test, *P* > 0.05, Table 1).

I. nil and *I. setosa* were systemically infected by SPFMV-10-O infection, and the infection caused mottling and vein-clearing of the upper leaves, respectively. 10-O causes chlorotic spots on a few inoculated leaves of *C. amaranticolor* and *C. quinoa*. The other tested plants did not develop symptoms and were not infected by 10-O, as indicated by negative RT-PCR assays.

Sequence divergence and discrimination between SPFMV-10-O and SPFMV-S. The sequence of the 1781 3' nt bp fragment containing the N1b gene, the CP gene, and the 3' untranslated region from the 10-O isolate was determined (DDBJ/EMBL/GenBank accession number AB438224), and the CP gene sequence (nucleotide positions 613 to 1557 encoding 315 amino acids) was compared with the corresponding region of SPFMV-S (DDBJ/EMBL/GenBank accession number D86371) and SPFMV-O (DDBJ/EMBL/GenBank accession number D16664). The 10-O sequence was 91.1 and 99.3% identical to SPFMV-S and SPFMV-O, respectively, at the nucleotide level, and 95.6 and 99.4%, respectively, at the amino acid level. Based on these data, we designed an RFLP analysis that would distinguish between the 10-O and SPFMV-S isolates.

Digestion of the 10-O amplicon yielded 3 fragments, 238, 214, and 36 bp in size,

whereas the amplicon from SPFMV-S contained only one *Alu*I site, which generated two fragments, 452 and 36 bp in size, respectively (Fig. 2).

Cross-protection tests of 10-O. Plants inoculated with 10-O and then challenged with SPFMV-S produced storage roots that either were asymptomatic or had only mild skin discoloration (Fig. 1). The disease severity ratings of these plants were slightly higher than those of plants inoculated with 10-O only, but were significantly lower than those of plants inoculated with SPFMV-S only (Table 2). RFLP analysis did not detect SPFMV-S in storage roots of plants infected with 10-O and then challenged with SPFMV-S. RFLP analysis readily detected SPFMV-S and 10-O in mixtures of sap from leaves of 10 plants infected with each virus. However, in leaves of 10 plants infected with 10-O and then challenged with SPFMV-S, RFLP analysis did not detect SPFMV-S at all.

Transmission of 10-O by aphids. 10-O was transmitted by aphids, but the rate of transmission was less (2 of 9 *I. batatas* cuttings tested) than for SPFMV-S (5 of 9 cuttings tested). However, further testing is required to determine if these differences are significant.

DISCUSSION

In this study, we found a mild isolate of SPFMV (10-O), which protects *I. batatas* from SPFMV-S infection. RT-PCR-RFLP revealed that SPFMV-S did not latently infect any 10-O-infected *I. batatas*. In contrast, when *I. batatas* was preinfected by inoculation with SPFMV-O (15,26) and subsequently challenged with SPFMV-S, the two viruses coinfect, resulting in russet crack symptom expression and severe skin discoloration (data not shown).

Table 1. Disease severities and yield of storage roots of *Ipomoea batatas*

Experiment ^a	Year	Day		Inoculum	Average weight of a storage root (g) ^b	Number of roots in a stock ^b	Average yield of a stock	
		Planting	Harvest				Weight (g) ^b	% to noninoculated
A	2000	8 Jun	26 Oct	10-O	208.9	2.7	564.0	96
				— ^c	218.1	2.7	589.0	—
B	2000	9 Jun	7 Nov	10-O	215.0	3.6	774.0	97
				—	211.1	3.8	802.0	—
C	2001	29 May	30 Oct	10-O	183.4	3.5	639.0	98
				—	204.8	3.2	654.0	—
D	2001	29 May	19 Nov	10-O	210.2	3.9	819.0	104
				—	208.3	3.8	790.0	—
E	2001	16 May	26 Oct	10-O	252.3	2.8	698.0	95
				—	246.0	3.0	738.0	—
F	2002	15 May	5 Nov	10-O	237.4	2.7	641.0	100
				—	279.1	2.3	642.0	—
G	2002	15 May	11 Nov	10-O	180.0	3.6	648.0	105
				—	176.3	3.5	617.0	—
H	2003	13 May	13 Nov	10-O	225.9	3.3	745.5	99
				—	243.3	3.1	754.2	—
I	2003	15 May	29 Oct	10-O	137.5	3.9	536.3	92
				—	176.5	3.3	582.5	—
J	2003	15 May	13 Nov	10-O	180.0	2.9	522.0	95
				—	183.9	3.0	551.7	—

^a For each treatment, 30 cuttings of the *I. batatas* cv. Tosabeni were used.

^b Not significant at the 5% level by Student's *t* test.

^c —, Not inoculated.

Occasionally, mild viruses may affect the phenotypic expression and nutrient uptake in infected plants. *Gentiana scabra* inoculated with a mild isolate of *Bean yellow mosaic virus* showed an increase in the number of flowers (23). Similarly, *G. scabra* produced more flowers after infection with a mild isolate of *Cucumber mosaic virus* (20). Sayama et al. reported that ascorbic acid accumulates in the fruits of tomato infected with a mild isolate of *Cucumber mosaic virus* (21). The yield of fresh storage roots produced by 10-O-infected cuttings was almost equal to that of healthy plants. 10-O appeared to have little effect on the storage roots of *I. batatas*, as the average weight of a root and the number of roots per plant were not significantly different. Further study is needed to evaluate the effects of 10-O infection on the quality and nutrient composition of sweetpotato.

Transmission tests suggested that 10-O could be transmitted between *I. batatas* by aphids in the fields. Some mild isolates of other viruses appeared to have poor aphid transmissibility (10,11,22). Lack of transmissibility is a desirable trait in mild viruses, as it minimizes the risk of natural spread. However, spreading of 10-O in fields might be limited because of its narrow host range. Even if 10-O infects other plants, our data suggest that the damage would not be serious due to the low pathogenicity of the virus.

Based on the nucleotide sequence of its 3' noncoding region and CP gene, 10-O is genetically more similar to SPFMV-O than to SPFMV-S. As the amino acid sequence identity of the CP of 10-O with the CP of SPFMV-O was 99.4%, 10-O could be classified as an isolate of SPFMV-O. However, the nucleotide sequence of the 5' terminal region of 10-O was more similar to

SPFMV-S than to SPFMV-O (S. Yamasaki et al., *unpublished*). We are currently determining the complete nucleotide sequence of 10-O and attempting to develop infectious clones. In further studies, genetic recombinants among these isolates could reveal which gene or region is responsible for the mild symptom phenotype and the cross-protection ability.

I. batatas was rarely infected with 10-O by sap inoculation. From a practical point of view, large numbers of the infected cuttings should be prepared at one time to use for commercial production. We graft-

inoculated *I. nil* with 10-O to prepare 10-O-infected cuttings. We confirmed that 10-O could be maintained in *I. batatas* in subsequent generations once it was inoculated, and that the mild characteristic of the infection was stable in the infected roots. Therefore, if farmers are provided with the 10-O-infected roots or cuttings, they would be able to maintain, propagate, and cultivate the 10-O-infected roots or cuttings by themselves.

In Japan, cosmetic appearance of vegetables and fruits including sweetpotato is very important for marketing, because consumers

Fig. 2. Differentiation of the mild isolate 10-O and *Sweet potato feathery mottle virus*, severe strain (SPFMV-S) by reverse transcription-polymerase chain reaction (RT-PCR) followed by *AluI* digestion. Products of PCR were generated using primers 490-RT, 490-5', and 490-3'. Amplified products were digested with *AluI*. Total RNA extracted from *Ipomoea batatas* that had been infected with 10-O (lane 2), SPFMV-S (lane 3), and inoculated with 10-O and then challenged with SPFMV-S (lane 4).

Fig. 1. Storage roots of *Ipomoea batatas* (cv. Tosabeni): **A**, healthy; **B**, infected with mild isolate 10-O; **C**, 10-O infected and challenge-inoculated with *Sweet potato feathery mottle virus*, severe strain (SPFMV-S); and **D**, SPFMV-S infected.

Table 2. Cross-protection of mild isolate 10-O against the severe strain of *Sweet potato feathery mottle virus* (SPFMV-S) infection in the field

Experiment	Year	Day		Preliminary inoculation	Challenge inoculation	Roots tested	Disease incidence	No. of plants with symptoms ^a				Disease severity ^b
		Planting	Harvest					0	1	2	3	
A	2000	8 Jun	26 Oct	10-O	SPFMV-S	30	16.7	25	5	0	0	5.6
				— ^c	SPFMV-S	30	100	0	0	0	30	100
				10-O	—	30	20.0	24	6	0	0	6.7
B	2000	9 Jun	7 Nov	10-O	SPFMV-S	30	40.0	18	10	2	0	15.6
				—	SPFMV-S	30	100	0	0	6	24	93.3
				10-O	—	30	50.0	15	13	2	0	18.9
C	2001	15 May	15 Oct	10-O	SPFMV-S	30	23.3	23	5	0	2	12.2
				—	SPFMV-S	30	100	0	0	7	23	92.2
				10-O	—	28	21.4	22	6	0	0	7.1
D	2001	29 May	30 Oct	10-O	SPFMV-S	30	16.7	25	3	2	0	7.8
				—	SPFMV-S	30	100	0	3	4	23	88.9
				10-O	—	30	6.7	28	2	0	0	2.2
E	2001	29 May	19 Nov	10-O	SPFMV-S	30	30.0	21	8	1	0	11.1
				—	SPFMV-S	30	100	0	3	8	19	84.4
				10-O	—	30	20.0	24	5	1	0	7.8
F	2001	16 May	26 Oct	10-O	SPFMV-S	30	30.0	21	7	2	0	12.2
				—	SPFMV-S	30	100	0	1	7	22	90.0
				10-O	—	30	10.0	27	3	0	0	3.3
G	2002	15 May	5 Nov	10-O	SPFMV-S	79	22.8	61	15	3	0	8.9
				—	SPFMV-S	80	93.8	5	8	16	51	80.4
				10-O	—	40	15.0	34	6	0	0	5.0
H	2003	13 May	13 Nov	10-O	SPFMV-S	49	12.2	43	4	2	0	5.4
				—	SPFMV-S	34	97.1	1	2	0	31	93.1
				10-O	—	41	9.8	37	4	0	0	3.3

^a Symptoms were categorized by disease indices as follows: 0, no symptoms; 1, slight skin discoloration; 2, mild skin discoloration; 3, russet crack disease or severe skin discoloration. All roots were scored 5 months after putting inoculated cuttings into soil.

^b Disease severity = $\Sigma(\text{disease index} \times \text{number of plants}) / (3 \times \text{total number of roots}) \times 100$.

^c —, Not inoculated.

consider that good appearance is an indication of good quality and taste. Russet crack or skin discoloration in sweetpotato drastically decreases their commercial value. Although sweetpotatoes with russet crack symptoms have very low commercial value, 10-O-infected storage roots were almost equal to virus-free roots in apparent marketability. We anticipate that the ability of 10-O to protect against russet crack, especially over several generations of crop production, will save considerable commercial value of sweetpotato in Japan.

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