Cry, the Resistance Locus of Cowpea to Cucumber Mosaic Virus Strain Y

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ABSTRACT

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Most cowpea cultivars are resistant to infection by cucumber mosaic virus strain Y (CMV-Y) that causes a hypersensitive reaction followed by the development of necrotic local lesions. It has been anticipated that resistance to CMV-Y depends upon the existence of a resistance (*R*) gene in cowpea. To define the nature of the *R* gene, we screened 38 cowpea cultivars to isolate susceptible ones. The cultivar PI 189375 was identified as susceptible based on symptoms and the presence of progeny virions on noninoculated upper leaves. Protoplasts prepared from both 'PI 189375' and the resistant cultivar Kurodane-Sanjaku supported virus multiplication. This result suggested that there was no difference between the capacities for virus multiplication in both cultivars at a single-cell level, implying an expression of the specific *R* gene in intact 'Kurodane-Sanjaku' plants. F_1 and F_2 populations were then constructed by reciprocal crosses between 'Kurodane-Sanjaku' and 'PI 189375'. Reactions of F_1 and F_2 populations to CMV-Y inoculation demonstrated that the *R* gene was inherited as a single dominant gene. We designated this resistance locus as *Cry* (cowpea *R* gene to CMV-Y).

Additional keyword: gene-for-gene theory.

Cucumber mosaic virus (CMV) is one of the most agronomically important viruses in the world as it causes considerable losses in CMV-infected crops. CMV has a larger host range than most viruses and is transmitted by aphids in a nonpersistent manner (22). Control of CMV-mediated diseases often depends on the use of resistant cultivars.

Most strains of CMV, including the yellow strain (strain Y, [CMV-Y]) induce the hypersensitive reaction (HR) followed by the development of necrotic local lesions on inoculated cowpea (*Vigna unguiculata* (L.) Walp.) (26). Resistance to a given pathogen is often correlated with the HR, i.e., localized and induced cell death in the host plant at the site of infection leading to protection of the whole plant (13). The gene-for-gene theory for the genetic basis of HR-mediated disease resistance was first proposed by Flor (7). Resistance is only expressed when a plant bears a resistance (*R*) gene that corresponds to an avirulence (*avr*) gene in a potential pathogen. Since the first *avr* gene was isolated from a bacterial pathogen, more than 30 *avr* genes have been characterized to date (12,15). Several *R* genes have been isolated (29) since the first report of isolation of *Pto*, the resistance locus to *Pseudomonas syringae* pv. *tomato*, from tomato (16). The best studied interaction between *R* and *avr* genes in plant-virus interactions is *N* gene-mediated resistance to tobacco mosaic virus (TMV) (8). The *N* gene encodes a protein of 131.4 kDa as the *R* gene product that has a homologous region to the mammalian interleukin-1 receptor and the *Drosophila* protein (30) and interacts with the TMV 126-kDa replicase protein that is the *avr* gene product (21). Several other *R* genes have been identified and characterized recently (4,20).

In the cowpea-CMV interaction, it was suggested that RNA 2 of CMV-Y coding for one of the replicase complex proteins is required for HR induction in cowpea (5,9), whereas the corresponding cowpea *R* gene has not been studied extensively. To investigate the molecular basis of the HR between cowpea and CMV-Y, we had to isolate a cultivar showing systemic infection upon the inoculation of CMV-Y. In 1952, Sill and Walker (27) reported that they observed a cowpea plant that occasionally became infected systemically upon the infection of CMV-Y in the cultivar Black. Subsequently, Sinclair and Walker (28) demonstrated that the resistance of the cowpea cultivars Black, Black Eye, and Dixie Queen was inherited as a single dominant trait. These studies, however, were based on observations of a spontaneous mutant, and it is uncertain whether or not they reflected the true nature of the resistance in cowpea. Furthermore, the progeny seeds used in their research are no longer available (P. Ahlquist, *personal communication*). To understand the cellular mechanism that results in the HR in the cowpea-CMV-Y system, a cowpea cultivar that showed stable susceptibility to CMV-Y infection was needed. In this paper, we report the isolation of a susceptible cultivar of cowpea, PI 189375, and the genetic characterization of resistance in the cowpea-CMV combination.

MATERIALS AND METHODS

Virus. CMV-Y was maintained in, and purified from, tobacco plants (*Nicotiana tabacum* L. cv. Ky57) as described earlier (11). Purified virions were suspended in 10 mM potassium phosphate buffer, pH 8.0, and used for inoculations. Total viral RNAs were extracted according to Karasawa et al. (11).

Plant materials. All the cowpea cultivars, except 'Kurodane-Sanjaku', that has been used extensively in our laboratory so far were received from the National Institute of Agro-biological Resources (NIAR), Tsukuba, Japan. Cowpea seedlings were grown in a growth chamber (day, 12 h at 27°C; night, 12 h at 25°C) to test the reactions to CMV-Y inoculation. Primary leaves of 10- to 14-day-old, completely expanded seedlings were used for the assay by rubbing them with CMV inoculum at a concentration of 0.1 mg/ml of virions after dusting leaves with Carborundum (#600).

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Inoculated plants were maintained under the conditions described above for about 1 week and scored for reaction phenotype.

Reciprocal cross experiments between 'Kurodane-Sanjaku' and 'PI 189375' (NIAR accession no. 00035400) were conducted by artificial fertilization. Both cultivars were grown to mature plants in a greenhouse under natural light, and hybridization was performed as described (3). In addition, F_1 generation plants were successfully selfed to produce F_2 progeny.

Isolation and inoculation of cowpea protoplasts. Cowpea seeds were surface-sterilized with 70% ethanol for 1 min and 2% NaClO solution for 20 min and then washed three times with sterilized, distilled water. These seeds were germinated on plates of 1% agar at 25°C, followed by the transfer of germinating seeds onto 1% agar plates of Murashige and Skoog medium with $^1/_2\times$ concentration of salts, except for the use of vitamins at 1× concentration. Seven days after transfer, young hypocotyls were thinly sliced and suspended in CPW solution (0.5 mM KH_2PO_4, 1 mM KNO_3, 1 mM $MgSO_4$, 1 μM KI, 0.1 μM $CuSO_4$, 1 μM $CaCl_2$, 0.1 M glycine, and 30 mM mannitol, pH 5.7) for about 1 h for the induction of plasmolysis. CPW solution was changed to an enzyme cocktail consisting of 1 volume of enzyme solution (2% Cellulase Onozuka R-10 and 0.05% Pectolyase Y-23 [wt/vol, Yakult Honsha Co., Tokyo], and 0.05% Casamino Acids [wt/vol, Difco Laboratories, Detroit] in CPW solution, pH 5.7) and three volumes of W5 solution (125 mM KCl, 145 mM NaCl, 5 mM $CaCl_2$, and 5 mM glucose, pH 5.7). Free protoplasts were obtained by shaking at 70 rpm for 2 h at 25°C. Protoplasts were purified by pelleting (70 × *g* for 2 min) and resuspending of the protoplasts in an appropriate volume of W5 solution two times, followed by a wash with 275 mM KCl.

Purified protoplasts of 2×10^6 were resuspended in 0.5 ml of 275 mM KCl and added to a mixture of 10 μg of purified CMV-Y total RNAs and 0.5 ml of 40% (wt/vol) polyethylene glycol 1,500. Inoculation mixtures were incubated on ice for 30 min and then washed in W5 solution three times. The inoculated protoplasts were cultured in W5 solution at a concentration of 1×10^5 cells/ml under continuous illumination by white light at 25°C.

Protein analysis. To investigate the viral accumulation in inoculated cowpea plants, 10 mg of leaf tissue were homogenized in five volumes of 10 mM potassium phosphate buffer, pH 8.0. The supernatant was obtained by low-speed centrifugation and was mixed with equal volume of Laemmli's sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (14). For protoplasts, 1×10^5 cells were collected by centrifugation at 600 × *g* for 5 min and resuspended in 10 μl of 10 mM phosphate buffer plus 10 μl of SDS-PAGE sample buffer. Each sample was boiled at 100°C for 5 min just before use and subjected to electrophoresis in 10% SDS-polyacrylamide gels. Western blot analysis was performed as described by Shirako and Ehara (25).

Random amplified polymorphic DNA (RAPD) analysis. To analyze the heterozygosity of F_1 and F_2 generations from crosses between 'Kurodane-Sanjaku' and 'PI 189375', we utilized RAPD analysis. Genomic DNAs were extracted from about 1 g of leaf tissue by the cetyltrimethylammonium bromide (CTAB) method (19). Amplification reactions were in a volume of 20 μl containing 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2 mM $MgCl_2$; 0.001% gelatin; 10 μM each of dATP, dCTP, dGTP, and dTTP; 10 pmol of a single 10-base primer (Operon Technologies Inc., Alameda, CA); 20 ng of genomic DNA; and 0.5 units of *Taq* DNA polymerase (Perkin-Elmer Cetus, Foster City, CA). Amplification was performed on a DNA Thermal Cycler (Perkin-Elmer Cetus) programmed for 45 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C, followed by 1 cycle of 7 min at 72°C. Amplified DNA fragments were analyzed in 2% agarose gel electrophoresis in Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide, and observed under UV light according to Sambrook et al. (23).

RESULTS

Screening and sensitivity of susceptible cultivar to CMV-Y. To find cultivars susceptible to the CMV-Y infection, we inoculated CMV-Y (0.1 mg/ml) onto primary leaves of 38 cowpea cultivars. Only one cultivar, PI 189375, uniformly failed to show the HR on inoculated leaves and developed systemic symptoms on inoculated and noninoculated leaves (Table 1). The first visible symptom appeared at 2-days postinoculation (dpi) as a vein necrosis in the inoculated leaves, and then necrosis developed in the whole leaf, occasionally after yellowing at 4 to 5 dpi (Fig. 1A). Noninoculated leaves showed a vein clearing at 4 dpi with small numbers of brown spots (Fig. 1B) and, subsequently, developed a blight with an apical necrosis. The inoculated 'PI 189375' plants died at 7 to 10 dpi. Symptom development in 'PI 189375' was clearly different from those reported for the susceptible line described by Sill and Walker (27).

The cultivars PI 339583 (NIAR accession no. 00035391) and TVU 456 (NIAR accession no. 00035421) showed some complex responses to CMV-Y infection. Most of their seedlings developed necrotic local lesions upon infection, but a small number of plants became systemically infected (Table 1). Because these unexpected phenotypes might have resulted from mutants, we excluded these cultivars from further experiments. In 'PI 255792' (NIAR accession no. 00035408), we could not detect either symptoms or viral coat protein (CP). All other cultivars displayed the HR, followed

TABLE 1. The reaction of 39 cowpea cultivars to cucumber mosaic virus strain Y

Cultivar name	NIAR accession no.	Symptom[a]
Kurodane-Sanjaku	–	LL
Black Eye 5	00046747	LL
Col/Kagawa/1967	00035360	LL
Col/Tokushima/1967	00034361	LL
Daruma	00035363	LL
Dr Saunder Upright	00035369	LL
I-369	00047594	LL
I-807	00047595	LL
I-9012	00047598	LL
Juuhachi Sasage	00035355	LL
Juuroku Sasage	00035354	LL
Kuro Sanjaku	00035356	LL
Manrichiang Tou	00035365	LL
New Era Crey	00035368	LL
PI 124609	00035370	LL
PI 165486	00035372	LL
PI 165941	00035373	LL
PI 186467	00035396	LL
PI 188702	00035398	LL
PI 189230	00035392	LL
PI 189374	00035399	LL
PI 189375	00035400	S
PI 221731	00035430	LL
PI 255765	00035401	LL
PI 255781	00035404	LL
PI 255788	00035406	LL
PI 255791	00035407	LL
PI 255792	00035408	–
PI 255910	00035380	LL
PI 338582	00035390	LL
PI 339583	00035391	L(S)
Sanjaku Sasage	00035353	LL
TVU 1484	00035424	LL
TVU 36	00035418	LL
TVU 456	00035421	L(S)
U-1709	00047604	LL
U-1728	00047605	LL
White Wonder Trailing	00035366	LL
Witsemberg Upright	00035367	LL

[a] LL = necrotic local lesion, S = systemic infection, L(S) = most of the plants developed local lesions but a few plants were infected systemically, and – = neither symptoms nor viral coat protein were detected.

by the development of necrotic local lesions similar to those on 'Kurodane-Sanjaku '(Fig. 1C).

Viral multiplication in inoculated 'PI 189375' was measured by Western blot analysis of viral CP and compared with that in the resistant cultivar Kurodane-Sanjaku. Samples were prepared as described in Materials and Methods each day after inoculation. As shown in Figure 2, 'PI 189375' accumulated considerable amounts of CP both in inoculated and noninoculated leaves. Viral CP was detected as soon as 1 dpi as a trace amount in inoculated leaves and increased rapidly at 3 dpi. CP accumulation in these leaves decreased after 4 dpi. This may have resulted from the collapse of infected leaf tissues coincident with the spreading of necrosis at 4 dpi as described above. CP was also detected in noninoculated leaves by 5 dpi. We could not detect CP in any experiment in inoculated, as well as noninoculated, leaves in the resistant cultivar Kurodane-Sanjaku (Fig. 2).

To confirm that the systemic symptoms on 'PI 189375' were not due to the mutation of CMV-Y during the multiplication of the virus, we performed back-inoculation experiments. Inoculated leaves at 3 dpi and systemically infected leaves at 4 dpi of 'PI 189375' were homogenized in five volumes of 10 mM potassium phosphate buffer, pH 8.0, and the homogenates were used to inoculate separately the primary leaves of 'Kurodane-Sanjaku'. Only necrotic local lesions appeared on the inoculated leaves for both inocula, indicating the systemic infection in 'PI 189375' was not due to the mutation of the virus (data not shown).

Multiplication of CMV-Y in protoplasts of resistant and susceptible cultivars. As described above, inoculation experiments indicated that 'PI 189375' was susceptible to infection by CMV-Y. To reevaluate the ability of cells of the resistant and susceptible cowpea cultivars to support viral multiplication, we prepared protoplasts from the hypocotyl tissues of 'PI 189375' and 'Kurodane-Sanjaku' and inoculated them with CMV-Y. Protoplasts (2×10^6) were inoculated with 10 µg of CMV-Y RNAs and assayed for virus titer every 12 h for 48 h. Figure 3 shows representative results from these experiments with each lane containing all the soluble proteins from 2.5×10^4 protoplasts. In protoplasts from both cultivars, we could detect the viral CP 12-h postinoculation, with a gradual increase in CP up to 48 h. Thus, there were no clear differences in the multiplication efficiency of CMV-Y in the protoplasts from the two cultivars. These results indicated that both cultivars were able to support the CMV-Y multiplication equally well at a single-cell level, and that the expression of the resistance was because of differences at a whole-plant level.

The nature of the *R* gene. To genetically characterize the inheritance of resistance and susceptibility of cowpea cultivars to infection by CMV-Y, we made reciprocal crosses between 'PI 189375' and 'Kurodane-Sanjaku' and scored the segregation of resistance among the F_1 and F_2 populations after inoculation of CMV-Y onto primary leaves. As shown in Table 2, all of the F_1 progeny showed resistance to CMV-Y, and the F_2 population segregated to give a good fit to inheritance for a single dominant gene (3:1 R/S) in a total of 330 F_2 plants. Furthermore, backcrosses of F_1 plants to 'PI 189375' showed a 1:1 segregation of BC^1F_1 plants. We confirmed the ability of resistant and susceptible F_1 and F_2 progeny to propagate the virus equally well as both parents by the Western blot. These plants showed complete agreement of apparent resistant/susceptible phenotypes with the detection of viral CPs in inoculated and noninoculated leaves (data not shown). In our inoculation experiments, however, all F_1 plants developed somewhat larger necrotic lesions than those appearing on 'Kurodane-Sanjaku'. Furthermore, some of F_2 plants also developed somewhat or much larger necrotic local lesions (data not shown). These plants did not support detectable levels of viral accumulation in noninoculated or inoculated leaves. The inheritance of the size of necrotic local lesions was not consistent with that for the resistance. These findings suggest that another locus affected the appearance of lesions that does not have any effect on the segregation of the resistance.

'Kurodane-Sanjaku' and 'PI 189375' showed obvious differ-

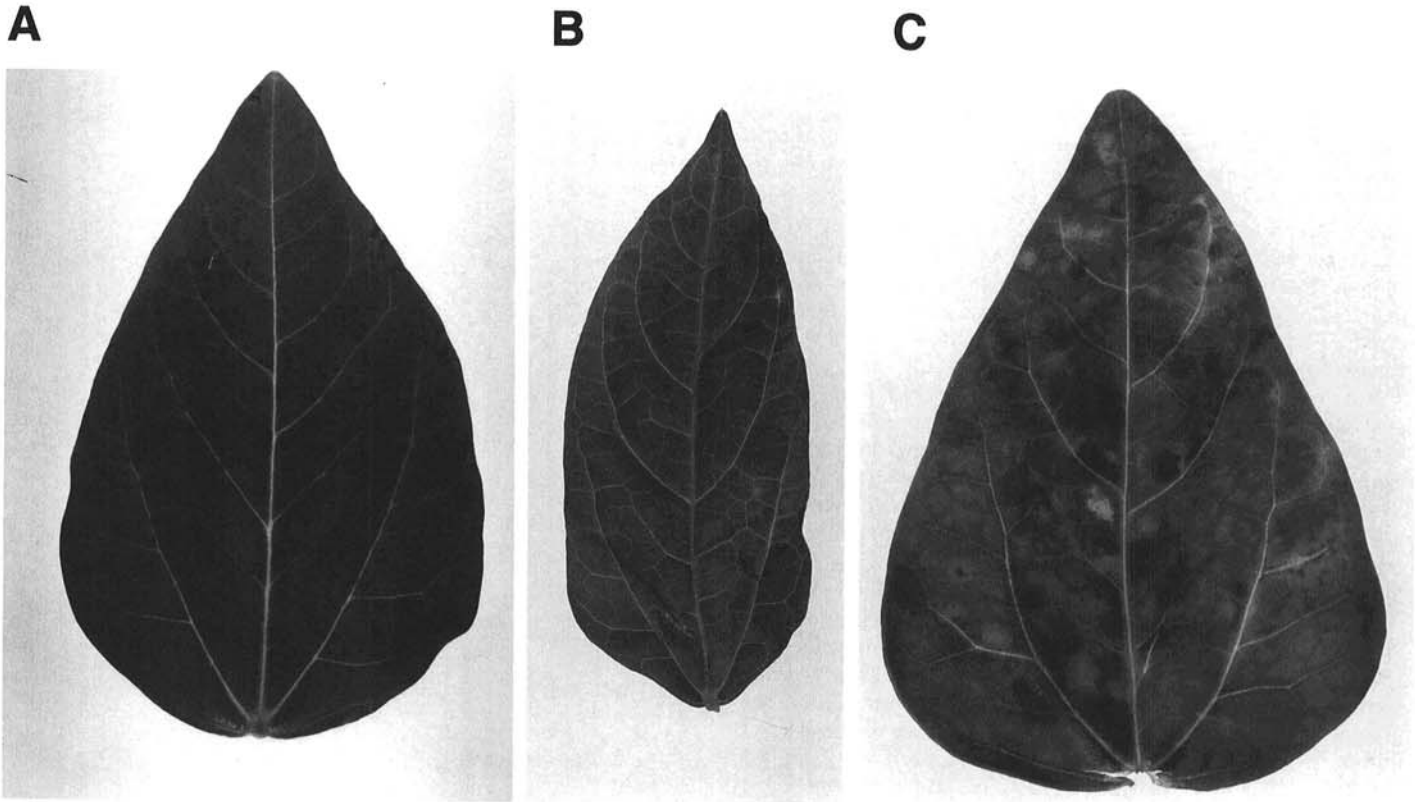

Fig. 1. A and B, Symptoms exhibited on the susceptible cowpea cultivar PI 189375 after inoculation with cucumber mosaic virus strain Y (CMV-Y). **A,** An inoculated leaf 4-days postinoculation (dpi). **B,** A noninoculated upper leaf 5 dpi. **C,** Symptoms exhibited on the resistance cowpea cultivar Kurodane-Sanjaku after inoculation with CMV-Y. Photograph shows an inoculated leaf 2 dpi.

ences in several phenotypes such as the color of flowers and seed and the length of pods. These traits did not cosegregate with the resistance in the progeny. We, thus, adopted the RAPD analysis to confirm that they were the hybrids. Genomic DNAs were extracted from leaf tissues of each plant, including both parents, by the CTAB method (19) and used as template DNA for RAPD analysis. We tested 20 primers (Kit A) supplied by Operon Technologies Inc. of which nine primers showed polymorphism between both parents as shown in Figure 4. Primer OPA-06, 5′-GGTCCCTGAC-3′, produced the most distinguishable bands between parents (950 bp for 'Kurodane-Sanjaku' and 900 bp for 'PI 189375'), so we used this primer to screen the F_1 population (Fig. 5). All of the tested F_1 plants showed two DNA bands derived from both parents, indicating that the F_1 progenies we obtained were hybrids between 'Kurodane-Sanjaku' and 'PI 189375'. Unfortunately, primers including OPA-06 did not show polymorphism corresponding to the segregation of the resistance in the F_2 generation.

Taken together, we concluded that the resistance to CMV-Y in cowpea plants depends on the existence of the locus that segregates in a single dominant manner. We designated this locus as the *Cry*, cowpea *R* gene to CMV-Y.

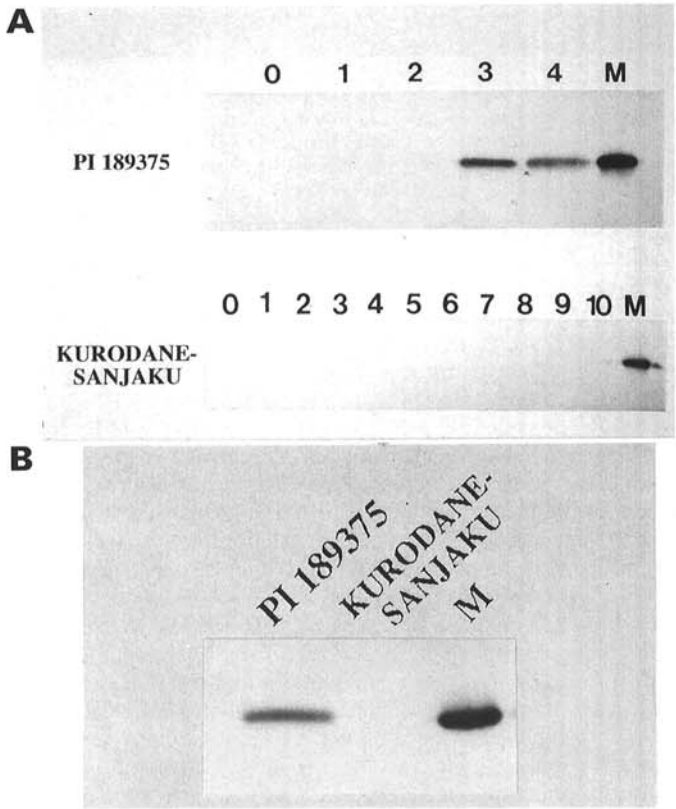

Fig. 2. Western blot analysis of cucumber mosaic virus (CMV) coat protein (CP) in both the resistant ('Kurodane-Sanjaku') and susceptible ('PI 189375') cowpea cultivars. **A,** Time course of CMV accumulation in inoculated leaves of both cultivars. Lane numbers correspond to the days after inoculation. M shows CMV strain Y (CMV-Y) CP (24.5 kDa) as a marker. **B,** Accumulation of CMV-Y CP in noninoculated upper leaves of both cultivars 5-days postinoculation. M shows CMV-Y CP (24.5 kDa) as a marker.

DISCUSSION

In the past few years, several *R* genes have been isolated from tomato (10,16), tobacco (30), *Arabidopsis* (2,18), and flax (6) and characterized to define the molecular basis of the gene-for-gene theory. These *R* genes are important for breeding of disease-resistance cultivars, as well as for advancing plant molecular biology. CMV is one of the most important viruses in the world and, thus, it is anticipated that genetic resources for breeding for resistance to it will soon appear. Available cowpea cultivars usually respond hypersensitively to infection by most strains of CMV, suggesting the usefulness of cowpea as a source of resistance to CMV. In the 1950s, Sill and Walker (27) and Sinclair and Walker (28) reported that a susceptible cowpea line was obtained in the course of studies involving inoculation of several thousand plants of 'Black' with CMV and that the resistance of the cowpea cultivars Black, Black Eye, and Dixie Queen were dominant and governed by a single gene. Subsequent analysis, however, has not been conducted. In addition, their results cannot be reexamined, because the seed stock used in their studies has not been maintained. Thus, it was necessary to again isolate a CMV-susceptible cowpea line.

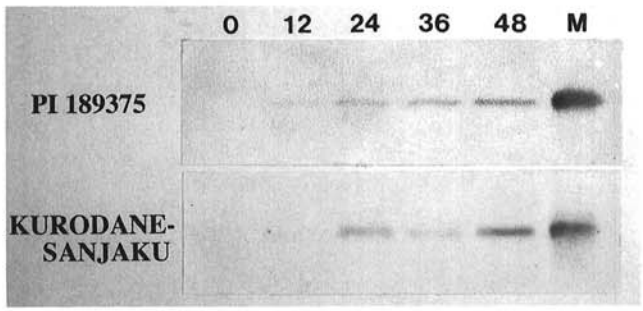

Fig. 3. Time course of cucumber mosaic virus strain Y (CMV-Y) coat protein (CP) accumulation in protoplasts of the resistance ('Kurodane-Sanjaku') and susceptible ('PI 189375') cultivars. Protoplasts (2×10^6 cells) were inoculated with 10 µg of purified CMV-Y RNAs and cultured for 48 h. CP accumulation was measured by Western blot analysis every 12 h as indicated above each lane. Each lane was loaded with a crude protein extract from 2.5×10^4 protoplasts. M shows CMV-Y CP as a marker.

TABLE 2. Segregation of cucumber mosaic virus strain Y resistance in the F_1, F_2, and BC^1F_1 populations from the resistant ('Kurodane-Sanjaku') and susceptible ('PI 189375') parents

Hybrid	Number of plants			χ^2 ratio (expected ratio)	
	Resistant	Susceptible	Total	Value	Probability
F_1 (female × male)					
'PI 189375' × 'Kurodane-Sanjaku'	10	0	10		
'Kurodane-Sanjaku' × 'PI 189375'	26	0	26		
F_1 total	36	0	36		
F_2 (F_1-selfed)					χ^2 (3:1)
'PI 189375' × 'Kurodane-Sanjaku'	125	42	167	0.0020	0.950–0.975
'Kurodane-Sanjaku' × 'PI 189375'	122	41	163	0.0066	0.900–0.950
F_2 total	247	83	330	0.0040	0.900–0.950
Backcross F_1 (female × male)					χ^2 (1:1)
'PI 189375' × F_1[a]	11	10	21	0.0476	0.750–0.900

[a] F_1 seeds were derived from a cross between 'Kurodane-Sanjaku' and 'PI 189375'.

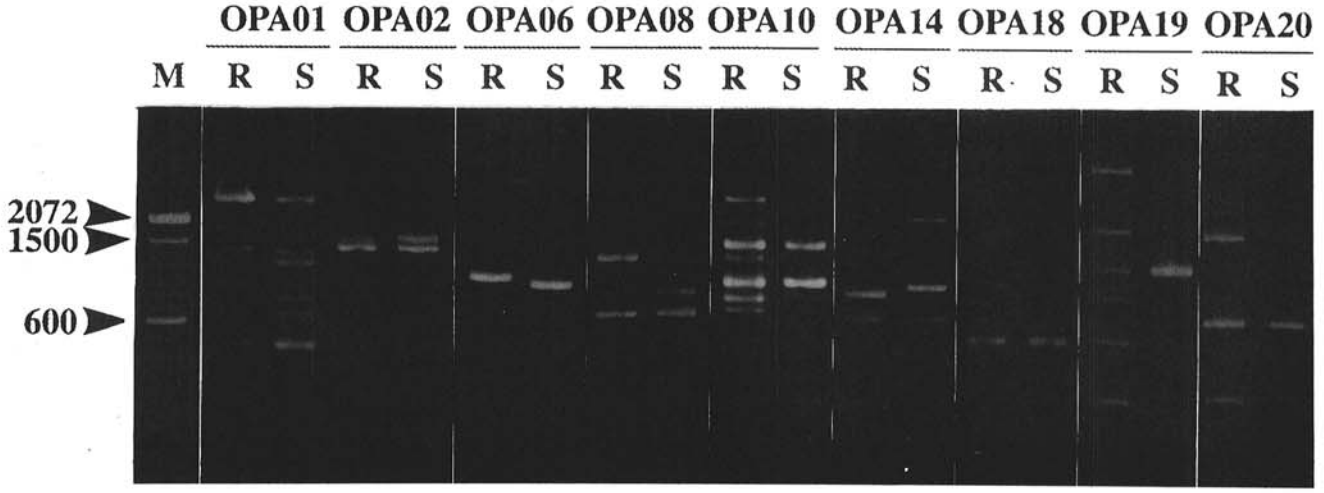

Fig. 4. Random amplified polymorphic DNA (RAPD) analysis of two cowpea cultivars. Genomic DNAs of resistant ('Kurodane-Sanjaku', indicated by R) and susceptible ('PI 189375', indicated by S) cultivars were extracted and used as templates for the polymerase chain reaction amplification using 10-mer primers indicated above each lane. Seven microliters of a total 20-µl reaction was electrophoresed in each lane of 2% agarose-Tris-borate-EDTA gel. Amplified DNA bands were stained with ethidium bromide and observed under UV light. Lane M is a DNA size marker (100-bp ladder; Gibco BRL, Gaithersbury, MD).

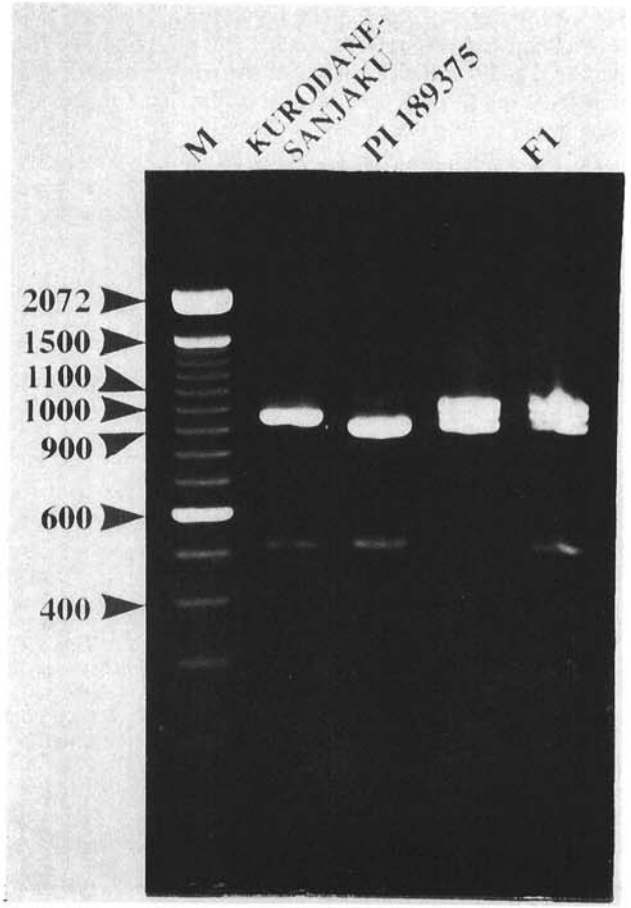

Fig. 5. Random amplified polymorphic DNA (RAPD) analysis of parents and two representative F_1 progeny plants using the OPA-06 primer, 5′-GGTCCCTGAC-3′. Each parent generated one of 950- or 900-bp DNA fragments and F_1 hybrids had both fragments. M is a 100-bp DNA size marker (Gibco BRL, Gaithersburg, MD).

To characterize the molecular basis of the resistance of cowpea to the infection of CMV-Y, we needed a susceptible cultivar that supports the systemic accumulation of CMV-Y. At first, we screened the cowpea stocks available in Japan. Among the cultivars tested, 'PI 189375' was the only one to show stable susceptibility to CMV-Y infection (Table 1). This cultivar showed systemic infection, usually followed by the death of the plant from the inoculated leaves to apical tissues within a week, suggesting rapid multiplication of the virus throughout the plant (Fig. 1). It is possible that these severe symptoms were associated with an HR as with the *Nx*/*Nb* resistance genes of potato and potato virus X infection (1,24). However, this idea was rejected by the confirmation of viral multiplication in inoculated and noninoculated upper leaves (Fig. 2). In addition, when the first leaves of 1-month-old seedling were inoculated, the inoculated 'PI 189375' plants showed systemic mosaic symptoms, accumulated the viral CP both in inoculated and noninoculated leaves, and did not die (data not shown). This accumulation of the progeny virions in inoculated plants has never been demonstrated in the resistant cultivar Kurodane-Sanjaku until 10 dpi (Fig. 2), and we concluded that 'PI 189375' was infected systemically with CMV-Y. As described in Results, the symptoms observed on 'PI 189375' were different from the ones reported on 'Black' by Sill and Walker (27). Typical symptoms they observed were epinasty and indistinct mottle on inoculated primary leaves. Thus, it suggests that different mechanism(s) may underlie the symptom expression among susceptible cowpea lines.

In virus and host interactions that lead to an HR, there are many reports that the HR is not expressed in protoplasts (8). Protoplasts prepared from the resistant cultivar Kurodane-Sanjaku did not show cell death upon the infection with CMV-Y and supported the multiplication of the virus to levels similar to those in protoplasts derived from the susceptible cultivar PI 189375 (Fig. 3). Although data was not shown, the rates of infection of protoplasts for both cultivars were similar in our preliminary experiment. These results suggest that the resistance to CMV-Y is not expressed at the single-cell level and that the difference in virus accumulation in intact plants inoculated with CMV-Y is not determined by differences in viral multiplication within a cell.

To determine the inheritance of the resistance, we conducted reciprocal crosses between 'Kurodane-Sanjaku' and 'PI 189375' and inoculated CMV-Y onto primary leaves of F_1 plants and their selfed F_2 progeny. All of the F_1 plants tested showed resistance. In a total 330 F_2 plants, resistance segregated 3:1 R/S (Table 2) indicating that this resistance locus segregates in a single dominant manner. This conclusion was supported by the results of backcross experiments (Table 2). The progenies were confirmed as true hybrids by RAPD analyses (Figs. 4 and 5). Taken together, we conclude that the resistance of cowpea to the infection of CMV-Y is governed by a single dominant gene and designate the locus *Cry*.

As mentioned in Results, there are some phenotypic differences between 'PI 189375' and 'Kurodane-Sanjaku'. These differences

did not cosegregate with resistance in F_1 and F_2 populations. 'Kurodane-Sanjaku' has a solid black seed, a long-sized pod (more than 50 cm in length), and showed resistance to CMV-Y infection; whereas 'PI 189375' has a white seed, a medium-sized pod (15 to 20 cm in length), and showed susceptibility to CMV-Y infection. Some of the other cultivars showed similar morphological characteristics, except for the reaction to CMV-Y. As the resistance to CMV-Y was not linked to morphological characters noticed, molecular biological approaches will be necessary to identify the *Cry* locus. RAPD analysis is a powerful tool to map the marker near a target gene (17). Our results (Figs. 4 and 5) indicate that the two cultivars used in this study have a considerable difference in their genomic DNA organization. Hereafter, we will need to carry out the several backcrosses with 'PI 189375' to construct near-isogenic lines for the isolation of the RAPD marker closely linked to *Cry*.

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