Use of Resistant *Cucumis metuliferus* for Selection of Nitrous-Acid Induced Attenuated Strains of Papaya Ringspot Virus

Shyi-Dong Yeh and Ying-Huey Cheng

Department of Plant Pathology, National Chung Hsing University, Taichung Taiwan, Republic of China.

We thank Dr. R. Provvidenti of Cornell University for providing seeds of different genotypes of *C. metuliferus* and reviewing the manuscript. This study was supported by grants from National Science Council and the Council of Agriculture, Taiwan, Republic of China.

Accepted for publication 21 June 1989 (submitted for electronic processing).

ABSTRACT

Yeh, S.-D., and Cheng, Y.-H. 1989. Use of resistant *Cucumis metuliferus* for selection of nitrous-acid induced attenuated strains of papaya ringspot virus. Phytopathology 79:1257-1261.

Papaya ringspot virus (PRV) HA 5-1 and HA 6-1 are two nitrous-acid induced mild strains that cause infection without conspicuous symptoms in papaya and *Cucumis metuliferus* line Acc. 2459. These strains were able to overcome the resistance governed by a single dominant gene *Wmv* in *Cucumis metuliferus* line PI 292190, in which resistance was defined by immunity to the parent severe strain PRV HA and susceptibility to the mild strains as manifested by systemic mosaic and necrosis symptoms. Moreover, the systemic and necrosis symptoms caused by HA 6-1 were found in all F_1 test plants of Acc. 2459 × PI 292190, and perfectly followed the segregation of the *Wmv* gene in the F_2 population, indicating that the symptoms are direct interactions between the mild virus strain and the *Wmv* gene. The possibility of using the resistant line of *C. metuliferus* to screen useful attenuated strains of PRV for cross protection was investigated. Virus in crude sap from susceptible *C. metuliferus* Acc. 2459 infected with PRV TM, a local strain prevalent in Taiwan, was treated with nitrous acid; enrich-propagated in plants of line Acc. 2459; and then transferred to plants of the resistant line PI 292190. Virus isolates that induced systemic symptoms on line PI 292190 were selected and followed by repeated serial dilutions on the same line. The isolates were then transferred to papaya to determine their pathogenicity. Among 20 isolates selected, six induced mild symptoms on papaya; and one, designated as PRV TM-1, caused infection without conspicuous symptoms. Under greenhouse conditions, papaya inoculated with PRV TM-1 were completely protected against PRV TM when challenge inoculations were at 23, 45, or 90 days after preimmunization. The results indicated that the resistant *C. metuliferus* can be used as a selective host for screening attenuated virus strains of PRV from artificial induction.

Additional keywords: artificial induction, *Carica papaya,* cross protection.

Papaya ringspot virus (PRV), a member of the potyvirus group, occurs commonly wherever papaya (*Carica papaya* L.) is cultivated and usually spreads rapidly with destructive consequences (1,7). It is transmitted mechanically and by aphids in a nonpersistent manner. Hosts of PRV are limited to the families of Chenopodiaceae, Cucurbitaceae, and Caricaceae. PRV is serologically indistinguishable from watermelon mosaic virus 1 (WMV-1), which has been reclassified recently as the type W strain of PRV (7,10,12). Severe crop losses coupled with the unavailability of PRV-resistant papaya varieties, the difficulty of virus eradication, and the restrictive host range of PRV make cross protection an attractive method of controlling this virus.

The mild strains PRV HA 5-1 and HA 6-1, obtained from the nitrous-acid mutagenic treatment, infect papaya without conspicuous symptoms and offer a high degree of protection against severe strains (11). They have been used widely to inoculate papaya seedlings for control of PRV by cross protection in Taiwan (9,13).

Cucumis metuliferus (Naud.) Mey. Acc. 2459, commonly known as horned cucumber or jelly melon, is very susceptible to PRV and has been used as an excellent propagation host for the virus (5,12). A particular genotype, *C. metuliferus* line PI 292190, has been reported immune to infection by PRV, and its resistance is conferred by a single dominant gene *Wmv* (5,6). When this resistant line is inoculated with severe strains of PRV or WMV-1, virus can not be detected by double-antibody-sand-

wich enzyme-linked immunosorbent assay (DAS ELISA) or local lesion bioassay (5,12). However, in this report we found that PRV HA 5-1 and HA 6-1 could overcome the resistance in line PI 292190, inducing symptoms of systemic mosaic and necrosis. The interactions between the mild strains of PRV and the resistant *Wmv* gene were further studied by genetic analysis in F_1 and F_2 populations of Acc. 2459 × PI 292190. Also, based on the assumption that the ability of PRV mutants to overcome the resistance gene might be correlated to the mildness in papaya, *C. metuliferus* line PI 292190 was used to select artificially induced mild strains of PRV useful for cross protection trials.

MATERIALS AND METHODS

Reactions of severe and mild strains of PRV on different genotypes of *Cucumis metuliferus*. The susceptible line *C. metuliferus* Acc. 2459, the resistant line PI 292190, and the F_1 plants derived from crosses between them, were propagated from seeds under greenhouse conditions. PRV HA, a severe strain originated from Hawaii (3), and PRV HA 5-1 and HA 6-1, two mild virus strains generated from nitrous-acid induction (11), were propagated in plants of line Acc. 2459. To investigate reactions of severe and mild strains of PRV on different genotypes of *C. metuliferus*, test plants of Acc. 2459, PI 292190, and F_1 progeny of their crossing at the four- to five-leaf leaf stage were mechanically inoculated with PRV HA, HA 5-1, and HA 6-1, each separately. Inoculated plants were kept under greenhouse conditions (23–38 C, without supplemental lights) for at least 1 mo for observation. Virus infection was recorded by symptom development and by double-antibody-sandwich enzyme-linked immunosorbent assay (DAS ELISA), using antibody specific for PRV (2,3).

An F_2 population of the cross between line Acc. 2459 and line PI 292190 was also included in this study. Each F_2 plant was vegetatively propagated from cuttings to produce two clones of identical genotype. One clone from each F_2 plant was inoculated with PRV HA 6-1 and the other clone was inoculated with HA. Symptom development and infection of the virus were recorded as described above.

Nitrous-acid mutagenic treatment. Nitrous acid, a powerful chemical mutagen for plant RNA viruses (4,8), was used to treat PRV TM, a severe mosaic-inducing strain prevalent in Taiwan. Leaf tissue of line Acc. 2459, 3–4 wk after inoculation with PRV TM, was ground in distilled water (1 g/ml) and strained through cheesecloth. The crude sap was then centrifuged in a Beckman JA-20 rotor at 8,000 rpm for 10 min, and aliquots of the supernatant were treated with 0.4 M sodium nitrite and 0.1 M sodium acetate (adjusted with acetic acid to pH 6.0) at 25 C for 30 min (11). The reaction was stopped by adding an equal volume of 0.1 M phosphate buffer (pH 7.0) and the mixtures were immediately introduced to plants of line Acc. 2459 for propagation of possible mutants. Viruses from infected plants at 14 days after inoculation were mechanically transferred to the resistant line PI 292190. Infection in the resistant line PI 292190 was judged by symptom development and also checked by DAS ELISA using antiserum to PRV (2,3).

Selection and characterization of virus isolates infecting resistant *C. metuliferus*. *C. metuliferus* line PI 292190 was shown to be immune to PRV TM in the present study (see Results) as previously reported (12), thus, any systemic infection detected by symptoms and ELISA was assumed to be induced by mutants generated by the nitrous-acid treatment (see results for controls). Because the virus treated with nitrous acid was first propagated in plants of line Acc. 2459, the possible mutants selected by line PI 292190 might arise from a mixture of different types of mutation. Thus, viruses in infected tissue of line PI 292190 were further separated by repeated fivefold serial dilutions on line PI 292190. Viruses in infected plants at the dilution end point (5^{-4}–5^{-5}) were mechanically transferred to papaya (Tainung No. 2) seedlings to determine their pathogenicity. Inoculated papaya plants were kept in the greenhouse and their reactions were recorded. To ensure the presence of the virus, each experimental step was checked with DAS ELISA using antiserum to PRV (2,3).

Evaluation of a mild strain induced by nitrous acid treatment and host selection. A total of 20 isolates were selected from nitrous acid treatment and passage through the resistant *C. metuliferus* line PI 292190. All isolates caused systemic infection in this line, one particular isolate, designated as PRV TM-1, caused infection without conspicuous symptoms on papaya. This isolate did not incite local lesions on *Chenopodium quinoa*, and thus was further purified by several repeated fivefold serial dilutions on line PI 292190. The putative pure isolate PRV TM-1 NH2 was evaluated for its stability and cross-protection effectiveness in papaya.

Tests of cross protection were conducted under greenhouse conditions from November 1986 to June 1987. Papaya seedlings (Tainung No. 2) at the five- to six-leaf stage were mechanically inoculated with PRV TM-1 NH2 prepared from infected tissue of *C. metuliferus* line Acc. 2459 (10 ml of 0.01 M potassium phosphate buffer, pH 7.0, per gram of tissue). DAS ELISA was used to confirm the infection by TM-1 NH2 20 days after inoculation. Separate challenge inoculations were performed mechanically with the severe parent strain PRV TM at 23, 45, or 90 days after the protective inoculation. The challenge inocula

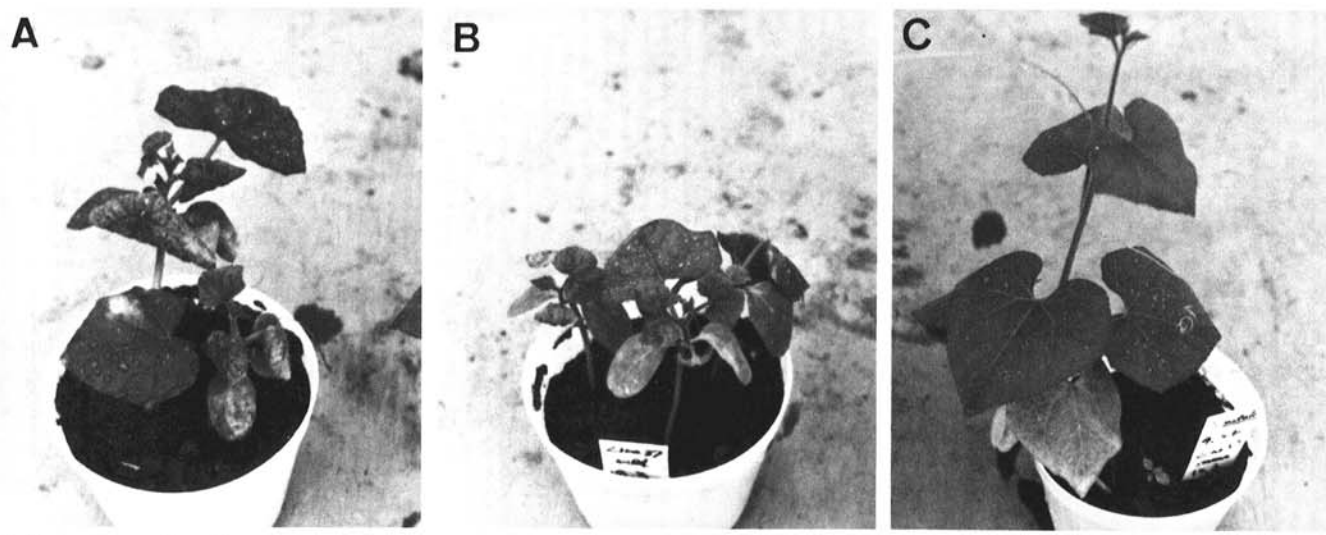

Fig. 1. Systemic mosaic and necrosis symptoms caused by the mild strain PRV HA 6-1 on **A,** F_1 plants of *C. metuliferus* PI 292190 × Acc. 2459 and, **B,** plants of *C. metuliferus* line PI 292190, and, **C,** no symptoms on a plant of *Cucumis metuliferus* line PI 292190 after inoculation with the severe strain PRV HA.

were prepared from PRV TM-infected *C. metuliferus* line Acc. 2459 (10 ml of 0.01 M phosphate buffer, pH 7.0, per gram of tissue) and their infectivity was determined by the local lesion assay on *C. quinoa*. Protection or superinfection was judged from the appearance of symptoms in the test plants kept in the greenhouse at least for 3–4 mo after the challenge inoculation.

RESULTS

Reactions of different genotypes of *C. metuliferus* to severe and mild strains of PRV. Reactions of PRV HA, HA 5-1, and HA 6-1 in the susceptible *C. metuliferus* line Acc. 2459, the resistant line PI 292190, and the F_1 plants of Acc. 2459 × PI 292190 are summarized in Table 1. The results confirmed that PRV HA infects papaya and line Acc. 2459 with severe systemic mosaic, but does not infect line PI 292190 and the F_1 progeny of Acc. 2459 × PI 292190 (5,12). PRV HA 5-1 and HA 6-1 have been reported causing symptomless or mild infection in papaya and *C. metuliferus* line Acc. 2459 (9,11), but in this study they incited symptoms of systemic mosaic and necrosis on plants of line PI 292190 and the F_1 plants of Acc. 2459 × 292190 (Fig. 1). The systemic infection of PRV HA 5-1 and HA 6-1 in the resistant genotypes of *C. metuliferus* was confirmed by strong positive reactions in DAS ELISA when antiserum to PRV was used. Moreover, papaya plants inoculated with the virus prepared from systemically infected plants of line PI 292190 or F_1 progeny showed typical mild or symptomless infection of PRV HA 5-1 or HA 6-1.

Reactions of F_2 population of Acc. 2459 × PI 292190. Reactions of the severe PRV HA and its nitrous-acid induced mild strain PRV HA 6-1 on clones of F_2 population of Acc. 2459 × PI 292190 were also investigated (Table 2). A total of 128 F_2 plants were tested in spring and summer of 1986. Among them, 97 plants were immune to PRV HA and 31 plants produced severe mosaic. All the clones corresponding to the 97 plants that were immune to PRV HA reacted to PRV HA 6-1 with severe mosaic and necrosis symptoms; and those severely infected by PRV HA with mosaic symptoms reacted to PRV HA 6-1 with mild vein-clearing or symptomless infection that were confirmed by DAS ELISA by using antiserum to PRV. The reactions of severe mosaic and necrosis with HA 6-1 were perfectly matched to the segregation ratio of 3:1 for the *Wmv* gene (Table 2).

PRV isolates generated by nitrous acid induction and host selection. PRV treated with nitrous acid was first propagated in the susceptible *C. metuliferus* line Acc. 2459, and symptoms of systemic mosaic were observed 8–10 days after inoculations. The virus was then transferred to the resistant line PI 292190. Several plants developed systemic mosaic and necrosis, mosaic with yellowing, or mosaic with stunting 5–8 days after inoculation. From three different trials a total of 20 plants of the resistant line PI 292190 were systemically infected with PRV as shown in Table 3. However, systemic infection was not observed when the untreated PRV TM was cultured in plants of line Acc. 2459 and transferred to the line PI 292190 in the same way (Table 3). The virus in the plants of line PI 292190 was further screened by repeated serial dilutions on the line PI 292190. The putative pure lines of the virus were then transferred to the papaya seedlings. Among these, 13 isolates caused typical severe symptoms of mosaic and stunting on papaya, six isolates caused mild mottling without stunting, and one particular isolate, designated as PRV TM-1, caused systemic infection on papaya without conspicuous symptoms.

Cross-protection effectiveness of the mild strain PRV TM-1 in papaya. PRV TM-1 did not incite local lesions on *C. quinoa* and so it was further purified six times by repeated serial dilutions. Two isolates designated as NH1 and NH2 were selected for their long-term stability. They did not cause severe symptoms on papaya for 3 mo and PRV TM-1 NH2 was used for cross-protection tests.

Before each challenge inoculation, mild infection caused by PRV TM-1 NH2 was confirmed by positive reactions in DAS ELISA. The virus titer in each challenge inoculum was extremely

TABLE 1. Host reactions of papaya ringspot virus (PRV) HA and its nitrous-acid induced mutants HA 5-1 and HA 6-1 on papaya and different genotypes of *Cucumis metuliferus* under greenhouse conditions

	Reactions after inoculation with[a]					
	PRV HA		HA 5-1		HA 6-1	
Host	Symptoms	ELISA	Symptoms	ELISA	Symptoms	ELISA
Carica papaya						
Tainung No. 2	SM	+	0	+	0	+
Cucumis metuliferus						
Line Acc. 2459	SM	+	0	+	0	+
Line PI 292190	0	−	SMN	+	SMN	+
F_1 of Acc. 2459 × PI 292190	0	−	SMN	+	SMN	+

[a]SM = Severe mosaic, SMN = Systemic mosaic and necrosis, 0 = No conspicuous symptoms. At least 10 plants were used for each treatment. Antiserum to PRV was used in ELISA (2,3).

TABLE 2. Reactions of clones of identical genotypes from an F_2 population of *Cucumis metuliferus* Acc. 2459 × PI 292190 to a severe strain of PRV HA and its nitrous-acid induced mild strain PRV HA 6-1[a]

	No. of original plants inoculated with HA 6-1		No. of cutting plants inoculated with HA	
Trials	Systemic mosaic & necrosis	Symptomless or vein-clearing	Immune	Severe mosaic
I (April, 1986)	41	14	41	14
II (July, 1986)	56	17	56	17
Total	97	31	97[b]	31[c]
Expected segregation ratio for *Wmv* gene (3:1)	96	32	96	32
Goodness of fit		(P value = 0.950)		

[a]Each F_2 plant was vegetatively propagated from cuttings to generate two clones of identical genotype. One clone was inoculated with PRV HA 6-1 and the other was inoculated with PRV HA.
[b]All the clones derived from the original plants, which showed systemic mosaic and necrosis symptoms with HA 6-1 were immune to PRV HA. Systemic infection or immunity was confirmed by DAS ELISA.
[c]All the clones derived from the original plants which showed symptomless or vein-clearing with HA 6-1 showed severe mosaic with HA.

TABLE 3. Selection of papaya ringspot virus (PRV) isolates systemically infecting *Cucumis metuliferus* line PI 292190 by nitrous-acid mutagenic treatment and host passage through *C. metuliferus* line PI 292190

	No. of virus isolates systemically infecting line PI 292190	Symptoms on papaya[a]		
Trials		Severe mosaic and stunting	Mild mottling without stunting	No conspicuous symptoms
1	5/18[b]	3	1	1
2	10/24	6	4	0
3	5/20	4	1	0
Total	20/62	13	6	1
Untreated control	0/60[c]	...	...	...

[a]After repeated fivefold serial dilution on line PI 292190, the putative mutants were mechanically introduced to papaya.
[b]Number of plants systemically infected per total number of plants tested.
[c]When the untreated PRV TM was cultured in *C. metuliferus* line Acc. 2459 and transferred to line PI 292190, no systemic infection was recorded from a total of 60 plants tested.

high, since the same inoculum induced more than 200 local lesions per leaf on *C. quinoa*. Papaya plants infected with PRV TM-1 NH2 were completely protected against PRV TM when challenge inoculations were made 23, 45, or 90 days after the protective inoculation, respectively. Each challenge treatment included 15 plants preinoculated with PRV TM-1 NH2, and none of the challenged plants developed severe symptoms 120 days after the respective challenge inoculations. However, papaya plants mock-inoculated with buffer first and reinoculated with PRV TM at the same time intervals developed severe symptoms 10–20 days after the inoculation (Fig. 2A and B).

DISCUSSION

Reactions of different genotypes of *C. metuliferus* to the severe strain PRV HA and its nitrous-acid induced mild strains PRV HA 5-1 and HA 6-1, which were defined as mild strains according to their pathogenicity on papaya, clearly showed that the resistant line PI 292190 was immune to PRV HA but was severely infected by the mild strains with systemic mosaic and necrosis symptoms. Positive reactions in ELISA with PRV antiserum and mild infection on papaya using inocula prepared from the systemically infected tissues of resistant line PI 292190 and F_1 progeny of Acc. 2459 × PI 292190 indicated that systemic mosaic and necrosis symptoms were associated with infection by the mild strain of PRV.

The ability of PRV HA 6-1 to overcome the resistance of *Cucumis metuliferus* was perfectly associated with the segregation of the *Wmv* gene in F_2 population, indicating that the systemic mosaic and necrosis symptoms induced by the PRV HA 6-1 are direct interactions between the mild strain and the *Wmv* gene. Similar 3:1 segregation was also observed when PRV HA 5-1 was introduced to the plants of F_2 seedlings (data not shown). Clones of identical genotypes from individual F_2 plants were not used to analyze the reactions to PRV HA and HA 5-1.

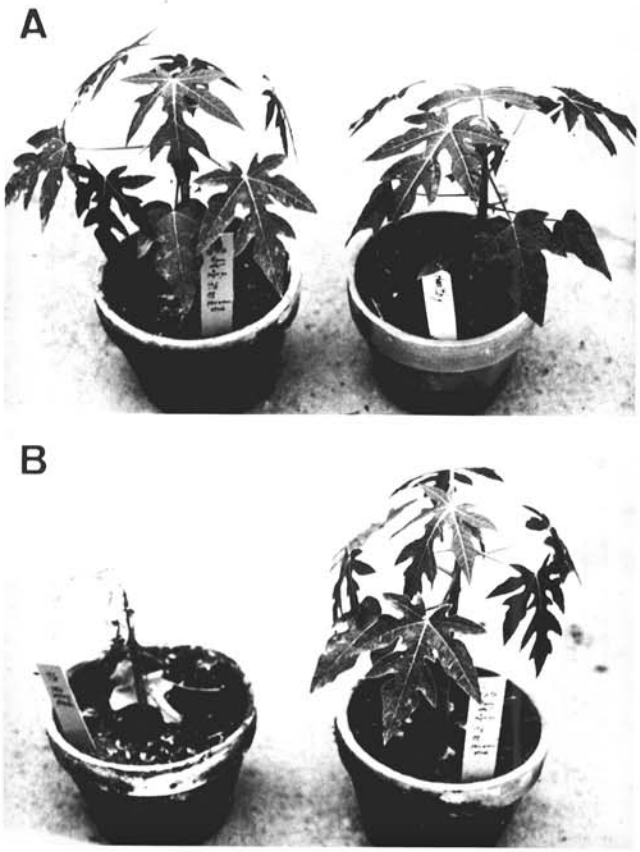

Fig. 2. Cross-protection effectiveness in papaya of the mild strain PRV TM-1 NH2 against the most prevalent severe strain PRV TM from Taiwan. **A,** Left, a papaya plant infected with PRV TM-1 NH2 showing no conspicuous symptoms; right, healthy control. **B,** Left, a papaya plant mock-inoculated with buffer and reinoculated with PRV TM showing severe mosaic and stunting; right, a papaya plant first inoculated with PRV TM-1 NH2 and challenged with PRV TM at 23 days after the protective inoculation showing no reduction in growth 45 days after the challenge inoculation.

Both PRV HA 5-1 and HA 6-1 were mild strains derived by nitrous acid induction, and caused symptomless or mild infection in papaya (11). However, this study showed that these strains have the ability to overcome the resistance in *C. metuliferus* line PI 292190, which the parent severe strain can not infect. The mutation in PRV HA 5-1 and HA 6-1 may occur in such a way that the virulence toward papaya is diminished, but the ability to overcome the resistance conferred by the *Wmv* gene is acquired. Thus the *Wmv* gene in *C. metuliferus* and the mildness of symptoms in papaya caused by PRV HA 5-1 and HA 6-1 provide an excellent system for studying host-pathogen interactions.

Based on the assumption that the the mildness of the virus for infecting papaya is correlated to the ability to overcome the resistance in *C. metuliferus*, it became possible to use the resistant line PI 292190 to screen the nitrous-acid induced mutants that infect the resistant line systemically and might be as mild as PRV HA 5-1 and 6-1 for infecting papaya. When virus after nitrous acid treatment was directly transferred to line PI 292190, no systemic infection was observed (data not shown). The virus after mutagenic treatment was therefore propagated in the susceptible line Acc 2459 first and in this case mutants that could infect the line PI 292190 systemically had a chance to become established. The passage through the line PI 292190 could select the possible mutants very efficiently from millions of virions in the population. This unique system provides a good alternative for the selection of attenuated stains after nitrous acid treatment in addition to the single-lesion-isolation method (11).

In this investigation, PRV isolates that could infect the resistant line PI 292190 systemically were easily selected. Because no plants of PI 292190 were systemically infected when the untreated PRV TM were processed in the same way (Table 3), we strongly believe that these virus isolates are mutants derived from nitrous acid mutagenesis. However, most isolates still produced severe symptoms on papaya. A few isolates did cause atttenuated symptoms of mild mottling on papaya without reduction in plant size, but they were not mild enough for practical application. The techniques used for selection and subculture do not rule out the possibility that the virus obtained might be a mixture of different mutants even after repeated serial dilutions. Complementation of and interactions between different mutations might be one of the reasons to explain the severe infection in papaya.

Because only one out of 20 isolates selected caused mild infection in papaya, another possible explanation for this is that the ability to overcome the resistance in *C. metuliferus* is not necessarily correlated to the mildness in papaya. Even though our untreated controls did not result in any systemic infection in the line PI 292190, the possibility of host passage effects in addition to nitrous-acid mutagenesis for obtaining the mild strain can not be completely ruled out.

Nevertheless, the isolate PRV TM-1 was selected for its ability to infect the resistant line PI 292190 and its putative pure isolate PRV TM-1 NH2 caused mild infection in papaya without conspicuous symptoms. This isolate also caused mild infection with vein cleaning or vein banding in line Acc. 2459 and infected F_1 plants of PI 292190 × Acc. 2459 with systemic mosaic and necrosis symptoms (data not shown). These observations coupled with the inability to produce local lesions on *C. quinoa* are similar to the characteristics of the mild strains PRV HA 5-1 and 6-1 (11). There is a possibility that the loss of pathogenicity on papaya, inability to produce local lesion on *C. quinoa*, and the ability to overcome *Wmv* gene may actually arise from the same virus mutation.

Under greenhouse conditions, papaya plants preinoculated with PRV TM-1 NH2 were completely protected against the severe parent strain PRV TM. Also, PRV TM-1 NH2 protected papaya plants against PRV TM and PRV TW, two prevalent severe strains

in Taiwan, much better than PRV HA 5-1 did (Yeh, S.D., *unpublished data*). PRV HA 5-1 and 6-1 have been used widely in Taiwan for control of PRV by cross protection (9,13); however, breakdown did occur under some field conditions. One explanation for this is that the severe strain in Taiwan may not be closely related to PRV HA 5-1, which was generated from a Hawaii strain (11). Thus, PRV TM-1 NH2 may be a better choice than PRV HA 5-1 as a protective strain in Taiwan. Further evaluation for its long-term stability and effects on horticultural properties of papaya is needed to evaluate its potential for practical application.

In summary, this study showed that the PRV-resistant *C. metuliferus* can be used as a selection host for screening attenuated strains of PRV after nitrous-acid treatment. The mechanism involved in this unique system needs to be studied further.

LITERATURE CITED

1. Cook, A. A. 1972. Virus diseases of papaya. Fla. Agric. Exp. Tech. Bull. 750. 19 pp.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Gonsalves, D., and Ishii, M. 1980. Purification and serology of papaya ringspot virus. Phytopathology 70:1028-1032.
4. Mundry, K. W. 1959. The effect of nitrous acid on tobacco mosaic virus: Mutation not selection. Virology 9:722-726.
5. Provvidenti, R., and Gonsalves, D. 1982. Resistance to papaya ringspot virus in *Cucumis metuliferus* and its relationship to resistance to watermelon mosaic virus 1. J. Hered. 73:239-240.
6. Provvidenti, R., and Robinson, R. W. 1977. Inheritance of resistance to watermelon mosaic virus 1 in *Cucumis metuliferus*. J. Hered. 68:56-57.
7. Purcifull, D. E., Edwardson, J., Hiebert, E., and Gonsalves, D. 1984. Papaya ringspot virus (revised). No. 292 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst. Assoc. Appl. Biol. Kew, Surrey, England.
8. Siegel, A. 1965. Artificial production of mutants of tobacco mosaic virus. Adv. Virus Res. 11:25-60.
9. Wang, H. L., Yeh, S. D., Chiu, R. J., and Gonsalves, D. 1987. Effectiveness of cross-protection by mild mutants of papaya ringspot virus for control of ringspot disease of papaya in Taiwan. Plant Dis. 71:491-497.
10. Yeh, S. D., and Gonsalves, D. 1984. Purification and immunological analysis of cylindrical inclusion protein induced by papaya ringspot virus and watermelon mosaic virus 1. Phytopathology 74:1273-1278.
11. Yeh, S. D., and Gonsalves, D. 1984. Evaluation of induced mutants of papaya ringspot virus for control by cross protection. Phytopathology 74:1086-1091.
12. Yeh, S. D., Gonsalves, D., and Provvidenti, R. 1984. Comparative studies on host range and serology of papaya ringspot virus and watermelon mosaic virus 1. Phytopathology 74:1081-1085.
13. Yeh, S. D., Gonsalves, D., Wang, H. L., Namba, R., and Chiu, R. J. 1988. Control of papaya ringspot virus by cross protection. Plant Dis. 72:375-380.