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Control of Papaya Ringspot Disease by Cross Protection

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ABSTRACT

Papaya ringspot disease is considered as the most destructive disease of papaya in Thailand. Papaya ringspot disease caused by papaya ringspot virus (PRV) which belongs to potyvirus group. Efforts to select naturally occuring mild strain PRV began in the late 1986 by field collection and serial dilution isolation were successful. Eight isolates of mild strain PRV were obtained from this study. Papaya seedlings inoculated with these mild strains remained symptomless or showed mild mottling with no reduction in plant size and leaf-shap. The mild strains designated as PRV-C1 and PRV-F1 gave better protection than the others against challenge inoculation with a severe strain (PRV-SD) under greenhouse conditions. Protection was also observed when the pre-immunized papaya seedlings with the mild strain PRV-F1 were transplanted into farmer fields. The results revealed that the natrually collected mild strain of PRV could be used for cross protection of papaya ringspot virus.

INTRODUCTION

Papaya ringspot virus (PRV), a member of potyvirus group (Purcifull *et al*,. 1984) is the major limiting factor for papaya production in Thailand (Nimmanpatcharin, 1985). Papaya ringspot disease was first reported in the northeastern part of Thailand in 1975 (Srisomchai, 1975). Recently, the disease was observed in at least 29 provinces throughout Thailand (data not shown). Several unsuccessful attempts have been conducted to develop effective control methods for PRV. A roguing program has been campaigned and practiced in the northeastern part of Thailand with a very limited successful (Sirithorn *et al*., 1989). The healthy papaya seedings became infected with PRV after replanting for 3 months. The unsuccessful of roguing program may account for the disease is endemic and the eradication of the naturally infected hosts, such as cucurbits, from the area is not possible (Kositratana *et al*., 1988). At the present time, PRV-resistant papaya cultivars have not been available to the farmer although the tolerant cultivars have been introduced into Thailand. Host range of PRV is limited to genera in the dicotyledonous families caricaceae, chenopodiaceae, and cucurbitaceae (Purcifull *et al*., 1984). PRV is transmitted by aphids in a nonpersistent manner, which this character the control of this disease through insecticide spray is not possible. Thus, the unavailability of PRV-resistant papaya cultivars and the restricted host range of PRV make cross protection to be selected method for controlling this disease (Yeh and Gonsalves, 1984) in the immediate work plan of the project.

The purpose of this study was to search for a mild strain (or strains) of PRV and used as cross protectant for control of papaya ringspot disease.

MATERIALS AND METHODS

Selection of Mild strain of PRV

Field collection: The attempt to obtain mild strains of PRV was made by selection of nutural ocurring isolates from papaya plants with the mildest symptoms in heavily infected papaya orchards in Ratchaburi province. Seventy-four samples were collected, mechanically inoculated to papaya seedlings "KAEK DAM" and kept in the insect-proved screenhouse. Isolates were evaluated by observing symptom development. An indirect enzyme-linked immunosorbent assay (ELISA) (Clark and Adams,

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1977) and Dot-immunobinding assay (Gumpf *et al.*, 1984) were employed to check for the presence of PRV.

Serial dilution for mild strain isolation: From our preliminary work, it was shown that PRV isolates from a location in the Central Region of Thailand (Ratchaburi) did not produced local lesion on a indexing host (*Chenopodium quinoa* willd.) as its previously reported by Yeh and Gonsalves (1984). Serial dilution technique was employed in order to isolate the individual strain from the other. Crude sap of papaya infected with isolates of PRV was diluted into two-folds serial dilution, and each dilution was inoculated to 10 papaya seedlings at the four-to-six leaf stage. Inoculated plants were rinsed immediately with tap water and kept in the greenhouse for further investigation. Mild strain isolates were evaluated by symptomless development. Inoculated papaya seedlings which shown symptomless were further invertigated for the presence of the virus by ELISA and Dot-immunobinding assay (DIBA) tests.

Cross Protection Effectiveness of the Mild Strains

Under greenhouse conditions : Papaya seed lings at the four-to-six leaf stage were mechanically inoculated with mild strain PRV prepared from infected tissue of papaya leaf (10 ml of 0.05 M potassium phosphate buffer, pH 7.2, per gram of tissue) and challenged by inoculation with the severe strain PRV. An indirect enzyme-linked immunosorbent assay (ELISA) (Kositratana *et al.*, 1987; Lommel *et al.*, 1982) was used to confirm the infection by the mild strain. Challenge inoculation was performed mechanically with the severe strain (PRV-SD). The challenge inocula were extracted from PRV-infected papaya as above. To determine the effect of time of challenge inoculation on cross protection, papaya seedlings preimmunized with each mild strain were mechanically challenge inoculated with PRV-SD at 15,20, 25,30 and 40 days after the initial inoculation, on the last three fully expanded apical leaves (Yeh and Gonsalves, 1984). Four from eight of isolated mild strains, namely PRV-B2, -C1, -F1, and -G1, were tested for cross protection effectiveness. The test plants were kept in a screenhouse for four months and observed periodically.

Farmers' field trials : The mild strain PRV-F1 was used in this study. The virus was maintained and propagated in zucchini plants (*Cucurbita pepo*) and kept in a screenhouse. The infected leaf was served as an inoculum for pre-immunization of papaya seedlings. Papaya seedling of KAEK DAM, Thailand's most popular commercial cultivar, were grown under screenhouse conditions in plastic bags 8 x 10 inch (20.32 x 25.40 cm) containing a commercial mixed soil. Six to ten seedlings were planted in each bag. At the four-to-five leaf stage, papaya seedlings were mechanically inoculated with PRV-F1 prepared from infected leaves of *C. pepo* by hand rubbing onto the carborundum dusted leaves. Inoculated seedlings were kept in a screenhouse and assay for infection by an indirect enzyme-linked immunosorbent assay (ELISA) as previously described, after inoculation for 3-4 weeks. Plants with positive ELISA reading were transferred and transplanted into farmer's testing fields. Seedings raised under the same conditions and mock-inoculated with 0.05 M potassium phosphate buffer (pH 7.2) only, were served as controls.

The field tests were conducted from November 1987 to April 1988 in Ratchaburi and from November 1988 to July, 1989 in Suphan Buri provinces, where papaya is commercially grown and PRV is the serious problem.

1. Ratchaburi province. The testing plot was located in Amphur Wat Phleng, where the severely infected papaya orchards surrounded the testing plot. Pre-immunized and non-immunized papaya seedlings were planted in between the rows of farmers' papaya plants which the severe symptoms caused by PRV were already observed. Seedlings were planted in a randomized block design, consisted of two treatments, pre-immunized seedlings and control seedlings. Each treatment consisted of 75 plants and in the total of 450 plants. Tests plants that became severely infected were not rogued.

2. Suphan Buri province. The testing plot was located in the serverly infected area with the heavily infected plot adjacent to the testing plot. Pre-immunized and non-immunized seedlings were planted in a randomized block design with each treatment consisted of 120 plants. The total of 720 plants were used in this test.

RESULTS AND DISCUSSION

Selection of Mild Strain of PRV

Field collection of seventy-four samples from papaya plantation located in Damnoen Saduak, Ratchaburi province caused severe symptoms on papaya 15 days after inoculation except three samples that showed mild symptom or were symptomless. These three samples were further isolated by serial dilution in order to isolate a mild strain PRV from field population.

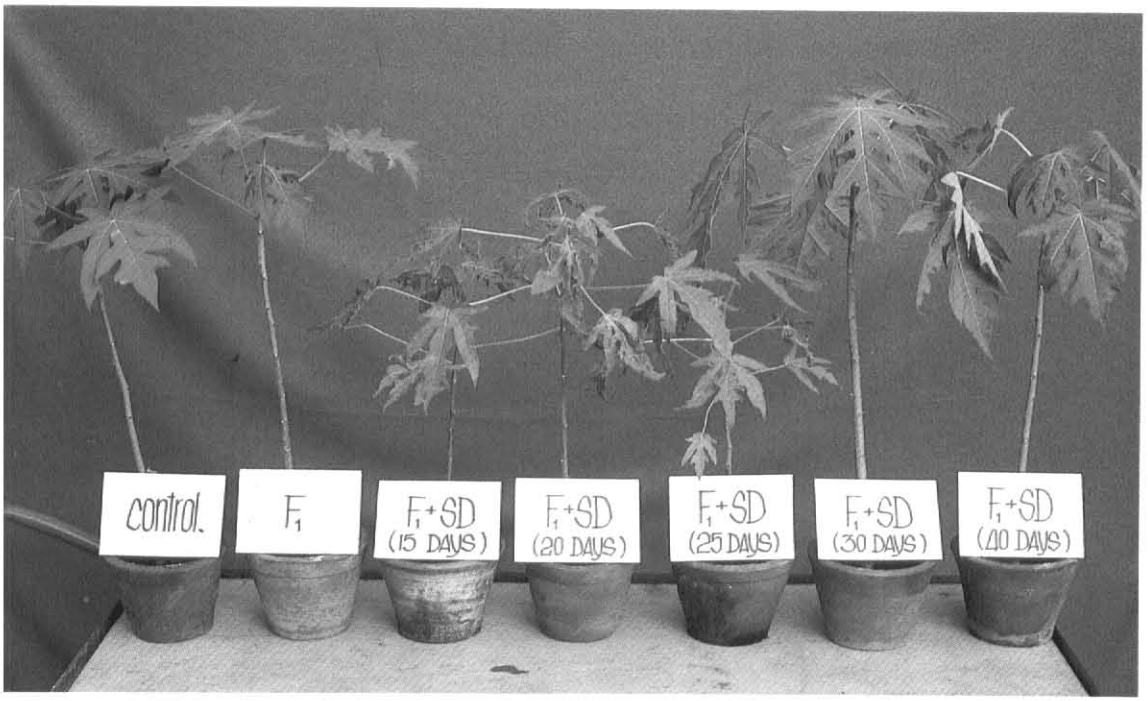

Figure 1 Cross protection effectiveness of PRV-F1 (mild strain) against PRV-SD (severe strain) in papaya : (left to right) healthy control plant; plant infected with PRV-F1 alone; plant preimmunized with PRV-F1, then challenged with PRV-SD at the different time intervals of 15, 20, 25, 30 and 40 days after the initial inoculation, respectively.

Serial dilution for mild strain PRV isolation: A total of 300 papaya seedlings were inoculated with serially diluted crude sap, for each of selected symptomless samples. Various degree of symptom severity appeared on these papaya seedlings. Attention was focused on papaya seedlings that did not show any prominent symptom but were ELISA and DIBA positive. The symptomless plants with the presence of PRV were re-inoculated through serial dilution for several cycles. Eight isolates of PRV were selected and shown sufficiently mild symptoms or symptomless for practical application. The mild strains PRV were maintained in papaya seedlings and kept the stock of inoculum in the form of $CaCl_2$-dehydrated tissue under refrigeration. At the present time, our selected mild strains of PRV were still caused symptomless after test inoculations to papaya seedlings for many cycles during the past four year.

Cross Protection Effectiveness of the Mild Strains Under greenhouse conditions: The results of cross protection in papaya seedlings among symptomless strains, PRV-B2, -C1, -F1 and -G1, and the severe strain, PRV-SD after challenge inoculation at different time intervals are shown in Table 1. The degree of effectiveness was observed among mild strains of PRV. When challenge inoculation were performed before 20 days after initial inoculation, protection was slightly low. However, when the time intervals were increased to 25,30 or 40 days, severe symptoms of PRV-SD were either delayed or not expressed. A high proportion (up to 90 %) of the test plants remained symptomless even 100 or 120 days after challenge inoculation when the time interval was increased to 25,30, and 40 days (Figure 1). Cross protection effectiveness was affected by the time of challenge inoculation (Yeh and Gonsalves, 1984). The results indecated that if the challenge inoculation was made before 20 days after initial inoculation with mild strain, a high number of test plants shown severe symptoms was observed. The different degree of cross protection effectiveness was also markly observed in papaya seedlings which preimmunized with PRV-C1 and PRV-F1, only 10-20 % showed severe symptoms even 100 or 120 days after challenge inoculation when the time interval was increased to 30 or 40 days.

Farmer fleld trials :

In Ratchaburi province, the testing plot was

Figure 2 Cross protection effectiveness test under field conditions in Ratchaburi province;
A: Test plants were grown in between farmer's papaya plants,
B: Close-up of pre-immunized plant with mild strain PRV-F1, 5 months after transplanting show normal growth and no leaf malformation;
C: unprotected, control plants in the same plot show severe mosaic, stunting and leaf malformation.

surrounded by diseased papaya orchards and moreover the border rows were severely infected with severe strain of PRV. This provided a high disease pressure to pre-immunized papaya plants. Non-immunized plants developed severe symptoms two months after transplanting, and reached 75 % of severe infection by the fourth months after transplanting (Tabel 2). Pre-immunized plants developed severe symptoms 2.5 %, 5 % and 6 % after transplanting for 4, 5 and 6 months, respectively. Non-immunized

Table 1 Cross protection effectiveness of different isolates of mild strain PRV against a severe strain (PRV-SD) in papaya seedlings after mechanical challenge at different time intervals [1]

Days after challenge inoculation	Papaya plants (no.) that showed severe symptoms after challenge at days				
	15	20	25	30	40
PRV-B2					
0	0	0	0	0	0
20	4	6	6	4	2
40	8	6	6	6	2
60	10	8	6	8	4
80	10	8	8	8	6
100	16	8	8	8	8
120	18	10	12	10	8
PRV-C1					
0	0	0	0	0	0
20	0	0	0	0	0
40	2	2	2	2	2
60	6	2	2	2	2
80	8	6	2	2	2
100	8	8	2	2	2
120	12	8	6	4	2
PRV-F1					
0	0	0	0	0	0
20	6	6	4	2	2
40	8	6	6	2	2
60	12	10	6	4	2
80	12	10	6	4	2
100	14	12	8	4	2
120	16	16	10	4	4
PRV-G1					
0	0	0	0	0	0
20	0	2	2	2	0
40	6	4	4	4	2
60	12	8	6	4	2
80	14	14	10	6	6
100	18	14	14	6	6
120	18	16	16	8	6

1 In each treatment 20 papaya seedlings were used. Ten papaya seedlings inoculated with PRV-SD (severe) at different time intervals after mock inoculation with buffer showed severe symptoms 20 days later. Papaya seedlings infected with mild strain PRV alone did not show severe symptoms during the tested period.

plants showed severe infection about 75 % whereas the other control plants did not show any symptom. This result was possibly due to the transmission of mild strain PRV from pre-immunized plants to control plants by aphid vector that enable them to cross protect against the severe PRV (Wang *et al.*, 1987). However, we were unable to clarify this point due to the lack of specific diagnostic tool to distinguish mild strain PRV from severe strain PRV. Pre-immunized plants gave good protection against natural infection of severe starin PRV in this location whereas the overall performance of the control plants was poor (Figure 2). Unfortunately, we were unable to continue the experiment because the farmers changed their crop in the testing plot. Under these conditions, pre-immunized plants were able to grow normally at least for six months after trainsplanting. This data lead us to presume that the pre-immunized plants should be able to provide the good fruit quality and yield at least from the first flowering set whereas the non-protected plants showed severe symptoms before the flowering stage. With this available data, we currently distributed the mild strain PRV especially PRV-F1 to papaya growers in several provinces including

Table 2 Cross protection effectiveness of mild strain papaya ringspot virus (PRV-F1) under field conditions in Ratchaburi province from November 1987 to April 1988.

After transplanting	Severe disease incidence (%)	
(month)	Pre-immunized[1]	Control[1]
4	2.48	74.51
5	4.95	74.51
6	6.08	75.50

1 average from 225 plants

Ratchaburi, Kanchanaburi, Nakhon Pathom and the northeastern part of Thailand.

In Suphan Buri province, the overall results were not dramatically distinct between pre-immunized plants and control plants as that in the Ratchaburi experiment. Disease expression in pre-immunized plants were delayed and the symptom expression level was lower than that of the control. In this case, the test plants grew under water and temperature stresses for a long period (3-4 months) in the hot season. Nearly all of test plants had severe symptoms within 6 month after transplanting. However, the protected plants showed mild mosaic symptoms and the flowers were able to develop fruit set whereas the control showed yellowing, more severe mosaic and oily streak on stem and petiole after the test plants received water in the first month of the rainy season. We were unable to collect the data on fruit quality and yield from the testing plot.

The failure of cross protection at Suphan Buri province could be attributed to several factors. Papaya plants poorly grew and developed which the results from lack of irrigated water and farm management. The test plot was a open field type and closed to infected papaya orchards. The aphid vectors may have better chance to transmit the disease from the nearby infected field. The inter-cropping with a high stem barrier crop such as corn and banana may help to reduce the challenge pressure (Sirithorn *et al.*, 1989; Yeh *et al.*, 1988).

In these two on-farm experiments, pre-immunized papaya plants were able to delay and reduce the symptom expression which caused by severe strain PRV (Yeh *et al.*, 1988). Several unexpected problems were faced. For example, we were unable to collect the full data for statistical analysis from farmer's fields. In the opposite view, we feel strongly that on-farm experiments gave the direct technology transfer to farmer eventhough we could not fullfill our academic acheivement on the statistical analysis. We were unable to do this kind of experiment on campus field trial because the papaya ringspot disease was not observed around the Kamphaeng Saen campus.

More studies are needed to evaluate the other four isolates of mild strain PRV, to monitor the mild strains in the field, to compare the effectiveness of cross protection under different conditions of farm management, to correlate the failure of cross protection to the alate aphid population, plant vigor and inoculum pressure, and most important in the present time, to develop a mass inoculation method for the production of pre-immunized papaya seedlings.

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LITERATURE CITED

Clark, M.F. and A.N. Adams. 1977. Characteristics of the microplate method of enzyme-liked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.

Gumpf, D.J., W. Kositratana and G.Y. Zheng. 1984. Dot-immunobinding assay for virus detection. Phytopathology 74:847. (Abstr.)

Kositratana, W., V. Korpraditskul, S. Attathom, N. Thaveechai, S. Burikam and O. Chatchawankanphanich. 1988. Production and multiplication of disease-free planting stock of papaya, pp. 45-53. *In*: KU-ACNARP Cropping Programmes and Agricultural Topic Research Technical Report 1987-1988, Faculty of Agriculture, Kasetsart University, Bangkok.

Kositratana, W., S. Attathom, V. Korpraditskul, R. Hongprayoon and R. Visessuwan. 1987. Production and multiplication of disease-free planting stock of papaya, pp. 72-81. *In* KU-ACNARP Cropping Programmes and Agricultural Topic Research Technical Report 1986-1987. Faculty of Agriculture, Kasetsart University, Bangkok.

Lommel, S. A., A.H. McCain, T.J. Morris. 1982.

Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology 72:1018-1022.

Nimmanpatcharin, R. 1985. Partial purification, serology and ultrastructure studies of papaya ringspot virus. M.S. Thesis, Kasetsart University, Bangkok, Thailand.

Purcifull, D.E., J. Edwardson, E. Hiebert and D. Gonsalves. 1984. Papaya ringspot virus (revised). No. 292. *In* : Descriptions of plant viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol. Kew, Surrey, England.

Sirithorn, P., A. Hirunsalee, S. Ruaysoongnern, N. Sonoamuang, V. Nilbunpot, and S. Boonkaew. 1989. Study on suitable control measures of some important diseases of papaya in the Northeast. Final Report submitted to USAID/KKUDI, Khon Kaen, Thailand. 119 p.

Srisomchai, T. 1975. Studies on papaya ringspot virus, pp. 228-232. In : Annual Report of the Northeast Regional Office of Agriculture, Tha Phra, Khon Kaen, Thailand.

Wang, H.L., S.D. Yeh, R. J. Chiu, and D. Gonsalves. 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.

Yeh, S. D., D. Gonsalves, H. L. Wang, R. Namba and R. J. Chiu. 1988. Control of papaya ringspot virus by cross protection. Plant Dis. 72:375-380.

Yeh, S.D. and D. Gonsalves. 1984. Evaluation of induced mutants of papaya ringspot virus for control by cross protection. Phytopathology 74: 1086-1091.