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Response of Papaya Cultivars to Inoculation with the SMN Papaya Ringspot Viral Strain

LOONG-SHENG CHANG[1]

Abstract

Field trials with a wide range of papaya *(Carica papaya)* cultivars inoculated with the severe mottle and necrosis (SMN) papaya ringspot virus (PRV) were evaluated with no control of natural aphid populations. It revealed significant differences in disease severity, ELISA readings, and plant growth rate. Survival rate and symptom development significantly differ among genotypes. 'Sunrise,' 'Mardi' and 'Ostream' were mostly susceptible to SMN strain of PRV. There were no marketable yield in the study. Different sources of 'Cariflora' lines performed similarly. These cultivars tolerated the SMN viral strain, in agreement with reported tolerance in Florida. Monoclone antibodies were applied to distinguish viral strains in healthy looking plants seven months after planting. The extracted sap of healthy-looking plants was inoculated into susceptible hosts to detect plants responding to specific viral infection. This procedure showed that infection of healthy-looking plants was due to severe virulent strains of SMN and severe mottle and deformed (SMD) ringspot virus.

Introduction

Papaya ringspot virus (PRV) is the most serious disease reducing papaya tree life in Taiwan and other papaya production areas (1, 2, 8, 9, 10, 14, 16, 20). The viral pathogen (PRV) enters papaya plants by aphid inoculation (10, 11), and once established in the tissue, the virus symptoms include systemic mottle and necrosis of the leaves, stunt of trees, and eventually death of the tree within one to two years (3, 11, 13). Prior to plant death, leaf losses due to mottle, necrosis, and deformation results in greatly reduced fruit production and fruit quality.

Cook and Zettler (8) inoculated 90 papaya lines and found all to be susceptible after inoculation. Conover (4) reported significant differences in susceptible of 66 papaya cultivars to papaya distortion virus. 'Cariflora,' a cultivar bred in Florida is tolerant to papaya ringspot virus in South Florida and Caribbean (7). Wang (17) evaluated 53 papaya cultivars in the field without artificial inoculation. FL-77-5 and 'Costa Ricoh' lines were selected and used to develop the Tainung No.5 cultivar which is tolerant to papaya ringspot viral disease. Although papaya cultivars differ in their level of tolerance to PRV, no highly tolerant selec-

[1]Department of Horticultural Science, National Taiwan University, Taipei, Taiwan, R.O.C.

tion has yet been identified (5, 6, 16). Cross protection by inoculation with less virulent strains of PRV has been used to a limited extent since 1980 in Taiwan (18, 19, 20). Instead, for commercial production of papaya in Taiwan, trees are protected from aphid inoculation by cultivation under net house. The fruit quality is inferior in these systems because of insufficient light levels.

Four papaya ringspot viral strains have been characterized in Taiwan as (1) severe mottle and necrosis (SMN), (2) severe mottle and deformation (SMD), (3) severe mottle (SM), and (4) mild mottle (M) (12, 13). The most virulent strain was SMN papaya ringspot virus. It generally causes susceptible papaya host stunting and eventually death of trees. As discussed, previous research has tested the response of papaya cultivars to PRV. However, these studies were conducted with a mild strain of PRV (4). Limited information on the horticultural traits and host/strain interaction after inoculation of different virulent strains. The objective of this investigation was to evaluate the response of papaya to inoculation of SMN strain of papaya ringspot virus.

Materials and Methods

In summer 1991, 22 papaya cultivars of diverse background, two *Carica pubesens* and one *C. goudotiana* were inoculated in a randomized complete block design at the greenhouse of National Taiwan University, Taipei, Taiwan. Germplasm was received from a variety of sources. 'Florida' was collected from a commercial planting in Taiwan. 'Cariflora' was received from University of Florida. 'Hcar 197' and 'Yuen Nong #1' were from Hilo, Hawaii. 'FL-77-5' was from Feng-shen Tropical Horticultural Experimental Station. The inoculum was papaya severe mottle with necrosis strain (SMN) of PRV preserved in papaya plants maintained in the greenhouse. A sterilized cotton ball was dipped in extracted SMN sap (mixture of 1 gram leaf tissue/5ml of potassium phosphate buffer of pH 7.5 with 450 mesh celite) and rubbed on young leaf veins and the stem of the plant. Artificial inoculation was performed 1.5 months after germination. Seedling numbers ranged from 15 to 120. Plants were evaluated four weeks after inoculation. Healthy looking plants were inoculated once more, two weeks after first evaluation. More than two thousand papaya seedlings were inoculated on August 20, and about one thousand seedlings were subjected to a second inoculation. More than six hundred plants remained symptomless by the middle of October, 1991. The 326 randomly-selected papaya seedlings were planted in a randomized complete block design in November, 1991, Taichung, Taiwan. Plants were arranged in a 1.5m in-row and 4m between row spacing.

No attempts were made to avoid aphid inoculation in the field. Growth data were taken 12 weeks after planting. Leaf tissue samples (5g) were harvested to monitor virus by ELISA during mid-April and June of 1992. The symptomless papaya plants were subjected to monoclonal antibodies number 14 (McAb-14) and McAb-46 seven months after planting to identify the viral strain in these tolerant plants. Leaf extracts were also inoculated into susceptible two-month-old 'Sunrise' papaya seedlings to distinguish the viral strains associated with symptom development, and to confirm that tolerance was not due to cross protection of mild strains.

Results and Discussion

There were significant differences among the cultivars of papaya seedlings in plant height, leaf number, leaf area, plant height increment and cross sectional area following SMN viral strain inoculation (Table 1). Progeny from 'Cariflora,' 'HCAR 197,' 'Yuen Nong #1,' 'FL-77-5R,' and 'Florida' had greater visual viral rating, and the more vigorous growth. They repre-

Table 1. Mean values of plant height, leaf number, leaf area, cross sectional area, and viral rating of 21 papaya cultivars after artificial inoculation of SMN PRV in the field.

Papaya cultivar	Plant height[z] (cm)	Leaf no.[yz]	Leaf area[yz] (cm^2)	Cross sectional area (cm^2)	Plant height increment[w] (cm)	Viral rating[xz]	Cross sectional area increment[w] (cm^2)
Golden Beauty	56bcde	16ab	251ab	11.6ab	9.8abc	2.5	2.7abcd
Exotica	53ab	12cde	167ab	8.0b	7.5bcd	2.0	1.2e
Khag dum Sirikal	78a	16bc	376ab	14.1ab	11.3a	2.7	2.3abcde
Cariflora	67abc	16abc	351ab	15.7a	9.9ab	2.7	3.3a
T.N. no.5	58bcde	12cde	148b	8.6ab	10.0ab	2.1	2.8abcd
Sabah	61bcde	19a	313ab	10.3ab	9.0abc	2.6	2.4abcde
Indonesia	55bcde	14abcde	345ab	8.6ab	9.0abc	2.3	1.7cde
HCAR 197	62bcd	15abcd	309a	15.0ab	11.0a	2.8	3.1abc
Costa Ricoh	66ab	10e	175ab	8.3ab	10.1ab	1.9	2.0abcde
Kaek Dum	68ab	19ab	257ab	12.5ab	9.7abc	2.7	1.9abcde
FL-77-5Y	57abcde	15abcd	267ab	12.7ab	7.9bc	3.0	2.6abcde
FL-77-5R	67abc	17ab	230ab	14.0ab	8.6abc	2.7	3.3a
Line 2 Solo	53de	14bcde	201ab	10.1ab	7.5bcd	1.7	2.3abcde
Yuen Nong no.1	63bcd	19ab	340ab	14.2ab	8.6abc	2.9	2.5abcde
Honey Gold	60bcde	15abcd	336ab	14.2ab	9.8abc	2.6	3.3ab
Batu Pahat	55cde	14abcde	203ab	13.8abcde	8.7abc	2.0	1.7bcde
Waimanalo 77-23	62bcd	10de	190ab	10.2de	9.4abc	2.2	1.5de
Ostream 370	62bcde	12cde	250ab	8.6ab	6.8cd	2.0	1.8bcde
Mardi	49e	14ab	252ab	7.6b	2.7e	2.0	1.1e
N.T.U.	62bcde	17ab	395a	2.8ab	9.8abc	2.8	2.6abcde
Florida	54cde	17ab	337ab	13.9ab	9.3abc	3.0	2.8abcd

w: Plant height increment and cross-sectional area increment calculated as (growth on early April, 1992-growth on February, 1992).
x: Viral rating: 1 = dead or very severe mottle and necrosis; 2 = deformed and severe mottle; 3 = severe mottle; 4 = healthy or mild mottle.
y: Different letters within the column were significantly different at 5% level using Duncan Multiple Range Test. Data were recorded five months after planting.
z: The data were assessed 12 weeks after planting in the field.

Table 2. Correlation of coefficients of PRV infection leaf area, increment of leaf length, increment of cross sectional area, plant height, leaf number, and viral rating[z] following inoculation with PRV in papaya seedlings of 21 stocks.

Traits	Viral rating	Increment[x] of leaf length (ILL)	Leaf area	Increment[y] of cross sectional area (ICSA)	Plant height
Viral rating					
ILL	0.61****[w]				
Leaf area	0.42****	0.30***			
ICSA	0.52****	0.30***	0.48****		
Plant height	0.49****	0.26***	0.34***	0.66****	
Leaf number	0.57****	0.36***	0.43****	0.56****	0.39***

w: **** significant at p = 0.0001.
x: The leaf length on April minus the leaf length on February of 1992.
y: Cross sectional area on April minus cross sectional area on February.
z: Viral rating 1 = dead or very severe mottle and necrosis; 2 = deformed and severe mottle; 3 = severe mottle; 4 = healthy or mild mottle.

Table 3. The ELISA index of the antigenic specificity of monoclonal antibodies differentiated by different PRV strains in indirect ELISA test.

Monoclonal antibody no.	SMN	SM	M	SMD[z]
McAb-14	++++[y]	+	+	++++
McAb-46	++++	++	++	+++

y: ELISA INDEX: + 0.1-0.5, ++ 0.6-1.0, +++ 1.1-1.5, ++++ greater than 1.5.
z: SMN: severe mottle with necrosis type; SM: severe mottle type; M: mild mottle type; SMD: severe mottle with deformation.

sented the more tolerant families after planting in the field. Progeny from *C. goudotiana,* 'FL-77-5Y,' 'Line 2 Solo,' 'Golden Beauty' had moderate visual disease rating. Progeny from 'Exotica,' 'Batu Pahat,' 'Indonesia,' 'Kaek Dum,' 'Mardi,' 'Waimanalo,' 'Ostream' had definite symptoms, with less growth, and the least visual viral rating. These plants were susceptible during the flowering or fruiting stage through the end of harvest season (Table 1, Table 5).

Our survey included *C. goudotiana,* and *C. pubecens.* None of the progeny of these two papaya species in this study were tolerant to SMN viral strain. Mekako and Nakasone (15) had demonstrated the progeny of an interspecific hybrid between *C. goudotiana* and *C. papaya* were resistant to distortion ringspot virus. However, the SMN viral strain in our study might be different from the distortion virus in Hawaii, or more virulent than the Hawaiian viral strains. The non-inoculated *C. cauliflora* plants were assessed late in the field at Fengshen area, Taiwan. The *C. cauliflora* leaves were mottled and had ringspot symptom after natural infection in the field. Further tests are needed to determine whether the resistance of *C. cauliflora,* is due to the specific interaction of host/viral strain.

Growth rate was not severely retarded by viral infection 2 months after planting in the field (Table 1). However, the viral strain significantly retarded susceptible plants with regard to plant height increment or the cross sectional area increment (Table 1). Tolerant families had higher growth rates compared to those of susceptible families. Viral rating in the field six months after planting was positively correlated with plant height, leaf area, the increment of leaf length and the cross sectional area (Table 2). Tolerant plants grew well with less growth retardation due to viral infection. The healthy-looking papaya plants in the tolerant family fruit 3 months after planting. Susceptible plants remained stunted with few flowers and most fruits were aborted. Any symptoms that were evident on tolerant plants became less severe in summer, when young leaves developed normally. Perhaps the viral symptoms were masked by warm temperatures. Khun (12) developed monoclone antibodies that effectively specify viral strains of PRV (Table 3). McAb-14 and McAb-46 were applied to specify the possible viral strains in tolerant plants during June of 1992. ELISA index was high among tolerant plants (Table 4). It appeared that tolerant plants had higher concentrations of virus (SMN, or SMD), but still performed well in the field. Plants of susceptible cultivars were dead by the end of 1992. These complex infec-

Table 4. ELISA readings of monoclone antibodies to differentiation of PRV strains among selected papaya lines.

		ELISA readings[x]	
Papaya lines	Genotype	antibody-14 (McAb-14)	antibody-46 (McAb-46)
Line 2 Solo	0409	1.642	0.258
Line 2 Solo	0520	1.737	1.328
NTU	0201	1.256	0.276
NTU	0531	1.719	0.526
HCAR 197	0624	0.930	0.343
HCAR 197	0449	1.056	0.211
Cariflora	0619	1.709	0.660
Cariflora	0432	1.737	0.548
Yuen Nong no. 1	0625	1.572	0.710

x: ELISA assays done on June, 1992.

Table 5. ELISA reading, disease index, survival plant number, tree yield, and total soluble content (Brix) among reinfected papaya cultivar *in the field* conditions.

Papaya cultivar	ELISA	Survival plant no.[y]	Survival rate (%)	Tree yield (Kg)[z]	Total soluble solids (Brix)
Golden Beauty	0.174	1	7	0.25	5.2
Exotica	0.220	1	11	0.59	11.6
Khag Dum Sirikal	0.088	1	17	4.41	7.8
Cariflora	0.252	20	77	4.33	10.5
Kapoho	0.163	1	50	0.52	7.2
Sunrise O. P.	0.227	0	0	0.00	--
Tainung no.2	0.139	0	0	0.00	--
Batu Pahat	0.123	2	20	3.16	5.6
Sabah	0.422	3	33	3.48	7.4
Indonesia	0.275	2	20	0.91	8.9
HCAR 197	0.126	16	73	2.86	10.7
Costa Ricoh	0.096	2	20	1.39	7.9
Goudotiana (HCAR 168)	0.015	0	0	0.00	--
Kaek Dum	0.262	7	54	0.60	8.3
FL-77-5Y	0.181	8	47	0.90	8.5
FL-77-5R	0.197	18	56	3.62	9.3
Tainung no.5	0.184	5	62	1.36	6.8
Line 2 Solo	0.559	12	67	2.76	10.9
Yuen Nong no.1	0.242	16	70	3.42	7.5
Waimanalo 77-23	0.313	3	23	0.75	9.4
Honey Gold	0.172	3	20	1.22	8.2
Ostream	0.249	0	0	0.00	--
Mardi	0.215	0	0	0.00	--
NTU	0.111	7	88	1.22	8.2
Thailand	0.420	1	33	3.10	6.5
Florida	0.375	4	100	9.89	8.8
L. S. D.	0.168			3.38	4.2

y: ELISA assays done on June, 1992. Survival plant number count made in the end of harvested season.
z: Fruits were harvested from the end of July, 1992 to March 1993, and total soluble solids were means of harvested fruits during that period.

tions might have resulted from the secondary infection by aphids in the field. The susceptible 'Sunrise' plants showed symptoms of SMN and/or SMD 4 weeks after inoculation with buffer-extracted papaya sap from tolerant plants. Thus, even though tolerant plants grew nearly normally, they had large accumulations of SMN in their tissues. Selections were made among most tolerant cultivars and individuals with the best performance. Further studies are needed to investigate the viral replication and translocation rate among different tolerant genotypes.

The cultivars significantly differed in their tolerance to SMN following a year in the field (Table 5). The papaya ringspot symptoms were more severe

following one year in the field compared to 6 months in the field (Table 1 vs. Table 5). The ELISA index was positive among papaya cultivars, except *C. goudotiana* for these plants. The fruit load among papaya cultivars were significantly different. 'Sunrise,' 'Mardi,' 'Ostream,' were mostly susceptible to SMN strain of PRV. There were no marketable yield in the study.

Different sources of 'HCAR 197,' 'Florida,' and 'Cariflora' had similar viral rating, and growth data, performing similarly in fruiting habit, fruit shape, and tree type. These cultivars tolerated the papaya ringspot virus well under our conditions, in agreement with reported tolerance in Florida. A continuing process of improvement of papaya resistant to PRV depends upon a continued effort to increase the frequency of the desirable alleles in the breeding population of papaya.

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