



(: :)

(CPV)

MA-13C5

CPV- CRSV-4 ICRSV

(RT-PCR)

CPV-CPG RNA2

bp

(CRSV-4)


B

(CRSV)

Ophiovirus (CPV)

:

***Citrus psorosis virus*, Causal Agent of Ring Pattern Disorder in Thomson Navel Trees in East of Mazandaran**

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Abstract

Citrus psorosis virus (CPV) is causal agent of one of the important citrus diseases in the world. A disorder was observed on Thomson Navel orange trees on sour orange or citrange rootstocks, with ring spot symptoms on mature fruits, especially around the stylar end. It had often caused expanded yellow ring lesions on mature leaves. Severe fruit abscission was occurred in the infected trees. Leaf samples were collected from spring flashes of the infected trees and tested by TAS-ELISA, using *Citrus psorosis virus* (CPV) 13C5 monoclonal antibody and CPV infections were confirmed. RT-PCR was done with four pair primers closely related to ICRSV, CRSV-4, CPV-RNA2 and CPV-CPG on leaves of thirty infected Thomson Navel orange trees. The samples only amplified by CRSV-4 primers and produced a fragment about 218bp. This fragment length size was similar to the fragment obtained from CPV ring spot isolates from Florida and Argentina. Seven isolates were selected for biological indexing and based on the indicator plants divided into two groups. Based on the serological, molecular and biological results, the isolates can be settled into *Citrus psorosis virus*, B type group, without bark scaling symptoms.

Key words: *Citrus psorosis virus* (CPV), *Ophiovirus*, Ring spot isolates, Biological indexing.

:

B

(Swingle and Webber, 1896)

Klotz, 1973; Roistacher, 1991;)

.(Fawcett, 1936)

.(Whiteside *et al.*, 1989; Duran-Vila and Moreno, 2000

Derrick *et al.* (1988)

Ophiovirus

Ophioviridae

Fawcett and Bitancourt, 1943; Roistacher, 1991; Whiteside)

nm

.(*et al.*, 1989

B A

RNA

.(Milne, 2005)

kDa

Fawcett and)

.(Derrick *et al.*, 1988)

CRSV-4

.(Cochran, 1942; Wallace, 1957

B

A

.(Danos, 1989; Frison and Taher, 1991)

Wallace and Drake, 1968;)

A

.(Roistacher, 1993; Da Graça *et al.*, 1991

B A

Navas-Castillo)

(*et al.*, 1991; Roistacher, 1993; Martín *et al.*, 2005

B

()

Djelouah *et al.*, 2000; Roistacher *et al.*,)

.(2000; Alioto *et al.*, 1999

.(Alioto *et al.*, 2001; Garnsey and Timmer, 1980)

.(Klotz, 1973; Roistacher, 1991; Whiteside *et al.*, 1989)

(Ebadi, 2007)

A

Weathers and)

(Harjung, 1964

Citrus nobilis Lour ×) Kinnow

(*C. deliciosa* Tenora

CRSV (*Indian citrus ringspot virus*, ICRSV)

(Sharma *et al.*, 2004; 2009)

Flexiviridae

Mandarivirus

(Adams *et al.*, 2004; Rustici *et al.*, 2002; Thind *et al.*, 1998)

()

()

Citrus ring pattern virus

Dehyar and Habashi, 1974; Ebrahimi *et al.*,)

(1988; Bove, 1995

(Timmer and Garnsey, 1980; Roistacher, 1991)

AgriTest



Fig.1. Ring spot symptoms on leaves and fruits of Thomson Navel orange in East of Mazandaran

°C :

) / (TAS-ELISA)

(pH9.5 MgCl₂ / 13C5

((Milne)

(Martin *et al.*, 2004) Alioto *et al.* (1999)

ELISA Reader-) / 13C5

(Stat Fax2100, USA (pH9.6 NaHCO₃ Na₂CO₃)

()

CPV (MaxiSorp® Plate, Nunc, Denmark)

: °C

)

/ KH₂PO₄ / Na₂HPO₄

/ NaN₃ NaCl / KCl

pH7 / (pH7.4 Tween-20

()

(

/ Na₂HPO₄)

Gomphrena globosa *Chenopodium quinoa* NaCl / KCl / KH₂PO₄

PVP-10000 Tween-20 / NaN₃

(pH7.4

± °C

(A1)

(Ahlawat and Pant, 2008; Sharma *et al.*, 2009) (Blank)

/ 13C5

[C. *paradisi* Macf. × *P. trifoliata* (L.) Raf.(cv 'Swingle')] °C

Citrus sinensis × *Poncirus trifoliata*]

[(cv 'Troyer')

(Boehringer Mannheim Co., Germany)

Citrus paradisi (cv 'Duncan') ×] /

MMuLv-Reverse Transcriptase
(dATP, dTTP, dGTP, dCTP) dNTP mix

[*Citrus reticulata* (cv 'Dancy')
Citrus limon (L.)]
[Burm.f. (cv 'Eureka')]

(Garcia *et al.*, 1997)

(cDNA) DNA

U:5'-CCAACTGGATGAAAT-3',] ICRSV
(Nagpal *et al.*, 2005) [D:5'-GAGCCAAGCGTTCAGA-3'
U:5'-ACAATAAGCAAGACAAC-3',] CRSV
RNA₂ (Garcia *et al.*, 1997) [D:5'-CCATGTCACCTTCTATTC-3'
D:5'- GAAAGTAGTCATCCTT-3',] CPV
(De La Torre *et al.*, 2002) [U:5'-GACAACTCCTCCTCC-3'
D:5'- TCTGTTTTGTCAACAACACACTCC-3',] CPV
(Barthe *et al.*, 1998) [U:5'-GCTCCTGGAAAAGCTGATG-3'
(Primus25-MWG Biotech, Germany)

(Roistacher *et al.*,)
(2000; Sarachu *et al.*, 1988

RNA : (RT-PCR)
(Vivantis Co., USA) GF-1
/ ml
RNase / ml

RNA- %
Binding
12000g

DNase I
RNase Inhibitor

(
RNA
(cDNA synthesis) DNA
RNA
(C-primer)
RT-PCR 5x
(Denaturation)
(annealing)

M6 M5

()
()

M7

()

()



(M4 و M3 و M2 و M1)

Fig.2. Yellow spots on Clementine mandarin leaf, inoculated with the first group isolates (M1, M2, M3 and M4).



(M4 و M3 و M2 و M1)

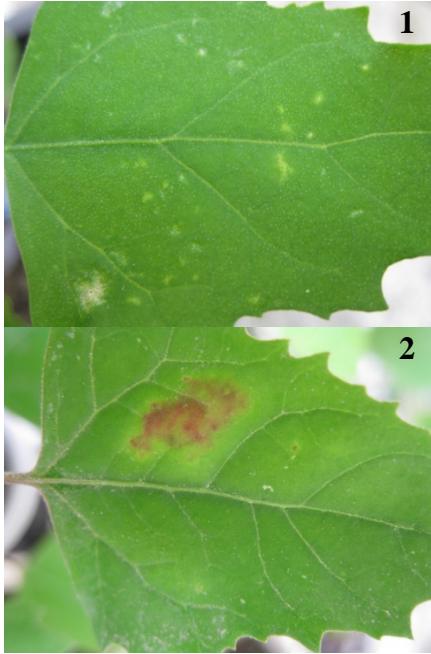
Fig.3. Leaf edge necrosis of Eureka lemon, inoculated with the first group isolates (M1, M2, M3 and M4)

:
:
/
/
/
/
13C5

()
M7 M1

M4 M3 M2 M1

B
A
(Timmer *et al.*, 1978; Garnsey and Timmer, 1988)



Chenopodium quinoa

: (M4 M3 M2 M1)

(M7 M6 M5)

Fig. 5. Inoculated leaves of *Chenopodium quinoa*. 1: white necrotic local lesions, inoculated with the first group isolates (M1, M2, M3 and M4); 2: red necrotic local lesions, inoculated with the second group (M5, M6, and M7)



Gomphrena

globosa

Fig.6. Necrotic local lesions on the leaves of *Gomphrena globosa*, inoculated with *Citrus psorosis virus* isolates in East of Mazandaran



(M7, M6, M5)

Fig.4. Yellow spots on Troyer citrange leaf, inoculated with the second group isolates (M5, M6, and M7)

Chenopodium quinoa

M4 M3 M2 M1

M7 M6 M5

()

Gomphrena globosa

()

ICRSV

Gomphrena globosa

A

B

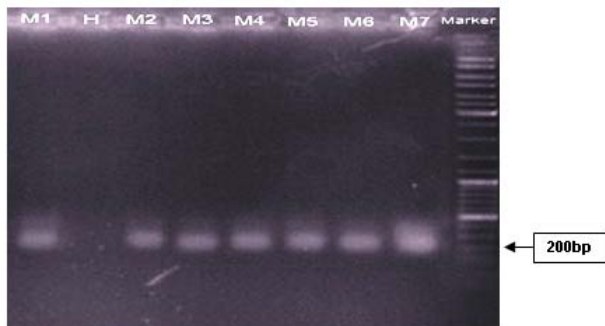
(Danos, 1989)

B

A

(Frison and Taher, 1991)

(Derrick *et al.*, 1988; Ebadi, 2007; Da Graça *et al.*, 1993)



M7 M1) CRSV
: H
(: Marker

Fig.7. The obtained fragment from collected isolates of the orange trees with ring spot symptoms, amplified by the specific primer of *Citrus psorosis virus*-CRSV isolate (M1 to M7, the seven collected isolates; H, healthy samples collected from trees without any symptoms; Marker, molecular marker ladder with 100 bp intervals)

Roistacher, 1991; Da Graça *et al.*, 1993; Sharma *et al.*,)
(2009

13C5

RNA1

A B
(Da Graça *et al.*, 1993) CRSV

CRSV
(Roistacher, 1993; Da Graça *et al.*, 1991)

Chenopodium quinoa

Da Graça *et al.*,)
(1993; Sarachu *et al.*, 1988

(Da Graça *et al.*, 1991; Vogel and Bove, 1980) CRSV-4
B

:
(CRSV)
() bp
bp

(Garcia *et al.*, 1997)

(Nagpal *et al.*, 2005)

(De La Torre *et al.*, 2002) RNA2

(Barthe *et al.*, 1998) RNA3

Table 1. Indexing of seven *Citrus psorosis virus* isolates, obtained from Thomson Navel trees in East of Mazandaran

Isolate	Biological group	Host and symptoms				Sampling site and the symptoms on sampled Thomson Navel orange trees
		Clementine mandarin	Duncan grapefruit	Eureka lemon	Troyer citrange	
M1	1	cl	cl	tns	-	Neka, without any bark scaling symptoms, with chlorotic patterns and ring spots on mature and immature leaves and fruits, especially around the stylar end, fruit abscission
M2	1	cl	cl	tns	-	Neka, without bark scaling symptoms, with chlorotic patterns and ring spots on mature and immature leaves and fruits, especially around the stylar end, fruit abscission
M3	1	cl	cl	tns	-	Neka, without any bark scaling symptoms, with chlorotic patterns and ring spots on mature and immature leaves and fruits, especially around the stylar end, fruit abscission
M4	1	cl	cl	tns	-	Ghaemshahr, without any bark and leaf symptoms, with ring spots along with gumming on fruit, fruit abscission
M5	2	-	-	-	cs	Ghaemshahr, without any bark and leaf symptoms, with ring spots along with gumming on fruit, fruit abscission
M6	2	-	-	-	cs	Sari, without any bark scaling symptoms, with chlorotic patterns and ring spots on mature leaves and fruits
M7	2	-	-	-	cs	Sari, without any bark scaling symptoms, with chlorotic patterns and spots on mature leaves and fruits

:(-) :cs :tns :cl

cl: Chlorotic lesions, tns: tip necrosis of leaves, cs: Chlorotic spots, (-): without any symptoms

(

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