STUDIES ON SOME ISOLATES OF CITRUS TRISTEZA VIRUS (CTV)

By

NASHWA MOHAMED AHMED ABD EL-MOHSEN
B.Sc. Agric. Sc. (Agricultural Microbiology), Ain Shams University, ١٩٩٩
M.Sc. Agric. Sc. (Agricultural Virology), Ain Shams University, ٢٠٠٤

A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science (Agricultural Virology)

Department of Agricultural Microbiology
Faculty of Agriculture
Ain Shams University

٢٠١٣

Approval Sheet

STUDIES ON SOME ISOLATES OF CITRUS TRISTEZA VIRUS (CTV)

By

NASHWA MOHAMED AHMED ABD EL-MOHSEN

B.Sc. Agric. Sc. (Agricultural Microbiology), Ain Shams University, ١٩٩٩
M.Sc. Agric. Sc. (Agricultural Virology), Ain Shams University, ٢٠٠٤

This thesis for Ph.D degree has been approved by:

Dr. Mohamed Haroun Abd El-Mageed ……..
Prof. of Plant Pathology, Faculty of Moshtohor Agriculture, Banha University.

Dr. Khaled Abd El-fatah El Dougdoug ……
Prof. of Agricultural Virology, Faculty of Agriculture, Ain Shams University.

Dr. Rania Ahmed Younis ……
Associate Prof. of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Sohair Ibrahim El-Afify ……
Prof. Emeritus of Agricultural Virology, Faculty of Agriculture, Ain Shams University.

Date of Examination: ١ / ١٢ / ٢٠١٢

STUDIES ON SOME ISOLATES OF CITRUS TRISTEZA VIRUS (CTV)

By

NASHWA MOHAMED AHMED ABD EL-MOHSEN

B.Sc. Agric. Sc. (Agricultural Microbiology), Ain Shams University, ١٩٩٩
M.Sc. Agric. Sc. (Agricultural Virology), Ain Shams University, ٢٠٠٤

Under the supervision of:

Dr. Sohair Ibrahim El-Afifi
Prof. Emeritus of Agricultural Virology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Rania Ahmed Younis
Associate Prof. of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Ahmed Shawky Gamal El-Din
Head of Research of Agricultural Virology, Virus and Mycoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center

ABSTRACT

Nashwa Mohamed Ahmed Abd El-Mohsen. Studies on Some Isolates of Citrus Tristeza Virus (CTV). Unpublished Ph.D Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, ٢٠١٣.

In this study, investigation was conducted at various locations within the citrus growing orchads in Qalubia governorate (Qaha & Kanater). ١٩٢ budwood samples thought to be infected with citrus tristeza virus (CTV) were collected from sweet orange orchards. Two positive trees from the total numbers were CTV isolates and classified into mild to moderate severe via the symptoms on Mexican lime plants.

CTV isolates in Egypt were studied, through modes of transmissions, biologically by host range plants indexing on indicator plants and by aphids, serological detection using polyclonal and monoclonal antibodies, electron microscopy, polymerase chain reaction for cp٢٧ gene, tissue culture and cross protection for virus control. The results showed that none of the CTV-infected trees, even though grafted on sour orange, showed decline or obvious tristeza symptoms. All CTV isolates induce strong vein clearing, leaf cupping, seedling yellow and tolerant when indexed on specific woody indicators seedlings: Cleopatra Mandarin, Mexican lime, sweet orange, sour orange and Duncan grapefruit. ELISA tests for all indexed plants confirmed the presence of CTV.

The vector represent worldwide for CTV and exist in Egypt are both aphids (*A.gossaypii*, *M. persicae*), in semipersistent maner. The higher transmission rates after ٢٤ hr acquisition period for aphids on infected Mexican lime plants by severe CTV isolate are ٤٠, ١٠٪ by *A.gossaypii*, *M. persicae* respectively. None of CTV

characteristics symptoms on Mexican Lime was appeared with mild isolate after different acquisition periods.

Coat protein gene detection in severe and mild isolates by PCR amplification of the cp٢٧ gene with specific primers produced a fragment with the same expected size (٥٨٠ bp) for both isolates as revealed by electrophoresis in a ١% agarose gel.

The effect of ultrastructure of sweet orange (*Citrus sinensis* (L.) infected with moderate severe isolate of Citrus tristeza virus, as well as the difference between moderate severe and mild isolates were illustrated upon examination of ultrathin sections of infected Mexican lime (*Citrus aurantifolia* (L.) by transmission electron microscopy.

Attempt to provide resistance to the disease, a protocol for shoot tip grafting used to recovery infection of Mexican lime plants by moderate severe isolate of CTV, volkamariana seeds used as a rootstock, has shown the best effect on virus elimination through tissue culture technique. Success of cross protection by mild isolate CTV preimmunization to prevent infection with moderate severe isolate to Mexican lime plants.

Key words:

Citrus tristeza virus; Closterovirus; Cytopathology; microscopic examination; Inclusion bodies, aphid transmission, Cross protection, Shoot tip grafting, cp٢٧ gene.

Acknowledgment

I kneel humbly to **ALLAH** thanking **HIM** for showing me the right path, without **HIS** help my efforts would have gone astray.

I am greatly indebted to **Prof.Dr. Sohair I. El-Afifi**, Prof. of Agric. Virology, Microbiology Department, Faculty of Agriculture, Ain Shams University. I owe her and lot that words are poor to express. She suggested the point of investigation, checked every work stage and revised the thesis to prepare it in its final form. May **ALLAH** reward and bless her.

I would like to express my deepest gratitude and appreciation to **Dr. Rania A. Younis**, Associate Prof. of Genetics, Genetics Department, Faculty of Agriculture, Ain Shams University, for her supervision.

I would also like to thank **Dr. Ahmed S. I. Gamal El-Din**, Chief of Research of Virology, Virus and Mycoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center for suggesting the point, his supervision and encouragement as well as stimulating the discussions through out this work.

Thanks are also due to all staff members of Agric. Microbiology Department, Faculty of Agriculture, Ain Shams University.

Finally, thanks to the staff members of the Virus and Mycoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center.

Thanks are also due to my family, for their great patience and encouragement.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

A	Angstrome…….……..……	A°
B	Bais pair…….…...	bp
C	*Citrus tristeza closterovirus*…….....	CTV
	Centigrade…….....	C
	Complementary DNA……..	c-DNA
	Coat protein gene ٢٧……...	cp٢٧
	Cultivar(s)……..	cv(s)
D	Double abtibody sandwich-ELISA…….	DAS-ELISA
	Degree……	°
	Diethylpyrocarbonate…….……..	DEPC
	Diameter……..….	diam
	Deoxyribonucleic acid……..	DNA
	Deoxyribonuclease…….….	DNase
	Dideoxynucleotidetriphosphates……..….	dNTPs
	Dithiothreitol……..	DTT
E	Enzyme linked immunosorbent assay……..…	ELISA
	Ethylenediaminetetraacetic acid…….	EDTA
G	Gram(s)……..	g
	Gravity, centrifugal speed…….….	xg
H	Hour(s)……...	H
I	Immunoglobulin G……	IgG
K	Kilobase…….….	Kb
M	Monoclonal antibody……..	MAbs
	Micro…….……..	µ
	Microgram(s)…….……	µg
	Microliter(s)……	µl
	Micromolar…….……	µM
	Milligram(s)……	mg
	Milliliter(s)…….….	ml
	Millimolar……	mM

	Minute…….	min
	Molar……..	M
N	Nanogram(s)……..……..	ng
	Nanometer(s)…….....	nm
	Normal……	N
	Nucleotid(s)……....	nt
P	Polyclonal antibodies……..	PAbs
	Page……..…	p
	Pages……	pp
	Picomole(s)……	pmol
R	Ribonucleic acid……..……	RNA
	Ribonuclease……	RNase
S	Second(s)……..…….....	s
	Shoot-tip micrografting technique……..…	STG
	Species……..	spp.
U	Ultraviolet……..…	uv
	Unite……..…..	U
	٥`-untranslated region of genome……..…..	٥`-UTR
	٣`-untranslated region of genome……..…..	٣`-UTR
V	Volume……...	V
	Volume per volume……..	V/V
W	Weight per volume……...	W/V

CONTENTS