

STUDIES ON SOME ISOLATES OF CITRUS TRISTEZA VIRUS (CTV)

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Approval Sheet

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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 Unpublished Ph.D Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, ٢٠١٣.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 orchards 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 cp^Ψ 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 cp^{YV} gene with specific primers produced a fragment with the same expected size (280 bp) for both isolates as revealed by electrophoresis in a 1% 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, cp^{YV} gene.

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LIST OF ABBREVIATION

A	Angstrome.....	A°
B	Bais pair.....	bp
C	<i>Citrus tristeza closterovirus</i>	CTV
	Centigrade.....	C
	Complementary DNA.....	c-DNA
	Coat protein gene $\Psi\Psi$	cp $\Psi\Psi$
	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).....	μ g
	Microliter(s).....	μ l
	Micromolar.....	μ M
	Milligram(s).....	mg
	Milliliter(s).....	ml
	Millimolar.....	mM

IX

	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
	5'-untranslated region of genome.....	5'-UTR
	3'-untranslated region of genome.....	3'-UTR
V	Volume.....	V
	Volume per volume.....	V/V
W	Weight per volume.....	W/V

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