

Advanced Studies on some Geminiviruses Affecting Kidney Bean

By

AMRO AHMED ABD-ELRAHEEM

B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 1999

M.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 2006

THESIS

**Submitted in Partial Fulfillment of the
Requirements for the Degree of**

DOCTOR OF PHILOSOPHY

In

**Agricultural Sciences
(Plant Pathology)**

**Department of Plant Pathology
Faculty of Agriculture
Cairo University
EGYPT**

2015

APPROVAL SHEET

Advanced Studies on some Geminivirus Affecting Kidney Bean

**Ph.D. Thesis
In
Agric. Sci. (Plant Pathology)**

By

AMRO AHMED ABD-ELRAHEEM

B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 1999

M.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 2006

APPROVAL COMMITTEE

**Prof. Dr. KHALED ABDULFATTAH MEGHAWRI ALI E
DOUGDOUG.....**

Professor of Microbiology, Fac. Agric., Ain Shams University

**Prof. Dr. ALY MOHAMED MAMOUN ABD-EL SALAM
.....**

Professor of Plant Pathology, Fac. Agric., Cairo University

**Prof. IBRAHIM Abd-El-MONIEM MOHAMED IBRAHIM
.....(Late)**

Professor of Plant Pathology, Fac. Agric., Cairo University

**Prof. Dr. OM-HASHEM MOHAMED EL-BANNA
.....**

Professor of Plant Pathology, Fac. Agric., Cairo University

Date: / / 2015

SUPERVISION SHEET

Advanced Studies on some Geminiviruses Affecting Kidney Bean

**Ph.D. Thesis
In
Agricultural Sciences (Plant Pathology)**

By

AMRO AHMED ABD-ELRAHEEM
B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 1999
M.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 2006

SUPERVISION COMMITTEE

Dr. OM-HASHEM MOHAMED EL-BANNA
Professor of Plant Pathology, Plant Pathology Dept., Fac. Agric., Cairo University

Dr. IBRAHIM Abd-El-MONIEM MOHAMED IBRAHIM
Professor of Plant Pathology, Plant Pathology Dept., Fac. Agric., Cairo University

Dr. HAMED MAHMOUD MAZYAD
Professor Emeritus of Plant Pathology, Pl. Path. Res. Instit., ARC, Egypt

Name of Candidate: Amro Ahmed Abd-Elraheem **Degree:** Ph.D.

Title of Thesis: Advanced studies on some Geminiviruses affecting kidney beans

Supervisors: Dr. Om-Hashem Mohamed El-Banna

Dr. Ibrahim Abd-Elmoniem Mohamed Ibrahim

Dr. Hamed Mahmoud Mazyad

Department: Plant Pathology

Approval: 25 / 3 /2015

ABSTRACT

Symptomatic leaf samples were collected from bean fields cultivated in different governorates and tested by PCR using Geminivirus degenerate primers and *Squash Leaf Curl Virus* (SLCV) specific primers. All bean varieties grown in surveyed fields were found susceptible to Geminivirus infection and the dominant Geminivirus affecting bean plants was SLCV. Percentage of infection was higher at Nili season than that at the Summer season. *Squash leaf curl virus* (SLCV) was isolated from naturally infected common bean plants grown in Egypt and transmitted from naturally infected common bean onto twenty two species and varieties belonging to six different families i.e. *Moraceae*, *Solanaceae*, *Cucurbitaceae*, *Fabaceae*, *Chenopodiaceae*, and *Malvaceae* using viruliferous whitefly (*Bemisia tabaci*). Results revealed that SLCV could be transmitted to 16 out of 22 species tested and positively back inoculated to beans from these hosts. Six different commercial varieties of bean were evaluated for SLCV infection at three different growth stages. The evaluation was performed by whitefly inoculation in insect proof green house. Two commercial varieties (Tema and Giza 6) were found to be tolerant to SLCV infection in all tested growth stages. The disease severity of the viral infection varied in the other four susceptible varieties at the different growth stages. It was observed that infection percentage and disease severity were decreased with increasing growth stage. Significant difference in the percentage of yield loss in inoculated plants at age 15d and 25d from planting compared with control was recorded. On the other hand percentage of yield loss in plants inoculated at 35d stage was non-significant. The coat protein gene of SLCV was PCR amplified from infected common bean plants. SLCV-CP was cloned in pJET cloning vector and directly sequenced. The sequence alignment and phylogenetic analysis showed a relatively high diversity among the three different isolates that the identity ranged from 89 to 94%. Nucleotide sequencing of the complete genome of the virus was as follow, DNA-A (2667 bp) and DNA-B (2621 bp). The obtained sequences were submitted into the GenBank with accession numbers KJ624994 and ٤. The full nucleotide sequence of DNA-A, DNA-B and all open reading frames (ORF) of the SLCV-bean was aligned and compared with eleven different isolates of the SLCV available in the GenBank. The phylogenetic analysis of the complete nucleotide sequence revealed that SLCV is related to other isolates of SLCV from other governorates in Egypt (Cairo and Ismailia) as well as isolates from Lebanon (SLCV-LB2), Palestine (SLCV-Pal), Jordan (SLCV-JO) and Israel (SLCV-IL) sharing high identities ranging from (90% to 97%).

Key words: Common bean, virus incidence, *Squash Leaf Curl Virus*, PCR detection, cp sequence analysis, phylogenetic analysis, Egypt

ACKNOWLEDGEMENT

Deep thanks to God supporting me in this work.

I would like to express my gratitude to my supervisor *Prof. Dr. Om-Hashem Mohamed El-Banna* Plant Pathology Department, Faculty of Agriculture, Cairo University for the useful comments, remarks and constructive guidance through this work.

I would like to express my gratitude to *Prof. Dr. Ibrahim Abd-Elmoniem Mohamed Ibrahim* for supervision and constructive guidance over the past years.

I would like to express my gratitude to *Prof. Dr. Hamed Mazyad* , ex-director of Plant Pathology Research Institute, ARC for supervision and helping, guidance and caring to facilities available .

Thanks to all members of Virus and Phytoplasma Dept. Plant pathology intit. ,A.R.C ,specially my colleagues *Dr. Ahmed Kamal* and Misses *Reham Gamal* in molecular biology Lab 2 for their valuable assistance in the molecular virology experiments.

Finally I would also like to thank my family and everybody made this work possible.

CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
1. Family Geminiviridae.....	3
2. Genome Organization of Begomoviruses	4
3. <i>Squash Leaf Curl Virus</i> (SLCV) Genome Organization...	5
4. <i>Squash Leaf Curl Virus</i> (SLCV) Incidence	7
5. <i>Squash Leaf Curl Virus</i> (SLCV) Symptoms	8
6. Host range of <i>Squash Leaf Curl Virus</i> (SLCV).....	9
7. Transmission of <i>Squash Leaf Curl Virus</i> (SLCV).....	10
8. Whitefly Maintenance and Plant Inoculation.....	11
9. Disease Severity.....	14
10. PCR Detection of Geminivirus Using Degenerate Primers.....	15
11. Detection of SLCV Using Specific Primers.....	16
12. <i>Squash leaf curl virus</i> (SLCV) sequence analysis.....	19
MATERIALS AND METHODS	23
1. Samples Collection and Geminivirus Incidence on Beans.....	23
2. Isolation and propagation of virus.....	23
3. Whitefly Maintenance and Plant Inoculation.....	24
4. Host Range of the Virus Isolate	24
5. Response of Bean Cultivars to Virus Infection	26
6. Total Nucleic Acid Extraction.....	28
7. Polymerase Chain Reaction (PCR) for detection of geminivirus and SLCV.....	29
8. Amplification of DNA-A and DNA-B of SLCV.....	30
9. Cloning and Sequencing for the Amplified DNA Fragments...	32
a. Cloning in pJET 1.2/blunt Cloning Vector	32
1. Preparing PCR product for ligation.....	32
2. Ligation.....	33
3. Transformation.....	34

4.Isolation of Plasmid DNA.....	34
5.Enzyme digestion analysis of cloned DNA	36
b. Cloning in pGEM-T Easy Vector	36
1.Ligatin.....	37
2.Transformation.....	38
3.Isolation of Plasmid DNA.....	38
4.Enzyme digestion analysis of cloned DNA	38
10.DNA Sequencing.....	39
11. Sequence analysis.....	39
RESULTS	40
1. Geminivirus incidence on Bean fields.....	40
2.Virus isolation.....	40
3.Host range of the virus isolate.....	42
4.Effect of bean plant growth stage on <i>Squash Leaf Curl virus</i> infection.....	48
5. Effect of Squash Leaf Curl virus infection on bean plant yield.....	49
6. Molecular detection of SLCV by polymerase chain reaction.....	53
7.Partial Sequence analysis of SLCV.....	54
8.Amplification of the SLCV full genome.....	57
9.Sequence analysis and Genomic organization of SLCV.....	57
Discussion	67
SUMMARY	74
REFERENCES	80

LIST OF TABLES

No	Title	Page
1.	Primers used in PCR detection and Full genome organization.....	31
2.	<i>Squash Leaf Curl Virus</i> (SLCV) incidence in different beans fields at different governorates checked by PCR.	41
3.	Host range of SLCV Bean Isolate by whitefly mediated transmission employing a 48-hrs followe by a 48-hrs inoculation acces period.....	43
4.	Effect of growth stage of six bean cultivars on infection percentage and disease severity of SLCV.....	51
5.	Effect of SLCV infection at three different growth stages on (pod yield g.)	52
6.	Nucleotide sequence identities (%) between SLCV-Qaliobeya (Egypt) DNA-A and other SLCV isolates available in the genebank.	64
7.	Nucleotide sequence identities (%) between SLCV-Qaliobeya [Egypt] DNA-B and other SLCV isolates available in the genebank.....	65

LIST OF FIGURES

No	<i>Title</i>	Page
1.	Genome organization of <i>Squash Leaf Curl Virus</i> (SLCV).....	6
2.	Cages prepared for Whitefly propagation(A) and population of whitefly on bean eaves(B).....	25
3.	Squash Leaf Curl Virus Disease severity index (DSI)	27
4.	pJET1.2/blunt Vector Map according to Thermo- Fermentas, USA	33
5.	pGEM-T Easy Vector Map according to Promega, USA.....	37
6.	Symptoms induced by SLCV on Fabaceae plants.....	44
7.	Symptoms induced by SLCV on Cucurbitaceae plants.....	45
8.	Symptoms induced by SLCV on Solanaceae plants.....	46
9.	Symptoms induced by SLCV on marshmallow leaves	47
10.	Symptoms induced by SLCV on <i>Chenopodium quinoa</i>	47
11.	PCR detection of SLCV using the AC/AV core degenerate primer pair specific for Geminiviruses and primer pair specific for SLCV .	53
12.	Digestion analysis for the pJET-SLCV-CP recombinant plasmid...	55
13.	Partial coat protein sequence produced by degenerate primers Ac-Av core.....	55
14.	Phylogenetic analysis for the three different clones of the SLCV when aligned with each other [a] and when aligned with 11 different isolates of SLCV available in GenBank[b].....	56
15.	Amplification of DNA-A and DNA-B full genome of SLCV [a]. Digestion analysis of the DNA-A and DNA-B carrying pGEM-T Easy cloning vector using EcoRI restriction enzyme [b].....	58
16.	SLCV Full genome sequence for DNA A.....	62
17.	SLCV Full genome sequence for DNA B.....	63
18.	Phylogenetic trees based on a multiple sequence alignment of the complete DNA-A (a) and DNA-B (b).....	66

INTRODUCTION

Phaseolus vulgaris, (the green bean, kidney bean, or common bean), is an herbaceous annual plant in the *Fabaceae* family which can be harvested and eaten immature, still in the edible pod, or when mature, shelled, and dried (Van Wyk 2005).

The annual cultivated area is between 9–18 thousand hectares, yielding 15,000 tons of dry seeds and 150,000 tons of green pods (Central Administration of Agriculture in Egypt CAAE, 1994). Egypt is one of the tenth countries producing green beans (270 million ton) and exports about 12 thousand tons of green Beans from October to June (FAO 2010).

Under Egyptian conditions bean plants are vulnerable to infection by bacterial, fungal and viral diseases (Abo-Elyousr 2006; El-Mougy *et al.*, 2007), among the main causes for poor yields in common beans are Geminiviruses (Brown, 1990).

Geminiviruses have emerged as serious pathogens of agronomic and horticultural crops. Begomovirus genus is the largest genus of this family that infects dicotyledons plants and cause devastated crop production (Jones, 2003

and Mansoor *et al.*, 2003). They are exclusively transmitted in a persistent manner by the whitefly *Bemisia tabaci*.

Squash leaf curl virus (SLCV) is a bipartite begomovirus of family Geminiviridae. The virus affects various species of cucurbits, common bean and marsh-mallow (Hill *et al.*, 1998; Al-Musa *et al.*, 2008 and Hanley-Bowdoin *et al.*, 2013). SLCV was recorded for the first time on *Phaseolus vulgaris* plants in America and the Caribbean Basin by (Brown, 1990). The virus cause leaf curling, yellow mottling, and reduced fruit set on squash plants (Idris *et al.*, 2006) and cause leaf curling, vein necrosis and stem necrosis on inoculated common bean plants (El-DougDoug *et al.*, 2009).

A leaf curl disease with symptoms typical to many begomoviruses was observed on bean fields. The disease caused downward leaf curling, stunting and made the plants unproductive in case of severe infection.

The objectives of the present study were

1. Detect SLCV associated to bean crop in Egypt.
2. Identify and characterize SLCV associated to bean plants using both biological and molecular tools.
3. Assessing the yield damage produced by SLCV on common bean.

REVIEW OF LITERATURE

Phaseolus vulgaris is a member of family *Fabaceae*. It is known as Common, Snap, Kidney, French or Haricot beans (Singh, 1999 and Bisby *et al.*, 2011). The Kidney bean is a tender annual, cultivated as a food crop *Phaseolus vulgaris* L. in many parts of the world including the temperate, sub-tropical tropical zones (Purseglove 1988).

The bean plant come in two types; dwarf or bushy type and pole or climbing type (El-Tohamy *et al.*, 1999 and Singer *et al.*, 1996). Bushy varieties have a short growing period and they are commonly grown in Egypt. *P. vulgaris* is one of the most important food crops in Egypt and consumed as a cooked vegetable Plant either as dry seeds or green pods. It plays an important role in human nutrition as a cheap source for protein, carbohydrates, vitamins and minerals and is considered one of the most important vegetable crops cultivated in Egypt for local market and exportation (Abdel-Hakim *et al.*, 2012).

1. Family Geminiviridae

Based on their genome arrangement, insect vector, and host range geminiviruses are classified by the International Committee on Taxonomy of Viruses (ICTV) into seven genera: *Becurtovirus* (2 Species), *Begomovirus* (192 Species), *Curtovirus* (3 Species), *Eragrovirus* (1

Species), *Mastrevirus* (29 Species), *Topocuvirus* (1Species), and *Turncurtovirus* (1 Species) (Fauquet *et al.*, 2003; Varma and Malathi, 2003; Sopid, 2009; Varsani *et al.*, 2014).

2. Genome Organization of Begomoviruses

Begomoviruses have either a bipartite genome, with components known as DNA-A and DNA-B, or a monopartite genome resembling DNA-A.

DNA-A contain six open reading frames (ORF): AV1 (known as AR1; coat protein, CP) and AV2 (known as AR2; AV2 protein or movement protein, MP) on the virion-sense strand; AC1 (known as AL1; replication protein, Rep), AC2 (known as AL2; transcriptional activator, TrAP), AC3 (known as AL3; replication enhancer, REn) and AC4 (known as AL4; AC4 protein) on the complementary-sense strand. DNA-B contains two ORFs encoding proteins involved in movement: BV 1 (known as BR1; nuclear shuttle protein, NSP) on the virion-sense strand and BC1 (known as BL1; movement protein, MPB) on the complementary-sense strand (Seal *et al.*, 2006; Stanley et al., 2005).

Based on phylogenetic studies and genome arrangement, begomoviruses have been broadly

divided into two groups, the Old World viruses (Eastern Hemisphere, Europe, Africa, Australasia) and the New World viruses (Western Hemisphere and the Americas) (Padidam *et al.*, 1999; Paximadis *et al.*, 1999; Rybicki, 1994).

Begomovirus genomes have a number of characteristics that distinguish Old World and New World viruses. All New World begomoviruses are bipartite, whereas both bipartite and monopartite begomoviruses are present in the Old World. In addition, DNA-A of bipartite begomoviruses from the New World lacks an AV2 ORF (Rybicki, 1994; Stanley *et al.*, 2005).

3. *Squash leaf curl virus* (SLCV) Genome

Organization

Squash leaf curl virus (SLCV) is a bipartite begomovirus of family Geminiviridae. The DNA-A encodes five open reading frames (ORFs) which are positionally conserved with those of other begomoviruses; one ORFs, AV1 in viral sense and four ORFs, AC1, AC2, AC3 and AC4 in complementary sense. DNA-B encodes two ORFs, including BV1 in viral sense and BC1 in-complementary sense (Fig.1).

During the last three decades, numerous whitefly-transmitted begomoviruses have emerged as devastating pathogens, particularly in the tropics and subtropics, causing huge economic losses and threatening crop production (Idris *et al.*, 2006).

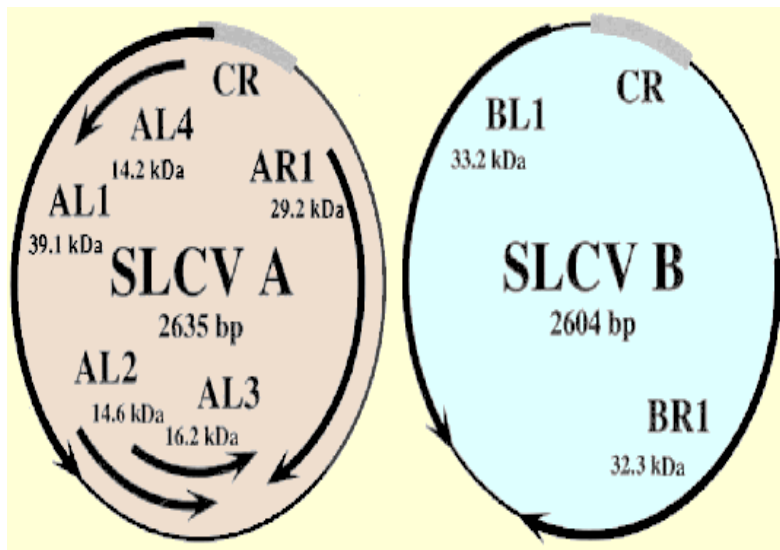


Fig.1. Genome organization of SLCV based upon the nucleotide sequence of SLCV as determined by Lazarowitz & Lazdins (1991). Circles represent the individual DNA components (A and B), arrows denote the location and polarity of viral ORFs, and stippled regions denote limits of the Common Region (CR) sequence conserved in the two-genome components. Sizes of proteins encoded by individual ORFs are expressed in kilo Daltons (kDa).