# Advanced Studies on some Geminiviruses Affecting Kidney Bean

**By**


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**B.Sc. Agric. Sci. (Plant Pathology), Fac. Agric., Cairo Univ., 1999**
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# APPROVAL SHEET

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SUPERVISION SHEET

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**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, Solanaeae, 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 ع.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

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# 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 vulgars* 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 (Purseglove1988).

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 p rotein, 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).

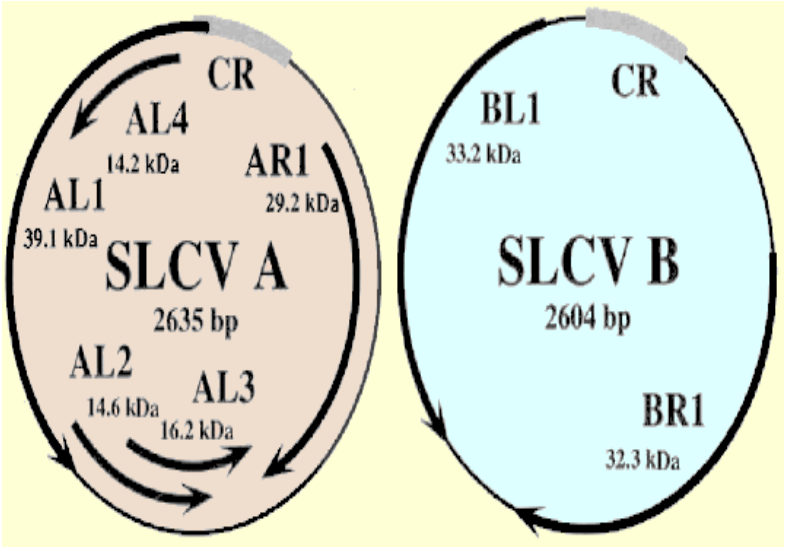


**Fig.1.** Genome organization of SLCV based upon the nucleotide sequence of SLCV as determined by Lazarowitz & Lazdins (1991). Circles represent the individual DNA components (A and B), arrows denote the location and polarity of viral ORFs, and stippled regions denote limits of the Common Region (CR)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