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RESEARCH

The co- infection of cucurbits with criniviruses and ipomoviruses: possible adverse effect on virus diagnosis and breeding for resistance

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ABSTRACT

Backgrouns: Recently whitefly-transmitted viruses (WTV) have emerged as a major economic threat to the cucurbit industry in the Middle East and countries in the Mediterranean basin. Whitefly-transmitted viruses to cucurbits include viruses in the *Begomovirus*, *Crinivirus,* and *Ipomovirus* genera. Induced symptoms are mostly of the yellow-type disease symptoms. Previous observation indicated that co-infection of cucurbits with these viruses was very common and may lead to severe problems in virus diagnosis.

Objectives: The purpose of this study is to characterize biologically, serologically, and molecularly one *Crinivirus* viz. *Cucurbit yellow stunting disorder virus* (CYSDV) and one *Ipomovirus* viz. *Cucumber vein yellowing virus* (CVYV) from infected cucurbits. Further, the study is concerned with illustrating the adverse effect of mixed infection with these viruses on diagnosis and breeding for virus resistance in cucurbits.

Methods: CYSDV was isolated through *Bemisia tabaci* insects; whereas, CVYV was isolated through mechanical inoculation. The two viruses were purified using the electro elution technique devised by the author of the present study. Rabbit immunization was used for induction of antisera for CYSDV and CVYV. RT-PCR was used to amplify coat protein genes for the two viruses using specific primers. DAS-ELISA and immuno-blotting were used for evaluating the induced antisera.

Results: Antisera for CYSDV and CVYV were produced efficiently and used for virus diagnosis through DAS-ELISA, DBIA, and TBIA. RT-PCR confirmed the nature of the two viruses. However mixed infection was noticed for CVYV and CVYV upon using duplex RT-PCR.

Conclusion: Mixed infection with WTV is common and complicates diagnosis and breeding for resistance. Antisera for diagnosis of WTV should be homogenous and should be produced through recombinant protein system for successful evaluation of resistance.

Keywords: Co-infection; CYSDV; CVYV; RT-PCR; DAS-ELISA; Immuno blotting

BACKGROUND

Viral diseases of cucurbits are an important limitation in production. Viral diseases can cause massive damage to cucurbit crops up to total loss. More than 35 viruses have been isolated from cucurbits (Provvidenti, 1996).

Whitefly transmitted viruses (WTV) have emerged as a major economic threat to the cucurbit industry in the Middle East and countries in the Mediterranean basin (Hassan and Duffus, 1991; Abou-Jawdah *et al.,* 2000; Cuadrado *et al*., 2001; Yakoubi *et al.,* 2007).

The WTV infecting cucurbits were recorded in Egypt and the Middle East and mostly include viruses in *Begomovirus*, *Ipomovirus,* and *Crinivirus,* genera. The *Begomovirus Squash leaf curl virus* (SqLCV), causing leaf curl symptoms, was introduced into Egypt a decade ago (Abdel-Salam *et al*., 2006a; Idris *et al*., 2006a).

Cucumber vein yellowing virus (CVYV), *Ipomovirus*, *Potyviridae*, is the causal agent of a severe disease of cucurbits, characterized by vein clearing, vein yellowing followed by leaf chlorosis, yellowing and necrosis of older leaves. CVYV infection may cause fruit cracking.

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Virions are filamentous particles and the genome is a single stranded, positive-sense, RNA molecule (Lecoq *et al*., 2000). CVYV is transmitted both mechanically and by the whitefly *Bemisia tabaci* in a semi-persistent manner (Mansour and Al-Musa, 1993). CVYV was originally identified in Israel (Cohen and Nitzany, 1960), Jordan (Al-Musa *et al*., 1985), Turkey (Yilmaz *et al*., 1989) Spain (Cuadrado *et al*., 2001), Portugal (Louro *et al*., 2004), France (Lecoq *et al*., 2007), Tunisia (Yakoibi *et al*., 2007) and Egypt (Abdel-Salam, 2012).

CYSDV*, Crinivirus, Closteroviridae,* is transmitted in a semi-persistent mode by *Bemisia tabaci*. It induces severe yellowing symptoms that begin as interveinal mottle and intensify as leaves age. Particles are flexible rods 750-800 nm long (Liu *et al*., 2000). The genome consists of two molecules of single stranded RNA with positive polarity designated RNA1 and RNA 2 (Célix *et al*., 1996). CYSDV was first reported by Hassan and Duffus (1991) in the United Arab Emirates and has since spread out to many countries around the Mediterranean basin including Egypt (Abdel-Salam *et al*., 1997; Wisler *et al*., 1998; Abdel-Salam, 2012; El-Rahmany *et al*., 2014), Spain (Celix *et al*., 1996), Lebanon (Abou-Jawdah *et al*., 2000), Portugal (Louro *et al*., 2000), Morocco (Desbiez *et al*., 2000), France (Desbiez *et al.*, 2003), and Tunisia (Yakoibi *et al*., 2007). CYSDV was reported for the first time in Northern America in Texas in 1999 (Kao *et al*., 2000) and caused outbreaks in California, Arizona and Northern Mexico (Brown *et al*., 2007, Kuo *et al*., 2007).

Virus-yellowing diseases of cucurbit crops including cucumber, squash, melon, and watermelon and transmitted by the whitefly (*B. tabaci*) incite severe economic losses worldwide (Navas-Castillo *et al*., 2011; Abrahamian and Abou-Jawdah, 2014).

The present study investigates the first incidence of CVYV in Egypt. The study also demonstrates the presence of mixed infection with both CVYV and CYSDV in cucumber, melon, pumpkin, and squash plants; where both viruses are transmitted by the whitefly species *B. tabaci.* The inclusion of these observations on integrated disease control strategies is discussed.

Several investigators have pointed out that mixed infection with both CVYV and CYSDV results in synergism leading to changing in symptom patterns, and dramatic increase in CYSDV levels in infected plants (Gil-Salas *et al*. 2011, 2012). This in turns might affect judging the degrees of resistance upon testing resistance of cucurbit varieties to CYSDV infection. Additionally unnoticed mixed infection with both viruses can lead to the production of polyclonal antisera with antibodies for both viruses which in turns drastically complicate diagnosis as well as breeding for resistance-evaluation results. The implications of these observations on integrated disease management strategies are discussed.

MATERIALS AND METHODS

Virus isolation and propagations:

CYSDV was isolated from different cucurbit plants grown at the experimental farm of the Faculty of Agriculture, Cairo University in Giza governorate in Egypt. Non-viruliferous *B. tabaci* insects were fed onto infected tissues then transferred onto healthy ones using 24 hr and 48 hr-acquisition and inoculation feeding periods, respectively.

CVYV was isolated from watermelon plants grown in Giza, Kafr-El-Sheikh, and Qalyubia governorates. For primary isolation, non-viruliferous whiteflies were fed on symptomatic tissues then transferred onto healthy watermelon seedlings. After primary symptom development, CVYV was serially transferred three consequtive times on other healthy watermelon seedlings through mechanical inoculation using 0.1 M phosphate buffer, pH 7.0, as an inoculation buffer.

CYSDV was propagated onto healthy cucumber, while CVYV was propagated in watermelon. The two viruses were kept in separate rooms in insect-proof greenhouse. Additionally leaf tissues for each virus were frozen at –86°C until used for analysis.

Virus purifications:

The two viruses were purified using frozen tissues according to the technique described by Abdel-Salam (1999). A cold mixture of chloroform and butanol were used for sap clarification. Polyethylene glycol and NaCl were used for virus concentration. Virions were electro-eluted in ISCO Blue Tank, ISCO INC, Lincoln, USA, using 4 mA per cell and tank buffer with buffer concentration equals to 20 folds of suspension buffer. Virus suspensions were frozen, and thawed and measured spectrophotometrically. Virus concentrations was estimated using an extinction coefficient ($A_{0.1\%,\ 1cm,\ 260\ nm}$) value of 2.4 for CVYV (Shukla *et al.,*1998) and a value of 3 for CYSDV (Liu and Duffus , 1990).

Serological studies:

Production of polyclonal antisera for CYSDV and CVYV:

For each virus, two New Zealand white rabbits were given six weekly intramuscular injections (1 ml, each) of the purified virus. First injection was emulsified with an equal volume of Freund's, complete adjuvant, while incomplete adjuvant was used for the other five consecutive injections. The animals were bled weekly starting one week after the last injection for a period of 12 weeks. For both viruses, induced antisera or IgG were cross absorbed with healthy non-diluted sap (20%, v/v) to remove non-specific antibodies according to Abdel-Salam (1999).

Serological tests

Dot and tissue blotting immunoboinding assay (DBIA, TBIA):

The procedure of DBIA described by Abdel-Salam et al. (1997) was followed. For TBIA, nitrocellulose membranes (NCM**),** washed in distilled water and air dried, were tissue blotted then blocked with 5% (w/v) non-fat dry milk (NFDM) and 1% (w/v) bovine serum albumin, prepared in water, for 1 h at 37^{o}C. After descanting the blocking solution, NCM were incubated overnight at 4^{o}C in IgG, diluted 10^{-3} in phosphate buffer saline, PBS. NCM were washed three X, 10 min each, in PBS containing 0.5% Tween (PBST), pH 8.0. NCM were then transferred to goat anti-rabbit alkaline phosphatase conjugate (GARAP) diluted at 10^{-4} in PBST containing 5% NFDM and 2% (w/v) polyvinyl pyrrolidone and left for 3 h at 37^{o}C or overnight at 4^{o}C. NCM were washed six X, 5 min, each at 37^{o}C. NCM were air dried and stained with Naphthol/Fast Red complex as described before (Abdel-Salam *et al;* 1997).

Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA):

The DAS-ELISA procedure described by Clark and Adams (1977) was followed with some modification including an additional blocking step with 5% non-fat dry milk and 1 % bovine serum albumin after the IgG coating and washing steps. Extraction buffer used for sap extraction composed of 0.1 M sodium citrate, pH 6, containing 2.5 mM EDTA and 2 % Triton X 100. Samples were extracted at a dilution 1/20 (w/v). IgG fractions for induced antisera were separated with caprylic acid as described by Abdel-Salam (1999). IgG-alkaline phosphatase conjugation was prepared according to Converse and Martin (1990). IgG concentrations used for plate coating was tested at a range between one to four µg/ml, whereas for both viruses, IgG-enzyme conjugate was tested at rage of dilutions between 1/500 up to 1/3000. Symptomatic

leaves with yellows symptoms of several cucurbit species were collected from the experimental farms of the Faculty of Agriculture, Cairo University at Giza, Egypt.

Molecular studies:

Oligonucleotide primers for CYSDV and CVYV:

The specific oligonucleotide primers CYSCPf (5' ATGGCGAGTTCGAGTGAG AATAA 3') and CYSCPr (5' ATTACCACAGCCACCTGGTGCTA 3') were designed by Rubio et al. (2001) to amplify the full length of the coat protein (CP) gene, 756 bp, of CYSDV. For CVYV, specific oligonucleotide primers (CV (+): 5' AGCTAGCGCGTATGGGGTGAC 3'; CV (-): 5' GCGCCGCAAGTGCAA-ATAAAT 3′) were designed by Cuadrado *et al.* (2001) to amplify 449 bp of the CP of CVYV.

Total RNA extraction and reverse transcription polymerase chain reaction (RT-PCR):

Total RNAs of the viruses understudy were extracted from 100 mg of infected, symptomatic leaves using RNeasy plant mini kit, Cat No. 74903, Qiagen Sciences, Meryland, USA. Complementary DNA (cDNA) was reverse-transcribed from 2 µL of total RNAs (0.15 /ml) in a total reaction volume of 20 µl (Cat No. M510A Promega, Madison, WI, USA). The reaction mixture was incubated at 42°C for 1 h followed by heating at 94^{o}C for three min then frozen.

For duplex PCR for CYSDV and CVYV, PCR was performed, using GOTaq Flexi DNA polymerase kit (Promega Cat No. M8301, Madison, WI, USA). For 25 µl reaction mixture, 2 µl of the synthesized cDNA, 2.5 µl of 10X reaction buffer, 2.5 mM $MgCl_2$, 1.25 U Taq polymerase, 0.2 mM for each dNTP, 0.4 µmol for each forward and reverse primer, for each virus, were mixed in the presence of diethylpyrocarbonate (DEPC)-treated water. The mixture was incubated first at 94^{o}C for 3 min, followed by 30 cycles of denaturation at 92^{o}C for 30 s, primer annealing at 53^{o}C for 1 min, and primer extension at 72^{o}C for 1 min per cycle with a final extension cycle at 72^{o}C for 5 min. DNA amplicons were evaluated by electrophoresis with 2% agarose gel prepared in TAE buffer and stained with 0.5 µg/ml ethidium bromide.

RESULTS

Symptomatology:

Symptoms caused by CVYV infection to cucumber in the field are mostly expressed as vein yellowing (VY) on young leaves which turns to general yellowing and necrosis on older leaves (Fig. 1-A). Vein yellowing symptoms were induced by mechanical inoculation in the greenhouse (Fig. 1-B). Infected melon plants with CVYV show vein yellowing on small leaves (Fig. 1-C) then VY was replaced by bright yellowing and brown necrotic areas with limited margins. Infected melon fruits show bright chlorotic areas which coalesce together to larger yellow patches which become necrotic causing fruit cracking (Fig. 1-D). Generally infected fruits are small and malformed and the produced crop is of low yield and quality. Similar VY symptoms are expressed on infected watermelon plants (Fig. 1-E). Upon disease progress leaves show general yellowing and necrotic areas are common. Symptoms on CVYV-infected zucchini squash are difficult to observe and the VY symptoms are expressed only on small leaves (Fig. 1-F. Older leaves show general yellowing and brown necrosis.

Infected cucumber, melon, watermelon, and pumpkin with CYSDV show similar disease expression including interveinal chlorosis on older leaves where smaller leaves appear normal (Fig. 2-A). Upon disease progress, the entire leaves become yellow but the veins remain green. Formed fruits are small and deformed (Fig. 2-B). Infected Plants appear stunted with stiffened stems.

Fig.1: Symptoms induced by CVYV infection on of cucumber (A, B), on melon (C, D), on watermelon (E), and on squash (F).

Fig. 2: Symptoms induced by CYSDV on cucumber at early (A) and progressed disease stages (B).

Mixed infection with more than one virus is a common phenomenon in the field. Field observation showed mixed infection with CYSDV and CVYV in both cucumber (Fig. 3-A) and muskmelon (cantaloupe) (Fig. 3-B). Dual infection of cucumber with both viruses led to the formation of chlorotic deformed fruits (Fig. 3-A).

Fig.3: Mixed infection of both CYSDV and CVYV on (A) cucumber and cantaloupe (B)

Chemical studies:

Virus purification:

Purified virus preparations of CVYV and CYSDV and their spectophotometric parameters are illustrated in Table (1).

Table 1: Virus yields and particle physical properties of isolates of CVYV and CYSDV in Egypt

Virus/ isolates	A_{max}	A_{min}	$A_{max/min}$	$A_{260/280}$	Virus yield*
CVYV/Giza	260	240	1.23	1.65	0.30
Kafr-El-Sheikh	260	240	1.17	1.57	0.50
Qalyubia	260	240	1.23	1.71	0.56
CYSDV/ Giza	260	240	1.08	1.47	1.16

*measured as mg/g fresh tissues.

Serological studies:

Immunoblotting assays using DBIA and TBIA:

Antisera for CYSDV and CCYV were used to detect both viruses in cucurbits in the field using DBIA and TBIA as illustrated in Figures 4 and 5, respectively.

DAS-ELISA:

Results indicated that the optimum concentrations for the IgG used for coating the plates was 2 µg/ml for CYSDV and 1 µg/ml for CVYV; whereas the optimum dilution for IgG-enzyme conjugate for both viruses was at 1/2000 dilution. The induced antisera of both CVYV and CTSDV were able to detect both viruses in cucumber, squash, watermelon, melon, and pumpkin. The optical density (OD) at 405 nm in tested samples was three times more than the corresponding healthy controls.

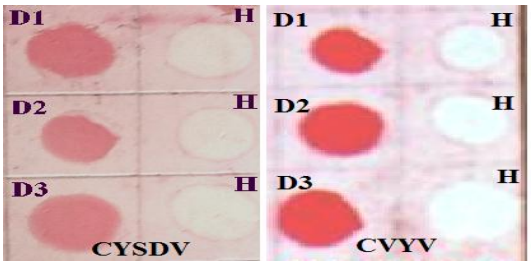


Fig. (4): DBIA applied on nitrocellulose membrane (NCM) showing the reactions of the induced antisera (AS) against some infected cucurbits in the field with their corresponding healthy (H) samples. D1 = infected cucumber; D2 = infected melon; D3 = infected watermelon. Samples were diluted 1/10 in PBST. CYSDV- and CVYV-IgG AS were used at 1/2000 dilution. GARAP conjugate was used at 1/10,000 dilution. NCM was stained with Naphthol/Fast Red complex

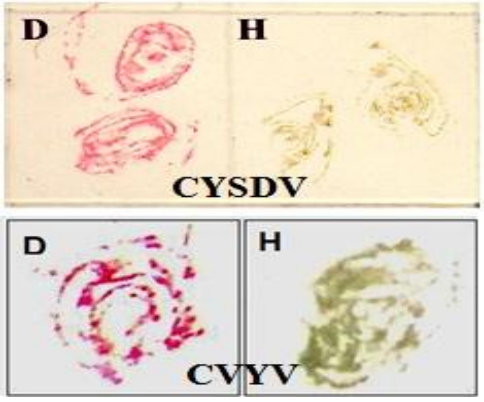


Fig. 5: TBIA applied on NCM showing the reactions of the induced antisera for CYSDV and CVYV against infected cucumber in the field. IgG antisera for both viruses were used at 10^{-3} dilution while GARAP was used at 10^{-4} dilution. NCM was stained with Naphthol/Fast Red complex

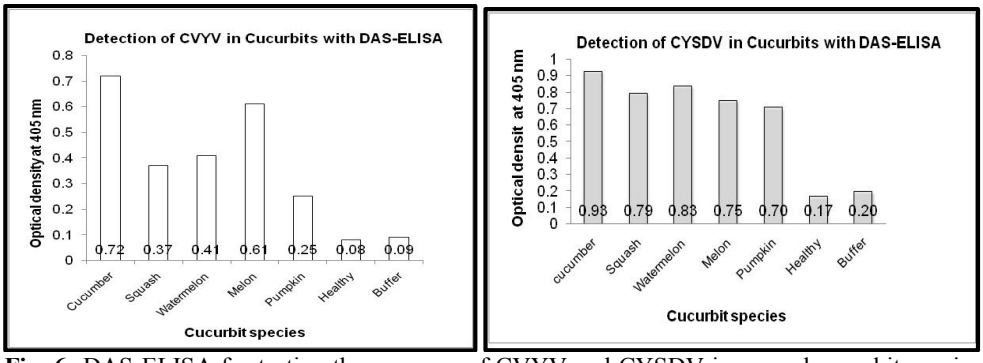


Fig. 6: DAS-ELISA for testing the presence of CVYV and CYSDV in several cucurbit species. For each virus, 15 leaves for each of cucurbit species were tested. Each experiment was repeated thrice and average of OD in each cucurbit species was used in data analysis

Molecular studies:

Results of duplex RT-PCR (Fig. 7) using primers for the CP of both CYSDV and CVYV amplified amplicons at 756 and 449 bp, respectively; indicating the presence of mixed infection with both viruses in the tested samples of cucumber, melon, pumpkin and squash. Results in Fig. (7-B) showed that upon testing three plants, one was solely infected with CYSDV, a second with both viruses, and a third plant free from both viruses.

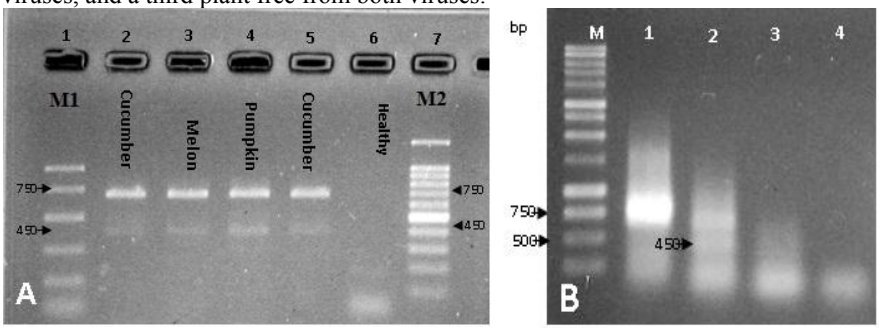


Fig. 7: Agarose gel (2%) electrophoresis of duplex RT-PCR of amplified 449 bp of CVYV and 756 bp of CYSDV CP genes using CV+/CV- and CYSCPf/CYSCPr primers, respectively. In (A), M1 (lane 1) and M2 (lane 7) are PCR markers and 100 bp DNA ladder, respectively; lanes 2, 3, 4, and 5 are infected cucumber, melon, pumpkin, and cucumber, respectively. Lane 6, healthy cucumber. In (B), M = 1kb DNA ladder; lanes 1, 2, 3, are different tested squash samples; 4, healthy squash

DISCUSSION

The present study aimed at characterizing some of the viruses causing yellowing diseases in cucurbit in some governorates in Egypt. The viruses, understudy, included one crinivirus, viz. CYSDV and one Ipomovirus, viz. CVYV. The study involved isolation, propagation, purification and antiserum production for CVYV and CYSDV. Additionally, this study is illustrating the adverse effect of mixed infection with some of these viruses on diagnosis and breeding for virus resistance in cucurbits.

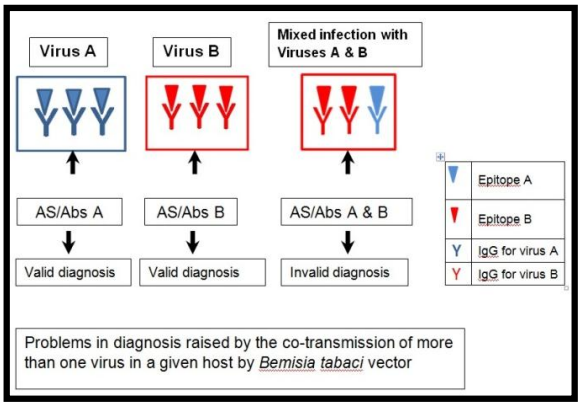
The descriptive symptoms induced by both viruses in this study are typical to those cited in the literature for CYSDV (Abdel-Salam *et al;* 1997, Abou-Jawdah *et al.,* 2000; Yakoubi *et al*., 2007; Abdel-Salam, 2012) and CVYV (Yakoubi *et al*., 2007; Abdel-Salam, 2012).

The two viruses were partially purified using the electro elution (EE) technique for purifying and concentrating biomolecules (Abdel-Salam, 1999). Both viruses had A_{max} and A_{min} at 260 and 280 nm, respectively; indicating purity of the resulting virus nucleoproteins. Purified CYSDV had A $_{260/280}$ ratio of 1.47 in the range of similar ratios (1.44-1.61-1.73) described for other closteroviruses (Brunt *et al.,* 1996). Three isolates of CVYV from Giza, Kafr-El-Sheikh, and Qalyubia were purified and all had almost similar A $_{260/280}$ ratios of 1.65, 1.57 and 1.71. These ratios, however, were different from A_{260}/A_{280} ratio of 1.243 found by Mansour and Hadidi (1999) for purified CVYV from cucumber. Such variations in ratios may be attributed to the purification method itself. The presence of contaminated host proteins may increase the A_{280} value and hence lower the A_{260}/A_{280} ratio. The EE technique depends on separation of biomolecules on surface-electric charge and osmotic pressure through semi-permeable membrane. Probably the success of such a technique is the evasion of the ultra-speed

centrifugation and its sheering forces on bimolecular entities. The EE technique was utilized for the first time in purifying *Cotton leaf curl mosaic virus* (Abdel-Salam, 1999). Later on it was used in purifying many plant viruses such as *Banana bunchy top virus* and *Faba bean necrotic yellows virus* (Abdel-Salam *et al*. 2004), *Banana streak virus* (Abdel-Salam *et al*., 2005), *Beet necrotic ringspot ilarvirus* (Abdel-Salam *et al*., 2006b), *Squash leaf curl virus* (Abdel-Salam *et al.*, 2006a), and *Prunus necrotic ringspot virus* (Abdel-Salam *et al*., 2008).

Both locally induced antisera detected CYSDV and CVYV in various cucurbit species in the field with DAS-ELISA. Both CYSDV and CVYV antisera had optimum IgG-enzyme conjugate at 1/2000 dilution in DAS-ELISA; being much better than lower dilution (≈ 1/200) used in commercial kits in the market.. Each of DBIA and TBIA were able to detect several species of cucurbits for infection with each of CYSDV and CVYV. Both immuno- blotting techniques have been used for detection of many plant viruses (Abdel-Salam, 1999; Abdel-Salam *et al*, 2004; Tzanetakis *et al*., 2004; Abdel-Salam *et al*., 2005, 2008). Trials made to test specificity of the induced antisera, using DAS-ELISA, to detect CYSDV with CVYV antiserum failed. Similarly CYSDV antiserum failed to detect CVYV in watermelon (data not shown). Such latter results may entail high specificity of the two induced antisera. However, this has turned out to be misleading as it will be discussed below in the molecular analysis with RT-PCR section.

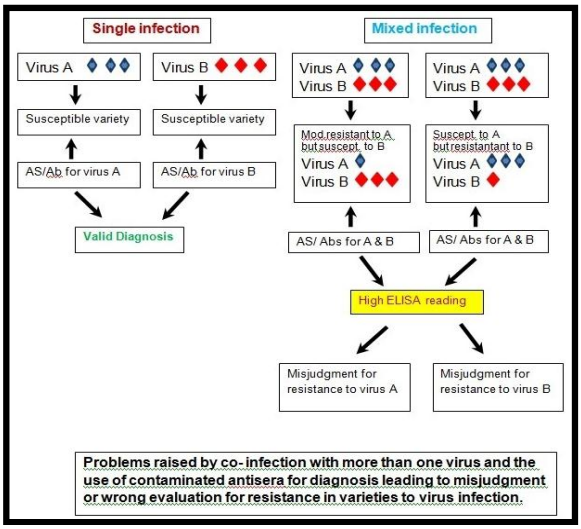
Duplex RT-PCR using primer pairs for testing possible mixed infection of cucurbit species with CYSDV and CVYV indicated that all the tested cucumber, melon, pumpkin, and squash plants were dually infected with CYSDV and CVYV. This mixed infection is attributed to the ability of *B. tabaci* whitefly to transmit both viruses semipersistently. Mixed infection in cucurbits with whitefly transmitted viruses is not uncommon (Celix *et al.,* 1996; Jansssen and Cuadrado, 2001; Yakoubi *et al*., 2007, Abdel-Salam, 2012; Amer, 2015). Mixed infection with CVYV and CYSDV can lead to synergism and drastic increase in CYSDV concentration in cucumber compared with single infection with CYSDV (Gil-Salas *et al*., 2011, 2012). Abrahamian *et al*. (2013) reported double or triple infection of *Cucurbit chlorotic yellows virus* (CCYV), a crinivirus, and CYSDV in 18 out of 20 plants reported infected with CVYV by RT-PCR in Lebanon. Infection of cucumber with *Squash leaf curl virus*, (SLCV), a begomovirus, yields neither symptom expression nor yield reduction. However co infection of cucumber with SLCV in the presence of other criniviruses as CYSDV and/or CCYV developed yellowing symptoms and considerable reduction in cucumber yield (Abrahamian *et al*., 2015). Many investigators have pointed out that mixed infection between unrelated viruses often results in viral synergism (Pruss *et al*., 1997; Mukasa *et al*., 2006; Untiveros *et al*., 2007). Karyeja *et al.* (2000) showed that Single infection with *Sweet potato feathery mottle virus,* a potyvirus or *Sweet potato chlorotic stunt virus*, a crinivirus had very little effect on symptom expression in sweet potato. On the other hand, dual infection with both viruses broke the resistance of SPFMV-resistant cv. Tanzania. From the above discussion it is clear that single infection with a crinivirus has little effect on the infected plant. While co infection or mixed infection with unrelated virus can lead to serious sequences of synergism expressed in increasing the concentration of the crinivirus, increase of disease-symptom manifestation, and in some instances dismantling host resistance to viral infection (Untiveros *et al.,* 2007; Tzanetakis, *et al*., 2013; Abrahamian and Abou-Jawdah 2014).



For Screening for resistance in cucurbits against virus infection, serological methods, especially ELISA, are being used profusely. Molecular tools as RT-PCR, PCR, and nucleic acid hybridization are also used (Aguilar *et al.,* 2006; McCreight and Wintermantel, 2008) but with some limitation due to the high costs of such techniques. Thus, DAS-ELISA remains as one of the most accurate techniques for quantitative assay, diagnosis, and screening for resistance in plant varieties against virus infection.

Mixed infection with WTV in cucurbits is common and complicates diagnosis and breeding for resistance. For instance, presence of two cucurbit viruses in a single host used for purification and antiserum production would lead to the induction of antiserum (AS) with antibodies (Abs) for both viruses. This most likely is the case in the present research where RT-PCR detected both CVYV and CYSDV in the same tested samples for several cucurbit species (see Fig. 7). The failure of DAS-ELISA to detect both viruses in the same samples is probably due to the low sensitivity of DAS-ELISA comparing to RT-PCR. Probably the induced antiserum for CYSDV in the present study would contain antibodies for CYSDV and CVYV since this antiserum was the outcome of purified preparations from infected cucumber plants through whitefly transmission. With the fact that *B. tabaci* insects are capable of co transmission of CYSDV and CVYV semipersistantely, there is no guarantee that the CYSDV-infected cucumber plants used for whitefly transmission to healthy cucumber plant in the green house is free from infection with CVYV contaminating the preparation. On the other hand the antiserum prepared for CVYV is expected to be free from any contamination with CYSDV or other WTV since mechanical transmission to watermelon plants was used as a propagation source for purification. It is known that criniviruses are solely whitefly transmitted (Tzanetakis *et al*., 2013) and not mechanically transmitted. This, in turns, will exclude any contaminating crinivrus(s) that might contaminate the propagation materials of CVYV during purification. Several investigators are beginning to overcome this problem of mixed infection of criniviruses with related or unrelated viruses by producing recombinant coat protein antiserum for the criniviruses CYSDV (Steel *et al*., 2010; Hourani, and Abou-Jawdah, 2003) and CCYV (Kubota *et al*., 2011).

Mixed infection with more than one virus in a given host has serious consequences if this host was used for antiserum production. Such antiserum,with dual species of antibodies, if used in evaluation of resistance for a given variety or *Plant Introduction* (PI) against either one of the target viruses would lead to erroneous conclusion. The attached illustration indicates that upon casual co-infecting a given variety with two viruses and this host is moderately resistant to one of the two viruses and susceptible to the second, wrong evaluation or misjudgment of degree of resistance would happened.



CONCLUSION

Mixed infection with WTV is common in cucurbits and complicates diagnosis and breeding for resistance. Antisera for diagnosis of WTV and evaluation of resistance should be homogenous and should be produced through recombinant protein system. Molecular tools as PCR, immunocapture-PCR, and nucleic acid hybridization should be used infrequently to assist results of DAS-ELISA performed with recombinant antisera.

AUTHOR DETAILS

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REFERENCES

Abdel-Salam, A.M. 1999. Isolation and partial characterization of a whitefly-transmitted geminivirus associated with the leaf curl and mosaic symptoms on cotton in Egypt. Arab .J. Biotech. 2(2): 193-218.

Abdel-Salam, A. M. 2012. Occurrence of *Cucurbit yellow stunting disorder virus* and *Cucumber vein yellowing virus* in Cucurbits in Egypt. Epidemiology and management of whitefly-transmitted viruses- Cross-industry workshop, 15-17 Oct. 2012, Brisbane, Australia

Abdel-Salam, A.M., Abdalla, Nagla M., Soliman, Doaa Z.R., and Rezk, A.A. 2006a. The incidence of squash leaf curl begomovirus (SLCV) in Egypt. Arab J. Biotech. 9(2): 375-388.

Abdel-Salam, A.M., Abdel-Kader, H.S., and El-Saghir, S.M. 2005. Biological, serological, and molecular detection of *Banana streak Badnavirus* in vegetatively propagated banana plants in Egypt. Egypt. J. Virol. 2(1):255-268.

Abdel-Salam, A.M., Abdel-Kader, H.S., El-Saghir, S.M, and Hussein, M.H. 2004. Purification, serology, and molecular detection of Egyptian isolates of banana bunchy top babuvirus and faba bean necrotic yellows nanovirus. Arab J. Biotech. 7 (1):141-155.

Abdel-Salam, A.M., El-Shazly, Manal A., and Abdelkader, Hyam S. 2006b. Beet necrotic ringspot virus, a new ilarvirus infecting sugarbeet in Egypt. Biological, biochemical, serological, and genomic studies. Arab J. Biotech. 9(2): 395-414.

Abdel-Salam, A.M, Hassan, A.A., Merghany, M.M., Abdel-Ati, K.A., and Ahmed, Y.M. 1997. The involvement of a geminivirus, a closterovirus, and a spherical virus in the interveinal mottling and yellows diseases of cucurbit in Egypt. Bull. Fac. Agric., Univ. Cairo 48:707-722.

Abdel-Salam, A.M., Ibrahim, A.M.I., Abdel-Kader, H.S., Aly, A.M.E., and El-Saghir, A.M. 2008. Characterization of two isolates of *Prunus necrotic virus* (PNRSV) from peach and apricot in Egypt. Arab. J. Biotech. 11(1):107-124.

Abou-Jawdah, Y., Sobh, H., Fayad, A., Lecoq, H., Delecolle, B., and Trad-Ferre, J. 2000. *Cucurbit yellow stunting disorder virus*- a new threat to cucurbits in Lebanon. J. Plant Pathol. 82(1):55-60.

Abrahamian, P.E. and Abou-Jawdah, Y. 2014. Whitefly-transmitted criniviruses of cucurbits: current status and future prospects. Virus Dis. 25(1):26–38.

Abrahamian, P., Sobh, H., Seblani, R. and Abou-Jawdah, Y. 2015. Co-infection of two criniviruses and a begomovirus enhances the disease severity in cucumber. Eur. J Plant Pathol. 142:521–530.DOI 10.1007/s10658-015-0630-y.

Abrahamian, P., Sobh, H., Seblani, R., Samsatly, J., Jawhari, M., and Abou-Jawdah, Y. 2013. First Report of *Cucumber vein yellowing virus* on Cucumber in Lebanon. Plant Dis. 97(11):1516.

Aguilar, J.M., Abad, J., Aranda, M.A. 2006. Resistance to *Cucurbit yellow stunting disorder virus* in cucumber. Plant Dis. 90(5):583-586.

Al-Musa, A.M., Qusus, S.J. and Mansour, A.N. 1985. Cucumber vein yellowing virus on cucumber in Jordan. Plant Dis. 69:361

Amer, M.A. 2015. Serological and molecular characterization of *Cucurbit chlorotic yellows virus* affecting cucumber plants in Egypt. Int. J. Virol.11:1-11.

Brown, J.K., Guerrero, J.C., Matheron, M., Olsen, M., and Idris, A.M. 2007. Widespread outbreak of Cucurbit yellow stunting disorder virus in melon, squash, and watermelon crops in the Sonoran Desert of Arizona and Sonora, Mexico. Plant Dis. 91:773.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) (1996 onwards). `Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 16th January 1997.' URL http://biology.anu.edu.au/Groups/MES/vide/

Célix A., Lopez-Sesé A., Alwarza N., Gomez-Guillamón M.L., Rodriguez-Cerezo E. 1996. Characterization of cucurbit yellow stunting disorder virus, a *Bemisia tabaci* transmitted closterovirus. Phytopathol. 86: 1370-1376.

Clark, M.E. and Adams, A.N. 1977. Characterization of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

Cohen, S. and Nitzany, F.E. 1960. A whitefly-transmitted virus of cucurbits in Israel. Phytopathol. Mediterr. 1:44–46.

Converse, R. and Martin, R. 1990. Enzyme linked immunosorbent assay (ELISA). In:Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens (R Hampton, E Ball, S de Boer, eds.), pages 179-196, APS Press, Saint Paul, MN, USA,.

Cuadrado, I.M., Janssen, D., Velasco, L., Ruiz, L. and Segundo, E. 2001. First report of Cucumber vein yellowing virus in Spain. Plant Dis. 85:336.

Desbiez, C., Lecoq, H., Aboulama, S., Peterschmitt, M. 2000. First report of Cucurbit yellow stunt disorder virus in Morocco. Plant Dis. 84:596.

Desbiez, C., Lecoq, H., Girard, M., Cotillon, A.C., Schoen, L. 2003. First report of Cucurbit yellow stunting disorder virus in commercial cucumber greenhouses in France. Plant Dis. 87:600.

El-Rahmany, R.G., El-Attar, A.K., Zein, H.S., Abdallah, N.A., and Mazyad, H.M. 2014. Identification and characterization of the Egyptian isolate of the cucurbit yellow stunting disorder virus. Arab J. Biotech. 17(1):29-40.

Gil-Salas, F.M.,Peters, J.,Boonham, N.,Cuadrado, I.M., and Janssen, D. 2011. Yellowing Disease in Zucchini Squash Produced by Mixed Infections of Cucurbit yellow stunting disorder virus and Cucumber vein yellowing virus. Phytopathol. 101(11):1365-1372.

Gil-Salas, F.M., Peters, J., Boonham, N., Cuadrado, I.M., and Janssen, D. 2012. Co-infection with Cucumber vein yellowing virus and Cucurbit yellow stunting disorder virus leading to synergism in cucumber. Plant Pathol. 61:468-478.

Hassan A.A., Duffus J.E., 1991. A review of a yellowing and stunting disorder of cucurbits in the United Arab Emirates. Emirates J. Agric. Sci. **2**:1-16.

Hourani, H, Abou-Jawdah, Y. 2003. Immunodiagnosis of Cucurbit yellow stunting disorder virus using polyclonal antibodies developed against recombinant coat protein. J. Plant Pathol. 85:197–204.

Idris,A., Abdel-Salam,A. and Brown,J. 2006. Introduction of the New World *Squash leaf curl virus* to Squash (*Cucurbita pepo*) in Egypt: A Potential Threat to Important Food Crops. Plant Dis. 90:262.

Janssen, D. and Cuadrado, I.M. 2001. Whitefly problems escalate within Spanish cucurbit crops. ESWN Newsletter no. 11. Norwich, UK. John Innes Centre.

Kao J, Jia L, Tian T, Rubio L, Falk BW. 2000. First report of Cucurbit yellow stunting disorder virus (genus Crinivirus) in North America. Plant Dis. 84:101.

Karyeija, R.F., Kreuze, J.F., Gibson, R.W. and Valkonen, JP. 2000. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology 269(1):26-36.

Kubota, K., Usugi, T., and Tsuda, S. 2011. Production of antiserum and immunodetection of Cucurbit chlorotic yellows virus, a novel whitefly-transmitted crinivirus. J. Gen. Plant Pathol. 77:116–120. doi: 10.1007/s10327-010-0287-5.

Kuo, Y.W., Rojas, M.R., Gilbertson, R.L., and Wintermantel, W.M. 2007. First report of Cucurbit yellow stunting disorder virus in California and Arizona, in association with Cucurbit leaf crumple virus and Squash leaf curl virus. Plant Dis. 91:330.

Lecoq, H., Desbiez, C., Delécolle, B., Cohn, S. and Mansour, A, 2000. Cytological and molecular evidence that the whitefly-transmitted Cucumber vein yellowing virus is a tentative member of the family Potyviridae. J. Gen. Virol. 81:2289–2293.

Lecoq, H., Dufour, O., Wipf-Scheibel, C., Girard, M., Cotillon, A.C., Desbiez C., 2007. First report of Cucumber vein yellowing virus in melon in France. Plant Dis. 91: 909

Liu, H.Y. and Duffus, J.E. 1990. Beet pseudo-yellows virus: Purification and serology. Phytopathol. 80:866-869.

Liu, H.Y., Wisler, G.C. and Duffus,J.E. 2000. Particle lengths of whitefly-transmitted criniviruses. Plant Dis. 84:803–805

Louro, D., Vicente, M., Vaira, A.M., Accotto, G.P., Nolasco, G. 2000. Cucurbit yellow stunting disorder virus (genus Crinivirus) associated with the yellowing disease of cucurbit crops in Portugal. Plant Dis. 84:1156.

McCreight, J.D. and Wintermantel, W.M. 2008. Potential new sources of genetic resistance in melon to *Cucurbit yellow stunting disorder virus*. Proceedings of the IXth EUCARPIA

meeting on genetics and breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008.

Mansour, A. and Al-Musa, A. 1993. Cucumber Vein Yellowing Virus; Host Range and Virus Vector Relationships. J. Phytopathol. 137(1):73-78.

Mansour, A.N. and Hadidi, N. 1999. Cucumber vein yellowing virus: purification and serological studies. Dirasat. Agric. Sci. 26(26):8-14.

Mukasa, S.B., Rubaihayo, P.R., and Valkonen, J.P.T.2006. Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweetpotato plants. Plant Pathol.55: 458–467.

Navas-Castillo, J., Fiallo-Olive´, E., Sa´nchez-Campos, S. 2011. Emerging virus diseases transmitted by whiteflies. Annu. Rev. Phytopathol. 49:219–48.

Provvidenti, R. 1996. Diseases caused by viruses. In: Compendium of Cucurbit Diseases, (T. A. Zitter, D. L. Hopkins and C. E. Thomas, Eds.), pages 37-45, PS Press, St. Paul, Minn.

Pruss G., Ge, X., Shi, M.X., Carrington, J.C., Vance, V.B. 1997. Plant viral synergism: the potyviral genome encodes a broad range pathogenicity enhancer that transactivates replication of the heterologous viruses. The Plant Cell 9:859–868.

Rubio, L., Abou-Jawdah, Y., Lin, H.X., Falk, B.W. 2001. Geographically distant isolates of the crinivirus Cucurbit yellow stunting disorderirus show very low genetic diversity in the coat protein gene. J Gen Virol. 82:929–33.

Shukla, D.D., Ward, C.W., Brunt, A.A. and Berger, P.H. 1998. Potyviridae family. AAB Descriptions of Plant Viruses no. 366. http://www.dpvweb.net/dpv/showdpv.php?dpvno=366.

Steel, E., Barker, I., Danks, C., Coates, D., and Boonham, N. 2010. A tumefaciens-mediated transient expression as a tool for antigen production for cucurbit yellow stunting disorder virus. J Virol. Methods.163:222–228. doi: 10.1016/j.jviromet.2009.09.024.

Tzanetakis, I.E., Halgren, A.B., Keller, K.E., Hokanson, S.C., Maas. J.L., McCarthy, P.L., Martin, R.R. 2004. Identification and detection of a virus associated with strawberry pallidosis disease. Plant Dis. 88:383–90.

Tzanetakis, I.E., Martin, R.R., and Wintermantel, W.M. 2013. Epidemiology of criniviruses: an emerging problem in world agriculture. Front. Microbiol./Virol. 4:1-12.

Untiveros, M., Fuentes, S., and Salazar, L.F. 2007. Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with Carla-, Cucumo-, Ipomo-, and Potyviruses infecting sweet potato. Plant Dis. 91(6):669-676.

Wisler, G.C., Duffus, J.E., Liu, H.Y., Li, R.H. 1998. Ecology and epidemiology of whitefly-transmitted Closteroviruses. Plant Dis. 82:270–80.

Yakoubi, S., Desbiez, C., Fakhfakh, H., Scheibel, C.W., Marrakchi, M., and leciq, H. 2007. Occurrence of cucurbit yellow stunting disorder virus and cucumber vein yellowing virus in Tunisia. J. Plant Pathol. 89(3):417-420.

Yilmaz, M.A., Ozaslan, M. and Ozaslan, D. 1989. Cucumber vein yellowing virus in Cucurbitaceae in Turkey. Plant Dis. 73:610.

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