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MOLECULAR CHARACTERISTICS OF *ZUCCHINI YELLOW MOSAIC VIRUS* (ZYMV) ON CUCUMBER PLANTS AND COMPARISON BETWEEN MOLECULAR CHARACTERISTICS OF TWO ISOLATES OF *VIRUS* (ZYMV) THAT INFECTS CUCURBITS IN RIYADH REGION, SAUDI ARABIA

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ABSTRACT:

***Zucchini yellow mosaic virus* (ZYMV) is the most prevalent virus in cucurbits in Saudi Arabia. Samples of cucumber(*Cucumis sativus*)and Pumpkin (*Cucurbita moschata*) that show symptoms of virus infection were collected during 2009 growing season from field-grown cucumber and pumpkin in Riyadh Saudi Arabia. Based on reverse transcription polymerase chain reaction (RT-PCR) analysis using one primer pairs, the virus was identified as strain of ZYMV. The PCR assay revealed that the two Riyadh isolates, ZYMV1(cucumber) and ZYMV2(Pumpkin) were closely related to ZYMV. Serological and molecular data indicated that the two Saudi ZYMV isolates were biologically variable and presented a low molecular diversity. The Gen bank numbers were GU808330.1 and GU058058.1 for ZYMV1 and ZYMV2, respectively. The nucleotides sequence of the two isolates ZYMV1 and ZYMV2 presented molecular diversity that suggests that they did not share a common origin and they have adapted to their different hosts.**

INTRODUCTION:

Cucurbits are among the main vegetable crops grown in Saudi Arabia. Cucurbit are infected by several viruses such as *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV) that cause severe diseases and serious yield reduction in cucurbit crops. From the economic point of view, ZYMV considers the most important cucurbit virus in KSA in general and in Riyadh in particular (Al Shahwain, 1995). One of the reasons makes it very dangerous; ZYMV induces different types of symptom including yellow mosaic, mottle, blisters, stunting, leaf and fruit deformation (Desbiez & Lecoq, 1997). ZYMV, a species of the genus Potyvirus in the family *Potyviridae*, with a positive sense-single stranded RNA of about 9.5 kb (Gal-On, 2007). Strains of from distinct geographic origins exhibit biological diversity, particularly concerning their host, symptomatology and aphid transmission (Desbiez *et al.*, 2002). The virus was first reported in Italy and France by Lisa *et al.* (1981), and within a few years the virus had been reported from almost all cucurbit

producing countries worldwide including those of Europe, Asia, Africa, North and South America, and Oceania (Desbiez & Lecoq, 1997 and Gal-On, 2007).

The positive sense single-stranded RNA genome of ZYMV, like other potyviruses, was found to be translated as one polyprotein precursor containing the activity of viral RNA-dependent RNA polymerase (Hong *et al*., 1995).

This action has multifunctional activities involved in aphid transmission, cell-to-cell movement, systemic movement encapsidation of the viral RNA, and regulation of viral RNA amplification (Gal-On *et al*., 1992; Dolja *et al*., 1993;Varrelamann and Maiss, 2000 and Hong *et al*., 1995).

Since the 1970s, serological methods especially enzyme-linked immunosorbent assay (ELISA) have been used widely and successfully for detection of plant viruses and diagnosis of plant viral diseases (Clark and Adams, 1977). In the 1990s, nucleic acid-based methods such as reverse transcription (RT) and the polymerase chain reaction (PCR) began to be used in plant virus detection (Rowhani *et al*., 1995 and Thomson *et al*., 1995). Accordingly, several degenerate primers have been designed to recognize the conserved regions of viral genomes of many virus species or the whole virus genus or family(Tian *et al*., 1996 and Chen *et al*., 2001).

In this study, the biological and molecular methods were used to characterize the variability of ZYMV isolates from cucumber (*Cucumis sativus*) and pumpkin (*cucurbita moschata*) plants grown in Riyadh, Saudi Arabia.

MATERIALS AND METHODS:

Sample Collection:

Samples were collected during the 18-May-2009 growing seasons from field-grown cucumber (*Cucumis sativus*)and pumpkin (*cucurbita moschata*) plants grown in Riyadh, Saudi Arabia. Show symptoms such as: mosaic, yellowing, leaf distortion, hoestring, fruit deformation and yield reduction. Young leaves from some symptomatic plants were collected at random. All samples were kept in ice chests for transportation to the laboratory. Each plant sample was kept separately in a plastic bag at 4^{o}C until analyzed.

RNA Extraction:

Total RNA was extracted from dried (20 mg ZYMV-infected leaves using TRI-Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions) resulting pellets were dissolved in 125 µl RNase-free water.

Reverse Transcription Polymerase Chain Reaction (RT-PCR):

Total RNA from ZYM-infected plants was extracted using a phenol/chloroform protocol (Wadsworth *et al*., 1988). Three µl of RNA were submitted to reverse transcription in a final volume of 20 µl, using 2µl PCR buffer 10x(0.5 M Tris-HCl, 0.7 M KCl, 0.1 M $MgCl_2$, pH 8), 1µl DTT (100 mmol/µl), 1µl dNTPs (10 mmol/ µl), 0.5 µl RNase inhibitors enzymes (10 mmol/µl) and 2 µl Reverse-DAG primer (100 pmol/µl) (5

/- GCT CCA TAC ATA GCT GAG ACA GC -3/: nucleotide position 091206P203 on sequence L 3098 for one hour at 42°C with 0.5 µl ZYMV reverse transcriptase (200 mmol/µl). 5 µl of the RT reactions were used for PCR using a 5 µl PCR buffer 10x, 2 µl $MgCl_2$, 1 µl dNTPs (10mmol/µl), 0.5 µl Taq polymerase (5 unit/µl), 1 µl Reverse-DAG (100 pmol), and 1 µl Forward-DAG (100 pmol) (5/-TAG GCT TGC AAA CGG AGT CTA ATC/: 091206P204 on sequence L 7457 oligonucleotides encompassing the N-terminal part of the coat protein coding region and the C-terminal part of the polymerase (NIb) (primers designed by (Desbiez *et al*., 2002). PCR reactions were performed by a first denaturation of the samples at 94°C for 3 minutes followed by 35 cycles at 94°C for 30 seconds, 43°C for 30 seconds and 72°C for 30 seconds and a final elongation step at 72°C for 7 minutes. PCR products were controlled by electrophoresis on 1% agarose gel (Desbiez *et al*., 2002).

RESULTS:

RT-PCR:

Bands of about 1202 bp obtained for ZYMV1 (cucumber isolate)and ZYMV2 (pumpkin isolate) in PCR assay (Fig. 1). Accordingly, the molecular characteristics revealed that both isolates (ZYMV1 and ZYMV2) were closely related to ZYMV. The molecular characterization of the two Riyadh ZMYV isolates ZYMV1 and ZYMV2 is presented in Table 1. The isolates in Gen bank revealed that accession numbers was GU808330 and GU058058 for ZYMV1 and ZYMV2, respectively. The nucleotides locus were 804bp RNA and 98 bp RNA, for ZYMV1 and ZYMV2 respectively. The nucleotides sequence of the two isolates ZYMV1 and ZYMV2 presented molecular diversity that suggests that they did not share a common origin and they have adapted to their different hosts.

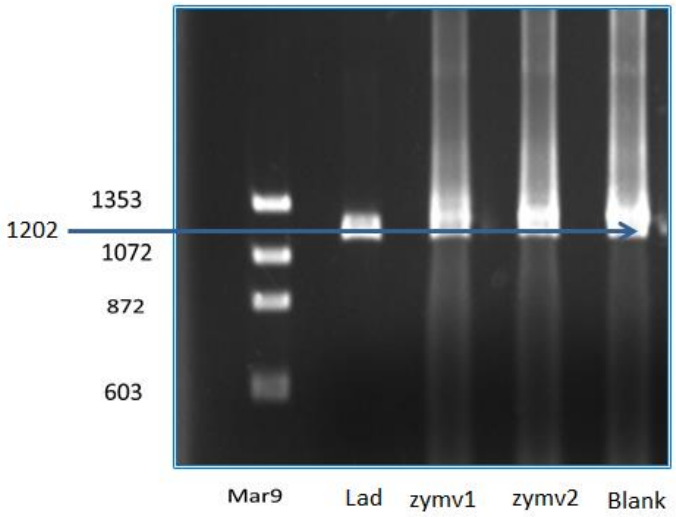

Fig. 1 : Poty primer for ZYMV1 in cucumber isolate and ZYMV2 in cucumber mosschata (pumpkin)" RT for RNA, using random primer then PCR for poty (primer :10Mm 4+ 10n poty).

Table 1: Molecular characterization of two ZYMV isolates ZYMV1 and ZYMV2.

Item	ZYMV1	ZYMV2
Host	*Cucumis sativus* (cucumber)	*Cucurbita moschata* (pumpkin)
GenBank (Accession)	GU808330	GU058058
Nucleotide (Locus)	804bp RNA	98 bp RNA
Nucleotide squences of the CP gene (Origin)	1 ttagtctcca gctatacctc atgcggagcg gatacatgtg tgctggccgt tatctaccgc 61 cctcccctca cttggtcttt gagcctgcca cgtgtaatgc tcccctcatg gactctgtcc 121 actgtggcct tggctggagc catgacttgg aggagggctt gataatgggt gaccattgat 181 gtgactggct ccggctcagg tgaataaatt gtggcagctg tcctcactta tgttgggaca 241 atgctggttc tcatggaaaa attgtgccgc gtctttcgaa gataacaaag aagatgtcac 301 tgccacgcgt gaaaggaaat gtgatacttg acattgacca cttgcttgag tataagccgg 361 atcaaattga gctatacaac acacgagcgt ctcatcagca attcgcctct tggttcaacc 421 aagttaaaac agaatatgat ttgaatgagc aacagatggg agttgtaatg aatggtttca	1 taggactggt tcgtcgtaca ggctcacacg agagcttccg acagcactac ggcactcctg 61 gacacttcat acactttacg cttgagaggt gggggggcc

DISCUSSION:

ZYMV causes marked yellowing of infected cucurbit plants. According to the molecular characteristics of ZYMV1 isolated from cucumber and ZYMV2 isolated pumpkin were closely related to ZYMV. The similarity of the CP nucleotide sequences of ZYMV isolates from other countries such as Germany suggests a common origin. In Saudi, seeds of many vegetables including cucumber are regularly imported from Europe which suggests that infected seeds may have been a pathway for introducing ZYMV. Although seed transmission of ZYMV was reported at a very low rate, it could cause epidemics (Desbiez and Lecoq, 1997). Although Saudi isolates of ZYMV were closely related regarding to the CP gene identity, they showed different phenotypes in host plants tested. Moreover, Saudi isolates of ZYMV had a different host range (Al Shahwain, 1995, Al Abrahaim, 2012, under publication data). Ali *et al*., 2009, point out that two Syrian isolates of ZYMV were closely related to the CP gene identity, they showed different phenotypes in host plants tested. Moreover, Syrian isolates of ZYMV had a different host range. However, Safaeizadeh (2008) compared the biological and molecular variability of ZYMV in Iran and reported that, the limited variability of ZYMV strains from Iran was neither correlated with their biological variability, nor with their geographical origin in the country.

In conclusion, Serological and molecular data tend to indicate that the two Saudi ZYMV isolates were biologically variable and presented a low molecular diversity. This suggests that they share a common origin but that they have subsequently adapted to their different hosts.

The present study reports comparative biological and molecular variability of ZYMV in Saudi Arabia for the first time. The information obtained in this study will be helpful to improve

control strategies for such destructive virus in KSA.

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الخصائص الجزيئية لفيروس موزاييك وإصفرار الكوسة الخضراء (**ZYMV**) على الخيار ومقارنة الخصائص الجزيئية والحيوية لعزلتين من الفيروس على القرعيات في منطقة الرياض بالمملكة العربية السعودية

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يعتبر فيروس موزاييك وإصفرار الكوسة من الفيروسات السائدة على القرعيات حيث تم جمع عينات من الخيار والقرع المصابة بالفيروس خلال موسم النمو مايو ٢٠٠٩ من منطقة الرياض– بالمملكة العربية السعودية. من خلال تقنية (RT-PCR) باستخدام زوج من البادئات تم التعرف على الفيروس. أظهرت تقنية PCR أن كلا العزلتين من الخيار والقرع ينتميا من قرب بفيروس موزاييك وإصفرار الكوسة الخضراء (ZYMV). وأظهرت النتائج الجزيئية للعزلتين أنهما يختلفا حيوياً بينما أقل إختلافاً من الناحية الجزيئية وكان رقم الجين البنكي GU808330.1 و GU 058058.1 لكل من عزلتي الخيار والقرع على التوالي. هذه الأختلافات الجزيئية تشير إلى أن العزلتين لا تشترك في الأصل بينما تأقلمت على العوائل المختلفة.