Ass. Univ. Bull. Environ. Res. Vol. 15 No. 2 October 2012

AUCES

BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF ZYMV ISOLATES FROM CUCUMBER (*CUCUMIS SATIVUS*) PLANT GROWN IN RIYADH, KSA

Jehan Saud Al Abrahaim

Princess Norah Bent Abdurrahman - Associated Professor In Microbiology (Virology –Bacteriology)

Jsaa242@hotmail.com

ABSTRACT:

Zucchini yellow mosaic virus **(ZYMV) is potyvirus with a worldwide distribution. The virus is the most prevalent in cucurbits. Samples of cucumber plants (*Cucumis sativus* L.) that show symptoms of virus infection were collected during the 18-May-2009 growing season from field-grown cucumber in Riyadh Saudi Arabia. Based on DAS-ELISA with a polyclonal antiserum against ZYMV and reverse transcription polymerase chain reaction (RT-PCR) analysis using two primer pairs , the potyvirus was identified as strain of ZYMV. The anti-ZYMV antibodies in dot-blot, confirming the identity of the isolated viral particles as a potyvirus immunogenically related to ZYMV. Nucleotides Sequence analysis confirms that the isolated virus belong to potyvirus and its gen bank is GU808330.1. This is the first report on ZYMV as causal virus infecting cucumber in KSA.**

INTRODUCTION:

As one of the most dangerous epidemic cucurbit viruses, *Zucchini yellow mosaic virus* (ZYMV) causes severe diseases and serious yield reduction in cucurbit crops including courgette (*Cucurbita pepo* L.), pumpkin (*Cucurbita maxima* L.), watermelon (*Citrullus lanatus* L.), melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.). 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). Moreover, for a given ZYMV strain, symptom expression varies according to the host species, growth stage at which infection occurs and environmental conditions (Desbiez & Lecoq, 1997).

***Zucchini yellow mosaic virus* (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). The virus was first reported in Italy and France by Lisa *et al.* (1981), and was found to be transmitted from infected plants to healthy ones by several kinds of aphids in a non-persistent manner (Gal-On *et al.*, 1995). Therefore, 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, 2002; Gal-On, 2007).

The positive sense single-stranded RNA genome of ZYMV, like other potyviruses, was found to be translated as one polyprotein precursor (Brigneti *et al*., 1998), 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; Hong *et al*., 1995).

Although, there have been several reports indicating the existence of different strains of ZYMV, all the known strains (or isolates) of ZYMV were originally isolated from cucurbit plants (Lee and Wong, 1998; Wisler *et al*., 1995; Kundu *et al*., 1997; Yoon and Choi, 1998).

As mentioned above, ZYMV strains display biological variability both in symptom expression and experimental host range (Desbiez and Lecoq, 1997). From the economic point of view, this feature of ZYMV makes it the most important cucurbit virus in kingdom Saudi Arabia (K.S.A) in general and in Riyadh in particular (AL Shahwain, 1995).

Therefore, it is very imperative to discuss the molecular information available on Riyadh isolates of ZYMV and employ them in more detailed studies and researches concerning this serious epidemic virus.

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; 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; Chen *et al*., 2001).

In the present study, we report the biological and molecular characteristics of ZYMV isolates from cucumber (*Cucumis sativus*) plant grown in Riyadh for the first time. It is expected that this study will contribute to the development of control strategies against ZYMV in Riyadh K.S.A.

MATERIALS AND METHODS:

Sample Collection:

Samples were collected during the 18-May-2009 growing season from field-grown cucumber (*Cucumis sativus* L.) in Riyadh, kingdom Saudi Arabia, show symptoms such as: mosaic, yellowing, leaf distortion, Shoe string, 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.

Virus Identification Using (ELISA):

The field-collected courgette samples were tested using antisera against isolated viruses. DAS-ELISA was carried out according to the manufacturer's instructions. Samples were considered positive when the absorbance value (405 nm) was three times higher than that of corresponding negative control. Plats were read after incubation for 1 h. at room temperature.

Host Range Studies:

The original isolates recovered from infected squash plants in the host range studied (from different locations) were maintained in cucumber (*Cucumis sativus* L.) by sap mechanical inoculation. For plant assays, 27 species from 6 families were inoculated with the virus isolates. Sap prepared from infected leaves which were ground in 0.01 M Sodium phosphate buffer, pH 7, and was rubbed onto leaves dusted with carborundum powder, A range of indicator plants were inoculated, while healthy control plants were inoculated only with buffer only.

Naturally infected-courgette leaf samples were ground in 10 mM sodium phosphate buffer, pH 7.0, at a dilution rate of 1: 10 (W:V) and inoculated on leaves previously dusted with carborundum. Inoculated plants were maintained in an incubator at 25^{o}C and observed daily for four weeks. To confirm virus infection, inoculated and non-inoculated leaves were tested about three weeks after inoculation by DAS-ELISA using ZYMV antiserum.

Dot-blot-ELISA:

Dot-blot-ELISA was carried out according to the method described by Banttari and Goodwin (1985) and Hibi and Satio (1985).

Preparation of reagents:

a-Coating buffer, pH 9.6:

-Na_2CO_3 = 1.59 g.

-$NaHCO_3$ = 2.93 g.

Dissolved in about 900 ml distilled. H_2O, adjusted pH to 9.6 and made up to 1000 ml with distilled water.

b-Tris–buffered saline (TBS), pH 7.5:

-Tris (0.02 m) = 4.84 g.

-NaCl (0.15 m) = 58.8 g.

Dissolved in 1900 ml distilled water, adjusted pH to 7.5 and made up the volume to 2000 ml with distilled water.

c-TBS-Tween:

-TBS = 1000 ml.

-Tween-20 = 0.5 ml.

d-Blocking solution:

-TBS = 100 ml.

-Non fat dried = 5 g.

-Milk powder.

e-Antibody buffer:

-TBS-T = 100 ml.

-Nonfat dried milk powder = 5 g.

f-HRP labeled goat antirabbit IgG:

Diluted in antibody buffer (1:5000) just before use.

g-Substrate buffer (0.5 M sodium citrate, pH 5.2) for HRP system:

-Trisodium citrate = 735 mg.

Dissolved in 30 ml distilled H_2O adjusted pH to 5.2 with 1N HCl and made up to 50 ml with distilled H_2O.

h-Substrate solution for HRP system:

Dissolved 6 mg DAB in 9 ml substrate buffer and added 1ml of 0.3% cobaltous chloride and 10 ml of 30% H_2O_2, mixed well and used it immediately.

i-Antisera:

RTBV–heterologous antiserum and CMV banana antiserum were diluted (1:5000) and (1:500) using antibody buffer respectively.

j-Antigens:

For Dot-blot-ELISA, virus infected ZYMV and healthy leaf and pseudostem tissues were extracted in carbonate buffer containing 0.01 M DIECA, subsequent dilutions of the antigens was made in carbonate buffer.

Antigen samples with a micropipette were applied on to the nitrocellulose membrane according to Labelling (Rajasulochana *et al.* 2008). The membrane was allowed for drying and then transferred to a petriplate and blocking solution added till the membrane was fully immersed. The membrane was kept constant in blocking solution for 3 hours at room temperature with intermittent shaking. The membrane was transferred from blocking solution to diluted antiserum in blocking buffer and kept at 37^{o}C for 1 hour. The antibody solution was discarded and washed the membrane thrice with TBS-T at 5 min. interval. The goat antirabbit antibodies labelled with HRP were added to the antibody buffer and placed the membrane in it under constant shaking conditions. The conjugate solution was discarded and the membrane was washed thrice with TBS-T at 5 min. interval. The substrate solution specific to enzyme was added and kept in shaking till sufficient colour was developed. The membrane was washed with water and then it was treated with 1.05% sodium hypochlorite solution for decreasing the back ground.

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 2 µl PCR buffer 10x (0.5 M Tris-HCl, 0.7 M KCl, 0.1 M $MgCl_2$, pH 8), 1 µl DTT (100 mmol/µl), 1 µl 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^{o}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 wer 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:

Host Range:

Some ZYMV isolates were able to systemically infect all tested cucumber plants inducing very severe symptoms such stunting, deformation and decline of the entire plant (Fig. 1). Able also to infect other species tested either locally or systemically (Table 1). On the other hand, the isolates could not infect the number of Solanaceae. Mosaic, blistering, leaf distortion and stunted growth were observed on *Cucumis sativus*.

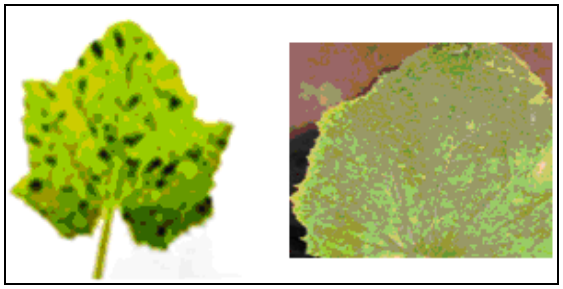

Fig. 1: Symptoms induced by ZYMV strain on *Cucumis sativus L.* leaves

Twenty eight species belonging to six families were inoculated with the virus isolates and were tested. It is evident that six strains varied in host reactions. These strains were different and distinguishable in the ability to infect specific hosts.

Dot-blot-ELISA:

Dot–blot-ELISA were performed using heterologous RTBV antisera. A set was evaluated using ZYMV infected samples of cucumber. ZYMV was detected in field-collected courgette samples. This Dot –blot ELISA technique was done for the first time on ZYMV in KSA.

Fig. 2: Detection of ZYMV in cucumber leaf samples using RTBV antiserum

RT-PCR:

Bands of about 1202 bp were obtained for ZYMV in the PCR assay, whereas no band appeared for the healthy control at that level while anther band at 1072 was observed in control sample (Fig. 3)

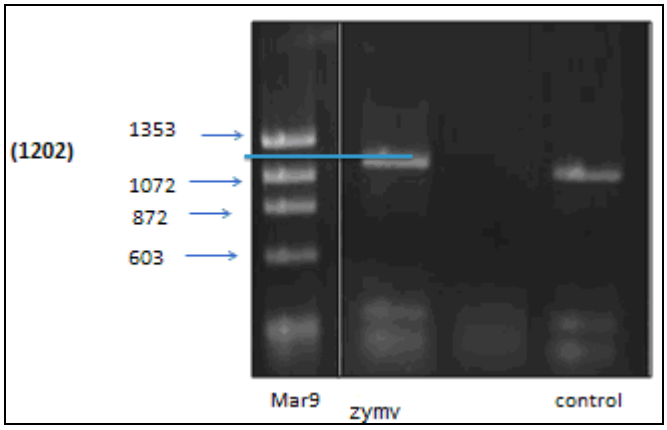

Fig. 3 : Poty primer for ZYMV in cucumber isolate RT for RNA, using random primer then PCR for poty (primer: 10 Mm 4+ 10 n poty) the Marker 1x Ladder

Table 1: Reaction of indicator plant species to ZYMV

Families	Test plants	Symptoms in leaves
Cucurbitaceae	*Cucurbita pepo* cv. Zucchini	M, b, id
	C. pepo cv. Khoy	M, b, id, ss
	C. pepo cv.Maragheh	M, b, id, ss
	Cucumis melo cv. Asgrown	B, m
	C. melo cv. Melon seed	B
	C. melo Local cultivar	B
	C. sativus cv. Dominus	M
	C. sativus cv. Ps	M
	Citrullus lanatus cv. Crimson Sweet	S, m, b
	Luffa acutangula L.	M
	Gomphrena globosa L.	Nll
Amaranthaceae	*Amaranthus paniculatus L.*	-
	Chinopodium quinoa L.	Cll
Chinopodiaceae	*C. amaranticolor* L.	Cll
	Spinacia oleraceae cv. keshtzar	-
	Phaseolus vulgarius cv. Khorran	Cll, m
	P. vulgarius cv. Daneshkadeh	-
Leguminoseae	*P. vulgarius* cv. Red Kideny	Cll, m
	P. vulgarius cv. Bountifull	-
	Vigna unguiculata	-
	Vicia faba	-
	Pisum sativum L.	-
Solanaceae	*Dautura metel* L.	-
	D. stramonium L.	-
	Nicotiana tabacum L.	-
	N. glutinosa L.	-
	N. benthamiana L.	-
Ranunculaceae	*Ranunculus sardous* L.	-

ss=Shoe string, id=leaf distortion, b=Blistering, s= Stunted growth, m=Mosaic
I= Latent infection, Cll= Chlorotic local lesion, Nll= Mecrotic local lesion. (Hosseni *et al.*, 2007).

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
481 tggttttggt gcattgaaaa tggcacgtca cccgacatta acggagtatg ggttatgatg
541 gacggtatga gcaggttgaa tatcctttga accaatagtt gaaatgcaaa gccacgctgc
601 gacaatatgc acactttcag atgcagcgga ggctatataa gatgagaatg caaggcacct
661 aatgccgagg tatggtttgc ttccaaacta cggaaagagt tggcacgatt gcttcgactt
721 ctcgaagtaa ttcaaactcg gaagggcccc gaactgttgc gaaatgaagg ccccctaac
781 aggttcttcc ggtgttggct tgat.

Figure 4: Nucleotide sequence of ZYMV isolates from Cucumber

The isolates in Gen bank revealed that accession numbers was GU808330 for ZYMV isolate. The nucleotides sequence of the ZYMV isolates revealed that, Nucleotide (Locus) was 804bp RNA. They presented low molecular diversity, this suggests that they share a common origin.

DISCUSSION:

It is clear that ZYMV causes marked yellowing of infected plants. Infected cucumber leaves develop vein clearing followed by general chlorosis marked with distinct, variable-sized, dark green spots. Severely affected leaves became filiform. Fruit develop large green protrusions and blisters and severe distortions. Although ZYMV was only recently described (Desbiez *et al.*, 2002), it is widely distributed. It occurs in Italy, France, Spain, Israel, Morocco, Germany, northeastern United States (Delkhosh *et al.*, 2000) and southeastern United States (Dougherty and Semler, 1993).

According to molecular characteristics, ZYMV isolates were closely related. The high 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. Some isolate was more aggressive in cucumber but could not induce any infection in Fabaceae. On the other hand, other isolate could not infect cucurbit or melon plants, and induced milder symptoms in courgette and watermelon. The CP coding region is a valuable determinant in the potyviruses classification (Shukla *et al.*, 1994). Direct connections were demonstrated between this region and the pathotype of potyviruses including ZYMV (Andrejeva *et al.*, 1999; Ullah

and Grumet 2002; Ullah *et al*., 2003; Bukovinszki *et al*., 2007; Gal-On, 2007). However, since the CP of ZYMV isolates had identical amino acid sequences, it is unlikely that this region could be responsible for the significant differences existing between the phenotypes of these isolates.

In conclusion, although the Saudi ZYMV isolates studied were biologically variable, they presented a low molecular diversity. This suggests that they share a common origin but that they have subsequently adapted to their different hosts.

The Saudi sites represent unique ecosystems, where ZYMV was introduced only recently. The tropical climate in the South part of the country allows cucumber to be present all year round without breaks in the virus cycle, and the insularity limits the introductions of virus from different origins.

Serological and molecular data tend to indicate that no new introduction of independent ZYMV isolates took place, and that the evolution of variability results from the emergence of mutants in virus populations and their spread or elimination in interaction with the isolates already present.

REFERENCES:

Al-Shanwan, I. M., O. A. Abdalla and M. A. Al-Saleh, (1995): Response of greenhouse-grown cucumber cultivars to an isolate of ZYMV. Plant Dis., 79(9): 898-901.

Andrejeva J., Puurand U., Merits A., Rubenstein F., Jarvekulg L. & Valkonen JPT (1999): Potyvirus helper component-proteinase and coat protein (CP) have coordinated functions in virus host interactions and the same CP motif affects virus transmission and accumulation. J. of General Virology 80, 1133–1139.

Banttari C. E., Goodwin P. H. (1985): Detection of potato viruses, S., X. and Y. by ELISA on nitrocellulose membranes (Dot-ELISA). Plant Dis.; 69: 202.

Brignti, G., Voinnet, O., Li., W.-X Ding, S. W. and Baulcombe, D. (1998): Viral path-ogenicity determinants are suppresoors of transgene silencing Nicotiana benthamiana. EMBO J. 222:6739-6746.

Bukovinszki A., Gotz R., Johansen E., Maiss E. & Balazs E. (2007): The role of the coat protein region in symptom formation on Physalis floridana varies between PVY strains. Virus Research 127, 122–125.

Chen, J., Chen, J., Adams, M. J., (2001): A universal PCR primer to detect members of the *Potyviridae* and its use to examine the taxonomic status of several members of the family. Arch. Virol. 146, 757–766.

Clark, M. F. and Adams, S.A.N. (1977): Characteristics of Micro Plates Method of Enzyme-linked-immunosorbent Assay for Detection of Plant Viruses. J. Gen. Virol., 34: 475-483.

Delkhosh, H., Rahimian, H. and Hayati, J. (2000): Identification of Causal Agent of Squash Fruit Deformation in Mazandaran Province. Proc. 14th

Iranian plant Protection Congress. Isfahan University of Technology.

Desbiez, C. and Lecoq, H. (1997): Zucchini yellow mosaic virus. *Plant Pathology* 46, 809–829.

Desbiez, C., Wipf-Scheibel, C. and Lecoq, H. (2002): Biological and Serological Variability, Evolution and Molecular Epidemiology of Zucchini yellow mosaic virus with Special Reference to Carib-bean Islands. Virus Res., 85: 5-16.

Dolja, V.V., Herndon, K. L., Pirone, T. P. and Carrington, J. C. (1993): Spontaneous mutagenesis of a plant potyvirus genome after insertion of a foreign gene. J. Virol. 67: 5968-5975.

Dougherty, W. G., and Semler, B. L. (1993): Expression of Virus Encoded Proteinases,

Functional and Structural Similarities with Cellular Enzymes. Microbiol. Rev., 57: 781-822.

Gal-On, A., Antignus, Y., Rosner, A. and Raccah, B. (1992): A zucchini yellow mosaic virus coat protein gene mutation restores aphid transmissibility but has no effect on multiplication. J. Gen. Virol. 73: 2183-2187.

Gal-On, A., Meiri, E., Huet, H., Hua, W. J., Raccah, B. and Gaba, V. (1995): Particle bombardment drastically increases the infectivity of cloned DNA of zucchini yellow mosaic potyvirus. J. Gen. Virol. 76: 3223-3227.

Gal-On A. (2007): Zucchini yellow mosaic virus: insect transmission and pathogenicity the tails of two proteins. Molecular Plant Pathology 8, 139–150.

Hibi, T., saito, Y. (1985): A dot immunobinding assay for the detection of tobaccomosaic virus in infected tissues. J. Gen Virology; 66: 1191-1194.

Hong, Y., Levay, K., Murphy, J. F., Klein, P. G., Shaw, J. G. and Hunt, A. G. (1995): A potyvirus polymerase interacts with the viral coat protein and VPg in yeast cells. Virology. 214:159-166.

Hosseni, S., Mosahebi, G. H., Koohi, M., and Okhovvaht, S. M. (2007): Characteri-zation of the Zucchini yellow mosaic from squash in Tahran Province. J. Agric. Sci. Technol. 9: 137-143.

Kundu, A. K., Oshima, K. and Sako, N. (1997): Nucleotide sequences of the coat protein genes of two japanese zucchini yellow mosaic virus isolates. Acta Virol. 41:297-301.

Lee, K. C. and Wong, S. M. (1998): Variability of P1 protein of zucchini yellow mosaic virus for strain differentiation and phylogenetic analysis with other potyviruses DNA Sequence 9:275-293.

Lisa, V., Baccardo, G., D., Agostino, G., Dellavalle, G., and D, Aquilio, M. (1981): Characterization of a Potyvirus that Causes Zucchini yellow mosaic. Phytopathology., 71:667-672.

Rajasulochana, P., dhamotharan, R. and Srinivasulu, P. 2008. comparison of Dac – Elisa and Dot-Blot-Elisa for the Detection of cucumber mosaic and Banana streak

Viruses Infecting Banana. J. of American Science, 4(3): 1545.

Rowhani, A., Maningas, M. A., Lile, L. S., Daubert, S. D., Golino, D. A., (1995): Development of a detection system for viruses of woody plants based on PCR analysis of immobilized virions. Phytopathology 85, 347–352.

Shukla DD, Ward CW & Brunt AA (1994): The Potyviridae. CAB nternational, Walling-ford (GB), 187–208

Thomson, K. G., Dietzgen, R. G., Gibbs, A. J., Tang, Y. C., Liesack, W., Teakle, D. S. and Stackebrandt, E. (1995): Identifi-cation of zucchini yellow mosaic potyvirus by RT-PCR and analysis of sequence variability. J. Virol. Methods 55:83-96.

Tian T., Klaassen V.A., Soong J., Wisler G., Duffus J.E., Falk B.W., (1996): Generation of cDNAs specific to Lettuce infectious yellows closterovirus and other whitefly-transmitted.

viruses by RT-PCR and degenerate oligonucleotide primers corresponding to the closterovirus gene encoding the heat shock protein 70 homolog. Phytopathology 86:1167-1173.

Ullah Z. & Grumet R. (2002) Localization of Zucchini yellow mosaic virus to the veinal regions and role of viral coat protein in veinal chlorosis conditioned by the zym potyvirus resistance locus in cucumber. Physiological and Molecular Plant Pathology 60, 79–89.

Ullah Z, Chai B, Hammar S, Raccah B, Gal-On A & Grumet R (2003): Effect of substitution of the amino termini of coat proteins of distinct potyvirus species on viral infectivity and host specificity. Physiological and Molecular Plant Pathology 63, 129–139.

Varrelamann, M. and Maiss, E. (2000): Mutations in the coat protein gene of plum pox virus suppress particle assembly, heterologous encapsidation and complementation in transgenic plants of Nicotiana benthamiana. J. Gen. Virol. 81:567-576.

Wadsworth G. J., Redibaugh, M. G. and Scandalios, J. G. (1998): A Procedure for the Small-scale Isolation of Plant RNA Suitable for RNA Blot Analysis. Anal. Biochem., 172: 279-283.

Wisler, G. C., Purcifull, D. E. and Hiebert, E. (1995): Characterization of the P1 protein and coding region of the zucchini yellow mosaic virus. J. Gen. Virol. 76:37-45.

Yoon, J. Y. and Choi, J. K. (1998): Nucleoide sequence of 3-terminal region of zucchini yellow mosaic virus (cucumber isolate) RNA. Korean J. Plant Pathol. 14:23-27.

الخصائص البيولوجية والجزيئية لفيروس موزايك وإصفرار الكوسة المعزول من نباتات الخيار النامية بمنطقة الرياض بالمملكة العربية السعودية

جهان بنت سعود بن راشد البراهيم

قسم الأحياء- كلية العلوم - جامعة الأميرة نوره بنت عبد الرحمن - المملكة العربية السعودية

يعتبر فيروس موزاييك وإصفرار الكوسة من الفيروسات التي تنتمي إلى Potyvirus واسعة الإنتشار عالمياً، كما يعتبر من الفيروسات السائدة على القرعيات. ولقد تم جمع عينات من نباتات الخيار التي لوحظت عليها أعراض إصابة بالفيروس خلال موسم النمو ١٨ مايو ٢٠٠٩ في منطقة الرياض– بالمملكة العربية السعودية. ومن خلال استخدام طريقة الإنزيم المرتبط الأليزا وساندوتش ثنائي مع سيرم مضاد متعدد طرز الاجسام المضادة وتفاعل البلمرة المتسلل بالنسخ العكسي (RT-PCR) ، وباستخدام زوج من البادئات تم التعرف على فيروس موزاييك واصفرار الكوسة وكذلك باختبارات اللطخة المناعية تم التأكد أن جزيئات الفيروس المعزولة هي Potyvirus ، وينتمي مناعياً إلى فيروس موزايك الكوسة. كما أنه من خلال تتابع القواعد النيتروجينية تم التأكد من أن الفيروس الذي تم عزله من الخيار ينتمي إلى Potyvirus ويحمل جين بنكي GU808330.1، ويعتبر ذلك أول تسجيل بالولايات المتحدة الامريكية لفيروس الزوكينيا والذي تم عزله من الخيار النامي بالمملكة العربية السعودية.