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TOBAMOVIRUS SPECIFIC PRIMERS REVEALED A CO-INFECTION OF TOBACCO MOSAIC VIRUS AND PEPPER MILD MOTTLE VIRUS ON TOMATO IN IRAQ

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ABSTRACTS

This study was conducted to investigate *Tobamoviruses* infecting tomatoes. Leaf samples (n=10) from symptomatic tomato plants were screened for *Tobamoviruses* using Enzyme Linked Immune-Sorbent Assay (ELISA) and group specific antibodies. One sample scored the highest absorbance value (3.1790) was selected. *Datura Stramonium* was leaf inoculated with the sample selected to confirm the infection. Total ribonucleic acid (RNA) was extracted from tomato leaves and a complementary cDNA strand was synthesized. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed using the primers TobUni1/TobUni2 targeting coat protein (CP) of the genus *Tobamovirus* genome. RT-PCR products were analyzed by agarose gel electrophoresis and sequenced. Nucleotide sequences obtained were included in the National Center for Life Informatics (NCBI) database under accession numbers (PP032058 and PP032061). The detection of 2 *Tobamoviruses* in a single tomato plant was confirmed when DNA fragments amplified shared 98% maximum nucleotide sequence identity with Tobacco mosaic virus (TMV) and Pepper mild mottle virus (PMMoV) CP gene retrieved from NCBI. Neighbor-Joining phylogenetic tree confirmed the relatedness grouped TMV and PMMoV sequences to those from Spain (MK087763.1) and Brazil (KT923121.1), respectively, suggesting a common origin. To the best of our knowledge, this is the first report of TMV and PMMoV mixed infection on tomato in Iraq, based on molecular approach. The two *Tobamovirus* co-infection may be a new threat to tomato production in Iraq.

Key words: local lesions, *Tobamovirus*, Mosaic, Group specific primers.

[*] **This article is taken from the first researcher's master's thesis.**

البرايمرات الخاصة بمجموعة Tobamovirus كشفت عن الإصابة المشتركة بفايروس موزائيك التبغ وفايروس تبرقش الفلفل الطفيف على الطماطه في العراق

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الخلاصة

أجريت هذه الدراسة للتحري عن فايروسات *Tobamoviruses* التي تصيب الطماطه. اذ تم فحص عينات الأوراق (العدد = 10) من نباتات الطماطه التي تظهر عليها أعراض الإصابة بفايروسات *Tobamoviruses* باستخدام اختبار الادمصاص المناعي المرتبط بالإنزيم (ELISA) والأجسام المضادة الخاصة بالمجموعة. تم أختيار عينة واحدة التي سجلت أعلى قيمة امتصاص (3.1790). تم اجراء عدوى على نبات الداتورة *Datura Stramonium* لتأكيد إصابة العينة. استخرج الحامض النووي الرايبي الكلي (RNA) من أوراق الطماطه ومن ثم تصنيع شريط cDNA المكمل. تم إجراء تفاعل البوليميراز المتسلسل للنسخ العكسي (RT-PCR) باستخدام البادئات TobUni1/TobUni2، مستهدفًا بروتين الغلاف(CP) لجينوم فايروس *Tobamoviruses*.تم تحليل نواتج RT-PCR بواسطة الترحيل الكهربائي على هلام الاكاروز ومن ثم تحديد تسلسلاتها النيوكليوتيدية. تم تضمين تسلسلات النيوكليوتيدات التي تم الحصول عليها في ضمن قاعدة بيانات المركز الوطني لمعلومات الحياة (NCBI) تحت أرقام الانضمام PP032058) و (PP032061)تم تأكيد اكتشاف اثنين من فايروسات *Tobamoviruses* في نبات طماطه واحد حيث أكدت مقارنة التسلسل النيوكليوتيدي أنه تم تضخيم قطع الحامض النووي من جين (CP) العائد لفايروس موزائيك التبغ (TMV) وفايروس التبرقش الطفيف على الفلفل (PMMoV) والتي سجلت اعلى نسبة تطابق نيوكليوتيدي بلغ 98% مع تسلسلات بنك الجينات المكافئة. NCBI أكدت شجرة العلاقات الوراثية من نوع الضامة بالتجاور تطابق تسلسلات TMV وPMMoV التي جمعتها مع تلك الموجودة في إسبانيا (MK087763.1) والبرازيل (KT923121.1) على التوالي، مما يشير إلى أصولها المشتركة. على حد علمنا، تعد هذه الدراسة هي التسجيل الأول عن الإصابة المشتركة بفايروسي TMV وPMMoV على الطماطه في العراق، بناءً على النهج الجزيئي والتي قد تشكل تهديداً جدياً لإنتاج الطماطه في العراق.

الكلمات المفتاحية: بقع موضعية، *Tobamoviruses* ، موزائيك ،برايمرات متخصصة بالمجموعة.

INTRODUCTION

The tomato (Solanum lycopersicum L., family Solanaceae) is a cash crop cultivated worldwide, including in Iraq (**Caruso *et al.*, 2022**). In Iraq, the tomato crop ranks first in terms of the most vegetable crops grown annually (**Ludemann *et al.*, 2024)**.

Despite the high productivity, this crop can be limited by many biotic and abiotic factors including plant pathogens **Adhab & Alkuwaiti, 2022**). About 200 different pathogens, were reported to impact tomato causing serious fruit losses, both in quality and quantity (**Panno *et al.*, 2021; Karačić *et al.*, 2024**). Virus diseases are the most devastating among others due to their rapid spread and the absence of a reliable controlling method. They are threatening tomatoes both in protected and open fields (**Panno *et al.*, 2021; Rivarez *et al.*, 2023**)

The genus Tobamovirus includes many species that are epidemic on tomatoes as they easily spread mechanically and through seeds means (**Lovelock *et al.*, 2022**). Therefore, the most practical controlling approach is to use reliable and cost effective diagnostic methods, to

minimize damage caused by viruses (**Mrkvová *et al*., 2022; Tatineni &Hein., 2023; Soni *et al*. 2024**). Tobamoviruses were investigated in Iraq based on biological and serological methods, but not molecularly confirmed **(Adhab *et al*., 2021**). PCR technology is the most efficient method for diagnosing plant diseases in Iraq **(Mohammed., 2023**) including plant viruses **(Mohammed *et al*., 2021**).

This study, therefor, was aimed to investigate *Tobamoviruses* infecting tomato and their genetic relatedness based on molecular approaches In Iraq .

MATERIALS AND METHODS

Bioassay

A comparison sample was prepared by Dr. Mustafa Adhab (College of Agricultural Engineering Sciences, University of Baghdad), and the Infected tomato leaves were ground with pre cooled deionized water using a porcelain mortar and pestle. Leaf extract was passed through a double layer gauze pad and the crude sap was collected in 250 ml flask. Sap inoculation was performed on *Datura Stramonium* and tomato leaves pre-dusted with carborundum. The sap was rubbed genially and leaves were washed with distilled water 1 min later. Inoculated plants were kept in an insect-proof environment and symptoms development were observed on a daily basis.

Serological testing

Tobamovirus -specific ELISA kit (Agdia, USA) was used following the standard protocol provided by the manufacturer company (Agdia, USA). Leaf samples (n=10) from symptomatic plants were tested. The absorbance value was estimated at 405 nm using an ELISA reader by Gen5 ELISA software.

Molecular diagnosis

Total RNA was extracted from tomato leaves using a commercial extraction kit (TransZol Up Plus RNA Kit) following the manufacturer's standard protocol (Transgenbiotech, China). The quantity and quality of RNA were measured using Nanodrop spectrophotometer (Thermo Scientific, USA) At room temperature. The cDNA was synthesized using the Synthesis Super Mix cDNA assay kit. following the manufacturer's recommended protocol (Transgenbiotech, China). Reverse transcriptase polymerase chain reaction (RT-PCR) was performed using the commercial kit PCR Super Mix EasyTaq, Transgenbiotech, China) using Tobamovirus primers, as shown in Table (1).

Table (1): Nucleotide sequences of the primers used.

Primer	Sequence 5'-3'		Amplicon
Tob uni	F	ATTTAAGTGGASGGAAAAVCAT	750 bp
	R	GTYGTTGATGAGTTCRTGGA	

Amplified DNA fragments were visualized by 1% agarose gel electrophoresis and sequenced (Macrogen, South Korea). Sequence analyses were performed using MEGA 11 (**Tamura *et al.*, 2021**) and SDTv 1.3 (**Muhire *et al.*, 2014**). Nucleotide sequences obtained were deposited in NCBI under the accession numbers PP032058 and PP032061.

RESULTS AND DISCUSSION

Bioassy

Tomato plants showed a systemic response about 14 days after infection, with the form of yellow mottling, which proves the success of the mechanical infection (Fig1A).
Datura. *stramonium* showed local lesions on the infected leaves 4-6 days of inoculation. (Fig1B).

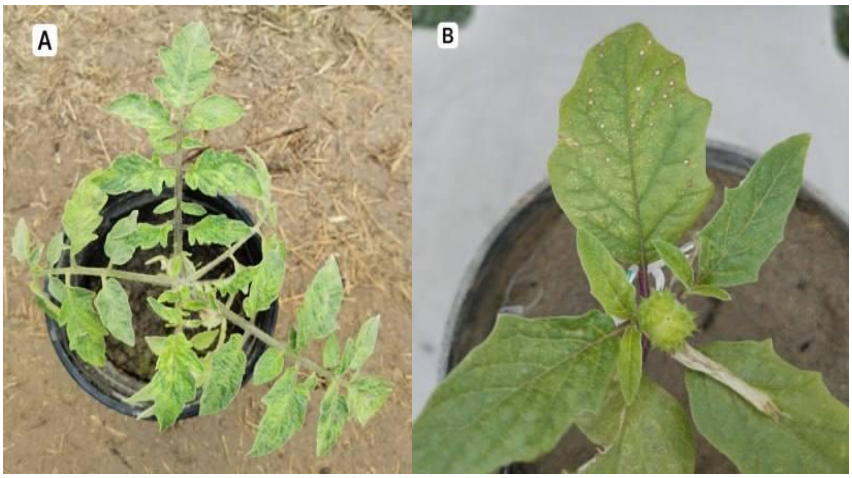

Figure (1): Bioassay on indicator plants inoculated with *Tobamovirus* infected tomato leaves. A: Mosaic symptoms on tomato, B: Necrotic local lesions on *Datura Stramonium.*

Molecular test

Gel electrophoresis showed Tob uni primer set could detect *Tobamoviruses* in tomato sample when amplify ~750 bp (Figure 2) (**Letscher *et al.*, 2002; Rodríguez *et al.*, 2022**)

Figure (2): Electrophoresis pattern on an agarose gel showing 750 base pairs DNA fragments (A) amplified by Tobamovirus specific primers (Tob uni) from infected tomato plants. M: DNA marker.

Sequence comparison confirmed that the DNA fragments were amplified from TMV and PMMoV CP gene when shared 98% maximum nucleotide sequence identity with the equivalent sequences retrieved from NCBI data base (Figure 3), suggesting that the sample was co-infected with both viruses.

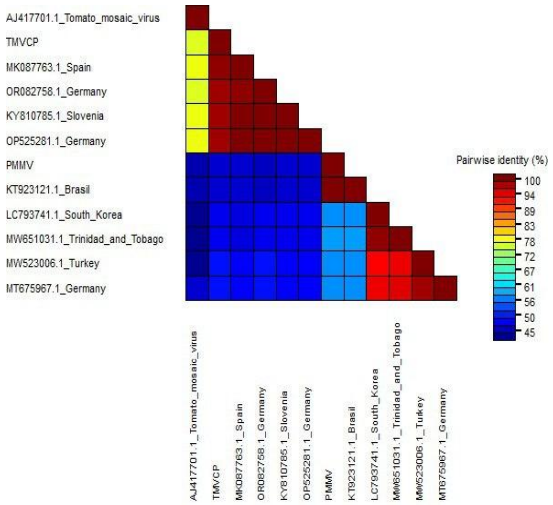

Figure (3): Nucleotide sequence identity percentages interpreted into colored matrix of part of the CP region of TMV and PMMoV with the equivalent GenBank sequences.

Neighbor-Joining phylogenetic tree, (Figure 4) grouped PMMoV and TMV to NCBI isolates from Brazil (accession code KT923121.1) and Spain (accession code MK087763.1), respectively suggesting a common origin of CP gene (**Tamura *et al.* 2021**).

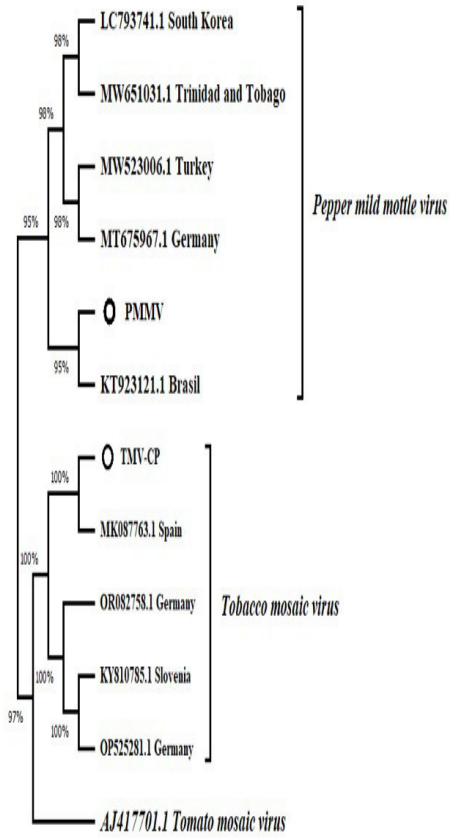

figure (4): Neighbor-Joining phylogenetic trees constructed from the nucleotide sequences of the CP gene of TMV and PMMoV viruses and their equivalent sequences from NCBI GenBank.

Serological testing

ELISA is a high accurate technique has been used to detect and estimate the concentration of many macromolecules due to (**Hussein et al., 2016**). ELISA technique, using antibodies specific to the genus Tobamovirus, enabled to detect the presence of one or more viruses belonging to this genus in the infected plants. However, virus species could not be identified. Group-specific antibodies enable to detect a wide range of *Tobamoviruses* rather than species-specific ones as the test can be more reliable, rapid and cost-effective (**Verma&Sing, 2020**).

The main advantage of applying group specific antibodies/oligonucleotides is to develop simultaneous tests. these can be useful for virus routine diagnosis and initial detection of infection enabling rapid action against virus spread. Besides, another advantage for group specific diagnosis is to minimize the risk of variant isolates to escape the test. The current study reported the co-infection of TMV and PMMoV for the first time on tomato in Iraq. The presence of these two viruses in a single tomato plant was confirmed through using *Tobamovirus* specific primer sets combined with sequence analysis, to resolve species identification.

CONCLUSION

Using specific-group primers is important in diagnosing co-infection with different species of virus in the same affected tomato plant (TMV and PMMoV).

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