



FIELD SURVEY AND MOLECULAR DETECTION OF VIRUSES ASSOCIATED WITH SQUASH MOSAIC DISEASE IN ERBIL PROVINCE

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Abstract

During August to November, 2021, symptoms of mosaic were observed in various squash (*Cucurbita pepo*) plants at different locations in Erbil province.

The survey showed mosaic diseases incidence in different squash varieties ranged 0% to 54.75%. Disease symptoms varied from malformation and distortion of leaves and fruits, vein clearing, vein banding, blistering, and shoestring. The highest infection rate was recorded in Grdasheer while the lowest was observed in Murtkha on Muzaffar variety.

RT-PCR confirmed the association of Water melon mosaic virus (WMV) and Zucchini yellow mosaic virus (ZYMV) associated with the symptomatic plants.

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Keywords: ZYMV, RT PCR, WMV, Squash viruses.

المسح الحقلّي والكشف الجزيئي عن الفايروسات المرافقة لمرض الموزائيك على القرع في محافظة أربيل

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الخلاصة

خلال الفترة (أغسطس - نوفمبر 2021)، لوحظت أعراض الموزائيك على اصناف مختلفة من محصول القرع (*Cucurbita pepo*) في مواقع مختلفة ضمن محافظة أربيل. اذ أظهرت نتائج المسح الحقلّي على نبات القرع وجود اعراض موزائيك واعراض اخرى مثل تشوه في الأوراق والثمار، شفافية وتحزم العروق، والانتفاخات واختزال نصل الورقة (اعراض رباط الحذاء). تراوحت نسبة المرض ما بين 0% إلى 54.75%. اذ سجلت أعلى نسبة إصابة في منطقة كردشير وأقلها في منطقة مرتكة على الصنف المظفر من نباتات القرع. شخص المسبب الموزائيك على نباتات القرع بواسطة تقنية RT-PCR اذ اظهرت نتائج الاختبار ان مسبب الموزائيك هما فايروس موزاييك الرقي (WMV) وفايروس الموزاييك الأصفر على قرع الكوسا (ZYMV).

كلمات مفتاحية: فايروسات القرع، نسخ العكسي - تفاعل البلمرة المتسلسل، فايروس موزائيك اصفر الزكيني، فايروس موزائيك الرقي.

Introduction

Squash *Cucurbita pepo* L. is one of the most important vegetable crops of the Cucurbitaceae family. It is cultivated in Iraq during spring (8). The family Cucurbitaceae includes 825 species and 119 genera. It is mostly found in tropical and subtropical areas of the world (2 and 10). Globally, the most important cucurbits are squash (*C. pepo* L.), cucumber (*Cucumis sativus* L.), melon (*C. melo* L.) and watermelon (*Citrullus lanatus*) (14).

Summer squash *Cucurbita pepo* is a widely-cultivated crop, easy to grow, with a short growing season both in temperate and subtropical environments (14). Like other crops, cucurbits are susceptible to many plant pathogens, including viruses causing serious losses (7). About 35 different viruses have been isolated from cucurbits (15 and 3).

The most prevalent and significant potyviruses on squash plants worldwide are Watermelon mosaic virus (WMV, formerly WMV-2), Papaya ringspot virus type watermelon (PRSV-W), and Zucchini yellow mosaic virus (ZYMV) (12).

This study aimed to survey of squash mosaic infections and their distribution in squash crop in Erbil province and to detect some viruses associated with the disease based on RT-PCR.

Materials and Methods

Disease survey and sampling: A field survey was carried out in nine squash growing areas at Erbil province for the autumn season in 2021 (Table 1) to estimate viral disease infections on squash within an area of 1-4 donums in each field. A random sampling was performed and diseased samples were collected separately. Percentage of disease incidence was calculated following (13).

$$\% \text{ infected plant} = \frac{\text{No. of infected plant}}{\text{Total no. plants}} * 100$$

Table 1 Field survey of mosaic symptoms on *C. pepo* in different locations in Erbil.

Location of survey	Field area (donum)	Squash variety	Planting date	Surveying date
Alana	1	Muzaffar	Early June	2 nd October
	1	Alexandrea	early June	2 nd October
Rayat	2	Muzaffar	Early June	2 nd October
Haji omaran	4	Alexandra	middle June	10 th October
Murtka	5	Muzaffar	Late July	15 th september
Quchablbas	4	Muzaffar	Late July	15 th september
	4	Alexandrea	Early august	15 th september
Smailawa	4	Alexandrea	Late July	30 th august
Grdasher	4	Muzaffar	Early April	30 th august
	4	Alexandrea	Late April	30 th august
Jdida	4	Alexandrea	Late June	30 th august
Gaing	4	Alexandrea	Late June	30 th august
	4	Alexandrea	Late June	30 th august
	4	Alexandrea+muzaf far	Half July	30 th august
	4	Muzaffar	Early June	30 th august
	4	Alexandrea	Early august	30 th august
Total	57	2		

Sample collection and preserving: Samples were collected in plastic bags, labelled and stored at -20°C in the laboratory of Plant Protection Dept./ College of Agricultural Engineering Sciences / University of Salahaddin.

Molecular detection (RT.PCR): A - RNA Extraction: The RNA was extracted using (FAWORGEN Total RNA Extraction kit (FAWORGEN Biotech COPR, S. Korea) following instructions of the manufacturer.

cDNA was synthesized using Adscript cDNA Synthesis Kit (Add Bio company, Korea). cDNA was generated in a total volume of 20 µl included 10 µl reaction buffer, 2.0 µl of 10mM dNTP mixture, 2.0 µl 10x olio dT₂₀, 4 µl virus RNA template, 1.0 µl Adscript enzyme solution and 1 µl of nuclease-free H₂O. The reaction conditions were priming for 10 min at 25°C, reverse transcription for 60 min at 50°C, RT inactivation for 5 min at 80°C and hold at for 1 min 12°C.

B-Polymerase Chain Reaction (PCR) amplification for partial polyprotein: Capsid gene of virus: PCR amplification of coat protein (CP) partial gene was performed using 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22. country), and species specific primer sets, (Table 2). A total of 50 µl PCR amplification reagent was prepared as follows: master mix 25 µl, forward primer 3 µl, reverse primer 3 µl, DNase free water 14 µl and cDNA template 5 µl. PCR program included 1 cycle initial denaturation for 5 min at 95 ° C, 35 cycles of denaturation for 35 second at 95 ° C, primer annealing for 40 sec at 57.5 or 60°C, extension for 1 min at 72°C and final extension for 10 min at 72°C steps.

Table 2 Primers sets used for virus detection.

Virus	Forward primer	Reverse Primer	DNA fragment size /bp	Annealing °C	References
ZYMV	5'-GCTCCATACATAGCTGAGAC-3'	5'-AACGGAGTCTAATCTCGAGC-3'	850	60	(5)
WMV	5'-AACTCGCTGCATCCGGA AAA-3'	5'-CGCAAATGCTAACTGTGACC-3'	1100	57.5	(9)

Results and Discussion

Disease survey: Field survey revealed disease symptoms occurred in 16 squash fields within 10 different regions (Table 2). Disease symptoms included yellowing, mosaic, line pattern, malformation and shoe string on the leaves and deformation on fruits of different varieties. The infection rate ranged 0-54.75% (Table 3).

Incidence of symptomatic plants varied widely, not only among regions but also among fields in the same region. It was high in regions 1 21.75 and low in region 4 scoring 21.75 and 1.75, respectively, in Gainj village. Whereas symptomatic plants were rarely found in Jdida and Alana with few or non in Murtkka. Similarly (17) confirmed in Samsun, Turkey, when detecting three cucurbit viruses that infections varied from one village to another.

Table 3 Percentage of virus infection in different locations, that is varies according to the area and according to the time of planting, and visiting time.

Location of survey	Field area (donum)	Squash variety	Number of plants surveyed	Infection percentage %
Alana	1	Muzaffar	100	11
	1	Alexandrea	100	7
Rayat	2	Muzaffar	200	22
Haji omaran	4	Alexandrea	400	11
Murtkka	5	Muzaffar	500	0
Quchablbas	4	Muzaffar	400	22.5
	4	Alexandrea	400	20.75
Smailawa	4	Alexandrea	400	33.75
Grdasher	4	Muzaffar	400	54.75
	4	Alexandrea	400	50.5
Jdida	5	Alexandrea	400	5.75

Gaing	4	Alexandrea	400	21.75
	4	Alexandria	400	12.25
	4	Alexandria+muzaffar	400	11.25
	4	Muzaffar	400	1.75
	4	Alexandria	400	6
Total	54	2		19.39

The infection percent varied from one area to another. In Murtkha fields no infection were recorded on the Muzaffar variety, planted in late June compared to other areas. The crop was planted inside tunnels and covered with white net during growing season (fig. 1). This procedure could protect cultivated crops from hot weather and mosaic virus insect vectors. Similarly, (6) used white coarse nets to protect pepper crops against Cucumber mosaic virus (CMV) and Potato virus Y (PVY) aphid transmission.



Figure 1 squash field that covered by white polyethylene net.

In Gainj village, lowest infection percent 1.75 was scored on Muzaffar variety in August 30th, planted in early June. that the low infection rate may be related to growing this squash variety in a wheat field Similarly, low infection rate, at 5.7% was scored on Alexandria variety, grown in wheat field in Jdida. The low 7% infection rate in a fields at Alana may be related to the field was infected with powdery mildew as the mosaic symptoms cannot be easily recognized. However, a study in the United States (4) confirmed powdery mildew infected cucurbit plants could resist ZYMV more than powdery mildew free plants.



Fig 2 the field were infected by powdery mildew.

All 8 symptomatic leaf samples collected from different were infected with both Zucchini yellow mosaic virus (ZYMV) and Watermelon mosaic virus (WMV), when

tested by RT-PCR (fig 3). Mixed infection of these two viruses in cucurbits was reported (11).



Fig 3 Gel electrophoresis pattern of DNA fragments amplified from eight symptomatic samples Lanes 1-4: WMV infected samples (1100 bp), lanes: 5-8 ZYMV (850 bp), C: negative control. Lane 1 100bp DNA marker (SMO Bio. Korea).

The successful amplification of RT-PCR enabled a rapid detection of both ZYMV and WMV in infected squash samples. Thus, RT-PCR can be a reliable test in viruses' diagnosis (16).

Molecular detection using reverse-transcriptase polymerase chain reaction showed both ZYMV and WMV are predominant in the most squash grown fields in Erbil. The obtained results are in harmony with that described by (1) who successfully detected ZYMV by RT-PCR.

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