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Short Notes: First Report of Cucurbite Aphid-Borne Yellows Virus in Iraq

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Abstract: Squash (*Cucurbita pepo*) fields in Mosul suffered from virus-like symptoms, such as vein clearing, severe mosaic, mottling, chlorosis, necrosis, stunting and fruit discoloration. The symptoms were associated with aphids presence in the infected fields. A metatranscriptomic analysis has been applied to detect the causal agent. The result showed that 559,024 mapped reads were related to *Cucurbit aphid-borne yellows virus* with full coverage to produce 5,662 bp consensus sequence. The virus sequence was deposited in National Center for Biotechnology Information (NCBI) under accession number OQ865059, and the isolate named Iraq. For the first time in Iraq, the infection of the virus has been reported in squash, highlighting the impact of such virus infection that spread epidemically across the squash and other cucurbits fields.

Keywords: Bioinformatics, Luteoviridae, *Polerovirus*, RNA seq, Squash genome.

Cucurbit aphid-borne yellows virus (CABYV) belongs to the genus *Polerovirus* in the family Luteoviridae, and was first reported in France in 1992. The virus causes a general yellowing of the older leaves, which become thick and leathery particularly in melon, cucumber and zucchini squash (Lecoq *et al*., 1992; Menzel *et al*., 2020), however, Iraq had not previously been reported to have the virus. In summer 2023, the major cucurbit-growing areas especially squash plants in Mosul Province, a virus-like symptom were observed, included vein clearing, severe mosaic, mottling, chlorosis, necrosis, stunting and fruit discoloration. The symptoms were associated with presence of aphids in the infected fields that showed reduction in the growth and yield of such plants consequently. Symptomatic leaves were collected from infected squash plant (Fig. 1B), and then cut into squares of 0.5×0.5 cm, and immersed in 5x volume of manually prepared RNALater in Eppendorf tube (2 ml) and sent to the DNA link company (Republic of Korea) for sequencing. The extracted RNA was subjected to whole genome sequencing (Platform: Novaseq 6000; Applications: WTS/mRNA). The total RNA sequence was 40,499,262 clean reads with 101 bases each. The reference sequence was constructed from 5040 suspected virus that downloaded from NCBI-GenBank, and concatenated to create one representative reference sequence and the entire length was 76,145,671 nt. The paired end reads of the whole RNA clean reads were mapped against the reference sequence using the map to reference tool in Geneious software (http://www.geneious.com/)(Kearse *et al*., 2012). The result shows that 559,024 reads mapped to *Cucurbit aphid-borne yellows virus* (X76931) with full coverage to produce 5,662 bp consensus sequence. The BLASTn, BLASTx and also ORF finder (NCBI) were used to confirm the nucleotide and protein sequences identity, and open reading frames respectively. Later on, the virus sequence was deposited in NCBI GenBank under the accession number OQ865059, and the isolate named Iraq. Six open reading frames (ORFs) are encoded in the CABYV genome, which shares 95.7% pairwise identity with the reference virus (X76931): ORFs 0, 1 and 1–2 are translated from genomic RNA, while ORFs 3 and 4 are transcribed from sub-genomic RNA. ORFs 3 and 4 overlap and are translated in different frames. A phylogenetic analysis showed that our isolate was clustered in between the French isolates X76931 and NC_003688, and the clade of Spanish isolates indicating the probability of a common ancestor (Fig. 2). For the first time in Iraq, the infection of the virus has been reported in squash, highlighting the impact of such virus infection that spread epidemically across the squash and other cucurbits fields.

Contributions of authors

Project supervision was provided by NQ, OA, and BI. Together with LA, OA performed the experiments and analyzed the data. All authors revised and approved the final version of the manuscript, which was written by NQ, OA, and BI.

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Conflicts of interest

There were no commercial or financial relationships that could possibly construe the research as conflicted.

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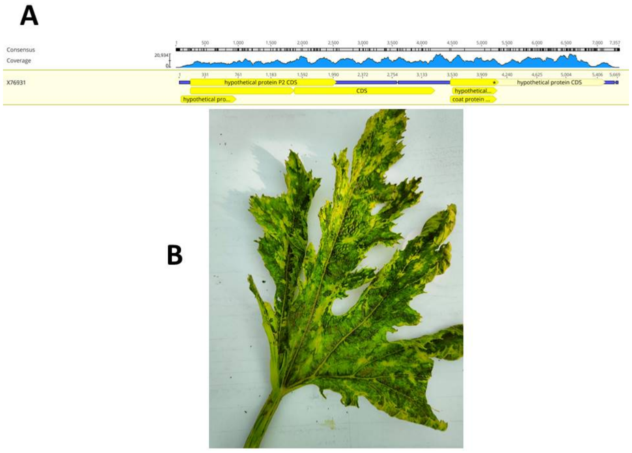

Fig. (1): A. Map to reference tool was applied in the Geneious prime software using *Cucurbit aphid-borne yellows virus* (X76931) as a reference sequence showing 559,024 assembled reads to generate 5,662 bp consensus sequence with six ORFs. B. Symptomatic squash leaf shows yellowing and mosaic symptoms.

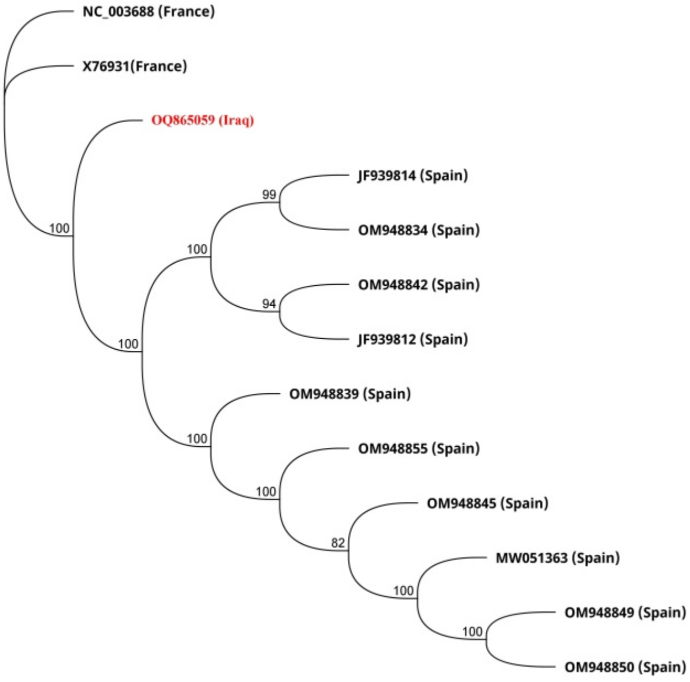

Fig. (2): Phylogenetic tree based on registered complete sequences of *Cucurbit aphid-borne yellows virus* isolates showing close relationship of the Iraq isolate and the French and Spanish isolates. The tree was generated with Geneious tree builder using the neighbour-joining method and The Hasegawa-Kishino-Yano (HKY) model in a bootstrap test.

التقرير الأول لفايروس اصفرار القرعيات المحمول بالمن في العراق

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عانت حقول الشجر في الموصل من أعراض شبيهة بالفيروس، مثل شفافية العروق، والموزائيك الشديد، والتبقع، والتنخر، والتقزم، وتغير لون الثمار. وارتبطت هذه الأعراض بوجود حشرات المن في الحقول المصابة. أجريت الدراسة للكشف عن العامل المسبب. وتم تطبيق تحليل بيانات الاستنساخ الكلية ,وأظهرت النتيجة أن 559,024 قراءة تم ارتباطها بفايروس اصفرار القرعيات المحمول بالمن. مع تغطية كاملة لإنتاج تسلسل كامل للفايروس يبلغ 5,662 زوج قاعده. تم إيداع تسلسل الفيروس في بنك الجينات تحت رقم OQ865059، وتم تسمية العزله العراق. يعد هذا البحث هو التسجيل الأول للفايروس بالعراق، مما يسلط الضوء على تأثير هذه العدوى الفيروسية التي انتشرت بشكل وبائي عبر حقول الشجر والقرعيات الأخرى.

الكلمات المفتاحية: برامج المعلوماتيه الحياتيه، *Polerovirus*, Luteoviridae , تحليل الرنا، جينوم الشجر.