REVEALING HIDDEN VIRUSES INDUCING SIMILAR YELLOWING SYMPTOMS OR REMAINING ASYMPTOMATIC IN CUCURBIT CROPS

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ABSTRACT

Cucurbit crops include melons, cucumbers, squashes, and gourds - all of which are very important in global agriculture and are increasingly at risk as a result of undetected or asymptomatic viral infections. These "stealth" infections often evade detection during visual inspections or conventional diagnostic tests, with yellowing symptoms that may mask nutrient deficiencies or other plant stresses, or remain asymptomatic, silently affecting crop health. This study investigates the presence of hidden viral infections in cucurbit crops, using advanced molecular diagnostics and high-throughput sequencing to identify and characterize viruses that induce yellowing symptoms or remain asymptomatic. The complexity of detection and management of latent viral threats is discussed here, and we emphasize that traditional diagnostic methods are highly limited. We emphasize the advances in molecular diagnostics like PCR and NGS that allow the identification of symptomatic and asymptomatic infections. Detailed case studies, in this case the CABYV prevalence, argue the need for thorough surveillance in combating the spread and impact of viruses. We further provide discussions on the biological and ecological aspects of viruses in terms of transmission with respect to vectors like aphids and environmental triggers that provoke latent viruses. It calls for the use of integrated pest management and virus-resistant cultivars as fundamental strategies in sustainable crop protection. Detection methods are improving, and proactive management practices can ensure better protection of cucurbit crops against the hidden viral threats that could impair yield and quality.

Keyword: PCR, NGS, CABYV, Detection

Introduction

Revealing hidden viruses in cucurbit crops—such as cucumbers, melons, squashes, and pumpkins—is crucial for maintaining crop health and productivity. These crops are vulnerable to various viruses that cause yellowing symptoms, which often resemble damage from nutrient deficiencies, environmental stresses, or even other pathogens. This overlap makes identifying virus infections challenging, particularly when some viruses remain asymptomatic under specific conditions (Harrison,1999; Abrahamian and Abou-Jawdah,2014; Zitter and Banik,1984;kumari et al.,2022). These "hidden" or latent viruses are of particular concern because they can persist undetected in crops, potentially spreading to other plants without showing any symptoms. Factors like temperature, humidity, and plant stress can trigger latent viruses to become symptomatic, leading to sudden outbreaks that catch farmers off guard. As a result, these hidden viruses can act as reservoirs, allowing infections to spread over large areas and threaten overall crop yields. Detecting these asymptomatic or latent viruses requires advanced molecular techniques, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS). These methods allow for precise identification of viruses within plants, even when symptoms are absent. Once identified, integrated pest management (IPM) strategies, along with resistant crop varieties, can be employed to manage these infections effectively. Addressing these hidden viral threats in cucurbits is essential to protect crop productivity, support food security, and reduce the need for chemical interventions in agriculture.

Yellowing symptoms in cucurbit crops

Cucurbit aphid-borne yellows (CABY) is caused by the Cucurbit aphid-borne yellows virus (CABYV), which was first identified in France in 1988. This virus likely existed in Asia and the Mediterranean for many years prior. CABY is prevalent in several regions, including Algeria, France, Greece, Iran, Lebanon, Spain, Tunisia, Turkey, and Sudan. During tests conducted in Tunisia from 2000 to 2004, 70% of cucurbits were found to be infected with CABYV. Additionally, 83% of melons in Spain were infected in 2003 and 2004, and 41% of cucurbits tested in France also showed infection. CABYV has a broad host range, capable of infecting various cucurbit species as well as several non-cucurbit crops and weeds. Known hosts include beets, chickpeas, faba beans, lettuce, passion fruit, and tomatoes. Among weed hosts are velvetleaf, spiny amaranth, shepherd's purse, wild mustard, common poppy, and black nightshade. Beets may have played a significant role as a reservoir host in recent outbreaks in the Netherlands and Belgium (Olszewski, N. E., et al. (2019).

Symptoms

The primary symptom of CABY in cucurbit plants, including cucumber, melon, and squash, is the yellowing of older leaves. In some cultivars, the entire plant may show this yellowing. Additionally, yellow mottling and interveinal chlorosis (where veins remain green) can occur. Affected leaves often become thick, leathery, and brittle. Typically, symptoms do not appear on the fruit, and CABYV infection does not usually impact fruit quality. However, CABY can cause flower abortion, which significantly reduces yield by leading to fewer fruits on each plant. This lower fruit load might result in larger fruits, potentially affecting marketability if they aren't harvested at the right time. Under high disease pressure, fruits may not develop adequately to reach desirable market sizes and can have a shorter shelf life. CABY symptoms resemble those associated with other yellowing viruses in cucurbits, such as Cucumber yellow stunting disorder virus (CYSDV), Beet pseudo-yellows virus (BPYV), Cucumber vein yellowing virus (CVYV), and Cucurbit chlorotic yellows virus (CCYV). This similarity may explain why the pathogen has gone undetected in the Mediterranean region for many years. Additionally, co-infections with multiple viruses complicate diagnostic challenges. CABY symptoms may also be confused with those caused by nutrient deficiencies, such as magnesium deficiency. To confirm that the symptoms are due to CABYV infection, affected leaf samples should be sent to a plant diagnostic lab for testing. While commercial ELISA tests can detect viral proteins, they may cross-react with related viruses. In contrast, RT-PCR tests, which identify specific RNA sequences of CABYV, are more accurate and sensitive than ELISA tests (Shahid, M., & Ali, A. (2020).

Biology and Disease Cycle

To acquire the virus from an infected plant and later transmit it to a healthy one, the aphid must insert its stylet (feeding tube) into the phloem vascular tissue. The aphids need to feed on an infected plant for several hours or even days to acquire CABYV, and a similarly extended feeding period is required to transmit the virus. In contrast, many other aphid-borne viruses can be acquired or transmitted after just seconds or minutes of feeding. While other aphid species, such as the green peach aphid (Myzus persicae) and the potato aphid (Macrosiphum euphorbiae), can also transmit CABYV, they are less effective than A. gossypii. CABYV is persistent, circulative, and non-propagative in aphids, meaning that once an aphid acquires the virus, it can transmit it for the rest of its life. The virus circulates within the aphid's body and accumulates in the salivary glands, where it is injected into the plant's phloem during feeding. After feeding on an infected plant, it takes one to three days for the aphid to be capable of transmitting the virus. CABYV does not multiply within the aphid and is not passed on to its offspring. The virus cannot be transmitted mechanically (through infected sap) and spreads throughout the cucumber plant via the phloem. CABYV has not been reported to be seed-transmitted, and the quality of seeds from infected plants remains unaffected. Younger plants experience higher disease levels and greater yield impacts when infected. The severity of the disease also depends on the population levels of the aphid vector and the availability of CABYV in nearby reservoir hosts. Infected weed species and other cucurbit crops, whether garden or commercial, can serve as sources of CABYV for greenhouse-grown cucumbers.

Distribution of aphid- and white-fly-transmitted viruses in melon and watermelon crops exhibiting yellowing symptoms

We investigated the distribution of aphid- and whitefly-transmitted viruses in melon and watermelon crops showing yellowing symptoms. To eliminate the possibility of other viruses in previously negative samples, we used a sequence-independent method to amplify and clone potential viral dsRNAs from three pooled samples of both crops. This revealed the presence of PABYV in watermelon and CmEV in melon. Our findings indicate that PABYV is a newly identified cause of yellowing disease in these cucurbits, leading to its inclusion in our epidemiological analysis. The detection of PABYV significantly increased the proportion of samples testing positive for viral infection, reaching 82.7% in melon and 85.7% in watermelon. Consequently, the percentage of undetected virus infections associated with yellowing symptoms fell to 23% for melon and 16% for watermelon. Notably, we found no evidence of PRSV, CCYV, or BPYV in either crop, across various locations or years. Overall, the occurrence of aphid-transmitted viruses was significantly higher than that of whitefly-transmitted viruses ($\chi2 = 409.7$, df = 1; $p < 0.001$). Specifically, CABYV (47.3% and 24.0%), PABYV (25.3% and 71.0%), and WMV (33.3% and 27.3%) were more frequently identified in melon and watermelon, respectively, while other viruses were found at much lower rates. CABYV and PABYV were the dominant viruses detected in both crops, with occasional mixed infections. The detection rate of CABYV varied seasonally, ranging from 77% to 19% in melon and 7% to 0% in watermelon. PABYV also showed significant variability, with prevalence in melon ranging from 10% to 42% and in watermelon from 35% to 83%. WMV was detected infrequently, with only sporadic occurrences in both crops. CYSDV and CVYV were present in low frequencies (6% and 1%, respectively) and showed fluctuations over the years. Mixed infections were common, especially with CABYV and PABYV co-occurring in 3% to 25% of melon samples and 10% to 33% of watermelon samples (Perring, T. M., et al. (2019). In cucurbit crops (e.g., cucumbers, melons, pumpkins, and squashes), asymptomatic viral infections are those in which the plants carry a virus but do not show visible symptoms. These hidden infections can be problematic because they allow viruses to persist undetected within fields or greenhouses, silently affecting crop quality, yield, and potentially spreading to other plants. Key Aspects of Asymptomatic Viral Infections in Cucurbits

1. Common Asymptomatic Viruses: Some viruses may not cause visible symptoms in certain cucurbit varieties or under specific environmental conditions. Common asymptomatic or mildly symptomatic viruses in cucurbits include: Cucumber Green Mottle Mosaic Virus (CGMMV): Often asymptomatic in some cucumber varieties but highly contagious. Cucumber Mosaic Virus (CMV): Some strains may not show symptoms immediately or may only cause mild leaf mottling under certain conditions. Squash Mosaic Virus (SqMV): Occasionally remains asymptomatic in specific squash varieties. Watermelon Mosaic Virus (WMV): May produce mild or no symptoms in some cultivars.

2. Factors Influencing Asymptomatic Expression: Plant Variety: Resistance and tolerance levels vary among cucurbit cultivars, with some capable of harboring the virus without displaying symptoms. Environmental Conditions: Temperature, light intensity, and water availability can influence whether symptoms appear. For example, some viral symptoms only manifest under heat or drought stress. Virus Strain: Different strains of the same virus can vary in virulence, causing mild symptoms or no symptoms in some cases. Plant Age: Younger plants may be asymptomatic and only show symptoms as they mature or come under stress.

3. Detection of Asymptomatic Infections: Because asymptomatic plants can still spread viruses, early detection is crucial for managing viral diseases in cucurbit crops. Detection methods include: Polymerase Chain Reaction (PCR) and RT-PCR: Highly sensitive molecular methods that detect viral DNA or RNA, revealing infections even without symptoms. Enzyme-Linked Immunosorbent Assay (ELISA): Serological testing to detect viral proteins, allowing for the identification of viruses within asymptomatic plants. High-Throughput Sequencing (HTS): A comprehensive technique to identify known and unknown viruses in plant samples.

4. Implications of Asymptomatic Infections: Spread of Infection: Asymptomatic plants can act as reservoirs, spreading viruses to other plants through vectors (e.g., aphids) or human handling. Reduced Yields: Even without visible symptoms, infected plants may have lower productivity, produce smaller or less flavorful fruits, or show increased susceptibility to stress. Delayed Diagnosis: Without obvious symptoms, infections can persist and spread widely before detection, making control efforts more difficult.

5. Management Strategies: Regular Virus Testing: Implement routine testing of plants for common viruses, especially in seed lots and nursery plants. Use of Virus-Free Seeds: Plant certified virus-free seeds to reduce the risk of seed-borne infections. Vector Control: Manage insect vectors that transmit viruses, such as aphids, beetles, and thrips, using integrated pest management (IPM) methods. Crop Rotation and Sanitation: Rotate crops to break viral life cycles and remove any infected plant material at the end of each growing season to reduce viral reservoirs.In conclusion to asymptomatic viral infections in cucurbits are challenging to manage due to the hidden nature of these infections. Regular monitoring, advanced diagnostic methods, and proactive management practices are essential for minimizing the impact of these hidden viruses on cucurbit crop health and yield. (Moury, B., & Poinssot, B. (2021).

Conclusion

Detecting hidden viruses in cucurbit crops, especially those that cause yellowing symptoms or remain asymptomatic, is essential to safeguard crop health, yield, and quality. These viruses pose unique challenges due to their ability to mimic nutrient deficiencies or environmental stresses and remain latent under certain conditions. Left unchecked, they can spread through fields as asymptomatic reservoirs, leading to potential outbreaks and economic losses for growers. Advanced molecular tools, such as PCR and next-generation sequencing, play a crucial role in early virus detection and identification, helping to distinguish viral infections from other crop issues. With the support of integrated pest management (IPM) strategies and the use of resistant varieties, farmers can better control viral spread, even when symptoms are not visibly present. By focusing on early detection, accurate diagnostics, and proactive management, the agricultural industry can mitigate the impact of these hidden viral threats, ultimately leading to healthier crops and more sustainable production practices for cucurbit farming.

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