

# **CONSTRAINTS, CHARACTERIZATION AND INCIDENCE OF VIRUSES AFFECTING WATERMELON AND PUMPKIN PRODUCTION IN UGANDA**

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## **Abstract**

Watermelons and pumpkins are cultivated in Uganda for their leaves, fruits, and seeds, thereby contributing to food, nutrition and income security. However, there is limited research and information on constraints and especially viruses affecting their production. This study assessed the current production constraints, the incidence, and molecular characterization of viruses affecting watermelon and pumpkin cultivation in Uganda. Two surveys were conducted in 9 sub-regions including Acholi, central, east central, Elgon, Lango, south western, Teso, west Nile and western sub-regions based on the importance and availability of watermelon and pumpkin fields.

The first survey was conducted in between July and November 2022 using a questionnaire administered to farmers on different management practices, strategies, and current production constraints. During the survey, symptomatic watermelon and pumpkin leaf samples were preferentially selected for virus genome characterization. According to the results, pests, diseases, drought, high transport and labour costs were the most important general constraints. Bacterial wilt, downy mildews, anthracnose, powdery mildews and virus diseases in this order were the most common and important disease constraints. While whitefly, aphids, melon fly and cut worm were reported as the most limiting pests in watermelon and pumpkin production. Mixing of several agrochemicals was observed in watermelon fields coupled with gross lack of knowledge of proper usage which could result in pesticide resistance, health and environmental hazards.

For virus identification and genome characterization, a total of 75 (60 symptomatic and 15 non-symptomatic) representative pumpkin leaf samples out of 450 (360 symptomatic and 90 asymptomatic) collected were pooled in 5 groups, each containing 15 leaf samples and sequenced using Illumina Hiseq sequencing platform at the Agricultural Research Council-Biotechnology Platform, Pretoria. The raw reads were assembled using Genome Detective 1.132 and for the first time, six viruses were identified in Uganda from pumpkin, including Pepo aphid-borne yellows virus (PABYV), Cucurbit yellow stunting disorder virus (CYSDV), Tomato leaf curl virus (ToLCV), Tobacco streak virus (TSV), Pepper leaf curl virus (PepLCV), and Moroccan watermelon mosaic virus (MWMV). Recombination analysis also showed that one MWMV isolate (Accession number: OM718861) is a recombinant with major parents from Kenya (Accession number: MH713899) and minor parent from this study (Accession number OM718859). Complete genome sequences of MWMV and TSV infecting pumpkin in Uganda were assembled and the TSV complete genome is the first record in Africa. Primers were designed from NGS data targeting the cylindrical inclusion (CI) and coat protein (CP) of MWMV, and CP of PABYV, and TSV respectively to confirm presence of the virus in individual samples pooled.

In watermelon one pool of 15 watermelon leaf samples were sequenced using high throughput sequencing (HTS) of total RNA. Viral contigs were assembled using the Genome Detective virus tool and BLASTn searches were conducted for complete and near-complete genomes. A total of two complete genomes of Pepo aphid-borne yellows virus (PABYV) and Moroccan watermelon mosaic virus (MWMV) were assembled while near-complete virus genome sequences of Watermelon crinkle leaf-associated virus (WCLaV), *Citrullus lanatus* cryptic virus (CiLCV), and Cucumis melo cryptic virus (CmCV) were identified. The identification and characterization of these viruses in watermelon are important for the development of sustainable disease management options.

The second field survey was conducted during the June to July season 2022 to document the distribution of MWMV, and PABYV in 10 sub-regions and determine any co-infections in Uganda using virus specific primers designed from the Next-generation sequencing data. These two viruses were detected in all the sub-regions during the first survey, are emerging viral pathogens that are very destructive to cucurbits and have been reported in the neighbouring countries. A total of 101 samples were analysed using reverse transcription polymerase chain reaction (RT-PCR) of which, 84.2% of all the samples collected were symptomatic and had an average disease severity, of 2.5 on a visual scale of 0-4. Disease incidence was highest in east central in watermelon compared to central sub-region while in pumpkins, virus-like diseases were observed in all the sub-regions except Teso and Acholi. The incidence of PABYV was low at 17.8% in all the sub-regions surveyed while that of MWMV was 37.0% of which four samples were dually infected by the viruses.

The use of resistant cultivars remains the most reliable and environmentally friendly method to increase the annual yields. To increase on the resistance base, an experimental evaluation of the level of resistance of local cucurbits (watermelon, pumpkin, cucumber and loofah) to the most widely occurring virus infecting cucurbits in Uganda was carried out. The source of virus inoculum was a pumpkin plant from the field at the National Crops Resources Research Institute (NaCRRI). Local cucurbit cultivars were raised in a screen house in a completely randomized design and inoculated with the MWMV isolate. The reactions to the virus inoculum (symptom expression and severity) were recorded for four weeks after inoculation. The severity was evaluated on a scale of 0 to 4. Six cucurbit cultivars including A3, A4, A6, A7, A8, and C1, showed a high average virus disease severity of 3, while A9, A10, and B1, showed low average virus-like disease severity. These three cultivars are potential sources of tolerance or partial resistance after confirmation by other methods.