VIROME PROFILING OF RIDGE GOURD AND DEVELOPMENT OF DIAGNOSTIC TOOLS

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Keywords

Virome, next generation sequencing, de novo assembly, phylogenetic analyses, recombination analyses.

Introduction

The Cucurbitaceae family includes ridge gourd, cultivated globally for its tender, nutritious fruits. Originating in India, it spans 9,920 hectares, yielding 3.17 lakh tonnes of dark green, nutrient-rich produce annually. Its nutritional profile boasts vitamins, carbohydrates, proteins, and flavonoids, making it a valuable crop for food and medicine. Traditional diagnostic methods like ELISA, PCR, and RT-PCR offer speed and accuracy in virus detection but are limited to known pathogens. To address this, virome profiling utilizing NGS technologies is proposed. NGS revolutionizes genome research, enabling rapid sequence annotation and assembly, facilitating novel biological discoveries. Its application in virome analysis allows for the detection of both known and unknown viruses, crucial in disease investigation.

Sela *et al*. (2013) identified a novel partitivirus, Citrullus lanatus cryptic virus, in watermelon plants co-infected with melon necrotic spot virus (MNSV), closely related to pepper cryptic virus 1 and raphanus sativus cryptic virus, proposing its classification within the family Partitiviridae. Chen *et al*. (2021) characterized Lagenaria siceraria associated mitovirus 1 (LsaMV1), a novel mitovirus, suggesting its membership in the family Mitoviridae, marking the first report of a Mitoviridae member in bottle gourd. Reddy *et al*. (2023) revealed the presence of seven viruses in chilli crops, including Pepper vein yellows virus (PeVYV) and BPEV, reported for the first time in India. Naganur *et al*. (2019) developed a standardized LAMP assay for rapid detection of Tomato leaf curl New Delhi virus (ToLCNDV) causing ridge gourd yellow mosaic disease (RgYMD), crucial for disease management in India.

Objectives

1. Virome profiling in ridge gourd *(Luffa acutangula* L).
2. Identification of novel viruses associated with the ridge gourd.
3. Validation of identified viruses associated with ridge gourd and development of nucleic acid-based diagnostics for novel viruses**.**

Methodology

Sample collection for virome profiling in ridge gourd: Ridge gourd samples will be collected based on visual symptoms. The infected leaves, will be collected across different parts of southern Karnataka (Chikkaballapura, Kolar, Bengaluru, Tumakuru). Collected samples will be stored at -80 ℃.

Total RNA extraction for NGS analysis: Total RNA will be isolated from various tissues using LiCl or TRIzol reagent, followed by analysis of purity, integrity, and size via agarose gel electrophoresis and nanodrop spectrophotometry.

Construction of mRNA and sRNA library and sequencing: Sidharthan *et al*. (2020) present a detailed protocol for mRNAome and sRNAome sequencing, crucial for comprehensive virome identification; mRNA-based methods offer longer contigs aiding in variant detection, while sRNA-based methods provide sensitivity to viral RNA.

Raw data pre-processing: The obtained raw data from mRNA and sRNA libraries will be analyzed by using various bioinformatic tools.

De novo assembly: Two de novo assembly approaches will be employed, individually assembling mRNA and sRNA libraries, and combining them for whole transcriptome assembly

Identification of viruses from potato mRNAome and sRNAome: Assembled contigs undergo standalone MEGABLAST against virus reference sequences, while host-unmapped reads from mRNA/sRNA libraries are MEGABLASTed against virus reference genomes after mapping to the potato genome for validation. Copy number estimation of identified viruses in each mRNA and sRNA library

Reconstruction of whole genome of identified viruses: Whole genomes of identified viruses will be reconstructed through alignment of viral contigs, filling gaps with raw sequence reads, and considering full-length consensus sequences as complete genomes.

Pairwise distance and phylogenetic analyses using reconstructed viral genomes: The complete genomes retrieved from NCBI along with the viral genomes reconstructed in this study will be aligned using CLUSTALW tool in MEGA11 software or Bio Edit sequence alignment editor.

Recombination analyses: Only viral sequences used for phylogenetic analyses will be used for the detection of recombinants. Recombination analysis is done by RDP5 tool.

Validation of identified viruses and development of diagnostics tool like RT-PCR and RT-LAMP, for novel viruses.

Results and Conclusions

Survey was carried out in in different locations in southern Karnataka (Chikkaballapura, Kolar, Bengaluru rural districts) and ridge gourd samples collected based on visual symptoms.. Collected samples stored at -80 ℃.

RNA extracted from all samples by using LiCl Method.

Samples were pooled and sent for sequencing.

Innovation in the project

This research seeks to characterize the viral population in ridge gourd plants, facilitating the creation of diagnostic tools and ensuring the production of disease-free planting materials. Ultimately, it aims to enhance quarantine and certification programs for ridge gourd.

Future Scope:

The NGS data generated in this study will serve as a resource for reliable indexing of ridge gourd viruses in quarantine stations and certification programmes. Identification of novel viruses in ridge gourd will help in monitoring and management of diseases.