Primary Results from Nanopore and Next-Generation Sequencing of Tomato Samples in Albania

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Abstract

This research study investigates the role of Oxford Nanopore technologies in detecting and characterizing viruses, mainly the Tomato Brown Rugose Fruit Virus (ToBRFV) in tomato plants grown in greenhouses in Albania. The work was funded by the National Agency for Science Research and Innovation in Albania (NASRI), through NanoAlb.

ToBRFV in Albania was reported for the first time in 2022 [1] and from this period there has been a significant spread which should be studied quickly and accurately.

The period for sampling was based on the characteristics of the tomato vegetation cycle and the development of ToBRFV, it was in greenhouses (September - October - November 2023). During the sampling, we aimed to carry out a selection of plants that showed spotting, mosaic and narrowing of the upper leaf blades [2].

According to the manufacturer's instructions, the nanopore sequencing experiment was conducted using the cDNA-PCR sequencing kit V14 (SQK-PCS114). The initial sample consisted of 500 ng of total RNA extracted from symptomatic tomato plants in Albania. The first run of Oxford Nanopore sequencing yielded 11.04k paired-end reads, which were reduced to 8.78k after quality filtering and clean-up. High-quality reads were de novo assembled using Geneious software, resulting in 2,500 cotings with lengths ranging from 40 to 22,400 nucleotides. A reference mapping against the Tomato Brown Rugose Fruit Virus (ToBRFV) sequence from GenBank revealed an 84% genome coverage, with 8 reads matching this virus. The read lengths varied from 120 to 860 nucleotides.

For comparison, next-generation sequencing (NGS) was performed on the total RNA from the same tomato sample, generating approximately 55 million paired-end reads. After the quality filtering process, the number of high-quality reads decreased to 2 million. These reads were also de novo assembled, producing 35,334 cotings, with lengths ranging from 50 to 57,863 nucleotides. A reference mapping against the ToBRFV genome retrieved from GenBank provided 100% coverage, with 34 contigs confirmed through a BlastX search via Geneious software [3]. The lengths of these contigs ranged from 50 to 4,800 nucleotides.

These results demonstrate the efficacy of Oxford Nanopore Technologies (ONT) for rapid detection and partial genome characterization, despite lower read depth and coverage compared to NGS. The study underscores the potential for using Nanopore sequencing in resource-limited settings, while highlighting the robustness of NGS for comprehensive viral genome analysis.

References

[1] Orfanidou, C.G., Cara, M., Merkuri, J. et al. First report of tomato brown rugose fruit virus in tomato in Albania. J Plant Pathol 104, 855 (2022). https://doi.org/10.1007/s42161-022-01060-y.

[2] Luria N, Smith E, Reingold V, Bekelman I, Lapidot M, Levin I, Elad N, Tam Y, Sela N, Abu-Ras A, Ezra N, Haberman A, Yitzhak L, Lachman O, Dombrovsky A (2017) A new Israeli tobamovirus isolate infects tomato plants harboring Tm-22 resistance genes. PLoS One 12(1):e0170429.

[3] https://www.graphstats.net/geneious-prime.