Nanopore for fast detection and characterization of plant pathogens

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Abstract

Nanopore sequencing technology (MinION, Oxford, UK), and its applications in basic and applied research have undergone significant growth since Oxford Nanopore Technologies (ONT) provided the first nanopore sequencer, MinION, in 2014 [1,2]. This technology relies on a nanoscale protein pore, or 'nanopore', that serves as a biosensor and is embedded in an electrically resistant polymer membrane [1]. Being a low-cost, accurate, fast, and easy in-situ handling technology, its' application for detecting and characterizing known and undescribed plant pathogens in different plant species and crops has gained a lot of space in agricultural research. The big advantage of such innovative technology resides in the supremacy of multiple detection and characterization of many pathogens and in a reduction of expenses of posting the DNA in foreign laboratories to perform the analysis, as well as the time of obtaining the sequences.

The ONT sequencing as a tool in plant virology has been relatively slow despite its promise in more recent years to yield large quantities of long nucleotide sequences in real - time without the need for prior amplification. Here, we present a protocol using the ONT Flongle platform that was applied on mini-dsRNA templates extracted from a range of symptomatic ornamental plants that could be used to search for new unreported pathogens for domestic surveillance of plant samples. The results of the ONT's application on dsRNA templates, compared with those obtained from total nucleic acid templates extracted from tissues of the same plants, as a valid diagnostician's toolkit that, together with the integration of high-throughput sequencing technology, showed to be a highly reliable and validated plant virus diagnostic method for known and unknown virus detection, as well as for other types of plant pathogens. The existence of these novels and previously reported viral entities in the tested plant material was ascertained through the application of nanotechnological diagnostic methods, *i.e*., qPCR, TaqMan PCR, LAMP in our laboratories (IAMB, Italy; and NanoAlb, Albania) and afterward registered in NCBI database.

References

[1] Deamer, D., Akeson, M. & Branton, D. (2016). Three decades of nanopore sequencing. Nat. Biotechnol. 34, 518-524.
[2] Jain, M., Olsen, H. E., Paten, B. & Akeson, M. (2016). The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biol. 17, 239.