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REAL-TIME ON-SITE DIAGNOSIS OF QUARANTINE PATHOGENS IN PLANT TISSUES BY NANOPORE-BASED SEQUENCING

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Nowadays, cases of accidental introduction of harmful organisms into floriculture and agricultural production in general are increasingly frequent; besides, this can have devastating effects in the case of quarantine pathogens, many of which are also hard to diagnose. Consequently, quarantine pathogens spread in floriculture plants causes serious damage not only to crop welfare but also to floricultural sector and the agricultural sector in general. This issue can now be solved through the development of Nanopore Third Generation Sequencing technology for pathogens detection. Such technology has the massive advantage of being portable and suitable for sequencing in the fields. In addition, it is able to identify microorganisms even at low concentration confirming results obtained by currently employed molecular methods, enabling diagnosis beyond the laboratory.

The present research is aimed to develop a novel diagnostic assay to detect quarantine pathogens in plant hosts, using three different bacteria as a case study: Erwinia amylovora, Ralstonia solanacearum and Xylella fastidiosa subspecies fastidiosa, multiplex and pauca. For each pathogen, it has been identified a host plant species from which total DNA has been extracted, afterwards it has been spiked with different amounts of the pathogen's DNA, such as 10 or 100 copies of the genome per microlitre, to determine the sensitivity of the diagnostic test. For each bacterium, a pair of specific primers was employed to produce amplicons, which were

afterwards sequenced. In the context of this research project, such DNA fragments were sequenced in order to prove the detection capability of MinION device even at a low number of copies of pathogens and with high specificity. The assay workflow first step consists of preparing specific pathogen amplicons, which are then sequenced employing the third-generation sequencer MinION, SQK-LSK112 (Q20+) kit and r10.4 flowcell. Subsequently, sequencing data acquired are analysed by a bioinformatic pipeline identifies the potential presence of the investigated pathogens within each of the samples. We will present pathogens detection results even at a concentration of 10 copies per microliter, furthermore we will demonstrate the assay ability to resolve even mixture of different subspecies of Xylella fastidiosa in the same sample.