High Throughput Sequencing in Seed Health Testing

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Introduction

High Throughput Sequencing (HTS), also termed Next Generation Sequencing (NGS), is an emerging technology in plant pathogen detection. HTS can generate massive amounts of DNA sequence data at very low cost. It is now technically and economically feasible to use HTS for the rapid detection of multiple target pathogens. Therefore, these new technologies have many potential advantages in seed health testing and further work is required to investigate the use of HTS in seed health reference methods.

HTS is an indirect method

Seed health methods should demonstrate viability of the target organism and its pathogenicity. HTS is an indirect method which does not demonstrate either viability or pathogenicity. HTS can also reveal sequences of non-pathogenic commensal organisms that have no impact on seed health. To ensure a correct assessment of the health status of a seed lot and avoid unwarranted phytosanitary regulations based solely on HTS results, these technologies should be used as a pre-screen and be combined with other assays, including a direct test, see www.worldseed.org/[...]/Indirect_Seed_Health_Tests_2013.pdf.

HTS method development and validation

HTS methods should be developed and validated in comparison with existing reference methods. Protocols should describe each part of the analytical process including seed sample preparation, steps to eliminate the risk of cross-contamination, a description of the sequencing technology, the bioinformatic tools used for data analysis and details on sequence identity cut-offs. HTS assays should be validated for each target pathogen and matrix in the scope of the method, using standard validation guidelines and performance criteria.

Interpretation of HTS data

The development of publicly available reference databases of sequences associated with seed transmitted pests is essential for harmonization of data interpretation. HTS assays should be used with a predetermined scope of pathogens for which a rationale for phytosanitary measures exists, see www.worldseed.org/[...]/pest-lists. Sequences obtained in HTS assays that are not present in the reference database should not per se lead to restrictions in movement.

Conclusion

HTS technologies are relatively new and have potential advantages but also limitations for use in seed health testing. As with real-time PCR, HTS should be used only as a pre-screen to identify negative seed lots; positive results should be followed up with a confirmatory direct test, see www.worldseed.org/[...]/Real-time_PCR_pre-screens_2018.pdf and https://www.ippc.int/static/media/files/publication/en/2017/05/ISPM_38_2017_En_2017-05-15.pdf.