MOLECULAR METHODS IN SEED HEALTH TESTING

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Introduction

Many different techniques based on diverse biological principles are used for seed health testing. Some core techniques have been used since the beginning of seed health testing such as isolation of suspect pathogens, and pathogenicity testing on susceptible plants (bioassays). These techniques have been complemented more recently, by serological and molecular methods. This poster presents current state of modern techniques, with a focus on molecular methods (PCR, real-time PCR), and outlines those that are in the pipeline for future seed health testing methods (sequencing). The current use of PCR and real-time PCR methods are reviewed and the latest changes in the use of these molecular techniques are presented in this poster.

Why use molecular methods in Seed Health Tests?

Why have PCR assays been introduced into SH methods?

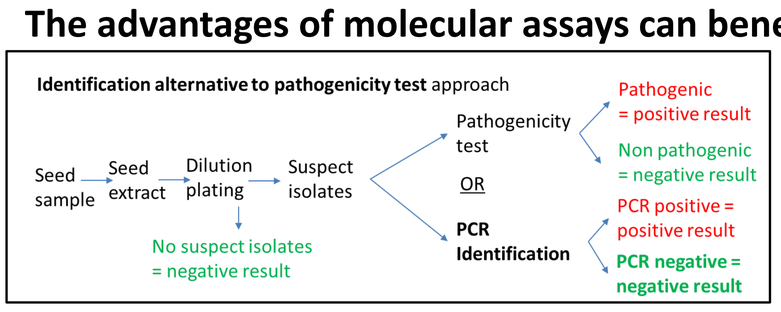
Rapid development of assays→ Large quantities of publicly available sequence data (GenBank) and simple low-cost PCR primer design. Only generic molecular biology skills and equipment are needed for assay development. Rapid analysis → decreases test lead-times meaning non-infected seed lots can be identified more rapidly than with direct tests

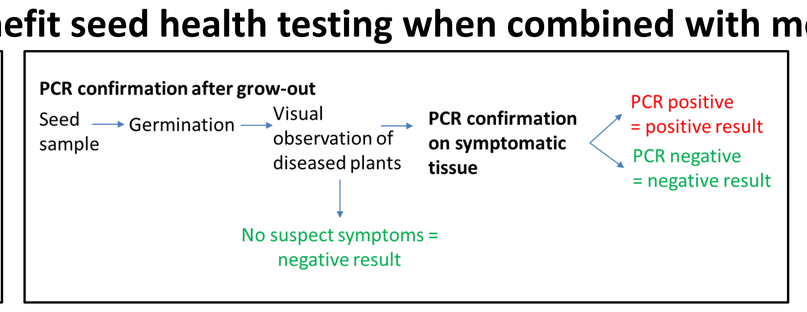
Application: Can be used in identification of isolates or symptoms or detection on seed-extracts as a pre-screen

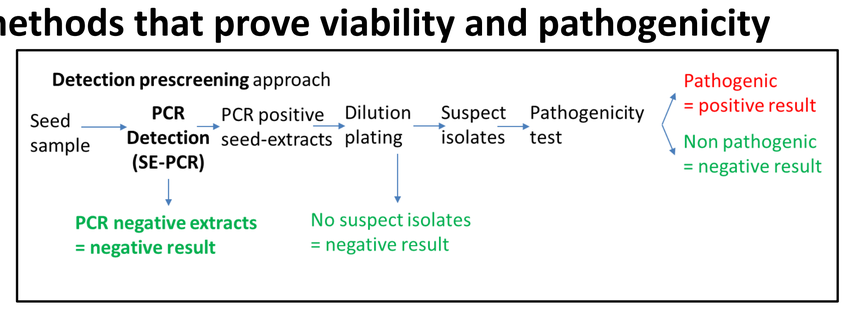
Drawbacks of molecular based assays

Specific PCR assays can be shown to have high correlation with pathogenicity during validation assays → However, no definitive proof of pathogenicity with a PCR result. PCR assays detect the presence of target DNA/RNA sequence and is not proof of intact genomes or viable pathogens → No distinction of dead/alive

The advantages of molecular assays can benefit seed health testing when combined with methods that prove viability and pathogenicity

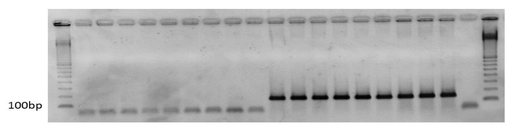


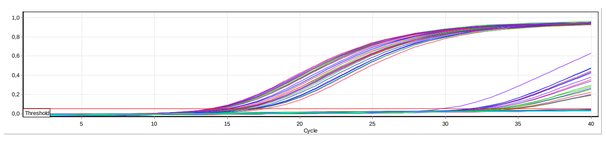


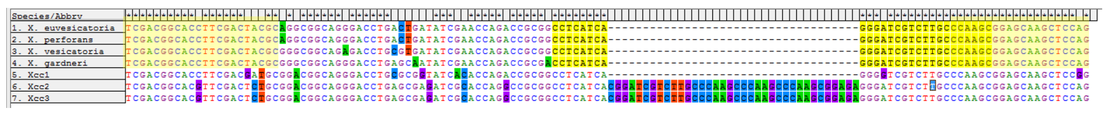


PCR, real-time PCR and Assay Design

- Most commonly used molecular method is the Polymerase Chain Reaction (PCR)
  - Imaging of the PCR product after gel electrophoresis (gel-based PCR). Use of DNA intercalant (Ethidium bromide)
  - Real-time detection of DNA amplification with DNA intercalant (SYBR-green) or hydrolysis probes (Taqman)
- PCR assay design
  - Primers can be made which are specific at different levels (pathovar, species….)
- Specific PCR assays can be rapidly designed based on sequence differences between target and closely related non-target species
- Different resolution levels can be designed to detect individual pathovar or more generic primers for groups of pathogens







Use of High-Throughput Sequencing (HTS) in seed health tests?

- Next generation DNA sequencing opens the way to low cost sequencing of DNA from seed extracts
- **Shotgun metagenomics** – Sequencing of an RNA/DNA extract. The complete sequencing of all genomes of microbial communities in a sample.
  - Large amounts of sequence data produced, not all pertinent for identifying pathogen species/pathovars
  - Interference with host DNA/RNA which will be sequenced and may make up most of the DNA extract
  - Useful for discovery of unknown microorganisms
- **Targeted Amplicon sequencing** – PCR amplification of a conserved molecular "barcoding gene". Sequencing of the PCR product and phylogenetic analysis based on the conserved gene.
  - Target gene to be sequenced can be chosen according to the level of resolution required (for example GyrB for bacterial pathovars, 16S for bacterial species)
  - The PCR step increases the proportion of microbial sequences in the sequenced sample
  - Requires some a priori knowledge of the targeted microorganisms

Examples of PCR in reference methods

- Confirmation of symptoms in grow-out tests with gel-based PCR:
  - ISTA rule 7-030: Detection of *Acidovorax valerianellae* in corn salad seed
- Identification of bacterial isolates obtained after dilution-plating on semi-selective media by gel-based PCR:
  - ISTA rule 7 -020: Detection of *Xanthomonas hortorum* pv. *carotae* in *Daucus carota* (carrot) seed
- Pre-screen detection by Seed-Extract PCR (SE-PCR):
  - ISTA rule 7-019a: Detection of *Xanthomonas campestris* pv. *campestris* in *Brassica* spp. seed

Perspectives

- Further development and collaborative validation of new PCR tests in international working groups to complement seed health methods
- Investigation into the performance characteristics of High-Throughput Sequencing technologies as a prescreen for seed health tests

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