The Challenges of DNA isolation in molecular diagnostics

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Pathogen detection using Molecular techniques is a precious tool for crop management in agriculture. Very often diagnostic methods are focused on the specificity towards the target pathogen, but before this step DNA/RNA needs to be available for processing. DNA extraction and purification is therefore crucial for the Diagnostic Success and presents multiple challenges. DNA extraction and purification from soil is yet rather suboptimal, the complexity and diversity of the matrix and the DNA sources make it a complex challenge where there is room for plenty improvement and standardization.

Pathogen Diagnostics in Agriculture

The importance of pathogen detection within agriculture has been proven over time. As diseases spread and new sub species develop. To maintain the security of our most important crops, pathogen detection is crucial. In work of Oerke et al. (1994) crop losses between 25 - 50% depending on the crop were reported. They estimated pre-harvest losses due to pests to be 42% of the potential value of the output. Also, in work of Strange et al. (2005) it was estimated that diseases alone made for 10% of the world's loss in harvest. Yet still, plant health remains overlooked compares to human and animal health. This can be seen in the limited information available on actual crop losses from pests and diseases. Showing how the importance of plant health is often overlooked (Flood, 2010).

Diseases can spread without any symptoms and infect large areas without showing. Making that once symptoms starts to show, large losses will already have occurred. Food security can be improved by early detection of these pathogens. Showing the presence of pathogens before the pathogen can spread to surrounding areas.

Molecular techniques such as Real-Time PCR can help to detect pathogens in early stages of infection and are therefore a crucial component of any crop-management system (Miller & Martin, 1988). As with a small amount of pathogen DNA, a fast and reliable detection can occur. Nonetheless, the right techniques should be chosen, depending on the pathogen and sample type, as different pathogens and samples need different treatments. For example, plant tissue samples need a different treatment than soil samples.

Soil pathogen Detection

Pathogen detection in soil gives valuable information about the presence of pathogens before any crops are affected. Unfortunately, the detection in soil comes with obstacles. The first challenge in the isolation of DNA out of soil comes from the sampling of the soil. Considering that the width of the fields and the depth at which the soil sample was taken can give opportunity for lots of variation in sampling (Wollum, 2018; Tate, 2020). Additional to the sampling difficulties of soil, the type of soil can have a large impact on the DNA extraction

process. The soil components itself can have large impact on the capturing of the DNA. As soils from different locations can have largely different components (Zhou et al, 1996). The most influential soil components are sand, clay, silt, organic matter (OM) and pH. The most problems however occur due to clay and OM (Young et al., 2014).

The presence of clay in the samples makes that more cations are present in the solutions during DNA extraction. The minerals present in clay such as Mg2+ or Ca+ are often highly cationic, making that the negatively charged DNA will bind to it.

Next to clay, OM is known to give problems in the DNA extraction and PCR processes. OM is known to lower the pH of soils, therefore the amount of OM is bound to the pH level of the soil (McCauley et al., 2009). A low pH once again makes far more cations in the solution as more proteins will get a positive charge. Additionally, soils with a high OM percentage have PCR inhibitors present such as humic acids (HA) and fulvic acids. During a PCR, HA is able to adsorb enzymes and interfere chemically or sterically with the enzymes active site.

The challenges found when isolating DNA from soil will remain an important research topic, as optimizing the DNA isolation out of soil could improve the overall detection of pathogens. This could elevate molecular diagnostics, making them even more reliable. Giving the opportunity to lower overall crop losses by earlier and better detection of pathogens.

References

Flood, J. (2010). The importance of plant health to food security. *Food Security*, *2*(3), 215–231. https://doi.org/10.1007/s12571-010-0072-5

McCauley, A., Jones, C., & Jacobsen, J. (2009). *Soil pH and organic matter* (Nutrient management module ed., Vol. 8). Montana State University.

Miller, S. A., & Martin, R. R. (1988). Molecular Diagnosis of Plant Disease. *Annual Review of Phytopathology*, *26*(1), 409–432. https://doi.org/10.1146/annurev.py.26.090188.002205

Oerke, E. C. (2005). Crop losses to pests. *The Journal of Agricultural Science*, *144*(1), 31–43. https://doi.org/10.1017/s0021859605005708

Strange, R. N., & Scott, P. R. (2005). Plant Disease: A Threat to Global Food Security. *Annual Review of Phytopathology*, *43*(1), 83–116. https://doi.org/10.1146/annurev.phyto.43.113004.133839

Tate, R. L., III. (2020). *Soil Microbiology*. Wiley.

Wollum, A. (2018). Soil Sampling for Microbiological Analysis. *SSSA Book Series*, 1–14. https://doi.org/10.2136/sssabookser5.2.c1

Young, J. M., Rawlence, N. J., Weyrich, L. S., & Cooper, A. (2014). Limitations and recommendations for successful DNA extraction from forensic soil samples: A review. *Science & Justice*, *54*(3), 238–244. https://doi.org/10.1016/j.scijus.2014.02.006

Zhou, J., Bruns, M. A., & Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. *Applied and Environmental Microbiology*, *62*(2), 316–322. https://doi.org/10.1128/aem.62.2.316-322.1996