A new diagnostic tool for botrytis in chickpeas – in-paddock biosensors - progress towards development of an in-paddock diagnostic device that is cheap, reliable, and accurate

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Key words

Botrytis, chickpea, biosensor, fungal pathogen, diagnostics

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Take home message

A novel, specific and highly sensitive molecular probe-based nano biosensor device and diagnostics protocol were validated for both *Botrytis cinerea* and *B. fabae* on field host crop samples.

The diagnosis of the *Botrytis* spp. was sensitive, specific, quantitative, fast and low cost and allowed the detection of inoculum prior to the visible appearance of disease symptoms.

The tools developed and protocols validated within this work may be applied to many other pathosystems, dependant on the development of target-specific probes and protocol optimisation.

Additional research is required to perform broader *in field* and *in industry* validation and to simplify sample preparation and DNA extraction to develop this prototype into a compact and portable device that can inform on the presence, distribution and quantity (inoculum load) of crop pathogens. If done, this would provide significant power to disease management decision making, more targeted (and potentially reduced) chemical usage and potentially improved grower returns.

Botrytis grey mould (BGM), caused by *Botrytis cinerea* and *B. fabae*, separately or within a complex, substantially reduces grain legume yield during environmentally conducive seasons. Fast, accurate and cost-effective diagnosis and quantification of the causal pathogen(s) can lead to greater success in application of integrated disease management approaches to reduce yield and profit losses.

Biosensors that use functionalised magnetic gold nanoparticles for molecular target enrichment have been recently developed to detect cancer biomarkers with extreme specificity, sensitivity and accuracy (Islam *et al.*, 2018). In this project we have partnered with experts from the Queensland Micro- and Nanotechnology Centre at Griffith University to adopt this diagnostics approach for the detection of plant pathogens, using *Botrytis spp.* as a case study.

To achieve this, biotinylated capture probes were developed based on the MRR1 and NEP1 genes *B. cinerea* and *B. fabae*, respectively. The probes were assessed for their specificity and sensitivity to detect the pathogens from field collected faba bean leaf samples using a functionalised magnetic gold nanoparticles biosensor assay (Bilkiss *et al.*, 2020).

Sampling of faba bean leaf samples was performed at four field sites in south-eastern South Australia (Millicent, Bool Lagoon, Frances and Mundulla) in October 2020 in a replicated manner. Foliar samples were collected at each site from symptomatic and asymptomatic tissues to provide robust quantifiable levels of target pathogens. Genomic DNA was extracted from five symptomatic and five asymptomatic samples from each site and was used for the biosensor assay.

The target DNA was directly adsorbed onto the electrode surface *via* gold-affinity interaction, and the amount of adsorbed DNA of each target species DNA was robustly and reproducibly quantified via chronocoulometric (CC) charge density change (Figure 1).

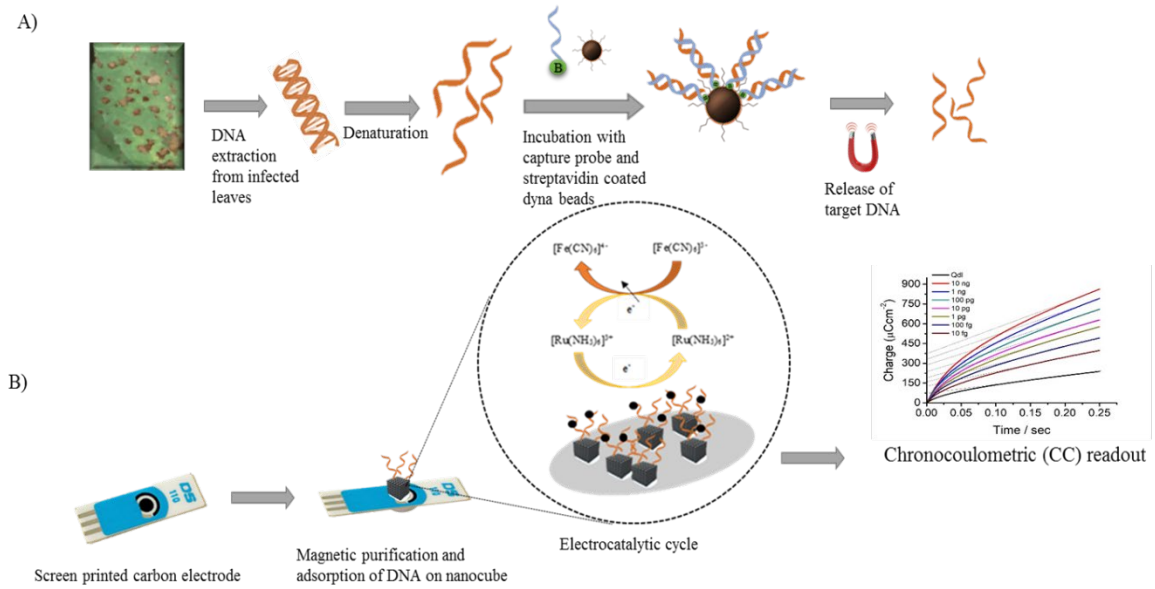

Figure 1. The two-step process for the electrochemical detection of *Botrytis* spp. from leaf samples. Where A = Magnetic isolation and purification of target *Botrytis* spp. DNA and B = Electrochemical detection and quantification of the adsorbed target ssDNA (Source: Marzia Bilkiss PhD thesis).

Based on the charge density changes observed, the capture probes were shown to be species-specific to either *B. cinerea* or *B. fabae*. The biosensor assay was able to detect single spores of *B. cinerea* and *B. fabae* from symptomatic and asymptomatic leaves, thus demonstrating its ability to detect and quantify the causative organisms prior to the visible appearance of the disease on plants and proving to be more sensitive than other published diagnostic methods for both species (Bilkiss *et al.,* 2019). This provides a diagnostic tool for *B. cinerea* and *B. fabae* that is highly sensitive, quantifiable, species-specific to each of *B. cinerea* or *B. fabae* and fast. The process from sample collection to result is ~45 mins and low cost at <$2 per sample.

The tools developed and protocols validated within this work are open for commercialization partnering through investor engagement. Further investment may be required to perform broader *in field* and *in industry* validation and to simplify sample preparation and DNA extraction to develop this prototype into a compact and portable device that provides an accurate and calibrated readout of pathogen loads.

References

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