Grower Summary

CP 195

Rapid genomics approaches for downy mildews on high-value crops

Final Report 2020

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Project leader:	Dr Thomas Wood, NIAB
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Thomas Wood

Programme Leader, Plant Pathology

NIAB

Signature T. Wood Date 17th March 2020

GROWER SUMMARY

Headlines

- New genomics resources have been generated for basil and column stock downy mildews. These can be used in fundamental and applied studies to improve knowledge and understanding to help manage these important pathogens.
- Additional genomes for downy mildew species affecting spinach and sugar beet will be completed imminently, providing new public resources for investigating these serious pathogens.
- The downy mildew genomes have been exploited to design new (or to refine existing) isothermal diagnostics (LAMP) assays that will enhance practical capability to reduce the impact of downy mildew through rapid detection.

Background

The aim of the project was to generate *de novo* hybrid genome assemblies based on Illumina short-read and Oxford Nanopore Technology long-read chemistries to provide new, novel genomics resources for *P. destructor* on chives*, P. lamii /* additional species on mint/sage*, P. belbahrii* on sweet basil and *P. petroselini* on parsley. Subsequently we proposed to conduct a preliminary phylogenetic investigation to establish relatedness between the four downy mildew (DM) species, design new isothermal amplification (LAMP) assays to provide diagnostic support for growers, and finally to ensure the new sequence data was made available through public repositories (Genbank).

The work program was organised to address the following objectives:

i: Obtain pure cultures of target DMs and extract DNA for sequencing

ii: Produce *de novo* draft genome assemblies of four target DM species

iii: Conduct comparative genomics and phylogenetic analyses to compare gene content, genome structure/organization

iv: Design three new isothermal assays for detecting target species

v: Sequence data and assay sequences deposited in public repository so the information can be accessed and utilized effectively

Due to the timing of the project occurring after the growing season, viable samples could only be obtained for *P. belbahrii* and not the three remaining target species. After discussion with the funders, three additional downy mildews, *Peronospora effusa* affecting spinach, *Peronospora farinosa* f. sp *betae* on sugarbeet and *Peronospora matthiolae* on the ornamental species column stock (*Matthiola incana)* were selected as alternatives for the new generation of genomics resources. The basis for the selection was that *P. effusa* and *P. farinosa* f. sp *betae* are closely related and affect economically important spinach and sugarbeet / table beet, respectively; sequencing them provided an opportunity to compare for differences that may confer host-specificity or specialisation, and differences in pathogenicity for *P. effusa* where other race-types have recently been sequenced[1]. *P. matthiolae* affects high-value, clonally propagated ornamental species, and is thought to be closely related to other economically important pathogenic downy mildews on Brassica species and model plant *Arabidopsis thaliana*.

Summary

Downy mildews (DMs) are obligate oomycete pathogens that cause serious economic losses in high value crops and ornamental species such as sweet basil, chives and parsley. Control of downy mildew disease is often confounded by a lack of genetic resistance, need to minimise residues in food, continued reduction in effective fungicides and issues with effective control due to fungicide resistance. Despite their economic importance, and an established knowledge base and accompanying genomics resources for a number of major and model oomycete plants pathogens (i.e. *P. infestans, P. ultimum* and *H. arabidopsidis),* there is still a paucity of relevant tools for many major crop downy mildew pathogens. In an effort to help address this, the project has utilised next-generation sequencing technologies to rapidly and accurately create novel genomics resources for key downy mildew pathogens affecting high value horticultural, arable and ornamental crops.

We have generated *de novo* genome assemblies for *P. belbahrii* (48 Mb) and *P. matthiolae* (112 Mb) which have begun to facilitate fundamental comparative genomics studies with other downy mildews, whilst also enabling us to design new LAMP diagnostics for specific detection of column stock downy mildew to aid growers. The new assay can effectively detect mildew spores or symptomatic *M. incana* leaf tissue.

A preliminary phylogenetic analysis was conducted by comparing newly generated (ITS Internal Transcribed Spacer region) sequences from *P. matthiolae* (column stock DM) with those from *P. belbahrii* (basil DM) and publicly available sequences for five other downy mildew species including those affecting mint, sage/rosemary and spinach. This analysis demonstrated that *P. matthiolae* is most closely related to *H. parasitica* (affecting brassicas, oilseed rape) and *H. arabidopsisdis* (pathogenic on *Arabidopsis thaliana)* whereas the remaining species cluster on a separate branch (node) indicating they are less closely related; *P. belbahrii* was more closely related to *P. salivia-officianalis* (Sage/Rosemary DM), than to *P. lamii* (mint DM) and *P. effusa* (Spinach DM).

Sequencing of *P. effusa* and *P. farinosa* f. sp *betae,* and additional samples of *P. belbahrii* and *P. matthiolae* was delayed by Covid-19 impacts on an external company. This has prevented the genome assembly and annotation, and the design of any additional LAMP assays for the Spinach and Sugarbeet downy mildew. The outstanding work and complete datasets shall be released as an Annex to the current report with links to relevant sequence repositories as soon as they become available.

All raw sequencing data will be made publicly available to ensure the greatest access for the research community.

Improved access to enhanced genomics resources generated during the project will greatly improve researchers' abilities to investigate differences in gene content and function underlying host-range, virulence and pathogenicity in important downy mildew species.

Financial Benefits

The new isothermal diagnostic developed for *P. matthiolae* has been shown to be effective for testing seed and plant material for DM infection. The assays could be utilized to screen for the presence of stock DM to help prevent new outbreaks and facilitate early disease detection to help reduce the impact on crops. The assays can be used freely for research purposes, but commercial testing can only be delivered through a licensed service or kit provider (which can require a minimum order before manufacture). LAMP assays kits are designed to be implemented at a low unit cost (<£100 including labor), with minimal operator training and without the requirement for specialized laboratory equipment. Extraction kits enabling preparation of crude templates have facilitated the use of LAMP assays for point of entry and near/in-field screening for plant pathogens. Semi-portable LAMP units retail at approx. £ 9 K whereas fully mobile LAMP units retail at approx. £14.5 k, making them accessible to outreach and extension service providers. Access to improved testing will have direct economic benefits by minimizing losses from unsaleable produce, by reducing crop-destruction to control disease through early detection, and also through a reduction in fungicide treatments.