PhD Studentship

Title **Application of small RNA to control downy mildew on pea plants**

Closing date: 3rd May

Interview date: 31st May

Supervisory team

Director of Studies:

Professor Mahmut Tör, School of Science and the Environment, University of Worcester [Link}

Supervisors:

Professor Yiguo Hong, School of Science and the Environment, University of Worcester [Link]

Dr Tom Wood, NIAB [Link]

Research Group: SERG [Link]

The Project

Applications are invited for a fully-funded, full-time PhD studentship for the project "**Application of small RNA to control downy mildew on pea plants**

Background

Introduction to oomycetes: Oomycetes comprise several hundred microbial species including biotrophic, necrotrophic and hemibiotrophic plant pathogens. They have superficial similarity to filamentous fungi but are distinct from them [1] in several aspects: the cell walls of oomycetes have been reported to be primarily ß-1-3 glucans and cellulose with little or no chitin, oomycetes' hyphae are coenocytic i.e. multinucleate with no division by septa, and their vegetative nuclei are in a diploid state [2]. Plant diseases caused by oomycete pathogens include seedling blights,

damping-off, root rots, foliar blights and downy mildews (DM). Collectively, oomycetes are estimated to cause tens of billions of annual losses in crops, due to their high evolutionary potential that enables host jumps, resistance to fungicides, and suppression or evasion of host *R*-genes. Some of the most economically devastating oomycete pathogens are *Phytophthora infestans* (tomato and potato late blight), *P. ramorum* (sudden oak death), *P. capsici* (stem and fruit rot of cucumber and pepper), *P. cinnamomi* (dieback in avocado and pineapple), *Plasmopora viticola* (grapevine DM), *P. halstedii* (sunflower DM), *Peronopsora vicia* (pea and broad bean DM), *Pythium ultimum* (damping off and root rot), *Bremia lactuca* (lettuce DM), and *Albugo candida* (white blister rust of crucifers) [3].

Peronospora vicia f.sp. pisi (PVP)-*pea system*. Pea *(Pisum sativum*) is one of the principal legume crops cultivated in the UK, with areas of 50 K Ha for marrowfat and blue peas, 34 K Ha for vining peas [4]. Peas and beans generate revenues in excess of £220 M UK trade in dried pulse and fresh vegetable sectors, with increasing quantities of the crop now utilised for human consumption. Despite their high value, pulse crops are difficult to grow compared to cereals and effective control of diseases can often limit productivity. This is particularly true of DM, caused by *Peronospora vicia f.sp. pisi* **(***PVP*), which can cause yield losses of up to 45-75 % in pea [4, 5].

***Methods to assay effector function* in planta.** One of the motivations for this proposal is that *PVP* and other obligate oomycetes are not amenable to genetic transformation, thus hindering genetic analysis. Several groups including ours have relied on alternative approaches to assay effector function *in planta* including: a) co-bombardment assays into plant cells using the *GUS* gene to indicate avirulence activity [6, 7], b) effector delivery from bacteria using Type III secretion [6, 8], and c) creation of stably transformed plants expressing effector genes under control of plant promoters [3]. However, these methods stripped the effector gene away from the pathogen where the expression level of a gene may not be comparable to that in the native background.

Cross-kingdom RNA silencing. Noncoding sRNAs (20-30 nucleotides, nt) are involved in the regulation of gene expression and defence in eukaryotes [9]. Different types of RNAs such as double stranded RNA (dsRNA) and small interfering RNA (siRNA) can trigger homologous RNA degradation or inhibit mRNA translation [10]. This process is known as RNA silencing, which plays a significant role in various biological processes including innate immunity [11] and development [12]. In plant-microbe interactions, plants and microbes can exchange RNA molecules, which then integrate into RNA silencing machinery in reciprocal recipient cells [13]

Movement of sRNAs from plants to pathogens has been explored using the host-induced gene silencing (HIGS) technique where the sRNAs are generally made from dsRNA in transgenic plants using *Agrobacterium* or virus delivery systems. HIGS has been successfully used to suppress essential genes in a few pathosystems including barley and wheat–*Blumeria* [14] and lettuce–*Bremia* [15]. Another approach for gene silencing in plants is based on exogenous application of sRNAs directly onto plants (referred to as spray-induced gene silencing, SIGS) [16]. This approach avoids the need to develop transgenic plants [17].

Aims and Objectives

We hypothesize that the sRNA method can identify genes that are involved in pathogenicity and development, and further the identification of targets for disease control.

The overall **aim of this proposal** is to use an sRNA approach to increase our understanding of plant – biotrophic oomycete microbe interactions and find target genes to control the pathogen. **We will use *PVP*-pea system with a sRNA-based genetic screen to identify genes and develop a method to target them for disease control.**

Objective 1: Optimize sRNA-mediated silencing in *PVP*.

Objective 2: Generate gene-specific sRNAs for highly regulated genes in spores, during germination, mycelial development and sporulation.

Objective 3: Identify genes showing a phenotype upon silencing.

Objective 4: Develop and optimize SIGS for disease control.

Indicative methodology

Student will have research training in Plant pathology methods including inoculations, maintaining isolates, spore germination assays, and plant tissue staining; Molecular biology techniques such as DNA and RNA isolation, RNA-seq, PCR, gene cloning and expression; bioinformatics tools including including sequence analyisis, data mining, phylogenetic tree construction, prediction of protein structure and function.

Student will have opportunity to work with different groups and laboratories. Supervisors have extensive experience in supervising students and collaborated and published joint papers before. Results obtained from this work will be published in internationally well- known journals and will be presented at international scientific meetings.

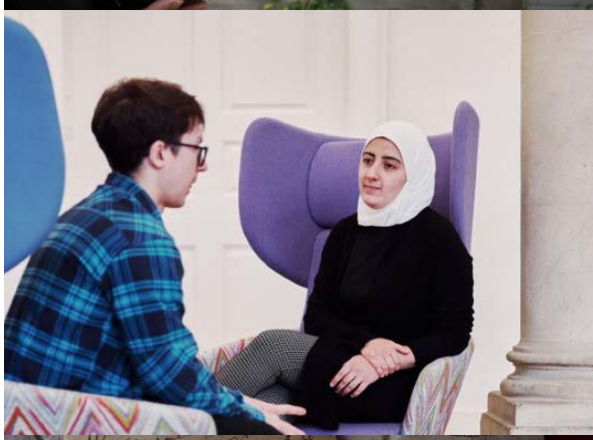
References: 1) Kamoun et al. (2015) MPP **16:** 413-434**; 2) Bilir et al.** (2019) MPP **20:** 1523-1534**; 3) Woods-Tör et al.** (2018) Front. Plant Sci. **9:**265; **4) Chang et al.** (2013) Journal of Crop Prot. **46:** 23-28; **5) Stegmark** (1994) Agronomie, EDP Sciences, **14**: 641-647; **6) Bailey et al.** (2011) MPMI **24**: 827–838**; 7) Allen et al.** (2004) Science **306:** 1957-60; **8) Fabro et al.** (2011) PLoS Pathog **7:** e1002348; **9) Qin et al.** (2017) Plant Physiol. **174**: 1067–1081; **10) Nejat, N. and Mantri, N.** (2018) *Critical Reviews in Biotechnology* **38**, 93–105; **11) Deng et al.** (2018) *PLoS Pathog* **14**, e1006756–22; **12) Li et al.** (2017) *Plant J.* **90**: 654–670; **13) Bilir et al.** (2022) *Front. Plant Sci.***13**: 951097; **14) Nowara et al**. (2010) Plant Cell **22:** 3130–3141; **15) Govindarajulu et al.** (2015) Plant Biotechnol. J.**13**: 875–883; **16) Koch et al.** (2013) *PLoS Pathog.* **12**: e100590; **17) Wang and Jin (2017)** Trends in Microbiol. **25:** 4–6.

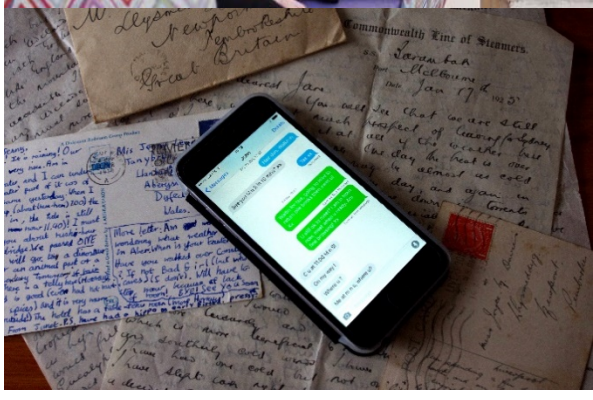
Details of the studentship

The studentship is offered for a 4-year period on a full-time basis. The studentship is campus based. During the period of your studentship you will receive the following:

- a tax-free bursary of £17,668 for 3 years
- a fee-waiver for 4 years (expectation that full time students complete in 3 years. If student enters year 4, bursary stops but fees waived)
- a budget to support your direct project costs including dissemination costs
- a laptop and other IT equipment and software as appropriate to the project
- use of the Research School facilities
- Spending time at NIAB and breeding companies and other research laboratories.

You will be expected to play an active role in the life of both the Research School and of your academic School. You will be given opportunities to gain experience in learning and teaching within the School under the guidance of your Director of Studies.





Application Process

To begin the application process for this studentship please go to http://www.worcester.ac.uk/researchstudentships and click 'apply now' next to the project you wish to apply for.
It is expected that applicants will have the following qualifications:

- A Masters in the area of molecular plant pathology, molecular plant-microbe interactions or molecular plant biology or equivalent professional experience.
- A First or Upper Second Honours Degree

It is also expected that applicants will be able to demonstrate the following:

- A sound understanding of and interest in both the project and the wider subject area
- Experience of relevant research methods and skills
- Ability to contribute to the research design of the project
- Proficiency in oral and written English
- Proficiency in IT relevant to the project, knowledge on how to use bioinformatic tools are desired.
- Ability to organise and meet deadlines
- Good interpersonal skills
- Ability to work independently
- Ability to work as part of a team
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The Interview

The interview will provisionally be held on 31st May in person or on Teams. Shortlisted candidates will be given at least 7 day's-notice of interview. In advance of interview, shortlisted candidates will be asked to submit a sample of their written work (such as a publication or a dissertation). Alongside the interview,

shortlisted candidates will also be asked to give a 10-minute presentation on their MSc work.

Research at the University of Worcester

Research at the University of Worcester has grown significantly over the last 10 years. The outcomes of the Research Excellence Framework 2014 (REF 2014) showed that Worcester was the most improved University in the UK based on Research Fortnight's "Research Power" measure. The University's continued progress was shown in the outcomes of REF 2021 which demonstrated that both the scale and quality of our research has further increased, with over 40% of our research recognised as world-leading or internationally excellent.

The University has been successful in winning funding from a wide range of major funders: Research Councils such as AHRC, BBSRC, ESRC and NERC; major charities such as the Leverhulme Trust, the Alzheimer's Society and the British Academy; health-research funders such as the NIHR, the Department of Health and local NHS Trusts; European funding through Horizon 2020 and Erasmus+; and funding from local, national and global businesses.

The University is focused on research which addresses real world challenges and provides solutions to these challenges:

- Human Health and Wellbeing
- Sustainable Futures
- Digital Innovation
- Culture, Identity and Social Exclusion
- Professional Education

The University continues to provide a robust infrastructure for research. It has a well-established Research School which houses its growing research student body and which provides a comprehensive programme of researcher development for staff and students. It has a well-established Research Office, responsible for research funding, governance and strategy. The University is committed to further developing its research profile, through a strategic approach to its support for and investment in research. Its fully-funded studentships are part of this investment.

Research School

The Research School is a focal point for all our research students. It provides:

- day-to-day support for our students, both administrative and practical, through our dedicated team
- a Research Student Study Space with both PCs and laptop docking station

- a comprehensive Researcher Development Programme for students and their supervisors
- a programme of student-led conferences and seminars

School of Science and the Environment

Science and the Environment at Worcester encompasses subject areas including Archaeology, Biology (including Animal Biology, Biochemistry, and Human Biology), Biomedical Science, Environmental Management and Sustainability, Forensic and Applied Biology, Geography (including Human Geography and Physical Geography), Human Nutrition, and Medical Sciences.

We offer top quality degrees in a friendly and supportive environment, using modern approaches to learning and teaching.

From sustainability to crime scene analysis, our world-leading research focuses on today's key challenges. Working in collaboration with different disciplines and with other universities, private industry and the public sector, our research is a means to achieve real-life benefits.

Our mission is to ensure science is accessible and that our research is relevant to society.

Research and Knowledge Exchange within the School of Science and Environment is at the core of what we do, ensuring society benefits from our translational and applied research, and that our undergraduate students benefit from research informed teaching. The Degree Courses we deliver and the knowledge exchange we undertake therefore embody the range and reach of our impactful research.

Research within the school focuses on 'Sustainable Futures' and 'Human Health and Wellbeing'; Areas of Challenge outlined in the University's Research and Knowledge Exchange Strategy (2020-2025). We also actively engage in other research areas and encourage blue skies thinking.

To ensure the continued delivery of high quality and impactful research at the University of Worcester we have invested significantly over the last decade to enhance our research facilities. Find out more here.

Widening Participation

As part of its mission statement the University is committed to widening participation for its higher degrees. Although most candidates will have an undergraduate and/or a Masters degree, the University is happy to accept applications from candidates with relevant professional qualifications and work related experience.

For further information or an informal discussion on this project, please contact Professor Mahmut Tör (Director of Studies) via email at m.tor@worc.ac.uk

Applications can be made here