Identification of avirulent alleles of pathogen effector genes in UK *Leptosphaeria maculans* (phoma stem canker) populations

Phoma stem canker is an important disease of oilseed rape (*Brassica napus*) worldwide and is often caused by fungal pathogen *Leptosphaeria maculans.* This disease can cause yield losses of up to 50% if it is not managed properly. Use of host resistance is an effective and environmentally friendly way to control this disease. Resistance against *L. maculans* relies on major resistance (*R*) gene-mediated resistance and quantitative resistance. *R* gene-mediated resistance to *L. maculans* is associated with a gene-for-gene interaction in which the product of a pathogen *Avr* gene (effector gene) is recognised by the product of a host *R* gene so that the pathogen is unable to infect the host (resistant reaction). *R* gene-mediated resistance to *L. maculans* is single-gene, race-specific resistance that is effective in protecting plants only if the corresponding avirulent allele of the *Avr* gene is predominant in the local *L. maculans* population. Therefore, there is a need to monitor pathogen population for effective use of host resistance.

Part of my project involved investigating avirulent and virulent alleles in *L. maculans* isolates. A set of eight differential cultivars with known *R* genes were used to detect the corresponding *Avr* genes in *L. maculans* isolates. In total, 36 *L. maculans* isolates were cultured onto V8 medium amended with 20 units/ml penicillin and 40 units/ml streptomycin and incubated at 20°C with 12h light/12h darkness to encourage pycnidial production. After 14 days of incubation, mature conidia were produced and conidial suspensions were made. The conidial concentrations were adjusted to 1×10^7 spores/ml using a haemocytometer. Cotyledons of 14-day old seedlings were wounded using a sterile needle and a 10 μl drop of conidial suspension was placed over the wound. At 14 – 17 days after inoculation, disease symptoms on each plant were scored on a 0-9 scale (0, no darkening around the wound; 9, large pale grey lesion with pycnidia). Mean scores of < 3.5 were assigned as avirulent phenotypes (i.e. the isolate is avirulent against the corresponding *Rlm* gene), while scores > 3.5 were assigned as virulent phenotypes. Results showed that 100% of the isolates were avirulent against *Rlm7*, suggesting that the resistance gene *Rlm7* is effective for control phoma stem canker in the UK. By contrast, all the isolates were virulent against resistance genes *Rlm3* and *Rlm9.* Only 6% and 28% of the isolates were avirulent against *Rlm1* and *Rlm4*, respectively. This suggests that the resistance mediated by *Rlm1* and *Rlm4* is partly ineffective.

The second part of my project was to assess the severity of phoma stem canker in field trials. I learned how to recognise phoma stem canker symptoms and how to score stem canker severity. I was involved in sampling and scoring of three field trials. From each plot, 20 stems were sampled and phoma canker severity was examined by scoring how far the pathogen-associated necrosis had spread across the cross-section of the stem. Stem tissues were scraped into Eppendorf tubes and freeze-dried for DNA extraction and pathogen identification. From the results of field experiments, I understand that cultivars with different resistance genes showed differences in stem canker severity. In general, cultivars with the resistance gene *Rlm7* had less severe stem canker than cultivars with other resistance genes.

Winnie in the field sampling plants for stem canker assessment

In addition to the planned work in my project, I had the privilege of being introduced to more molecular and microbiological techniques as part of further work which involved harvesting mycelium, DNA extraction from 281 stem samples and mycelium using the DNA Plantmite kit. From this work, I have improved my microbiological skills, especially aseptic techniques, enabling me to produce results with increased accuracy.

Through this 10 week period of research, I have gained a clearer idea of the sophistication of academic research and obtained great insights into the use of modern, advanced technology in molecular biology and plant pathology, which will aid me in my one year sandwich placement and future career. Having had the chance to work with a variety of postgraduate students, my interest in pursuing a masters or PhD has heightened. I would like to extend a heartfelt thanks to BSPP for the funding and my supervisor Dr Yongju Huang for the support and encouragement during this research project.

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