

Molecular mechanisms of mutation to virulence in *Leptosphaeria maculans* populations in the UK

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INTRODUCTION

- Phoma stem canker disease of oilseed rape caused by the fungus *Leptosphaeria maculans* is often controlled by deploying race-specific *R* genes in the UK.
- However, *R* gene-mediated resistance is often rendered ineffective in 2-3 years due to *L. maculans* population changes from avirulent to virulent.
- Due to this rapid adaptation of *L. maculans* populations to selection, they give rise to new virulent races through mutation or deletion of effector genes.
- In this study, the regional distribution of the *L. maculans* races in the UK and the molecular mechanisms of mutation to virulence are being investigated to provide effective management of the disease.

MATERIALS & METHODS

- Winter oilseed rape field experiments at different sites in two seasons.
- Single pycnidial isolates obtained from leaf lesions on cultivar Drakkar (susceptible, trap crop) and other cultivars (with *Rlm7* resistance gene)
- Changes in frequencies of avirulent *AvrLm1*, *AvrLm4* or *AvrLm7* alleles in *L. maculans* populations at different sites investigated by inoculation of conidial suspensions on cotyledons of a differential set of cultivars.

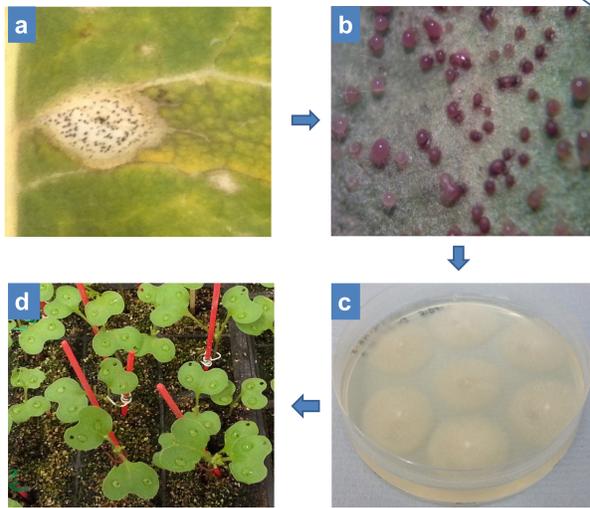


Figure 1: Procedure for single pycnidial isolation to obtain isolates of *Leptosphaeria* species. Leaf lesions (a) incubated in a Petri dish lined with filter paper. After 2-3 days of incubation the cirrhus produced from a pycnidium (b) was mixed with sterile water and pipetted on a PDA plate. *L. maculans* cultures observed after 4-5 days incubation (c). Conidial suspensions made from the isolates inoculated onto a differential set of cultivars (d).

L. maculans isolates virulent against *Rlm1*, *Rlm4* or *Rlm7* identified by cotyledon test method

Subcultured onto V8 agar media plates with cellulose discs

Mycelium harvested

DNA extracted

Corresponding regions of *L. maculans* *Avr* genes amplified in these isolates

Sequencing

RESULTS

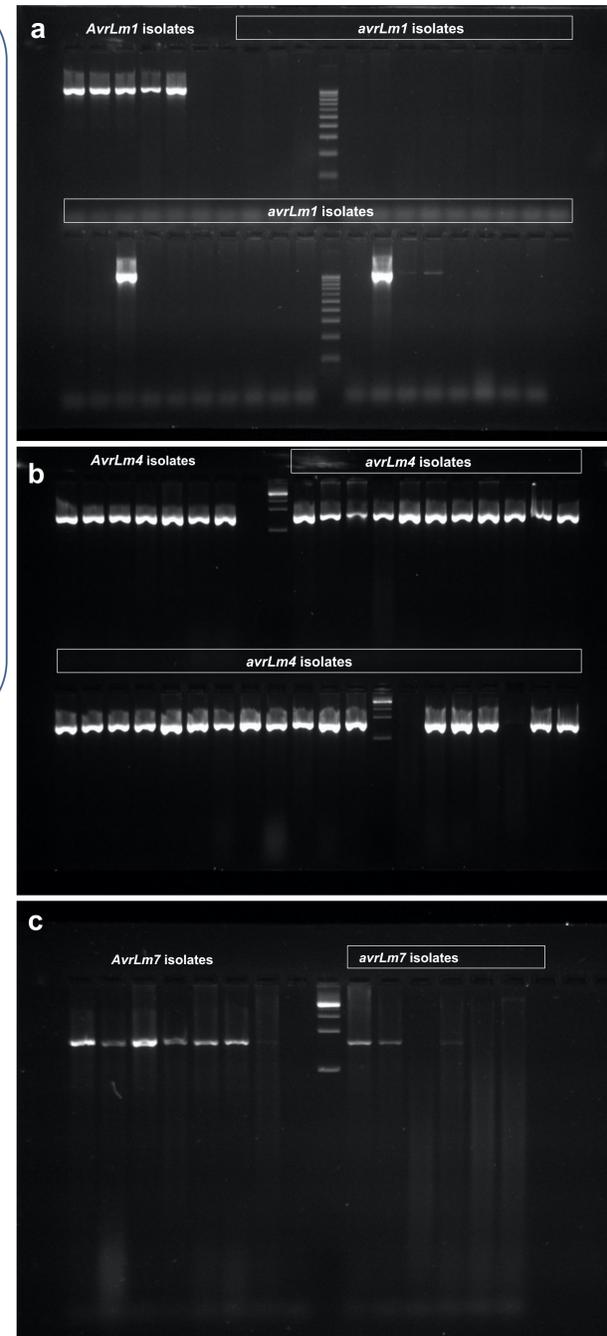


Figure 3: Gel images showing amplification of *AvrLm1* (a) *AvrLm4-7* (b, c) regions in *L. maculans* isolates containing avirulent and virulent alleles against *Rlm1* (a), *Rlm4* (b) and *Rlm7* (c) resistance genes.

- No amplification with the *AvrLm1* external primers observed in 86% of isolates carrying the virulent alleles of *AvrLm1*, indication of whole gene deletion.
- The other 13% were sequenced to investigate events leading to virulence in *AvrLm1*, found to be point mutations.
- Whole gene deletion was observed in 6% or 50% of isolates carrying the virulent alleles of *AvrLm4* or *AvrLm7*, respectively.

RESULTS

- Differences in frequencies of avirulent *AvrLm1* and *AvrLm4* alleles between sites and cropping seasons.
- All isolates from different sites were avirulent against *Rlm7* in the 2015/2016 season.
- In the 2016/2017 season, 6.8% of isolates were virulent towards *Rlm7*.

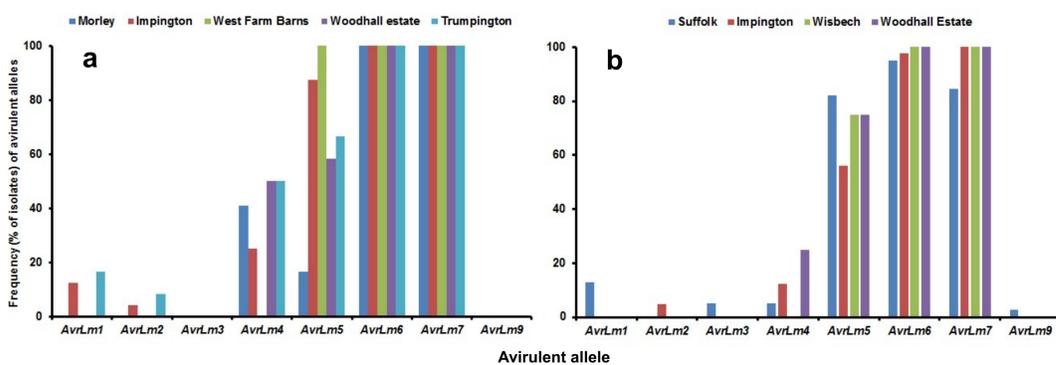


Figure 2: Mean frequencies (%) of avirulent alleles in *Leptosphaeria maculans* populations at different sites in the UK in 2015/2016 (a) and 2016/2017 (b) cropping seasons.

DISCUSSION

- The predominance of the avirulent *AvrLm7* allele in UK *L. maculans* populations suggests that the corresponding *Rlm7* resistance gene is still effective. There is a need to continue monitoring of regional distribution of *L. maculans* races in the UK for effective deployment of *R* genes.
- Whole gene deletion is likely the major molecular mechanism of mutation towards virulence in *AvrLm1* gene, and some isolates carried the virulent alleles of *AvrLm4* or *AvrLm7*. However, there is a need to investigate other molecular mechanisms of virulence in *AvrLm4* or *AvrLm7* genes in the UK.
- New information about molecular mechanisms of mutation to virulence in *L. maculans* can be used to optimise the use of novel resistance so that it does not break down quickly. This can be used to develop new strategies to increase the durability of resistance against the phoma stem canker pathogen *L. maculans*.

ACKNOWLEDGEMENTS

