



Inter-relationships between *Bipolaris sorokiniana* isolates involved in spot blotch, common root rot and black point in winter cereals

A dissertation submitted by

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Abstract

Foliar spot blotch (SB) and common root rot (CRR) are important cereal diseases caused by the fungal pathogen *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*), and the relationship between the causal agents responsible for the two diseases is poorly understood. This thesis investigated the population structure of *B. sorokiniana* in the Australian grain belt to determine the relationship between SB and CRR. We examined 126 single spore isolates of *B. sorokiniana* from basal and aerial tissue of wheat and barley, through amplified fragment length polymorphism analysis. Cluster analysis revealed two populations of the pathogen; one that consisted primarily of wheat CRR, and the other that consisted primarily of barley SB isolates. This indicated the isolates were possibly tissue and/or host specific. Genetic divergence among isolates according to geographic location was also evident. We further investigated the extent to which genetic differentiation occurs in Australian isolates during the infection process by the analysis of isolates from a single inoculated plant in the field, and inoculated wheat and barley genotypes. The question addressed was whether genetic divergence occurs among isolates during the infection process according to the host genotype. In the current investigation, genetic variation was evident among isolates from single lesions, even in controlled glasshouse conditions. Genetic variation was observed among isolates sampled from different genotypes after inoculation with the same original isolate. This indicates that although the haploid pathogen is considered to reproduce by asexual means only, genetic differentiation does occur in isolates at low levels even with a single infection event.

We determined whether the virulence of *B. sorokiniana* could be predicted based on the genetic variability identified in the AFLP analyses. A selection from each host and tissue source (barley SB, wheat SB, barley CRR and wheat CRR) was selected for phenotypic testing on leaf tissue, then on root tissue of wheat and barley differential sets. The spot blotch phenotype testing revealed both host and tissue specificity of isolates. The phenotypic common root rot assessment suggested the possibility of tissue specificity, but the screening method requires further development.

The most effective means for control of fungal pathogens is by use of genetic resistance when available. Two major quantitative trait loci (QTL) associated with spot blotch resistance have been identified on barley chromosomes 7H and 3H in a number of mapping studies. The QTL on chromosome 7H has been fine mapped, however fine mapping of the QTL located on chromosome 3H has proven difficult due to a low level of polymorphism in the region. Fine mapping of the 3H region is necessary to identify new markers closely linked to the QTL of interest for use in breeding programmes, and also potential identification of candidate genes. This was attempted in an ND24260 x Flagship doubled haploid (DH) population of 334 lines, which had previously been mapped using DArT markers. Seven PCR-based markers based on EST sequence data from an unrelated mapping study were screened across the population, as well as eight PCR-based markers from a second mapping study, which were designed based on single nucleotide polymorphism (SNP) data. Identification of markers more closely linked to the resistance region was unsuccessful, as none of the fifteen markers were found to be polymorphic. However

high resolution melt analysis may be used in future as a more thorough method for identifying polymorphism.

A third disease historically attributed to infection by *B. sorokiniana* is the grain defect black point, which occurs in both wheat and barley. The defect is undesirable in barley as it is believed to be associated with fungal contamination detrimental to beer production. Black point in wheat is well-studied, but little has been done to determine the cause of the defect in barley. Additionally, many of the studies investigating black point of wheat have produced contradictory results. Although this symptom has been attributed to *B. sorokiniana* as well as other pathogens, this association has been questioned in two aspects. On one hand, symptoms may appear even in the absence of detectable levels of the pathogen, and in many instances *B. sorokiniana*-colonised wheat grains did not exhibit BP symptoms. In this work we examined whether BP affected and BP unaffected barley grains exhibited different levels of *B. sorokiniana* colonisation by comparing the amount of DNA recovered from barley grains as a measure of fungal biomass. This is the first time this issue has been addressed in this manner. There was no compelling evidence that colonisation of seeds by *B. sorokiniana* is a sufficient condition for the production of BP symptoms under favourable environmental conditions.

Certification of Dissertation

I certify that the ideas, experimental work, results, analyses, and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award, except where otherwise acknowledged.

Signature of Candidate

Date

ENDORSEMENT

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- Appendix 6.0 (Excel File):** Results for new primers tested for potential use as species-specific primers on several isolates of *B. sorokiniana* and other fungal species
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