Identification and characterisation of a genetic marker for Ascochyta lentis virulence in lentils

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Ascochyta lentis is a necrotrophic fungal pathogen that causes ascochyta blight (AB) in lentil. This pathogen infects all above ground parts of the plant and can reduce grain quality and yield. Our research has focused on the discovery of virulence factors in *A. lentis*, and host specificity in two Australian lentil cultivars, Nipper and PBA Hurricane XT. Recent studies have found many isolates in the *A. lentis* population in Australia that differ in virulence towards these cultivars.

To identify the gene/s associated with virulence, the response of *A. lentis* progeny from a bi-parental population generated from the cross between *Al*Kewell (virulent on PBA Hurricane XT; avirulent on Nipper) and P94-24 (virulent on Nipper; avirulent on PBA Hurricane XT) was evaluated. QTLs in 96 progeny isolates were mapped based on disease response on PBA Hurricane XT and Nipper, and genotyping using ddRADseq. This analysis resulted in the identification of a major QTL, in a region containing a gene with the hallmarks of an effector and was considered the most likely candidate effector for determining virulence. Further investigation of this candidate gene showed major allelic differences between *Al*Kewell and P94-24, with 23 amino acid differences in protein sequence between the two strains for this effector candidate. The two protein forms were designated as K-type (*Al*Kewell) and P-type (P94-24). To validate this as a putative marker, a set of primers were developed to differentiate the two genes using PCR. All 96 bi-parental progeny and a set of *A. lentis* isolates from the SARDI *A. lentis* collection were evaluated and results confirmed that isolates virulent on PBA Hurricane XT or Nipper could be predicted using the PCR marker based on nucleotide polymorphism.

To characterise the proteins for effector activity, agroinfiltration on PBA Hurricane XT and Nipper was performed. Necrosis was observed on PBA Hurricane XT infiltrated with the P-type protein while the K-type protein did not elicit any response from the lentil cultivars tested. Sensitivity of PBA Hurricane XT to the P-type effector from P94-24, which is avirulent on this cultivar, suggests that the P-type effector is an avirulence (Avr) effector.

In this study, we have identified a virulence marker that can be used as a molecular tool to assess *A. lentis* isolates and populations for virulence, while the putative effector can be developed as a valuable resource for effector-guided breeding for AB resistance. It is proposed that two pathotypes in the Australian *A. lentis* population can be designated based on virulence and avirulence responses in these key Australian lentil varieties, and the presence of the K- or P-type effector. A better understanding of the molecular mechanisms of *A. lentis* pathogenesis and the nature by which the pathogen infects its host is key for the development of novel and durable strategies to manage AB in lentil.