

Genome sequencing and characterisation of diverse ascochyta blight species isolated from legumes

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Ascochyta blight disease in legume species is caused by a number of closely related ascomycete fungal pathogens from the class Dothideomycetes. *Ascochyta lentis* is a host specific pathogen of lentil and *A. fabae* specifically infects faba beans. *Ascochyta rabiei* (Syn: *Phoma rabiei*) is the sole ascochyta pathogen of chickpeas. In field peas ascochyta blight, or pea blackspot as the disease is commonly known, can be caused by a number of pathogens from several genera, including *Peyronellaea pinodes*, *Peyronellaea pinodella*, *Phoma koolunga* and *Ascochyta pisi*. *P. pinodes* and *P. koolunga* are the most serious and most commonly found organisms in Australian field pea blackspot epidemics. Other ascochyta species from legumes have been reported but there is a lack of taxonomic description and genomic sequence information available with which to identify fungi that may be present on legume crops and related species.

In this study we have isolated ascochyta blight fungi from field peas, from a *Vicia villosa* crop, and from *Vicia sativa* and *Lathyrus tingitanus* weeds along roadsides in Western Australia. Isolates have been characterised by growth on plates, and by spore size and morphology. We have assessed the capacity of ascochyta from lathyrus and vetch to cause disease symptoms on field peas and lentils and also in the host species from which they were isolated. One representative cultivar for each of the host species was used in seedling assays with the collected isolates. To classify the isolates we have carried out Illumina genome sequencing and have constructed a phylogenetic tree of these ascochyta blight species. Species include a novel *Phoma* species, *Ascochyta viciae-villosae*, *Ascochyta viciae*, *Phoma koolunga* and *P. pinodes* from field peas, and *Peyronellaea lethalis* from lathyrus. Genome assembly sizes ranged from 33 Mb to 48 Mb and can be accessed from the NCBI BioProject archive. Additional genomic sequences for *A. lentis*, *A. rabiei* and *A. fabae* were obtained from other CCDM projects. A Neighbour-Joining tree shows three clades of species. Clade 1: ‘*Ascochyta*’ includes *A. lentis*, *A. fabae*, *A. viciae* and *A. viciae-villosae*. Clade 2: ‘*Phoma*’ includes *A. rabiei*, *P. koolunga* and an unknown *Phoma* sp. from vetch. Clade 3: ‘*Peyronellaea*’ includes *P. pinodes*, *P. pinodella* and *P. lethalis*. Additional characterisation of the species described in the study was achieved by microscopic evaluation of diseased leaves from *in vitro* seedling infections. We observed two main types of pathogen-host interactions; a compatible reaction in which extensive mycelial colonisation of host tissues and brown, spreading necrosis were evident, and what appeared to be an incompatible reaction with red-brown discoloration of discrete host cells at the site of penetration attempts and restricted hyphal development.

Among the closely related *Ascochyta*, *Phoma* and *Peyronellaea* species there is a mix of host-specific and generalist fungi. The host response and susceptibility towards different pathogen species can be measured in traditional seedling assays but further details of the plant response can be revealed through microscopy. Genome sequences will assist future studies to identify virulence genes and effectors that underlie the molecular mechanisms that determine host specificity.