ABSTRACT

By

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The plant pathogen Fusarium graminearum is a cosmopolitan filamentous ascomycete fungus and the primary etiological species of Fusarium Head Blight (FHB) of wheat in North America, infecting the heads of the plants and contaminating the grain with mycotoxins. Direct infection of the heads is needed for development of FHB. An important source of inoculum for FHB is the aerial dispersal of forcibly discharged ascospores (sexual spores). Previous Trail lab results indicated that calcium signaling was involved in ascospores discharge, stimulating the examination of the role calcium channels and possible targets of calcium signaling play in the growth and development of F. graminearum.

In fungi, the high affinity calcium uptake system (HACS), consisting of the calcium channel Cch1 and the calcium channel/regulatory protein Mid1, and the low affinity calcium uptake system (LACS), for which only the transmembrane protein Fig1 is a known component, have been characterized. Previously, deletion of CCH1 resulted in significantly reduced vegetative growth with a more fluffy appearance than wild-type, macroconidia (asexual spores) production, and ascospore discharge, and a few abnormally developed ascospores. Here, deletion of MID1 resulted in phenotypes similar to the Δcch1 mutant but with a much higher rate of abnormal ascospore development. Results were similar but slightly more severe in a Δmid1 Δcch1 double mutant. Exogenous calcium partially rescued the phenotypes of all strains, suggesting an alternate route for calcium entry. As FIG1 is involved in low affinity calcium uptake in other fungi, the role of Fig1 in the calcium uptake was explored. As with the HACS mutants, loss of Fig1 resulted in significantly slowed vegetative growth rate, but with mycelium

appressed to the surface of the medium rather than fluffy, and reduced conidiation. Following induction of sexual development, Δfig1 mutants did not produce perithecia, and a microscopic examination led to the finding that sexual development halted after the production of perithecium initials. The LACS and HACS double and triple mutants' phenotypes were similar but more severe than the Δfig1 mutants and included reduced pathogenicity on wheat. Addition of calcium did not lead to any detectable phenotypic rescue. As perithecia did not develop, the function of FIG1 during ascus and ascospore development could not be determined.

To facilitate the examination of genes essential for sexual development, such as FIG1, a doxycycline inducible RNA interference (RNAi) system was adopted for use in F. graminearum. Vectors were constructed targeting a PKS3, a polyketide synthase gene needed to make a dark perithecium pigment, and MYO2, a myosin gene. Myosins are calcium regulated molecular motors that move along actin filaments, transporting membranous structures. Induction of RNAi during sexual development of strains with the MYO2 construct resulted in severely slowed ascus growth and altered vesicle trafficking and ascospore delimitation. A transformant containing the PKS3 construct was found to lack perithecium pigment without doxycycline induction, suggesting that either transcriptional read-through from upstream sequences or the influence of an enhancer drove expression of the RNAi construct.

These results indicate the importance of calcium uptake and signaling in the growth and development of F. graminearum and provide impetus for investigating the roles of other calcium signaling components. These results also show that doxycycline inducible RNAi can be effectively utilized in F. graminearum to investigate the function of genes involved in sexual development.