Hordeeae *Epichloë* endophytes and the formation of synthetic symbioses with cereal grasses

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Abstract

This thesis examined two classes of organism that live in symbiosis, grasses and fungi. Specifically it dealt with grasses of the tribe Hordeeae (Triticeae) in the subfamily Pooideae and *Epichloë* (*Epichloë* /*Neotyphodium*) fungi of family Clavicipitaceae.

Epichloë endophytes, particularly asexual forms, have important roles in pastoral agricultural systems in the Americas, Australia and New Zealand. Selected strains add value to grass-based forage systems by providing both biotic and abiotic stress resistance. Cereal grasses such as wheat, barley and rye are important to human and animal nutrition and indeed to the foundation and maintenance of Western civilisation. Modern Hordeeae cereal grasses such as wheat, barley and rye do not host *Epichloë* endophytes, although grasses of some genera within the tribe, such as *Elymus* and *Hordeum*, do so. Both organism classes, *Epichloë* endophytes and cereal grasses, are of great importance in their own contexts; this research examined the possibility of bringing them together in symbiosis with the ultimate goal of improving cereal production systems.

In this study, a screen of wild *Elymus* and *Hordeum* grasses in Gansu Province, China showed high levels of *Epichloë* infection. A diverse range of fungal genotypes was identified using SSR markers, and chemical screening revealed the production of alkaloid metabolites consistent with the range seen in *Epichloë*-infected pasture grasses of tribe Poae. Importantly, strains were identified that did not produce the mammalian toxins ergovaline or Lolitrem B, although less toxic intermediates such as the indole diterpene paspaline and ergot clavine alkaloids were identified. In addition, strains were identified that produced the insect deterrents/toxins peramine and loline.

Inoculation studies performed in this study demonstrated that cereal grasses could be successfully infected by artificial means using cultured *Epichloë* fungus, although infected plants generally had poor morphological phenotypes. While alkaloid production of synthetic associations was qualitatively the same as that of native associations, relative quantitative differences were observed between native *Elymus* and synthetic rye. Differences in infection frequencies and host phenotypes were observed between *Epichloë* strains. The choice of *Epichloë* strain used for inoculation profoundly affected the outcome of the symbiosis, ranging from no infection to stunted plants that died prematurely, infected dwarf plants through to normal phenotype plants. Host genotype was also observed to impact infection frequency and phenotype. Family differences in infection phenotype in outcrossing rye suggested a host genetic basis for the observed variation, while population differences in selfing rye indicated that genetics may not have been the sole driver. Consistent phenotypes were observed from the self-fertilizing cereals wheat and barley but, unlike rye, these were not amenable to recurrent selection. Finally, the infection of wheat alien addition/substitution lines showed that there is potential to select wheat-based germplasm with improved phenotypes. Thus, both *Epichloë* genotype and host genotype underpinned successful compatible symbiosis.

This work demonstrated that cereal grasses could be synthetically infected with *Epichloë* and that agriculturally useful metabolites were produced by these symbioses. The manifestation of infection phenotypes highlighted the necessity for careful selection of germplasm for inoculation and a need for selection and breeding of cereal grasses after infection.

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'One thing I have learned in a long life: that all our science, measured against reality, is primitive and childlike -- and yet it is the most precious thing we have.'
Albert Einstein

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