

The novel host barley produced a rapid defence response to *Epichloë* endophyte by elucidating gene regulation

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Epichloë fungal endophytes can have a crucial role in plant growth and development. These fungi are recognised as beneficial microorganisms that form symbiotic relationships with grasses and provide several advantages to the host, such as improving stress resistance and promoting growth. The advantageous traits identified in natural ecosystems can, and have been, exploited in managed ecosystems such as pastoral farming in countries including New Zealand, Australia and the Americas. In the current study, *Epichloë bromicola* strain WBE1, an endophyte isolated from *Hordeum brevisubulatum* (wild barley) was artificially inoculated into endophyte-free plants of *H. brevisubulatum* and *H. vulgare* (cultivated barley, novel host) by researchers at Lanzhou University, China, to study grass-endophyte compatibility. WBE1 successfully infected 54% of the endophyte-free plants (n=107) of wild barley and 10% of the cultivated barley plants (n=545). WBE1 colonisation enhanced the callus and lignin content in both grass hosts but also led to an increase in plant cell death. Cultivated barley showed more resistance to endophytic infection as its infection frequency was lower than wild barley. After 14 days, mycelial growth in both barley hosts was observed with the naked eye at the inoculation sites. The microscopy of endophyte infected leaf sheath tissue of wild barley showed that hyphae were branched and curved. In contrast, hyphae within leaf sheath tissues of cultivated barley were slightly curved and unbranched. Inoculation of both grass hosts by WBE1 induced the expression of early signalling molecules including

MAPK, which increased by 12 and 54% in cultivated barley compared with wild barley, 2- and 4-days post inoculation, respectively. Furthermore, O₂ increased in both wild and cultivated barley after inoculation by 11 and 19%, respectively; H₂O₂ increased in both wild and cultivated barley by 67 and 72%, respectively and NO content increased in both wild and cultivated barley by 21 and 18%, respectively. After inoculation, regulated genes related to the synthesis of secondary metabolites involved in phytopathogen detection were expressed earlier in cultivated barley compared with wild barley. NADP+1 oxidoreductase and alcohol dehydrogenase (NADP+) activities were down regulated in cultivated barley showing that the host was metabolically adjusted to environmental or physiological stress. The expression of signalling molecules and down-regulation of genes related to oxylipin biosynthesis, lipid oxidation, cellular response to environmental stimuli, oxidoreductase activity, and heme binding indicated that inoculation of cultivated barley by WBE1 triggered the host defence responses. This study investigated the gene transcription of an *Epichloë* endophyte strain across two different host plants, namely the original host and a novel host. The results showed that *Epichloë* can induce a defence response after inoculation, similar to that exhibited when being invaded by a phytopathogen and was expressed earlier in the novel host compared to the original host. The research results provide new ideas for further revealing the genetic foundations of the symbiotic relationship between *Epichloë* and their grass hosts.