

Decoding horizontal gene transfer mechanisms in *Epichloë*-grass symbiosis

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Epichloë endophytes establish mutualistic symbioses with host grasses, significantly enhancing host resistance to diverse biotic and abiotic stresses. Understanding molecular mechanisms of symbiosis and stress response regulation in *Epichloë*-host symbionts could provide us effective strategies to overcome the challenges posed by global climate change. Horizontal gene transfer (HGT) is a critical evolutionary mechanism for both fungi and plants to improve their survival, propagation and environmental stress adaptation. A glucanase gene in perennial ryegrass and a disease resistance gene (*Fhb7*) in wheat are speculated to have originally arrived from *Epichloë* endophytes based on the nucleotide sequence identity, suggesting that HGT may be a widespread event presented in *Epichloë*-plant symbionts. However, there is no conclusive evidence for the occurrence of HGT in *Epichloë*-plant symbionts, and the mechanism by which it occurs as well as its functional implications in symbionts remain unclear. *Achnatherum inebrians* belongs to the grass tribe *Triticeae* and is genetically close to the model grass *Brachypodium distachyon*, the symbiont of *A. inebrians* with which *Epichloë* exhibits remarkable stress tolerance. To obtain more definite evidence and reveal the mechanisms of HGT events in *Epichloë*-plant symbionts, we performed chromosome-level de novo genome assemblies of *A. inebrians* and *E. gansuensis* isolated from the symbiont through

a combined strategy integrating Illumina HiSeq sequencing, PacBio sequencing and Hi-C technology. The analysis results showed that the genome of *A. inebrians* is relatively small (~1.47 Gb) with low heterozygosity, which is conducive to the analysis of HGT events. Whole-genome resequencing of the *A. inebrians*-*E. gansuensis* symbiont generated over 50 Gb of sequencing data. Through the comparative genome analysis, three possible *E. gansuensis*-derived HGT reads were detected in the symbiotic *A. inebrians* genome, with the evidence of each read clearly flanked by definitive *A. inebrians* genome sequences. This finding strongly supports the occurrence of HGT events during the symbiotic interaction of *E. gansuensis* with *A. inebrians*. However, only three HGT-derived reads were identified in a quite massive sequencing data, suggesting that these HGT events may not be heritable. Intriguingly, functional annotation based on homology comparison revealed that the genes in HGT reads were enriched in biological processes related to DNA recombination and repair, transposon integration, and transcriptional regulation. Our results provide valuable genomic resources and a robust foundation for further dissection of symbiotic regulatory mechanisms mediated by HGT events between *Epichloë* endophytes and host grasses.