

Multi-omics elucidation of the phytohormone biosynthetic pathways in *Epichloë sinensis*

Yang LUO and Pei TIAN*

State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems;
College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, China

*Corresponding author: tianp@lzu.edu.cn

Epichloë sinensis is an endophyte species that forms a mutualistic symbiosis with *Festuca sinensis*, which improves host fitness by promoting growth and enhancing stress tolerance. Phytohormones play essential roles in regulating plant–microbial interactions. Previous research has found that *E. sinensis* is able to produce growth-promoting hormones, including auxin (IAA), cytokinins (CTK), abscisic acid (ABA), and gibberellins (GA). However, the underlying biosynthetic pathways remain uncharacterised. In the present study, *E. sinensis* strain 57A was used to systematically investigate phytohormone biosynthetic pathways based on genomic and transcriptomic analyses. Genome annotation identified 53 putative phytohormone biosynthesis genes, including key enzymes such as 6 aromatic amino acid aminotransferases, 1 indole-3-pyruvate decarboxylase, 14 aldehyde dehydrogenases, 5 nitrile hydratases, 1 ent-copalyl diphosphate synthase, and 6 cytochrome P450 monooxygenase. Comparative pathway analysis indicated that IAA in *E. sinensis* is primarily synthesised through the indole-3-pyruvic acid (IPyA) pathway, involving sequential reactions catalysed by aminotransferase, indole-3-pyruvate decarboxylase, and dehydrogenase. Additionally, *E. sinensis* may synthesise CTK through both the *de*

novo biosynthesis pathway and the tRNA degradation pathway. The former transfers the isopentenyl chain from dimethylallyl pyrophosphate or 4-hydroxy-3-methylbut-2-enyl pyrophosphate to the N6-amino group of adenosine monophosphate, while the latter generates CTK through the breakdown of prenylated tRNAs. Notably, the canonical GA and ABA biosynthesis pathways in *E. sinensis* appear incomplete, with absent homologs of alpha-ionylidenethane synthase *aba3*, ent-kaurene synthase, and ent-kaurenoic acid oxidase. This suggests that *E. sinensis* may utilise non-canonical or currently unknown pathways to produce GA and ABA, which warrants further investigation. Transcriptome analysis of *E. sinensis in vitro* showed that the majority of genes involved in IAA and CTK biosynthesis were expressed, which provides molecular evidence supporting our hypothesis regarding the phytohormone biosynthesis pathways in *E. sinensis*. In conclusion, this study provides the first comprehensive elucidation of phytohormone biosynthesis in *E. sinensis* and reconstructs the putative IAA and CTK biosynthetic pathways. These findings contribute to a deeper understanding of the *E. sinensis* endophyte and lay a theoretical foundation for further investigation into the symbiotic mechanisms.