Abstract

Exploring wild *Bromus-Epichloë* interactions in Uruguay: genetic diversity, eco-geographic distribution, and antiherbivore effects

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A comprehensive survey of *Epichloë* fungal endophytes forming symbioses with the wild C3 forage grass *Bromus auleticus* across a transitional climate–soil zone in Uruguay was conducted. Among 83 accessions, we detected 70% were infected with *Epichloë*. Using PCR with 29 genetic markers targeting *tefA*, mating type and alkaloid biosynthesis genes we identified nine distinct *Epichloë* genotypes that varied in potential alkaloid production. Of these nine genotypes, 1, 2 and 3 were most commonly observed. All genotypes contained the genes required for the synthesis of pyrrolopyrazines (e.g., peramine, a known insect-feeding deterrent). Two genotypes (designated 1 and 7) contained genes required for the synthesis of pyrrolizidines (e.g. loline alkaloids, known for their broad spectrum insecticidal and insect-deterrent activity), but only genotype 1 was predicted to produce *N*-formylloline, a compound with strong insecticidal properties. Seven genotypes (2, 3, 4, 5, 6, 8 and 9) contained genes involved in indole diterpene (IDT) synthesis, however, only five genotypes (2, 3, 4, 5, and 6) were predicted to produce early pathway IDTs such as paspaline or terpendoles. Genotypes 8 and 9 lacked essential early pathway genes were not predicted to produce IDTs. Genotypes 3 and 7 contained *EAS* genes required for ergot alkaloid synthesis; however, these genotypes were only predicted to produce chanoclavine, as genes required for downstream ergovaline synthesis were absent. None of the *Epichloë* endophytes from this survey contained genes required for the synthesis of the mammalian toxins ergovaline or lolitrem B. As genotypes 1, 2, and 3 were the most prevalent in the collection, six plant accessions, two from each of these genotypes, were selected to evaluate the protective effects of their different *Epichloë* genotypes against the aphid *Rhopalosiphum padi,* a generalist insect, in laboratory-controlled bioassays. Notably, only the plant accessions associated with genotype 1, predicted to produce *N-formylloline*, significantly suppressed *R. padi*. Endophyte presence and distribution were strongly influenced by environmental factors, particularly water availability, soil organic matter, and temperature extremes. Additionally, plants associated with endophyte genotypes 1, 2 and 3 were grown in two contrasting environments, displayed genotype-specific effects on host performance emerged under different soil fertility conditions. These findings highlight the ecological and agronomic potential of *Epichloë* endophytes that would lack mammalian toxicity and could provide a foundation for developing sustainable forages with enhanced insect resistance and environmental resilience without compromising livestock health.

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