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Beatriz R Vázquez de Aldana, Iñigo Zabalgogeazcoa (Eds)

Diversity of *Epichloë* in *Hordeum comosum* from Patagonia, Argentina

M. Victoria Vignale[1,2], Patricia D. McCargo[1,2], Cecilia Casas[3,5], M. Victoria Novas[1,2], Pedro E. Gundel[4], Hans Schnyder[5], Leopoldo J. Iannone[1,2]

[1]Instituto de Micología y Botánica (INMIBO), Buenos Aires (Argentina)
[2]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (Argentina)
[3]Facultad de Agronomia, Universidad de Buenos Aires (Argentina)
[4]Instituto de Investigaciones Fisiologicas y Ecológicas Vincualdas a la Agricultura (IFEVA) CONICET (Argentina)
[5]Lehrstuhl für Grünlandlehre, Technische Universität München, Freising (Germany)
leopoldoiannone@gmail.com

Hordeum comosum J. Presl is a native, perennial grass widespread in Patagonia and in the Andean region of South America. This species is an excellent—forage grass highly preferred by sheep. A previous local study[1], based on *tubB* and *tefA* phylogenies of isolates from northwestern Patagonia distinguished two hybrid lineages, one derived from *E. amarillans* x *E. typhina* and the other derived from *E. typhina* subsp. *poae* x *E. festucae* and identified as *E. tembladerae*.

The objective of this work was to study, at regional scale, the diversity and the potential toxicity to cattle of *Epichloë* sp. in *H. comosum* along a gradient of aridity.

An exhaustive survey of *H. comosum* covering an area of 60 000 km² in Patagonia Argentina, that included the previously studied area, was performed in January 2015. Four transects from extreme arid to sub-humid conditions (from 150 to 1200 mm annual precipitation) and collection sites on each transect were established according to their mean annual precipitation (Wordclim). Each site was classified according to its aridity index (AI =Annual precipitation/Potential evapotranspiration) and classified as: arid, AI<0.2; semiarid, 0.2<AI<0.5; or sub-humid, AI>0.5. In each site eight plants were collected. The incidence of endophytes in each population was established by checking the presence of the endophyte by microscopic observation of aniline blue stained culm piths and seeds of each plant. Endophytes were isolated on Potato Dextrose Agar (PDA) in darkness at 24 °C and single spore cultures were obtained for morphological characterization and DNA isolation[2]. The genetic diversity analyses and phylogenetic relationships of *Epichloë* sp. isolates were based on calmodulin gene (*calM*) phylogeny, mating type, and screening by PCR for presence of alkaloid biosynthesis genes (*perA*, *lolC*, *dmaW* and *idt genes: G*, *K*, *P*, *Q*, *F*, *B*, *E, J*).

The incidence of endophytes was variable, ranging from 0 to 100%. *Epichloë* sp. was detected in 27 of the 30 sites, with an average incidence across infected populations of 81%. Populations in semiarid environments presented higher incidence of endophytes (100%), whereas those in arid and sub-humid environments presented lower values (of incidence) (15-30%). Even though the isolates presented variability in morphological characteristics, *cal M* phylogeny indicated that most of the isolates correspond to *E. tembladerae*, which was detected in all the populations (*i.e.*: sites), with the exception of the previously *E. typhina* x *E. amarillans* isolate. Alkaloid gene profiling indicated that the *E. typhina* x *E. amarillans* hybrid was positive for perA, *lolC*, *dmaW* and most of *IDT* genes (*idtE*-). The isolates identified as *E. tembladerae* were negative for *lolC* and *dmaW* genes, all of them presented *perA* and the same *IDT* genes profile, being positive for *idtG*, *K*, *P*, *Q*, *F*, *B* and negative for *idtE* and *J*, with the exception of one isolate that was negative for all the screened IDT genes.

Our results show that in *H. comosum* chances of hosting *Epichloë* sp. decrease with aridity. However, the symbiosis is still maintained in some of the arid and semiarid environments, suggesting long-term benefits of the association. Although different endophyte taxa could be associated with *H. comosum*, *E. tembladerae* seems to be the prevalent species in this host and the diversity of endophytes is not associated with the environmental conditions of the populations. Only the *E. typhina* x *E. amarillans* hybrid could be toxic to cattle producing ergot alkaloids, whereas the *IDT* gene positive *E. tembladerae* isolates could only produce terpendole C. Both endophyte species detected could confer resistance to insects through the production of peramine or some loline in the case of the *E. typhina* x *E. amarillans* hybrid.

References

[1] Iannone L. *et al*. (2015) Journal of Arid Environment 115: 19-26.
[2] Iannone L. *et al*. (2009) Mycologia 101: 340-351.