Pyrrolizidine alkaloids in the Lolium-Festuca species complex

Poster
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Background

Structure of E/Z-thesinine-rhamnoside, a pyrrolizidine alkaloid (PA) produced by *Lolium perenne*

Koulman and colleagues (Phytochemistry 69, 2008) were the first to identify and isolate pyrrolizidine alkaloids produced by a grass (Poaceae). Thesine-rhamnoside consists of the pyrrolizidine-characteristic necine base and necic acids in addition to glycoside moiety.

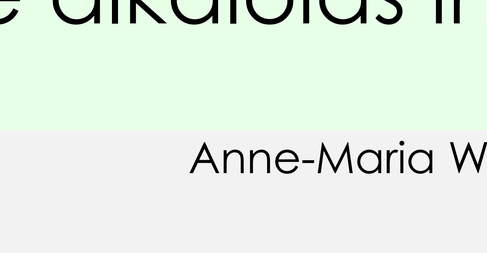
Thesinine-glycosides:

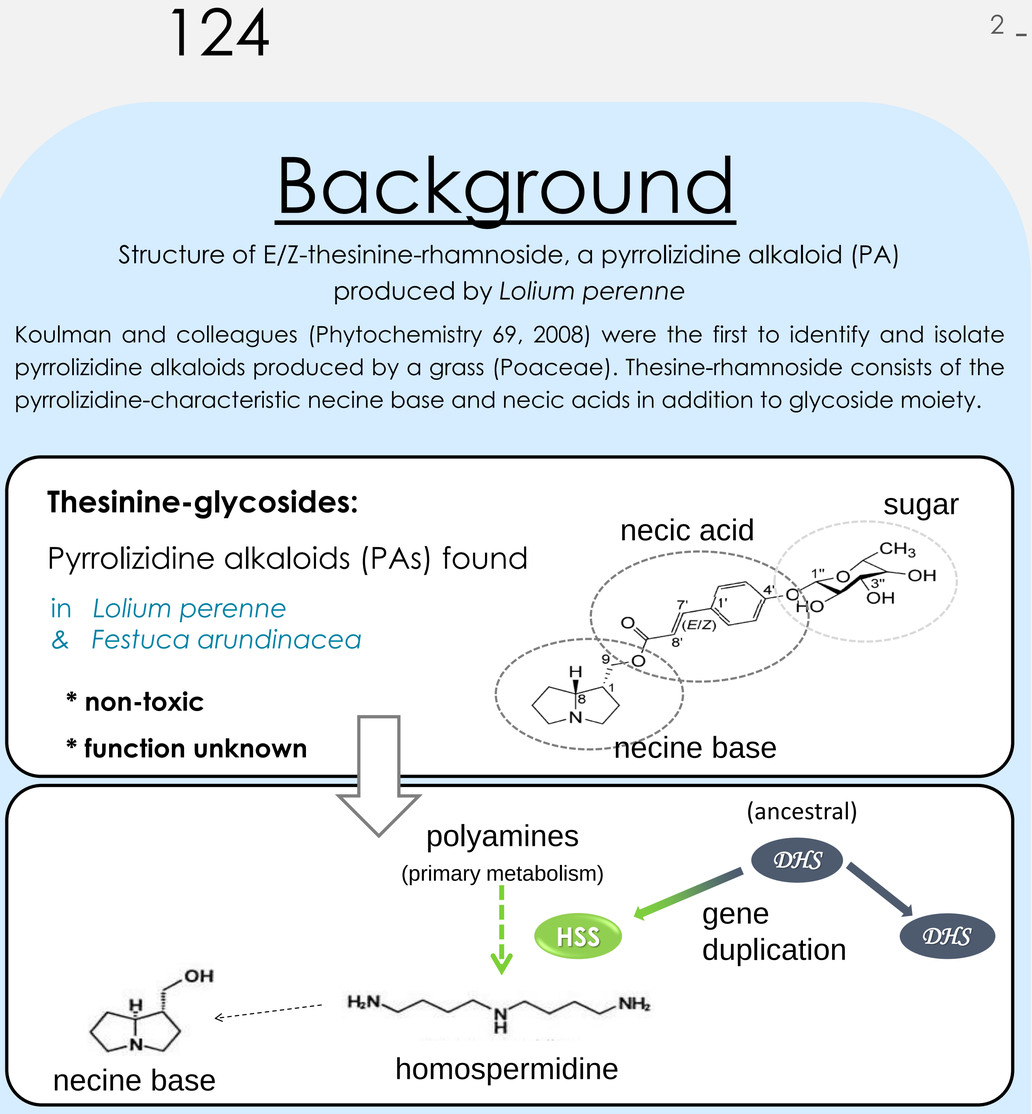
Pyrrolizidine alkaloids (PAs) found

in *Lolium perenne*
& *Festuca arundinacea*

*** non-toxic**

*** function unknown**





Function and evolution of homospermidine synthase (HSS)

The homospermidine synthase (HSS) catalyzes the production of homospermidine which is the first pathway-specific intermediate of PA biosynthesis. HSS evolved from a gene duplicate of the essential deoxyhypusine synthase (DHS) with which it still shares biochemical properties and sequence similarity. HSS (and DHS) have been identified from various PA-producing angiosperm species (Reimann et al., Plant Cell 2004).

Material & Methods

various Lolium and Festuca species:

- climate chamber & green house
- two or three harvesting time points
- three biological replicates

Sample material

Sample preparation

- Freezing of all the green parts
- Freeze-drying
- Pulverizing
- Extraction of alkaloids with 70% MeOH

Sample analysis

- LC Sample separation: RP C18 column [2.1*100mm, 1.8µm Particles]
- QToF-MS detection: positive Ionization, optimized range: 50-400 m/z, 4 Hz rate

Data evaluation

- Exact data calibration: Data Analysis 4.3 [Lithium Formate, Sigma Aldrich]
- automated Peak detection: Compass Pathway Screener 1.0 [Bruker, Bremen]
- multivariate statistical approach: Profile Analysis 2.1 [Bruker, Bremen]

Lolium perenne

QToF [Bruker, Bremen]

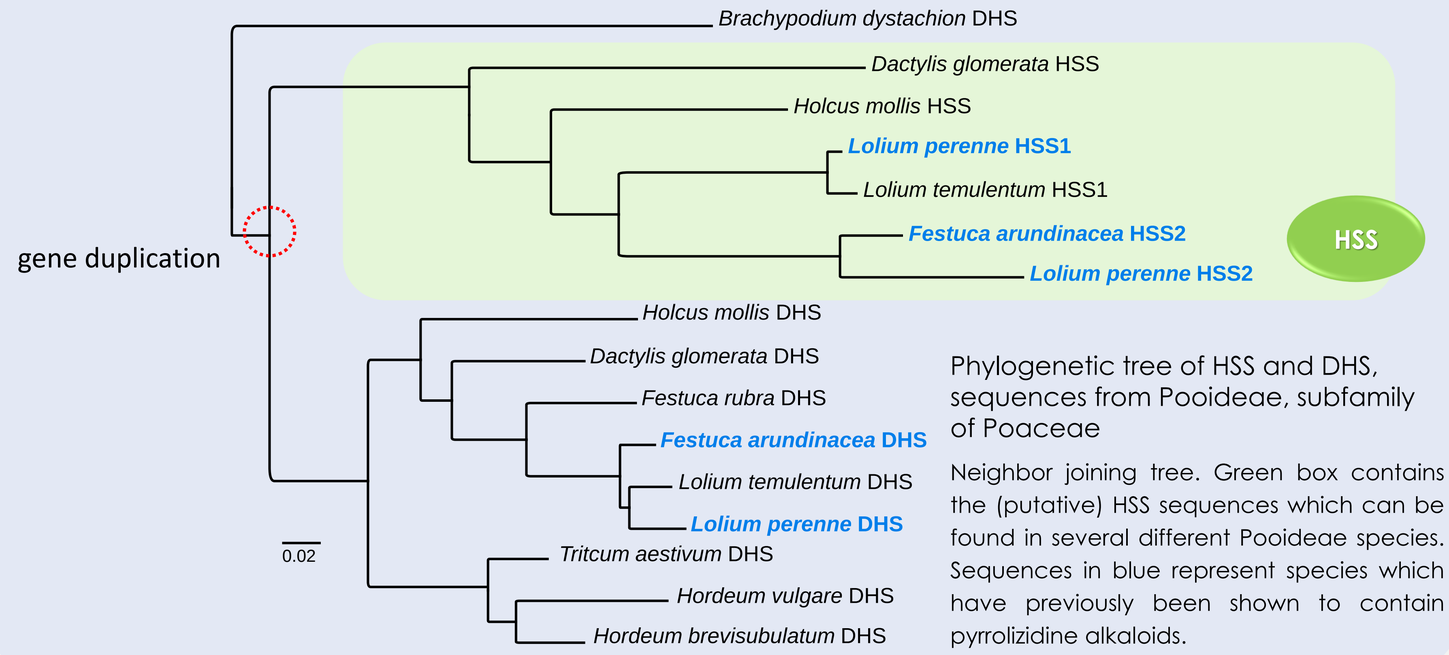
Summary

Although the HSS gene was sequenced from various Pooideae species, thesinine and thesinine-glycosides have only been detected in a few closely related species of the Lolium-Festuca species complex. And within this taxon thesinine-glycosides were only found sporadically.

References:
Reimann A, Nurhayati N, Backenköhler A, Ober D. Plant Cell 2004 16(10):2772-84.
Koulman A, Seeliger C, Edwards PJ, Fraser K, Simpson W, Johnson L, Cao M, Rasmussen S, Lane GA. Phytochemistry 2008 69(9):1927-32.
Tobias J. Demetrowitsch, Beate Petersen, Julia K. Keppler, Andreas Koch, Stefan Schreiber, Matthias Laudes, Karin Schwarz Bioanalysis (2015) 7(1), 103–112

Results

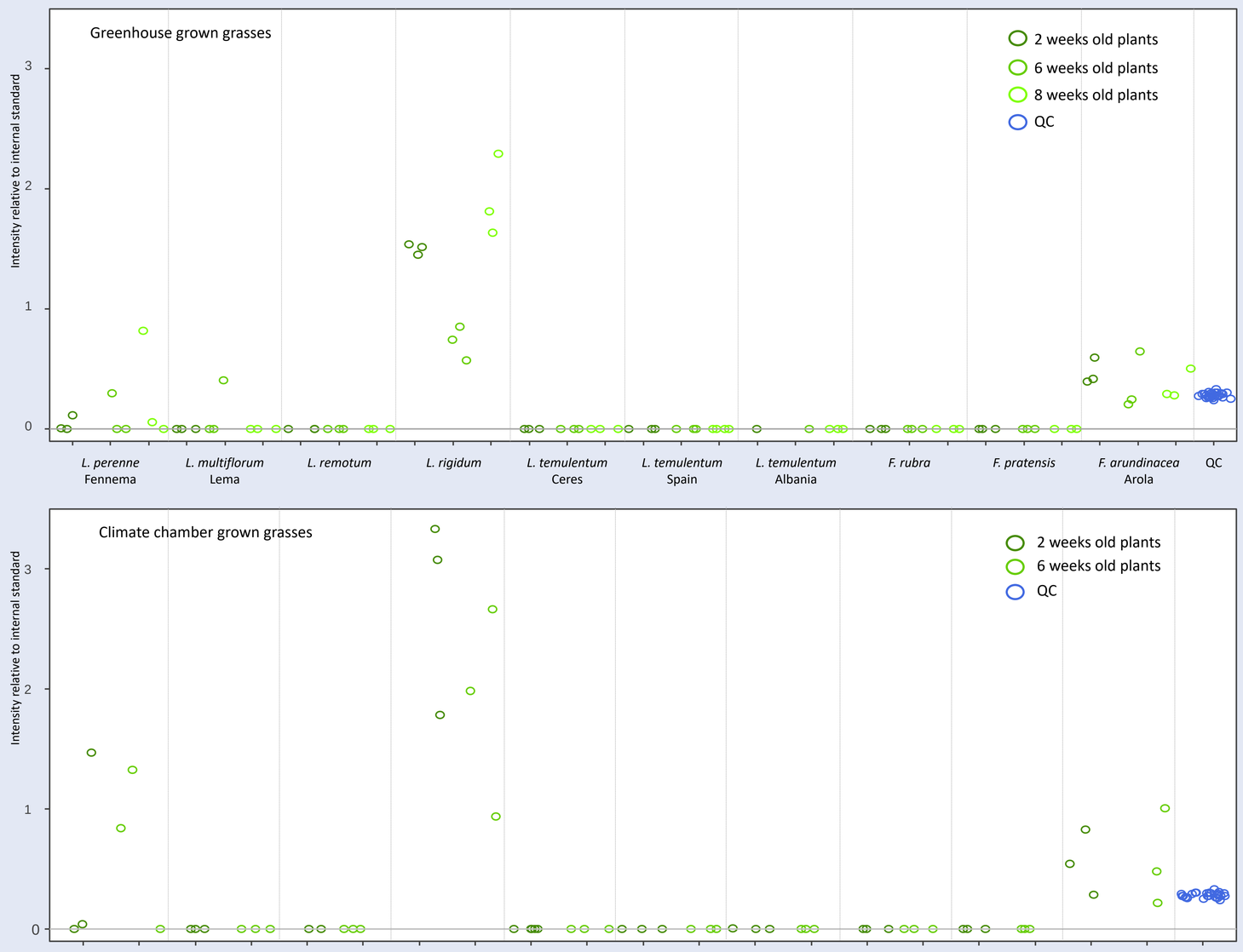
Molecular evolution of HSS within the Pooideae



Phylogenetic tree of HSS and DHS, sequences from Pooideae, subfamily of Poaceae

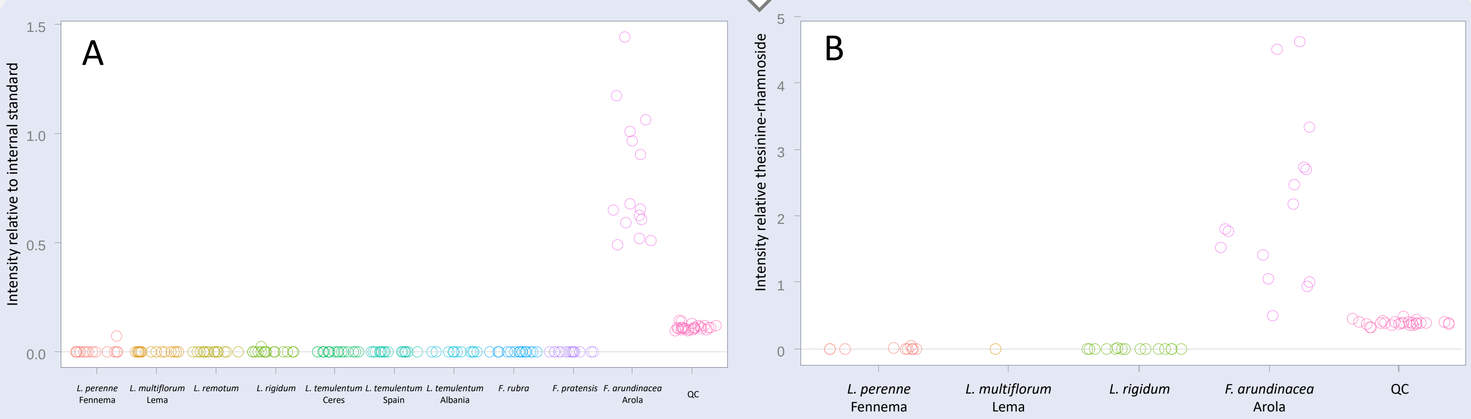
Neighbor joining tree. Green box contains the (putative) HSS sequences which can be found in several different Pooideae species. Sequences in blue represent species which have previously been shown to contain pyrrolizidine alkaloids.

Detection and quantification of thesinine-glycosides



Relative quantification of thesinine-rhamnoside in various Lolium and Festuca samples

Intensities of one stereoisomer of thesinine-rhamnoside (m/z 434) relative to the intensity of the added internal standard retrorsine (m/z 352, 0.375 µg/ml) measured by UPLC-QTOF-MS. QC approach verified a robust and repeatable analytical conditions (Demetrowitsch et al., Bioanalysis 2015.



Relative quantification of thesinine in Lolium and Festuca samples

Intensities of one stereoisomer of thesinine (m/z 288) relative to (A) the intensity of internal standard retrorsine (m/z 352, 0.375 µg/ml) (B) the intensity of thesinine-rhamnoside in the same sample measured by UPLC-QTOF-MS.

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