Analysis of genetic drift in perennial ryegrass populations via SNP marker and candidate gene approaches

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Introduction

Perennial ryegrass (*Lolium perenne* L.) is an out-crossing pasture grass species with importance in world-wide agriculture. Due to its high yield and tolerance to grazing and cutting as well as its high content of digestible nutrients, perennial ryegrass is a main component in forage mixtures. Additionally, it is intensively used in turf and lawn. “Persistence”, i.e. plants surviving well under typical, locally varying environmental conditions like intensity and duration of snow coverage and low temperatures during winters, is a very important trait in agricultural science and farming. Therefore, the Bavarian State Research Center for Agriculture (LfL) started an experiment on the improvement of persistence in perennial ryegrass in 1998 (Hartmann; cf. Fig. 1). The differences in persistence between *Lolium* cultivars resulted in a differentiation into winter hard and less winter hard entries (summary shown in Fig. 1). As a follow-up, this study aims to develop a selection method for “persistence” via monitoring genetic drift of different ryegrass varieties at sites with different environmental conditions. For this means, SNP alleles were quantified using the Illumina GoldenGate genotyping assay, while the same set of cultivars was evaluated phenotypically in the field since 2004.

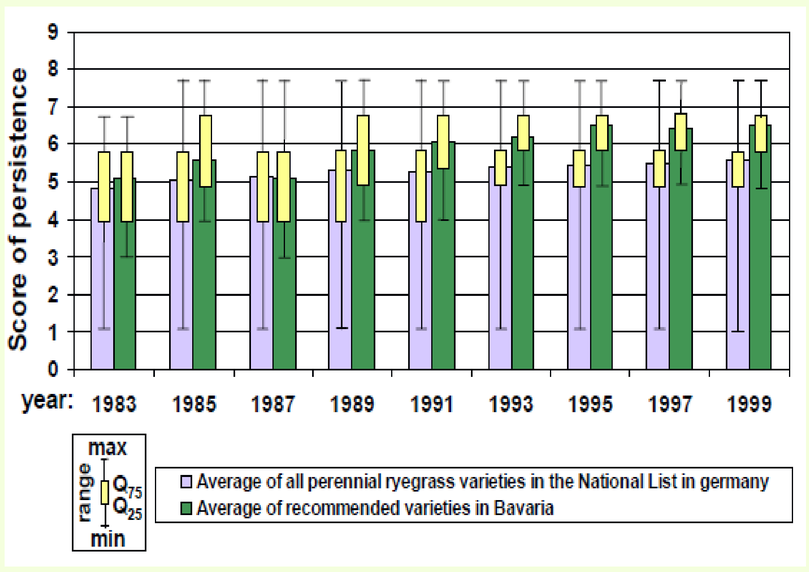

Figure 1: Differences in persistence between listed cultivars and varieties recommended for cultivation in Bavaria (Hartmann, Mitteilungen Arbeitsgemeinschaft Grünland und Futterbau 5 (2003), 163-165.

Material

- 19 forage and 4 lawn varieties of *Lolium perenne* L.
- among these varieties: winter hard and less winter hard entries
- five defined cultivation sites (Fig. 2; sites 1-4 sown in 2004; site 5 sown in 2005)
- DNAs extracted from pools of individuals used in SNP allele quantification assays

Strategy

The detection of possible changes in SNP allele compositions between original genotypes and genotypes in the trial field after four years of cultivation (two visually fittest varieties in trial locations vs. two visually least fit varieties) is performed via Illumina GoldenGate genotyping assay. The evaluation of phenotypic data (e.g. visible changes in variety appearance, Fig. 3) and the analysis of correlations to the genotyping results (Fig. 4) is used for investigating the “persistence” components leading to the development of selection methods for the trait “persistence”. Genetic drift, e.g. changes in the phenotypic scoring from “less winter hard” to “winter hard”, will be analyzed within and between the varieties at different sites, too.

Figure 2: Map of Germany showing the five experimental sites: (1) Detern, (2) Schmalenbeck, both Lower Saxony, moor; (3) Spitalhof, (4) Hötzelsdorf, both Bavaria, mountainous; (5) Malchow/Poel, Mecklenburg Western Pomerania; maritime.

Figure 3: Genotype mixtures of variety Guru from sites Hötzelsdorf (A) and Schmalenbeck (B) after four years of cultivation, with drastic phenotypic changes being visible at Schmalenbeck.

Status & Results

- field evaluations resulted in scores concerning the winter hardiness of the varieties (cf. Fig. 1) as a whole and of individual plants of them
- phenotypic data seem to indicate genetic drift in one out of the four chosen varieties: being less winter hard before the start of the field experiments, it is now phenotyped as winter hard
- evaluation of 192 SNP markers of the Illumina Golden Gate genotyping assay is being conducted in order to characterize the material before and after four years of cultivation, with some SNPs hinting at allele frequency shifts for certain varieties at certain sites (cf. Fig. 4)
- candidate gene approaches are being applied to investigate cold tolerance (via CBF/C-Repeat Binding factor) and *Fusarium* resistance

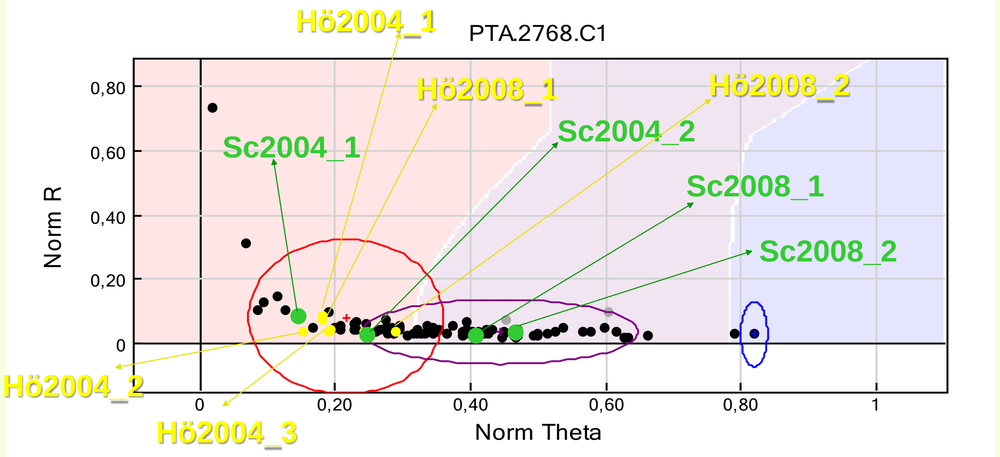

Figure 4: GenomeStudio SNP Graph of an individual marker showing allele compositions of samples from four varieties from trial sites 1-4 (cf. Fig. 2); **yellow dots**: variety Guru, sampled at Hötzelsdorf (Hö) in 2004 and 2008 (two/three bulks analyzed; no shift/differentiation); **green dots**: Guru sampled at Schmalenbeck (Sc) in 2004 and 2008 (two bulks analyzed each), with indicated shift in allele frequency between the two years.

Outlook

With the outlined strategy, we aim at providing breeders and farmers with recommendations for the selection and cultivation of the best, i.e. most persistent varieties for different environments for further increasing the efficiency and profitability of *Lolium* cultivation in permanent grasslands.

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