



Genetic drift in perennial ryegrass populations: Analysis of phenotypic data, Illumina genotyping assays and candidate genes

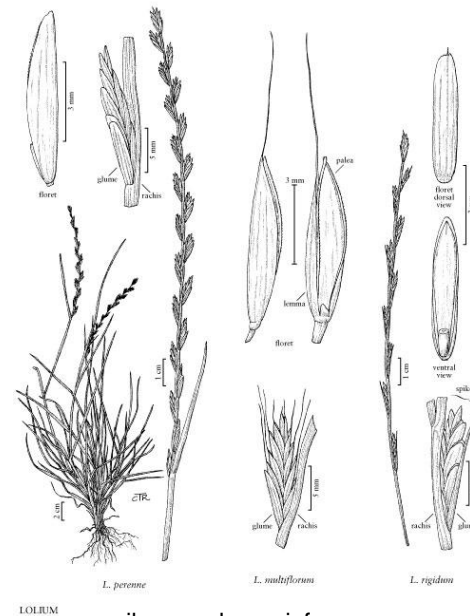


Gräserabbildungen: © COMPO GmbH & Co .KG



Outline

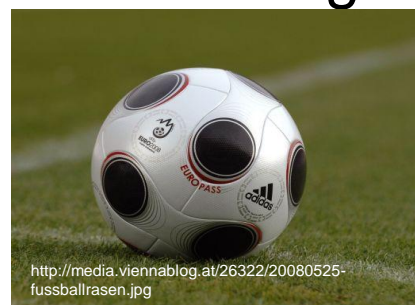
- Introduction
- Phenotypic data
- Illumina SNP genotyping assays
- Candidate gene approach
- Outlook





Introduction

- PhD project is dealing with: “Monitoring the effects of natural selection in populations of *Lolium perenne* L. and development of markers for the trait persistence”
- cooperation with Institute of Crop Science and Plant Breeding (IPZ) of the Bavarian State Research Center for Agriculture (LfL)



<http://media.viennablog.at/26322/20080525-fussballrasen.jpg>



http://www.ski-epic.com/2007_london_trip/p42b_london_hyde_park_italian_gardens.jpg

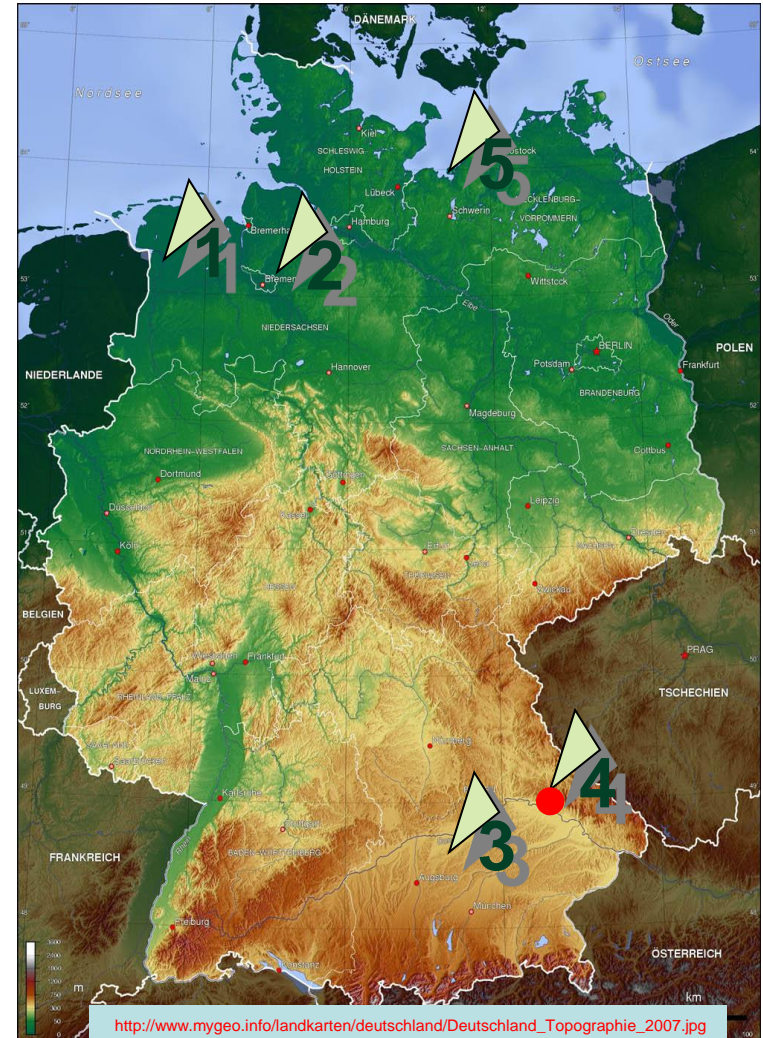


<http://www.doliwa-naturfoto.de/Bilder-Galerie/Tiere/Saeugetiere/Kuh/Kuh3/kuh3.html>



Introduction - Material

- experimental design: five locations, four years of cultivation, 19 forage varieties
- DNA extraction, phenotyping of the plots at the five sites and of the single plants in Steinach (●), Bavaria





Introduction - Material

- *Phenotyping of the plots:*
- data from sites Hötzelndorf, Spitalhof and Schmalenbeck (no data from Detern)
- e.g. traits: growth after winter, defects (diseases a.s.o.), denseness, were phenotyped
- each plot in four repetitions



Genotype mixtures and winter survival of variety 'Guru' from sites Hötzelndorf (A) and Schmalenbeck (B) after four years of cultivation, with drastic changes being visible at Schmalenbeck.





Introduction - Material

- *Phenotyping of the single plants:*
- 96 single plants of each of the five sites were transplanted to Steinach, Bavaria with 12 plants per row in 2008 → per variety and site eight rows with 12 plants each side by side arranged
- traits: e.g. diseases (rust, foliar diseases, *Fusarium* a.s.o.), heading date, mass production and others were phenotyped
- phenotyping from 2008 until 2011, this data available





Introduction - Phenotyping

- scheme of the Bundessortenamt (BSA), Germany used for phenotyping
- each trait was scored with a number (one to nine), '1' → trait is not formed, '9' → trait is formed clearly



rasengesellschaft.de



rasengesellschaft.de; Gray Leaf Spot infected *Lolium leaf* (Foto: Vincelli)



ShowYourPlant.com



Results - Analysis of phenotypic data

- two types of sets: plots 2004-2008 and single plants 2009-2011
- both data sets evaluated and analyzed, trends observed, hypotheses set and discussed in cooperation with Bavarian Institute, group of LfL/Dr. Hartmann



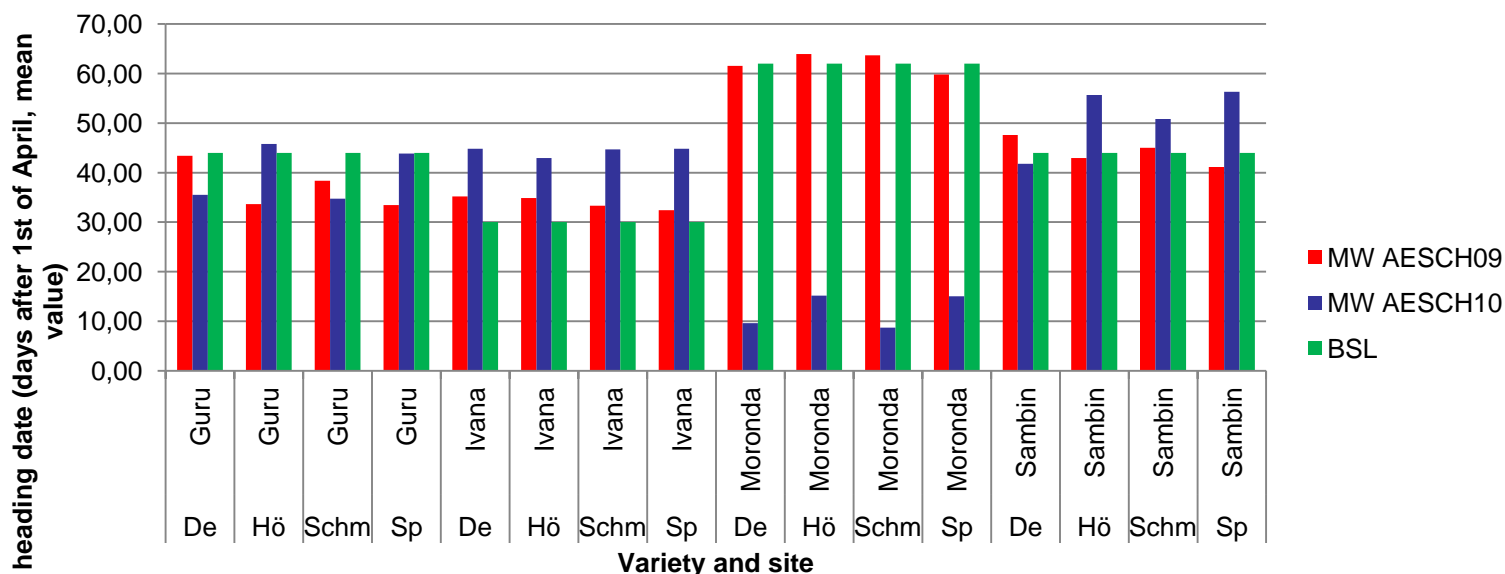
rasengesellschaft.de; Gray Leaf Spot infected *Lolium* leaf
(Foto: Vincelli)



Results - Analysis of phenotypic data – single plants

- data sets showing trends, but: phenotyping in 2010 and 2011 completely different compared to 2009, even with respect to standards of Bundessortenamt

Heading date (mean value) of 2009 vs 2010 vs BSL





Results - Analysis of phenotypic data – single plants

- unexpected effects have to be clarified → hypotheses:
 - climatic effects
 - mistakes in field practice (randomization...)
 - phenotyping suboptimal



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amnh.org



Results - Analysis of phenotypic data – plots

- data only for three sites available
- phenotyping at those sites done very differently → not every trait phenotyped at every site and with the same number of repetitions
- possible to observe trends and to evaluate the available data statistically, but comparison between north and south Germany will be not appropriate



locomnet.de

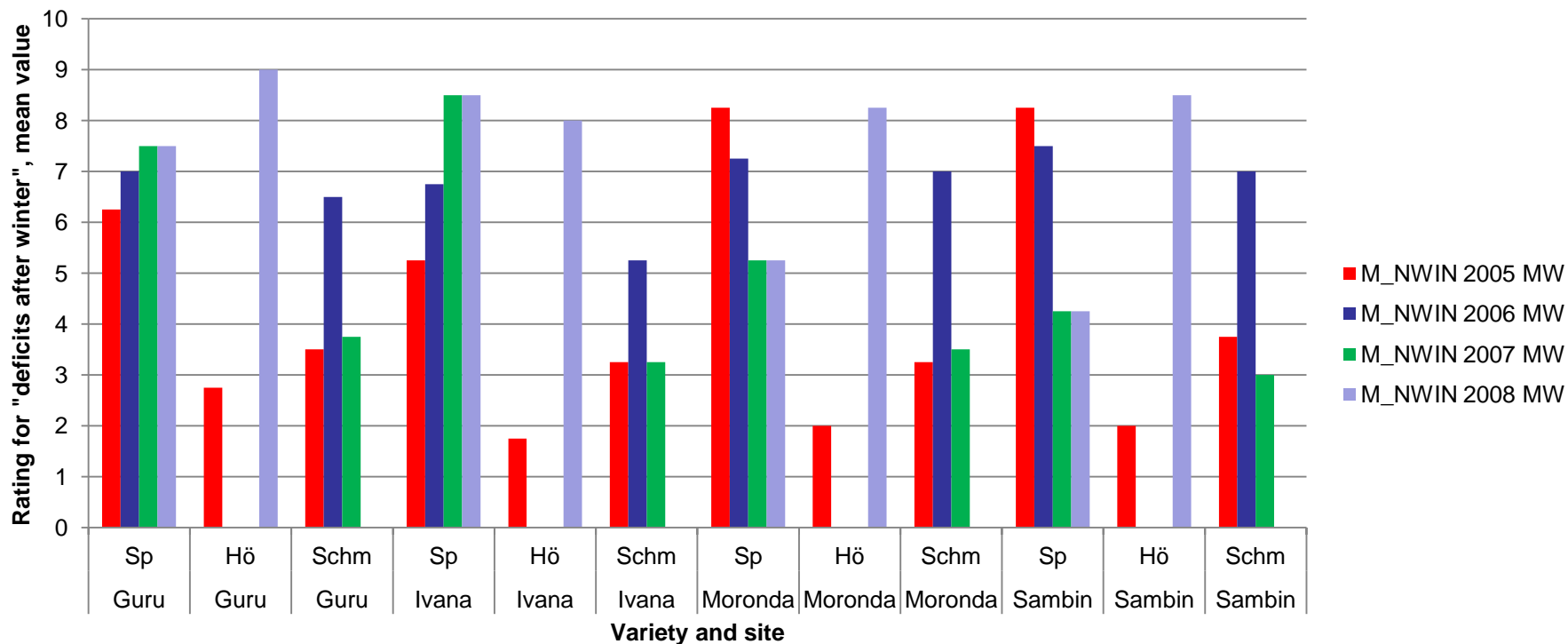


dominika.twoday.net



Results - Analysis of phenotypic data – plots

Phenotyping plots - deficits after winter 2005 - 2008



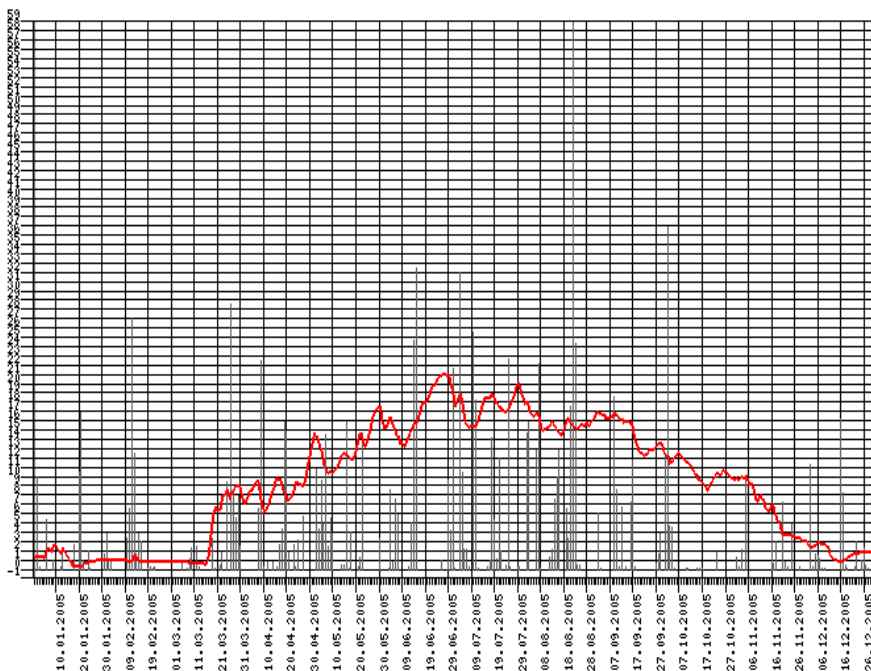


Results - Analysis of phenotypic data – climate

Agrarmeteorologisches Messnetz Bayern - Wetterdatenabruf

Grafik für Station 38, Spitalhof

— average temperature 20cm above soil
— rain sum



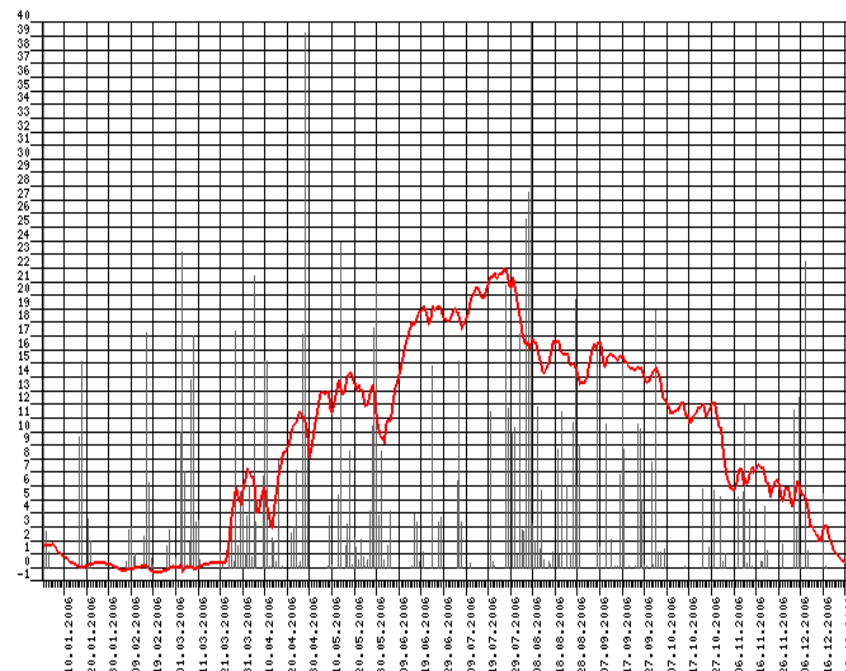
01.01.2005 – 31.12.2005

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Agrarmeteorologisches Messnetz Bayern - Wetterdatenabruf

Grafik für Station 38, Spitalhof

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— rain sum



01.01.2006 – 31.12.2006

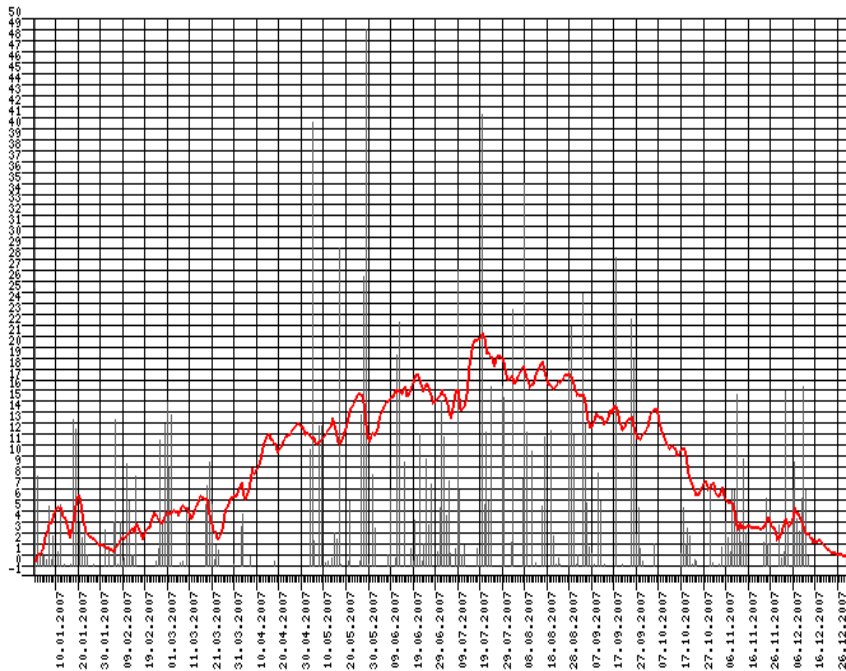


Results - Analysis of phenotypic data – climate

Agrarmeteorologisches Messnetz Bayern - Wetterdatenabruf

Grafik für Station 38, Spitalhof

— average temperature 20cm above soil
— rain sum



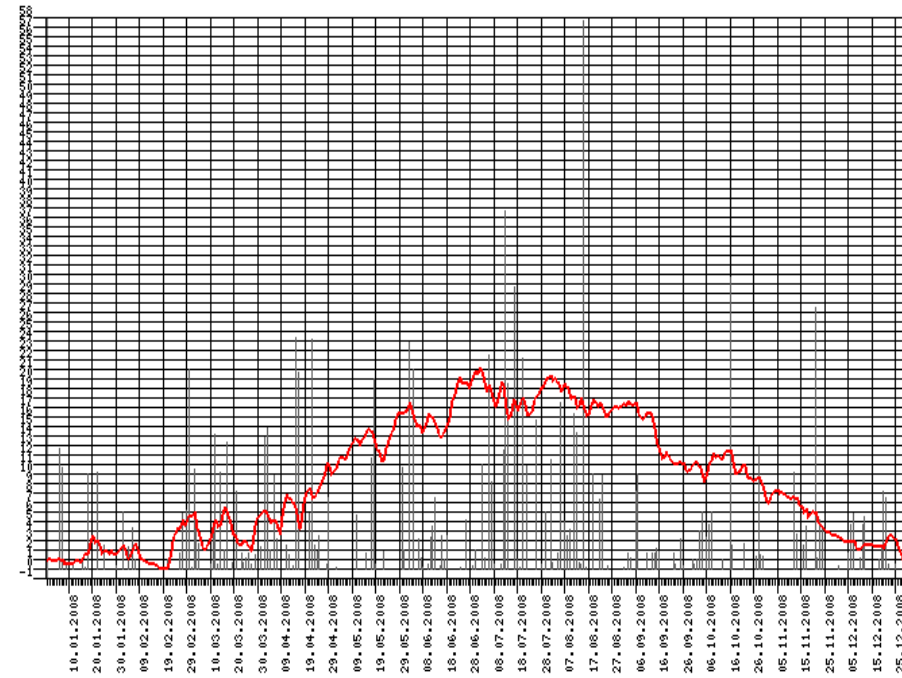
01.01.2007 – 31.12.2007

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Agrarmeteorologisches Messnetz Bayern - Wetterdatenabruf

Grafik für Station 38, Spitalhof

— average temperature 20cm above soil
— rain sum

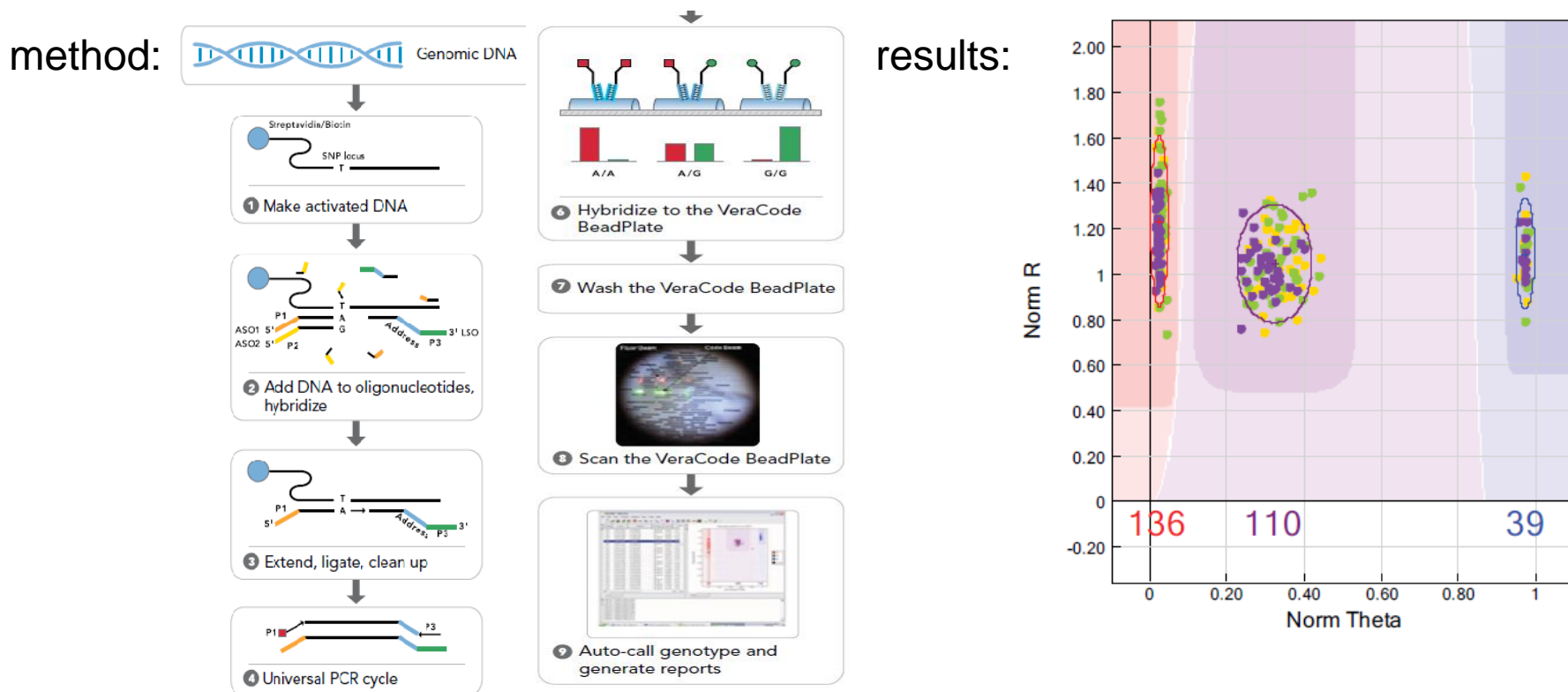


01.01.2008 – 31.12.2008



Results - Analysis of Illumina GoldenGate genotyping assay

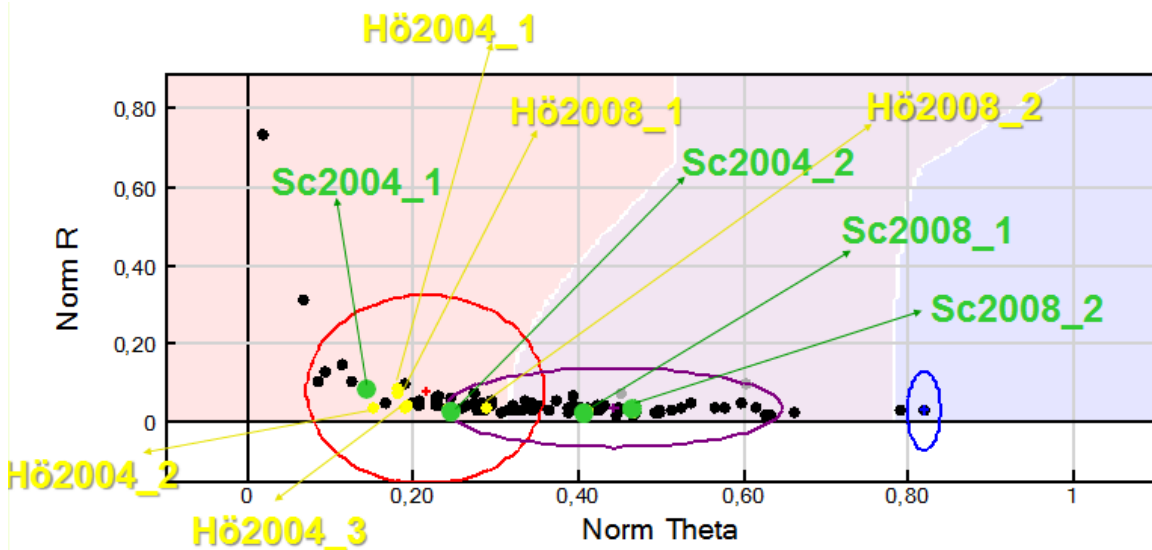
- analyses of bulked samples conducted by TraitGenetics





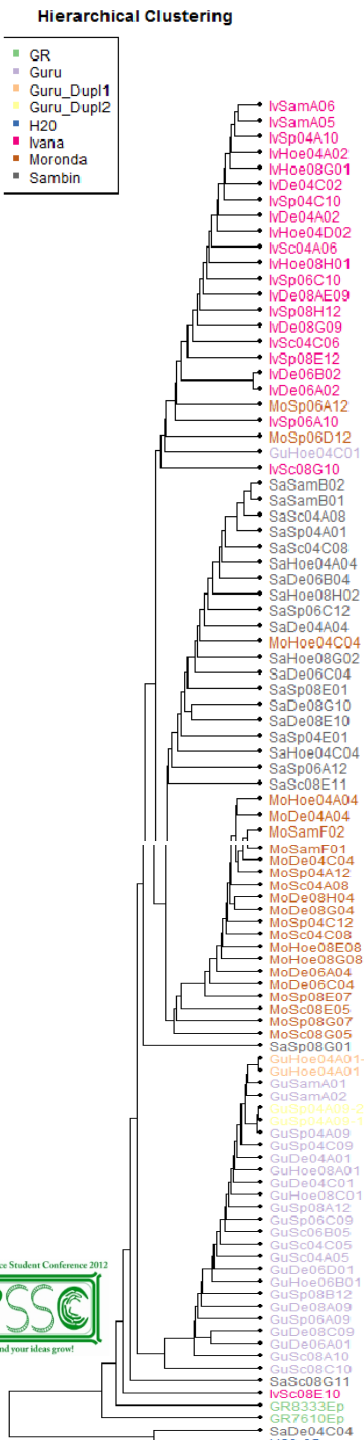
Results - Analysis of Illumina GoldenGate genotyping assay

- normalized theta value nearest zero \rightarrow homozygous for allele A
- normalized theta value nearest one \rightarrow homozygous for allele B



GenomeStudio SNP Graph of an individual marker showing allele compositions of samples from four varieties from four trial sites; **yellow dots**: variety Guru, sampled at Hötzelendorf (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.

Results - Analysis of Illumina GoldenGate genotyping assay



- results of Illumina GoldenGate assay checked with function “hierarchical clustering” of DiSTo2 → four different varieties clustered together; some outliers
- expectation that genotype mixtures could cluster according to the site of origin → not observed
- interesting: seed lots are clustering closely; Moronda Spitalhof and Hötzelstdorf → different compared to rest of Moronda entries



Results - Candidate gene approach

- in 2007 Tamura & Yamada published in Theor Appl Genet: “A perennial ryegrass *CBF* gene cluster is located in a region predicted by conserved synteny between *Poaceae* species”
- CBF genes = C-repeat binding factor
- CBF/dehydration-responsive element binding protein 1 (DREB1) regulon

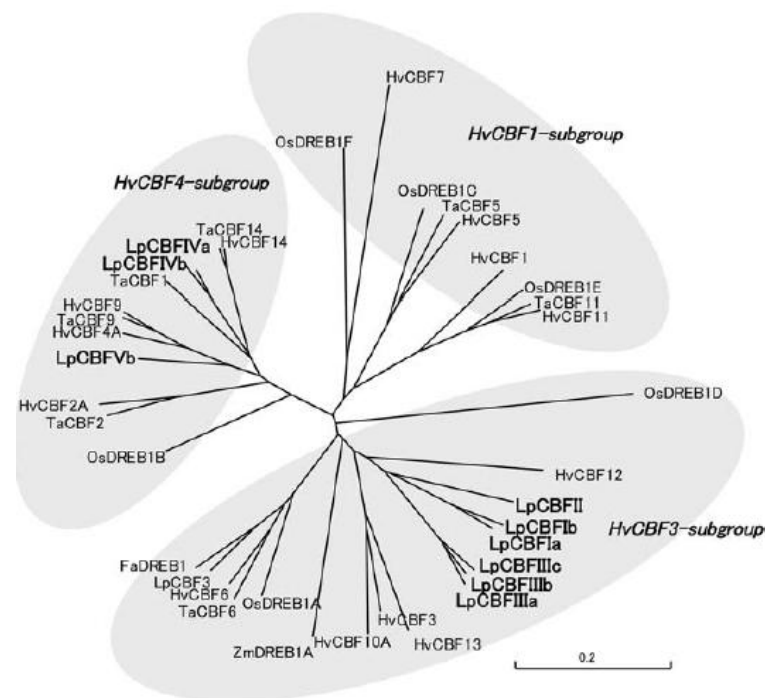


Fig. 2 Phylogenetic relationship of monocot CBF proteins. The full length of each protein was used for phylogenetic tree analysis. *Scale* indicates branch length. LpCBFVa was excluded from this analysis due to the lack of N-terminal amino acid sequence. The



Results - Candidate gene approach

- CBF/DREB 1 regulon → most important transcription unit involved in cold acclimation
- sequences available; new primer design etc.

LpCBF1-1F
LpCBF1-2F
LpCBF1-1R
LpCBF1-2R
LpCBF1-4R
LpCBF1-5R
LpCBF3-1F
LpCBF3-1R
LpCBF12-1F
LpCBF12-1R

CTCATCAAGCCATGGACATG
CTCACAGTCCACAGTCCACC
TGAGGATGGCTCTCTGT
TGGAGTCAAAGCGCGACGA
GAACTGCATCTGCTTGCATG
TCTCCTTGAACCTTGGTCCCTCC
AGCATCCAGAGCTTACCAAG
GCACTATCACATCACATAACCAG
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GAATCTACAGGCAGATCTCCAGC

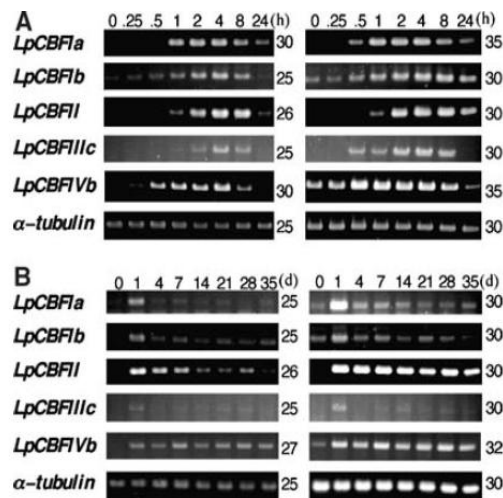
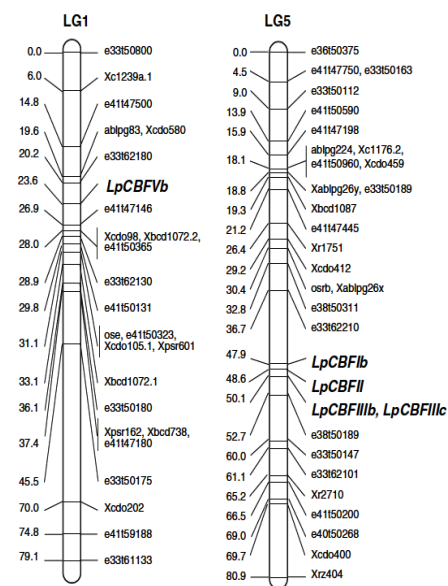


Fig. 3 Expression profile of *LpCBF* genes in response to low temperature (4°C) during a short term (24 h, under the light condition) (a) and a long term (35 days under the 8 h day length) (b).

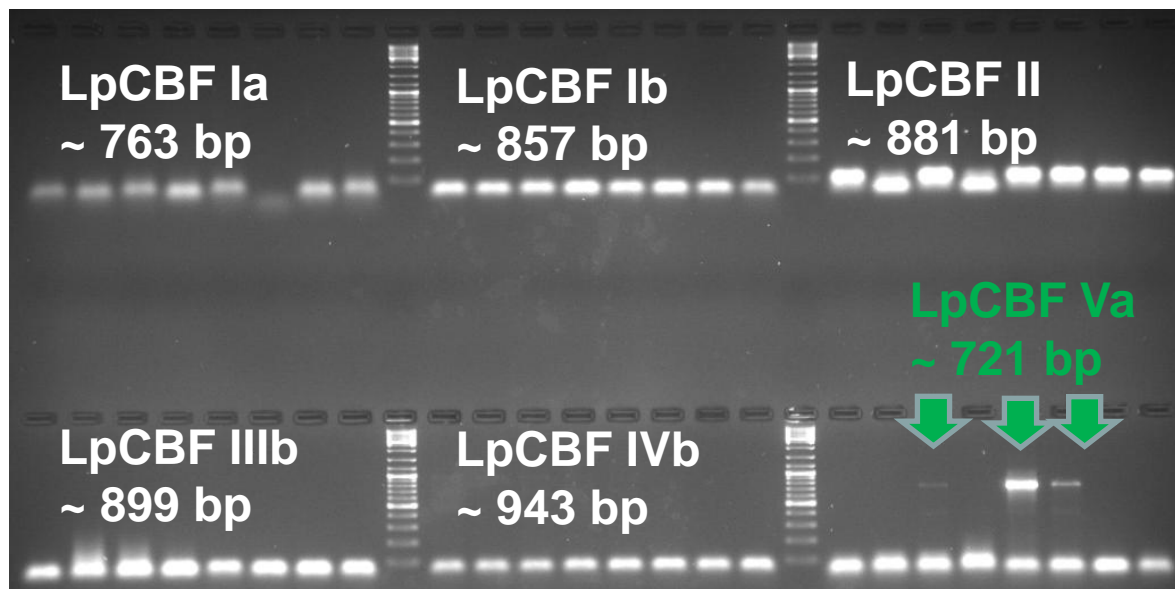
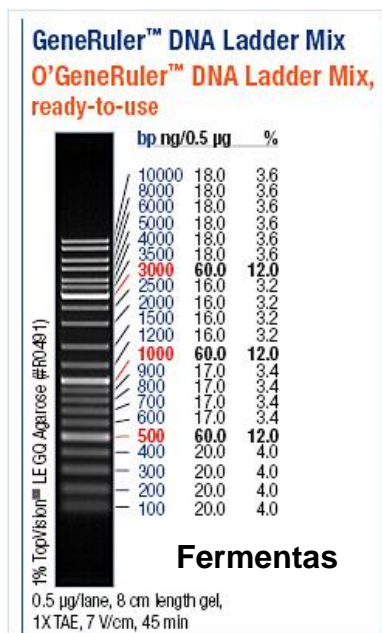
Fig. 4 Loci of the *LpCBF* genes on linkage group 1 and 5 of the p150/112 perennial ryegrass mapping population. Nomenclature of AFLP loci and RFLP loci is as described by Jones et al. (2002). The numerical value described to left of each marker indicates genetic distance (cM)





Results - Candidate gene approach

- problem: amplified PCR fragments too small compared to the fragment sizes calculated → work in progress





Results - conclusion lab work:

- Candidate gene approach is not working yet → work in progress 



- Illumina GoldenGate genotyping assay → correlation with phenotyping data started 





Outlook

- Analysis phenotypic data of the plots and of the single plants
- Correlation Illumina GoldenGate genotyping assay data with phenotypic data





Acknowledgements

- **funding: Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten.**
- **AG GED, AG PBP, AG EXT**
- **Dr. Klaus Dehmer, Dr. Stephan Hartmann (LfL, Bavaria)**





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Thank you for your attention!

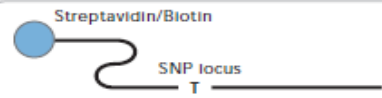


cutcaster.com



GENOMIC DNA (250 ng for single use, 2 ug for multiple uses)

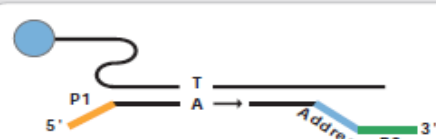
DAY 1



1 Make activated DNA



2 Add DNA to oligonucleotides, hybridize



3 Extend, ligate, cleanup

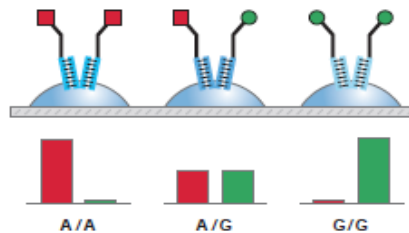


4 Universal PCR cycle at 1536-plex

DAY 2



5 Bind PCR product, elute dye-labelled strand, prepare for hybridization



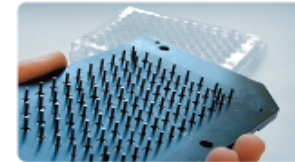
6 Hybridize to the Sentrix® Array Matrix or BeadChip

ASO - Allele-Specific Oligo
 LSO - Locus-Specific Oligo
 P1, P2, P3 - Universal PCR primers
 Address - Sequence unique targeting a particular bead type

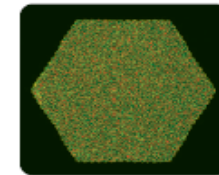


- Optional Stopping Point

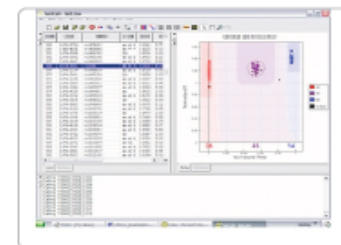
DAY 3



7 Wash and dry Array Matrix or BeadChip



8 Image Array Matrix or BeadChip



9 Auto-call genotypes and generate reports