

*Analysis of drought tolerance in
perennial ryegrass (Lolium perenne L.)
with methods of metabolite profiling
and systemic metabolite markers*

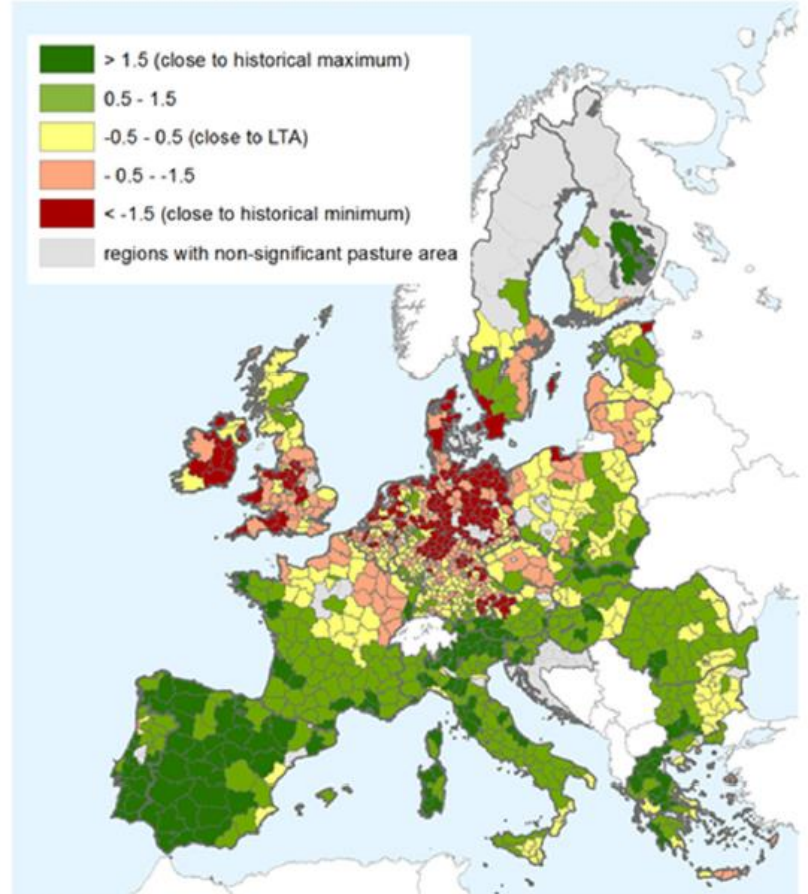
Wittmann J., Drettwan D., Westermeier P., Willner E., Hartmann S., Geyer R.
GPZ, Tulln, 11.02.2020



- 30% of agricultural land used as grassland
- *Lolium perenne*
 - most important forage grass
 - limited tolerance towards temporal drought
- Extensive breeding efforts, combined with the development of new tools for selection and prediction

Relative index of pasture productivity

Period of analysis: 1 May - 20 July 2018
Index based on Copernicus GEOV2 fAPAR 10-day product.
Historical archive (LTA) from 1999 to 2017



Source: EU joint research center



Genetic analysis of drought tolerance in perennial ryegrass (*Lolium perenne* L.) with phenological, physiological and molecular differentiation methods- DRYeGRASS

01.06.18 – 31.01.20



Gefördert durch:



aufgrund eines Beschlusses des Deutschen Bundestages





- lifespın was founded in April 2017 in Regensburg, Germany
- Experienced interdisciplinary team of 11 PhDs and 4 lab technicians
- More than 20 years experience in the development and application of NMR



NMR-analysis	Automated data analysis	Metabolomics/Diagnostics
with		
<i>lifespın</i> efficiency	<i>lifespın</i> software	<i>lifespın</i> databases and AI

- lifespın's methods are used in biotech, breeding, quality control, diagnostics and medical R&D



- ISO 9001:2015 certified
- NMR and lab capacities for >50.000 samples/a
- Bruker 600 MHz spectrometer with sample jet autosampler
- -80°C storage capacities for 70.000 samples

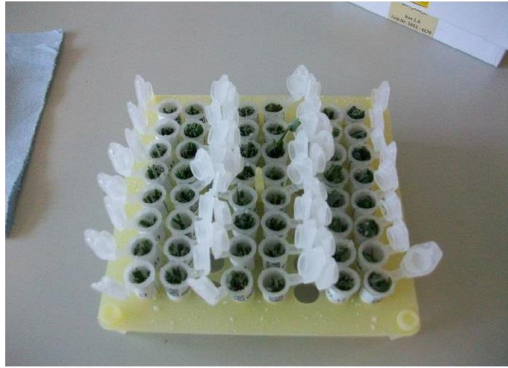




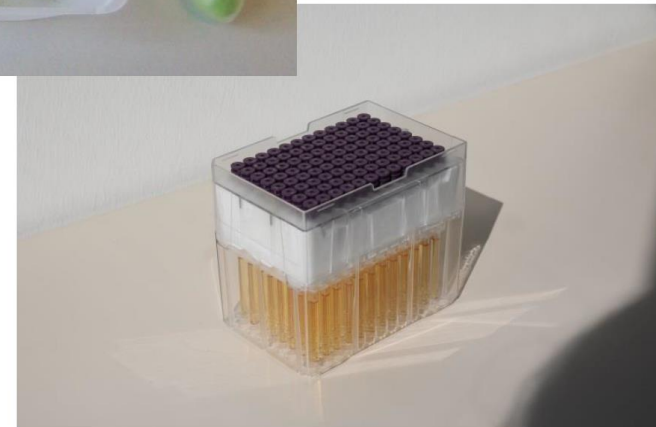
- *Development of a NMR based method for quantitative metabolite profiles in leaf tissue*
- *Analyses of ~1200 single plant samples*
- *Development of 2 software tools/models*
 1. *Multiparameter quantification = Metabolite profiler*
→ *Chemotype*
 2. *(Systemic) biomarker, e.g. combination of metabolites and their ratios, for drought tolerance*
→ *Screening for drought tolerance in an early stage, without stress experiments*

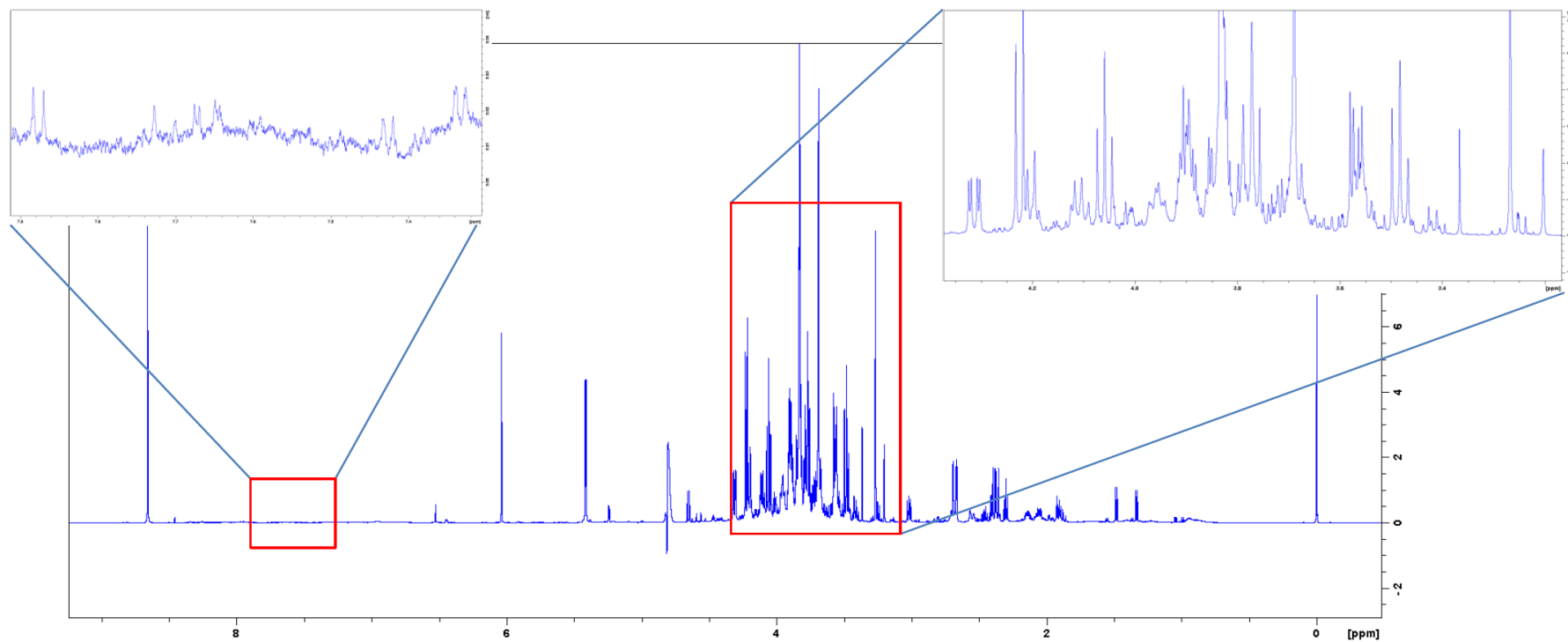


Automated and efficient sample preparation



- Highly standardized and efficient sample preparation, fully automated measurement and data analysis
→ throughput ~140 samples/d



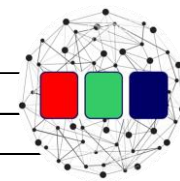


→ Samples from 1368 plants analyzed



- Continuously growing reference database
- Software identifies metabolites, deconvolutes signals and quantifies
→ quantitative metabolite profiles
- For the finale DRYeGRASS-Panel >200 metabolites checked, **60 are quantified**

Acetaldehyd	Cystein	Lysin
Aceton	Cystin	Malonsaeure
Acetylcholin	Essigsaeure	Methanol
Adenosin	Ethanol	Methionin
Aepfelsaeure	Ethanolamin	Milchsaeure
Alanin	Fructose	myo-Inositol
alpha-Ketoglutarsaeure	Fumarsaeure	Phenylalanin
Ameisensaeure	GABA (gamma-Aminobuttersaeure)	Prolin
Ameisensaeuremethylester	Galactose	Propanol
Arginin	Glucose	Propionsaeure
Asparagin	Glucuronsaeure	Pyroglutaminsaeure
Asparaginsaeure	Glutamin	Raffinose
Bernsteinsaeure	Glutaminsaeure	Saccharose
Betain	Glutarsaeure	Serin
Brenztraubensaeure	Glycerol	Shikimisaeure
Butanol	Glycin	Threonin
Buttersaeure	Histidin	Tryptophan
Chinasaeure	Isoleucin	Tyrosin
Cholin	Isopropanol	Uridin
Citronensaeure	Leucin	Valin



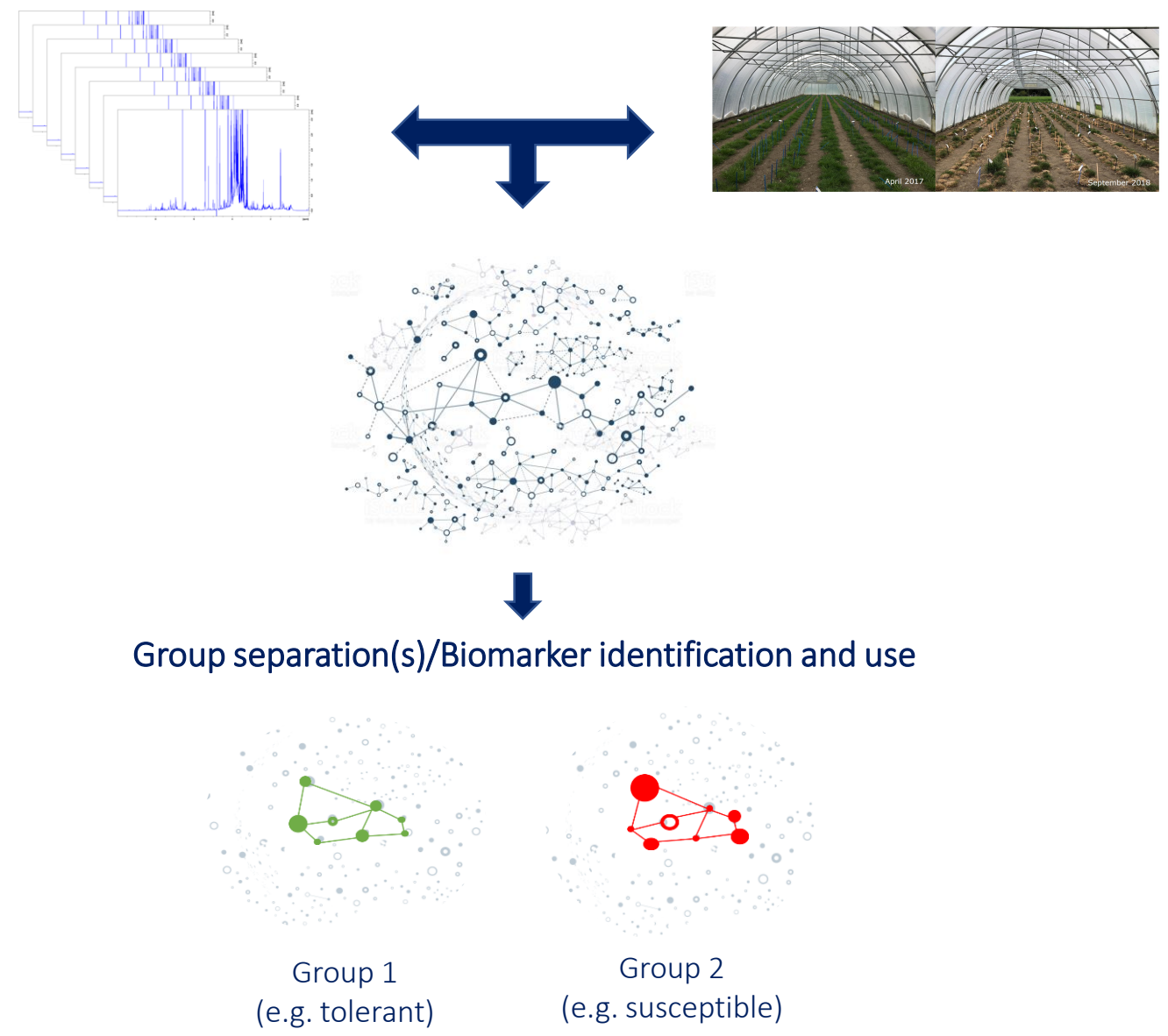


- Field trials: 2 years, 2 locations (Freising, Poel), 2 drought stress periods per year
- Sampling was performed 10 days after first cut in both years (before stress was induced)
 - *out of eight segregating, connected crossing populations*
 - *Including randomly distributed standard genotypes representing the population parents*





Metabolite profiles + field data + AI = marker profiles

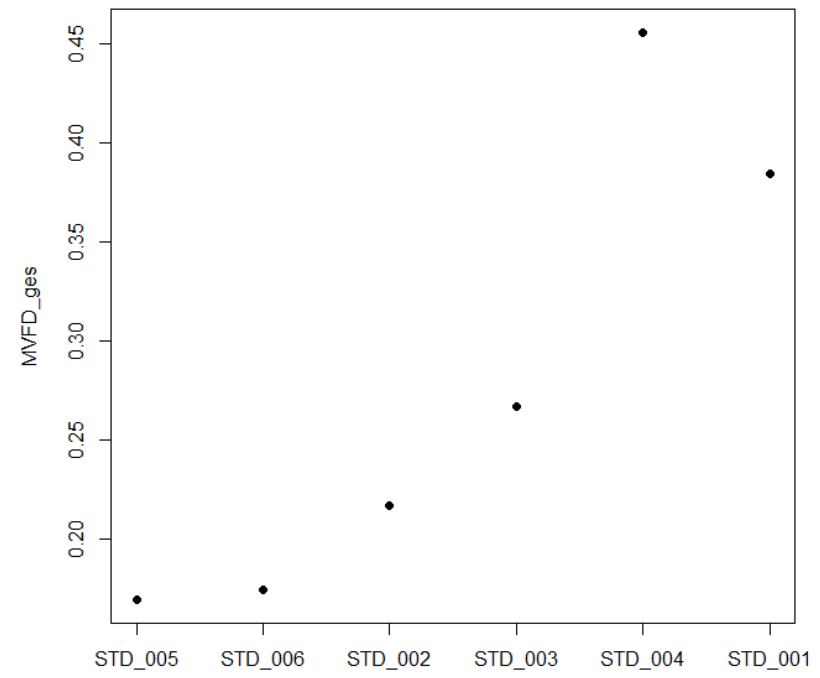
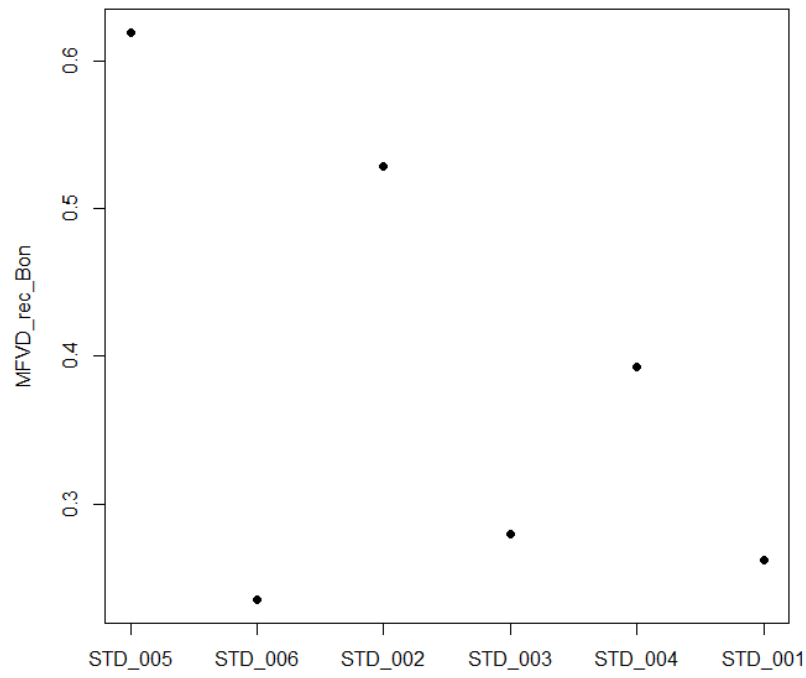
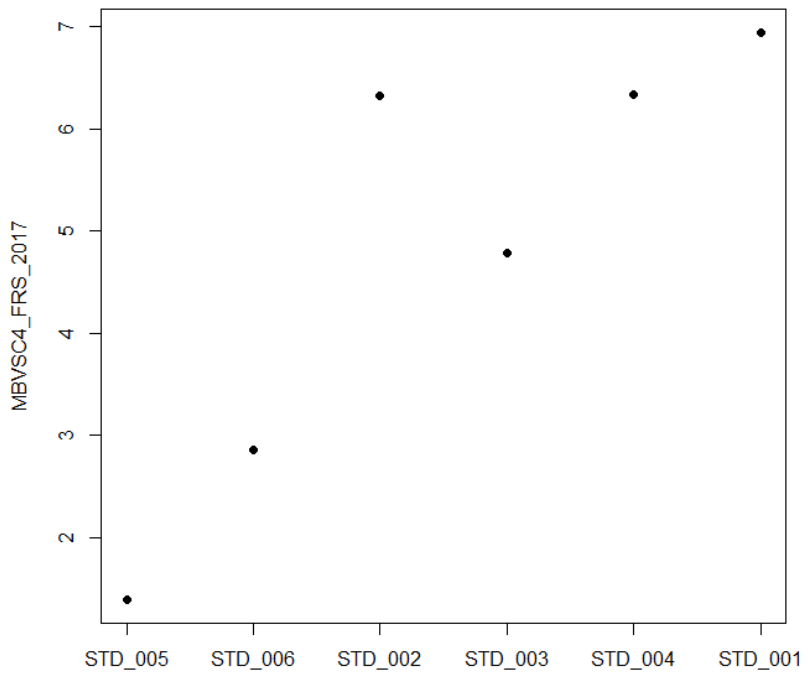




Complexity of the problem

- *What is drought tolerance?*
- *Which molecular mechanisms are active?*
- *Which additional variables are influencing the metabolite concentrations?*

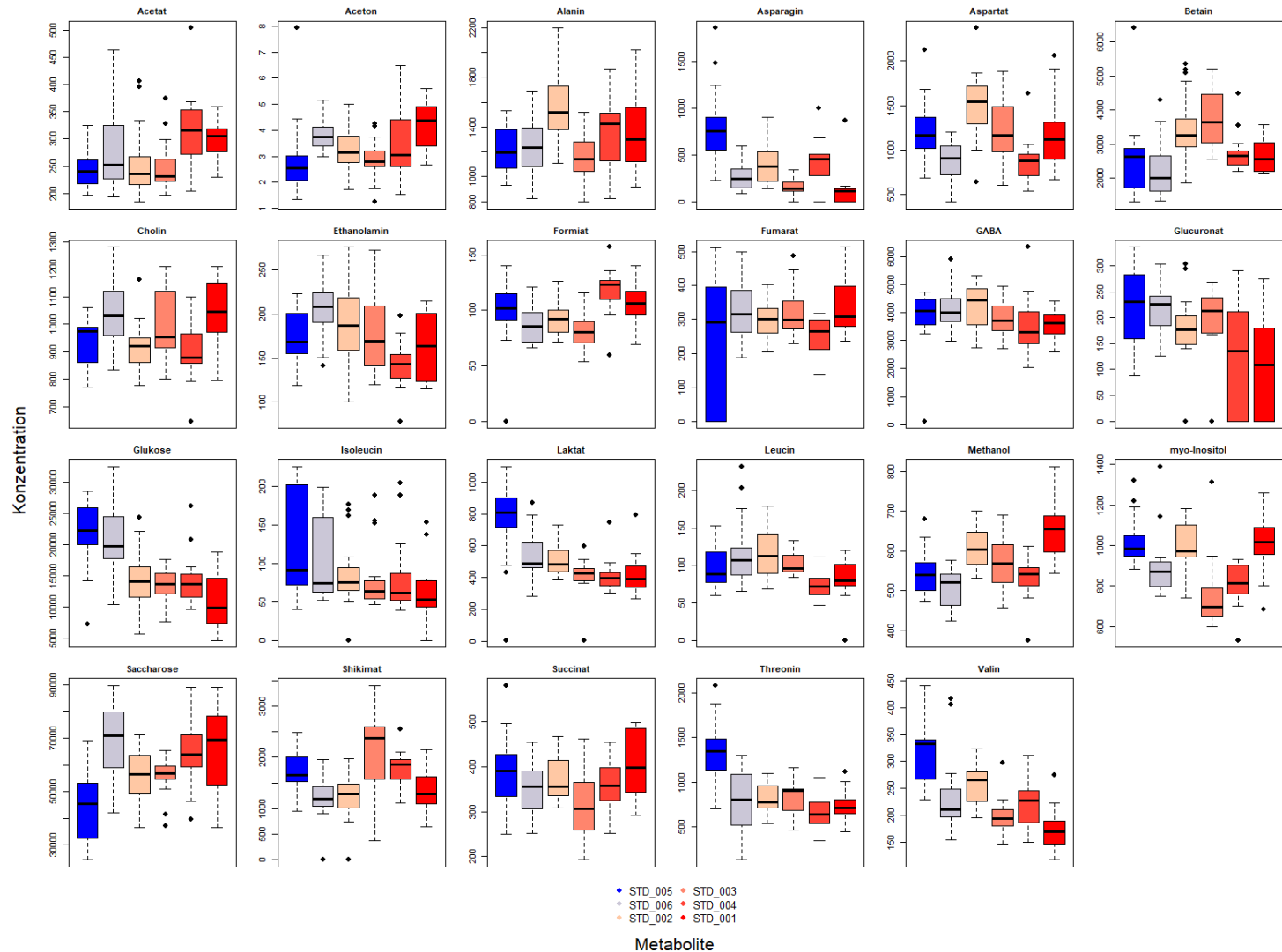
- *Chance or burden?*





Single metabolites vs tolerance – standard genotypes

- *Boxplots of selected metabolite concentrations depending on standard genotype (sorted by tolerance class)*

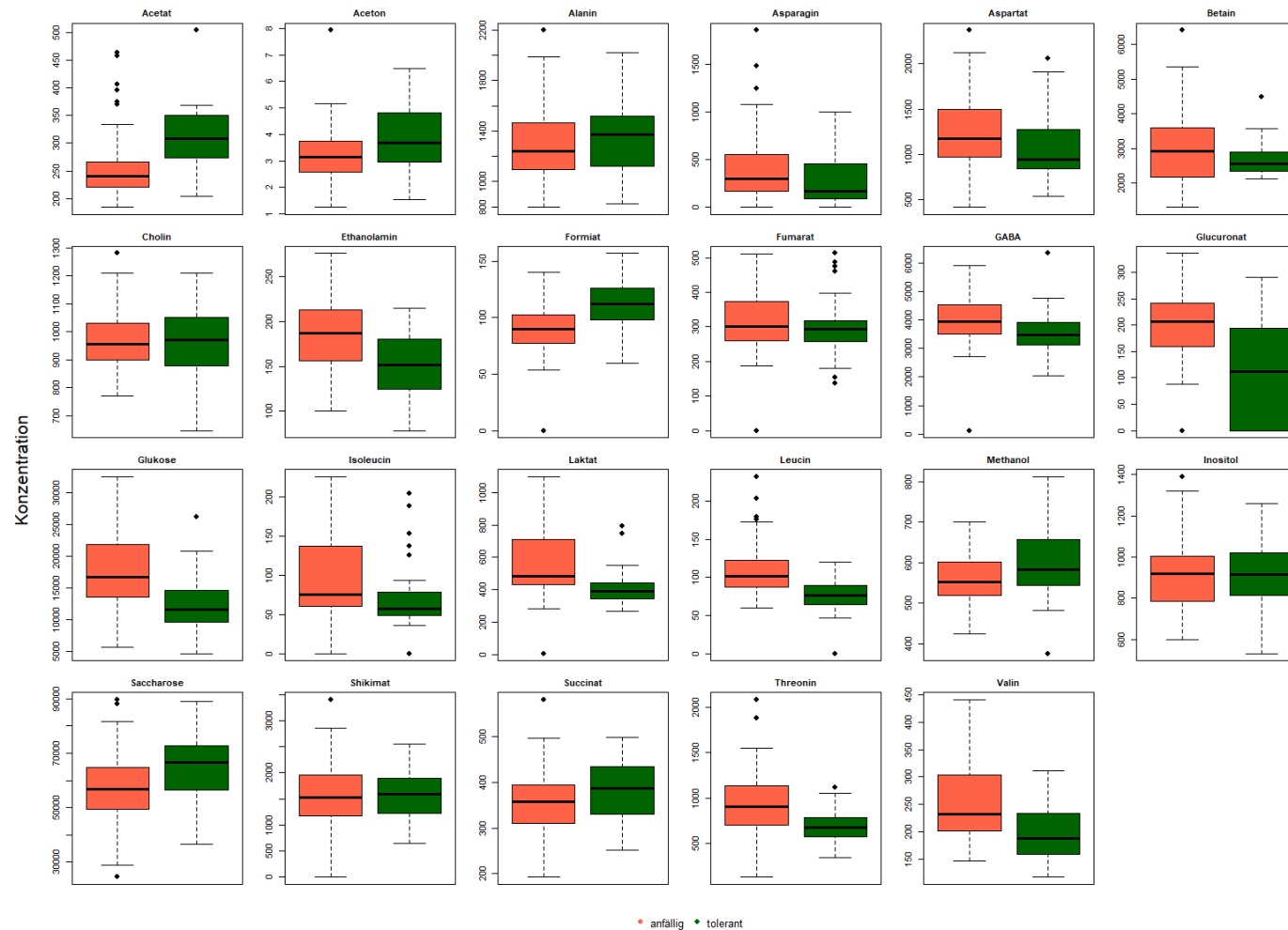


- Left to right:
- *Highly susceptible*
 - *Susceptibel*
 - *2x Moderately tolerant*
 - *2x tolerant*



Combination of field score and metabolites – standard genotypes

- Boxplots of selected metabolite concentrations depending on classification based on Total-Tolerance-Score (cut-off above the 2 moderate tolerant standards)

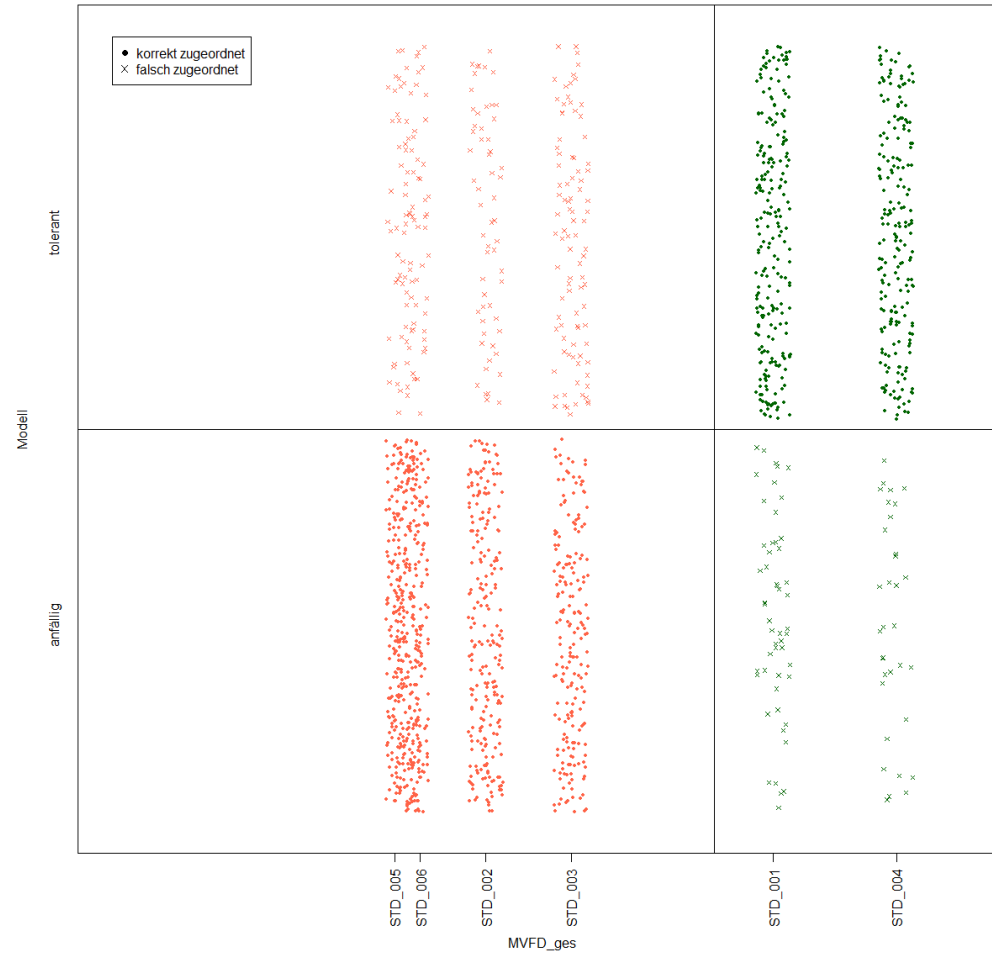


MVFD_ges, cutoff 0.35, Anzahl Tolerante: 34, Anzahl Anfällige: 71



Systemic metabolite markers – standard genotypes

- Classification performance based on Total-Tolerance-Score on an independent test set
- RandomForest (Acetate, Threonine, Isoleucine, Ethanolamine, Asparagine, Alanine)



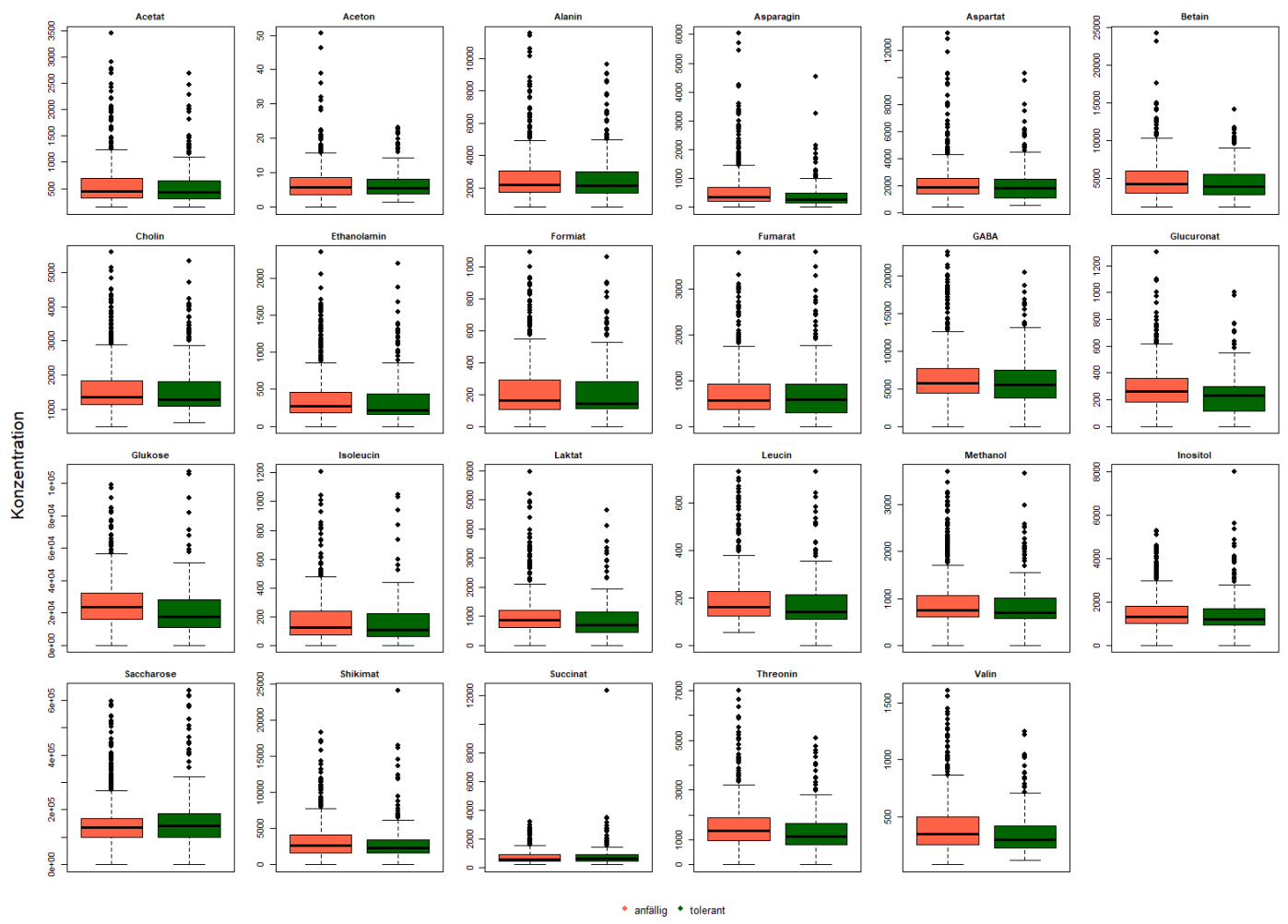
Correctly classified: 80%

TPR 84%

FPR 21%



Same story – considering all genotypes...



MVFD_ges, cutoff 0.35, Anzahl Tolerante: 189, Anzahl Anfällige: 487

Correctly classified: 57%

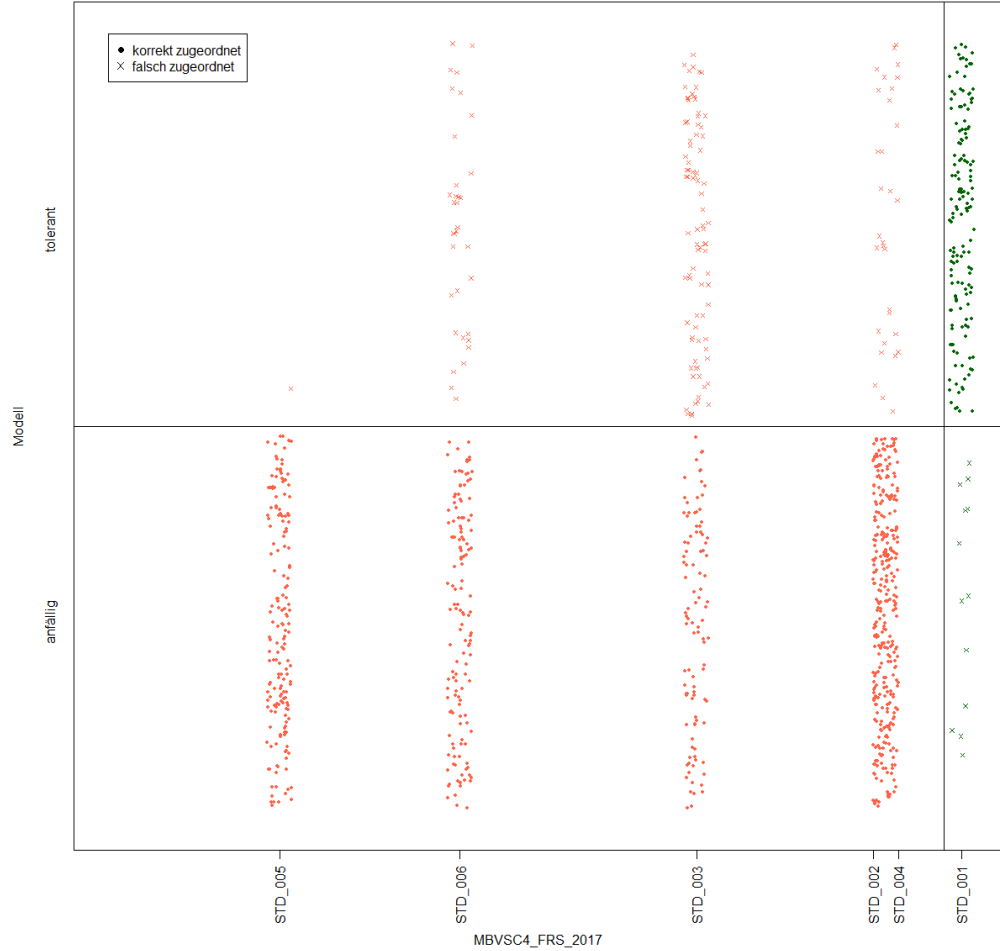
TPR 70%

FPR 48%



Step back again...

- Classification performance based on “production of mass pre 4th cutting/scored” on an independent test set
- RandomForest (Asparagine, Valine, Glucose, Inositol)



Correctly classified: 85%

TPR 93%

FPR 17%



- lifespin offers efficient tool and service for metabolite quantification (single target to complete metabolite profile)
- Metabolite profiles provide valuable information for biomarker research, understanding of mechanisms, relations between samples, genotypes, populations, ...
- Metabolite profiles combined with “AI” enable elite selection and metabolic prediction, however, many variables and influences are to be considered



Thank you

Gefördert durch:



aufgrund eines Beschlusses
des Deutschen Bundestages



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