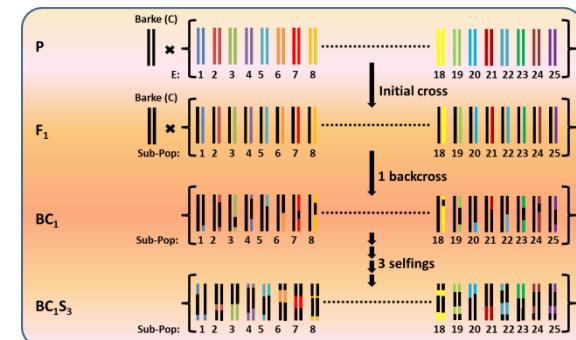


Methoden und Potenziale der Datenverarbeitung

Umsetzungsstrategien in Pflanzenbau, Pflanzenernährung und Pflanzenzüchtung



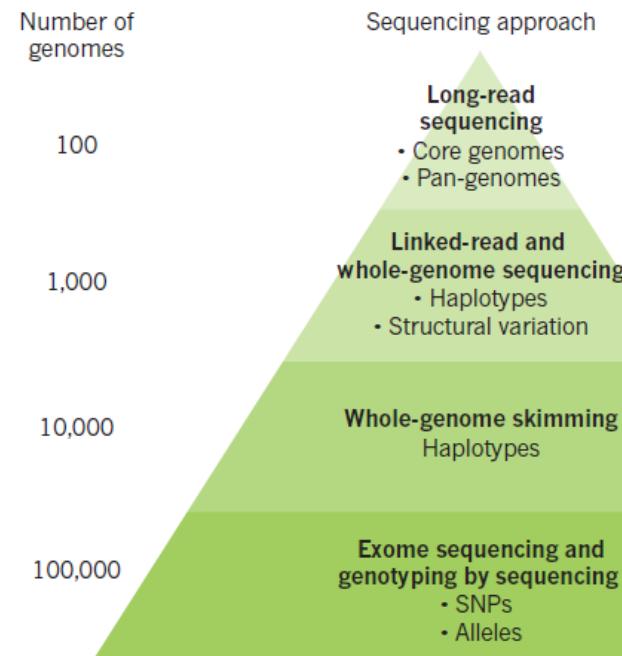
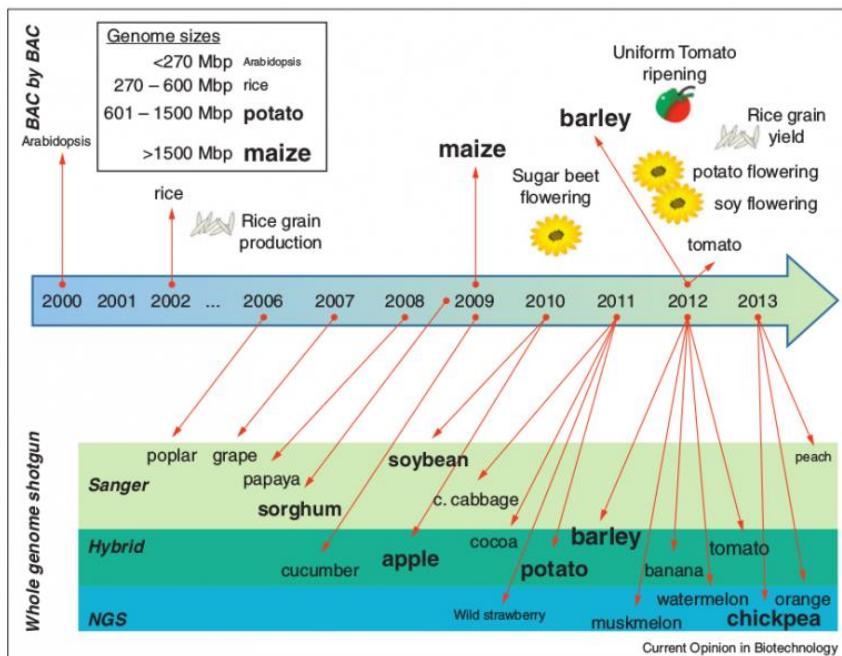
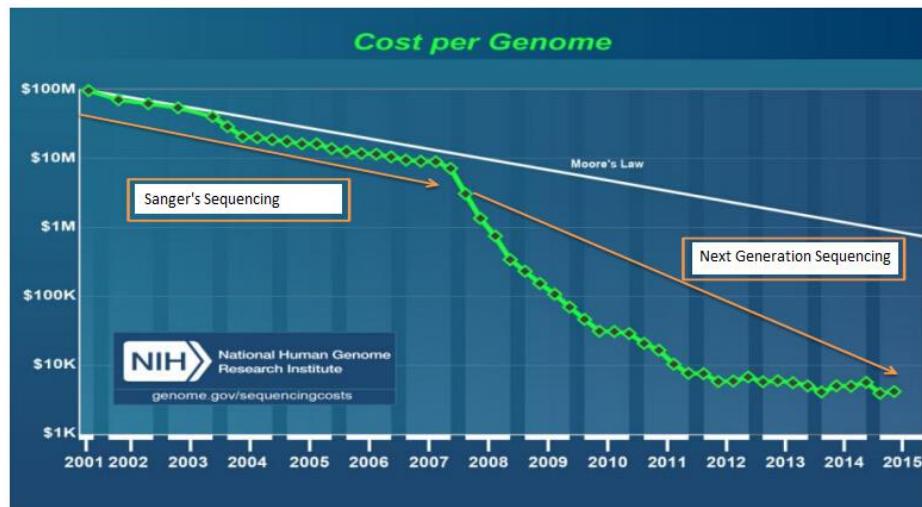
**Prof. Dr. Klaus Pillen
MLU Halle**



Methoden der Datenverarbeitung

1. Genomsequenzierung (NGS)
2. Genomische Prädiktion (GP)
3. Genom-Editierung (GE)
4. Hochdurchsatzphänotypisierung (HTPP)
im Feld und im Gewächshaus
5. Modellierung (*Machine Learning, ML*)
6. Datenintegration Systembiologie
(Genom → Transkriptom → Proteom → Metabolom → Phänom)

1. Genomsequenzierung: Sanger vs. NGS



1. Genomsequenzierung:

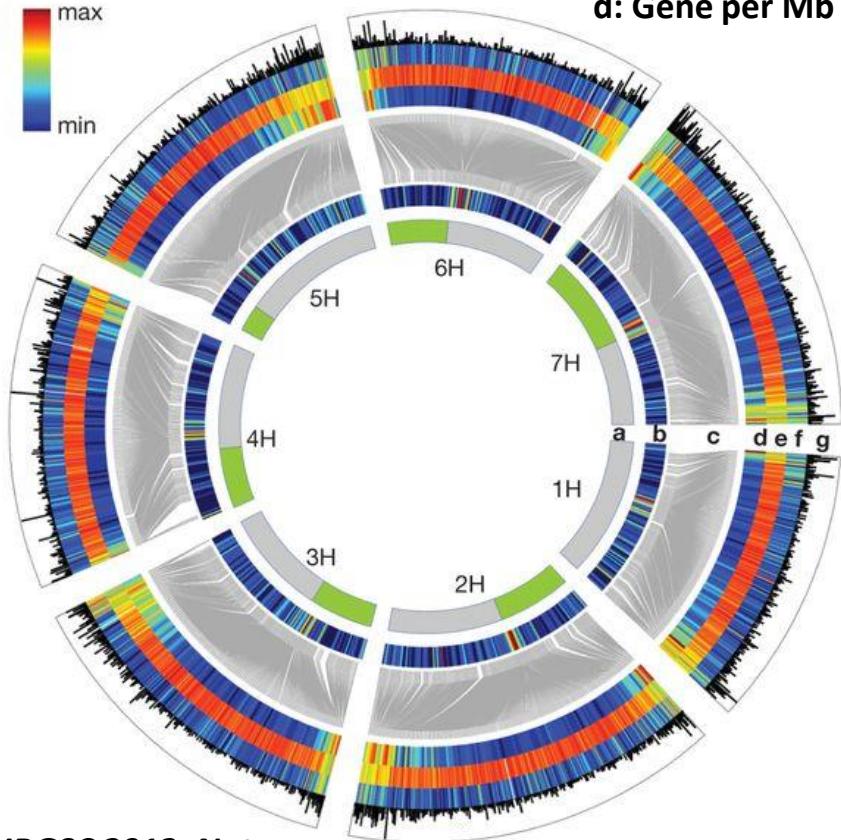
Nachweis von Allel-Fixierungen im Gerstengenom

Gerstengenom

Größe: 4,8 Gb

Kodierend: 65,3 Mb (=1,4%)

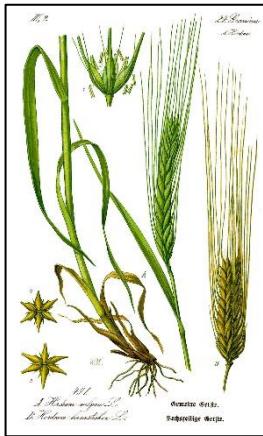
Gene: 39.734



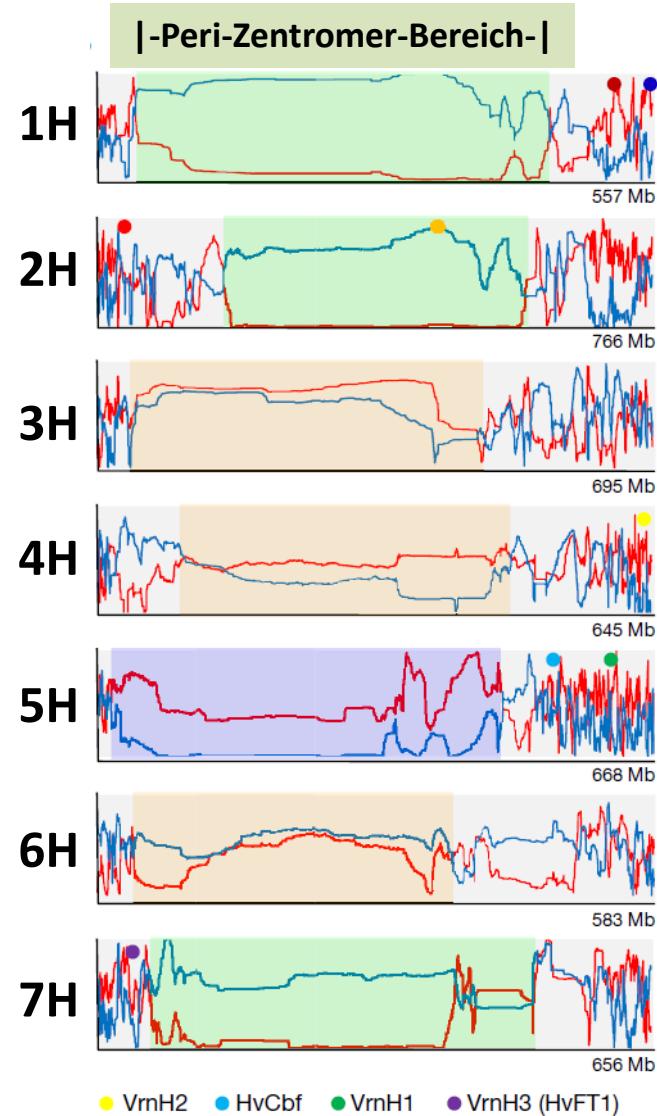
IBGSC 2012, Nature

Kreise:

- b: Gene per cM
- d: Gene per Mb



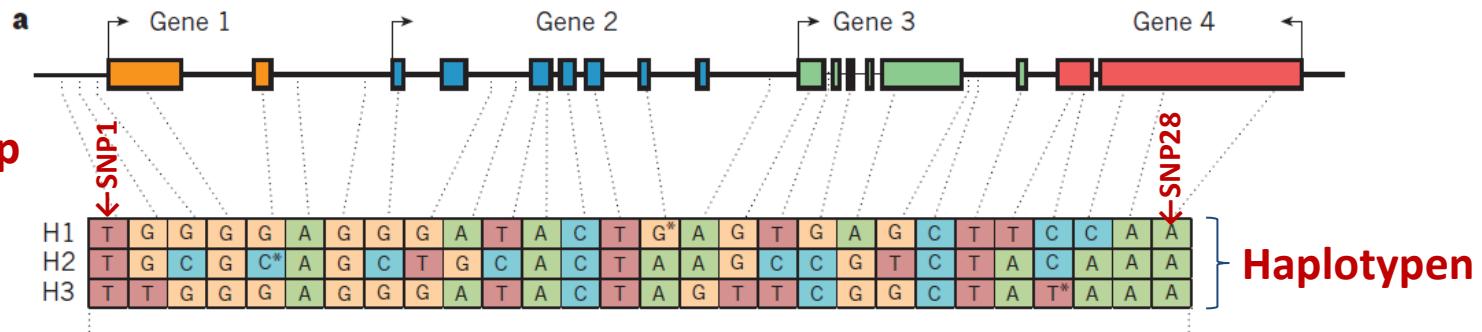
Diversität in:
– Wintergersten
– Sommergersten



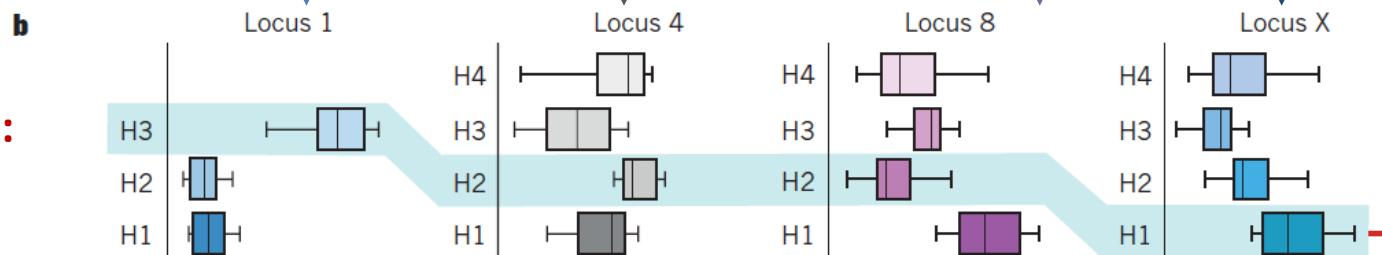
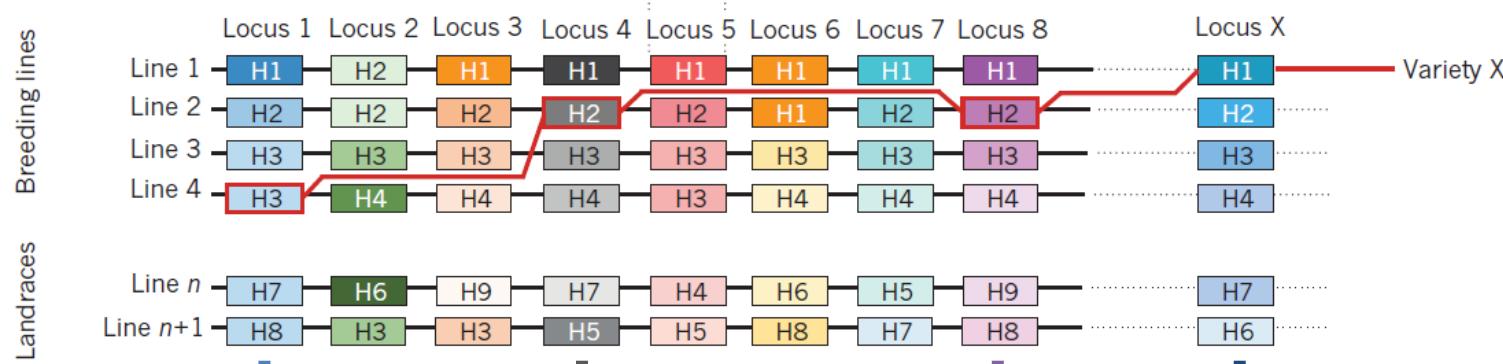
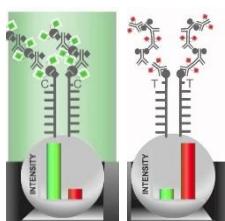
1. Genomsequenzierung: *Haplotype calling*



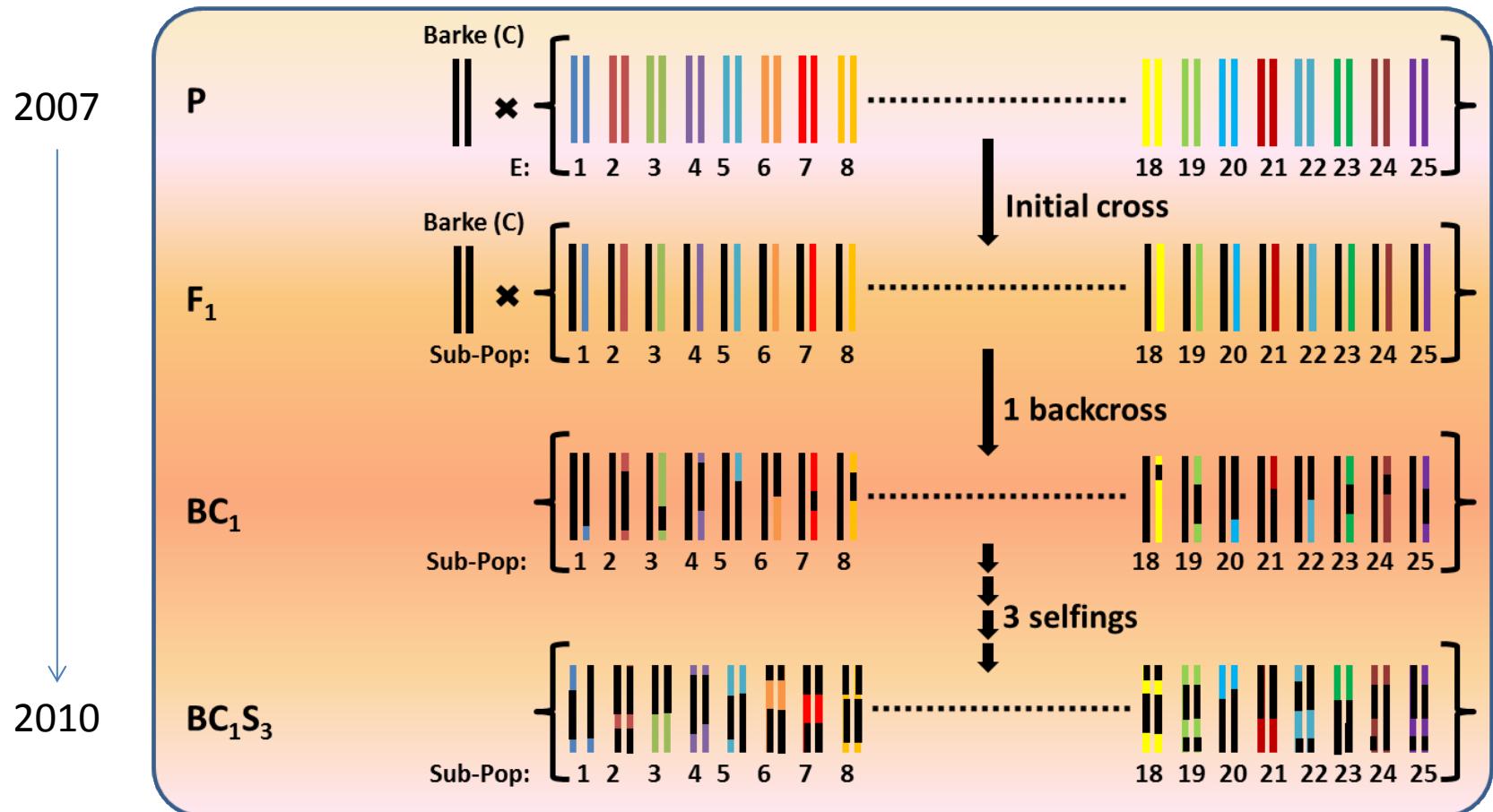
Locus 5 = 4 Gene mit 28 SNPs definieren 3 Haplotypen



iSELECT SNP-chip



Die nested association mapping (NAM)-Population HEB-25 besteht aus 1.420 Genotypen

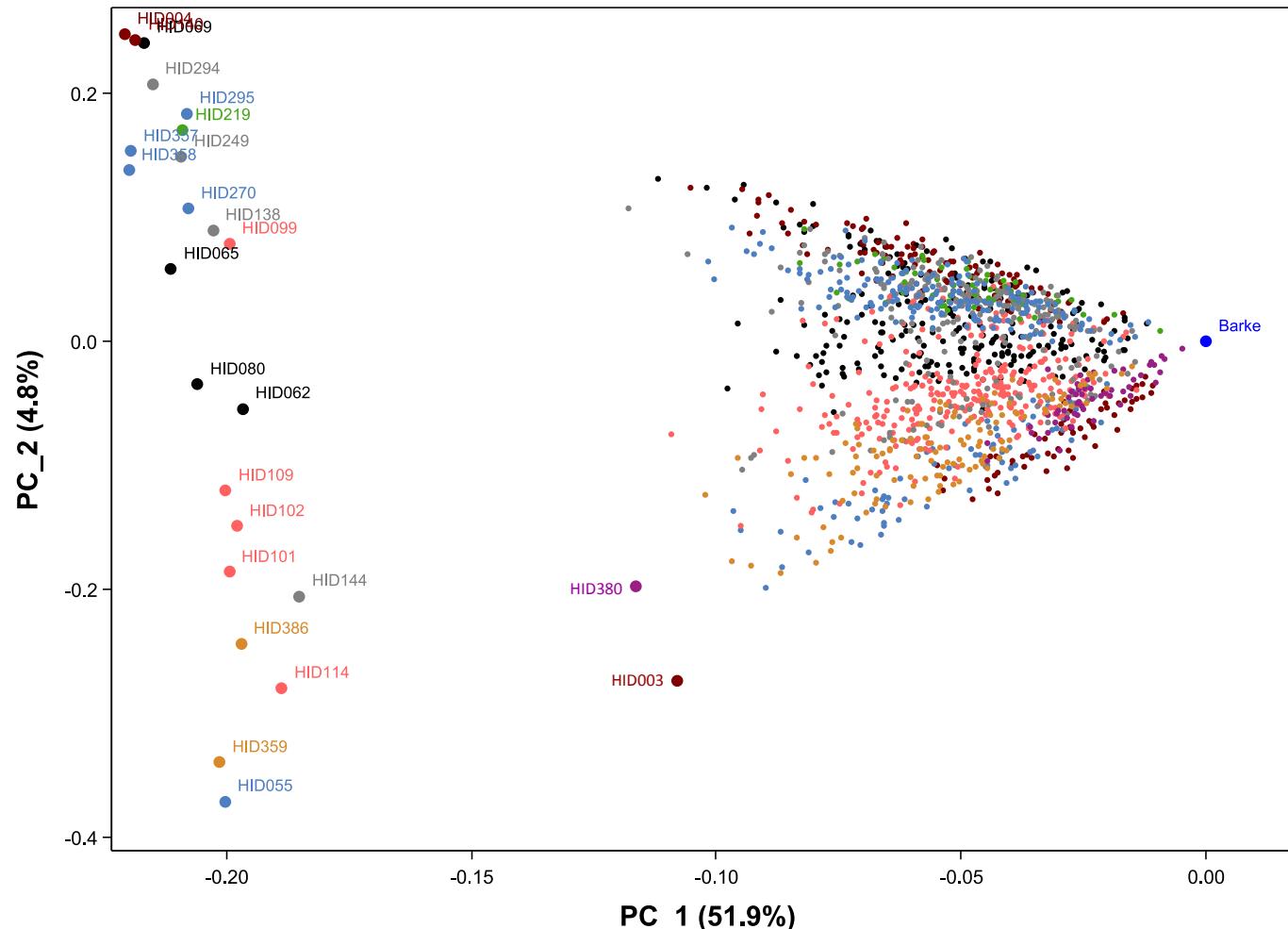


HEB-25 (Halle exotic barley) = 25 Familien mit 1.420 NAM lines in BC₁S₃

Erwartete Segregation pro Locus: 72% : 6% : 22% (Hv : het : Hsp)

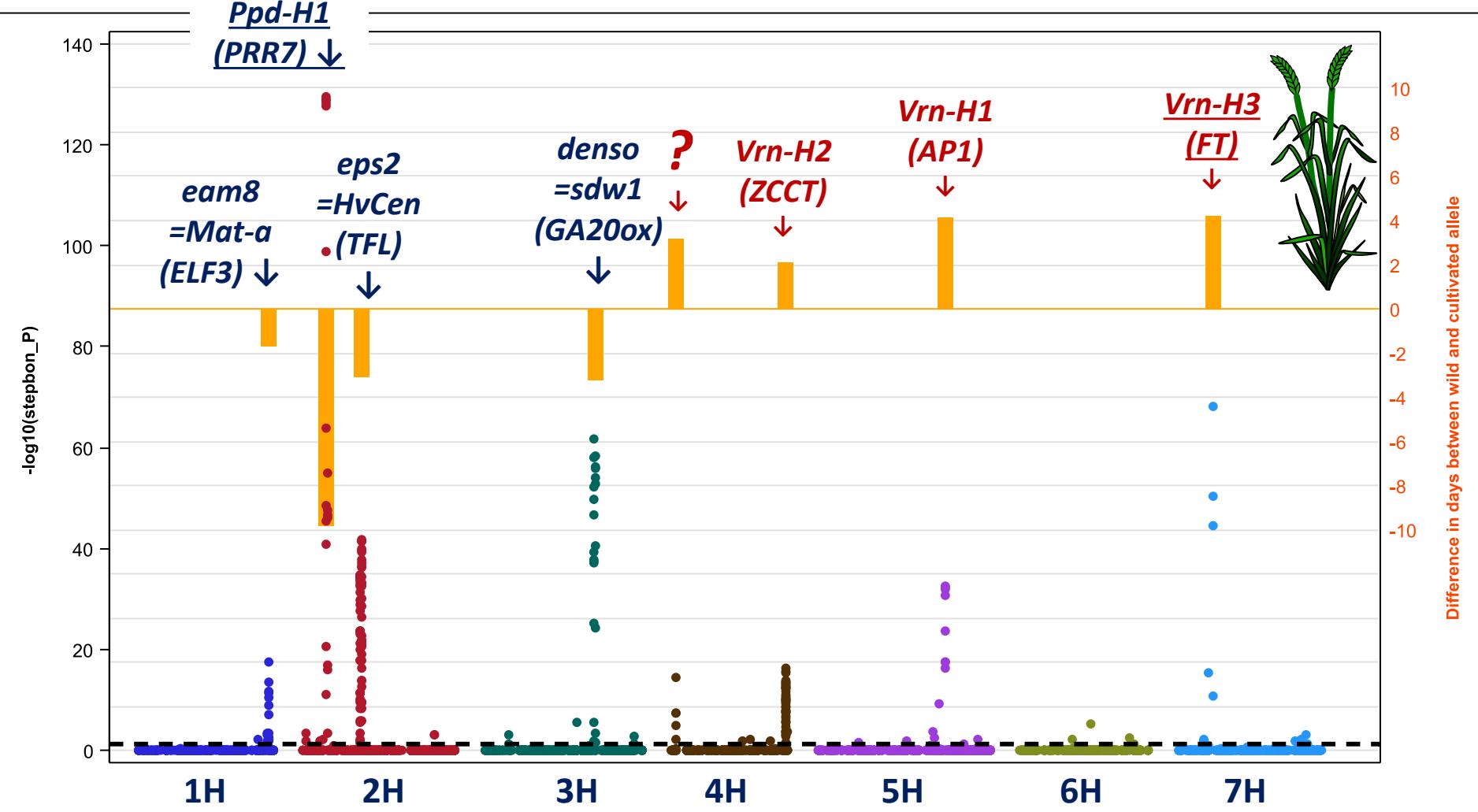
Maurer et al. 2015, BMC Genomics

Genetische Diversität von 1.420 HEB-Linien, Barke und 25 HEB-Donoren, geschätzt mit 5.727 iSELECT SNPs



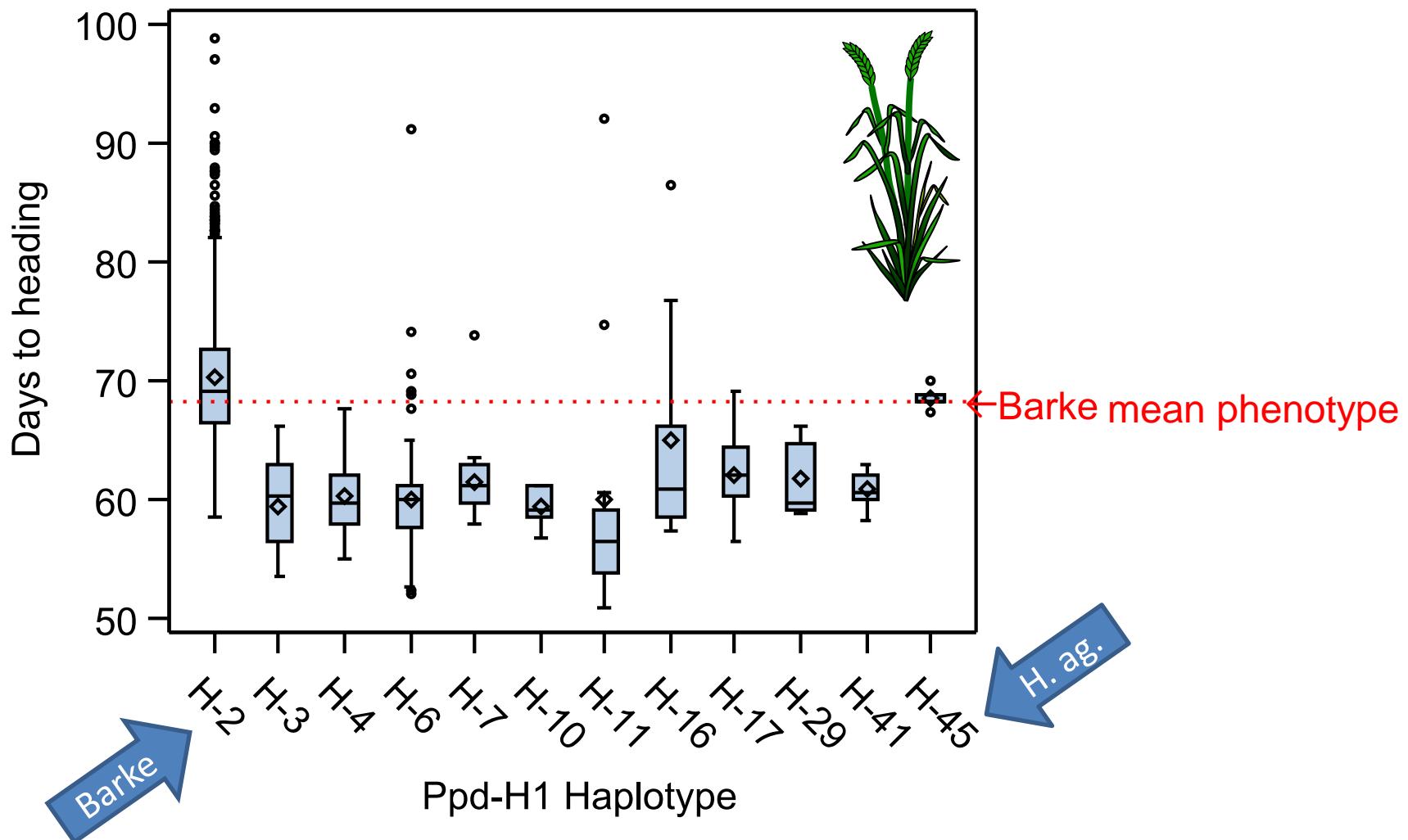
Maurer et al. 2015, BMC Genomics

Der GWAS Manhattan Bi-Plot lokализiert 8 Haupt-QTL, welche den Blühzeitpunkt in HEB-25 kontrollieren



Maurer et al. 2015, BMC Genomics

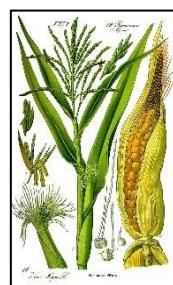
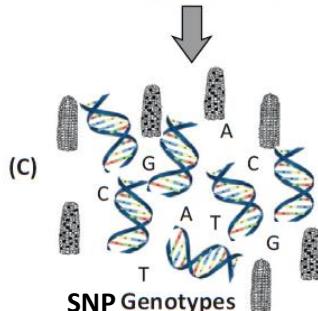
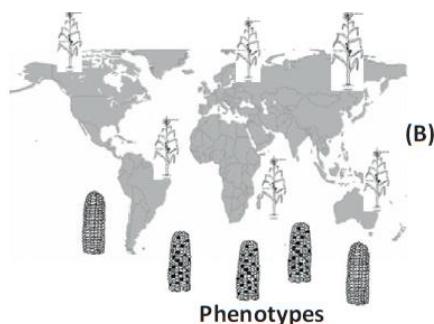
11 Ppd-H1-Haplotypen zeigen allelische Variation in der Blühzeitpunkt-Antwort ($\Delta_{MAX} = 10$ Tage)



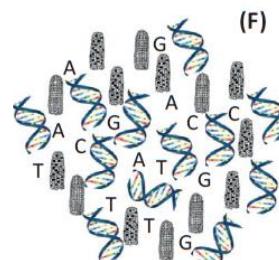
Maurer et al. 2015, BMC Genomics

2. Genomische Prädiktion (GP)

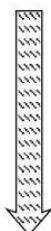
Trainingspopulation



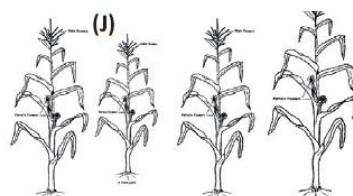
Selektion
nach GEBV



(K)
Bayes
Ridge
regression
GBLUP
.....
(A) Estimation GEBV

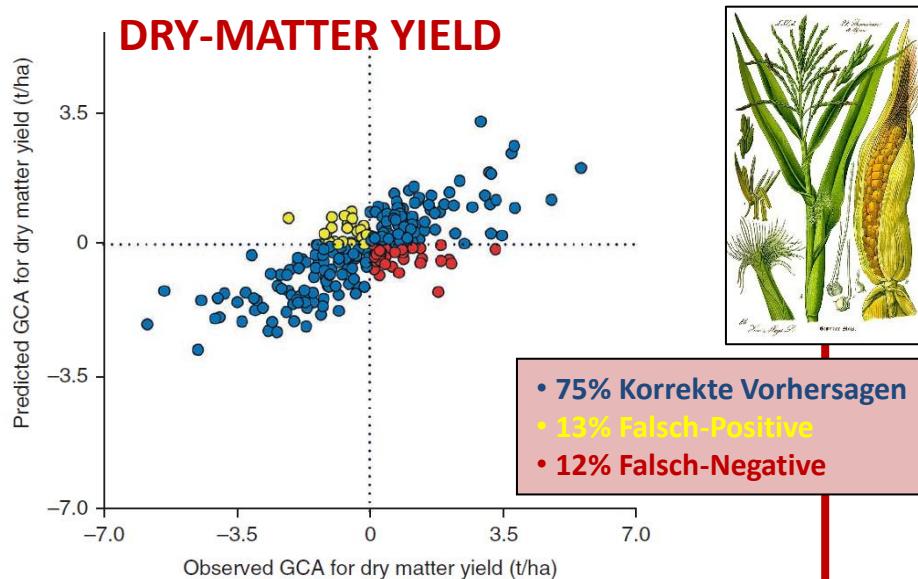


Selection of individuals based on
Genomic estimated Breeding Value (GEBV)



TRENDS in Biotechnology

2. Genomische Prädiktion (GP): Vorhersage des Mais-Ertrags mit SNPs bzw. Metaboliten



QTLs für 9 Metabolite, lokalisiert (rote Punkte) mittels GWAS

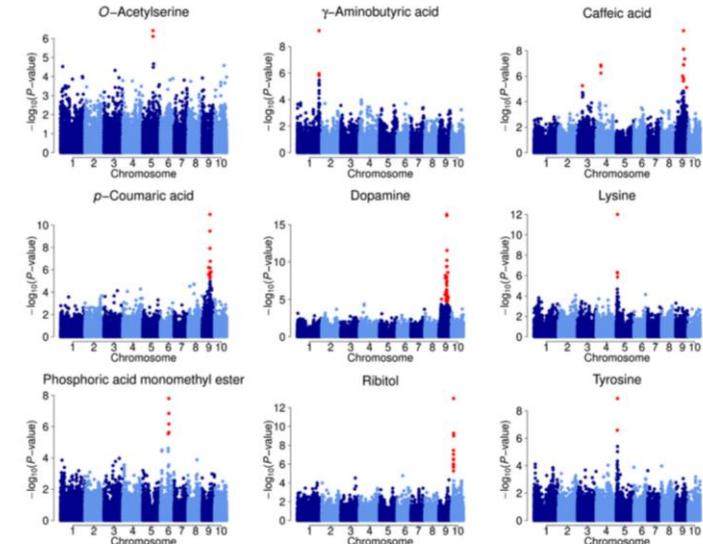


Table 1 Summary of whole-genome and metabolic prediction

GCA	h^2_{GCA}	w_M^2	SNPs (N=37k)		Metabolites (N=130)			
			$r_{(g,y)}$	$r_{(g,g)}$	s.d.	$r_{(g,y)}$	$r_{(g,g)}$	s.d.
Dry matter yield	0.89	0.73	0.74	0.78	0.07	0.48	0.60	0.11
Plant height	0.95	0.72	0.70	0.72	0.06	0.52	0.63	0.10
Dry matter concentration	0.96	0.72	0.78	0.80	0.07	0.66	0.79	0.06
Female flowering	0.98	0.71	0.80	0.81	0.06	0.67	0.80	0.07
Starch content	0.93	0.73	0.70	0.73	0.07	0.59	0.71	0.07
Sugar content	0.94	0.74	0.69	0.72	0.06	0.55	0.67	0.09
Lignin content	0.82	0.73	0.72	0.80	0.05	0.50	0.64	0.10

Predictive abilities $r_{(g,y)}$ and prediction accuracies $r_{(g,g)}$ averaged over all cross-validation runs and their s.d. are shown for models using either SNPs or metabolites. Heritabilities of the predicted traits (h^2_{GCA}) are given as well as the repeatabilities of the used metabolic profile (w_M^2) calculated as the weighted sum of the repeatabilities of the individual metabolites (see Online Methods).

Riedelsheimer et al. 2012, Nature

Riedelsheimer et al. 2012, PNAS

2. Modellierung: Machine Learning (ML)

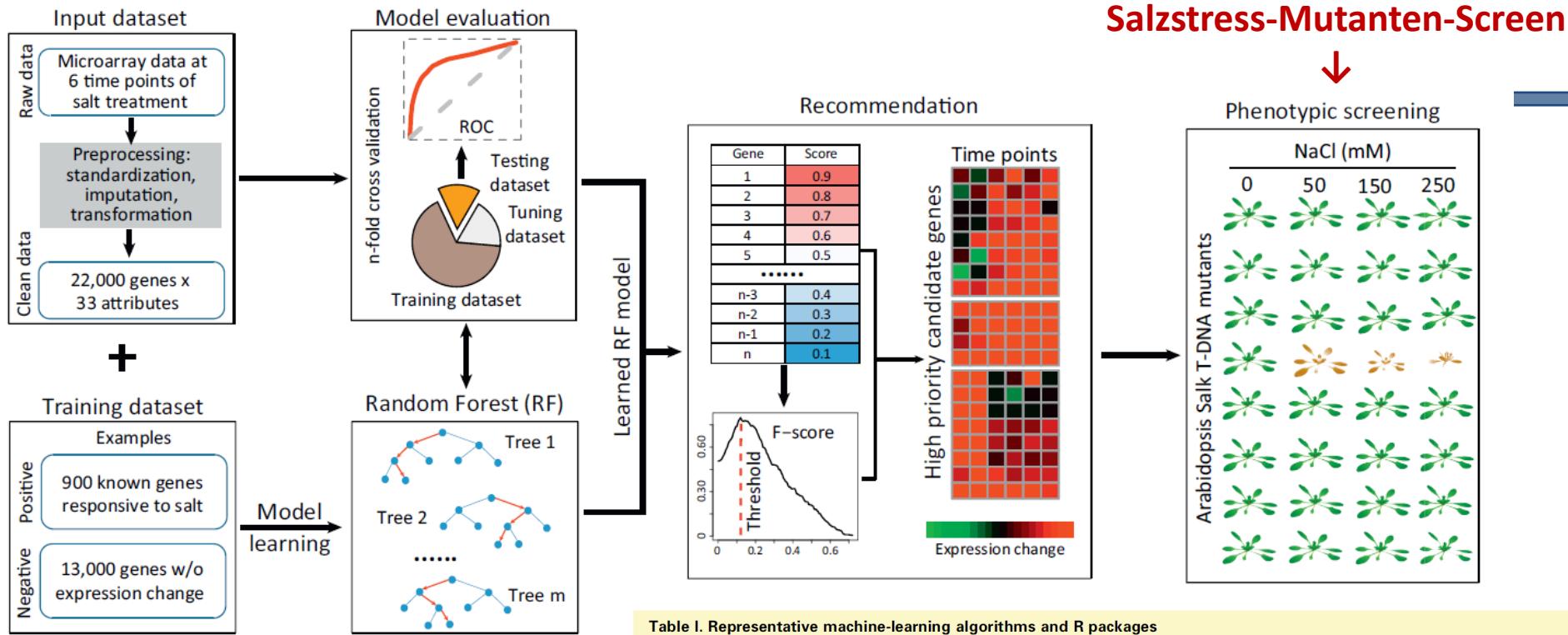
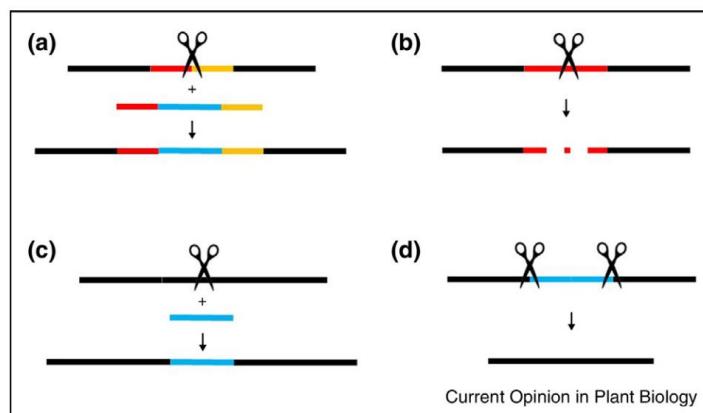
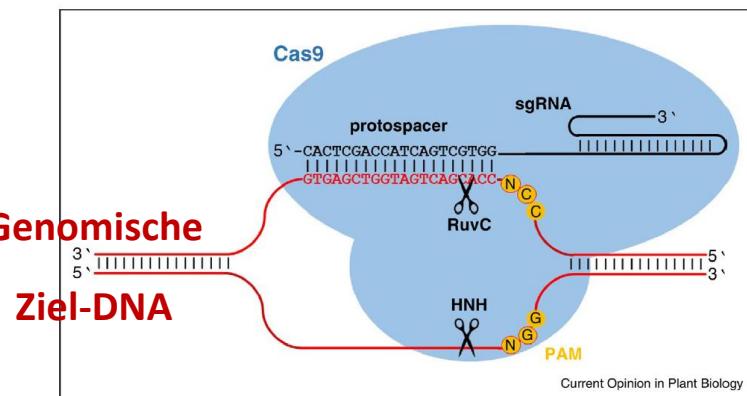


Table I. Representative machine-learning algorithms and R packages

Learning tasks	Algorithms and methods	Software packages in R
Classification	Nearest neighbor methods, linear discriminant analysis, quadratic discriminant analysis, logistic regression, naive Bayes classifier, support vector machine (SVM), classification trees, neural nets	knn, knn1, glm2, kernsvm, svmpath, CART, e1071, nnet, gbm, tree, randomForest, sda, rda, penalizedLDA
Regression	Least squares, linear models, ridge regression, additive models, generalized additive model, nearest neighbor methods, regression trees, project pursuit regression kernel methods, local regression, splines, wavelet smoothing, Bayesian models	lm, gam, knn, splines, locfit, mgcv, polspline, earth, cosso
Clustering	K-means clustering, spectral clustering, hierarchical clustering, self-organizing maps, association rules, multidimensional scaling, independent component analysis, local multidimensional scaling	Kclust, cluster, fastcluster, sparseBC, sparcl, pvclust
Feature selection	Best subset selection, forward selection, least angle regression, shrinkage methods: lasso, elastic net, group lasso, fused lasso, sure independence screening	Regsubsets, LAR, glmnet, elasticnet, glmpath, gllasso, Sparsenet, penalizedLDA
Dimensionality reduction	Principal component analysis (PCA), factor analysis, kernel PCA, partial least squares	pca, pls, mda, elasticnet, ipca
Ensemble learning	Boosting methods, bagging, random forest, additive regression	Adaboost, randomForest, ada, adabag, erboost, mrt
Network analysis	Gaussian graphical models, Bayes networks	bnlearn, JGL, GGMselect, lgsm, gRain, gRim, gRbase
Density estimation	Kernel density estimation	KernSmooth

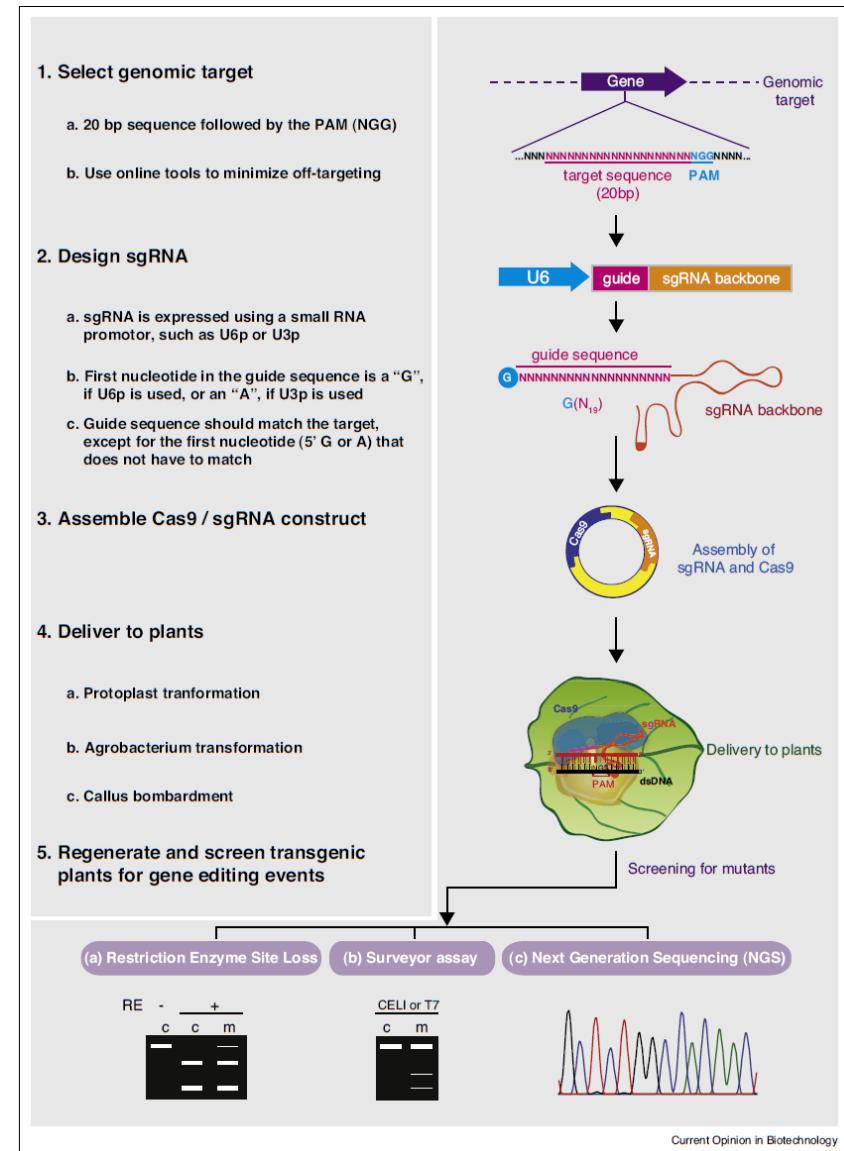
3. Genom-Editierung (GE)

Die CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats)



a) Homologe Rekombination (HR)
b-d) Nicht-homologe Endpaarung (NHEJ)

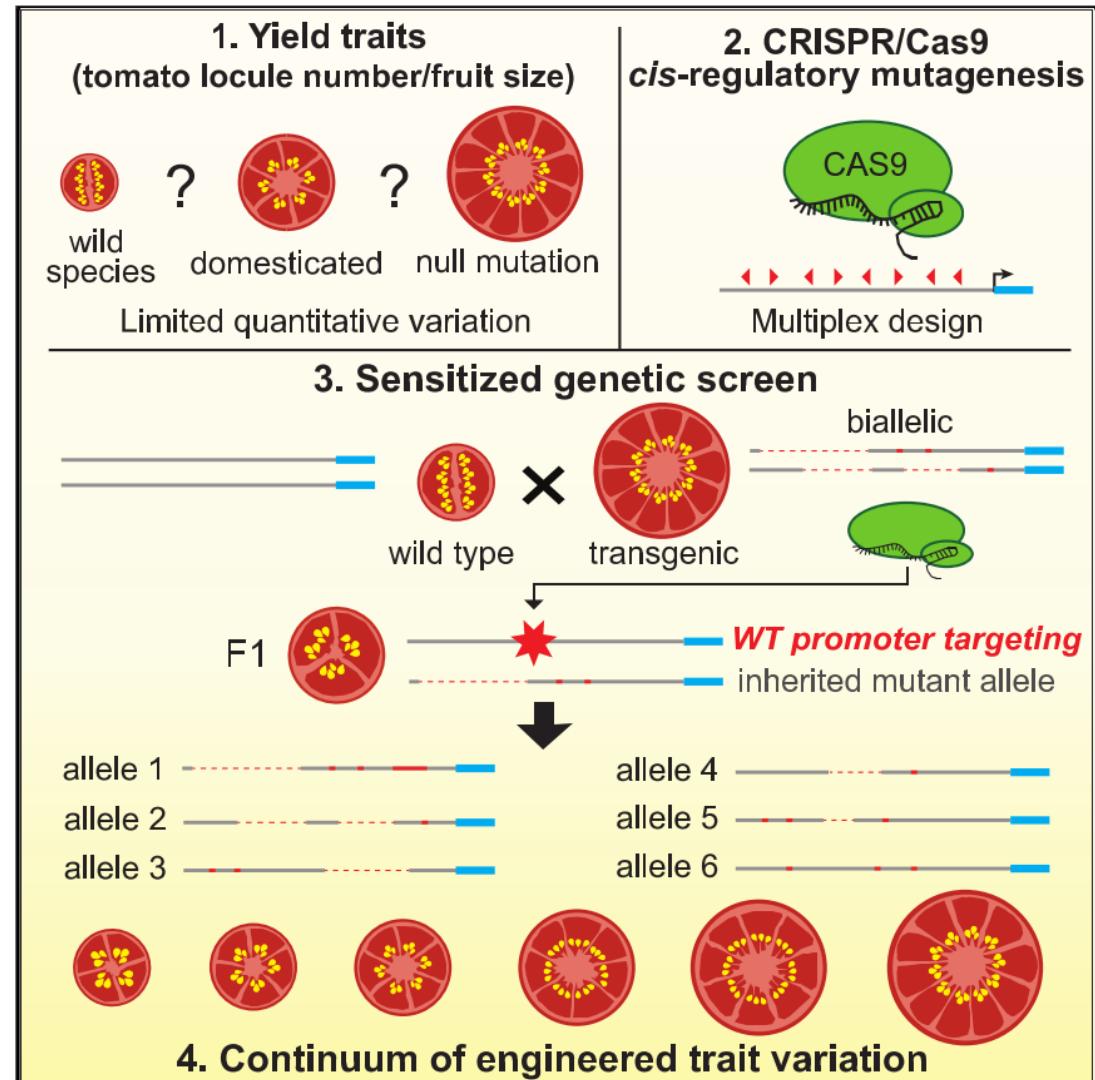
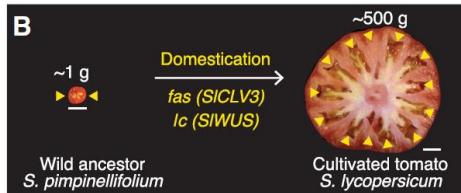
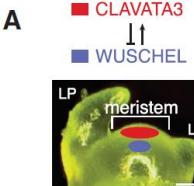
Puchta 2017, Curr Op Plant Biol



Belhaj et al. 2015, Curr Op Biotech

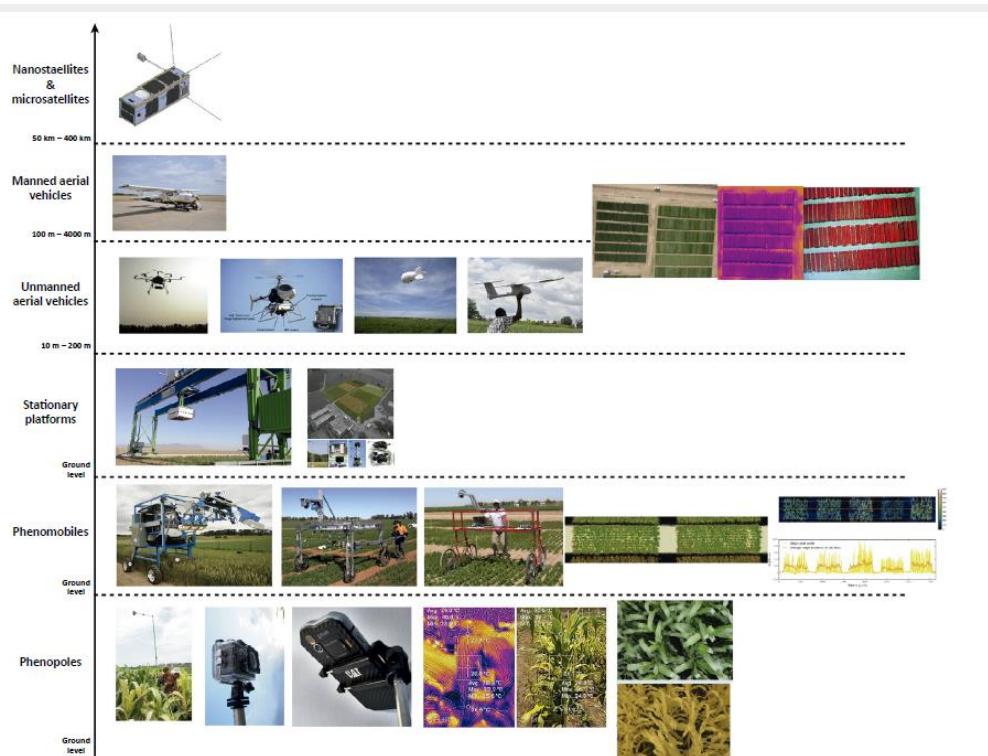
3. Genom-Editierung:

Zunahme der Tomaten-Fruchtgröße durch Variation der Promotorsequenz des *CLAVATA 3*-Gens

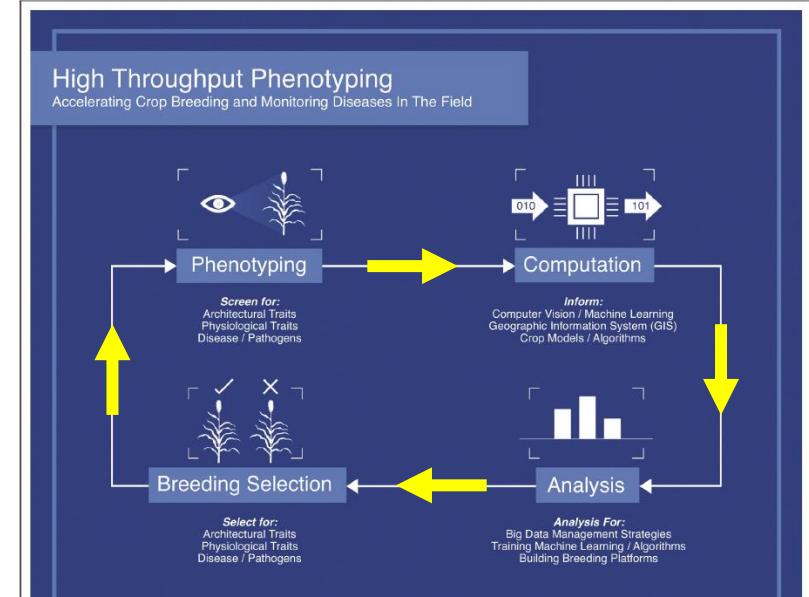


Rodriguez-Leal et al. 2017, Cell

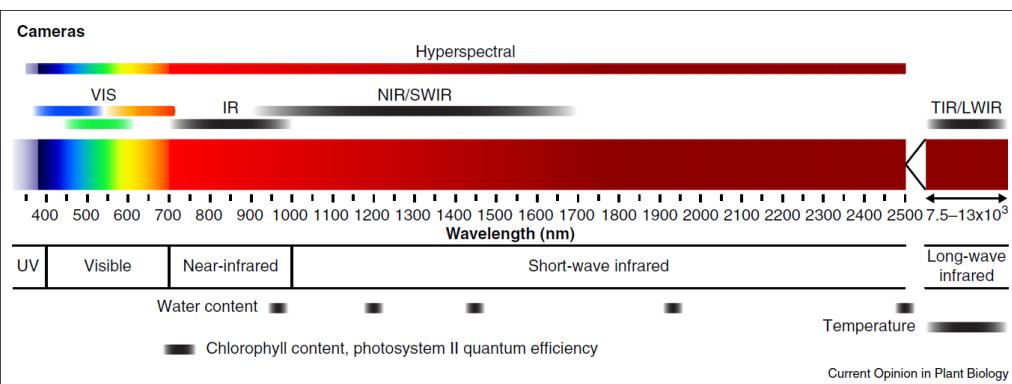
4. Hochdurchsatz-Phänotypisierung im Feld



Araus et al. 2018, Trends Plant Sci



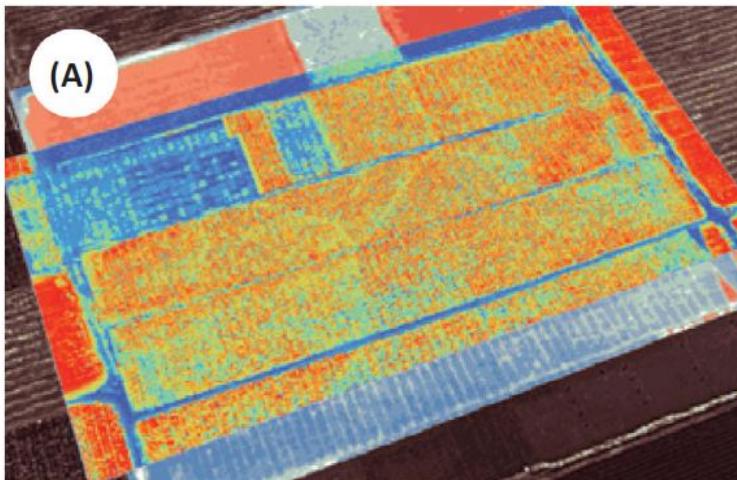
Shakoor et al. 2017, Curr Op Plant Biol



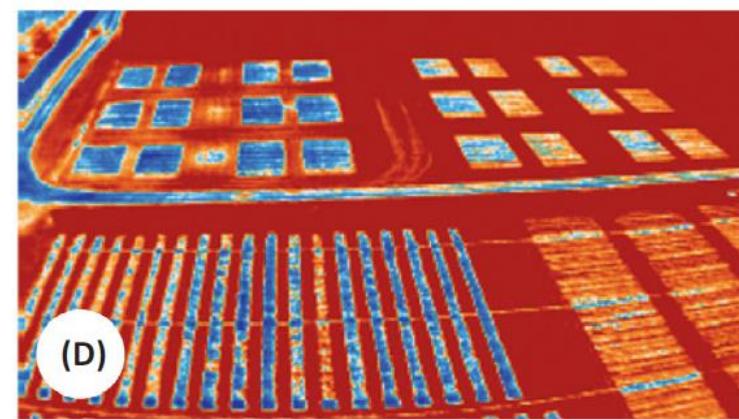
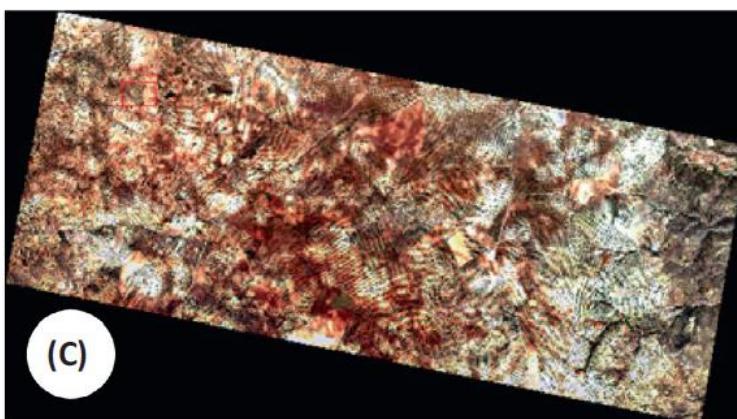
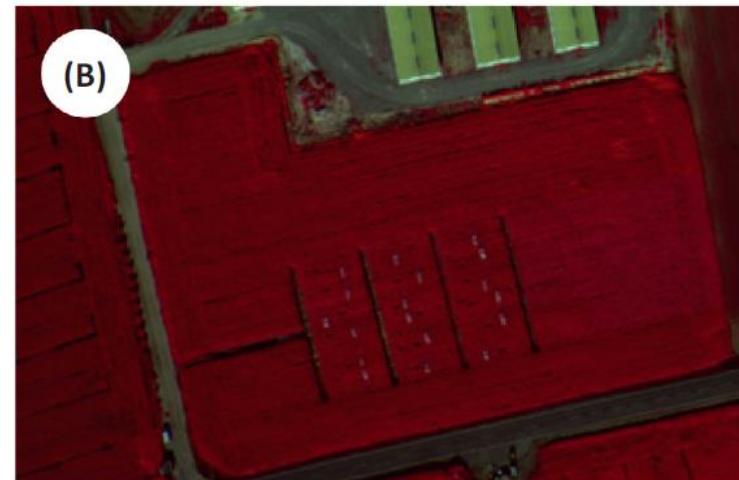
Falgren et al. 2015, Curr Op Plant Biol

4. Hochdurchsatz-Phänotypisierung im Feld

RGB/CIR Kamera (\rightarrow NDVI)
(Mono lens camera ADC Lite)



Multispektral-Kamera (\rightarrow Pflanzenentwicklung)
(Six lens camera miniMCA-6)



Hyperspektral-Kamera (\rightarrow Inhaltsstoffe)
*(Microhyperspec VNIR,
923 bands, 380-1000 nm)*

Thermal-Kamera (\rightarrow Blatttemperatur)
 $(8 - 14 \mu\text{m})$
 $-20 - 200 ^\circ\text{C}$

Araus et al. 2014, Trends Plant Sci

AgRover-Versuche Halle 2016, HEB-25

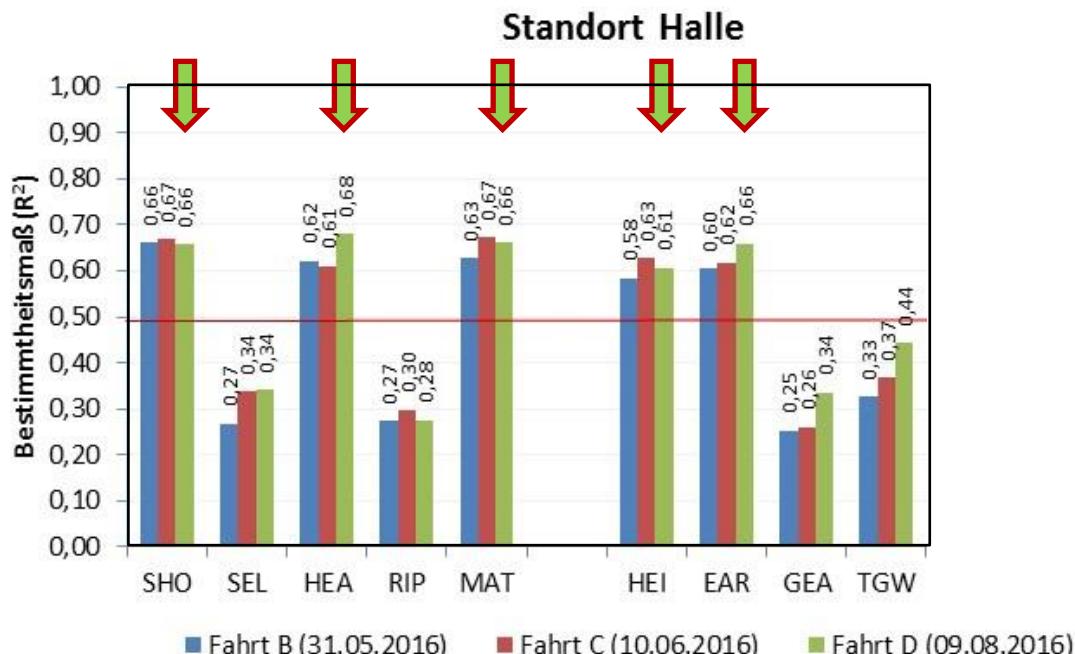


AgRover HEB-25



HySpex
SWIR 384 (1000-2500 nm)

Abk.	Merkmal
SHO	Schossen (BBCH 31)
SEL	Vegetative Phase
HEA	Blüte (BBCH 49)
RIP	Generative Phase
MAT	Reife (BBCH 87)
HEI	Pflanzenhöhe
EAR	Ähren pro qm
GEA	Kornzahl pro Ähre
TGW	Tausendkorgewicht

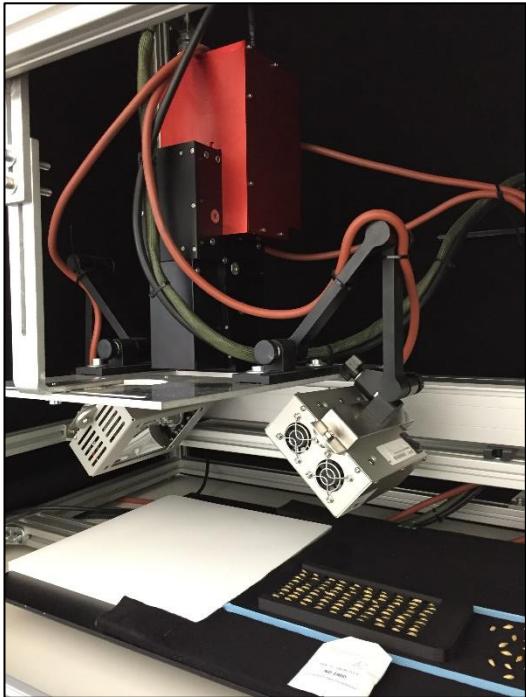


Bestimmtheitsmaße (R^2) für 9 agronomische Merkmale für die Messfahrten B (31.05.2016) und C (10.06.2016) und D (09.08.2016) am Standort Halle. Rote Linie: cut off bei $R^2=0,5$.

AgRover-Versuche Halle 2016, HEB-25

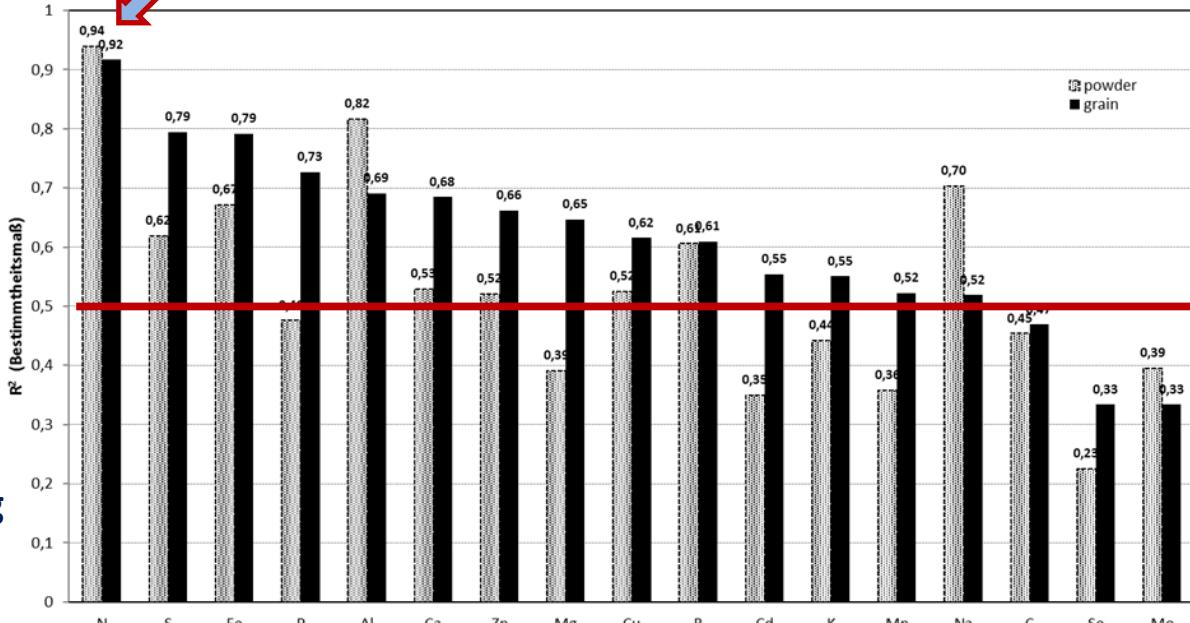
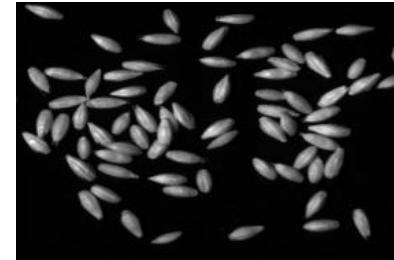
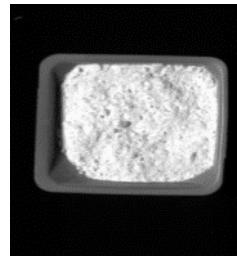


Hyspex HEB-25



HySpex
SWIR 384

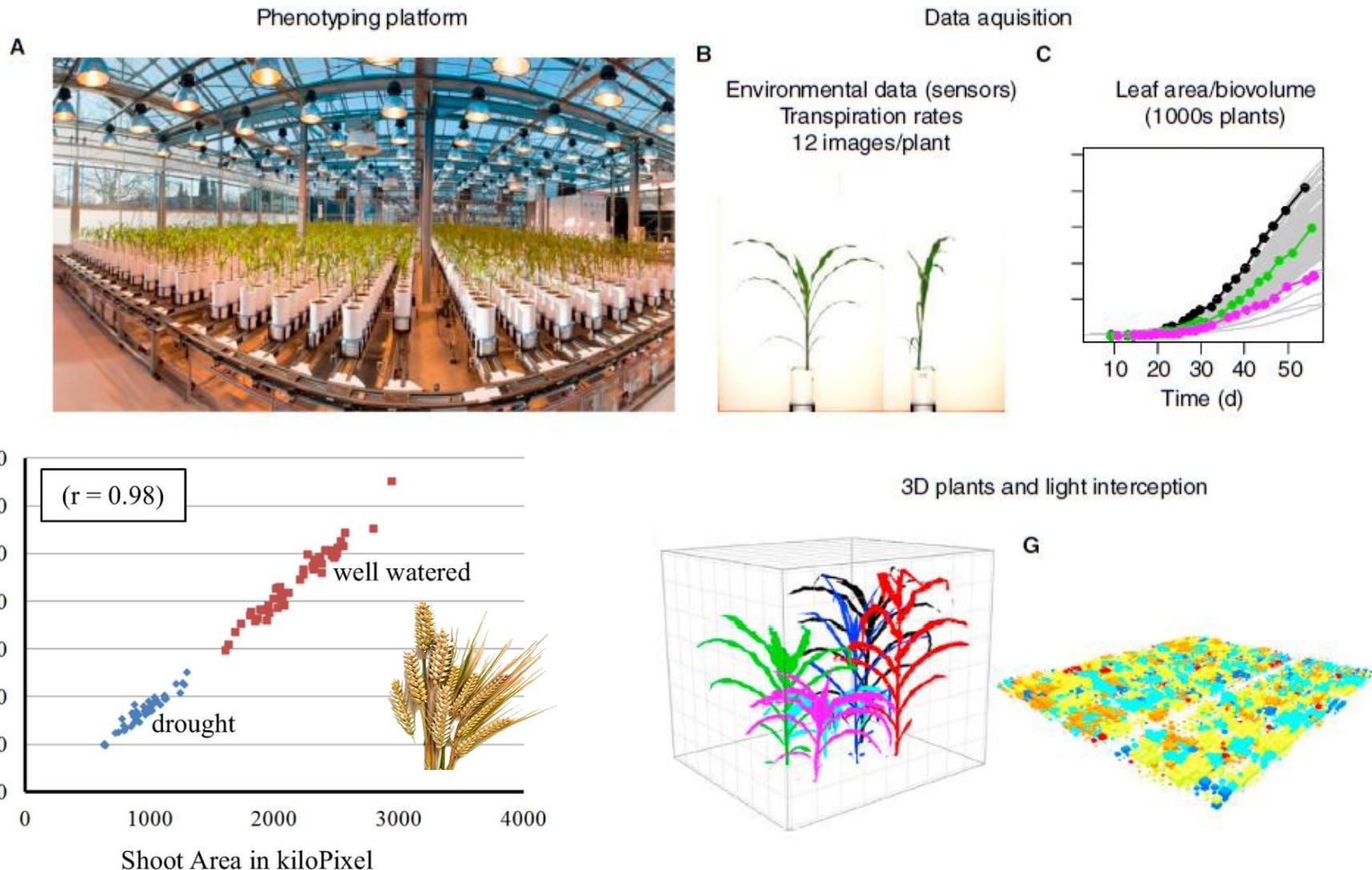
$$R^2_{(N)} = 0,94$$



Stationäre Hyspex-Messvorrichtung

Schätzgenauigkeit (R^2) der Gehalte von 17 Mikro- und Makronährstoffen im Ganzkorn bzw. im Mehl mittels Hyperspektralanalyse und nichtlinearer Modellierung, basierend auf CN-Analyzer bzw. ICP-OES-Analyse von Vergleichsproben. Bestimmung der Schätzgenauigkeit (R^2) mittels *leave one out* Validierung ($N=250$). Rote Linie: *cut off* bei $R^2=0,5$.

4. Hochdurchsatz-Phänotypisierung im Gewächshaus



Honsdorf et al. 2014, PLOS ONE

Tardieu et al. 2017, Curr Biol Rev

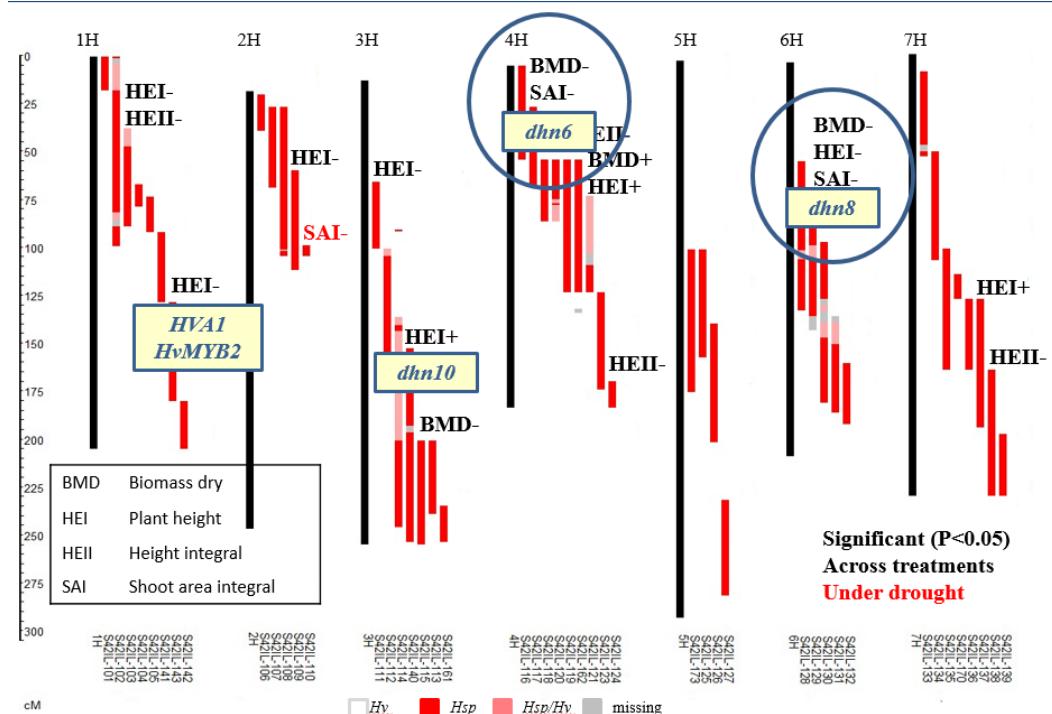
Hochdurchsatz-Phänotypisierung im Gewächshaus



THE UNIVERSITY
of ADELAIDE

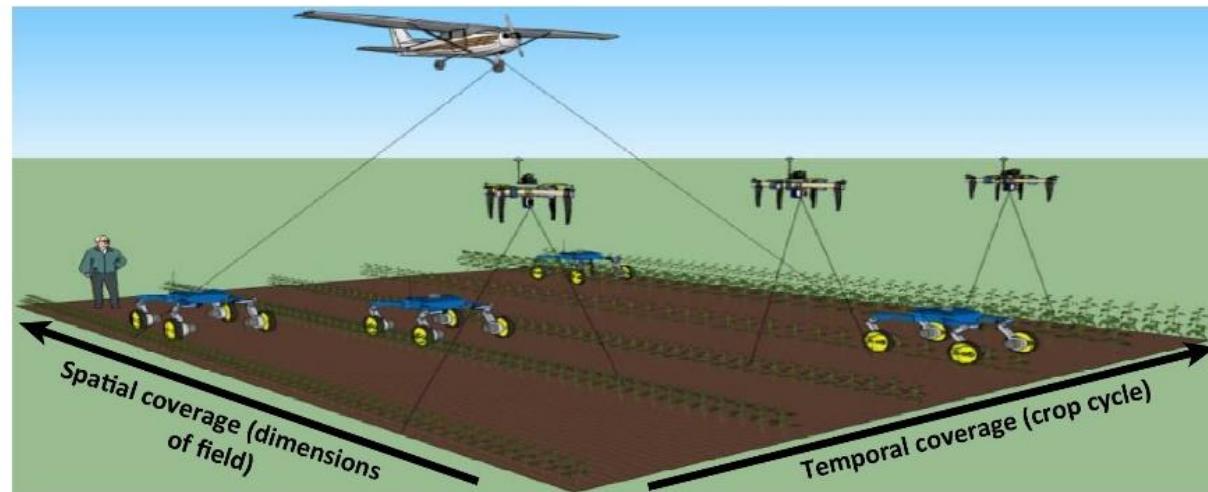


Australian
Plant Phenomics Facility



Honsdorf et al. 2014, PLOS ONE

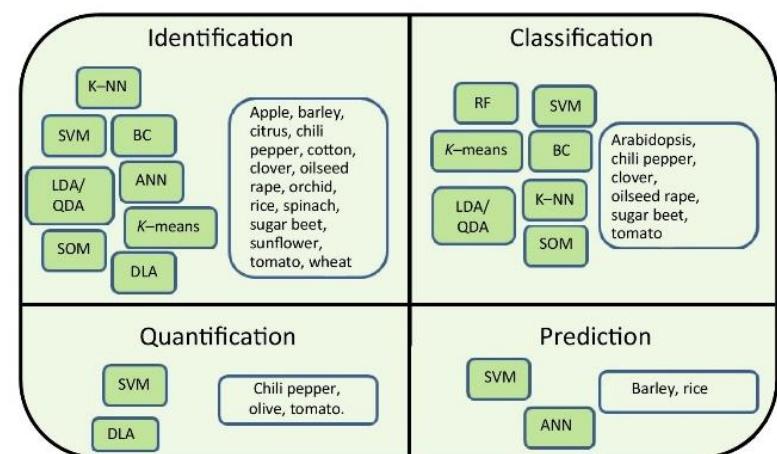
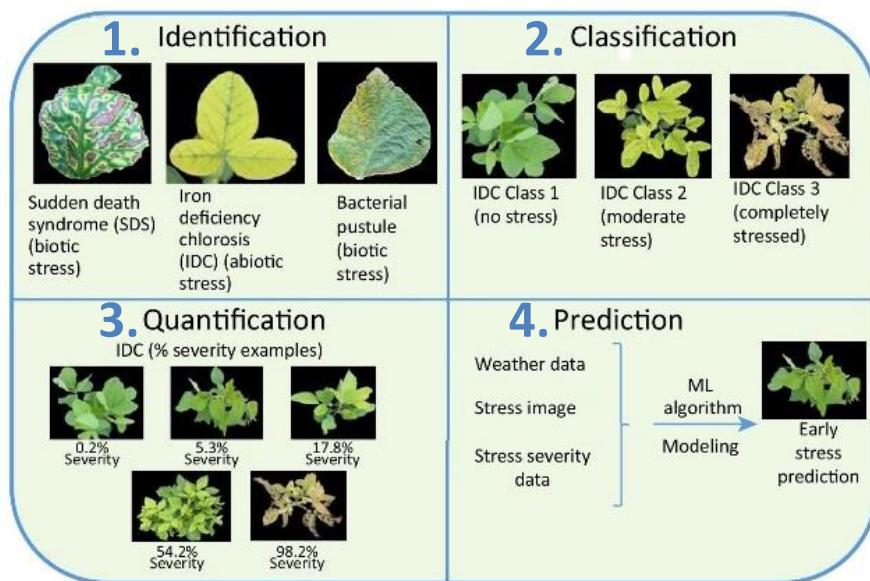
5. Modellierung: Machine Learning (ML)



Machine Learning Algorithms:

- BC, Bayes classifier;
- K-means, K-means clustering;
- K-NN, k-nearest neighbor classifier;
- LDA, linear discriminant analysis;
- RF, random forests;
- SOM, self-organizing map;
- SVM, support vector machine.
- ANN, artificial neural network;

(B)



Singh et al. 2016, Trends Plant Sci

6. Big Data-Integration Systembiologie

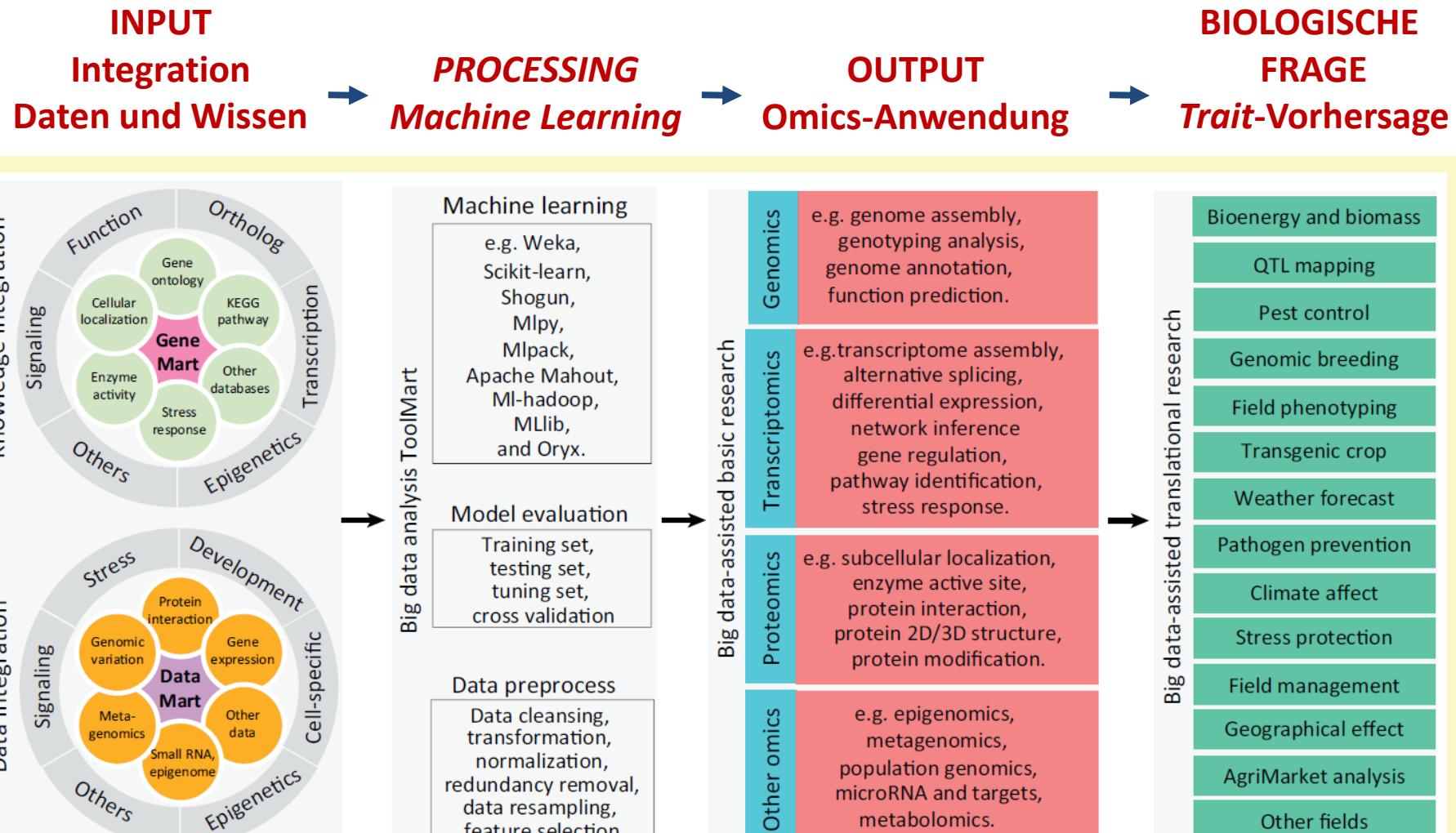
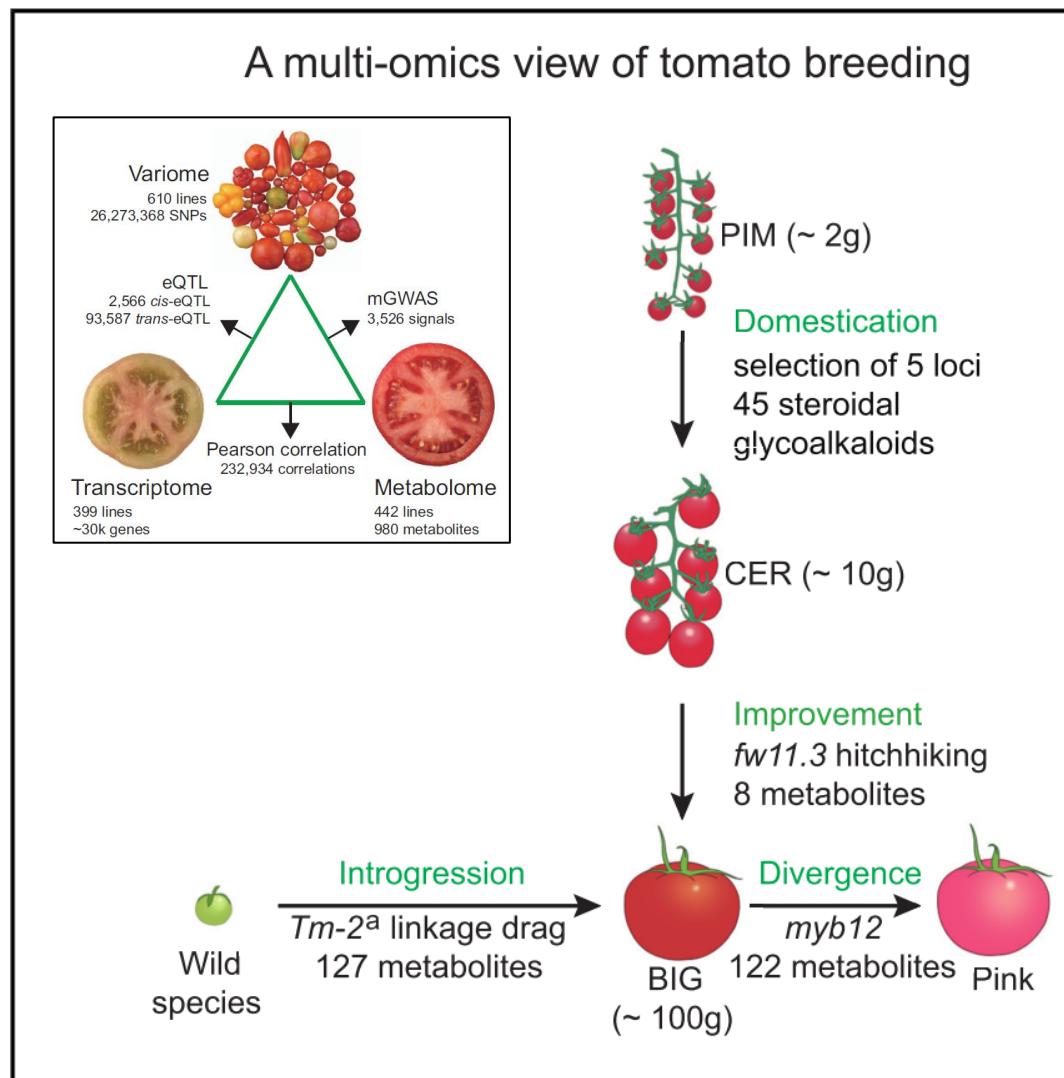


Figure I. A platform for machine learning-based Big Data analytics in plants.

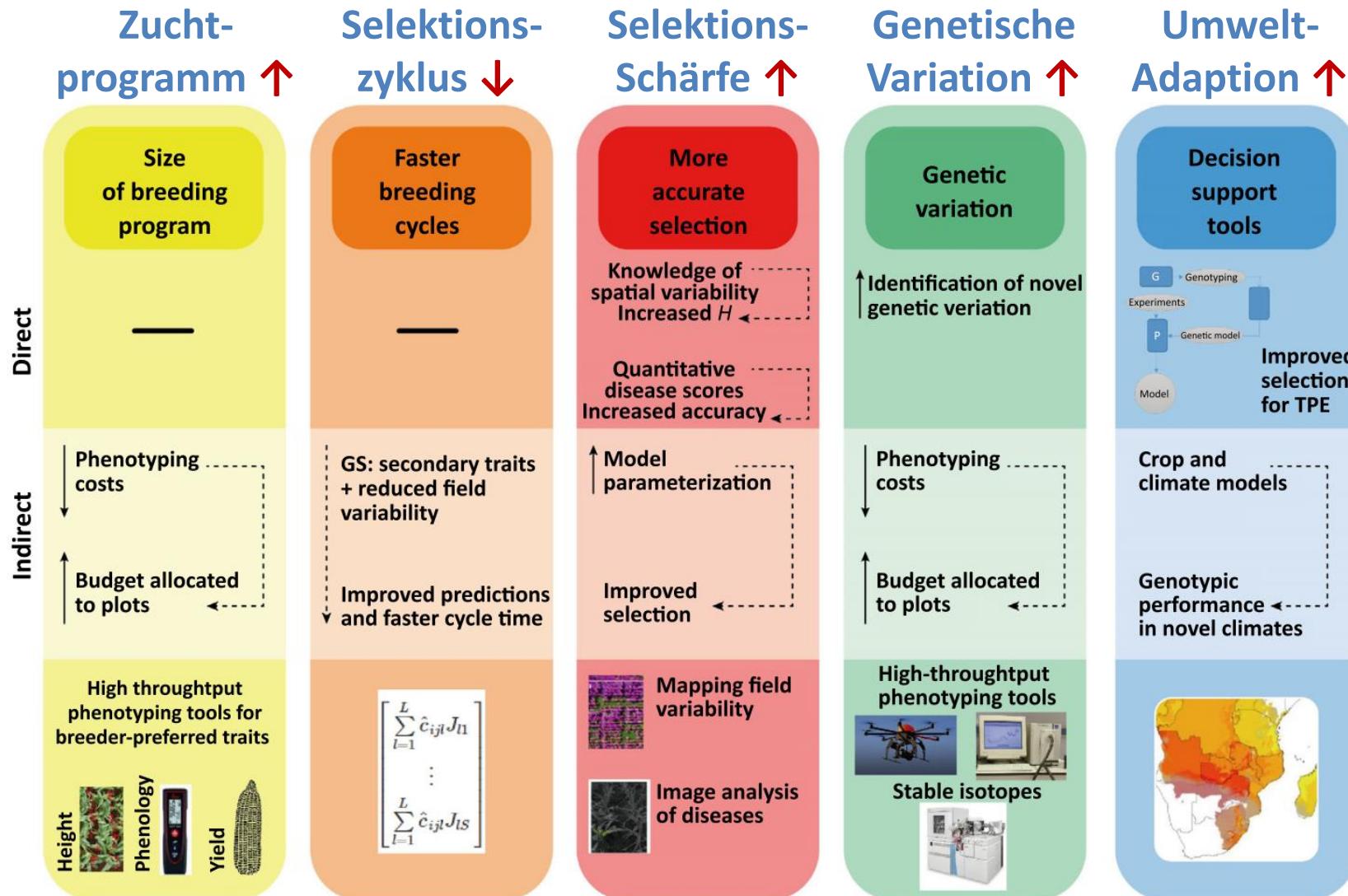
Ma et al. 2014, Trends Plant Sci

6. Datenintegration Sytembiologie: Phänom-, Transkriptom- und Metabolom-Analyse in Tomate



Zhu et al. 2018, Cell

Steigerung der Effizienz in der Pflanzenzüchtung



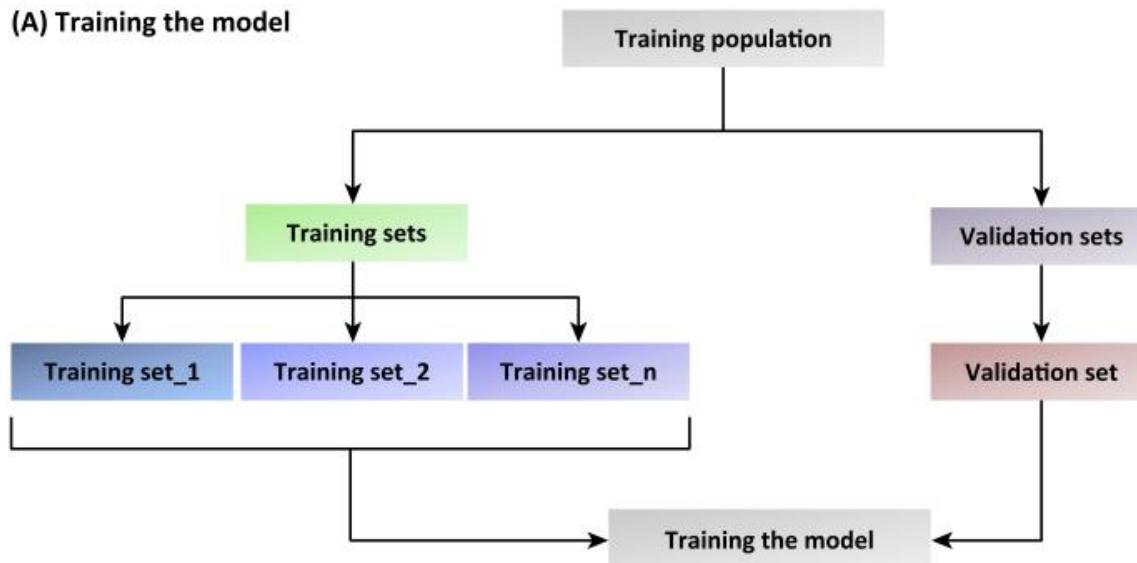
Araus et al. 2018, Trends Plant Sci

Potentiale der Datenverarbeitung

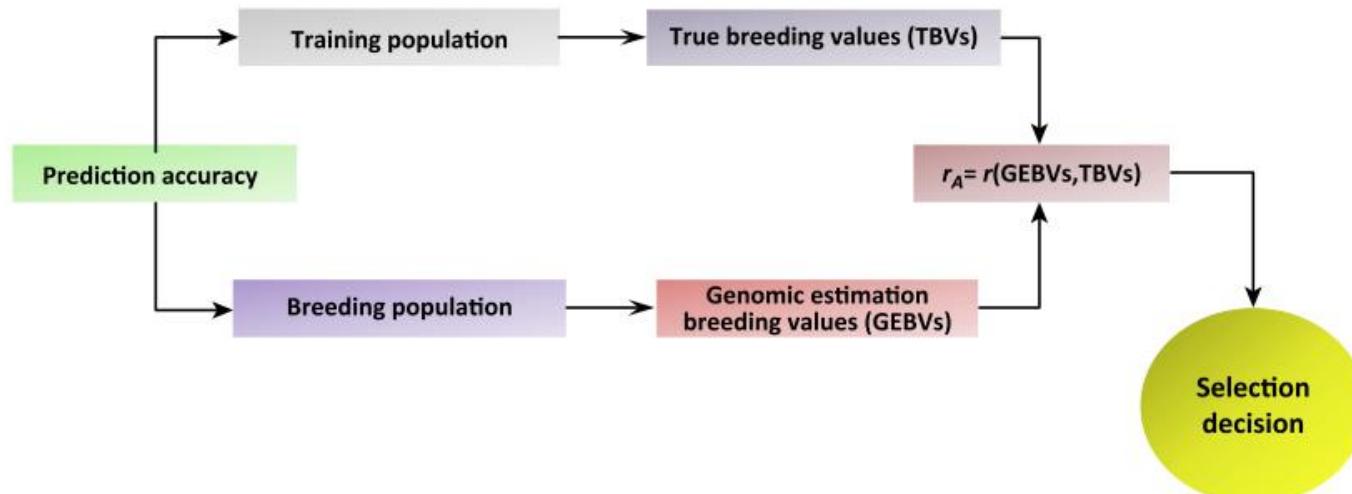
1. Genomsequenzierung (NGS)
 2. Genomische Prädiktion (GP)
 3. Genom-Editierung (GE)
 4. Hochdurchsatzphänotypisierung (HTPP)
 5. Modellierung
 6. Datenintegration Systembiologie
-
7. **Erhebliche Erweiterung des Verständnisses über Pflanzenwachstum und Ertragsbildung**
(→ molekulare sowie räumlich-zeitliche Modellierung)
 8. **Reduktion der Kosten der Datenerhebung**
(→ Genom & Phänom)
 9. **Steigerung der Effizienz in der Züchtung**
(→ Genomische Prädiktion, Selektionsgewinn pro Jahr)

Genomische Prädiktion (GP)

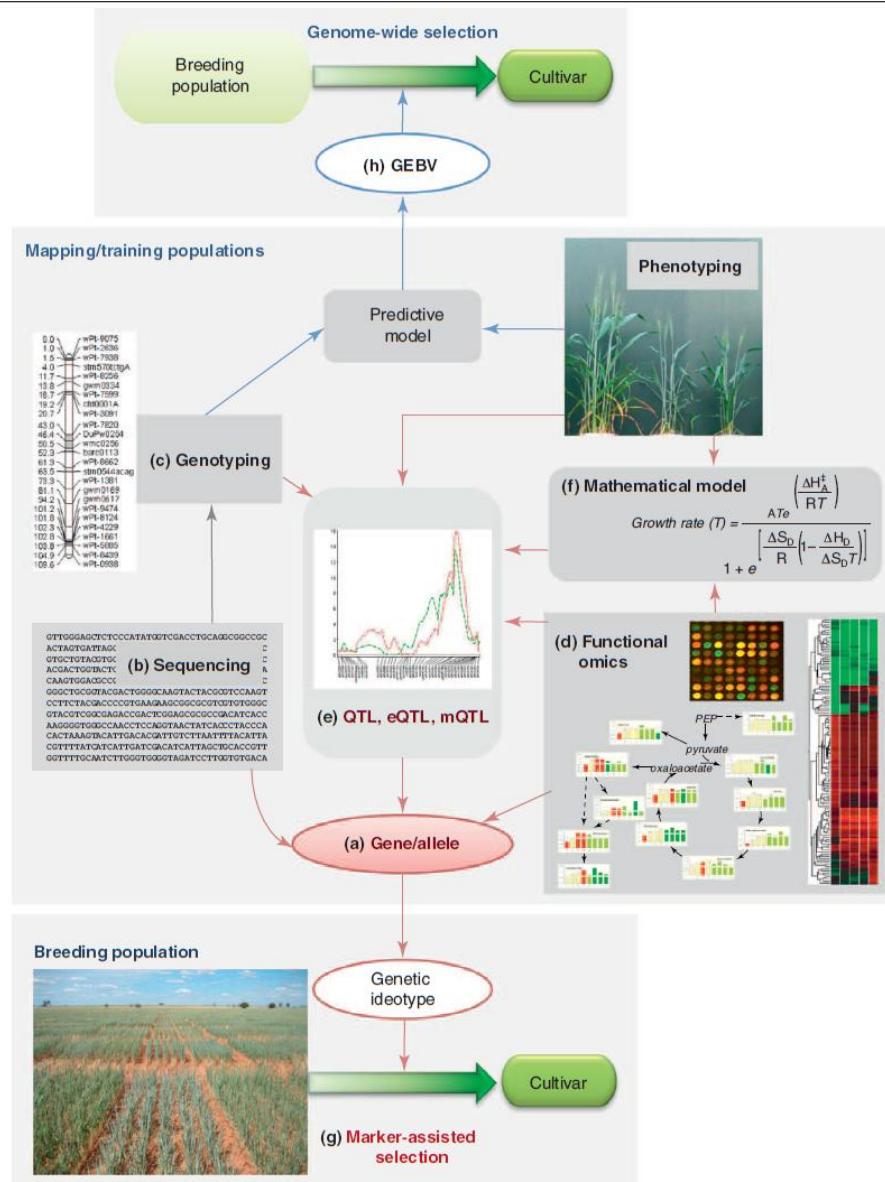
(A) Training the model



(B) The expected prediction accuracy (r_A)



Datenintegration (Klima, Boden, Düngung, Pflanzenschutz, Landtechnik, Genotyp)



Langridge & Fleury 2011, Trends Biotech

Genom-Editierung: CRISPR/Cas9 Mutationsgenerierung

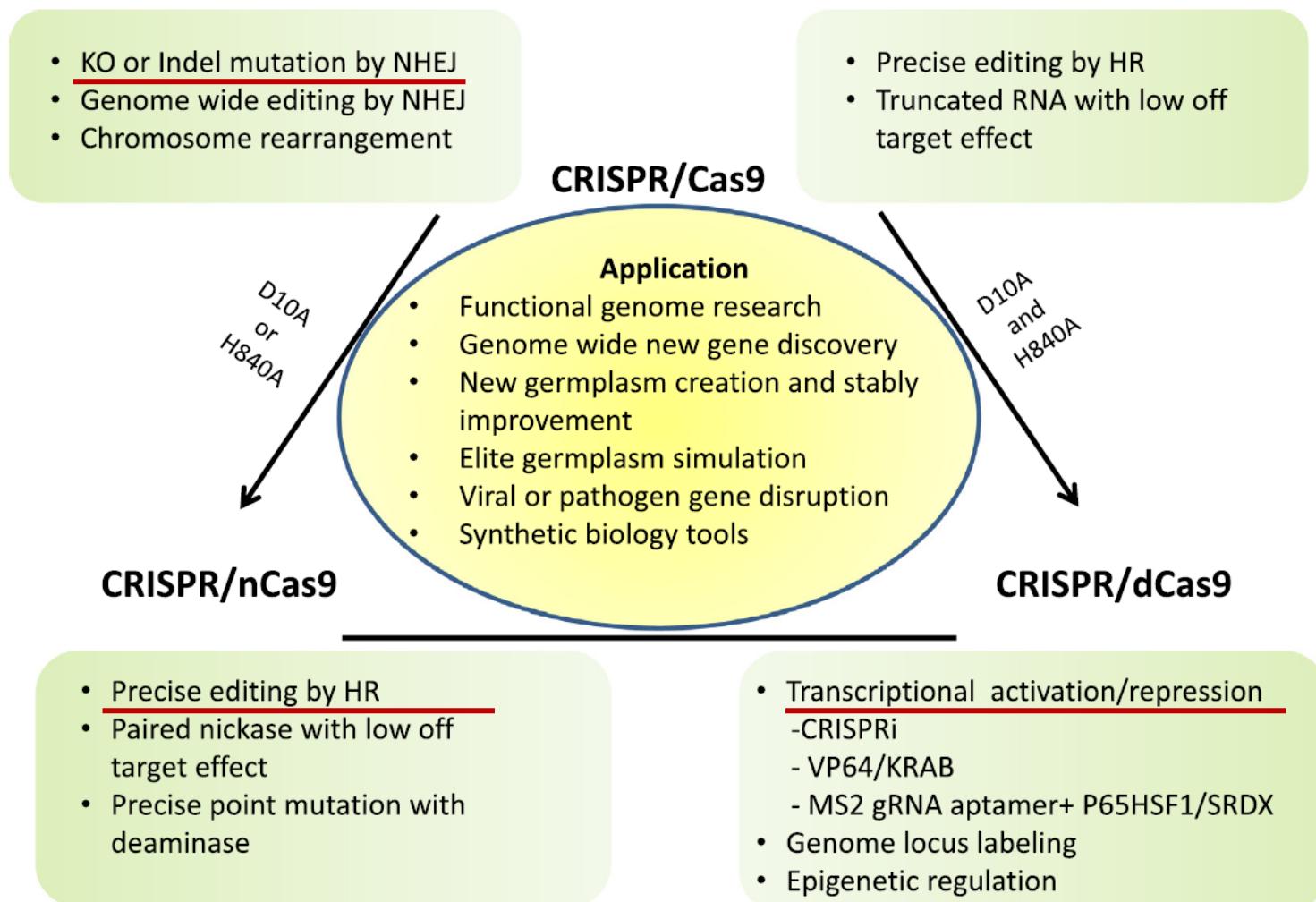
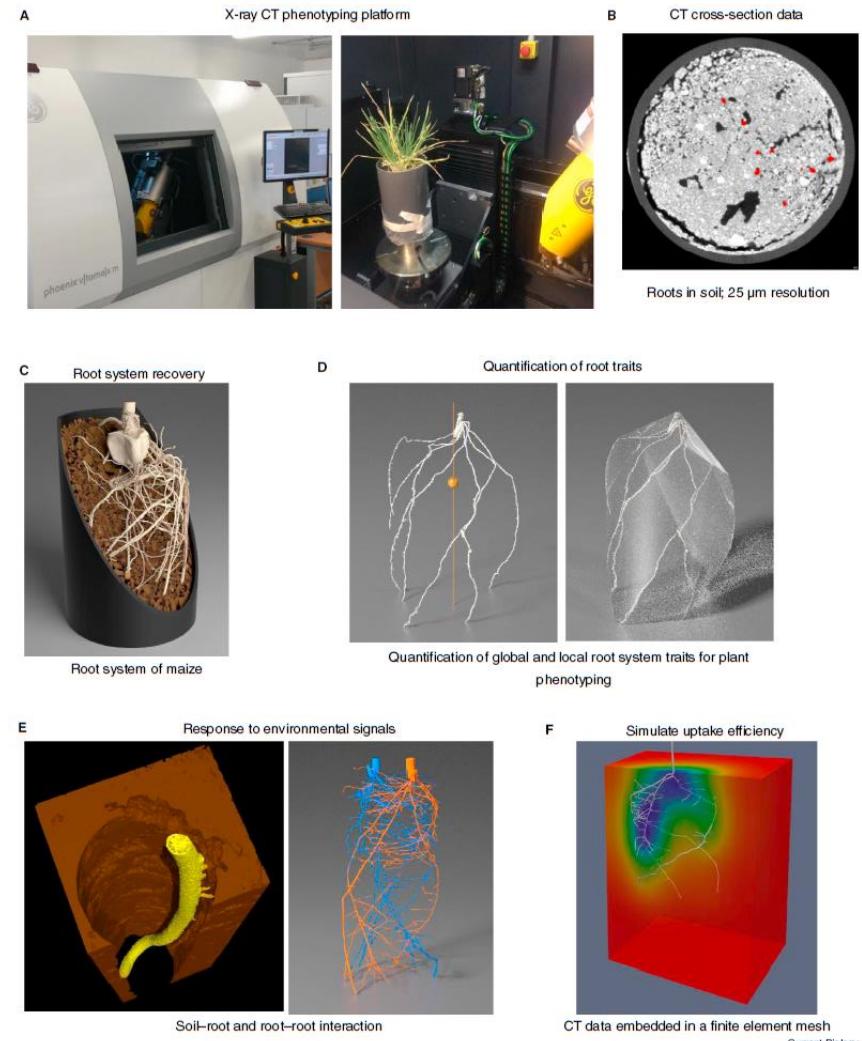
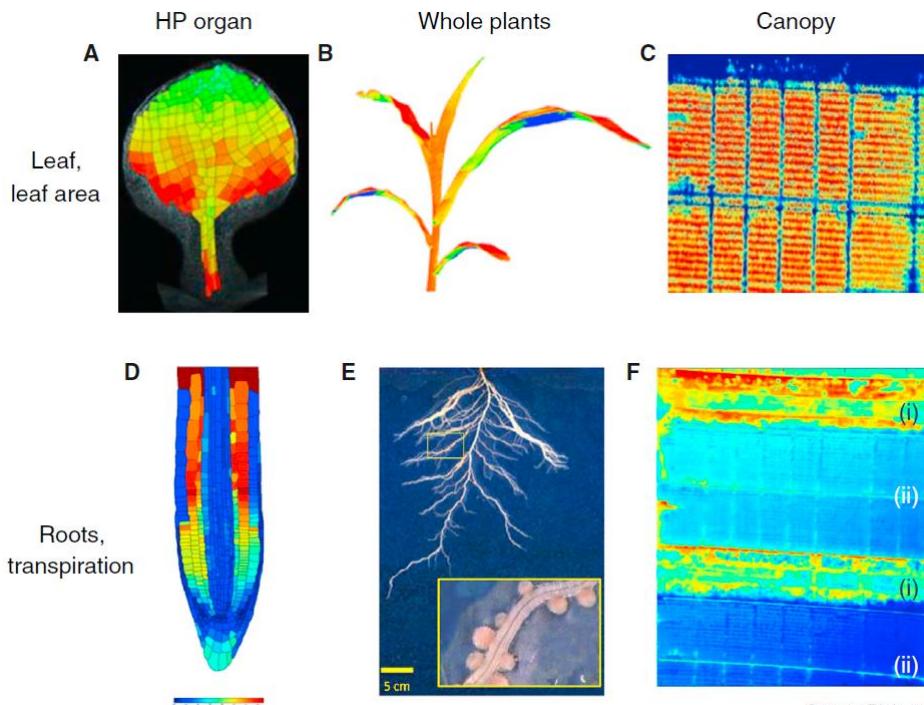


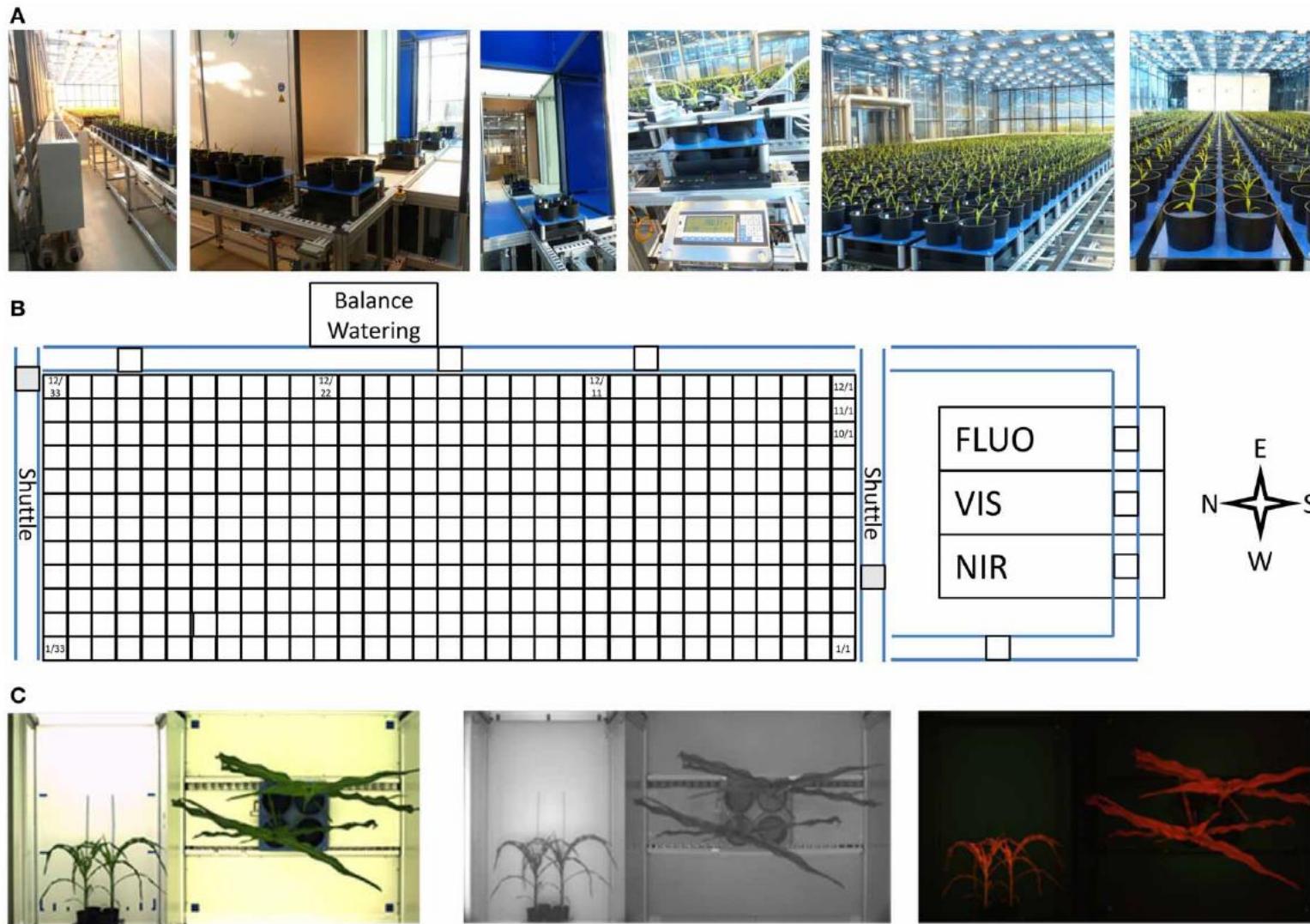
Fig. 1. Programmable RNA-guided genome DNA editing by CRISPR/Cas9 has been exploited in numerous diverse applications, some of which are shown.

Liu et al. 2017, Methods

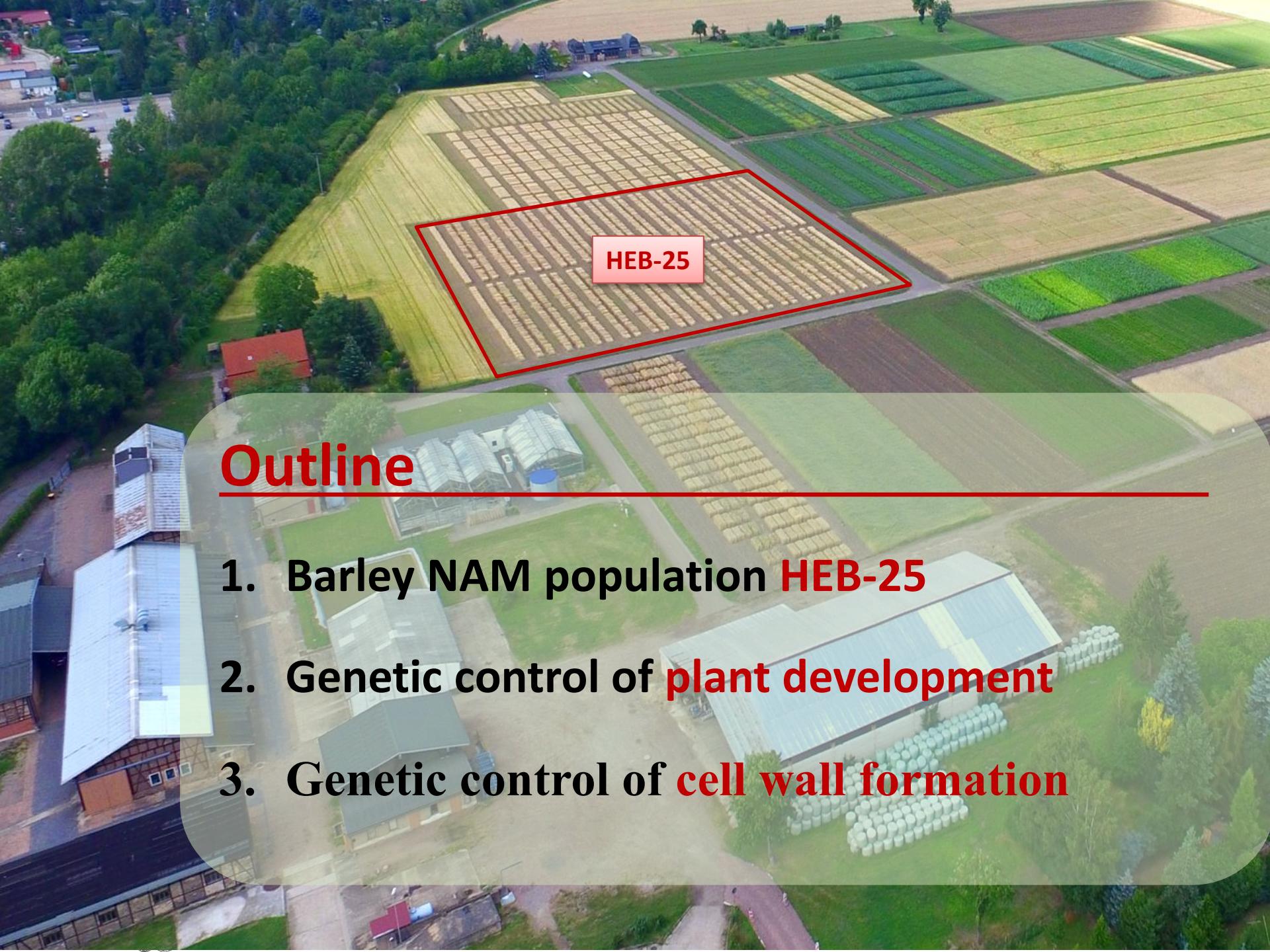
Hochdurchsatz-Phänotypisierung im Feld



Hochdurchsatz-Phänotypisierung im Gewächshaus



Junker et al. 2015, *Front Plant Sci*



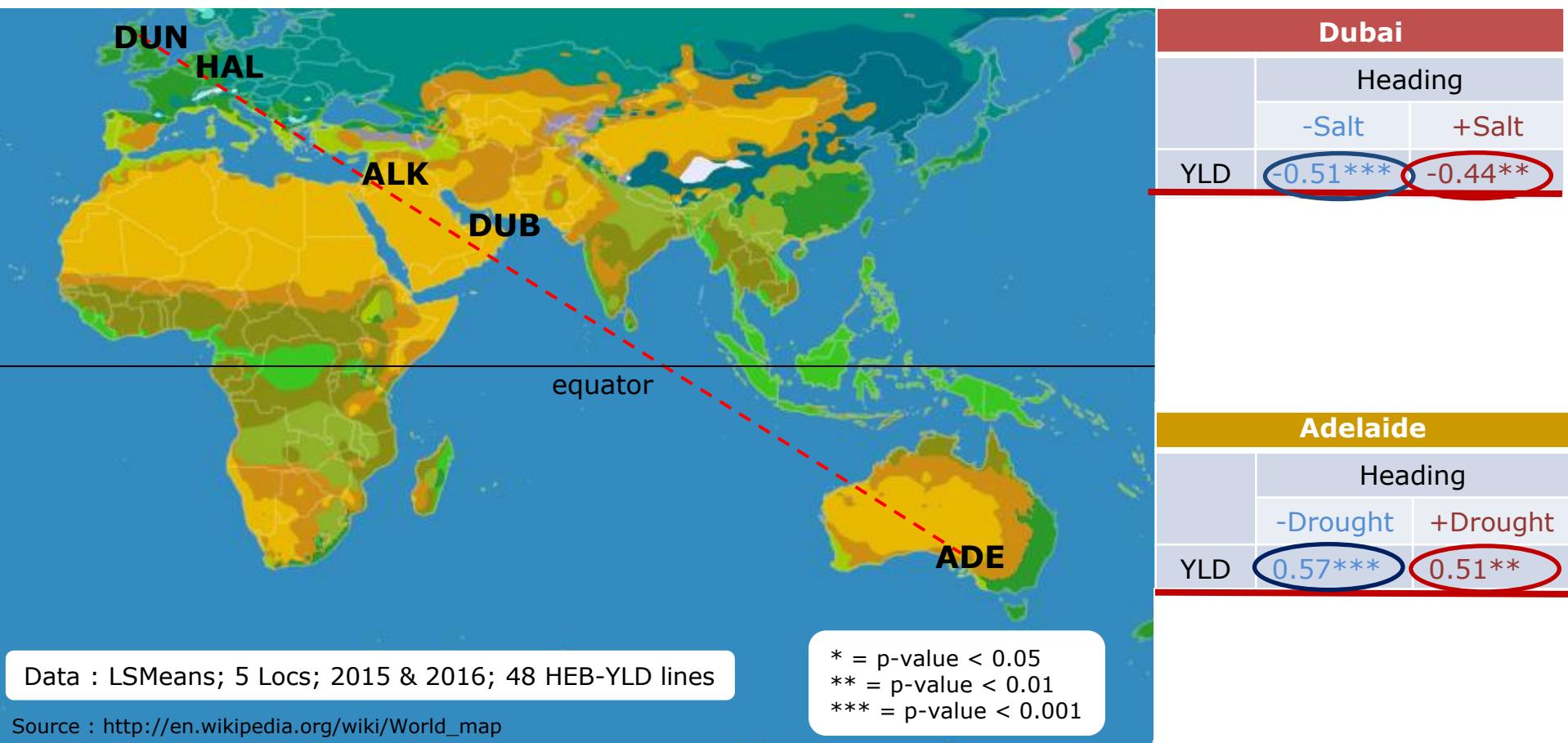
Outline

1. Barley NAM population **HEB-25**
2. Genetic control of **plant development**
3. Genetic control of **cell wall formation**

Correlation between heading & yield components

Dundee		Halle		Al-Karak	
	Heading		Heading		Heading
	+N fert.	-N fert.		+N fert.	-N fert.
YLD	0.59***	0.66***	YLD	0.32*	0.20
YLD	-0.30*	-0.72**			

HEA = Heading
YLD = Yield
EAR = # of ears
GNE = # of grains
TGW = Grain weight



Effects of *Ppd-H1* on heading and yield



Genomsequenzierung: *SNP calling*

Table 1

Summary of commercially available SNP genotyping platforms.

Platform	Provider	Assay type	Technology	Throughput	Multiplexing
Taqman	Applied Biosystems	PCR	Taqman probe	~1536/day	~256
SNPlex	Applied Biosystems	PCR	Capillary electrophoresis	~1536/3 days	~48
BioMark HD	Fluidigm	PCR	Microfluid-based chips	~96/3 h	~96
KASPar	LGC	PCR	FRET quenching oligos	~96/day	Not available
Axiom Biobank	Affymetrix	Hybridization	Oligo nucleotide array	~96/5 days	~650K
Infinium II	Illumina	Hybridization	Bead array	~128/5 days	~700K
GoldenGate	Illumina	Primer extension	Bead array	~172/3 days	~1536
iPlex	Sequenome	Primer extension	Mass spectrometry	~3840/2.5 days	~40

Table 2

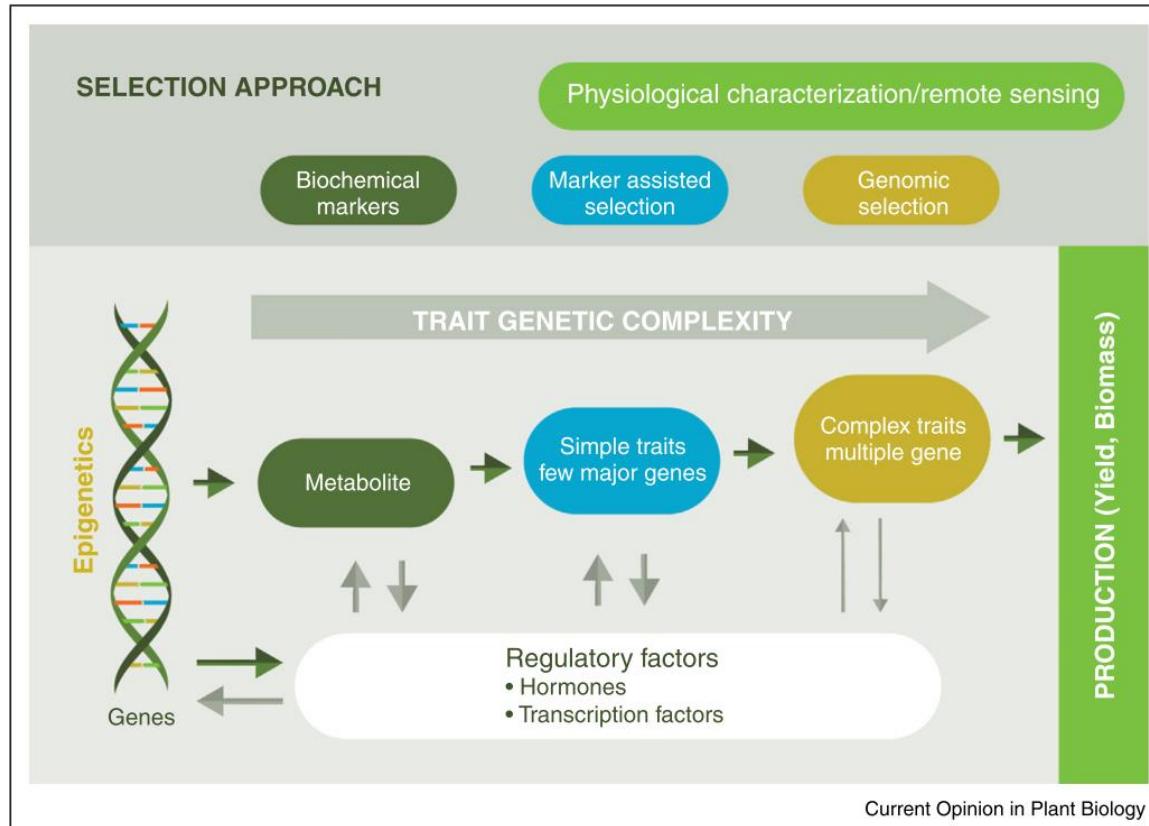
Representative GBS protocols published in peer-reviewed journals.

Method	Restriction enzyme	Insert size	Barcodes	Sequencing platform	Sequencing mode	Reference
RAD-seq (Restriction association DNA sequencing)	<i>SbfI</i> or <i>EcoRI</i>	Size-selection	~96	Illumina	Paired-end	[5]
MSG (Multiplex shotgun genotyping)	<i>MseI</i>	Size-selection	~384	Illumina	Single-end	[6]
GBS (Genotype by sequencing)	<i>ApeKI</i>	<350 bp	~384	Illumina	Paired-end	[4]
Double-digested RAD-seq	<i>EcoRI</i> and <i>MspI</i>	Size-selection	~48	Illumina	Paired-end	[42]
Double-digested GBS	<i>PstI</i> and <i>MspI</i>	<350 bp	~384	Illumina	Paired-end	[7]
Ion Torrent GBS	<i>PstI</i> and <i>MspI</i>	<350 bp	~384	Ion Torrent	Paired-end	[43]
SBG (Sequence-based genotyping)	<i>EcoRI</i> and <i>MseI</i>	Size-selection	~32	Illumina	Paired-end	[8]
REST-seq (Restriction fragment sequencing)	<i>PstI</i> and <i>MseI</i>	Size-selection	~305	Ion Torrent	Paired-end	[9]
Restriction enzyme sequence comparative analysis	<i>TaqI</i> and <i>TruI</i>	Size-selection	~96	Illumina	Paired-end	[10]

Kim et al. 2016, Plant Sci

Systembiologie

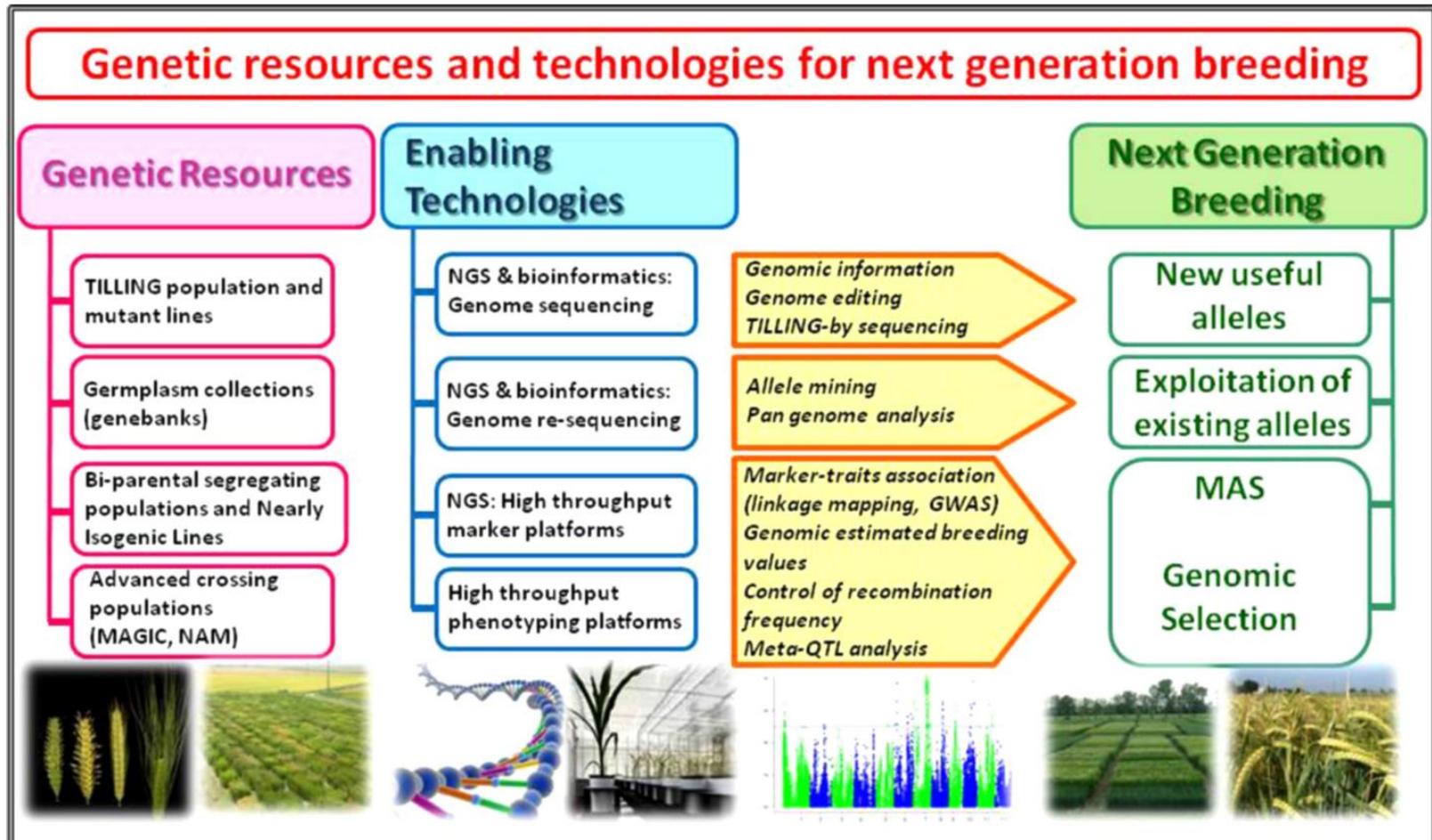
(Genom → Exom → Proteom → Metabolom → Phänom)



olds & Langridge 2016, Curr Op Plant Biol

Systembiologie

(Genom → Exom → Proteom → Metabolom → Phänom)



abaschi et al. 2016, Plant Science

Systembiologie

(Genom → Exom → Proteom → Metabolom → Phänom)

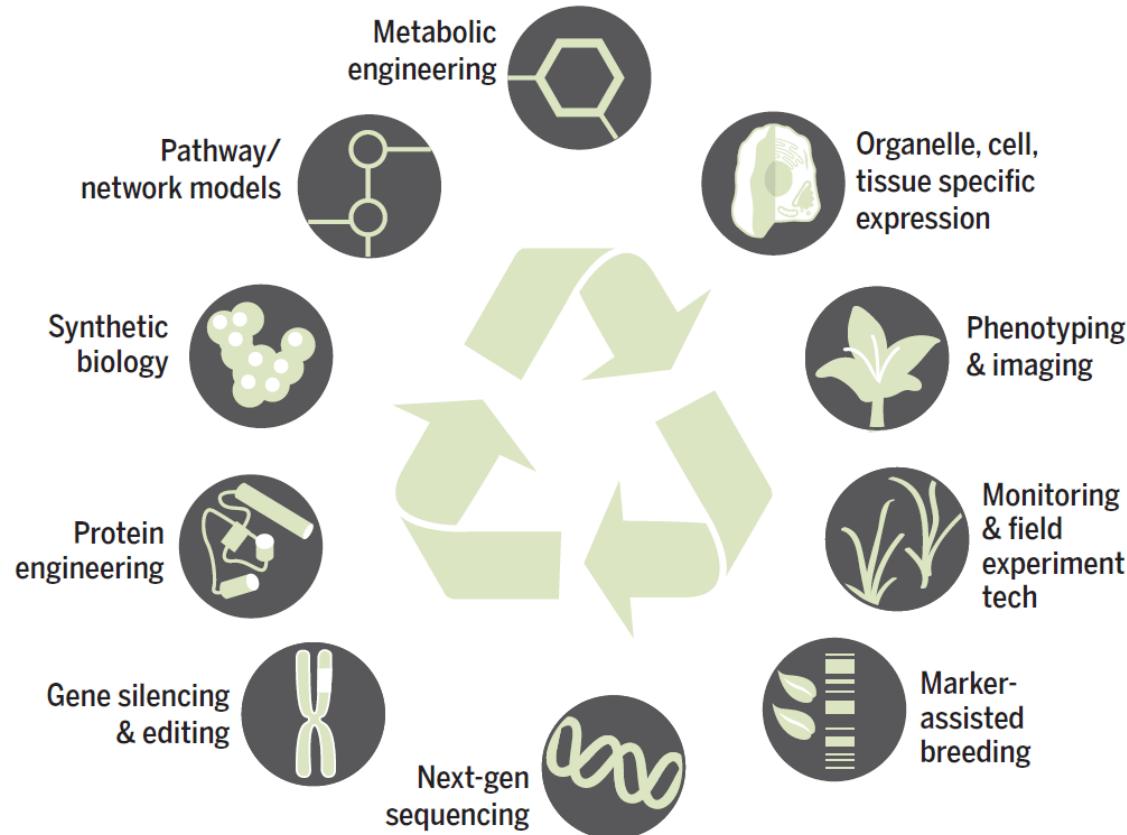


Fig. 1. Modern research tools support a cycle of discovery spanning atoms to ecosystems.

Jez et al. 2016, Science

Systembiologie

(Genom → Exom → Proteom → Metabolom → Phänom)

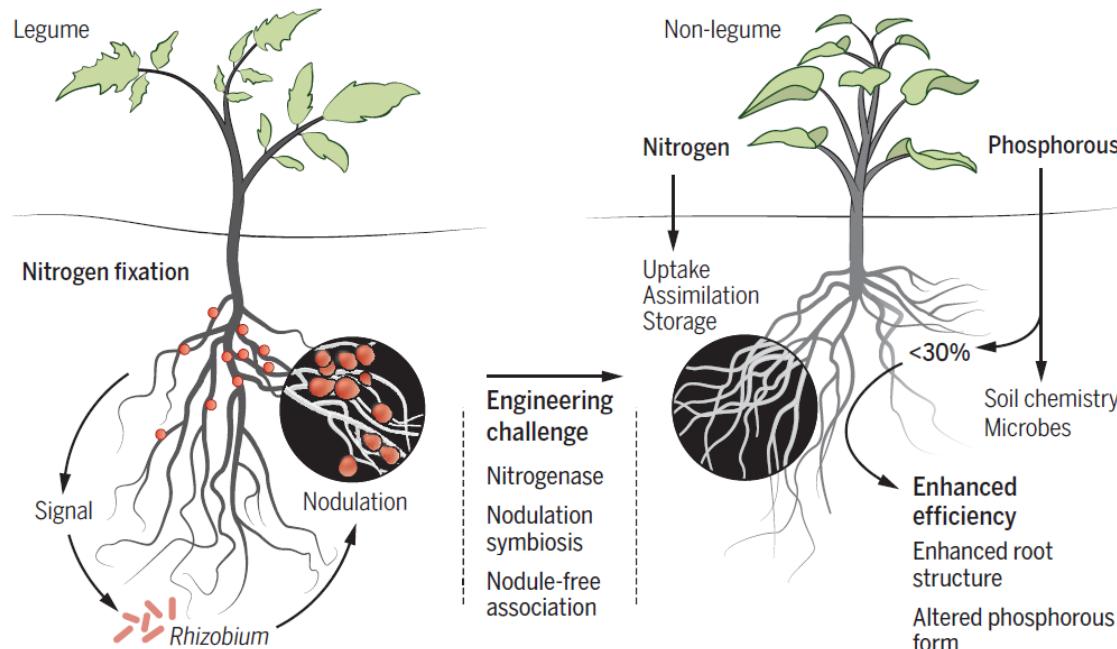


Fig. 2. Improving nitrogen and phosphorus use. (Left) Legume-Rhizobium symbiosis leads to nodule formation and nitrogen fixation. (Right) In nonlegume plants, nitrogen-related pathways are targets for breeding and genetic engineering. Different strategies for engineering nitrogen fixation are indicated. Most phosphorus is lost, but alterations of root structure and engineered pathways that select for phosphorus forms can enhance efficiency.

Systembiologie

(Genom → Exom → Proteom → Metabolom → Phänom)

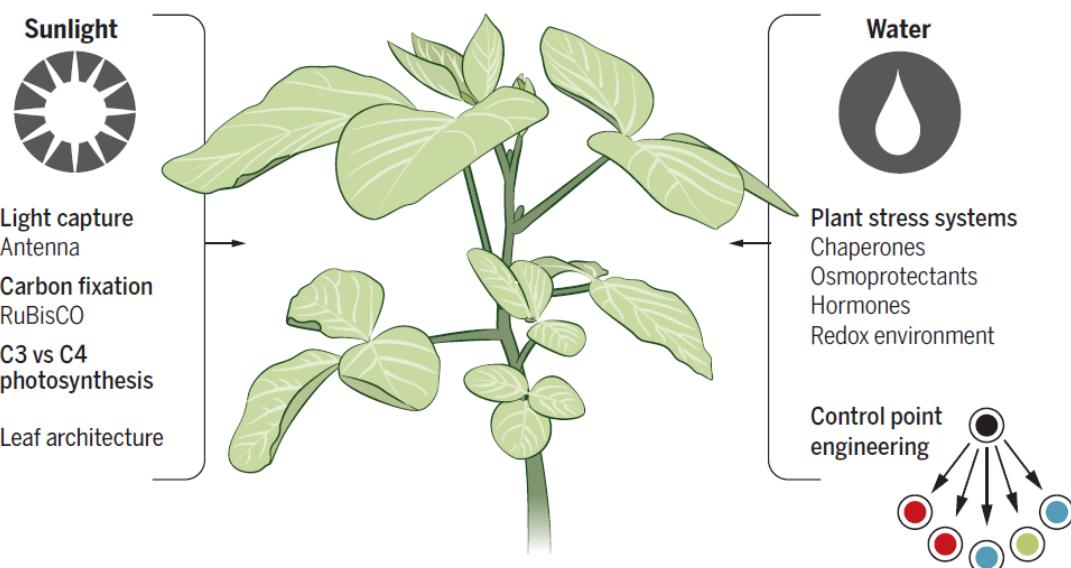
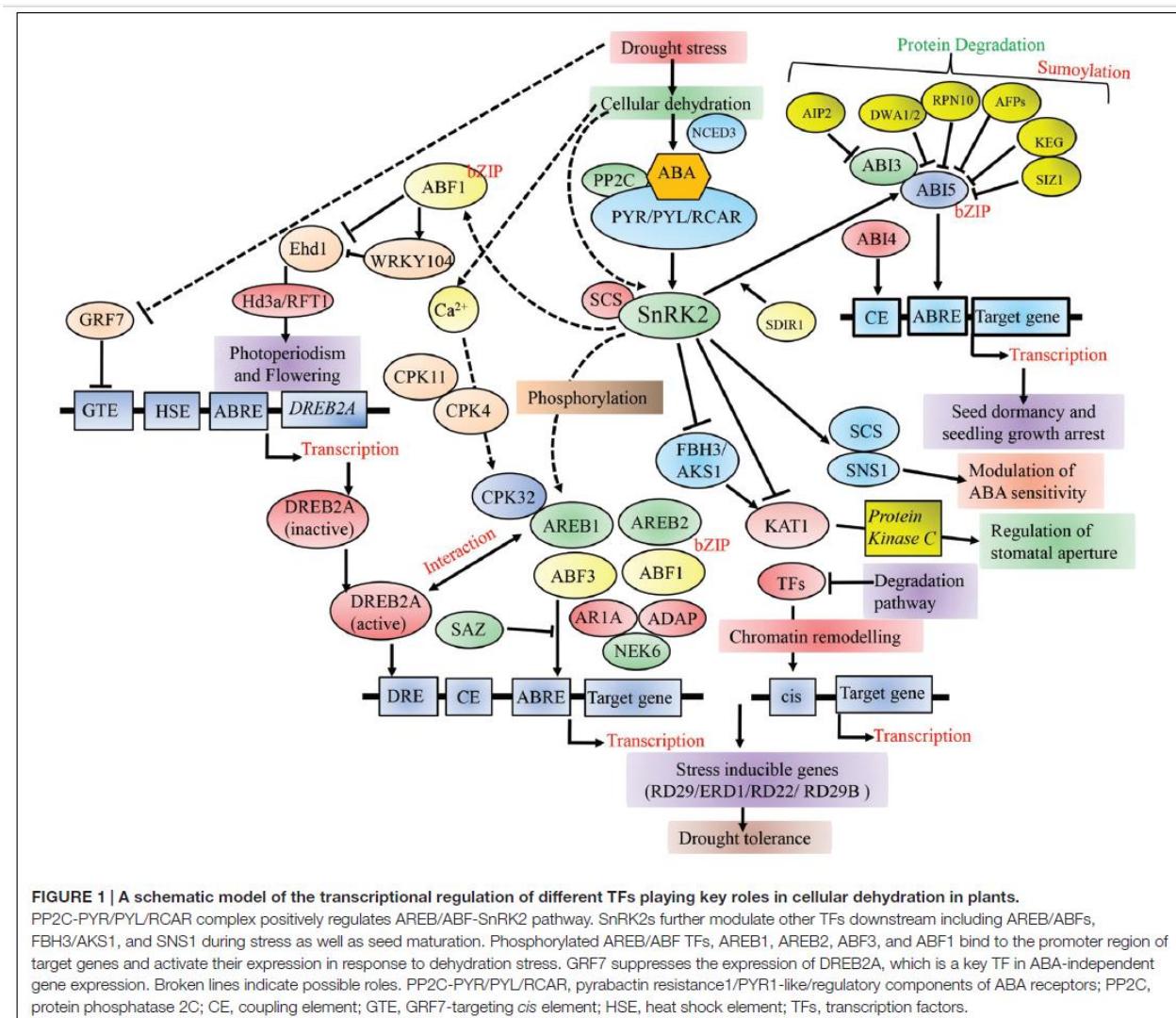


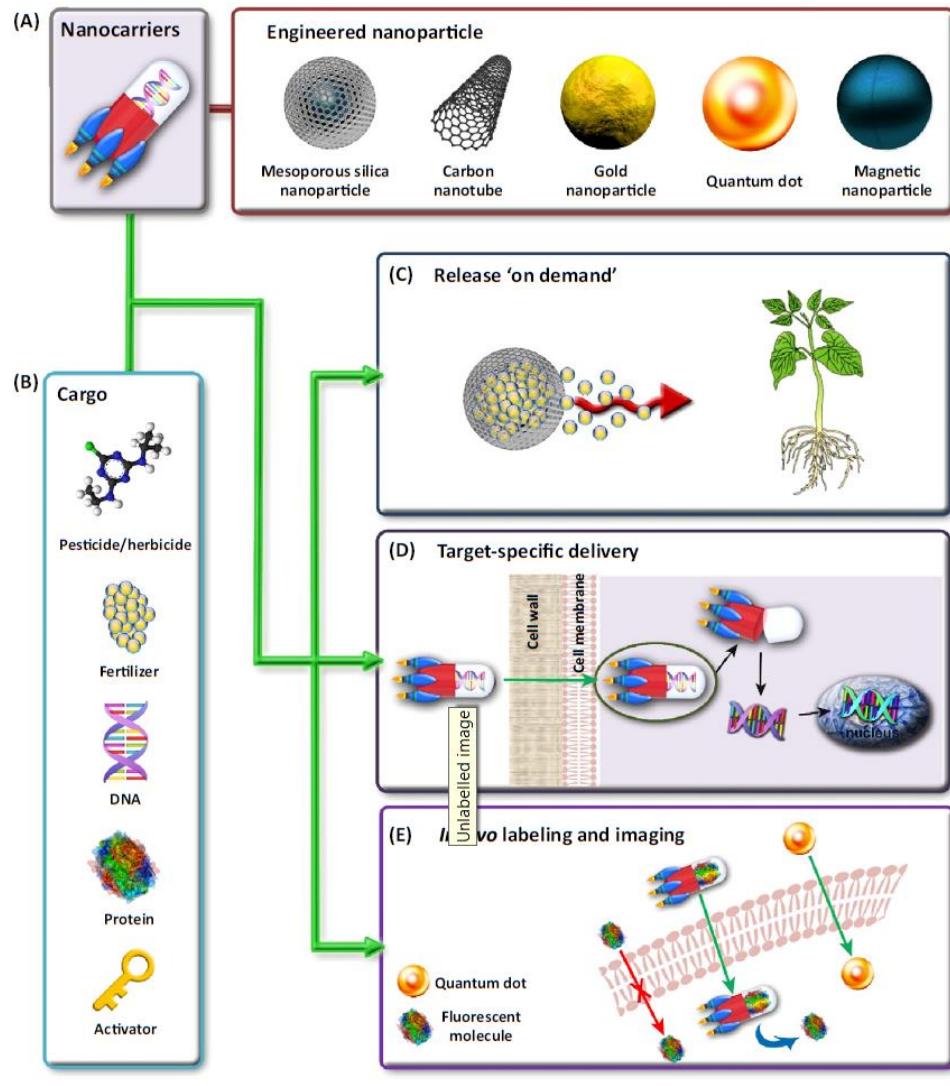
Fig. 3. Improving carbon fixation and water use. Breeding efforts to alter leaves and engineering of photosynthesis aim to improve the efficiency of carbon fixation. The multigenic nature of water-stress-related pathways requires researchers to target key control points that alter multiple other steps in various response/protective networks.

Systembiologie

(Genom → Exom → Proteom → Metabolom →)



Phyto Nano Technology

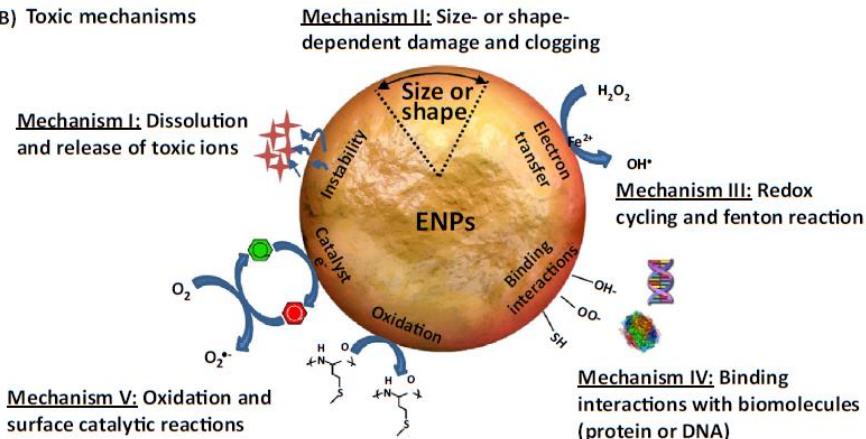


. 2016, Trends Plant Sci

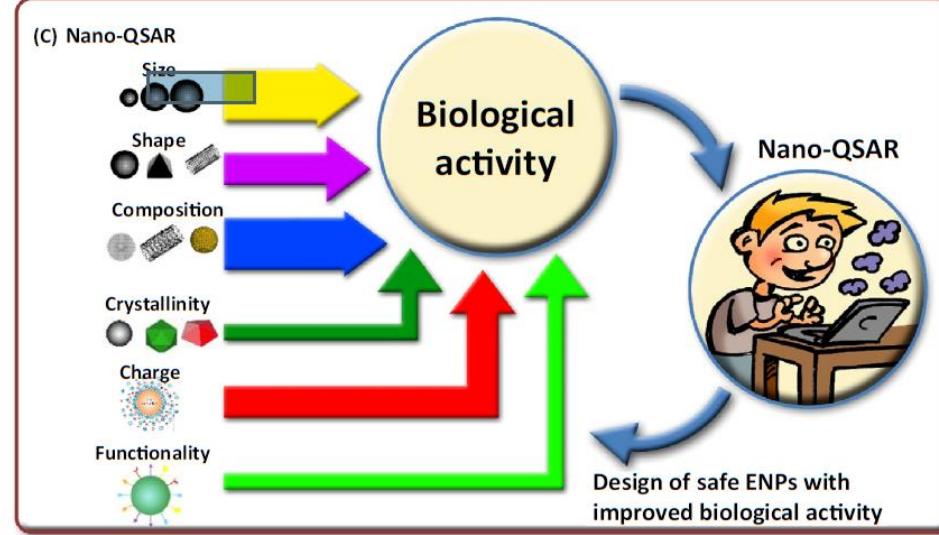
Phyto Nano Technology

	MSNs	CNTs	TiO ₂ NPs	Au NPs	Fe ₃ O ₄ NPs	QDs	Ag NPs	Observation frequency
Positive	****	****	****	**			*	• 1–2 •• 3–10 •••• ≥10
Nontoxic	••	••	•	••	•		•	
Negative	•	••	••	••	•	••	••••	

(B) Toxic mechanisms

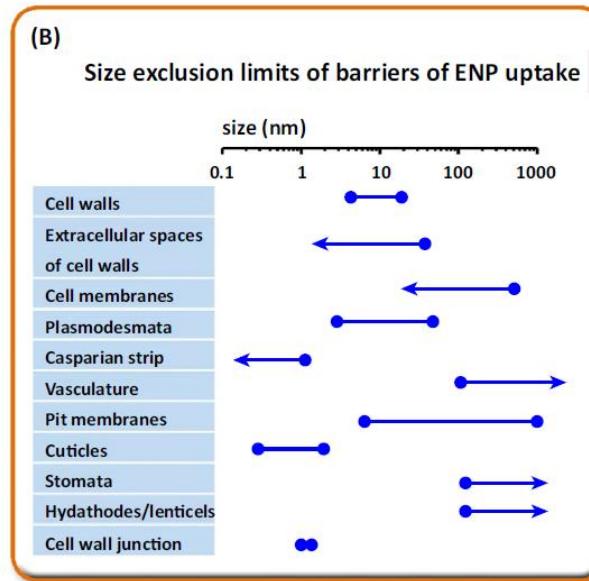
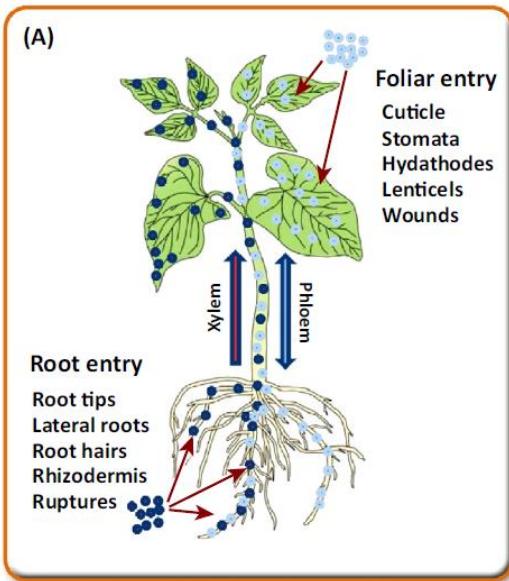


(C) Nano-QSAR



. 2016, Trends Pla

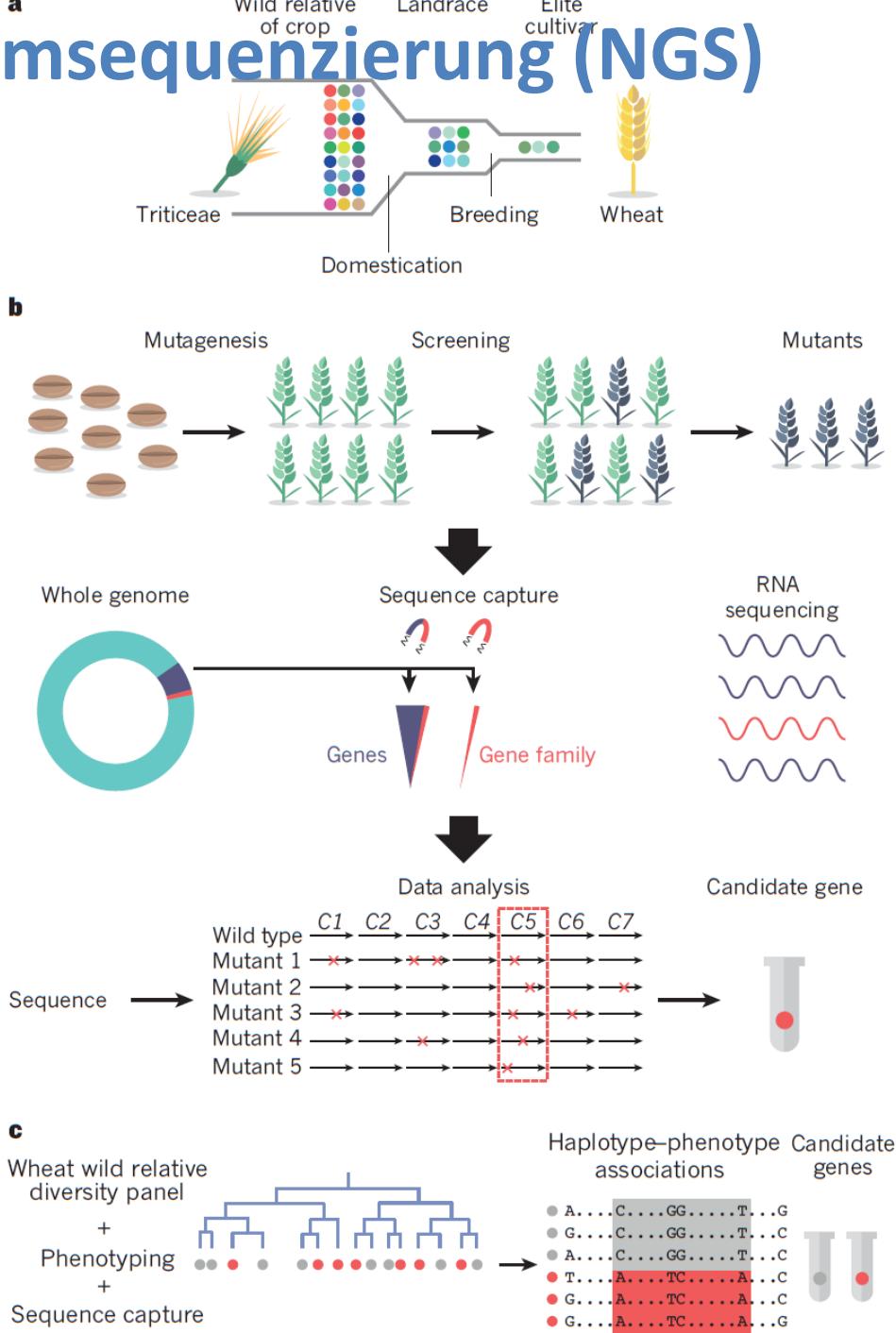
Phyto Nano Technology



Trends in Plant Science

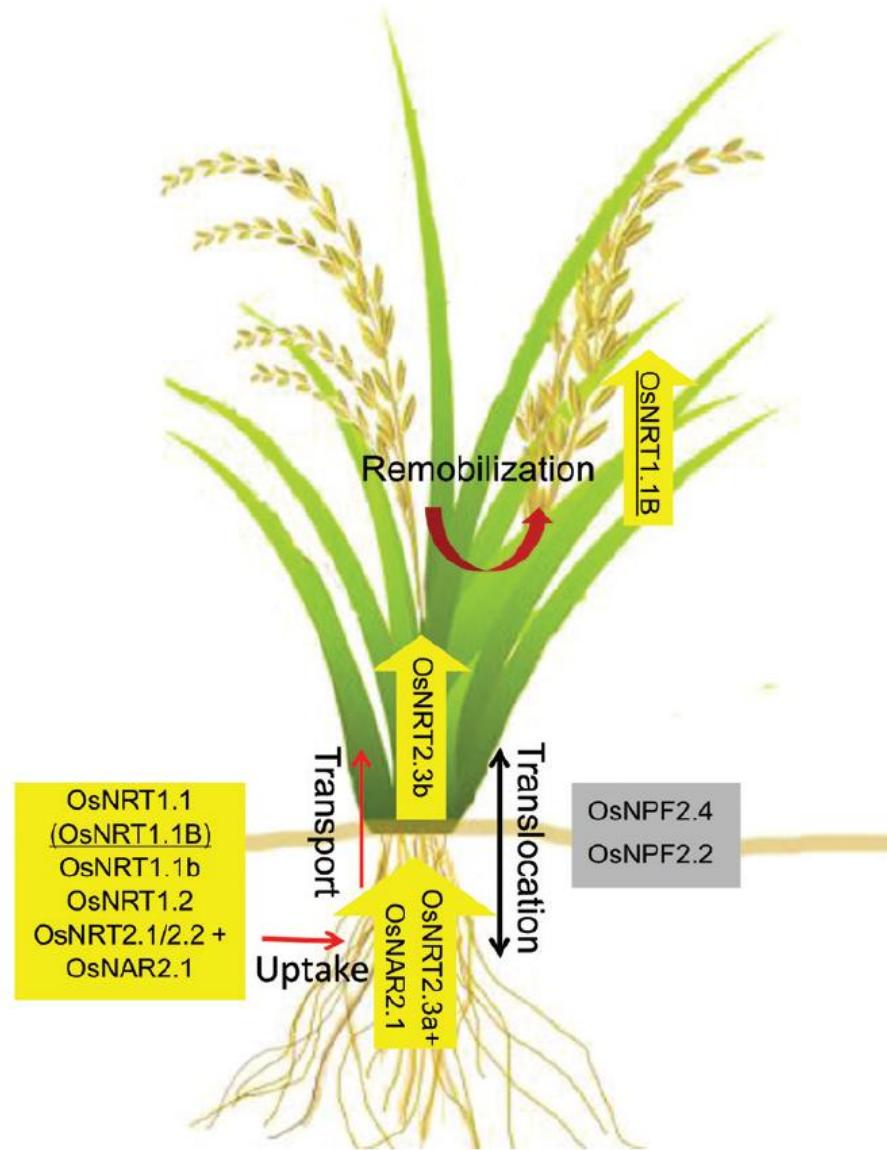
. 2016, Trends Plant Sci

Genomsequenzierung (NGS)



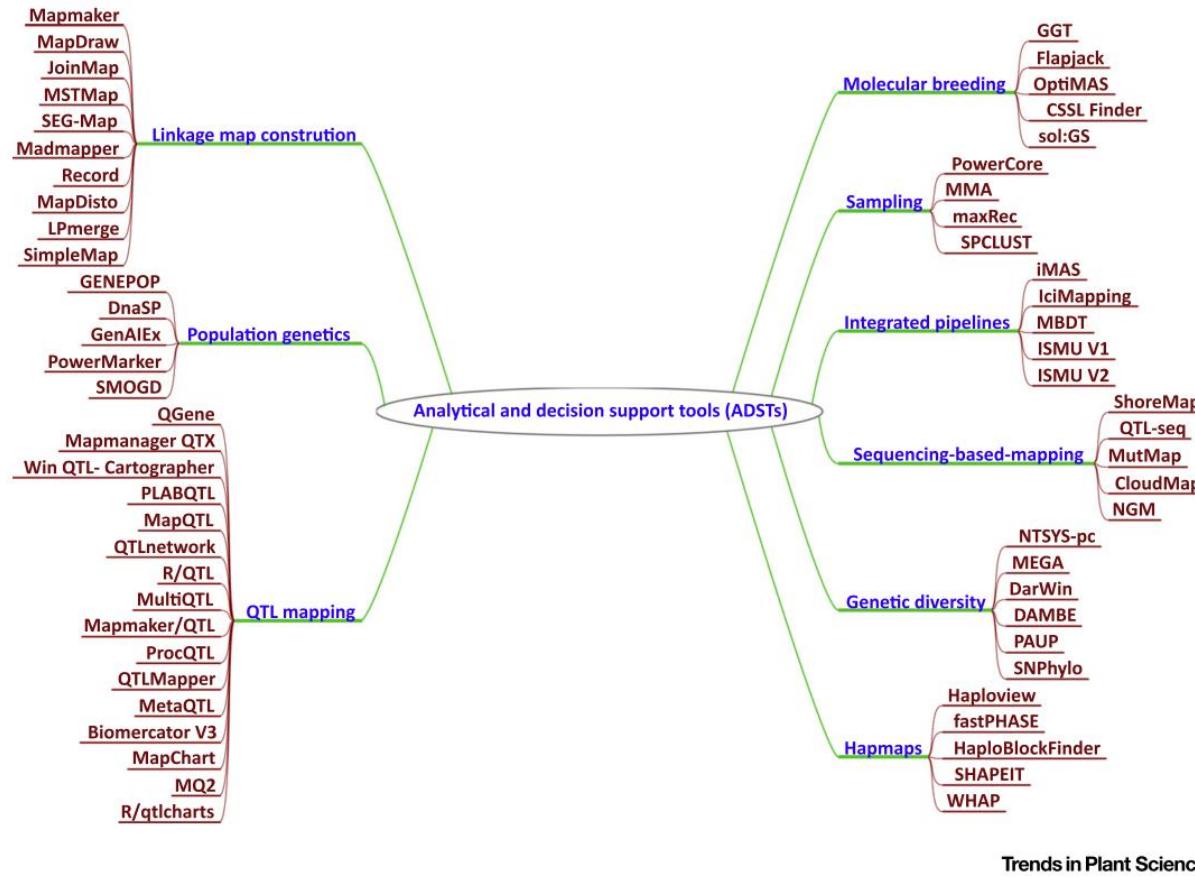
Bevan et al. 2017, Nature

Genomsequenzierung (NGS)



Fan et al. 2017, J Exp Bot

Genomische Prädiktion (GP)



Hochdurchsatz-Phänotypisierung im Feld

Attributes	Environmental Sensor Network	Autonomous Ground Vehicle (AGV)	Tractor-Based Phenotyping	Phenotyping Tower	Field Scanning Platforms	Unmanned Aerial Vehicles (UAVs)	Aerial Sensing (Zeppelin/Plane)	Spectral Satellite Imaging
Phenotyping Scale	Individual Plant / Plot	Individual Plant / Plot	Individual Plant / Plot	Individual Plant / Plot	Individual Plant / Plot / Field	Plot / Field	Plot / Field	Plot / Field
Sensor Payload Size	Small / Medium	Medium	Medium / Large	Small / Medium	Large	Small	Large	Large
Can Be Autonomous?	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Data Post-Processing	Light	Light	Moderate	Light / Moderate	Significant	Moderate	Moderate	Significant
Data Collection Interval	Continuous	Daily*	Daily*	Continuous	Continuous / Daily	Daily*	Daily*	Daily*
Platform Accessibility	High	Low	Medium / High	Medium	Low	High	Low	Low / Medium

*Dependent on environmental conditions

Current Opinion in Plant Biology

Hochdurchsatz-Phänotypisierung im Feld

Sensor	Examples of Potential Applications	Disease/Pathogen Assessment	References for Disease/Pathogen Assessment
RGB / Stereo RGB	biomass, morphology, height, leaf area and surface normal angles, disease symptoms, growth dynamics, yield traits, panicle traits, root architecture, germination rates, flowering time	Cotton: Bacterial angular (<i>Xanthomonas campestris</i>), Ascochyta blight (<i>Ascochyta gossypii</i>); Sugar beet: Cercospora leaf spot (<i>Cercospora beticola</i>), Sugar beet rust (<i>Uromyces betae</i>), Ramularia leaf spot (<i>Ramularia beticola</i>), Phoma leaf spot (<i>Phoma betae</i>), Bacterial leaf spot (<i>Pseudomonas syringae</i> pv. <i>aptata</i>); Grapefruit: Citrus canker (<i>Xanthomonas axonopodis</i>); Tobacco: Anthracnose (<i>Colletotrichum destructivum</i>); Apple: Apple scab (<i>Venturia inaequalis</i>); Canadian goldenrod: Rust (<i>Coleosporium asterum</i>); Potato: Late blight (<i>Phytophthora infestans</i>)	Camargo and Smith (2009); Neumann et al. (2014); Bock et al. (2008); Wijekoon et al. (2008); Sugiura et al. (2016)
3D Laser Scanner	plant architecture: height, leaf area, leaf angle distributions, canopy structure	Sugar beet: Cercospora leaf spot (<i>Cercospora beticola</i>); Oil palm: Basal stem rot (<i>Ganoderma lucidum</i>)	Roscher et al. (2016); Khosrokhani et al. (2016)
Multispectral	senescence evaluation, nutrient status, pigment degradation, photosynthetic efficiency, water content	Avocado: Laurel wilt (<i>Raffaelea lauricola</i>) Citrus: Citrus black spot (<i>Guignardia citricarpa</i>) Cassava: Cassava mosaic virus	de Castro et al. (2015); Pourreza et al. (2016);
Thermal Infrared (IR)	transpiration, heat stress, leaf senescence, leaf/canopy temperature, water stress, disease, and pathogen detection, evaluating fruit/vegetable maturity, and bruise detection	Sugar beet: Cercospora leaf spot (<i>Cercospora beticola</i>); Cucumber: Downy mildew (<i>Pseudoperonospora cubensis</i>), Powdery mildew (<i>Podosphaera xanthii</i>); Apple: Apple scab (<i>Venturia inaequalis</i>) Olive: Verticillium wilt (<i>Verticillium dahliae</i>)	Cherelle et al. (2004); Berdugo et al. (2014); Oerke et al. (2006); Oerke et al. (2011); Calderón et al. (2015)
Near Infrared (NIR) 700-1100nm	transpiration, water content heat stress, NDVI, leaf area index	Barley: Powdery mildew (<i>Blumeria graminis hordei</i>) Wheat: Powdery mildew (<i>Blumeria graminis f. sp. Triticici</i>)	Kuska et al. (2015); Cao et al. (2015)
Visual-Near IR (VNIR) 380-1000nm	vegetation indices, leaf structure, lignin/flavonoid contents, leaf and canopy water status, leaf senescence, chlorophyll fluorescence, vegetation indices	Barley: Net blotch (<i>Pyrenophora teres</i>), Brown rust (<i>Puccinia hordei</i>), Powdery mildew (<i>Blumeria graminis hordei</i>); Sugarcane: Orange rust (<i>Puccinia kuehnii</i>); Wheat: Head blight (<i>Fusarium graminearum</i>); Almond: Red leaf blotch (<i>Polystigma amygdalinum</i>)	Wahabzada et al. (2015); Apan et al. (2004); Bauriegel et al. (2011); López-López et al. (2016)
ShortWave IR (SWIR) 900-2500nm	water, lignin, cellulose contents, fluorescence, vegetation indices	Barley: Net blotch (<i>Pyrenophora teres</i>), Brown rust (<i>Puccinia hordei</i>), Powdery mildew (<i>Blumeria graminis hordei</i>); Apple: Apple scab (<i>Venturia inaequalis</i>); Maize: Phaeosphaeria leaf spot (<i>Phaeosphaeria maydis</i>)	Wahabzada et al. (2015); Delalieux et al. (2007); Adam et. Al (2017)
Fluorescence	photosynthetic status, quantum yield, non-photochemical quenching Fv/Fm, heat or drought stress, architecture leaf/plant health	Wheat: Leaf rust (<i>Puccinia triticina</i>), Powdery mildew (<i>Blumeria graminis f. sp. tritici</i>); Sugar beet: Cercospora leaf spot (<i>Cercospora beticola</i>); Bean: Common bacterial blight (<i>Xanthomonas fuscans</i> subsp. <i>fuscans</i>); Lettuce: Downy mildew (<i>Bremia lactucae</i>); Rice: Leaf scald (<i>Monographella albescens</i>)	Bürling et al. (2011); Cherelle et al. (2004, 2007); Konanz et al. (2014); Rousseau et al. (2013); Bauriegel et al. (2014); Brabandt et al. (2014); Tatagiba et al. (2015)

Source: Updated from Mahlein (2016). Reference list for Disease/Pathogen Assessment is not comprehensive



Modellierung (Pflanze, Bestand, Betrieb)

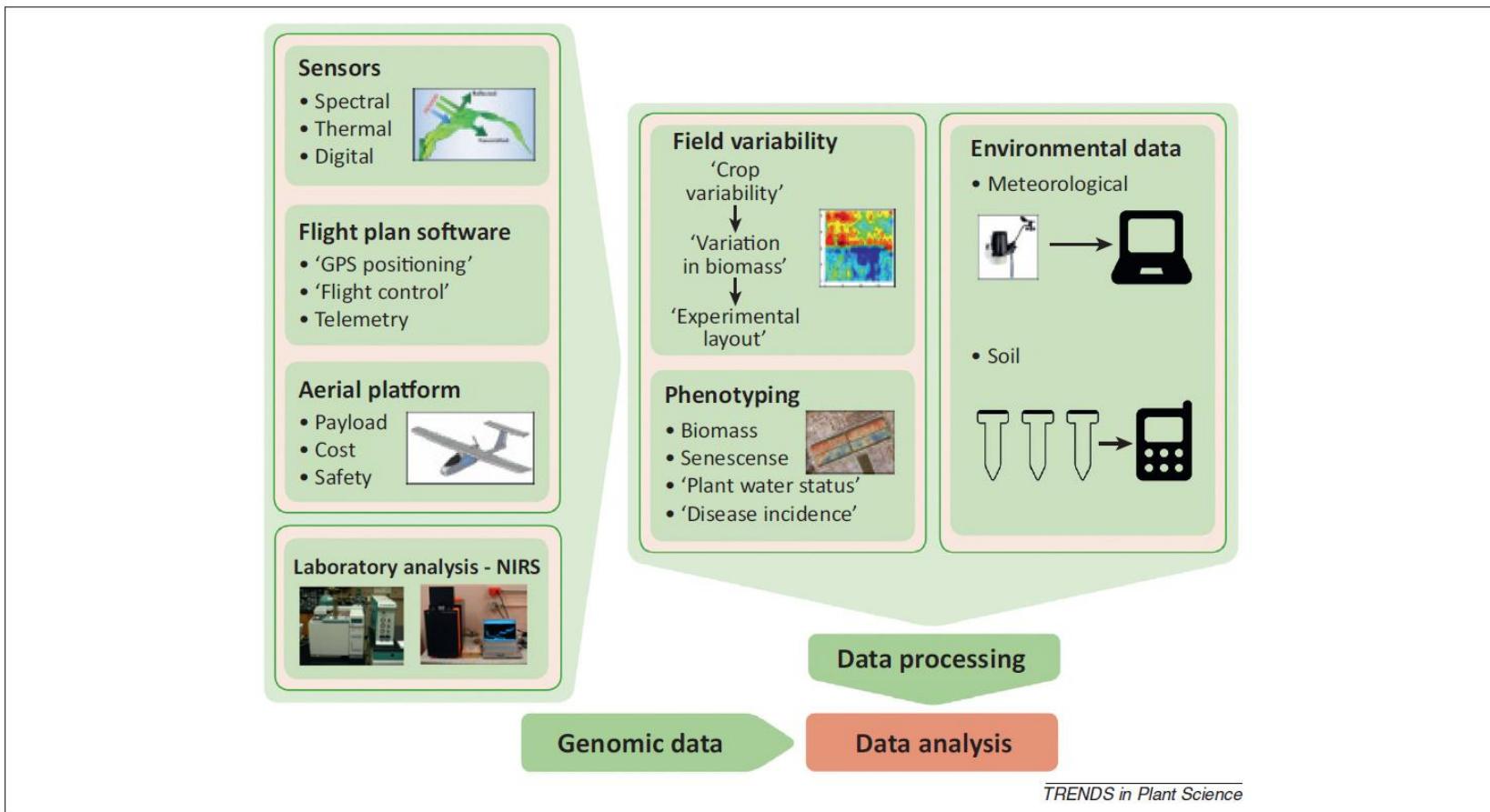
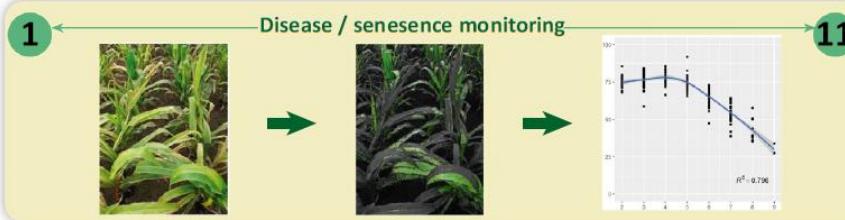
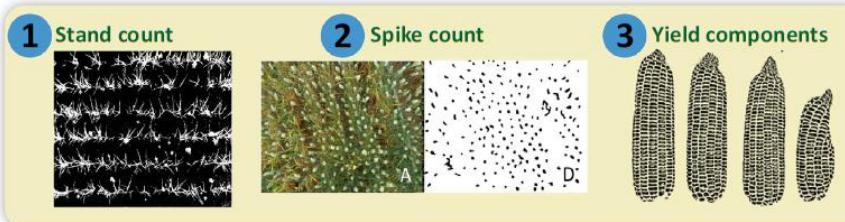
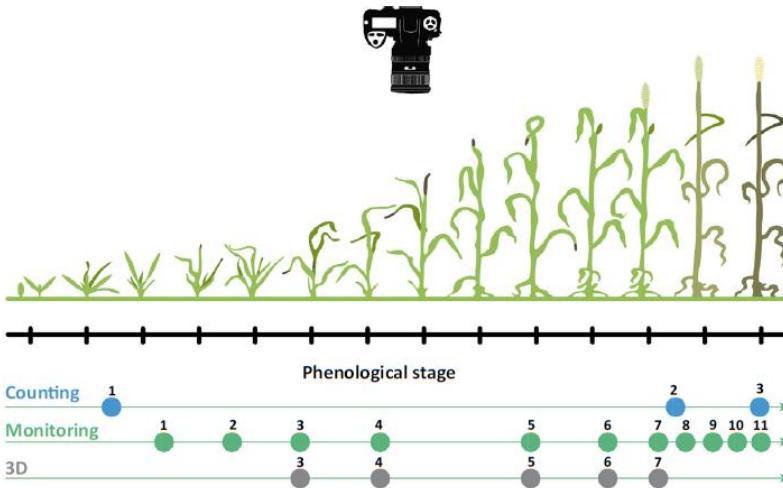


Figure 3. Summary of the different components of the breeding process where high-throughput phenotyping is involved. These include evaluation of key traits at the right moment, assessment of spatial variability, environmental characterization, and further integration of all the information.

Araus et al. 2014, Trends Plant Sci

Datenintegration (Klima, Boden, Düngung, Pflege, Genotyp)

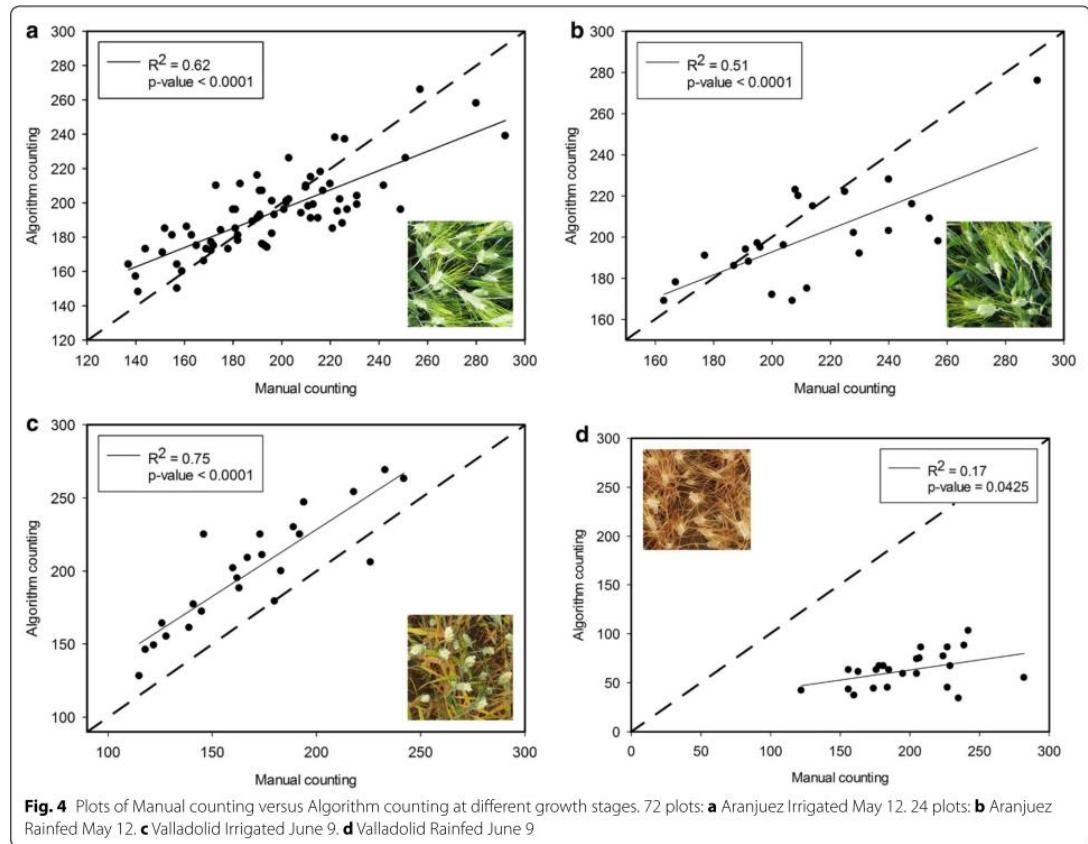


Datenintegration (Klima, Boden, Düngung, Pflanzenschutz, Landtechnik, Genotyp)

Traits	Tools					TRL (Technological readiness)				
	RGB	Multi/hyperspectral	LiDAR	Thermal	Fluorescence	1	2	3	4	5
Plant density @ emergence	High					Medium	Medium	Medium	Medium	Medium
Cover fraction	High					Medium	Medium	Medium	Medium	Low
Plant/canopy height			Medium			Medium	Medium	Medium	Medium	Medium
Ear density	High					Medium	Medium	Medium	Medium	Medium
Fruit/inflorescence size		Medium				Medium	Medium	Medium	Medium	Medium
Grain number and size						Medium	Medium	Medium	Medium	Medium
Leaf/plant glaucousness						Medium	Medium	Medium	Medium	Medium
Phenology (e.g., heading, anthesis...)		Medium		Medium		Medium	Medium	Medium	Medium	Medium
Lodging			Medium			Medium	Medium	Medium	Medium	Medium
Weed infestation						Medium	Medium	Medium	Medium	Medium
Diseases	High	Medium		Medium		Medium	Medium	Medium	Medium	Medium
Vegetation index monitoring			Medium			Medium	Medium	Medium	Medium	Medium
Green area index (GAI)			Medium			Medium	Medium	Medium	Medium	Medium
Senescence						Medium	Medium	Medium	Medium	Medium
Fraction of intercepted radiation			Medium			Medium	Medium	Medium	Medium	Medium
Leaf orientation						Medium	Medium	Medium	Medium	Medium
Leaf rolling						Medium	Medium	Medium	Medium	Medium
Chlorophyll content						Medium	Medium	Medium	Medium	Medium
Leaf/canopy temperature			Medium		Medium	Medium	Medium	Medium	Medium	Medium
Leaf/canopy chlorophyll fluorescense						Medium	Medium	Medium	Medium	Medium

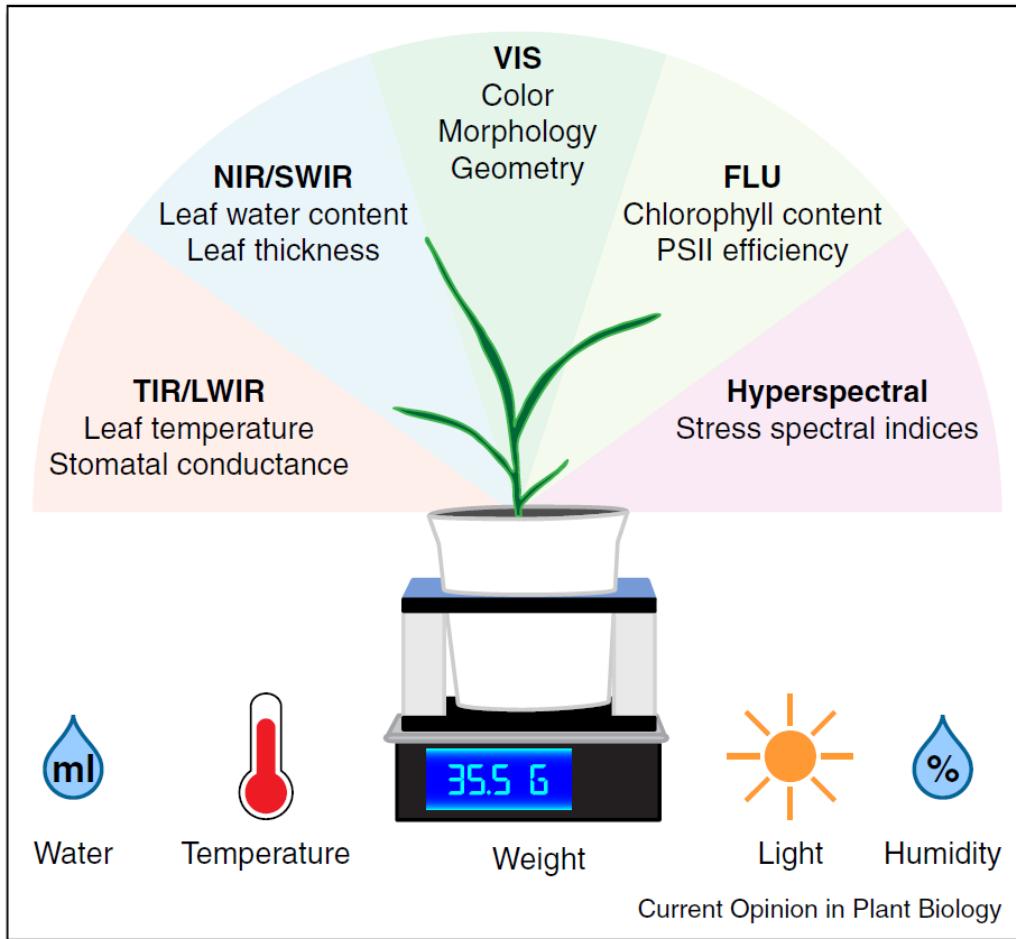
Araus et al. 2018, Trends Plant Sci

Datenintegration (Klima, Boden, Düngung, Pflanzenschutz, Landtechnik, Genotyp)

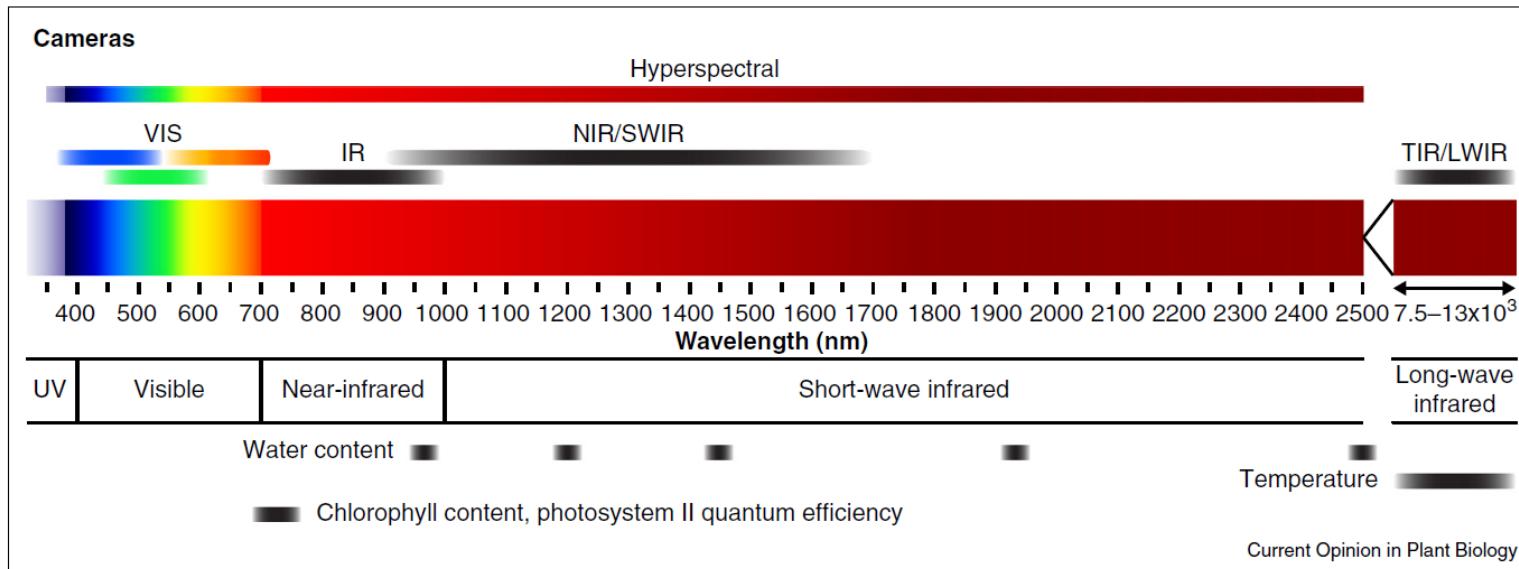


Plant Methods

High Throughput phenotyping (HTPP)



High Throughput phenotyping (HTPP)



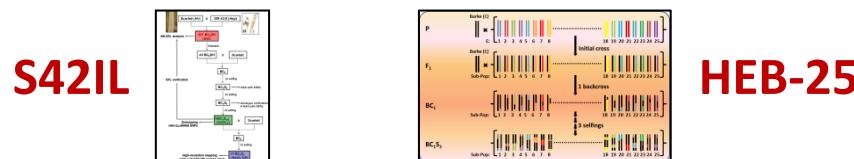
Falgren et al. 2015, Curr Op Plant Biol

IPAS Projekt BARLEY DIVERSITY

Projektstart: 01. März 2015 (effektiv)

Ziele:

1. Zwei Wildgerstenintrogressionspopulationen, S42IL und HEB-25, sollen in der Gerstenzüchtung verwendet werden, um die **Biodiversität** sowie die **Produktivität** unserer Elitegersten unter **Stickstoffmangel** und **Fruchfolge-Vorfruchtwirkungen** zu erhöhen.



2. Mittels des **nicht-invasiven**, mobilen Hyperspektral-Kamerasystems AgRover sollen **Pflanzenwachstum**, **Nährstoffkonzentration** und **Zellwandzusammensetzung** in Feldversuchen vorhergesagt und eingesetzt werden, um mit reduzierten Kosten merkmalsverbessernde Genotypen zu selektieren.

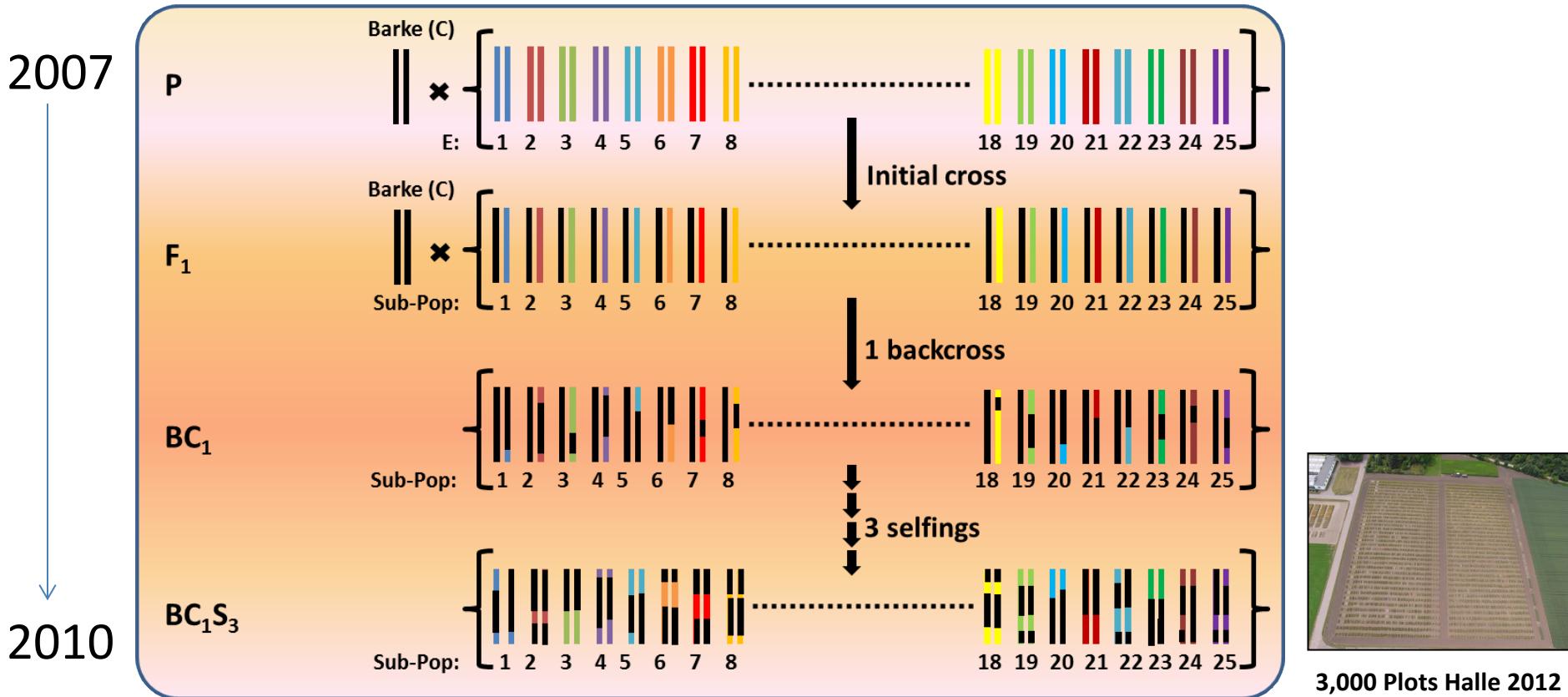


Innovation 1:

HEB-25

Die Wildgersten-Nested Association Mapping (NAM)

Population HEB-25



Maurer et al. 2015, BMC Genomics

Maurer et al. 2016a, J Exp Bot

Maurer et al. 2016b, Heredity

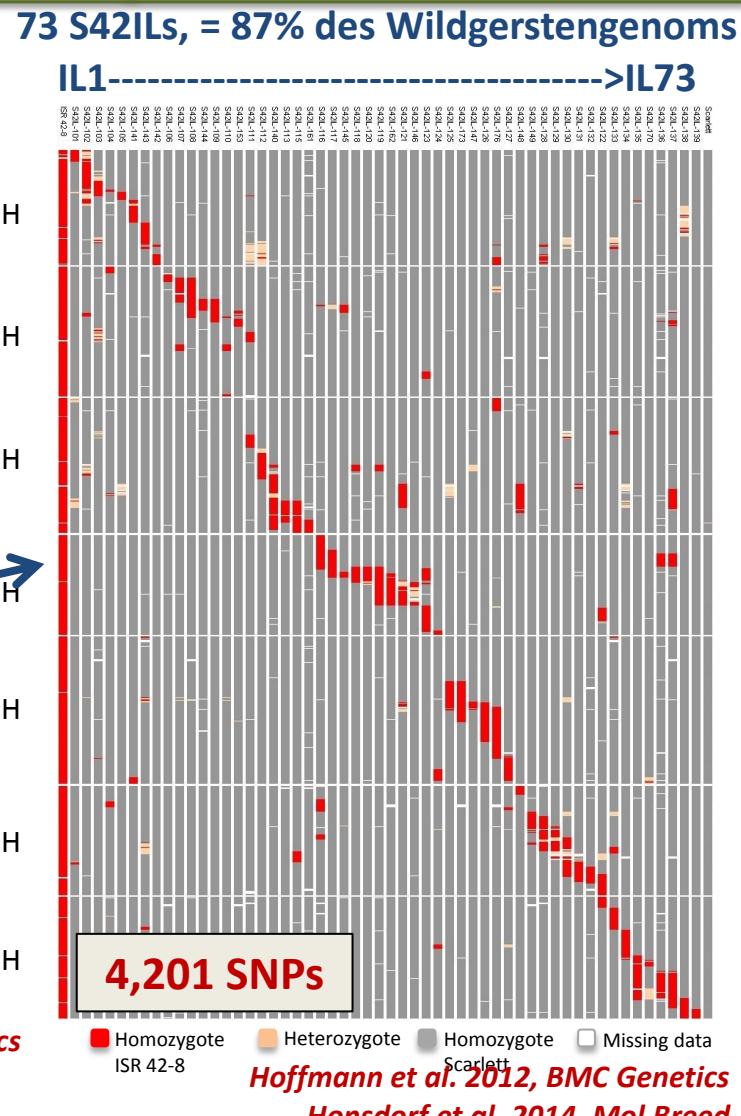
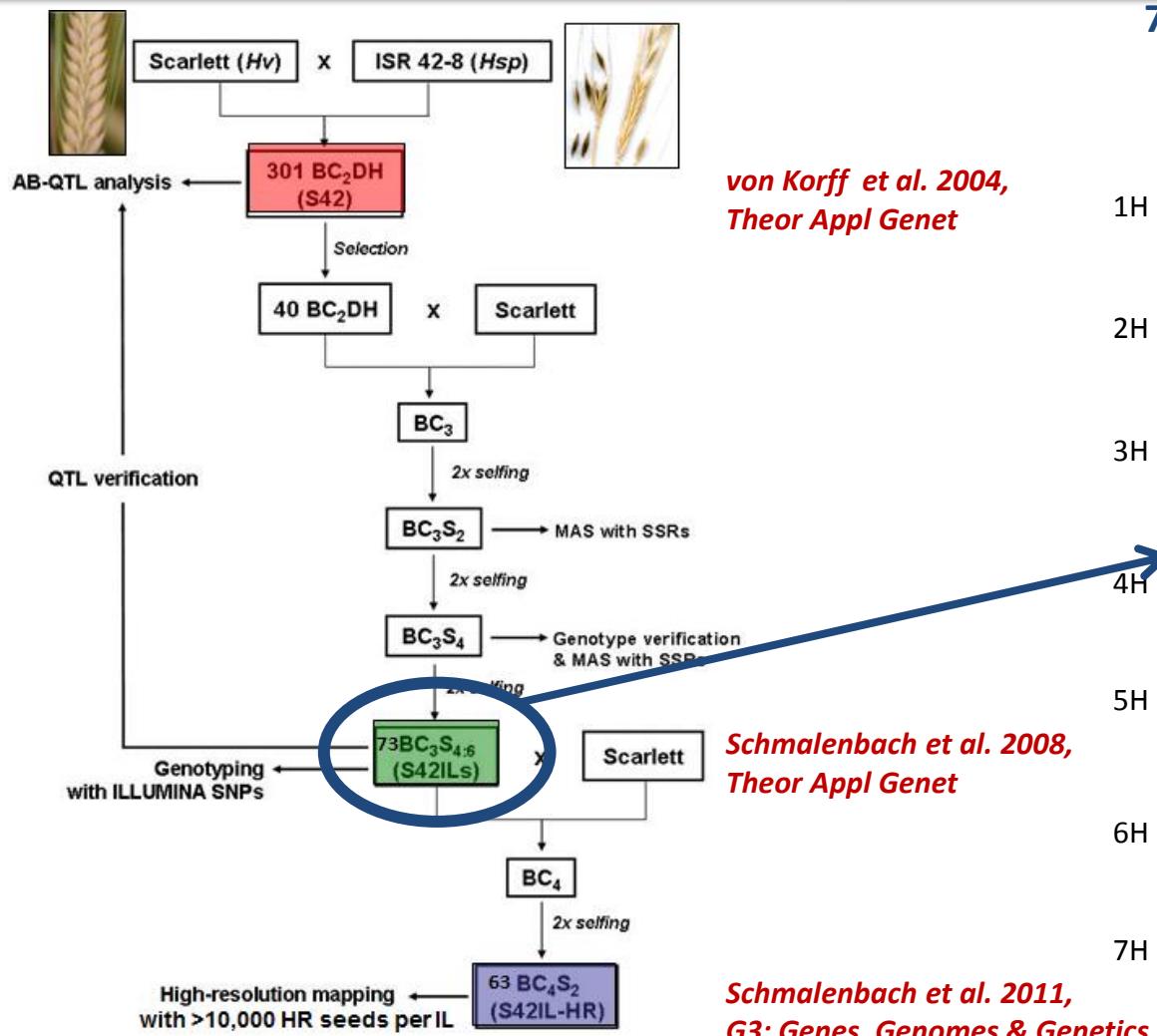
Saade et al. 2016, Scientific Reports

HEB-25 (Halle Exotic Barley) = 25 Familien mit 1,420 NAM Linien in BC₁S₃
Erwartete Segregation an jedem Locus: 72% : 6% : 22% (Hv : het : Hsp)

Innovation 2:

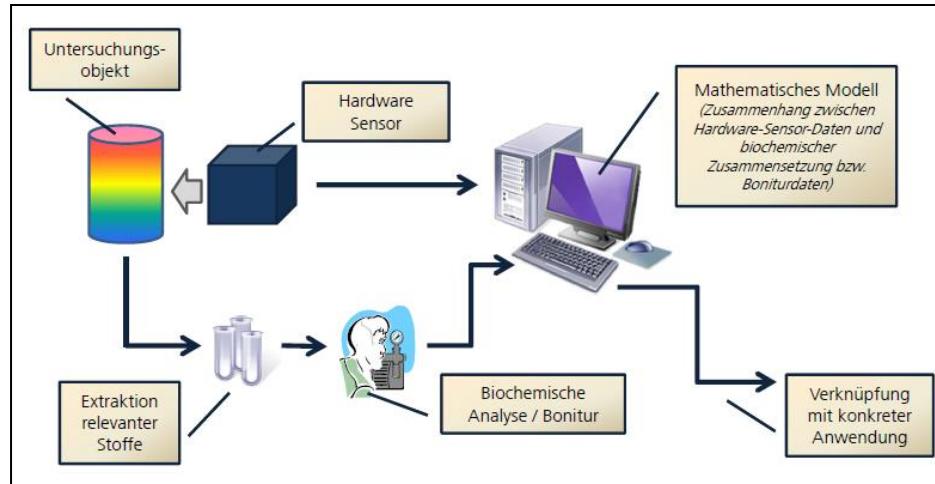
S42-IL

Die S42IL-Bibliothek, bestehend aus 73 Wildgersten-Introgressionslinien



Das hyperspektrale Messsystem AgRover zur Bestimmung von Nährstoffen und Kohlenhydraten im Feld

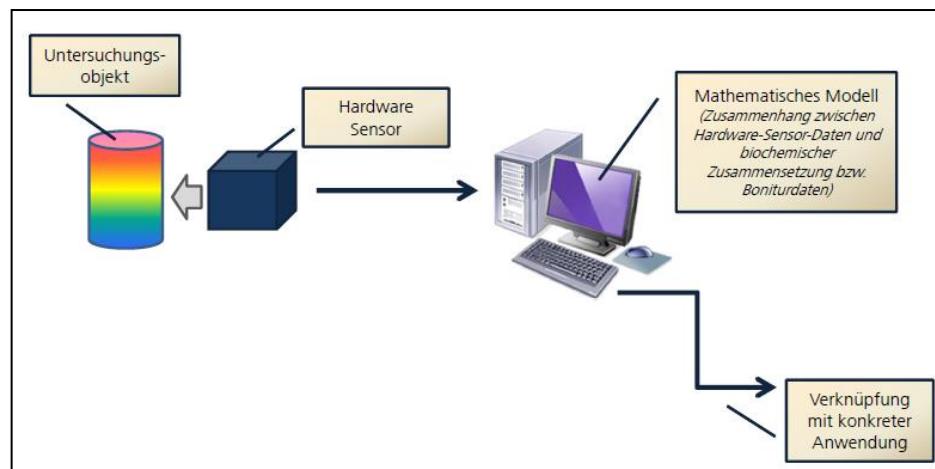
1. Kalibrations-Set (10% der Plots): HySpex + Naßlabor → Regressionsmodell



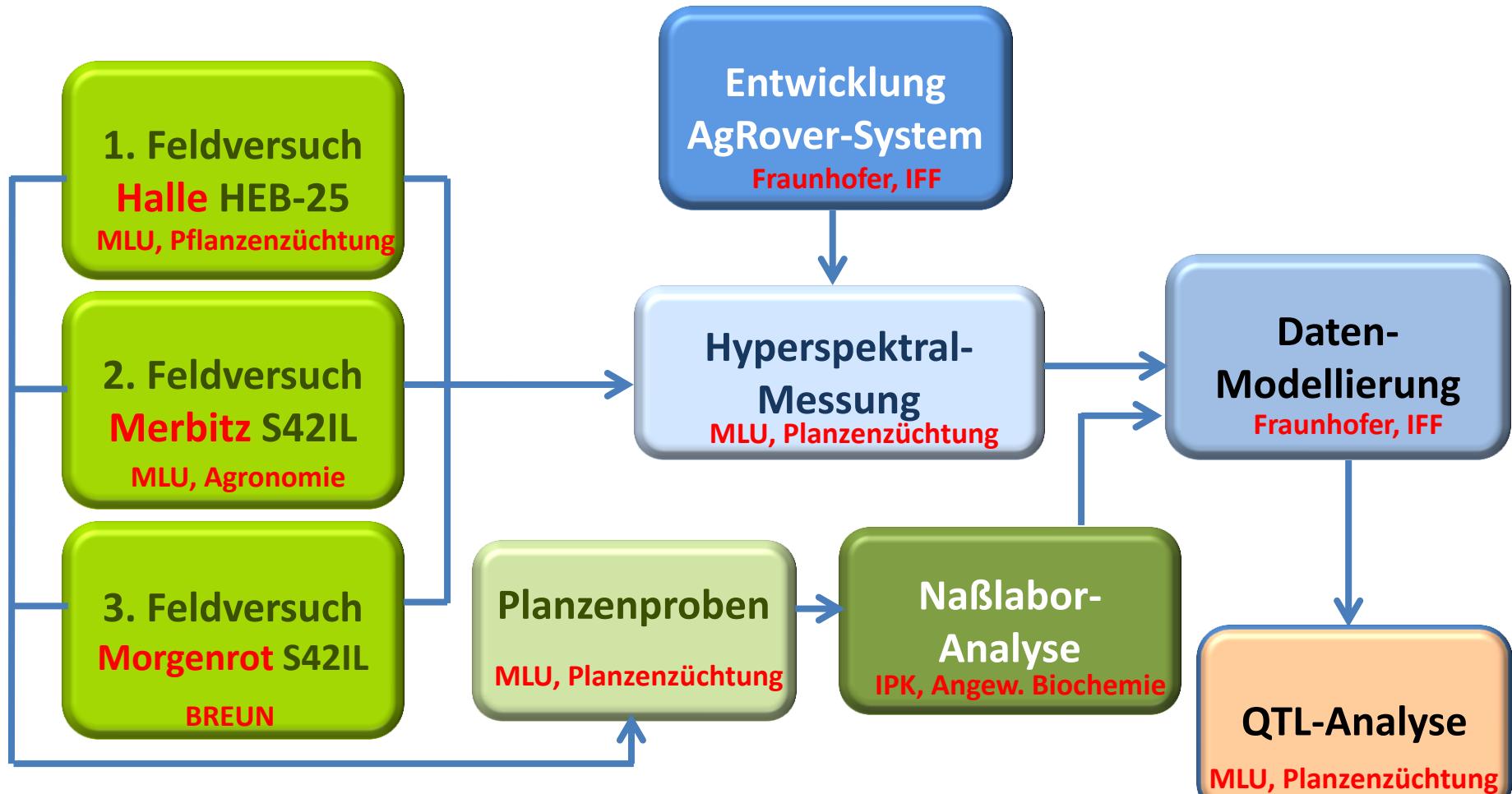
HySpex SWIR-384
Spektraler Bereich:
930-2500 nm
Kanäle: 288



2. Vorhersage-Set (90% der Plots): nur HySpex



Workflow BARLEY BIODIVERSITY



Feldversuche Halle 2015 & 2016, HEB-25



Halle HEB-25



Feldversuch Halle 2016 mit 3,000 Plots

Abkürzung	Merkmal
SHO	Schossen (BBCH 31)
HEA	Blüte (BBCH 49)
MAT	Reife (BBCH 87)
HEI	Pflanzenhöhe
EAR	Ähren pro qm
GEA	Kornzahl pro Ähre
TGW	Tausendkorngewicht

Merkmal	Einheit	N-Stufe		MW	CV	Korrelation N0 - N1	P NO vs. N1
		N0	N1				
SHO	Tage	2778	2797	59,8	7,21	0,76	***
		2797		57,2	7,18		
HEA	Tage	2778	2797	73,0	6,62	0,87	***
		2797		70,8	6,38		
MAT	Tage	2774	2794	113,0	4,88	0,74	***
		2794		110,0	5,22		
HEI	cm	2778	2794	66,6	16,75	0,79	***
		2794		70,9	16,84		
EAR	N0	2776	2796	560,8	33,78	0,47	***
				706,6	36,11		
GEA	N0	2649	2630	22,88	20,22	0,82	***
				23,66	19,74		
TGW	g	2767	2794	49,10	12,54	0,63	***
				49,02	11,79		

	SHO	HEA	MAT	HEI	EAR	GEA
HEA	0,82 ***					
MAT	0,66 ***	0,77 ***				
HEI	-0,27 ***	-0,10 ***	-0,17 ***			
EAR	-0,34 ***	-0,38 ***	-0,51 ***	0,16 ***		
GEA	0,12 ***	0,29 ***	0,30 ***	0,01 n.s.	-0,19 ***	
TGW	-0,27 ***	-0,21 ***	0,00 n.s.	0,16 ***	0,04 **	0,10 ***

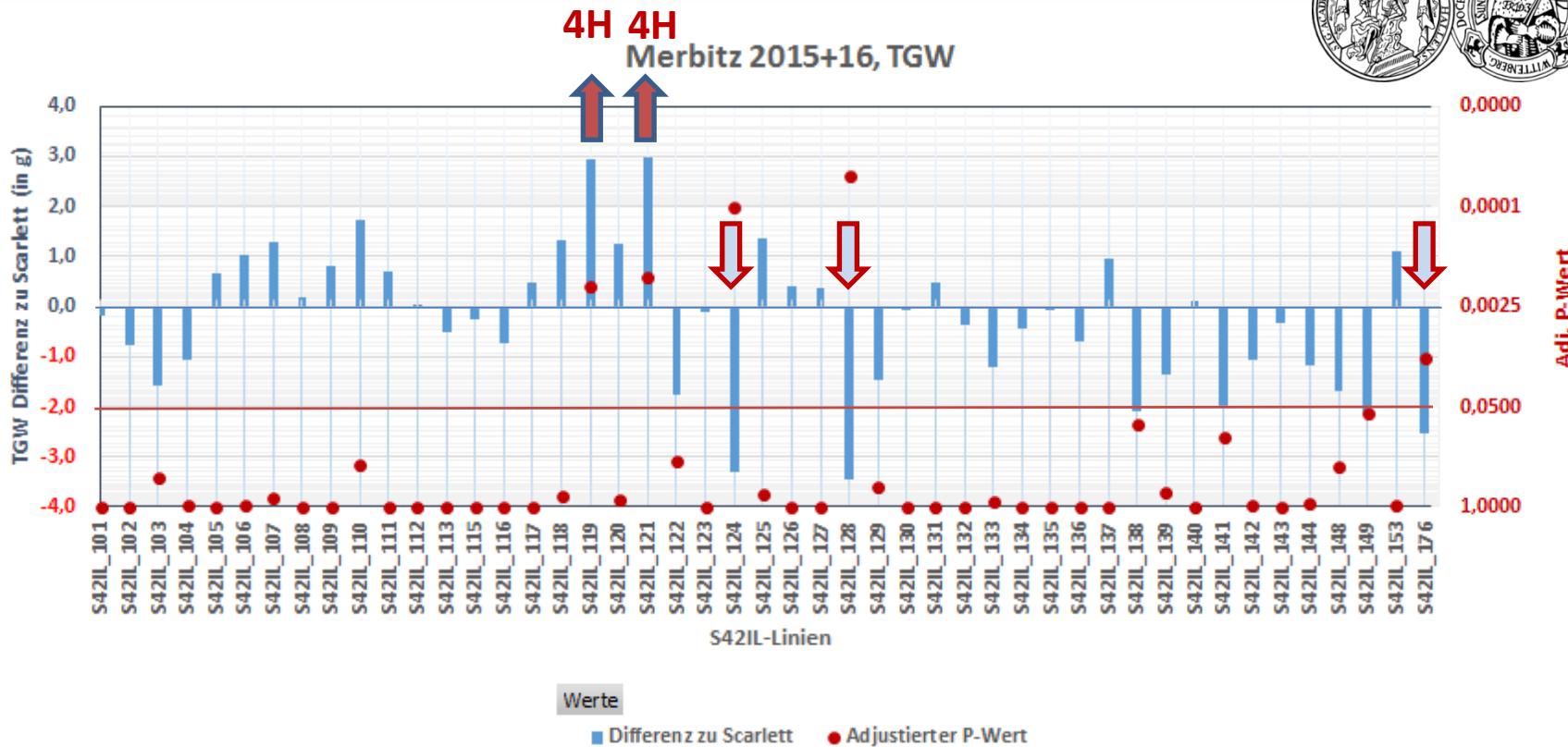
Maurer et al. 2015, BMC Genomics
Maurer et al. 2016a, J Exp Bot

Feldversuche Merbitz

2015 & 2016

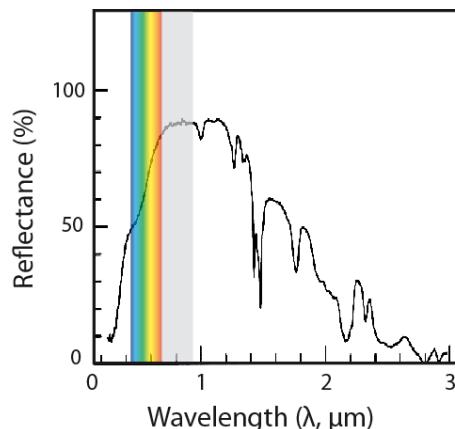
S42-ILs und TGW

S42-IL Merbitz



Signifikante Abweichungen (rote Punkte = adjustierte P-Werte) von 5 S42IL-Linien (IL-119, -121, -124, -128 und -176) als Differenz zu Scarlett für das Merkmal Tausendkorngewicht (TGW, blaue Säulen = Differenz zu Scarlett in g) am Standort Merbitz, gemittelt über Vorfrüchte und N-Stufen und Jahre. **Rote Linie:** cut off bei AdjP<0,05.

Das AgRover Kamerasystem



HySpec SWIR 384



Spektrale Kanäle: 288
Max. Framerate: 400 Hz
Auflösung räumlich: 384 px
Wellenlänge: 930-2500 nm

Kinect V2



3
30 Hz
RGB: 1920 x 1080 px
ToF: 512 x 424 px

AgRover

Hyperspektral
Kamera

Time of flight
Kamera (TOF)

RGB Kamera

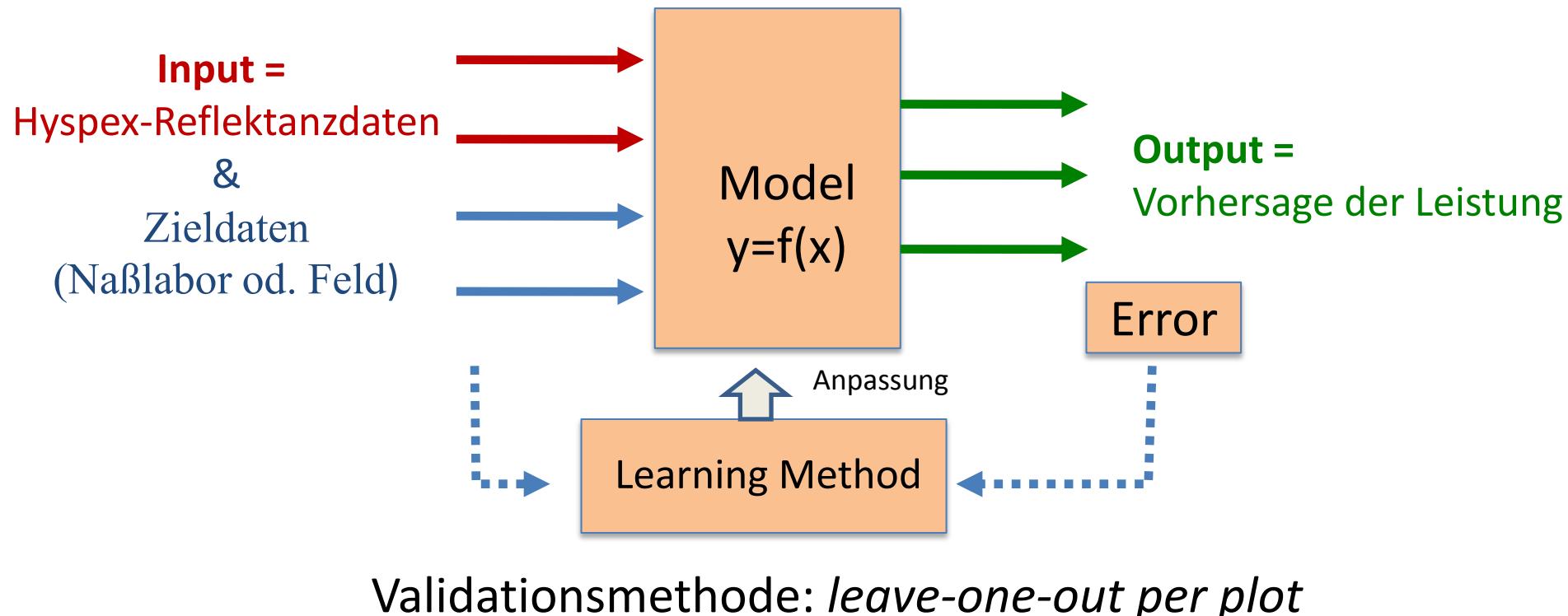
Hyperspektral
Bilder

Planzenhöhe

Pflanzen-
morphologie

Nichtlineare multiple Regressionsanalyse

→ *machine learning*



AgRover-Versuche Halle 2016, HEB-25

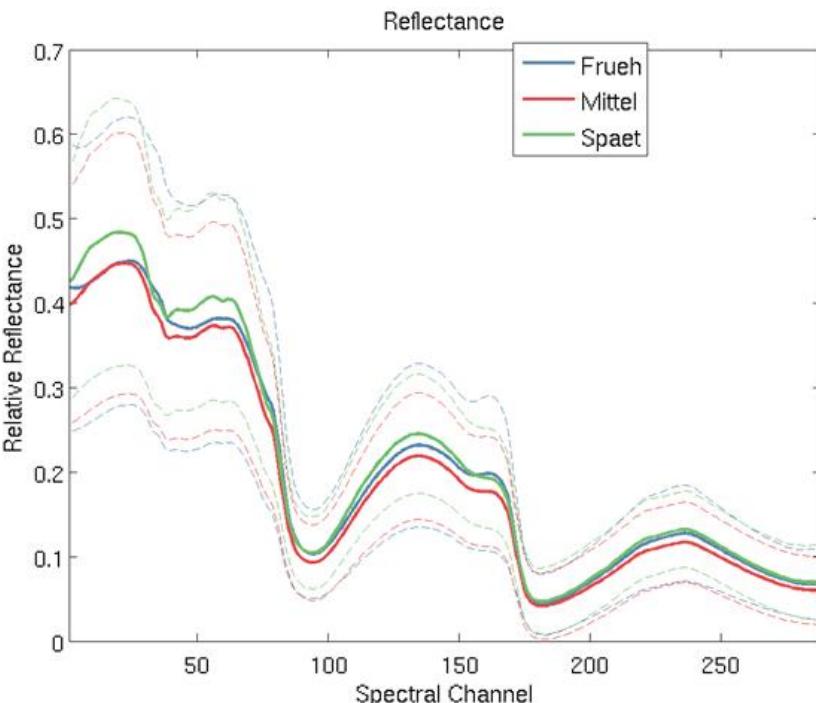


AgRover HEB-25



HySpex
SWIR 384

Ort	Fahrt A	Fahrt B	Fahrt C	Fahrt D	Daten-volumen	Blatt-proben	Ähren-proben	Korn-proben
Halle	03./04.05.16	23./31.05.16	07./10.06.16	27.07./09.08.16	2,79 TB	1.111	504	306
Merbitz	18.05.16	02.06.16	13.06.16	-	1,20 TB	288	-	96
Morgenrot	19.05.16	06.06.16	23.06.16	-	0,44 TB	144	-	48
Summe:					4.43 TB	1.543	504	450



Abk.	Merkmal
SHO	Schossen (BBCH 31)
SEL	Vegetative Phase
HEA	Blüte (BBCH 49)
RIP	Generative Phase
MAT	Reife (BBCH 87)
HEI	Pflanzenhöhe
EAR	Ähren pro qm
GEA	Kornzahl pro Ähre
TGW	Tausendkorngewicht

Spektrale Reflektanz von frühen, mittleren und späten HEB-Linien für den Zeitpunkt Schossen (1-Knoten-Stadium) mit deutlichen Reflektanz-Unterschieden in den spektralen Bändern 1-60 (= 930-1250 nm) und 130-160 (= 1640-1800 nm)..

AgRover-Versuche Halle 2016, HEB-25



AgRover HEB-25

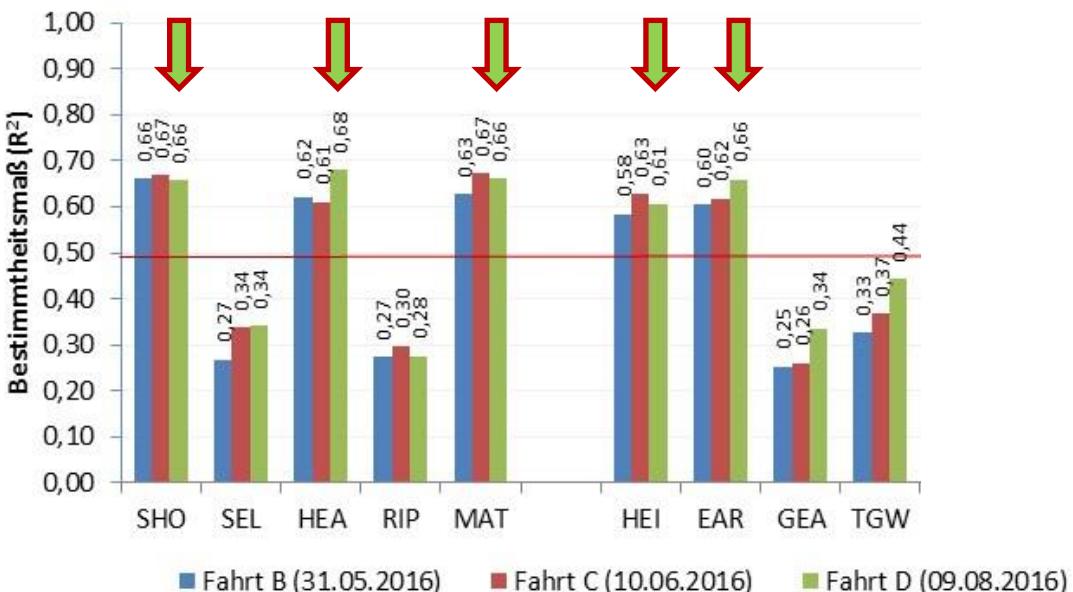


HySpex
SWIR 384



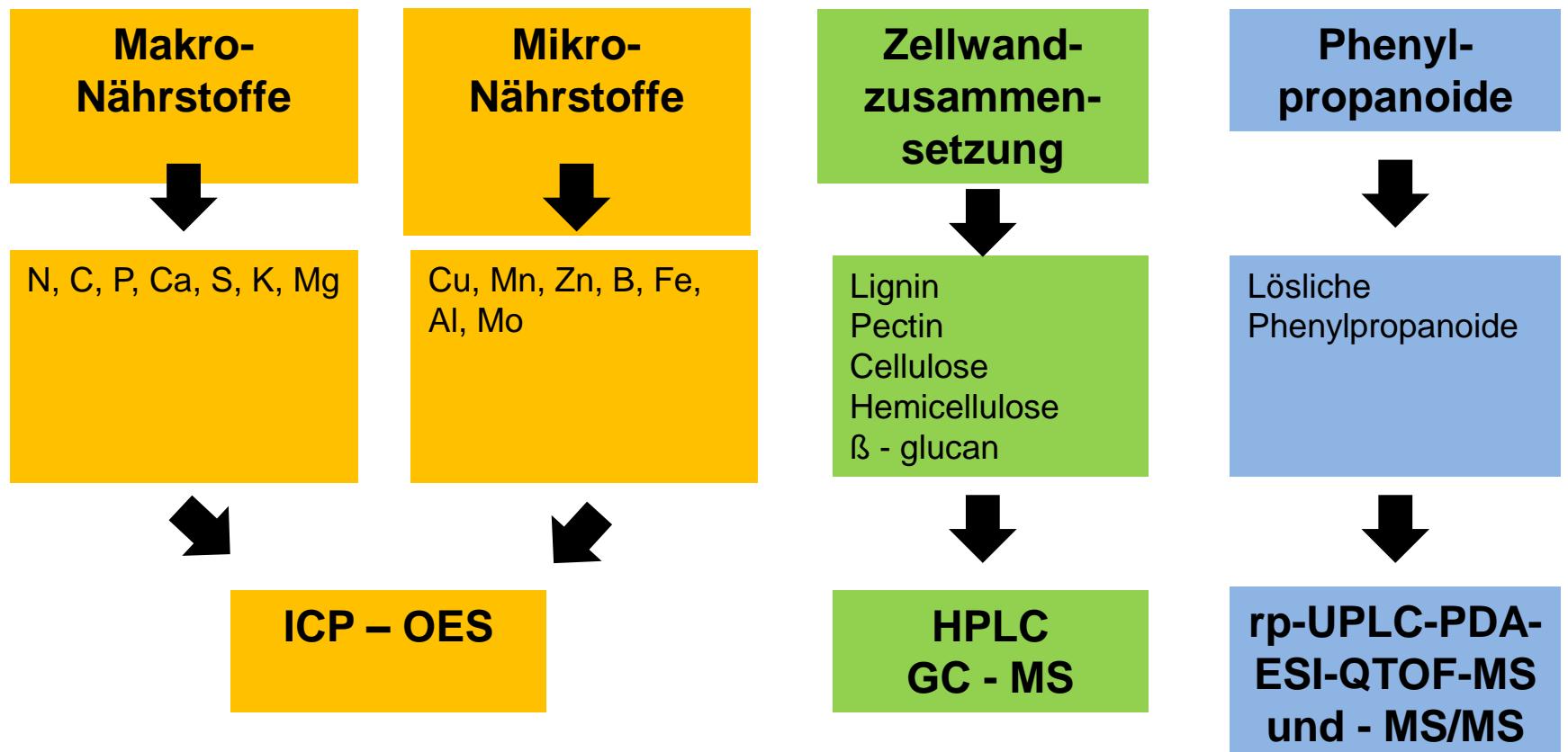
Ort	Fahrt A	Fahrt B	Fahrt C	Fahrt D	Daten-volumen	Blatt-proben	Ähren-proben	Korn-proben
Halle	03./04.05.16	23./31.05.16	07./10.06.16	27.07./09.08.16	2,79 TB	1.111	504	306
Merbitz	18.05.16	02.06.16	13.06.16	-	1,20 TB	288	-	96
Morgenrot	19.05.16	06.06.16	23.06.16	-	0,44 TB	144	-	48
Summe:					4,43 TB	1.543	504	450

Standort Halle



Bestimmtheitsmaße (R^2) für 9 agronomische Merkmale für die Messfahrten B (31.05.2016) und C (10.06.2016) und D (09.08.2016) am **Standort Halle**. Abkürzungen der Merkmale in **Tab. 1**. Die Reihung der Merkmale erfolgte nach den Merkmalskomplexen Entwicklung, Ertrag und Korn. **Rote Linie:** cut off bei $R^2=0,5$.

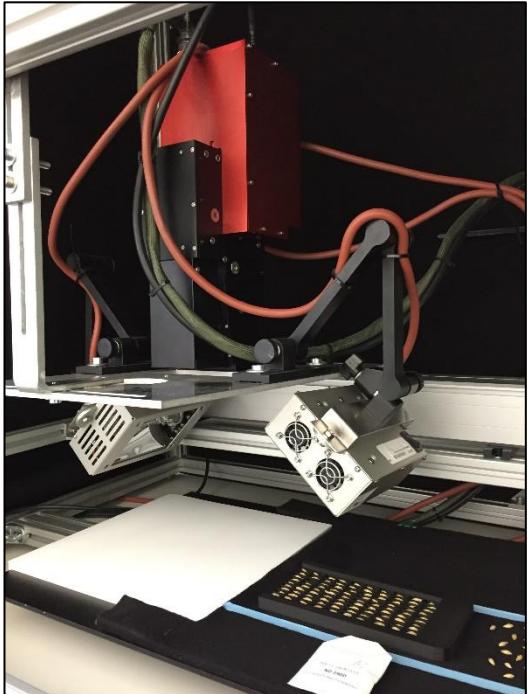
Naßlabor-Analyse zur Kalibration der Hyperspektral-Modellierung



AgRover-Versuche Halle 2016, HEB-25

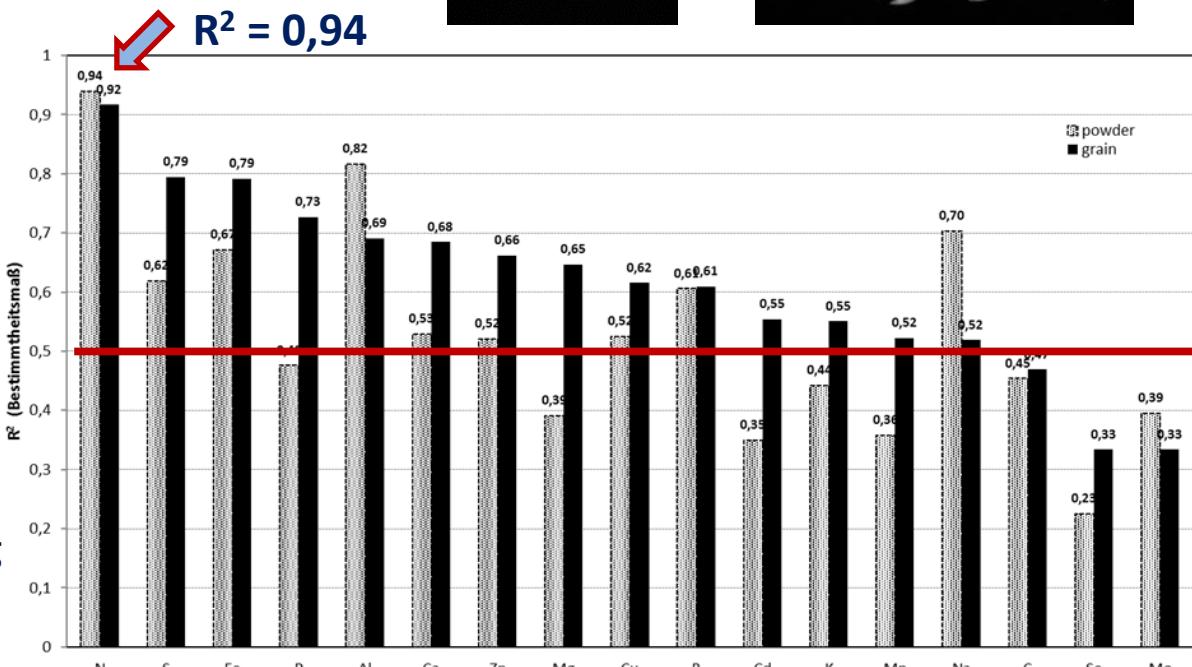
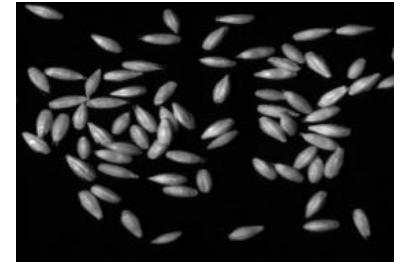


Hyspex HEB-25



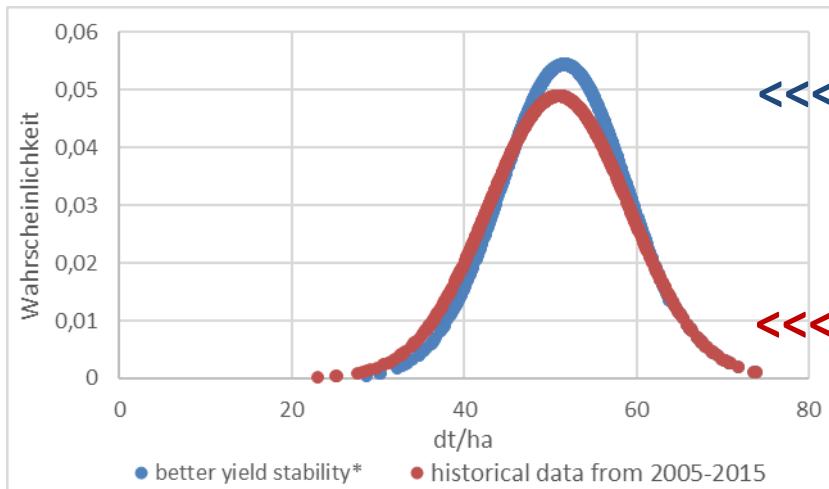
Stationäre Hyspex-Messvorrichtung

HySpex
SWIR 384



Schätzgenauigkeit (R^2) der Gehalte von 17 Mikro- und Makronährstoffen im Ganzkorn bzw. im Mehl mittels Hyperspektralanalyse und nichtlinearer Modellierung, basierend auf CN-Analyzer bzw. ICP-OES-Analyse von Vergleichsproben. Bestimmung der Schätzgenauigkeit (R^2) mittels *leave one out* Validierung ($N=250$). Rote Linie: *cut off* bei $R^2=0,5$.

Sozioökonomische Bewertung der Erhöhung der Biodiversität in der Pflanzenzüchtung



<<< Erhöhung der Ertragsstabilität um 20%
→ + 0,5 dt/ha Ertrag Sommergerste
→ + 3,5 Mio. € Sommergerstenproduktion

<<< historische Ertragsdaten D. 2005-2015

Simulierte Effekte erhöhter Ertragsstabilität durch eine Verbreiterung der genetischen Basis.
Annahme: Verringerung der negativen Differenz zum Mittelwert um 20 %.

Auf der methodischen Grundlage des *Total-Economic-Value* (TEV) kann die **Steigerung der Biodiversität im Genpool der Sommergerste in vier wirtschaftliche Dimensionen untergliedert werden**. Die Nutzbarmachung pflanzengenetischer Ressourcen führt zur:

1. Sicherung der landwirtschaftlicher Produktion,
2. Verringerung von Produktionsrisiken,
3. Verminderung der ökologischen Kosten durch Reduzierung des Produktionsmitteleinsatzes,
4. Bereitstellung einer optionalen Ressource für das Erreichen zukünftiger züchterischer Ziele.

Publikationen

1. Maurer A, **Draba V**, Jiang Y, Schnaithmann F, Sharma R, Schumann E, Kilian B, Reif JC, **Pullen K** (2015) Modelling the genetic architecture of flowering time control in barley through nested association mapping. **BMC Genomics**, doi:10.1186/s12864-015-1459-7
2. Maurer A, **Draba V**, **Pullen K** (2016a) Genomic dissection of plant development and its impact on thousand grain weight in barley through nested association mapping. **J Exp Botany**, doi: 10.1093/jxb/erw070
3. Maurer A, Sannemann W, Léon J, **Pullen K** (2016b) Estimating parent-specific QTL effects through cumulating linked identity-by-state SNP effects in multi-parental populations. **Heredity**, doi:10.1038/hdy.2016.121
4. Soleimani B, Sammler R, **Backhaus A**, Beschow H, Schumann E, **Mock HP**, von Wirén N, **Seiffert U**, **Pullen K** Genetic regulation of phosphorous efficiency in the wild barley introgression library S42IL (**submitted to Plant Breeding**)
5. **Brauch D**, Porzel A, Schumann E, **Pullen K**, **Mock HP** Changes in isovitexin-O-glycosylation during the development of young barley plants (**submitted to Phytochemistry**)

→ Weitere Publikationen in Vorbereitung

Schlußfolgerung

1. Die Wildgerstenpopulationen HEB-25 and S42IL zeigen genetische Variation für quantitative Merkmale (z.B. EAR und TGW) in den Feldversuchen 2015 and 2016 in Halle, Merbitz und Morgenrot.
2. Die Feldprüfung des AgRovers startete erst in der Vegetation 2016
3. Die hyperspektrale Modellierung von Nährstoffen, Metaboliten und agronomischen Merkmalen zeigen niedrige bis hohe Vorhersage-Genauigkeiten mit einer maximalen Güte von $R^2=0,68$ und $0,94$ zur Vorhersage des Blühzeitpunktes bzw. des N-Gehaltes.
4. Die AgRover-Feldprüfungen sollen in den Anbaujahren 2017 und 2018 fortgesetzt werden.
5. Die Schlagkraft der Spektralmessungen im Feld soll durch ein neues Copter-System mit Multispektralkameras erhöht werden.

Fortsetzungsantrag für die 2 Förderperiode (02/18 – 01/20)

Neue Ziele und Meilensteine (MS)

1. Ausdehnung der Feldversuche Merbitz (S42IL) und Halle (HEB-25) in das Jahr 2018 (MS #1 und #2)

- Weitere Prüfung des vorhandenen AgRover-Hyperspektralsystems (von 2016 bis 2018).
- Erhöhung der **statistischen Power** in der nachfolgenden GWAS.



2. Prüfung eines zusätzlichen MultiCoptersystems mit Multispektral- und Thermalkamera (MS #1 und #2)

- Erweiterung des Spektrums auf die Wellenlängenbereiche 450-1000 nm und 7,5-13 µm zur Messung der Entwicklung des Pflanzenbestandes sowie der **biotischen und abiotischen Stresstoleranz** unter N-Mangel.
- Steigerung der Schlagkraft durch Verwendung eines flugfähigen MultiCoptersystems



XR6 HexaCopter und Tetracam μ MCA Snap 6

Fortsetzungsantrag für die 2 Förderperiode (02/18 – 01/20)

Neue Ziele und Meilensteine (MS)

3. Hyperspektrale Vorhersage der Malzqualität (MS #3)

- Prüfung der hyperspektralen Modellierung der Malzqualität als indirekte Selektionsmethode in Kooperation mit Saatzucht BREUN.

4. Datenbankausbau (MS #4)

- Integration der erhobenen agronomischen, genomischen und Hyperspektral-daten in eine gemeinsame Datenbank (Optimas Data Warehouse)

5. Analyse der genetischen Regulation der untersuchten Merkmale (MS #5)

- Genomweite Assoziationskartierung (GWAS) von QTLs in den Populationen S42IL und HEB-25 mit einem neuen Gersten-50k SNP-chip.

6. Durchführung eines 3-faktoriellen Feldversuchs in Merbitz 2018 (MS #6)

- Prüfung selektierter S42IL- und HEB-25-Linien unter divergierenden N-Düngungs- und Pflanzenschutz-Anwendungen zur monetären Bewertung der Nutzung von Wildgerstenintrogressionslinien in der Pflanzenzüchtung.

Projektpartner

1. Anja Hanemann & Jens Weyen

Saatzucht Josef Breun GdbR (**BREUN**)



2. Andreas Backhaus, Christian Klück, Sebastian Warnemünde & Udo Seiffert

Fraunhofer-Institut für Fabrikbetrieb & –automatisierung (**IFF**)
→ Unterauftragnehmer für PB



3. Dominic Brauch & Hans-Peter Mock

Leibniz-Institut für Pflanzengenetik & Kulturpflanzenforschung (**IPK**)



4. Sarah Zahn, Barbara Koblenz & Olaf Christen

MLU Halle, Allgemeiner Pflanzenbau & Ökologischer Landbau (**AGR**)



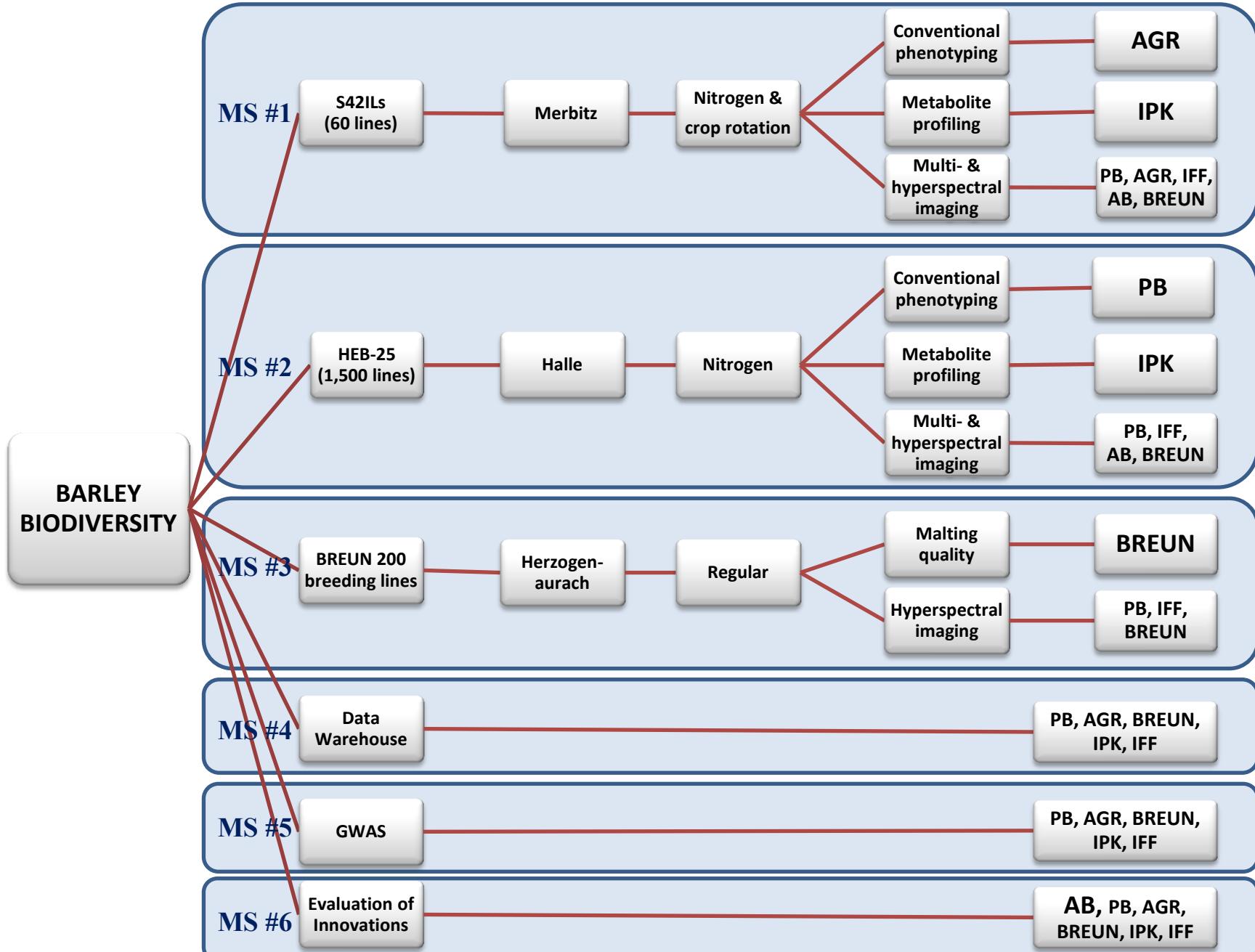
5. Johannes Ryll & Peter Wagner

MLU Halle, Landwirtschaftliche Betriebslehre (**AB**)

Finanzielle
Unterstützung



BMBF (IPAS)



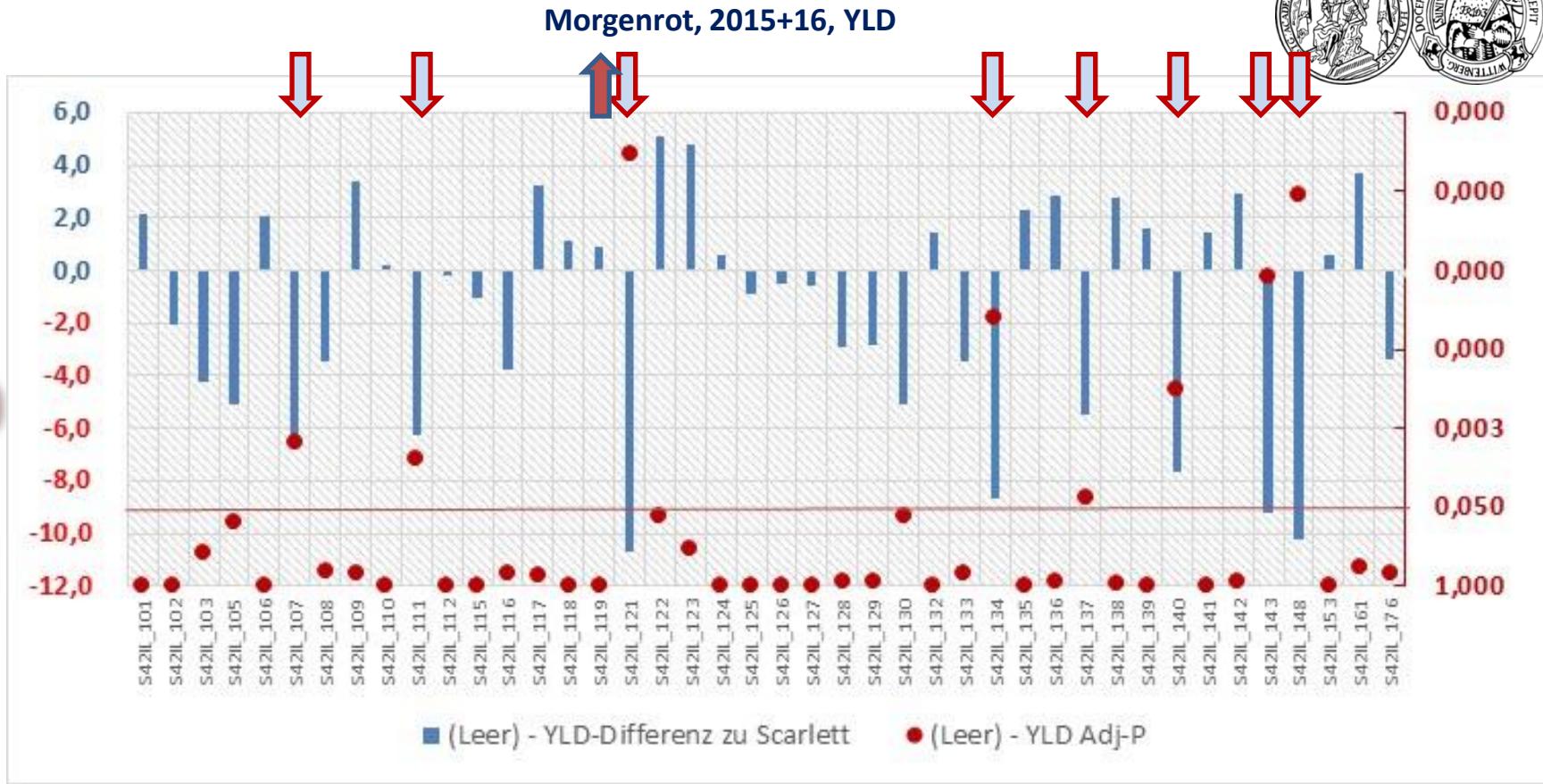
Feldversuche Morgenrot

2015 & 2016

S42-ILs



S42-IL Morgenrot



Signifikante negative Abweichungen (adjustierte P-Werte <0,05, rote Punkte) von 8 S42IL-Linien als Differenz zu Scarlett für das Merkmal Ertrag (YLD, blaue Säulen in dt/ha) am Standort Morgenrot, gemittelt über N-Stufen und Jahre. **Rote Linie:** cut off bei AdjP<0,05.

AgRover-Versuche Merbitz 2016, S42IL



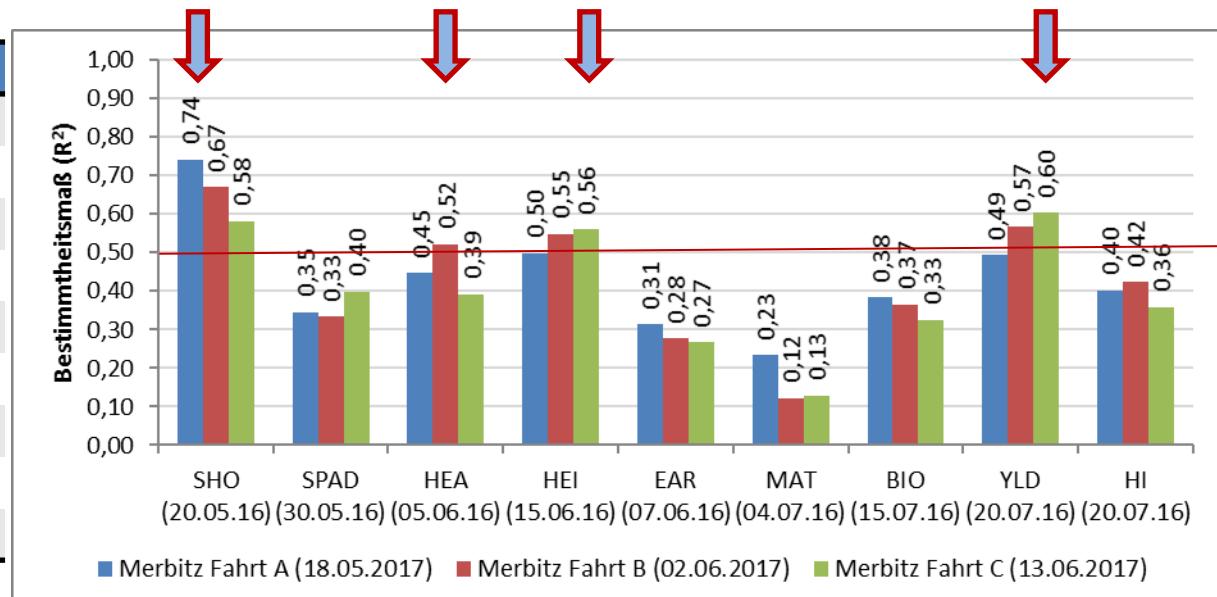
AgRover HEB-25



HySpex
SWIR 384

Ort	Fahrt A	Fahrt B	Fahrt C	Fahrt D	Daten-volumen	Blatt-proben	Ähren-proben	Korn-proben
Halle	03./04.05.16	23./31.05.16	07./10.06.16	27.07./09.08.16	2,79 TB	1.111	504	306
Merbitz	18.05.16	02.06.16	13.06.16	-	1,20 TB	288	-	96
Morgenrot	19.05.16	06.06.16	23.06.16	-	0,44 TB	144	-	48
Summe:					4,43 TB	1.543	504	450

Abk.	Merkmal
SHO	Schossen (BBCH 31)
SPAD	Chlorophyllgehalt
HEA	Blüte (BBCH 49)
HEI	Pflanzenhöhe
EAR	Ähren pro qm
MAT	Reife (BBCH 87)
BIO	Biomasse
YLD	Ertrag
HI	Ernteindex



Bestimmtheitsmaße (R^2) für 9 agronomische Merkmale für die Messfahrten A (18.05.2017, d.h. 44 Tage nach Saat), B (02.06.2017, d.h. 59 Tage nach Saat) und C (13.06.2017, d.h. 70 Tage nach Saat) am **Standort Merbitz**. Abkürzungen der Merkmale in **Tab. 1**. Die Sortierung der Merkmale erfolgte nach Datum der Messung (unterhalb des Merkmals), bzw. des Erreichens des Stadiums (SHO, HEA, MAT) bei 50% der S42IL-Linien. **Rote Linie:** cut off bei $R^2=0,5$.

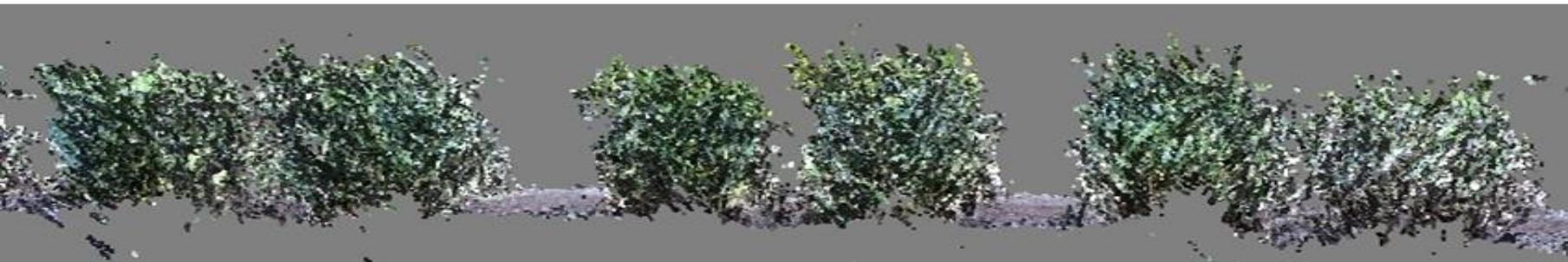
AgRover-Versuche Halle 2016, HEB-25

AgRover HEB-25



Kinect V2

Ort	Fahrt A	Fahrt B	Fahrt C	Fahrt D	Daten-volumen	Blatt-proben	Ähren-proben	Korn-proben
Halle	03./04.05.16	23./ 31.05.16	07./10.06.16	27.07./ 09.08.16	2,79 TB	1.111	504	306
Merbitz	18.05.16	02.06.16	13.06.16	-	1,20 TB	288	-	96
Morgen-rot	19.05.16	06.06.16	23.06.16	-	0,44 TB	144	-	48
Summe:					4,43 TB	1.543	504	450



Erfassung des 3-dimensionalen Wachstums der HEB-25-Pflanzen im Parzellenbestand zu den Messfahrten A (03.04.2016, oben) und B (23.05.2016, unten) in Halle..

R² (beobachtet : erwartet) = 77%

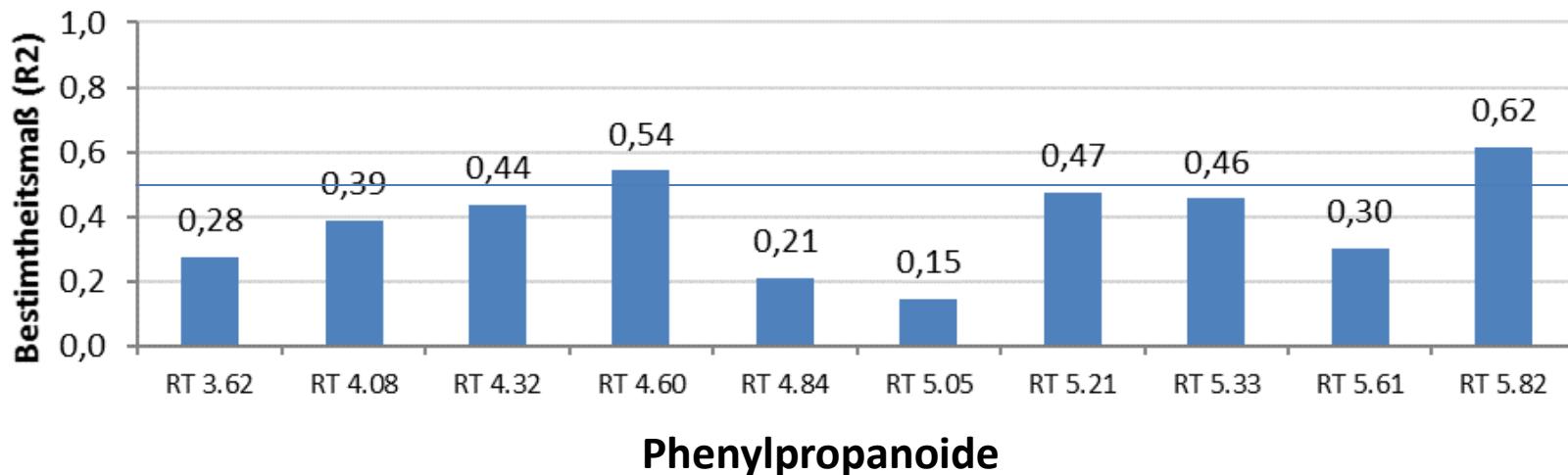
AgRover-Versuche Merbitz 2016, S42IL

AgRover S42IL



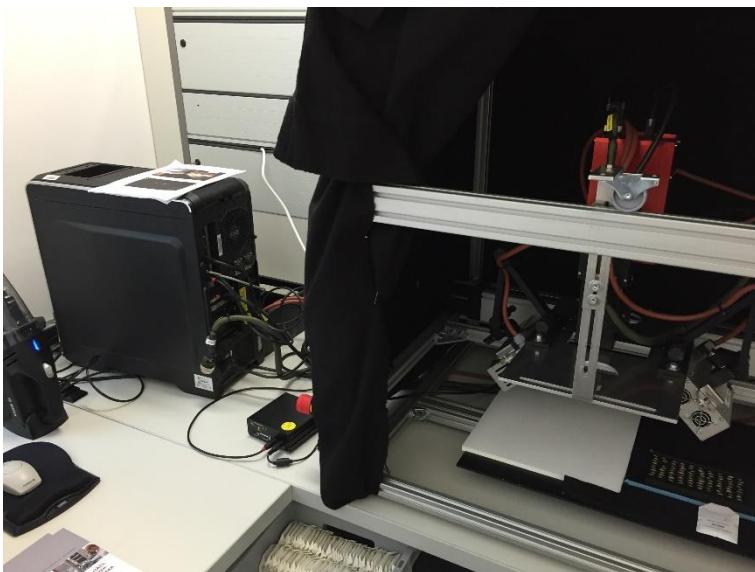
Ort	Fahrt A	Fahrt B	Fahrt C	Fahrt D	Daten-volumen	Blatt-proben	Ähren-proben	Korn-proben
Halle	03./04.05.16	23./31.05.16	07./10.06.16	27.07./09.08.16	2,79 TB	1.111	504	306
Merbitz	18.05.16	02.06.16	13.06.16	-	1,20 TB	288	-	96
Morgenrot	19.05.16	06.06.16	23.06.16	-	0,44 TB	144	-	48
Summe:					4,43 TB	1.543	504	450

Fahrt A Merbitz

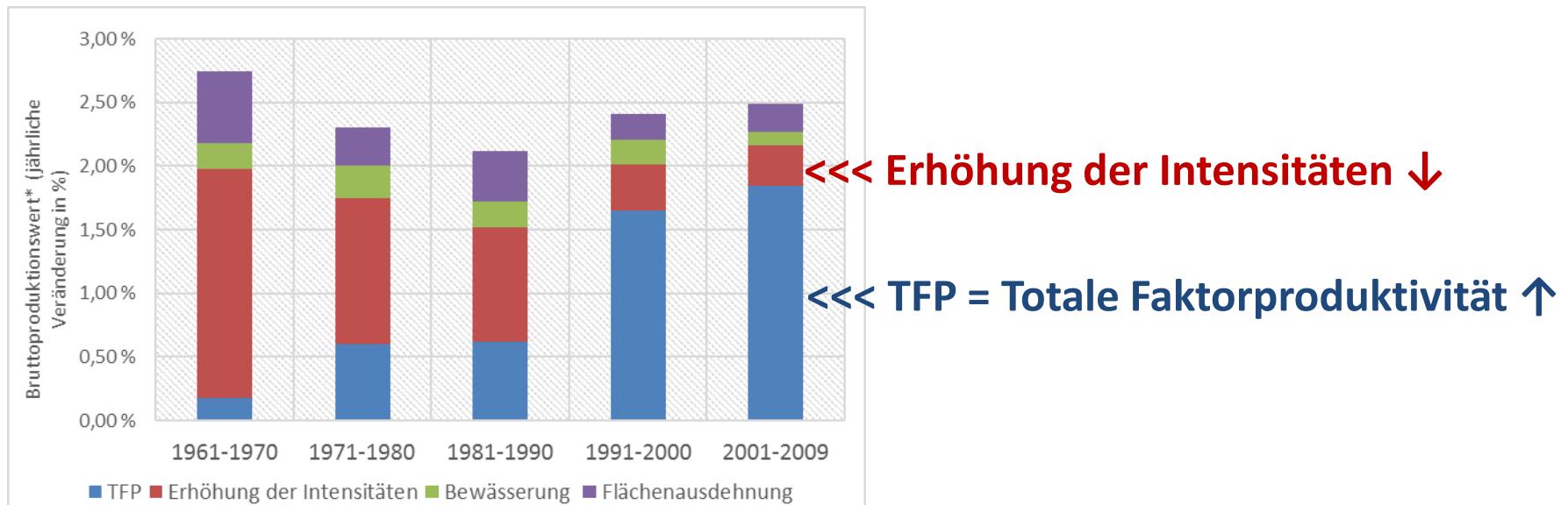


Bestimmtheitsmaße (R^2) für zehn Phenylpropanoide (RT) für die Messfahrt A (18.05.2017) am Standort Merbitz.

Blaue Linie: *cut off* bei $R^2=0,5$.

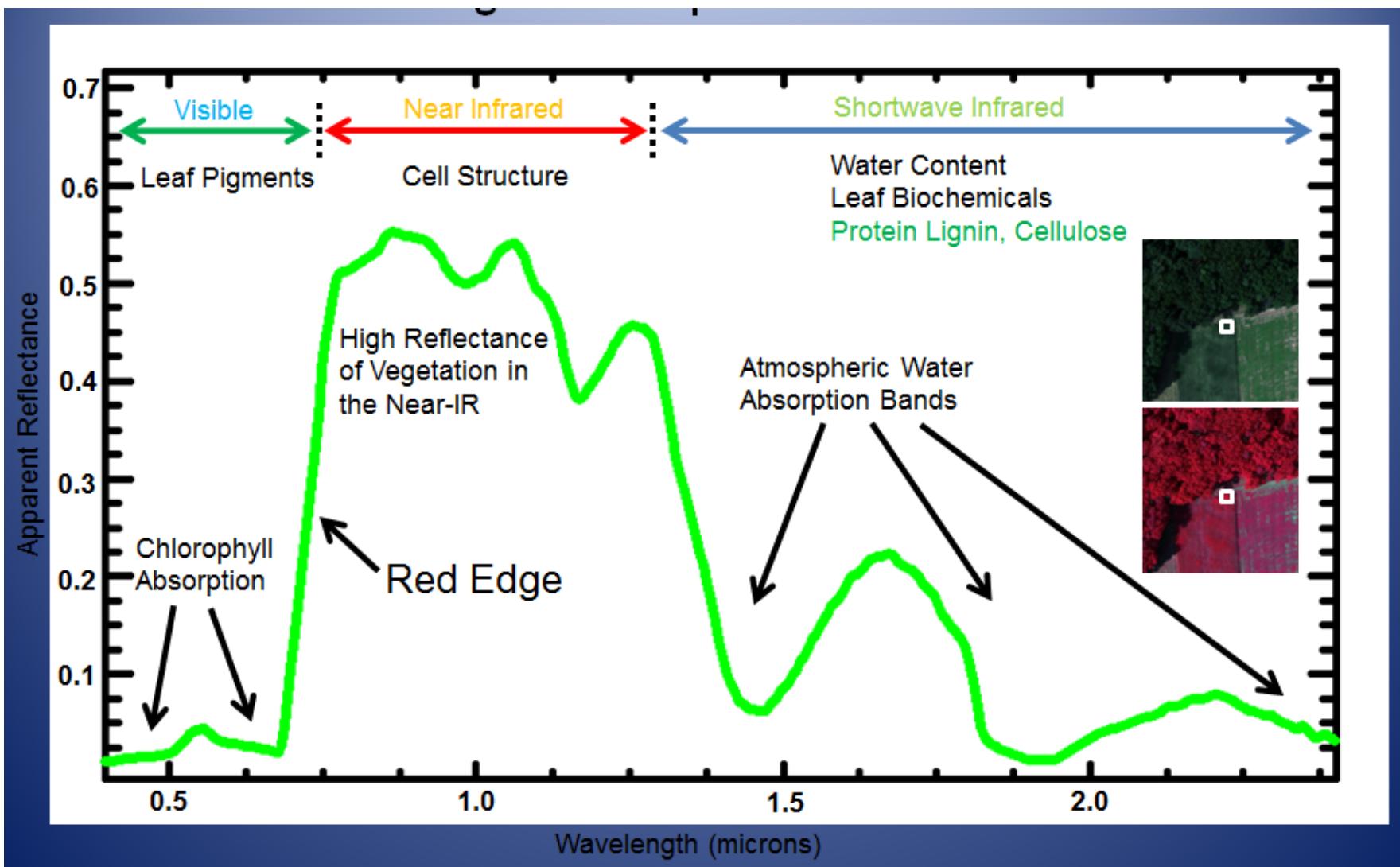


Sozioökonomische Bewertung von Innovationen in der Agrarwirtschaft



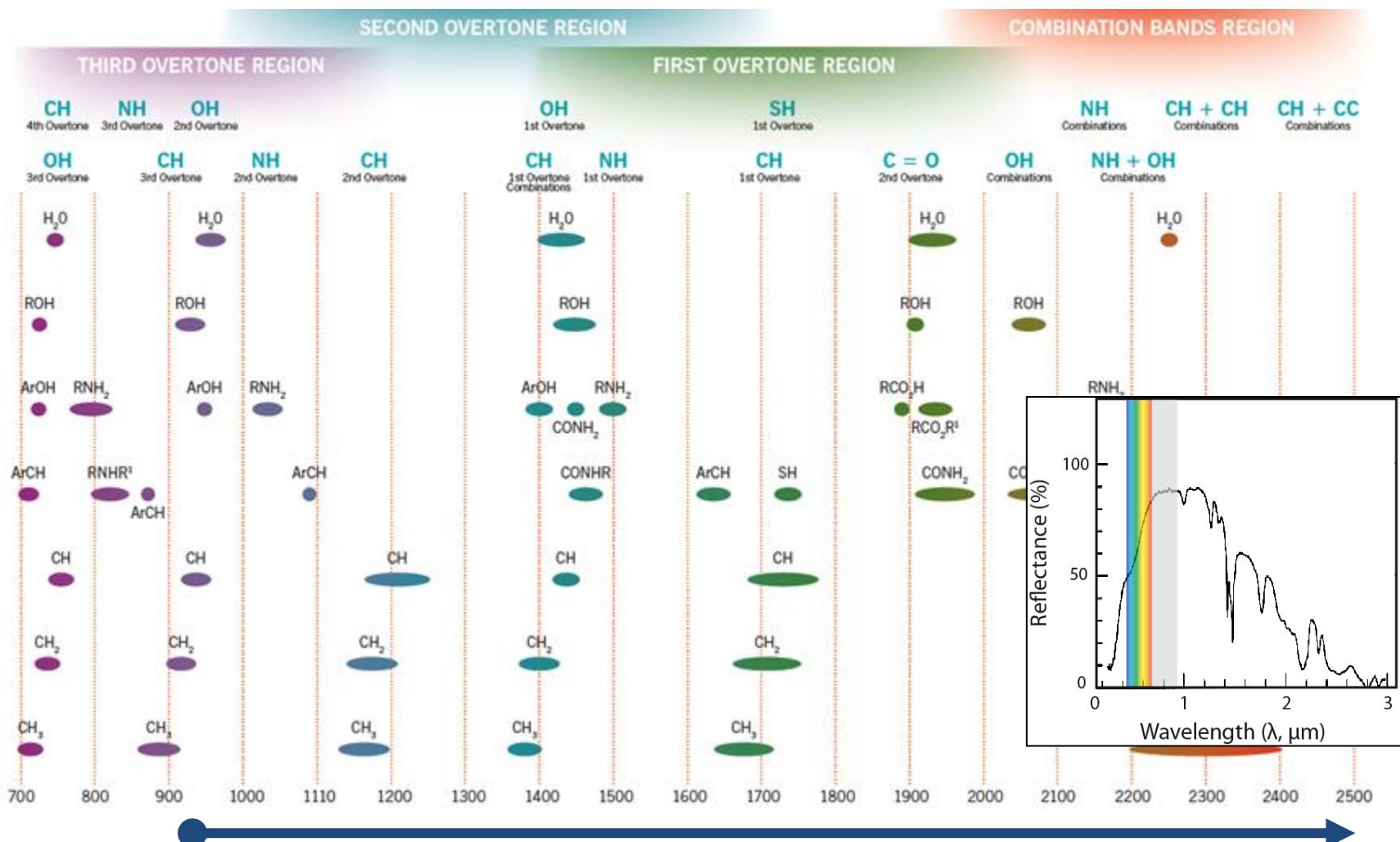
Wachstumsfaktoren der weltweiten Agrarproduktion, bewertet mit konstanten Preisen für das Jahr 2005 nach Fuglie (2012). Die **Totale Faktorproduktivität (TFP)** misst den Anteil des Wirtschaftswachstums, der nicht auf die Veränderung der Produktionsfaktoren Boden, Arbeit und Kapital zurückzuführen ist. Die TFP wird als Maß für den technischen Fortschritt interpretiert.

The vegetation spectrum



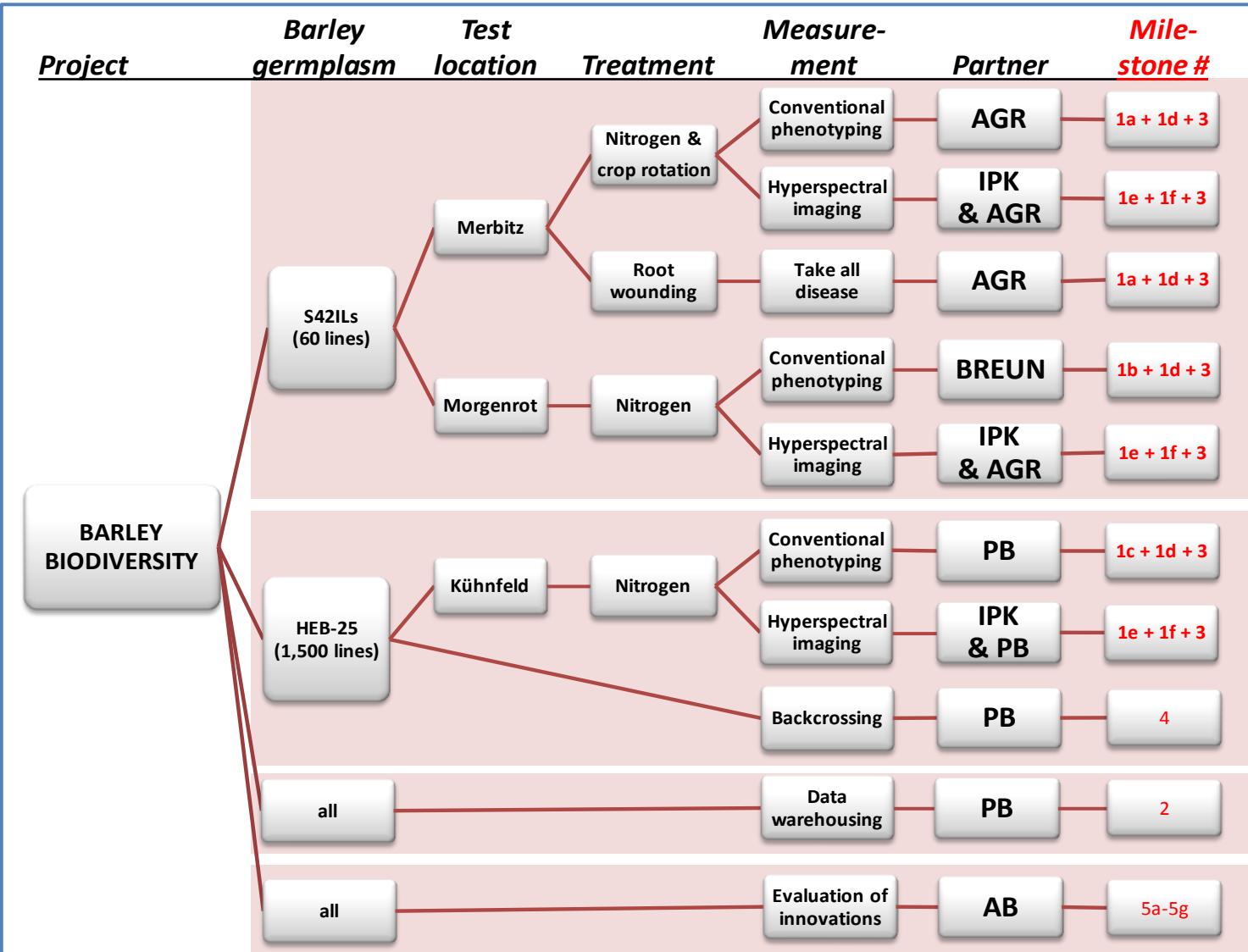
Source: www.markelowitz.com/Hyperspectral.html

Recognition of metabolites at particular wavelengths



Spectrum of HySpec SWIR-384 camera

Workflow BARLEY BIODIVERSITY



PB: Plant Breeding
 AGR: Agronomy
 IPK: Gatersleben
 AB: Agribusiness
 BREUN: Breun

Workflow for analysis of phenolics

- Extraction in 96 well format



Weighting of plant
material in 96 deep -
well - plate

1st Extraction step

80 % Methanol
Over night in fridge
centrifugation

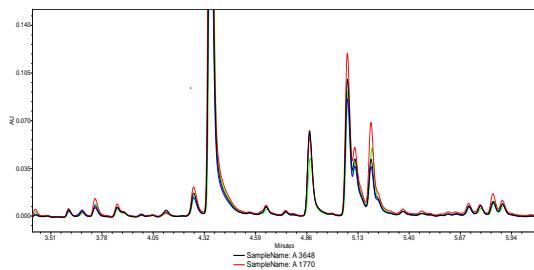


Transfer supernatant on
acroprep advance filter
plate
→ Filtration



100 % Methanol
Centrifugation
Filtration of
supernatant

2nd Extraction
with remaining
pellet



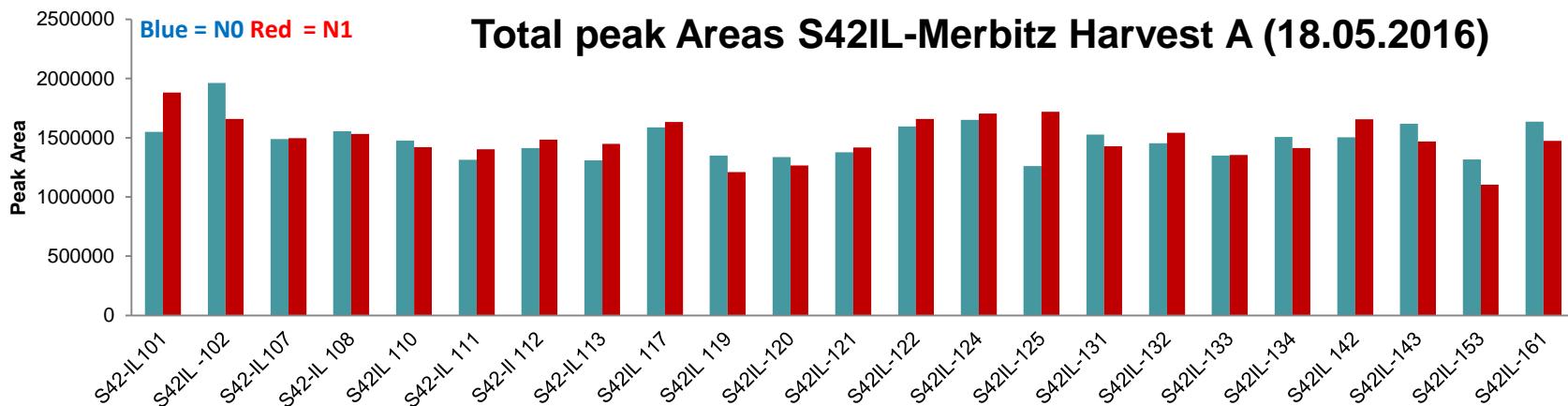
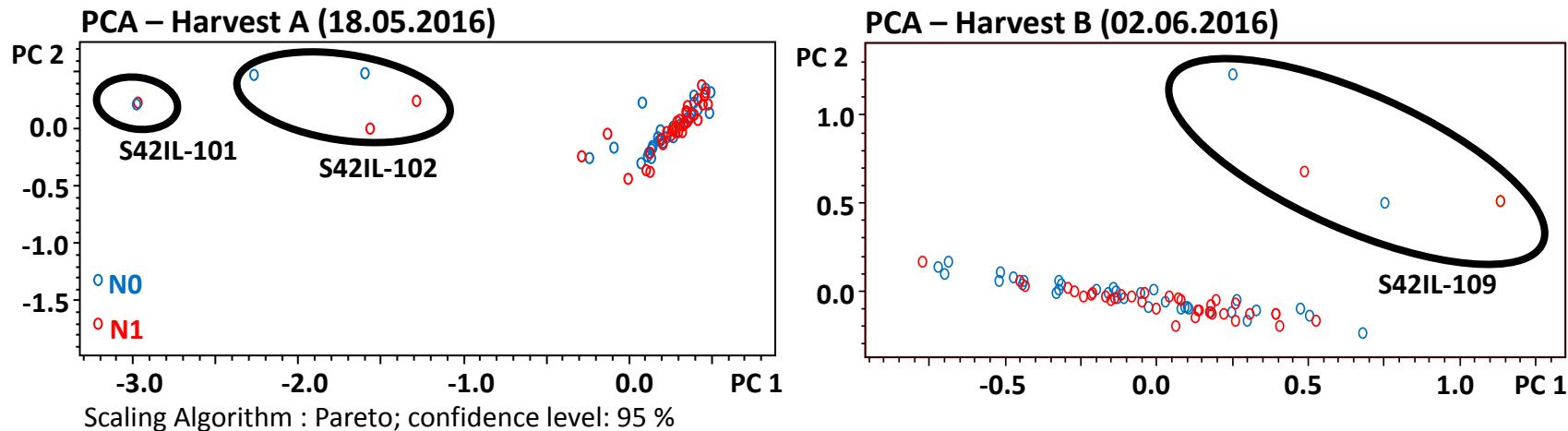
Datainterpretation

Sample preparation
rp-UPLC, PDA - detection



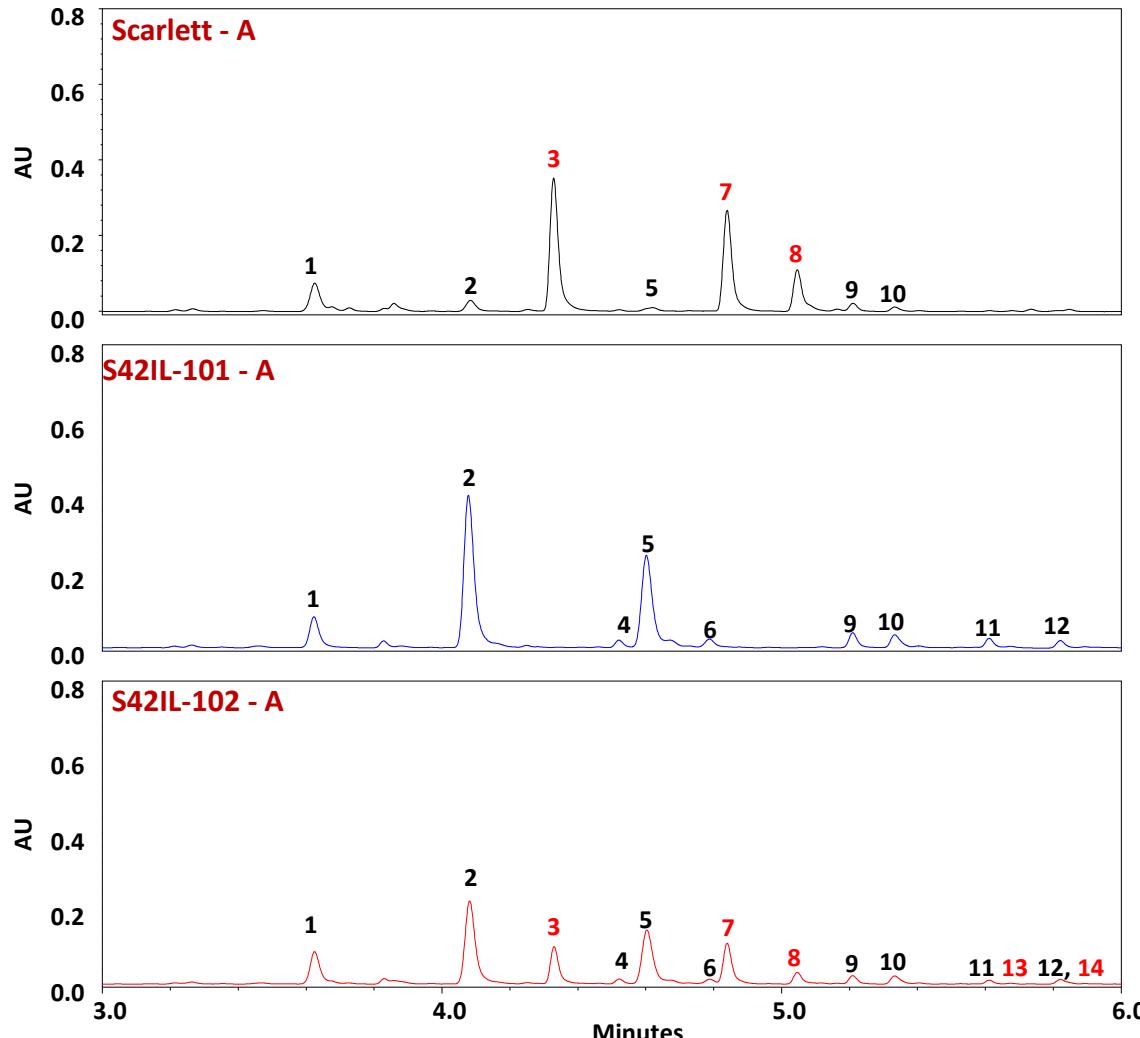
Merbitz – S42IL – 2016

- PCA analysis to visualize the differences of the abundance of the 10 most abundant phenylpropanoid peaks between N0 and N1



Annotation S42IL (A)

- Annotation was done by LC-MS/MS in negative ion mode



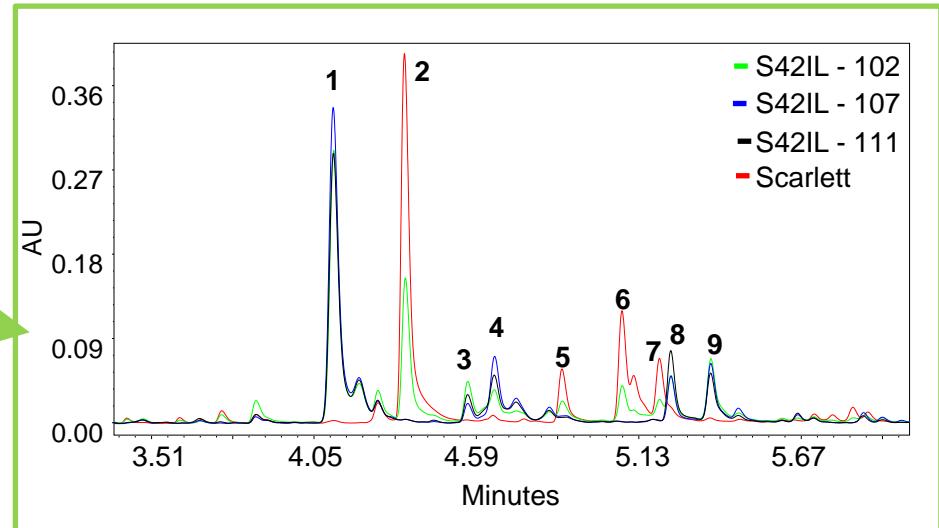
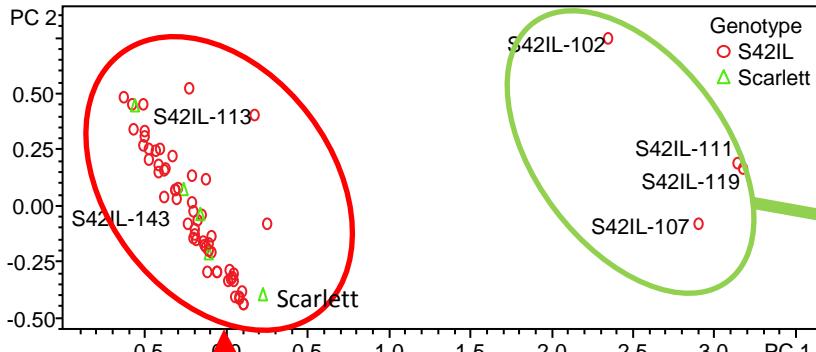
Compound	[M-H] ⁻	Annotation
1	367.104	Feruloylquinic acid (3FQA)
2	609.1466	Isoorientin-7-O-glc
3	609.1467	Isoorientin-2"-O-glc
4	447.0957	Isoorientin
5	593.1514	Isovitechin-7-O-glc
6	623.1647	Isoscoparin-7-O-glc
7	593.1508	Isovitechin-2"-O-glc
8	623.1618	Isoscoparin-2"-O-glc
9	815.2036	Sinapoyl-isoorientin-7-O-glc
10	785.1942	feruloyl-isoorientin-7-O-glc
11	799.03	sinapoyl-isovitechin-7-O-glc
12	769.2018	feruloyl-isovitechin-(7)-O-glc
13	799.2138	feruloyl-isovitechin-2"-O-glc
14	769.2018	feruloyl-isovitechin-2"-O-glc

- Profile of Scarlett dominated by 2"-O-glucosides
- S42IL-101 misses 2"-O-glucosides, potentially caused by a non-functional UDP-glycosyltransferase on chromosome 1H**

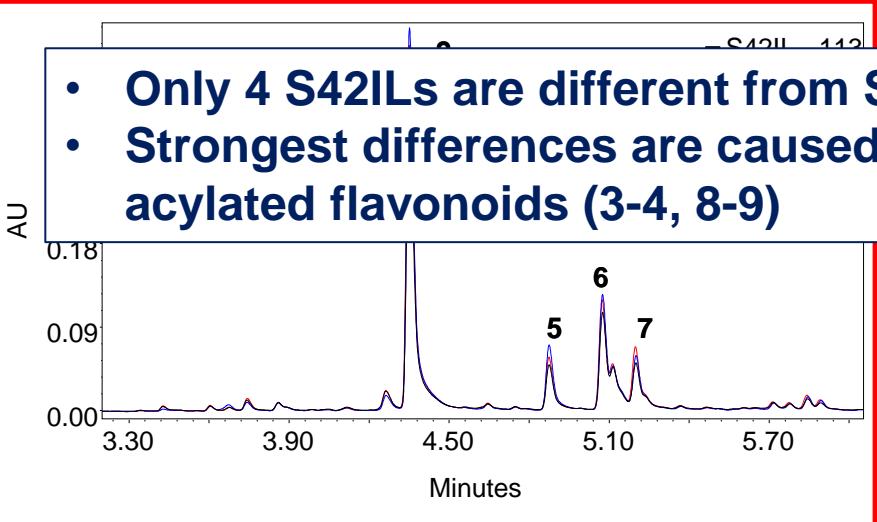
→Brauch et al. (submitted)

Phenolic Profile of S42IL

PCA – Plot of S42IL and Scarlett

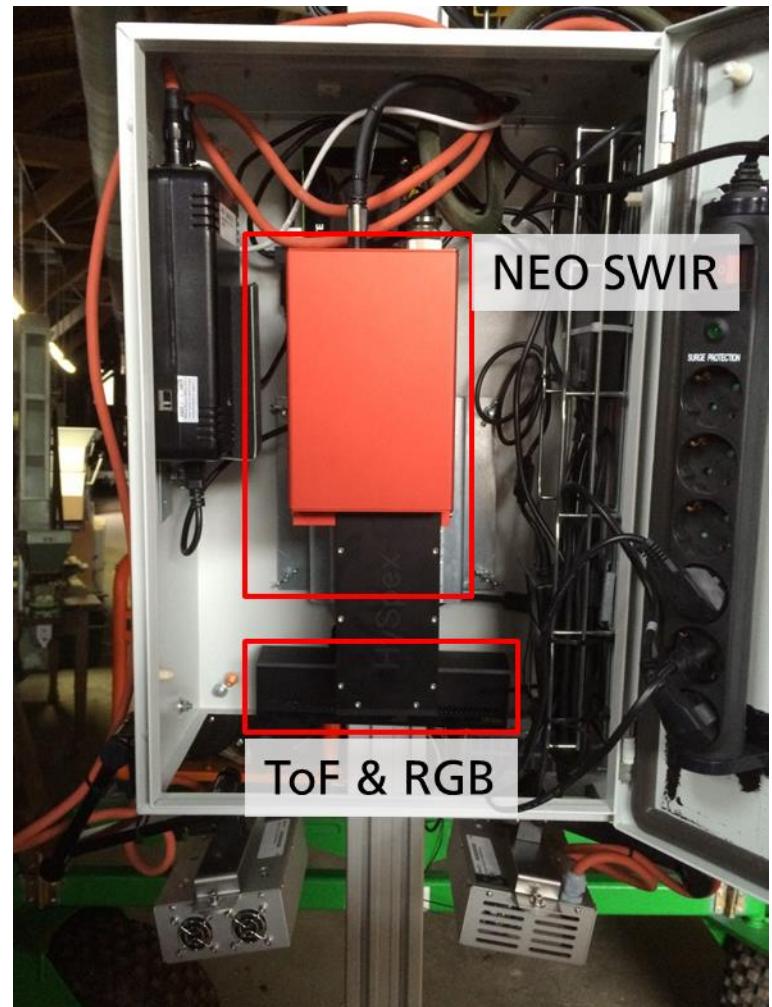


- Only 4 S42ILs are different from Scarlett in phenolic profiles
- Strongest differences are caused by new orientin isomers (1) and acylated flavonoids (3-4, 8-9)



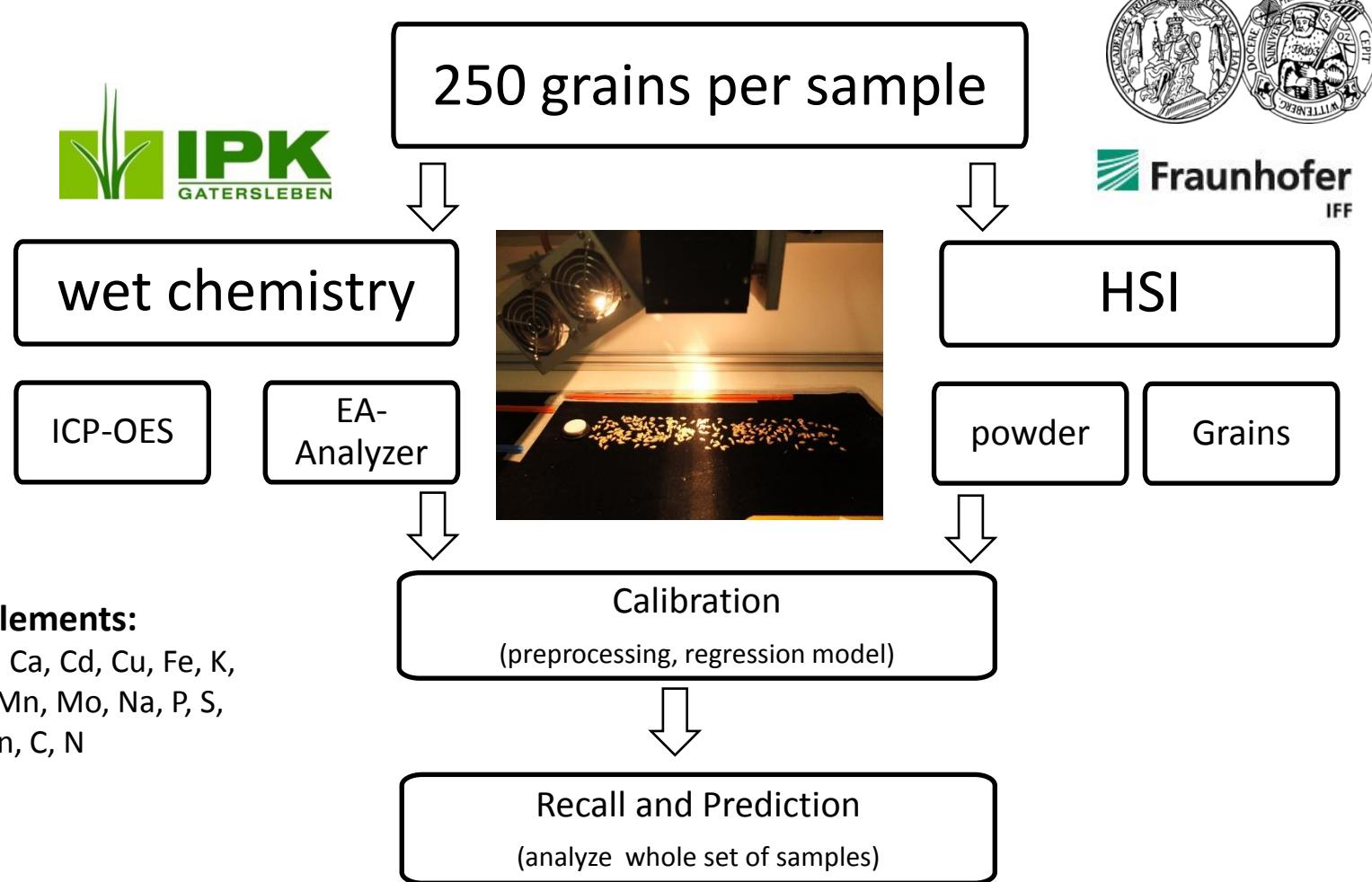
Peak No.	m/z	Annotation	MS1	A max
4	595.1002	Saponarin	433.1129 (-102.05)	209, 336
5	595.1682	2"- O - Glucosylisovitexin	433.1150 (-162.05)	270, 338
6	625.1782	7-O-Beta-D-glucosylscoparin	463.1247 (-162.05)	269, 349
7	787.2108	7-O-(6-feruloylglucosyl)iso/orientin	449.1085 (-338.1001), 611.1636 (-176.05)	270, 339
8	817.2196	7-O-(6-sinapoylglucosyl)isoorientin	449.1085 (-368.1110), 611.1636 (-176.05)	270, 341
9	787.2108	7-O-(6-feruloylglucosyl)iso/orientin	449.1085 (-338.1001), 611.1636 (-176.05)	270, 338

The field phenotyping platform AgRover, ready since December 2015



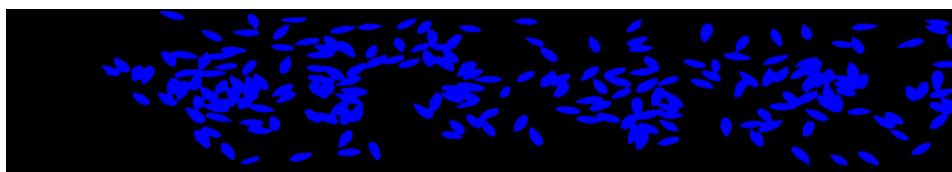
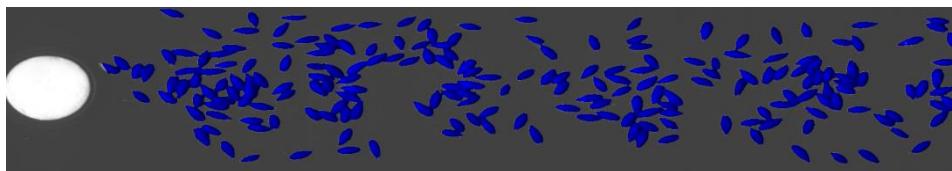
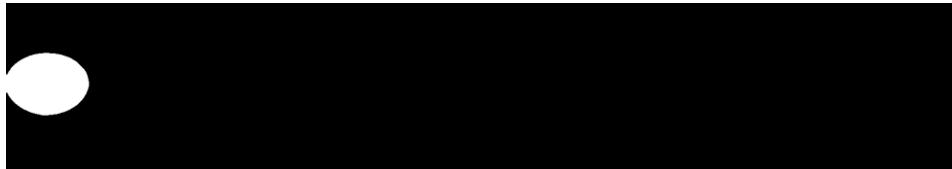
HySpec SWIR 384

Grain – Analysis



HSI – Pre-processing

Whole Grains



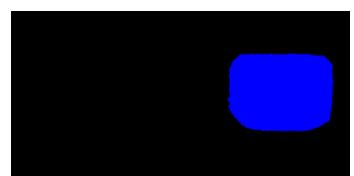
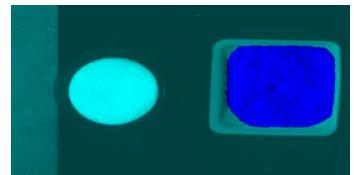
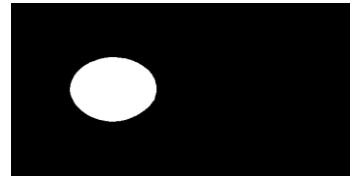
Overview of the picture
(one spectral channel chosen)

Auto detection of the **white
balance**

Segmentation mask to separate
background and sample

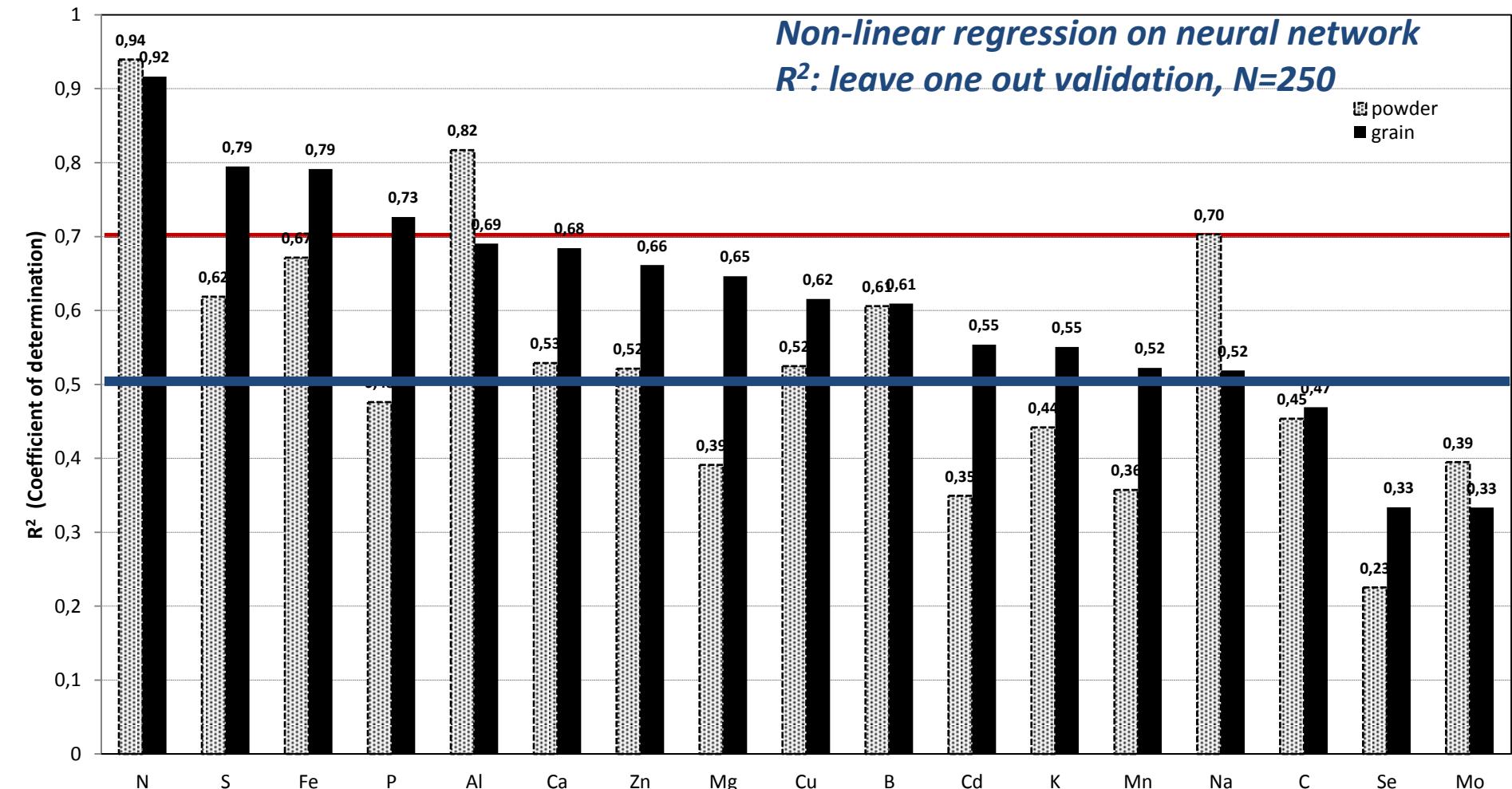
Result
Take all sample pixels to calculate
mean reflectance signature

Grain powder



HSI – Model accuracy - R^2

grain vs. powder



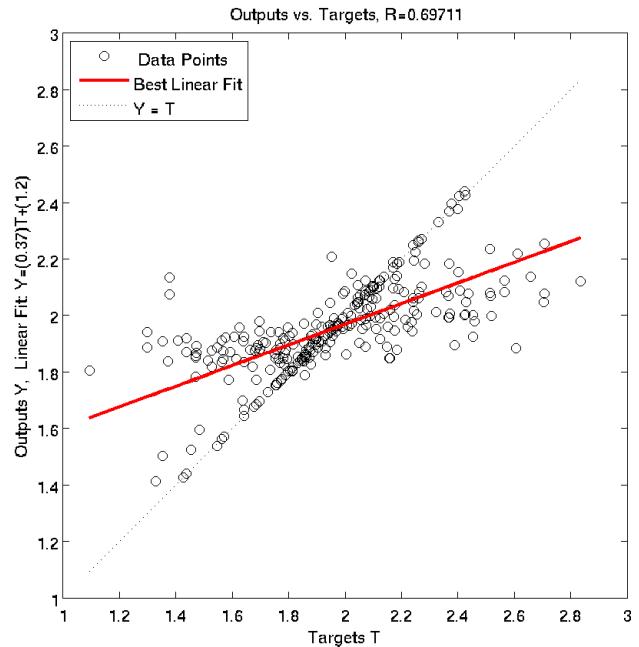
HSI – Nitrogen Model

grain vs. powder

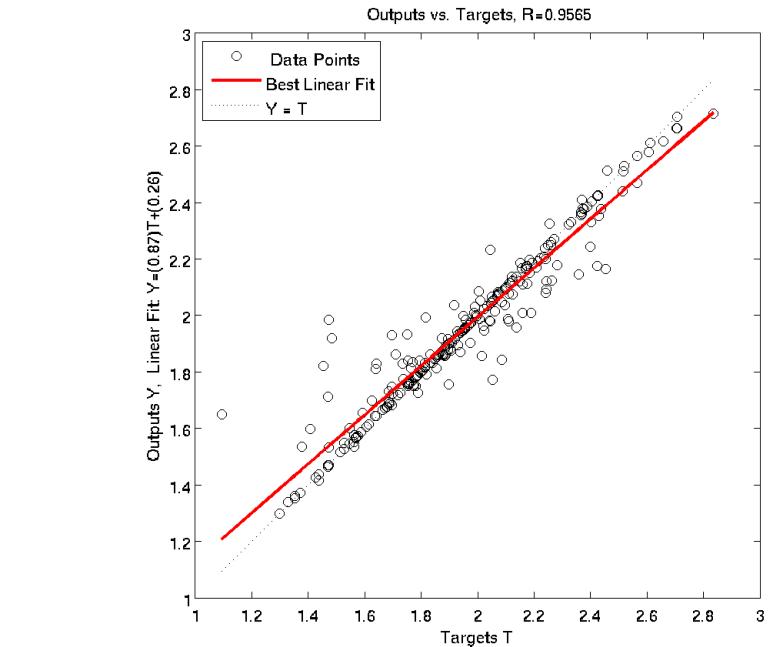
Model building:

Non-linear regression on neural networks

R²: leave one out validation, N=250



Whole Grain

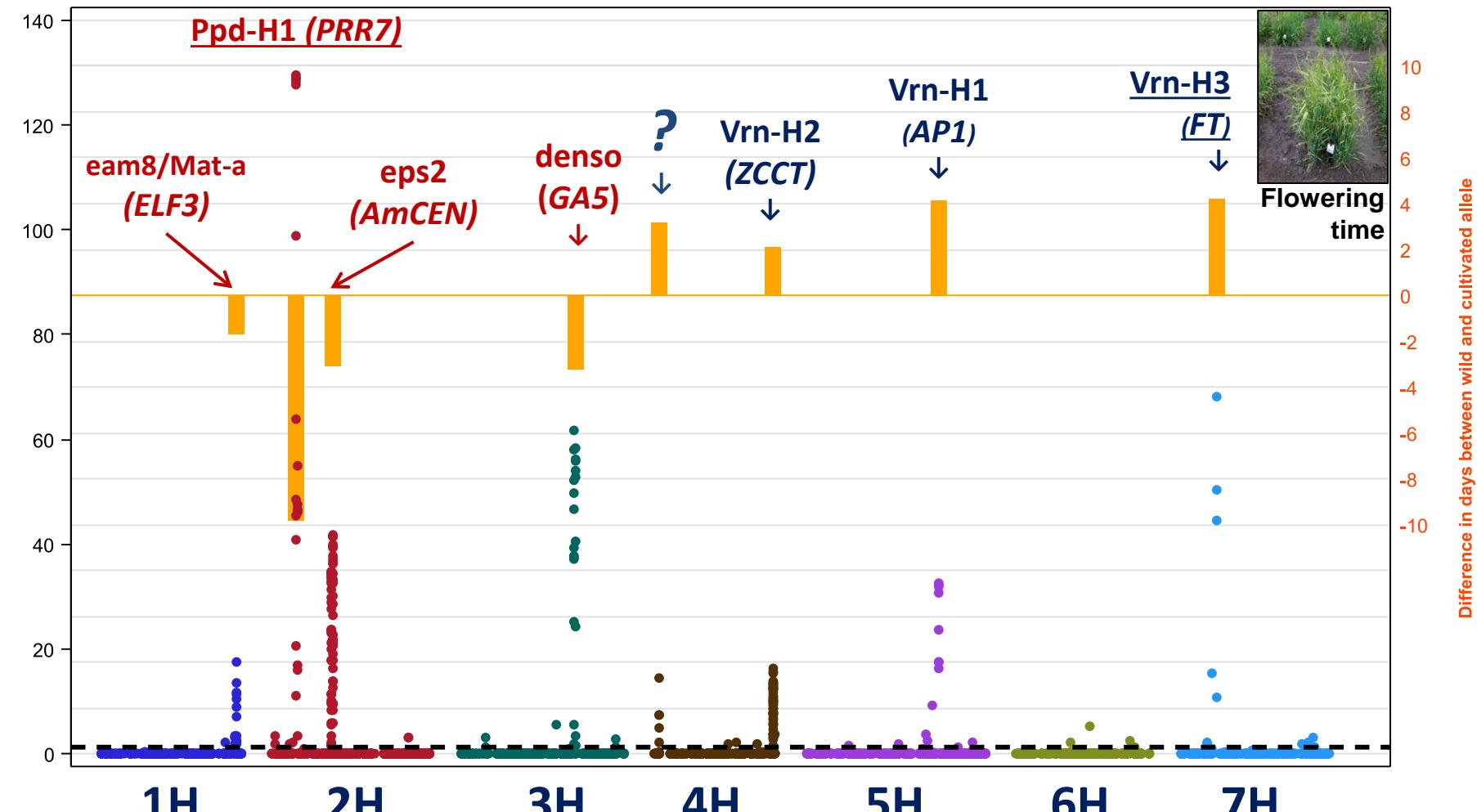


vs.

Grain Powder

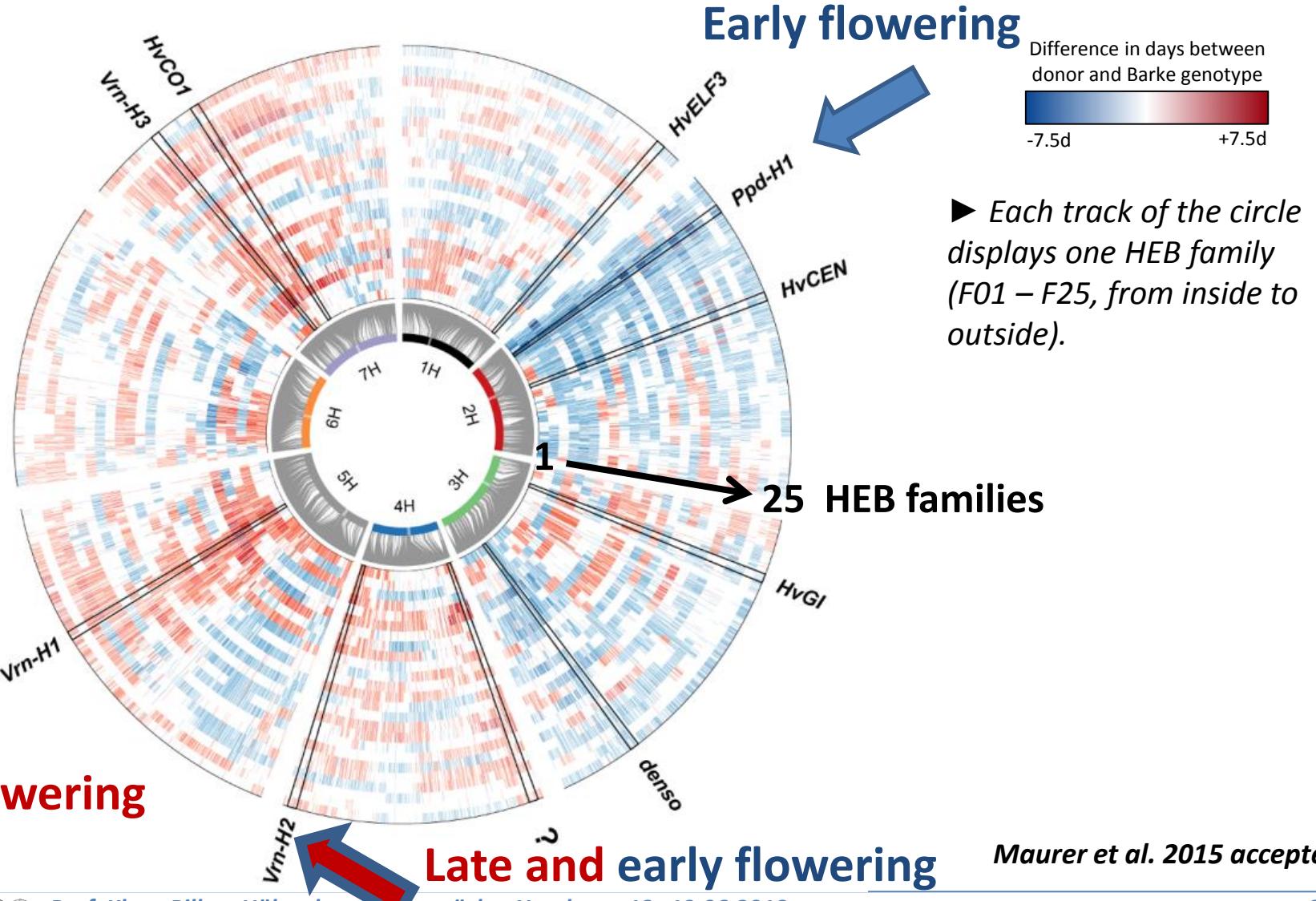
(based on 250 samples)

GWAS Manhattan plot indicates 8 major QTL controlling flowering time in HEB-25



Maurer et al. 2015 accept. BMC Genomics

Variation of gene effects between 25 HEB families



Genotype-phenotype association studies

GWAS for HEB-25

Genome-wide association study
(Multiple Regression)

$$\text{Trait} = \mu + \text{SNP} + \text{Family} + \text{Cofactors}_{1\text{cM}} + \varepsilon$$

Dunnett test for S42IL library

Compare each IL with control
'Scarlett'

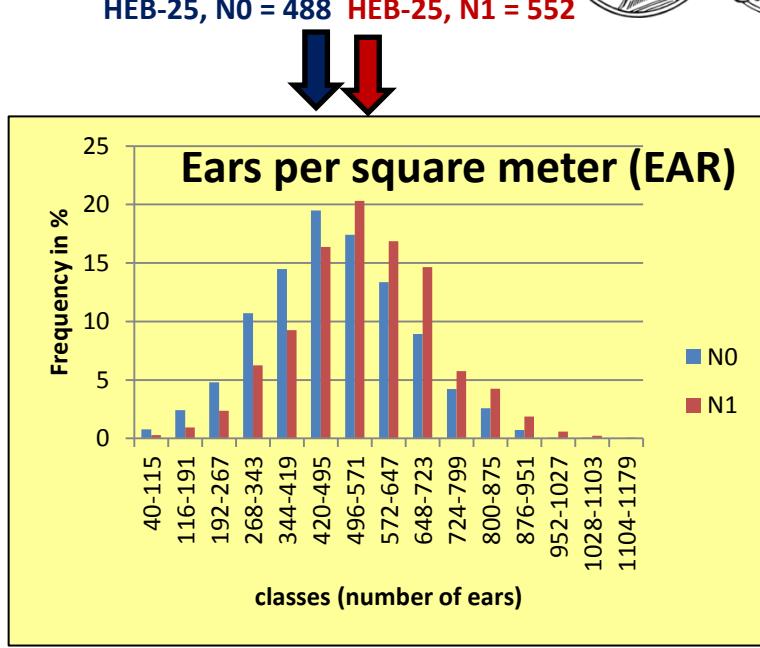
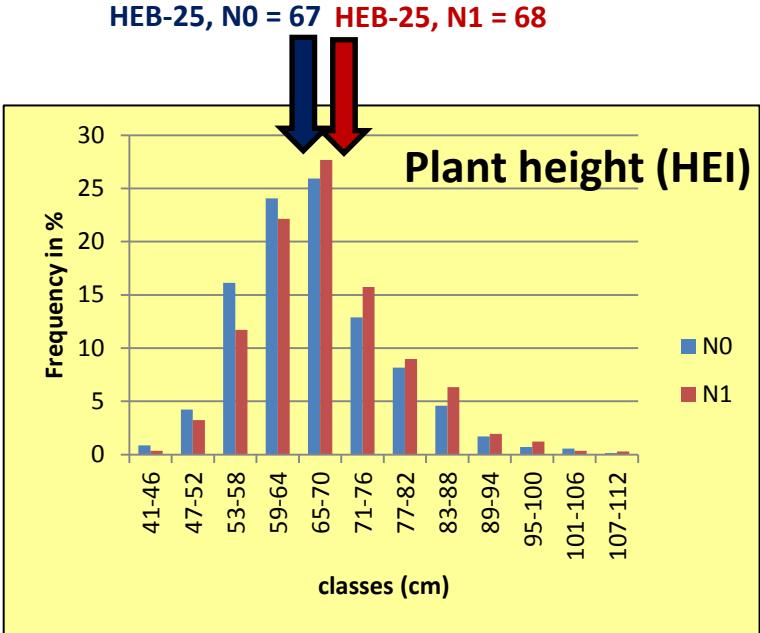


GWAS Leaf rust, field trial (Draba et al. unpublished)

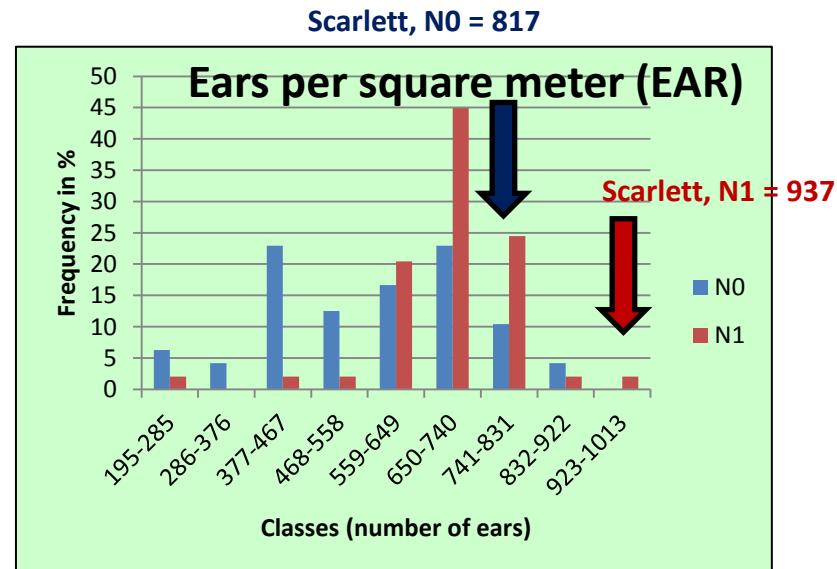
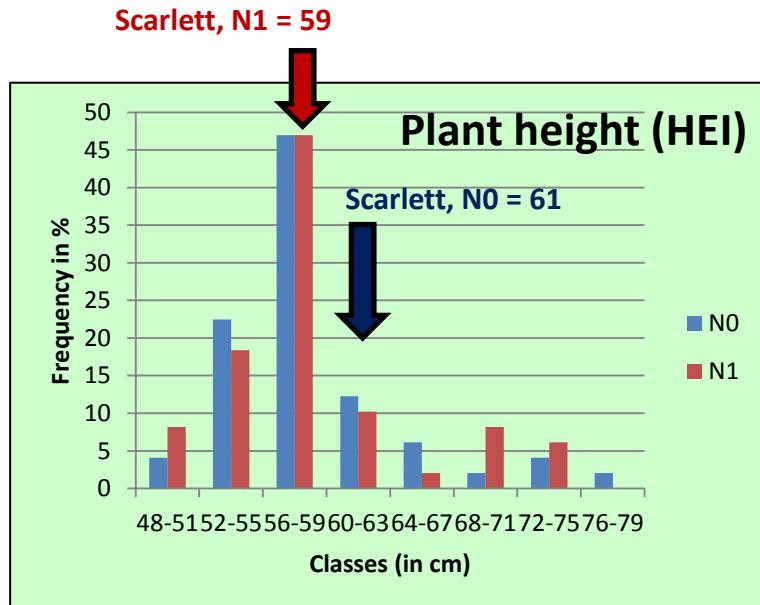
Field trials Halle and Morgenrot 2015



HEB-25
Halle 2015



S42-IL
Morgenrot 2015

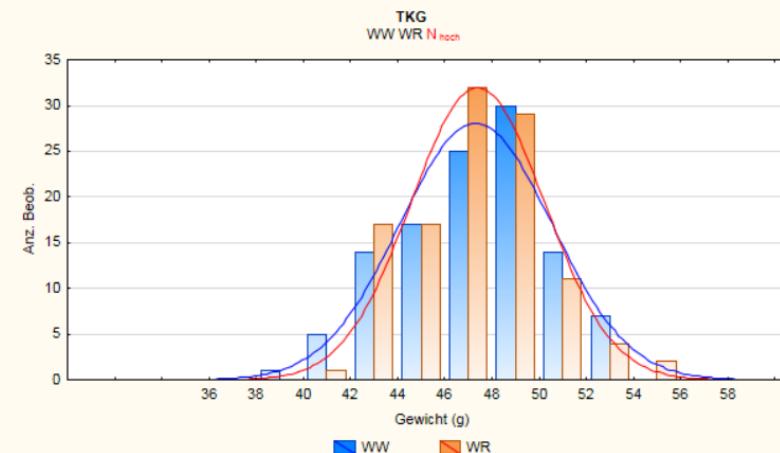
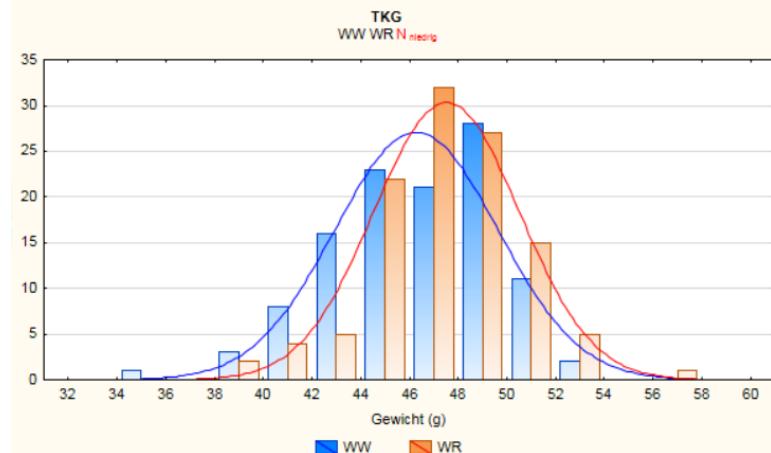
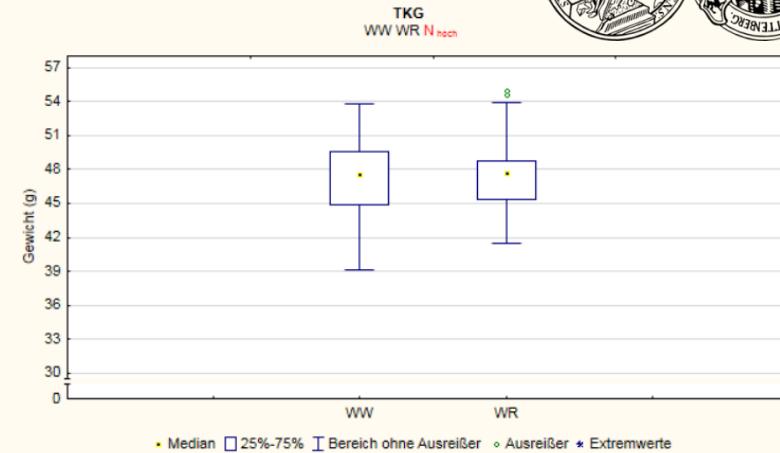
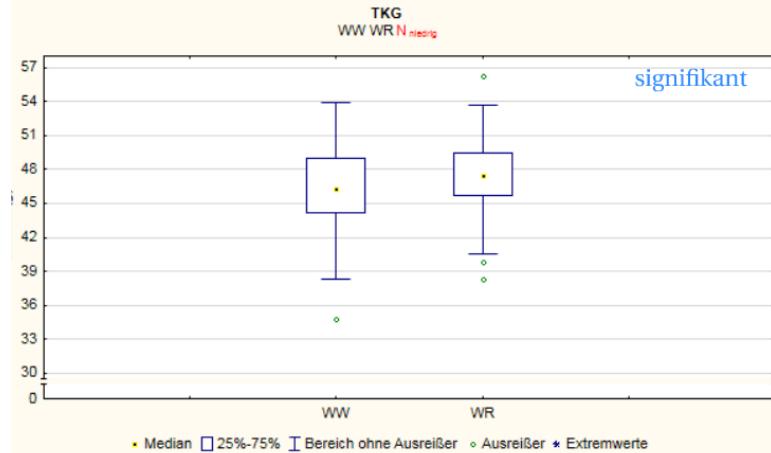


Field trial Merbitz 2015, Previous crop x thousand grain weight (TKG)

S42-IL Merbitz 2015



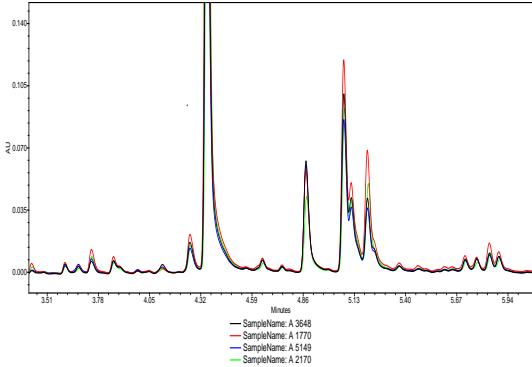
Previous crop: WW= Winter wheat; WR= winter rape seed



During the course of the project 3 questions, connected to 3 innovations, will be addressed

1. Can we identify and quantify the **effect of exotic barley introgression lines**, which are better adapted to grow under nitrogen stress than the elite barley controls Scarlett and Barke?
2. Can we **establish a mobile, field-based, hyperspectral imaging system (AgRover)** in order to increase spatial and temporal resolution for **non-invasive high throughput monitoring** of plant growth, nutrient content and cell wall composition?
3. Can we **quantify welfare effects**, related to the tested 3 innovations, on a **macro level** (socio-ecological efficiency) and on a **farm level** (economic efficiency)?

Workflow for analysis of phenolics



UV – Chromatogramms @
280 nm

- Creation of a peak list for the more abundant peaks
- Processing in Empower

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P		
Interspersions: A 104 B 104 C 1472 D 1472 E 1446 F 1451 G 193 H 193 I 106 J 111 K 178 L 1824 M 1824 N 1860 O 1862 P 1870																	
Name:	A 104	B 104	C 1472	D 1472	E 1446	F 1451	G 193	H 193	I 106	J 111	K 178	L 1824	M 1824	N 1860	O 1862	P 1870	
RT = 2.50 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 2.89 min	0	410	0	0	0	105	0	0	2121	0	0	0	0	0	0	0	
RT = 3.36 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 3.45 min	0	0	4907	707	8020	8050	12055	1605	930	7827	1830	10777	17280	18860	20765		
RT = 3.52 min	0	0	5321	5317	5545	8067	8065	0	3420	2962	932	4500	4348	1987	10996		
RT = 3.56 min	0	0	5956	5955	5955	8067	8065	0	4328	2962	2971	5900	4647	1987	4270		
RT = 3.61 min	486	0	4937	4934	304	2294	7000	4461	4695	628	5205	794	2002	6891	25729		
RT = 3.66 min	9376	6653	9003	0	0	7868	7793	0	5788	1395	1852	3650	3413	9457	13042		
RT = 3.70 min	0	2002	0	1971	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 3.74 min	20734	2521	19547	2185	0	0	23429	0	0	0	0	0	0	0	0	0	
RT = 3.85 min	13759	1759	10753	14848	52355	10252	12320	0	8567	3410	3060	10303	10854	5620	57646	15586	
RT = 3.91 min	0	1243	0	57	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 3.99 min	0	0	7442	936	2019	231	3021	956	3649	3630	5833	900	1785	7890	3238	9421	
RT = 4.20 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 4.26 min	33390	7437	28936	36726	65328	30901	37731	0	69808	29870	59471	44746	12303	34037	89391	32328	
RT = 4.30 min	69100	57989	70055	0	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 4.45 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 4.51 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
RT = 4.64 min	14481	1391	0	227	5754	0	0	0	4206	1721	3957	6076	64743	9795	127069	124123	18130
RT = 4.72 min	5340	5400	4036	4485	4333	5029	0	0	0	0	0	0	0	0	0	0	0
RT = 4.84 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 4.85 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 4.95 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.07 min	16731	16599	10007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.10 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.10 min	17255	12331	0	14030	13031	12996	13781	0	0	16251	119740	18524	19300	25741	8767	51065	170246
RT = 5.24 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.37 min	22317	10007	5623	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.47 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.57 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.72 min	9527	30303	16119	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.76 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT = 5.78 min	9530	3154	16110	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table with peak intensities for each treatment and genotype

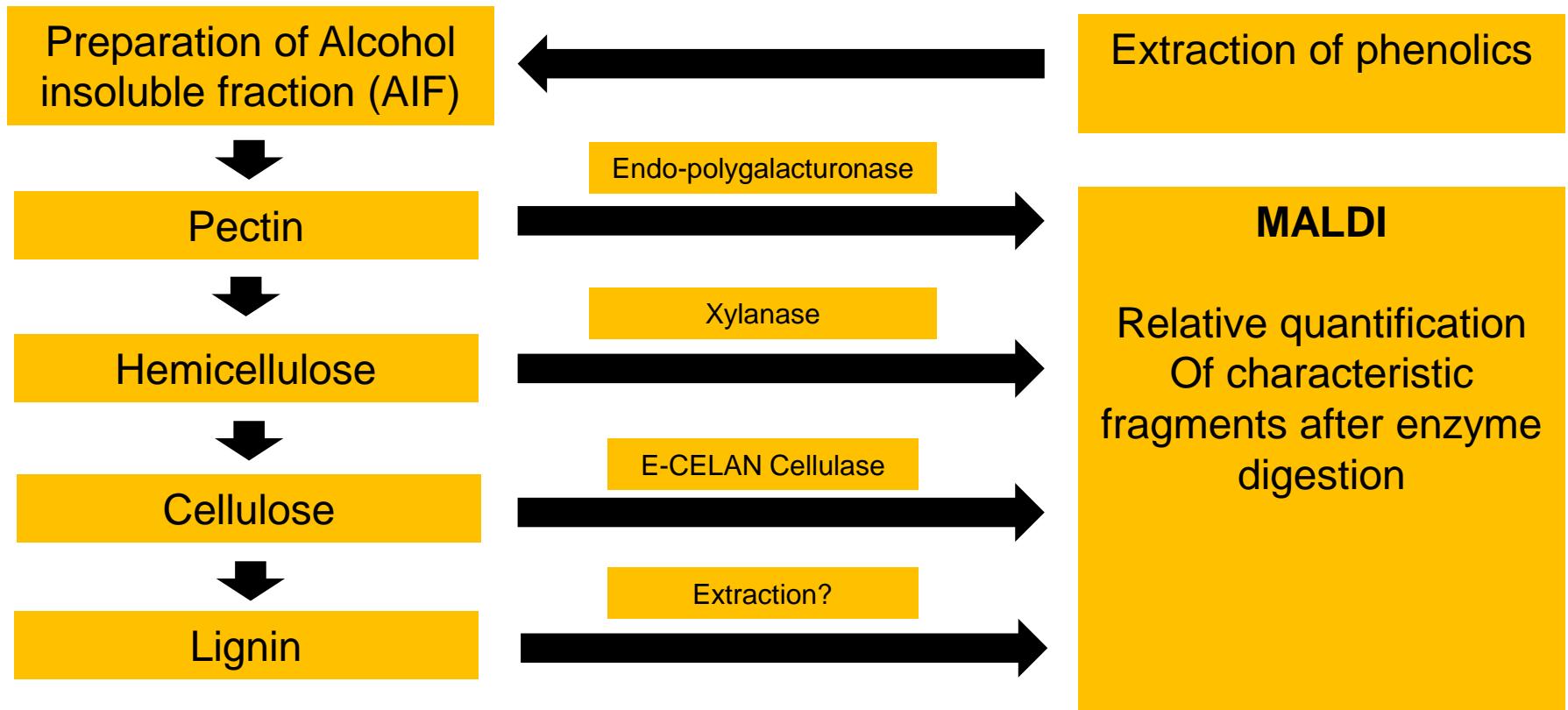


Identification of potential genes for the regulation of the phenylpropanoid pathway in barley

Mathematical Model

Analytical workflow

- 3045 leaf samples + 1500 spike samples → ca. 4500 samples
- For time saving high throughput method is needed



Field - experiment 2015

One harvest for testing and establishment of methods

- Establishment of high throughput methods
- Estimate effort for optimal organization of work
- Get overview of metabolic variation in the different genotypes

2 month old barley plants growing in Halle (HEB-25) and Merbitz (S42IL)

Harvest of the flag leaf
Pool of six leaf/genotype

Directly frozen in liquid N₂

Freeze drying of leaf material

Grinding of leaf material

Big Challenge: High sample amount

- Seven measurements at three different locations

	Repetition	No. of lot	10%	Standards	1 harvest Σ	Σ
Halle-Kühnfeld		total 2942	294		12	306
	N0	1471		147 3 Barke, 3 Scarlett		
	N1	1471		147 3 Barke, 3 Scarlett		

- High sample amount
- Sample preparation (freeze-drying, grinding, weighting)
- Methods for cell wall analytics, esp. extraction are very time consuming

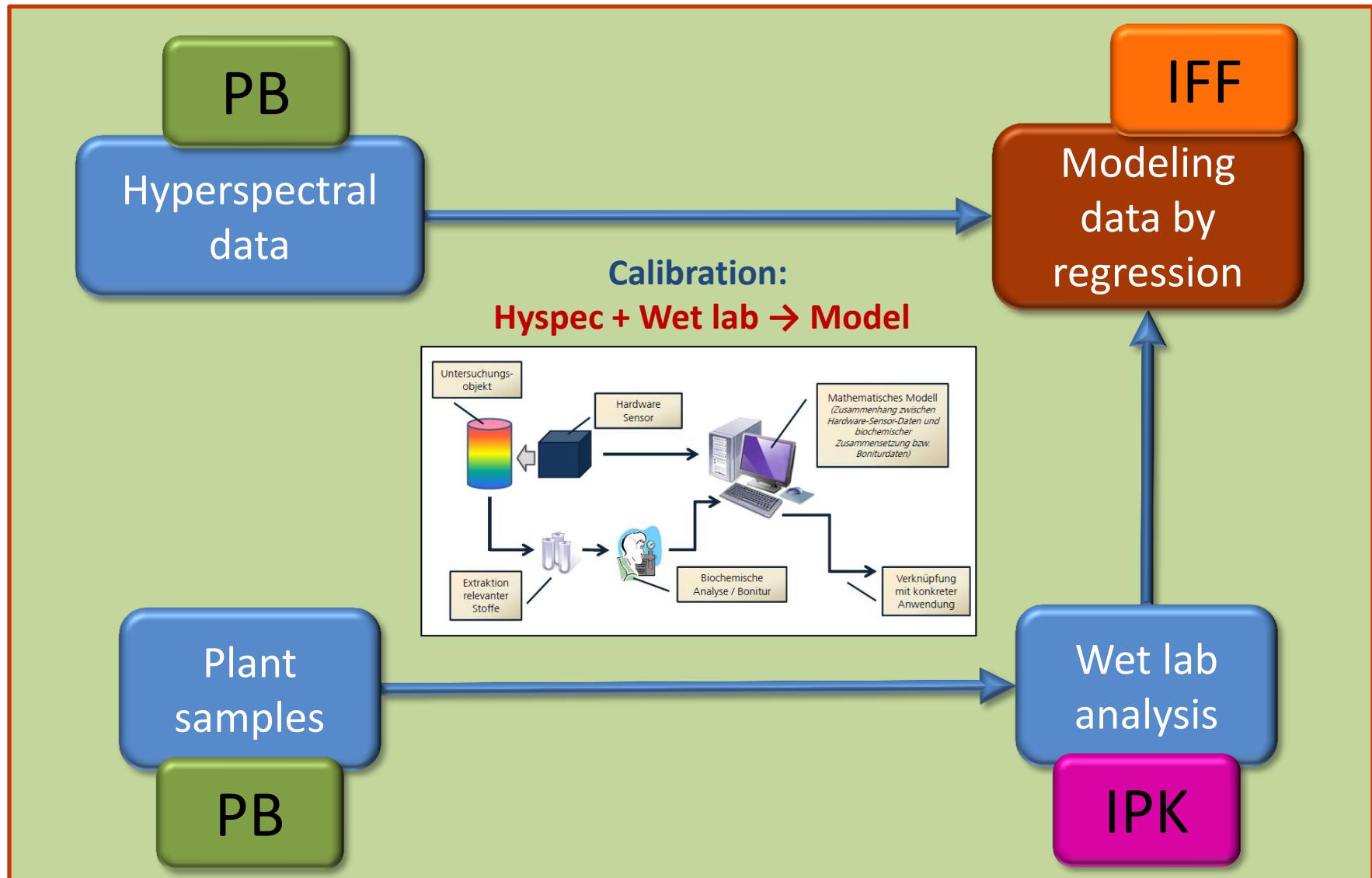
→ High throughput methods are needed

→ Avoiding of multiple sample preparation steps

	WR N1-C	60	6			
Morgenrot	gesamt	325	33		12	45
	N0-A (1)	59	6	Barke, Scarlett		
	N0-B (2)	55	6	Barke, Scarlett		
	N0-C (3)	38	4	Barke, Scarlett		
	N1-A (4)	60	6	Barke, Scarlett		
	N1-B (5)	59	6	Barke, Scarlett		
	N1-C (6)	54	5	Barke, Scarlett		
			Sum		435	3045

+ approximately 1500 spike samples

Modeling of hyperspectral data



Calibration phase of AgRover

Halle
(HEB-25)

- 3,000 plots
- 300 samples (10%) per run

= 1,800
samples

Merbitz
(S42IL)

- 720 plots
- 72 samples (10%) per run

= 432
samples

Morgenrot
(S42IL)

- 360 plots
- 36 samples (10%) per run

= 216
samples

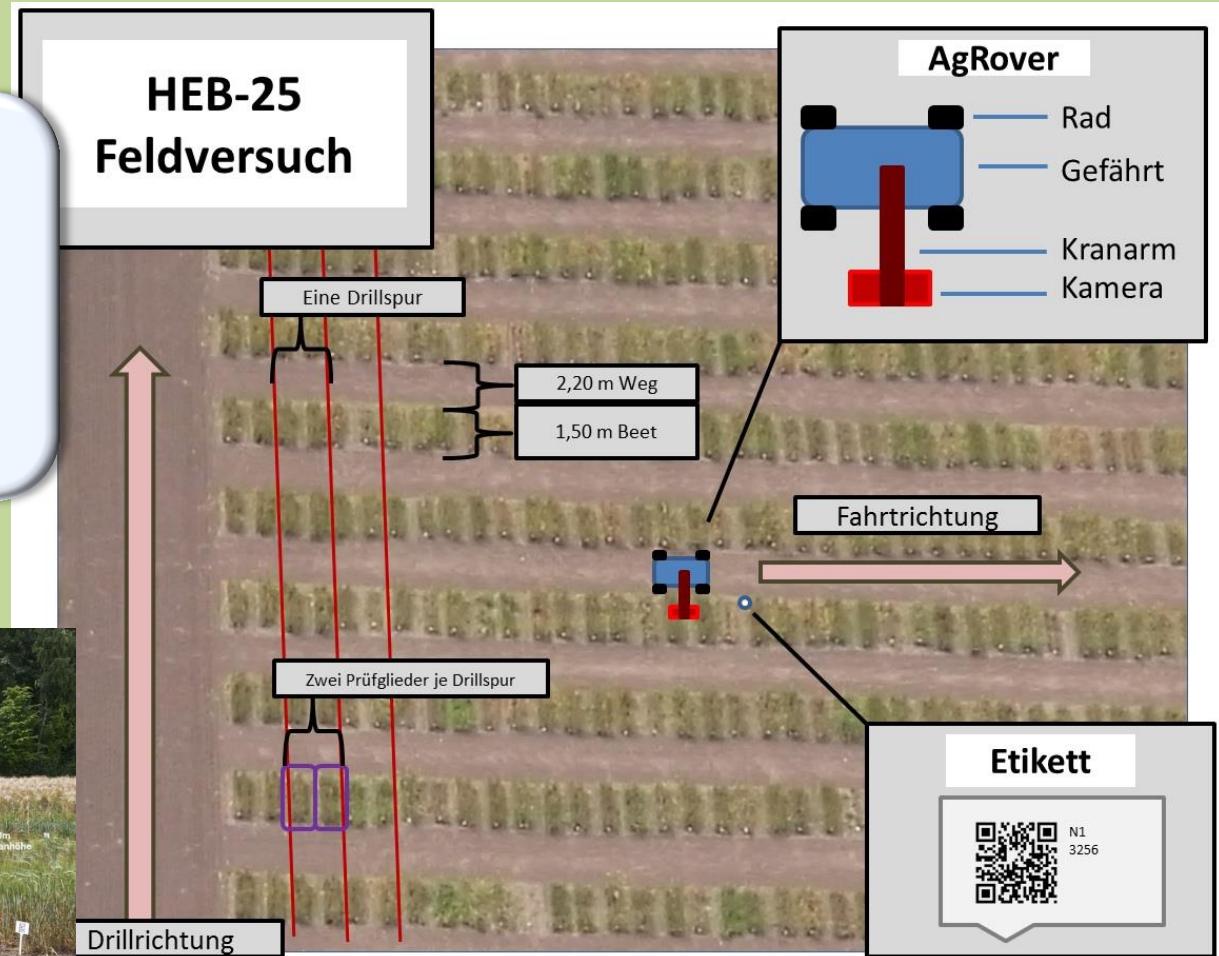
At least 2,448 wet lab samples for calibration

AgRover phenotyping



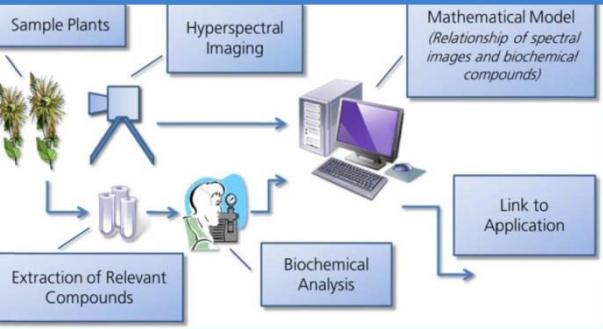
Hyperspectral imaging

- Plant growth
- Nutrient content
- Metabolites



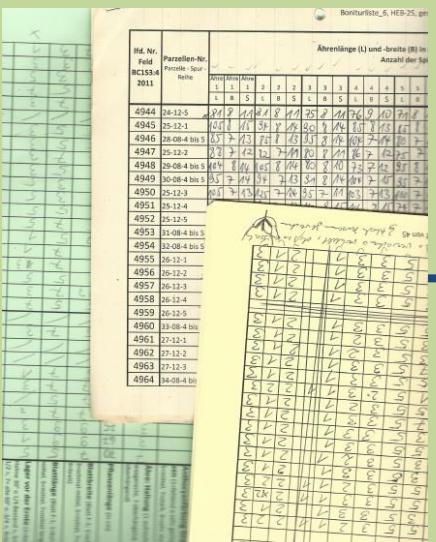
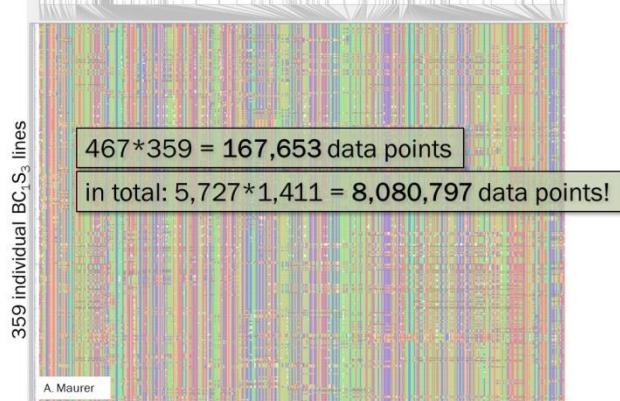
OPTIMAS data warehouse at MLU Halle

Hyperspectral imaging data

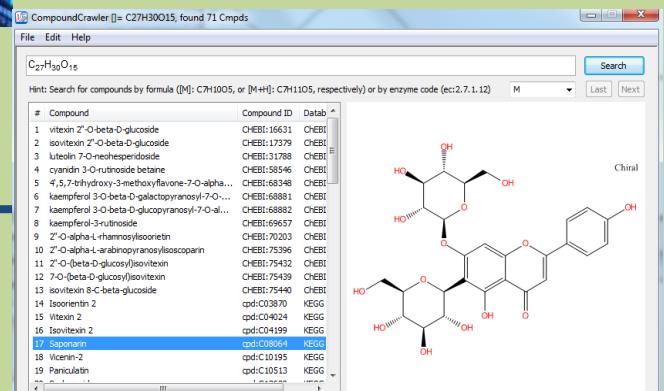


Genotype data

467 SNPs on chromosome 7H



Colmsee et al. 2012, BMC Plant Biol



Conventional phenotype data

Wet lab data

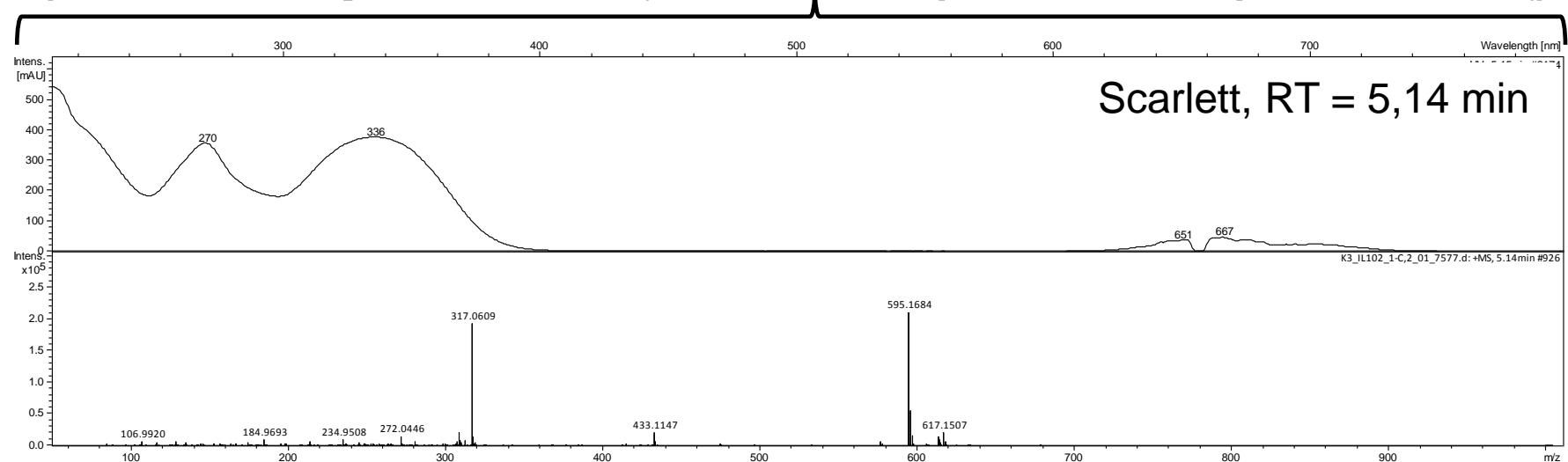
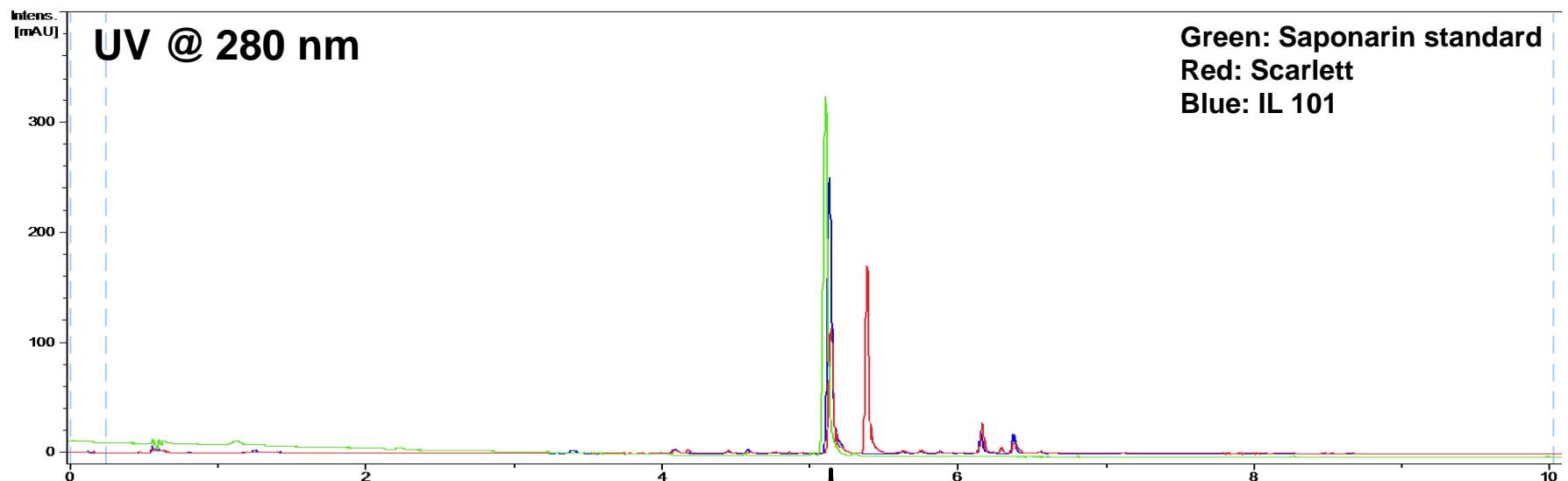
Conventional phenotyping



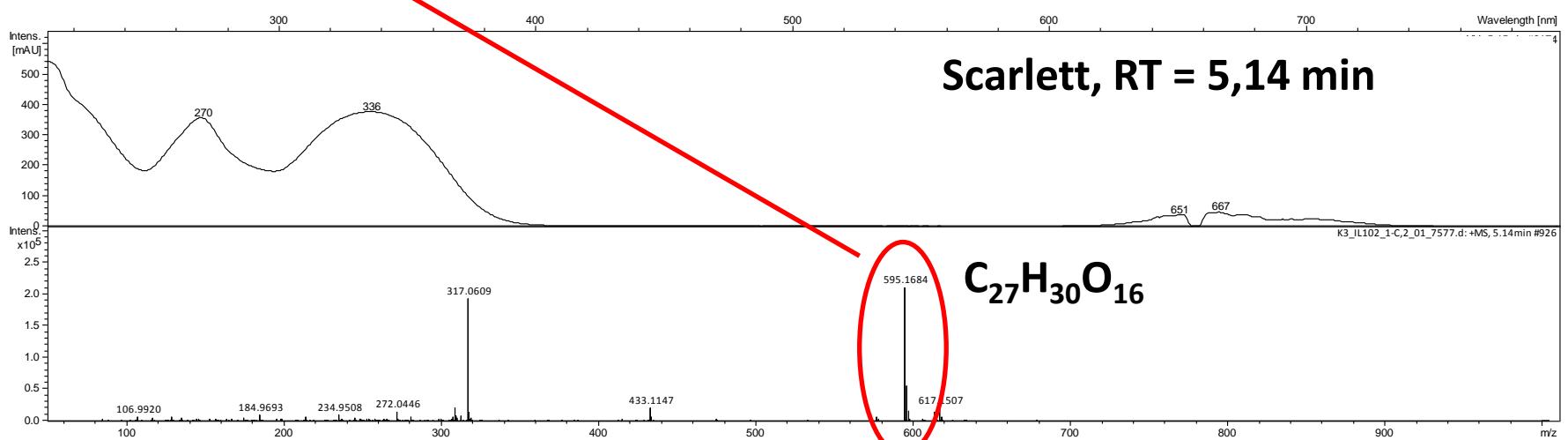
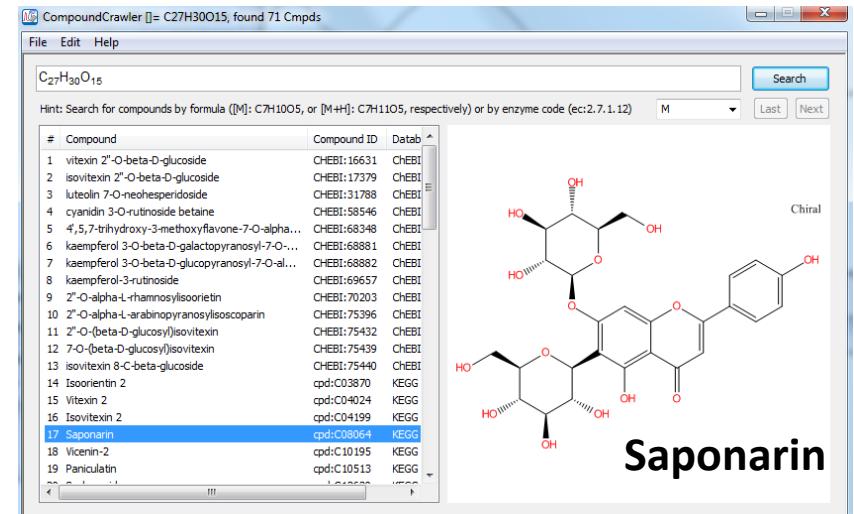
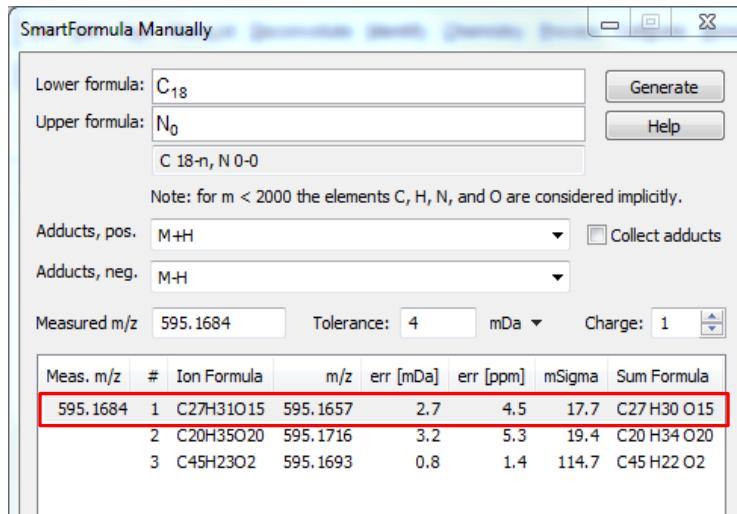
Conventional

- Life history
- Grain yield
- Grain morphology

Analysis of soluble phenylpropanoids

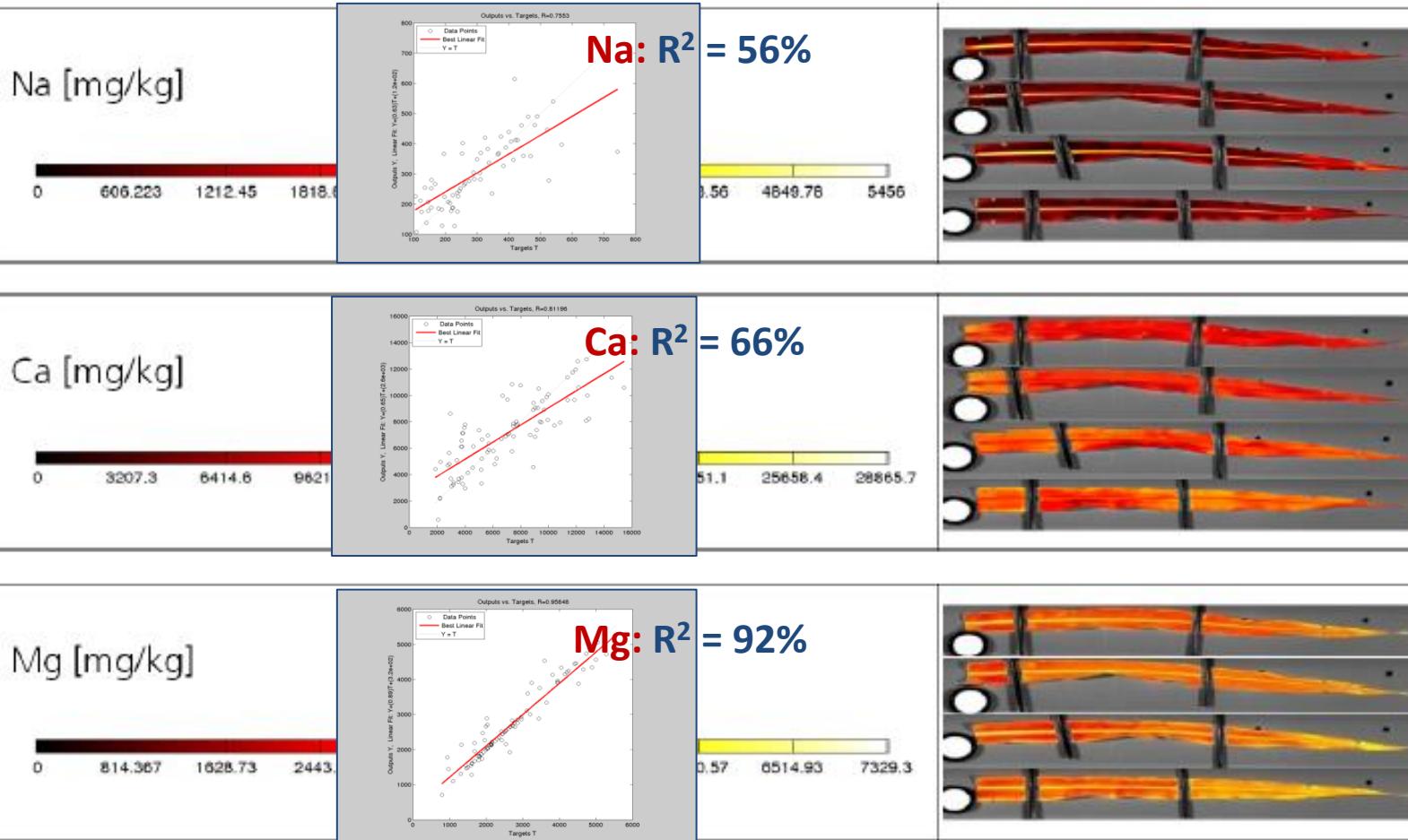


Wet lab analysis of soluble phenylpropanoids by LC-MS/MS

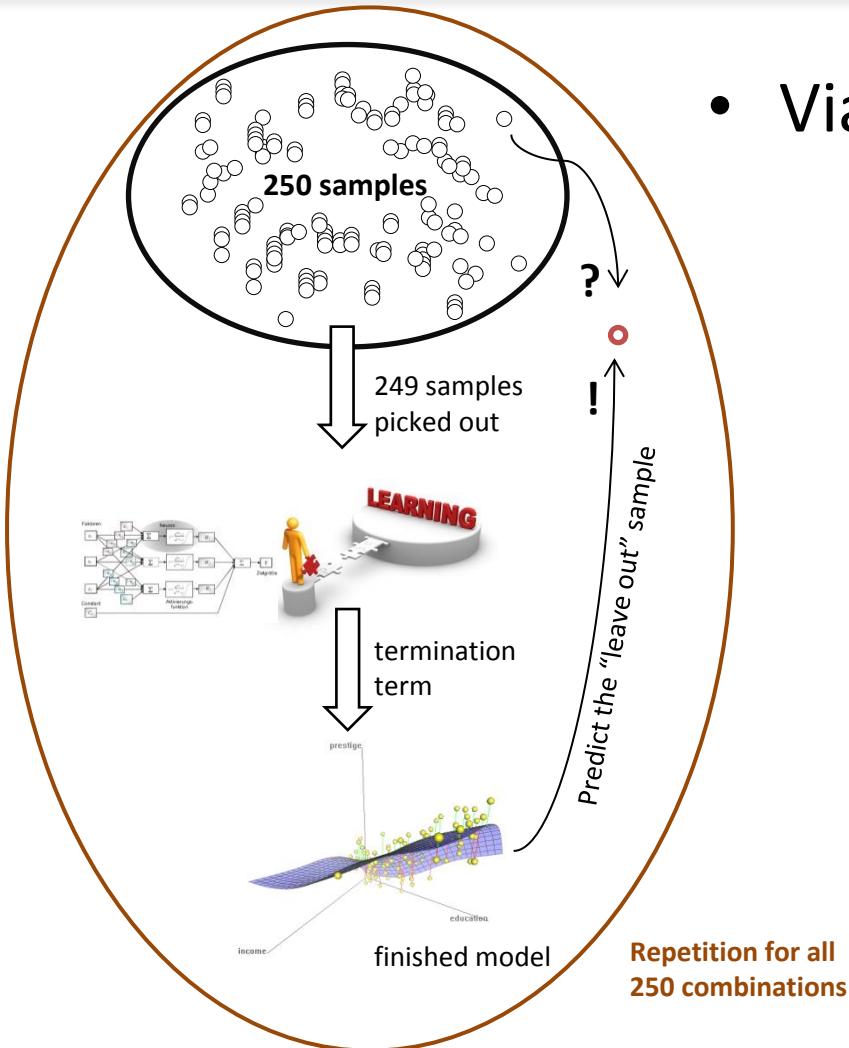


Hyperspectral quantification of Na, Ca and Mg across leaf blades

Predicted vs. Observed



HSI - Calibration



- Via “leave one out” validation
 - nonlinear regression based on neural network
 - trained on 249 samples
 - model training via iterated until the lowest error is found (learn)
 - Termination term means best model is found
→ predict one sample
- done this for each combination (250 models)

AgRover measurement schedule

At least six measurements

20th
April ► 04th
May ► 18th
May ► 01st
June ► 15th
June ► 29th
June

Monday: Halle Wednesday: Merbitz Friday: Morgenrot



Field trial 2015



- 1420 HEB lines
- cv. Barke
- 11 additional varieties
- Two nitrogen treatments

Halle-Kühnfeld			
N-Stufe	Ziel	Pflanzenverfügbarer N im Feinboden	N-Gabe zum Schossen (BBCH 31)
N0	30 kg N/ha	32 kg N/ha	0 kg N/ha
N1	90 kg N/ha	32 kg N/ha	60 kg N/ha

Morgenrot



- 49 S42 IL lines
- cv. Scarlett
- 9 additional varieties
- Two nitrogen treatments
- Three replications

Morgenrot mit Gabe von Wachstumsregulatoren

N-Stufe	Ziel	Pflanzenverfügbarer N im Feinboden	N-Gabe zum Schossen (BBCH 31)
N0	80 kg N/ha	82 kg N/ha	0 kg N/ha
N1	120 kg N/ha	82 kg N/ha	40 kg N/ha

Task #5

Task #5: Evaluation of socio-economic effects related to application of innovations (2015-2016) at AB

Task #5a) Literature research

A **literature research** will be carried out, studying effects of breeding progress on resource efficiency, land use, possible grass land re-use, wood re-use, net balance of CO₂ consumption, etc.

Task #5b) Analysis and monetary evaluation of field experiments in 2015 (Task#1a - #1e)

The **2015 field experiments** will be analyzed and **monetarily evaluated** in regard to nitrogen efficiency, yield, effect of preceding crops, pest management, seed density). The results will be compared with standard agricultural practices

Task #5c) Prediction of market acceptance of new barley cultivars

The potential **market acceptance of new barley cultivars** will be predicted based on current dynamics of market penetration of new barley cultivars.

Task #5d) Analysis and monetary evaluation of field experiments in 2016 (Task#1a - #1e)

The **field experiments in 2016** will be analyzed and **monetarily evaluated** in regard to effects of new genotypes on yield stabilization compared to standard barley cultivars.

Task #5e) Predicting socio-economic effects of innovations

Based on the estimated market adaptation rates, the **socio-economic effects** of the new barley genotypes will be predicted.

Task #5f) Expert interviewing

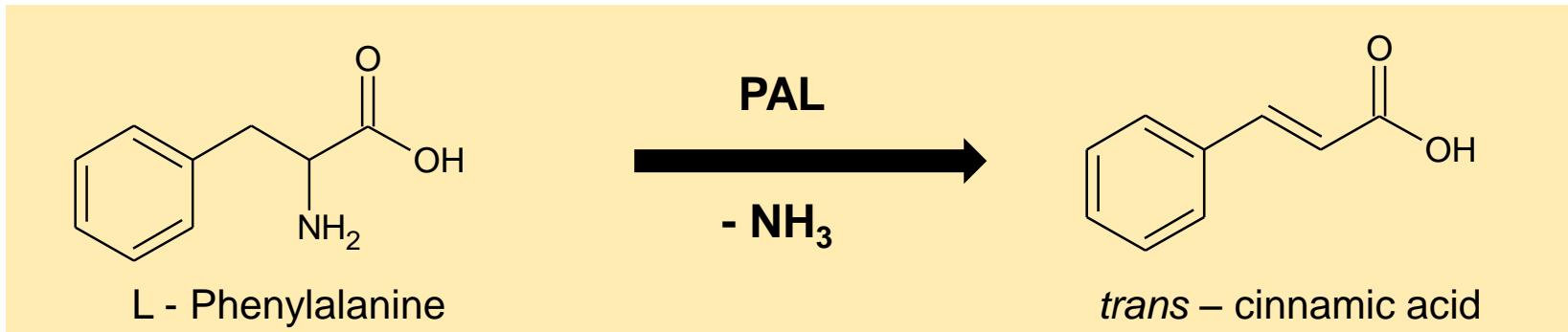
Experts from plant breeding companies will be **interviewed** in regard to quantifying advantages of the non-invasive assessment of quality and growth with the **AGROVER** hyperspectral imaging system. Also, possible effects of application of the AGROVER system on breeding costs, selection gain per year and time required to market a new cultivar will be interrogated.

Task#5g) Final documentation

Based on the assessments and interviews carried out, a **final documentation of the possible effects of both innovations**, the utilization of exotic barley germplasm and the utilization of the hyperspectral imaging system for all clients involved, barley breeders, and agricultural enterprises and consumers, will be assessed.



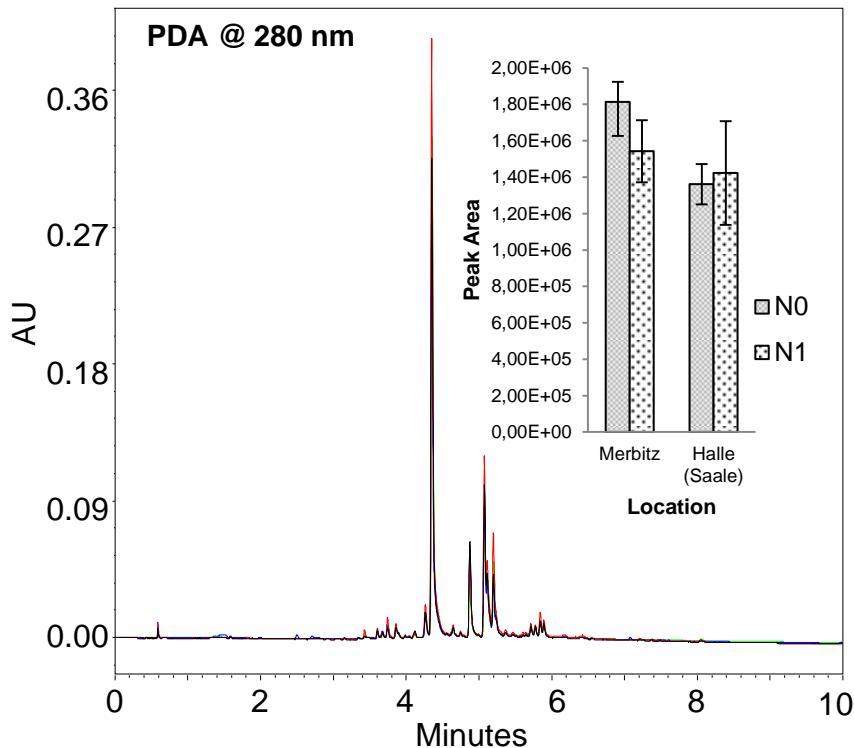
Influence of nitrogen on the phenylpropanoid pathway



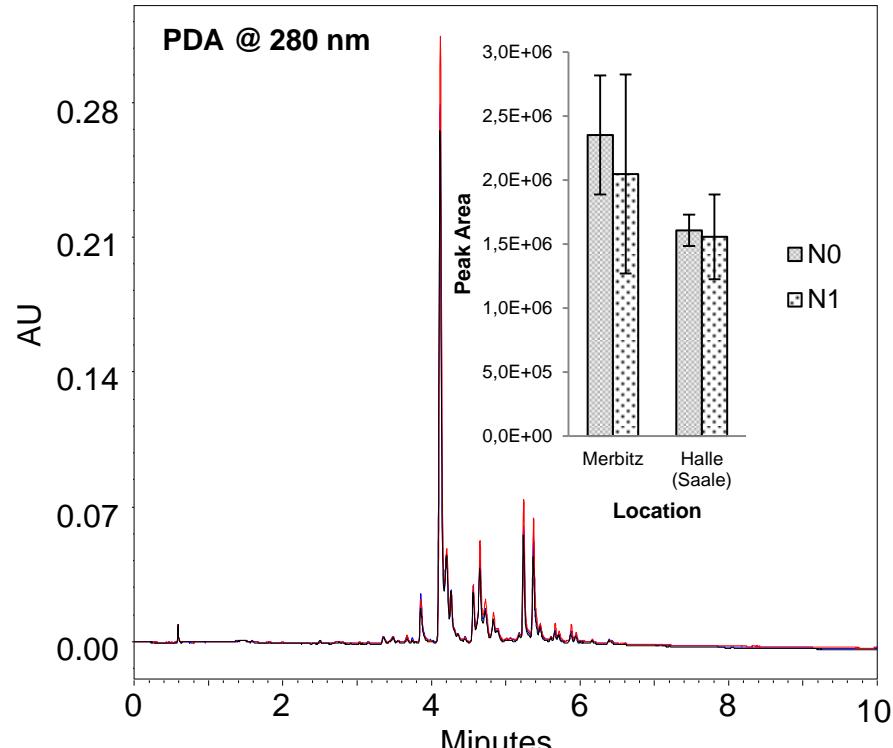
- Decreasing of plant growth, leaves' total nitrogen, amino acids, proteins, chlorophylls and carotenoids contents
- Nitrogen and phosphate deficiency leads to an accumulation of phenylpropanoids and lignification
 - E.g. 3-fold increase of anthocyanins under nutrient stress in tomato observed
- Flavonoid accumulation under nitrogen stress seems to be non - specific
 - N – deficient plants increase their availability of ammonia by enhanced deamination of phenylalanine

Differences in the phenolic profile of both locations

- Overlays of Barke and Scarlett of both locations showed **no qualitative difference in the phenolic profile between locations**



SampleName: A 3648_Scarlett_N0_Location_Halle
SampleName: A 1770_Scarlett_N0_Location_Merbitz
SampleName: A 5149_Scarlett_N1_Location_Halle
SampleName: A 2170_Scarlett_N1_Location_Merbitz



SampleName: A 1451_Barke_Location_Merbitz
SampleName: A 3622_Barke_Location_Halle(Saale)
SampleName: A 5121_Barke_Location_Merbitz
SampleName: A1911_Location_Halle(Saale)

Genotyping of HEB-25 with iSELECT 9k SNP chip

Single nucleotide polymorphism (SNP)



Barke: ...ACGT**A**CAGTCA...
Exote: ...ACGT**G**CAGTCA...



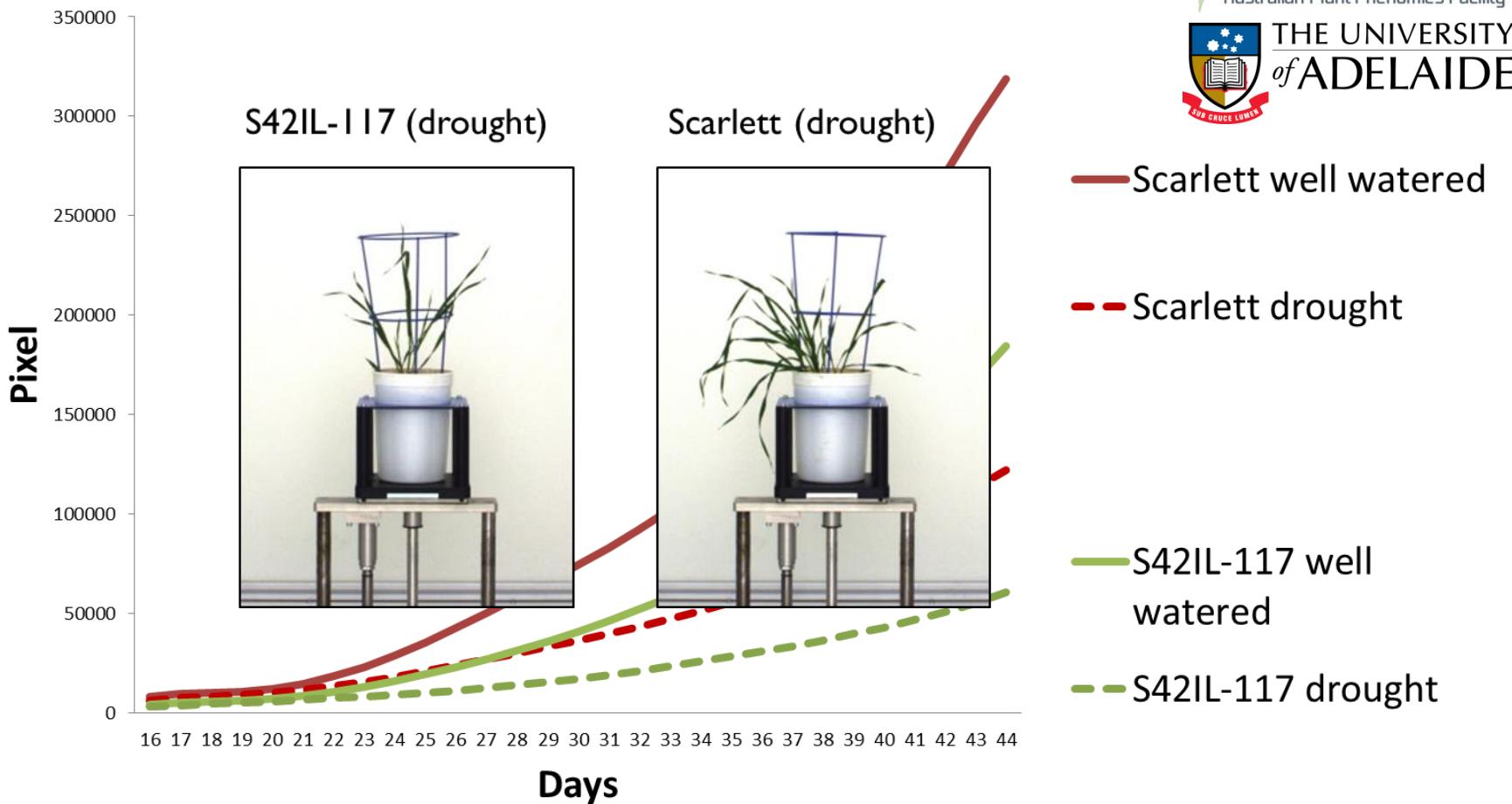
- Infinium iSelect 9k barley chip at **TraitGenetics**, Gatersleben, with 7,864 SNPs
- 5,727 SNPs are informativ in HEB-25
- Estimation of **genetic similarity (GS)** between lines based on DICE
- Calculation of **genetic diversity** with principle component analysis (PCA)



3 chips in Illumina BeadStation 500G

High-throughput phenotyping of S42IL growth under early drought stress

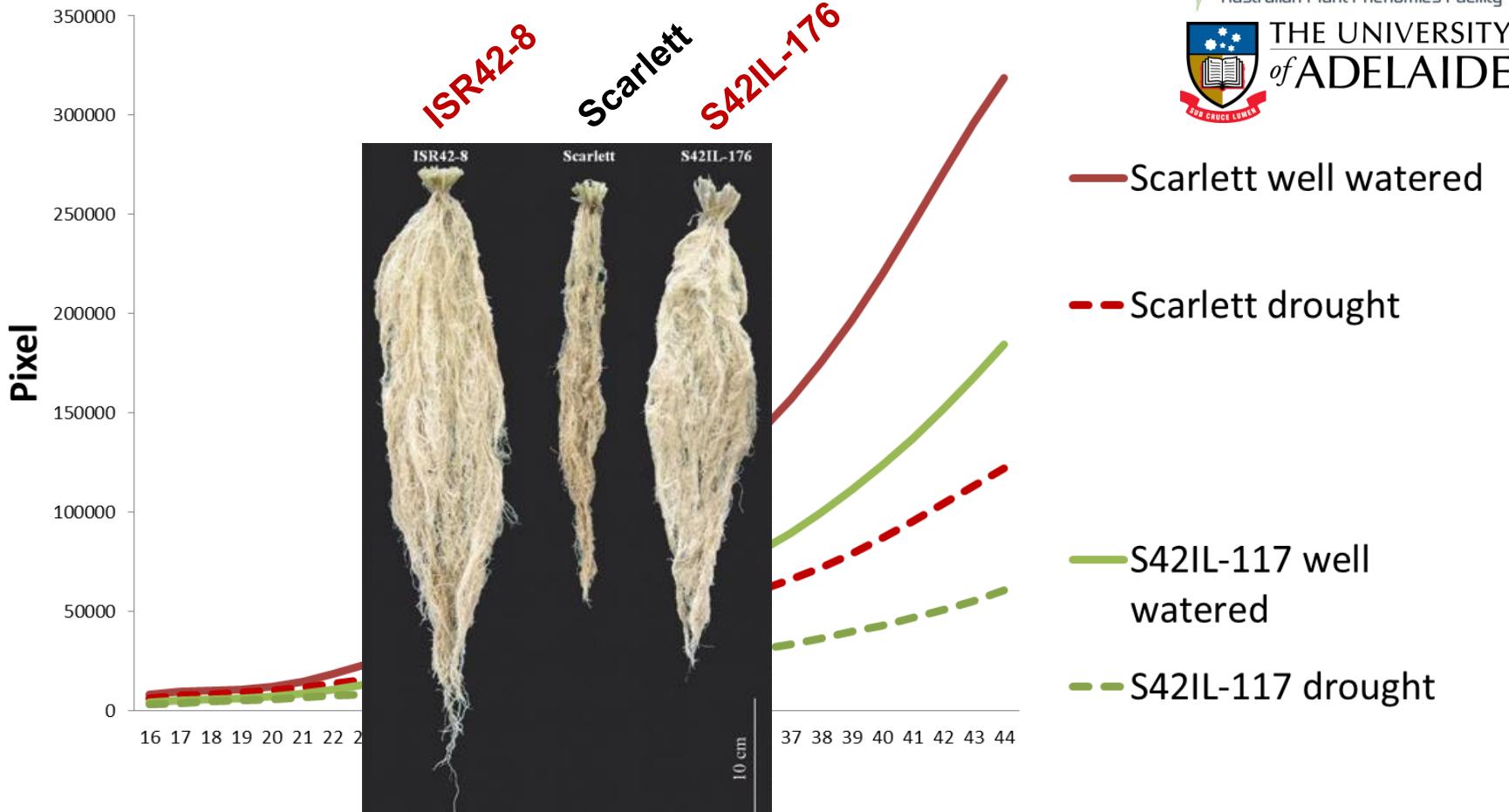
"Shoot Area" of Scarlett and S42IL-117



Honsdorf et al. 2014, PLOS ONE

High-throughput phenotyping of S42IL growth under early drought stress

"Shoot Area" of Scarlett and S42IL-117



Naz et al. 2012, Plant Breed

Honsdorf et al. 2014, PLOS ONE



Genome-wide association study (GWAS) in HEB-25

- Multiple linear regression (MLR)
 - Cofactor selection (stepwise regression, forward-backward selection)
 - correction for population structure and genetic background
 - Model: $Y = \mu + \text{SNP} + \text{HEB family} + \text{Cofactors} + \varepsilon$

Multiple testing: Bonferroni-Holm

- QTL: $P_{\text{BON-HOLM}} < 0.05$

Ref: Würschum et al. 2012, Heredity, 108: 332-340

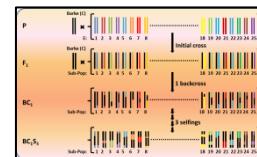
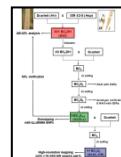
IPAS Projekt BARLEY DIVERSITY

Projektstart:

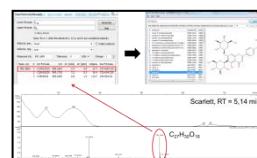
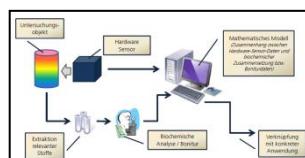
Effektiv: 01. März 2015

Ziele:

1. Zwei kürzlich erstellte Wildgersten-Introgressionsbibliotheken sollen geprüft werden, um a) die Biodiversität der Kulturgerste zu erhöhen und b) ihre Produktivität unter N-Mangel sowie unter zwei divergierenden Fruchtfolgen zu erhöhen.



2. Pflanzenwachstum, Nährstoffgehalte und Zellwandzusammensetzung sollen mittels eines nicht-invasiven Hyperspektral-Messsystems (AgRover) vorhergesagt und genutzt werden, um bessere Genotypen zu selektieren..



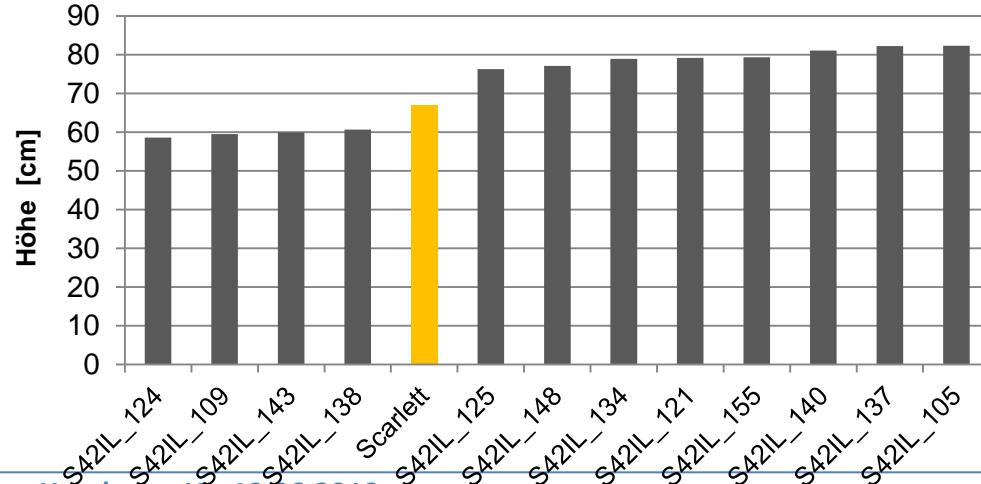
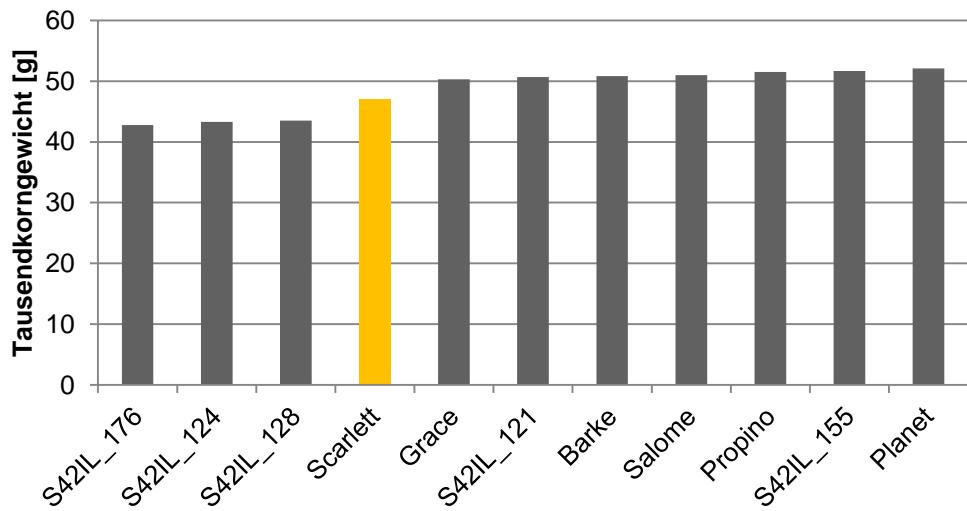
Field trial Merbitz 2015



Introgression Lines (ILs) significantly ($P<0.05$) deviating from Scarlett in thousand grain weight (TKG) or plant height (HEI)



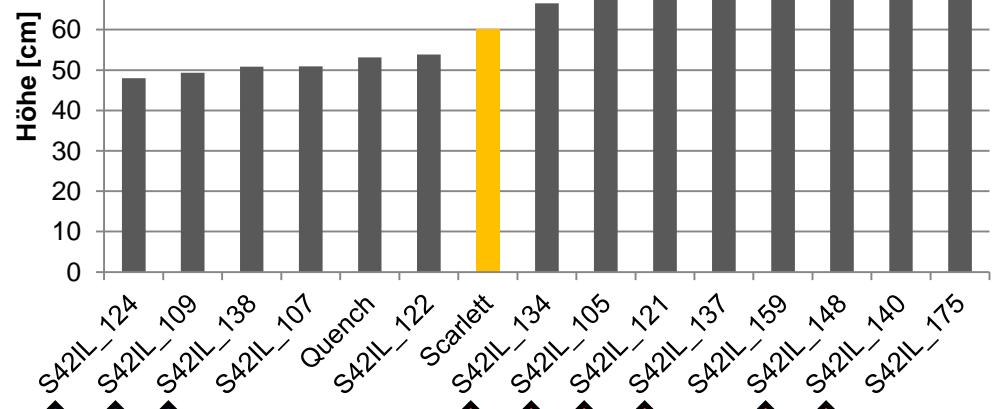
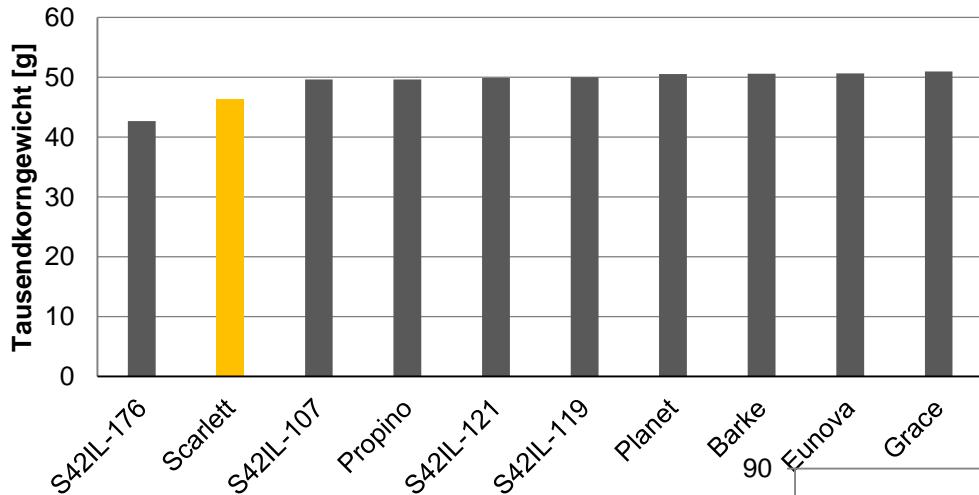
S42-IL Merbitz 2015



Field trial Morgenrot 2015



Lines significantly ($P<0.05$) deviating from Scarlett in thousand grain weight (TKG) or plant height (HEI)



Common IL effects
at both locations