CROP.SENSEe.net Network Sugar Beet
Sugar beet (*Beta vulgaris*): a biennial root crop
Physiological analyses during the early phases of root development

Morpho-physiological analysis during the early phases of root development. (A) Phenotype of the sugar beet tap root during the first 9 weeks after emergence (WAE). Bar, 10 mm. (B) Root diameter during the first 9 WAE. Vertical bars indicate SD.

Aims of the sugar beet network and their roles for CROP.SENSE.net

- Non invasive *in-vivo* measurements of sugar beet roots
- Establishing a RIL population segregating for root type and QTL mapping
- Developing sensors for early detection of leaf diseases and nematode infections
- Measuring abiotic stress before symptoms become visible
## Projects and participating groups

<table>
<thead>
<tr>
<th>Project</th>
<th>PI</th>
<th>Unit</th>
<th>Start</th>
<th>Funding</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant Architecture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZA1</td>
<td>Peter Schulze-Lammers</td>
<td>University Bonn, Institute of agricultural engineering</td>
<td>01.10.2010</td>
<td>BMBF</td>
<td>RADAR sensor technology for root growth monitoring</td>
</tr>
<tr>
<td>ZA2</td>
<td>Christian Jung</td>
<td>CAU Kiel Plant Breeding Institute</td>
<td>01.06.2012</td>
<td>BMBF</td>
<td>Populations segregating for tap root formation genetic maps, QTL-mapping, candidate genes</td>
</tr>
<tr>
<td>ZA3</td>
<td>Dagmar van Dusschoten</td>
<td>Forschungszentrum Jülich</td>
<td>15.04.2010</td>
<td>BMBF</td>
<td>3D Tomography of sugar beets in natural soils with MRI/PET</td>
</tr>
<tr>
<td><strong>Plant Stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZS1</td>
<td>Marcus Jansen, Uwe Rascher</td>
<td>Forschungszentrum Jülich</td>
<td>01.10.2010</td>
<td>BMBF</td>
<td>Phenotyping leaf invasions of pathogens with non-invasive sensor technology</td>
</tr>
<tr>
<td>ZS2</td>
<td>Heiner Goldbach</td>
<td>University Bonn INRES – Plant Nutrition</td>
<td>01.01.2012</td>
<td>EFRE</td>
<td>Non-invasive sensor technology on sugar beet leaves to estimate the density of <em>H. schachtii</em></td>
</tr>
<tr>
<td>ZS3</td>
<td>Heiner Goldbach</td>
<td>University Bonn INRES – Plant Nutrition</td>
<td>01.10-2009</td>
<td>BMBF</td>
<td>Tracing the development of stress symptoms by using volatiles as indicators</td>
</tr>
<tr>
<td>ZS4</td>
<td>Manfred Trimborn</td>
<td>University Bonn INRES – Plant Nutrition</td>
<td>01.03.2012</td>
<td>EFRE</td>
<td>Tracing the development of stress symptoms by using volatiles as indicators</td>
</tr>
<tr>
<td>external</td>
<td>Britta Schulz</td>
<td>Kleinwanzlebener Saat AG (KWS)</td>
<td>---</td>
<td>---</td>
<td>Cooperation with ZA3, ZS1, ZS2, ZS3, ZS4</td>
</tr>
</tbody>
</table>
ZA1: Sensing of sugar beet roots by Radar

- Using UWB-RADAR for non-invasive analysis of roots
- Set up of a test device suitable for rapid phenotyping
- Discriminating different root types by radar

Prof. Dr. Peter Schulze Lammers, Dr. Christian Peveling-Oberhag
Institute of Agricultural Engineering, University Bonn
Set-up of the UWB-Radar system

- RADAR-System for various linear and angular positions (linear- and rotational drive)
- Optimized back scatter characteristics
- Tests with different fake objects (metal plate, artificial plants), sugar beet and leaf beet, dry sand and Perlite as soil substrates
Beet root imaging by UWB Radar

Programming a user-interface based on MATLAB, combining data processing, parameter calculation and imaging

Contrast optimization of back scatter

Imaging of beets in 3D

sugar beet

leaf beet
ZA2: Use of non-invasive sensors for genetic characterization of root formation in sugar beet


Siegbert Melzer and Christian Jung
CAU Kiel, Plant Breeding Institute
Developing an F$_5$- RIL population

* 460 RILs including flowering RILs
Phenotyping and map construction

The two parents of the RIL population have been sequenced for SNP calling. 100 SNPs KASP markers have been designed and are currently used to generate a genetic map.
Results and deliveries

- A RIL population has been produced as a future resource for studying root characters and tap root development
- Numerous SNPs have been identified by sequencing the parents for mapping which are presently mapped as KASP markers
- Storage root QTL will be mapped in the beet genome
ZA3: 3D Tomography of sugar beet in natural soil with Magnetic Resonance Imaging (MRI) and Positron emission Tomography (PET)

- High resolution imaging by *Magnetic Resonance Imaging* (MRI) of sugar beet development grown in soil
- Qualitative sugar content dynamics in the beet belowground
- Quantitative water flow imaging within the developing beet
- Imaging of photoassimilate distribution patterns and quantification of transport and storage with *positron emission tomography* (PET)

Dr. Ralf Metzner and Dr. Dagmar van Dusschoten
FZ Jülich
3D Imaging of beet roots grown in soil
days after sowing

• Soil substrates have been identified for MRI studies that do not interfere with root structures (depletion of ferromagnetic particles)
• MRI protocols for the detection of 3D structures and the anatomy of beets in soil and their spatial and temporal development have been established (10 min/plant = potential for high throughput)

Frontiers in Plant Sciences (Metzner et. al., 2014)
high resolution imaging to measure the development of cambia, vascular tissues and storage parenchyma
Quantitative sugar imaging by MRI-chemical shift imaging (CSI)

Comparative studies for photoassimilate allocation and flow dynamics for three contrasting genotypes

Sucrose localization

Sucrose content

Arrowheads: Cambia of different growth rings
PET and MRI for relating photoassimilate allocation and tissue structure development during growth period

$^{11}\text{CO}_2$ radiotracer administered to the whole shoot

DAS = days after sowing
Greyscale: MRI tissue structure
False colours: PET maximum intensity of the radioactivity for 2h after tracer application
Outlook

• Study root development in contrasting genotypes by MRI – PET (in progress)
• Stress measurements
  • beet development after Cercospora infection (in progress)
  • beet development after drought stress
ZS1: Phenotyping disease severity of foliar pathogens with non-invasive sensor techniques

Marcus Jansen, Sergej Bergsträsser, Simone Schmittgen, Uwe Rascher
IBG-2 Plant Sciences, Forschungszentrum Jülich GmbH
A new sensor station to quantify disease severity by spectral images (Sensor-Prototype HyperART)

PS camera: 400 - 1000 nm (Resolution 2.8 nm) SWIR Kamera: 970 - 2500 nm (Resolution 10 nm)

Simultaneous Measurements of Reflectance und Transmittance

Literature: Patent application DE 10 2012 005 477.7. filed as March 2012
By: Forschungszentrum Jülich GmbH,
inventor: Sergej Bergsträsser
Sensor-Prototype HyperART: Non-invasive detection and quantification of *Cercospora* infestation on sugar beet leaves

- Discrimination between non-infected and *Cercospora*-infected leaf tissue
- Transmittance shows higher contrast between infected and surrounding tissue (circles)
- Combination of reflectance and transmittance shows distinct differences between infection site and surrounding tissue (arrows) and allows a more precise classification
- Future: investigate physiological processes, e.g. quantification of chlorophyll and water content

Classification of CLSI image differs:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic</td>
<td>33,9%</td>
<td>29,3%</td>
<td>31,8%</td>
</tr>
<tr>
<td>Non-symptomatic</td>
<td>66,1%</td>
<td>70,7%</td>
<td>68,2%</td>
</tr>
</tbody>
</table>

*Mahlein et al., 2013
Sugar beet growth during *Cercospora infestation* (in cooperation with ZA3)

- Taproot growth of a highly susceptible (HS) genotype was reduced 2 weeks after *Cercospora* inoculation.
- Similar *Cercospora*-induced effect was analyzed in a susceptible (S) genotype → distinct differences in growth pattern between HS and S.
- Root growth parameters (volume, diameter, overall ratio between root parts) as selection means for breeding resistant beets.

(A) 11, (B) 17 and (C) 25 weeks after sowing.
ZS2: Non-invasive sensor techniques on leaves of sugar beets for density estimations of *Heterodera schachtii*

- Developing a high throughput hyper spectral sensor technique under field conditions with non-invasive analyses of sugar beet leaves after infestation by the beet cyst nematode *Heterodera schachtii*
- Indirect quantification of nematode populations and establishing decision criterions for crop rotations and varieties

Dr. Kai Schmidt, Nemaplot, Bonn
Birgit Fricke, Uni Bonn
Dr. Matthias Daub, (JKI), Institut für Pflanzenschutz in Ackerbau und Grünland, Elsdorf

- NRW/EU funds for regional development (EFRE) -
Results ZS2: *H. schachtii* densities and suitable sensors

- Hyper spectral signatures of leaves are suitable for density predictions of *H. schachtii*
- General linear modeling to estimate densities of nematodes in the soil

Distributions of egg and larva densities per 100 g soil (EuL/100 g soil) on the experimental field in JKI-Elsdorf.
ZS3: Detection and differentiation of stresses analyzed through volatile indicators and analytic reference techniques

- Early and differential detection of drought stress with non-invasive and invasive measurement techniques
- Measuring of water balance, antioxidative system parameters and metabolites of the primary metabolism (reference analysis)
- Non-invasive measurements of gas emissions in the headspace under stress: nitrogen monoxide (NO), ethane (C\textsubscript{2}H\textsubscript{6}) and ethylene (C\textsubscript{2}H\textsubscript{4})

Prof. Dr. Heiner Goldbach, Dr. Manfred Trimborn, Dr. Monika Wimmer, Tina Kasal, Rita Krechel

INRES – Pflanzenernährung, Uni Bonn
Reference analytics sugar beet

- Standardized growth of plants to analyze drought stress
- *High throughput* analyses of 15 primary metabolites in leaves and roots of beets
- Differences under drought stress are indicated by metabolite patterns and changes in the water balance
- Parameter of the reference analysis are suitable for field and glasshouse experiments
Proline and Malondialdehyde as useful marker for drought stress and for membrane damages

Increasing concentrations of both molecules after drought stress

Stable concentrations in the controls
ZS4: Developing detection systems to measure VOC emissions from plants

• Early and differential detection of stress (biotic/abiotic) through VOC (volatile organic components) emission measurements
• Developing a system to measure VOC emission in the lab and in the field
• Plants used: tomatoes and sugar beets

*Prof. Dr. Heiner Goldbach, Tina Kasal*

*INRES – Pflanzenernährung, Uni Bonn*

- NRW/EU funds for regional development (EFRE) -
CROP.SENSe.net network sugar beet: Deliveries, conclusions and future prospects

- The anatomy of sugar beet roots and carbohydrate translocations can be examined non invasively in 3D
- A RIL population segregating for tap root development is available
- A new method to detect leaf pathogen infections has been established
- Parameter of abiotic stress are measurable in a high throughput manner
- Applications in plant breeding research and applied plant breeding
HT phenotyping meets HT genotyping

CROP.Sense.net Mission:

- Early and high throughput identification of yield, quality and stress related traits
- High accuracy trait prediction by sensor data
- Measuring genotype x environment interaction
- Phenotypic prediction of genotypes under different environments using quantitative models
HT phenotyping meets HT genotyping

Phenotypic variability

- Structured populations
  - F2
  - RIL
  - NIL
  - MAGIC

- varieties
- landraces
- Gene bank accessions

Phenotyping platforms

Genotypic variability

Genome (re)sequencing

Reference genome sequences

Transcriptome sequencing

Mapping and identification of relevant genes for breeding

Targeted modification of single genes

Selection of superior genotypes