QTL detection of multiple traits in sugar beet and fine mapping by sequencing (MBS)

Elena Orsini[1], Abdelnaser Elashry [1], Daniela Holtgräwe[2], Boas Pucker[2], Bernd Weisshaar[2]
[1] Strube Research GmbH & Co. KG, Söllingen, Germany
[2] Bielefeld University, Chair for Genetics and Genomics of Plants, Center for Biotechnology, Bielefeld

Motivation

Modern agriculture of sugar beet requires the breeding of multiple resistant traits in single-seeded cultivars. The aim of our project is the identification of candidate genes for tolerance toward the beet cyst nematodes (BCN) *Heterodera schachtii* and resistance to the leave pathogen *Cercospora beticola* (CB).
An $F_{2:3}$ population of 407 lines was obtained by crossing a monogerm DH line resistant to CB and a multigerm pollinator line tolerant to BCN. Thus, the population is segregating for multiple traits, including monogermity, multigermity and bigermity.

Nematodes

Phenotyping for nematode tolerance was performed in the greenhouse using metal containers each accommodating 100 plants from the $F_{2:3}$ population and 20 plants used as repeated checks. Ten plants per $F_{2:3}$ family were manually inoculated with 350 *H. schachtii* second stage juvenile larvae using a dispenser. Five weeks after inoculation the number of cysts per plant was counted under a stereoscope. The phenotypic data were analyzed using a mixed model approach where the metal containers were modeled as random incomplete blocks and the $F_{2:3}$ families as fixed effects.

Cercospora

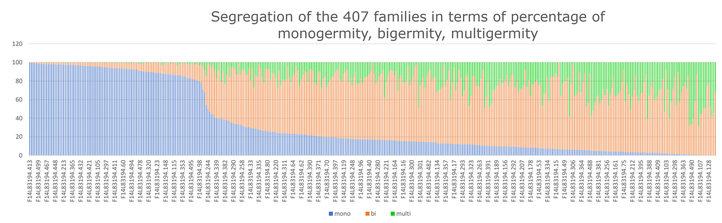
The 407 $F_{2:3}$ families were sown in 2017 in three locations in the South of Germany and in one location in the North of Italy. Plants were sown in a randomized incomplete block design in one or two field replications (Table 1). Each plot consisted of three rows and the third row was always sown with a hybrid susceptible to *Cercospora beticola*, in order to facilitate the natural fungal infection in the field. The scoring for cercospora leaf spot was performed per plot during the growing season of the plants using a scale from 1 (no infection) to 9 (entire defoliation).

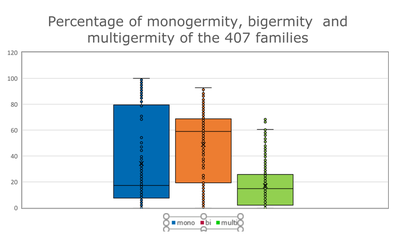
Trials	Sowing	Replications	Scoring in 2017
Gützingen (DE)	31.03.2017	1	29.08 ; 13.10
Mattenkofen (DE)	01.04.2017	2	19.09
Vierhöfen (DE)	03.04.2017	1	31.08. ; 19.09.
Ca Venier (IT)	10.03.2017	2	11.07 ; 18.07 ; 26.07 ; 2.08

Adjusted entry means per location were obtained using a two-dimensional model where spatial variation in the field was taken into account.

Monogermity, Bigermity, Multigermity

Phenotyping for seed traits occurred during seed production of the $F_{2:3}$ families. One family was 100% monogerm, 52 segregated for monogermity versus bigermity, 351 segregated for monogermity, bigermity, multigermity and 3 lines segregated for bigermity versus multigermity.



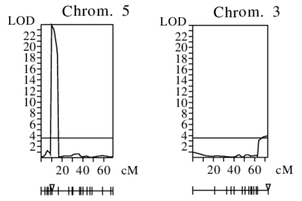


Genotyping

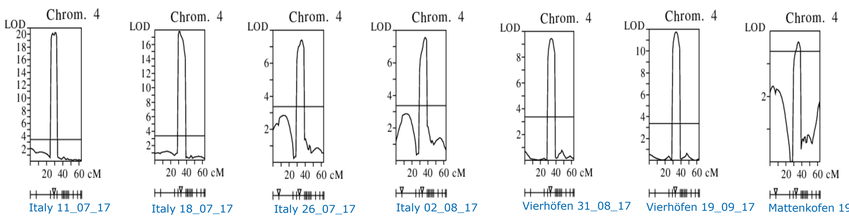
The parental lines were deep sequenced and the reads were assembled, aligned against the reference genome and variant detection was performed. More than 600.000 SNPs polymorphic between the parental lines and heterozygous in the F_1 individual were identified. 400 SNPs equally spread across the genome were developed as KASP markers and used for genotyping the F_2 lines for QTL mapping.

QTL mapping for nematode tolerance and cercospora resistance

Adjusted means of the $F_{2:3}$ families were used for QTL mapping using a genetic linkage map constructed with R/qtl. Composite interval mapping (CIM) was employed for QTL detection and a LOD threshold corresponding to an experiment wise Type I error rate of 0.05 was determined by using 1000 permutation runs. All QTL computations were performed with the software package PLABQTL.



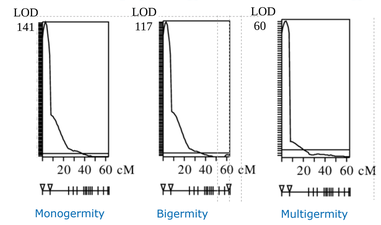
Trait	Chrom	Pos (cM)	LOD at QTL	Phenotypic variance
Adj number of cysts per $F_{2:3}$ family	5	10	24	22.83 %
	3	73	3.8	3.57 %



Another QTL on Chrom 3 was detected in Italy and in Gützingen and explained 5 % of the phenotypic variance

Trait	Chrom	Pos (cM)	LOD at QTL	Genetic variance	Phenotypic variance
Cercospora resistance (Adj means)	4	34-36	3-20	15-30%	10-20%

QTL mapping for Monogermity Bigermity Multigermity



Trait (%)	Chrom	Pos (cM)	LOD at QTL	Phenotypic variance
Monogermity	4	3	141	77.63
Bigermity	4	3	117	67.44
Multigermity	4	3	60	43.86
Multigermity	8	18	9	10.48

Ongoing work

DNA of either tolerant or susceptible F2 individuals is currently combined into pools and sequenced with Illumina technology. Allele frequencies for variants will be determined for both pools. Deviations in allele frequencies between the pools should identify the genomic loci responsible for the quantitative traits. In order to pinpoint to the causal gene, the candidate region will then be further analyzed with regard to e.g. functional gene annotation and the impact of variation positions in the diverse alleles.

Projektträger Jülich
Forschungszentrum Jülich
Bundesministerium für Bildung und Forschung
strube
The Seed. Est. 1877