GEFÖRDERT VOM

Bundesministerium
für Bildung
und Forschung

Veröffentlichung der Ergebnisse von Forschungsvorhaben im BMBF-Programm

Pflanzenbiotechnologie-Verbundvorhaben

Identifizierung und Validierung von wichtigen Marker/Merkmals Assoziationen für züchterisch wichtige Merkmale zur Entwicklung verbesserter Weizensorten (VALID) – Teilprojekt B

Förderkennzeichen:

0315947B

Zuwendungsempfänger:

Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK)

Corrensstr. 3

06466 Stadt Seeland OT Gatersleben

Projektkoordination:

Dr. Marion Röder

Laufzeit:

01.10.2011 bis 30.09.2015

Das diesem Bericht zugrundeliegende Vorhaben wurde mit Mitteln des Bundesministeriums für Bildung und Forschung unter dem Förderkennzeichen 0315947B gefördert. Die Verantwortung für den Inhalt dieser Veröffentlichung liegt bei dem Autor

Inhaltsverzeichnis

I. Short overview

1. Objectives of the project

Hexaploid wheat (*Triticum aestivum* L.) is in terms of hectares grown the most important crop species in Europe. Despite that, the increase in yield and other yield related parameters through plant breeding has been lagging behind other crops such as maize. Some reasons for that are (1) that wheat is a self-pollinating species; (2) it is a complex allopolyploid species that consists of three different but highly related genomes (A, B and D) which together have the largest genome of any major field crop and (3) the widely grown winter wheat has a long generation cycle (one year) which makes classical population development a lengthy procedure. Because of that, marker-assisted wheat breeding has been primarily focused towards improvement of disease resistance and quality parameters, based on mapping in segregating populations. Many of the relevant traits involving yield, yield components, quality characters, abiotic stress and disease resistance are quantitatively inherited and influenced by large numbers of quantitative trait loci (QTLs). Thus bi-parental segregating populations might not be optimal for the analysis of such traits. Because of that wheat might be better approached by association genetic studies where the wheat genome is scanned with large numbers of markers for their association with specific traits. Due to its long generation time, an association panel consisting of winter wheat varieties can be assembled much faster than populations can be developed. Furthermore, QTL from association studies might be applicable over a wider range of material than QTL from bi-parental crosses.

The purpose of this project was to validate associations derived from the GABI-WHEAT project. Firstly, we extended our phenotyping to additional diseases and, especially due to the changing environment, also to abiotic stress with the main emphasis on drought stress. Secondly, the investigated lines were genotyped to much higher depth with SNP markers. Thirdly, we started to develop and use a validation panel and specific populations for a number of significant marker-trait associations (MTAs) identified in the previous project. With this, we wanted to check how far association data can indeed be used for the development of significantly improved wheat varieties.

2. Prerequisites of the project

In the previous project GABI-WHEAT (project ID 0315067) a comprehensive dataset based on multi-environmental field data was developed for yield, other agronomic traits including resistance to fungal pathogens and quality traits. The phenotypic data were generated in replicated field trials at eight different locations and over several years, respectively, using a panel of 358 European winter wheat and 14 spring wheat varieties mainly released between 2000 and 2007.

The genotypic data consisted of a set of approx. 800 microsatellite markers and specific markers for major phenology traits (such as *Rht*, *Ppd* and others). The analysis of marker-trait associations was carried out by using a mixed linear model and a kinship matrix based on microsatellite markers to correct for population stratification.

3. Planning and course of the project

The project consists of four research areas:

Area 1: Expansion of the existing association panel and further phenotyping

We expanded our phenotypic screening based on lines from the original association panel. A more controlled testing for drought tolerance was carried out in rain-out shelters via a subcontract with the Julius Kühn Institute in Quedlinburg (Prof. Dr. Frank Ordon). Prof. Ordon and Dr. C. Balko evaluated 184 varieties (TROST panel) in three replications each, in the second and third year of the project. Characters recorded on the 184 genotypes grown under a rain-out shelter and controlled irrigation were flowering and maturity date, plant length, chlorophyll content and fluorescence, accumulation of proline, accumulation of soluble sugars, biomass and grain yield, yield components, harvest index, protein and starch content.

The new VALID panel consisted of 137 winter wheat varieties released in the years 2007 to 2010. This VALID panel was tested in repeated field trials together with 20 genotypes of the GABI-WHEAT panel by the companies KWS LOCHOW and Syngenta in the years 2012/2013 and 2013/2014. One field trial was repeated by Syngenta in 2014/2015.

Furthermore the VALID panel and the TROST panel were phenotyped for additional diseases (e.g. eyespot, take-all) and validation of previously acquired data for Fusarium, Septoria and tan spot was performed by the JKI in Braunschweig (Dr. Bernd Rodemann), based on a subcontract. This screening was performed mainly in year 2 and 3 of the project.

Area 2: Genotyping of the wheat lines and varieties with additional markers

In order to further increase the marker density in both wheat panels, TraitGenetics genotyped the entire set of 515 varieties (the original GABI-WHEAT panel of 384 lines and the new VALID-panel of 137 lines, some lines were duplicated) with two sets of wheat SNP markers: the 90k iSELECT ILLUMINA-chip (Wang et al. 2014) and a 35k Affymetrix chip (Axiom® Wheat Breeder's Genotyping Array, http://www.cerealsdb.uk.net/).

In order to obtain a comparable data set for the newly collected varieties with respect to population stratification, the 137 VALID lines were also genotyped with the same set of approximately 800 microsatellite markers as in the previous project.

Area 3: Validation of the identified marker-trait associations

The validation of interesting marker-trait associations was based on the data from the GABI-WHEAT project. In approach 1, we generated crosses between varieties with several favourable alleles for the respective trait and an elite line without these alleles. From these crosses, DH-lines were developed by the participating breeding companies. The individual DH-plants were genotyped with microsatellite markers that showed associations for the respective traits in GABI-WHEAT. Lines that are homozygous for the interesting allele at each interesting chromosomal region and combinations thereof were selected and seeds were multiplied. It is planned to grow these lines individually and as bulks in replicated field trials to phenotype yield, yield components and disease resistances. In the subsequent analyses the near isogenic bulks carrying the desired alleles will be compared to those without these alleles.

In approach 2, the partners started a backcross program for important yield-related marker-trait associations that are not caused by known genes (e.g. Rht genes, Ppd genes) and two loci associated with disease resistance. Furthermore, this endeavour provided much needed information regarding the optimization of marker-assisted backcross breeding for wheat based on association data and created the basis for map-based cloning of these loci in the future.

Approach 3 included the re-analysis of the identified main marker-trait associations based on the SNP genotyping data. Furthermore, it includes the validation of all identified marker-trait associations from the original GABI-WHEAT panel through the analysis of the data obtained from the newly generated VALID panel after two years of field experiments.

Area 4: Data analysis

Data analysis and data management was performed for the association analysis based on the experience from the previous GABI-WHEAT project. As in the previous GABI-WHEAT project, field data were pre-processed by the breeding companies for specific aspects regarding the experimental field design. Outlier-pruning was performed in interaction between the IPK, TraitGenetics and the breeding companies. Associating markers and traits and the analysis of the effects of interesting alleles was performed mainly by IPK and TraitGenetics. The project was extended on a cost-neutral basis for one year. One field trial had to be repeated and population development takes at least three years, not including field trials.

4. Scientific and technical state of the art at the beginning of the project

At the beginning of the project, association genetics had been suggested as an alternative to the lengthy process of traditional QTL-mapping in bi-parental populations. In association studies, it is assumed that alleles present in the investigated, optimally unstructured, material are mainly related by descent. Thus, for association studies, panels of lines are assembled, phenotypically characterized and subsequently analyzed with a large set of molecular markers. A careful analysis of each marker and allele in relation to the respective trait provides information whether a specific chromosomal region has a significant effect on the trait. If that is the case, the incorporation of this region by introgression into another line with another less favorable allele will improve the overall performance of the line.

First results on the use of association genetics in plants were reported for maize (Buckler et al. 2002). These studies demonstrated that microsatellite and SNP markers can indeed be used for the identification and localization of genes that are involved in quantitative traits (e.g. dwarf8 in maize) or selection constraints (Thornsberry et al. 2001). Association genetics has been performed with candidate genes for several traits in maize and other crops (Belo et al. 2008; Harjes et al. 2008; Cockram et al. 2008; Matthies et al. 2009; Haseneyer et al. 2010; Emebiri et al.2010). Using genome-wide marker sets, the extent of linkage disequilibrium has been studied in various plant species and genome wide association analysis was performed (Kraakman et al. 2004; Agrama et al. 2007; Breseghello and Sorrells 2006; Waugh et al. 2009; Atwell et al 2010) employing various statistical models (Yu et al. 2006; Zhao et al. 2007; Stich et al. 2008; for overview: Mackay and Powell 2007; Rafalski 2010). For wheat, some reports about the analysis of linkage disequilibrium (Maccaferri et al. 2005; Chao et al. 2007; Somers et al. 2007; Horvath et al. 2009) and a few examples for genome wide association mapping have been published (Breseghello and Sorrells 2006; Crossa et al. 2007; Tommassini et al. 2007; Peng et al. 2009; Neumann et al. 2010). The employed genotype panels in these projects were however relatively small (less than 100-200 lines) and only a limited number of markers were used.

However, over all studies remarkably little follow up studies have been published which demonstrate that interesting marker-trait associations can be confirmed and validated by crossing the respective chromosomal regions into another background. Thus, the verdict is still out whether association studies are in fact an approach that is superior to classical genetic analyses in segregating populations for crop plants.

5. Collaboration with others

There was a very close collaboration among all partners and subcontractors. Besides regular interactions between partners by electronic and telephone communication, biannual project meetings served for an intensive exchange of information and data and a strict planning of the practical course of the project.

Genotypic and phenotypic data related to resistance to Fusarium head blight were given to Prof. Reif of the Breeding Research Department at IPK Gatersleben in order to apply genomic selection tools on these data. These efforts resulted in a joint publication (Jiang et al. 2015).

5. Detailed Description

1. Detailed description of scientific results

A. The plant material

Three germplasm panels were used during the project (Figure 1):

- GABI-WHEAT panel: 358 European winter wheat varieties and 14 spring wheat varieties, released mainly in the years 2000 to 2007. Most varieties originated from Germany, France and UK, but also from North and Eastern European countries. This panel has been set-up in the GABI-WHEAT project and provided the initial marker-trait associations.
- VALID panel: 137 European winter wheat varieties, released mainly in the years 2007 to 2010. This panel served as an independent validation panel for MTAs detected in the GABI-WHEAT set.
- TROST: A subset of 184 varieties of the GABI-WHEAT panel. This panel was used for the evaluation of abiotic and some biotic stress tolerances.

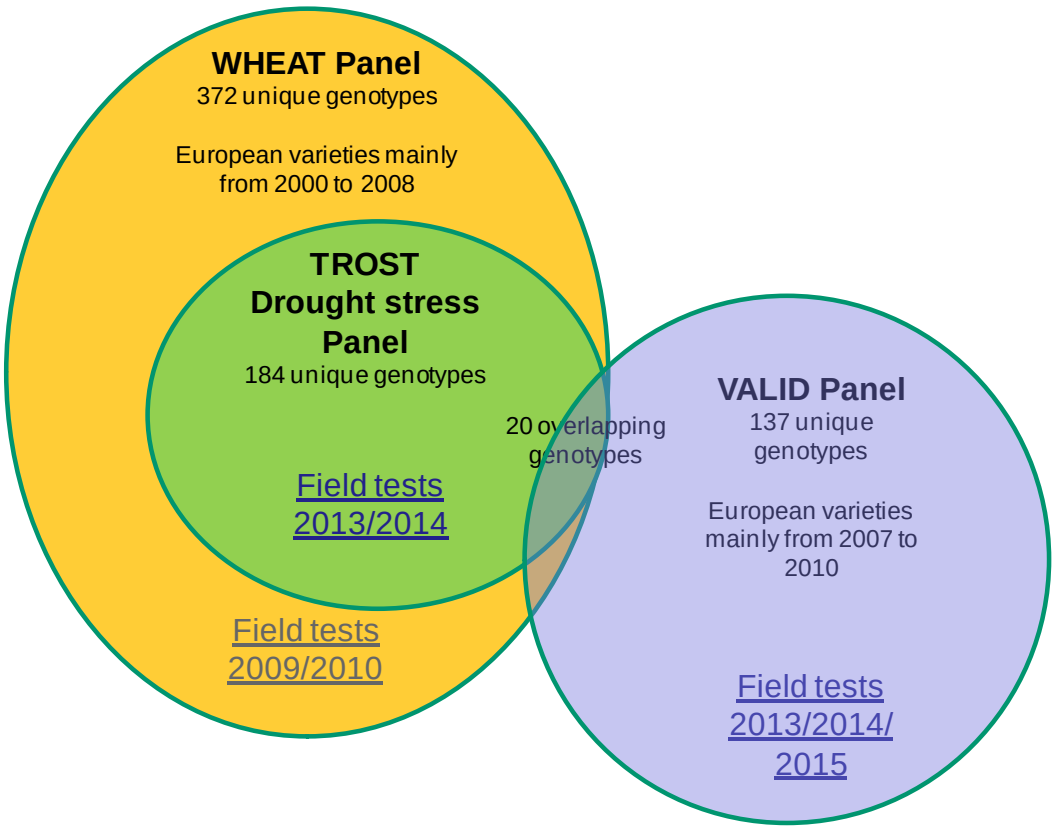


Figure 1: Overview of the germplasm panels used in the VALID project

B. Genotyping

The following marker sets were used to genotype all varieties of the GABI-WHEAT and VALID panel:

- Microsatellite markers: A set of 732 genome-wide SSR markers comprising 644 mapped and 88 unmapped markers.
- 90k iSELECT ILLUMINA SNP array: This array resulted in 21,200 markers polymorphic in the association panels of which 7,761 markers were mapped on a reference population ITMI-DH (Table 1).
- 35k Affymetrix SNP array: This array resulted in 20,052 markers polymorphic in the association panels of which 4,231 markers were mapped on a reference population ITMI-DH (Table 1).
- Candidate genes: A total of 26 candidate genes for plant height, flowering time, vernalization requirement, quality, resistance to biotic stresses, thousand-grain weight and yield were genotyped on all varieties (Table 2).

The two SNP arrays resulted together in a total of 41,252 polymorphic markers in the association panels of which 11,992 markers were mapped on the reference map ITMI-DH. In total, 16,688 different haplotype blocks (a haplotype block is defined as a set of markers that are in perfect linkage disequilibrium over all investigated lines) were detected among the varieties of the GABI-WHEAT and VALID panels (Figure 2), of which 4,811 haplotype blocks were detected with both marker sets while 6,459 and 5,418 haplotypes were detected with only one of the arrays. This demonstrates that the two arrays provide complementary data.

Table 1: SNP markers obtained by 90k iSELECT ILLUMINA SNP array and the 35k Affymetrix SNP array genotyping

	90k	**35k**	**Sum**
Total no. of markers	81,587	35,143	116,730
No. of polymorhic markers	21,200	20,052	41,252
No. of mapped markers	7,761	4,231	11,992

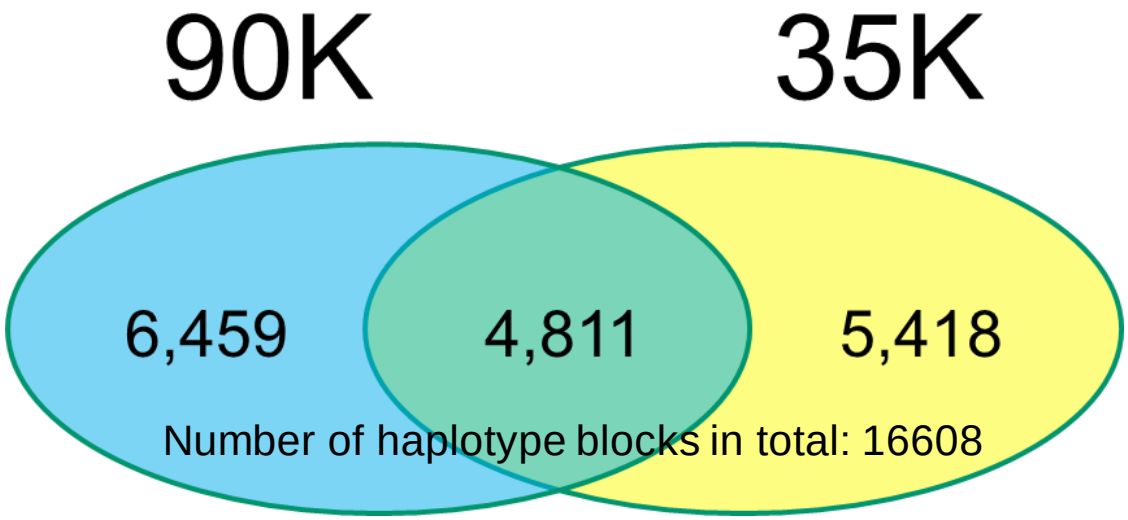


Figure 2: Haplotype-block analysis using SNP markers

Table 2: List of tested candidate genes

Gene/polymorphism name	**Abbreviation**	**Candidate for**	**Reference**
Matrix attachment region of the Bx7 gene	*Bx_MARB*	dough strength	Butow et al., 2004, Theor Appl Genet
HMW glutenin allele Glu-B1-1d (Bx-6)	*Glu-B1-1d*	flour quality	Schwarz et al., 2004, Theor Appl Genet
Eyespot resistance gene (linked marker)	*Pch1*	resistance to eyespot	Groenewald et al., 2003, Plant Breeding
Puroindoline-a	*Pina*	grain hardness	Gautier et al., 1994, Plant Mol Biol
Puroindoline-b	*Pinb*	grain hardness	Huang and Röder, 2005, J Agric Food Chem
Photoperiod response locus	*Ppd-D1a*	sensitivity to day length	Beales et al., 2007, Theor Appl Genet
Dwarfing gene (linked marker)	*Rht8*	plant height	Chebotar et al., 2001, Genetika
Dwarfing gene	*Rht-B1b*	plant height	Ellis et al., 2002, Theor Appl Genet
Dwarfing gene	*Rht-D1b*	plant height	Ellis et al., 2002, Theor Appl Genet
Linked marker	*SBCMV*	resistance to the soil borne cereal mosaic virus	Perovic et al., 2009, Mol Breeding

Linked marker	*Stb6*	resistance to Septoria tritici blotch	Chartrain et al., 2005, Plant Pathol
Subunit Ax2* of Glu-A1	*UMN19*	flour quality	Liu et al., 2008, Theor Appl Genet
Dx2 and Dx5 subunits of Glu-A1	*UMN25*	flour quality	Liu et al., 2008, Theor Appl Genet
Dy10 and Dy12 subunits of Glu-A1	*UMN26*	flour quality	Liu et al., 2008, Theor Appl Genet
Viviparous-1B gene	*Vp1B*	pre-harvest sprouting	Xia et al., 2008, Euphytica
Vernalization gene	*Vrn-B1*	growth habit	Zhang et al., 2008, Crop Sci
Vernalization gene	*Vrn-D1*	growth habit	Zhang et al., 2008, Crop Sci
Candidate gene TGW	*TAGW2-6A*	grain width and thousand grain weight	Su et al., 2011, Theor Appl Genet
Candidate gene TGW	*TAGW2-6B*	grain width and thousand grain weight	Qin et al., 2014, BMC Plant Biol
Candidate gene TGW	*TGS-D1-7D*	thousand grain weight	Zhang et al., 2014, Mol Breed
Sucrose synthase gene	*TaSus1-7A*	thousand grain weight and yield	Hou et al., 2014, Plant Physiol
Sucrose synthase gene	*TaSus1-7B*	thousand grain weight and yield	Hou et al., 2014, Plant Physiol
Sucrose synthase gene	*TaSus2-2A*	thousand grain weight and yield	Hou et al., 2014, Plant Physiol
Cell wall invertase gene	*TaCWI-4A*	thousand grain weight and yield	Jiang et al., 2015, Theor Appl Genet
Cell wall invertase gene	*TaCWI-5D*	thousand grain weight and yield	Jiang et al., 2015, Theor Appl Genet
Cytokinin oxidase gene	*TaCKX-D1-3D*	thousand grain weight and yield	Zhang et al., 2012, New Phytol

C. Phenotyping

- The GABI-WHEAT panel had already been phenotyped in the years 2009 and 2010 in a total of eight environments in Germany and France by KWS LOCHOW and Syngenta.

- The VALID panel and 20 varieties from the GABI-WHEAT panel (as controls) were phenotyped in field trials at the locations Bernburg (BER, DE), Wohlde (WHO, DE), Andelu (AND, FR), Saultain (SAU, FR) in 2013 and 2014 and at

the location Broue (FR) in 2015 (Figure 3, Table 3) by KWS LOCHOW and Syngenta.

- The TROST panel was tested in parallel in irrigated and non-irrigated field trials in Seligenstadt (SEL, KWS LOCHOW) and Biendorf (BAL, Syngenta) in the years 2013 and 2014.
- For further detailed assessment of drought tolerance parameters the TROST panel was tested in rain-out shelters with controlled irrigation (Figure 4) and in parallel in the field at the Julius Kühn Institute in Groß Lüsewitz in 2013 and 2014.

Figure 3: Locations of field trials in the VALID project

Table 3: Traits evaluated in field trials with the VALID panel

Location	YIE	HD	PH	Lod	SW	TGW	Sep	Prot	Sedi	STC
AND_2013	X	X	X		X	X	X	X		
BER_2013	X	X	X		X	X	X	X	X	X
SAU_2013	X	X			X	X		X		
WOH_2013	X	X	X		X	X	X	X	X	X
AND_2014	X	X	X	X	X					
BER_2014	X	X	X		X	X		X	X	
SAU_2014	X	X		X						
WOH_2014	X	X	X	X	X	X		X	X	
Broue_2015	X	X			X					

YIE = total grain yield, HD = heading date, PH = plant height, Lod = lodging, SW = specific weight, TGW = thousand grain weight, Sep = Septoria, Prot = protein content, Sedi = Zeleny sedimentation, STC = starch content

Figure 4: Rain-out shelter experiment in Groß-Lüsewitz (Foto. C. Balko)

Since the seasons 2012/2013 and 2013/2014 were atypical years with respect to drought stress (too much rain), the field experiments at both breeders locations could not be analyzed for their response to drought stress. Only the rain-out shelters in Groß-Lüsewitz could be evaluated since here (Figure 5), the desired stress was observed and respective traits could be measured (Table 4).

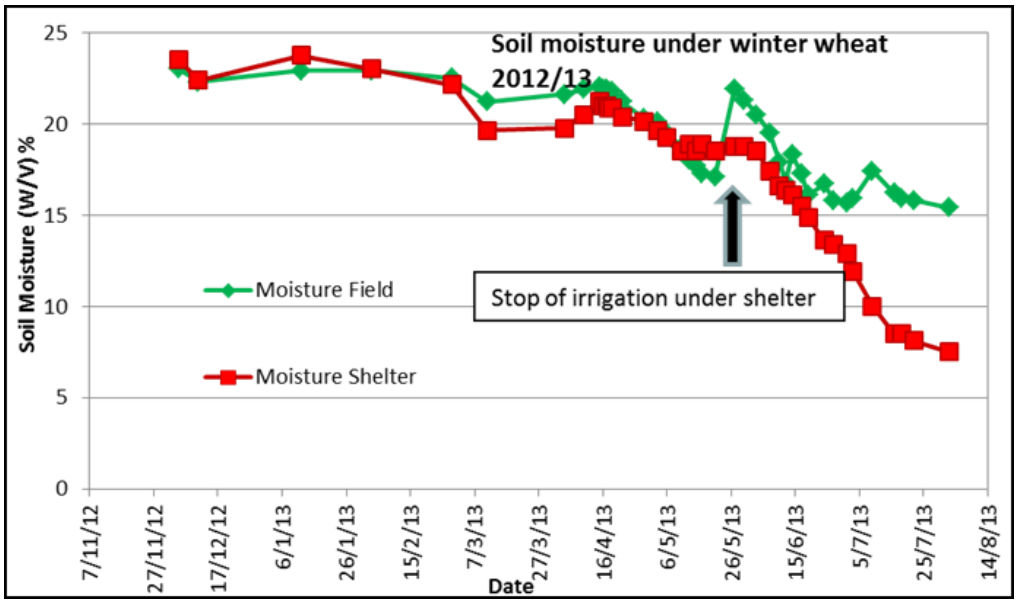


Figure 5: Soil moisture in the rain-out shelter and in the field in Groß-Lüsewitz

Table 4: Traits assessed in rain-out shelter experiments

	Abbr.	Trait	Unit
1	**HD**	Heading date	d after Jan 1
2	**FD**	Flowering date	d after Jan 1
3	**RD**	Ripening date	d after Jan 1
4	**PH**	Plant height	cm
5	**YLD**	Grain yield	g/plot
6	**TGW**	Thousand grain weight	g
7	**OBIO**	Biomass above ground	g/plot
8	**nE**	number of Ears/plot at harvest	
9	**nG**	number of Grains/plot at harvest	
10	**HI**	Harvest index	%
11	**ProC**	Accumulation of free proline	µmol/g DM
12	**TSS**	Total content of soluable sugars	µmol/g DM
13	**SPAD**	Chlorophyll content	rel. Units
14	**Y(II)**	Chlorophyll fluorescence	rel. Units
15	**PC**	Protein content	% FM
16	**STC**	Starch content	% FM

- At the JKI in Braunschweig disease resistance tests were conducted by Dr. B. Rodemann for a number of fungal pathogens:

- Resistance to *Fusarium* head blight (FHB) in the VALID panel in 2013 at Ahlum and at Hunzen and in 2014 at Ahlum and Heyen.
- Resistance to tan spot caused by *Pyrenophora tritici-repentis* was tested in the VALID panel in 2013 and 2014 in Ahlum and Lafferde in each year.
- Resistance to *Septoria tritici* blotch caused by *Zymoseptoria tritici* was tested in the VALID panel in 2013 in Ahlum. The additional field tests in 2013 and 2014 were lost due to environmental obstacles, such as flooding of the fields.
- Resistance to eyespot caused by *Oculimacula yallundae* (syn. *Pseudocercosporella herpotrichoides*) was tested in the TROST panel in the green house at location Hunzen in 2013 and at the two locations Ahlum and Heyen in 2014.
- Resistance to take-all caused by *Gaeumannomyces graminis* was tested in the TROST panel in the green house at location Braunschweig in 2013.

D. Data analysis and validation

Marker-trait associations (MTAs) were calculated by using a mixed linear model with correction for relatedness by a Loiselle kinship matrix and the software packages Genstat v.16 (employed by the IPK) and TASSEL v3.0 (employed by TraitGenetics). All phenotypic traits assessed in the GABI-WHEAT and VALID panel were calculated with the SSR-markers, the 90k iSELECT markers with a mapping position and the 35k Affymetrix markers with a mapping position (Table 5).

Table 5: MTAs calculated in the GABI-WHEAT panel

Trait	SSR	35k_mapped	90k_mapped
Fusarium resistance	X	X	X
Septoria resistance	X	X	X
Tan spot resistance	X	X	X
SDS sedimentation index	X	X	X
Starch content	X	X	X
Specific weight	X	X	X
Yield	X	X	X

Plot yield	X	X	X
Hagberg falling number	X	X	X
Heading date	X	X	X
Ear weight	X	X	X
Grain hardiness	X	X	X
Grains per ear	X	X	X
Protein content	X	X	X
Plant height	X	X	X
Thousand grain weight	X	X	X
Zeleny sedimentation index	X	X	X

Detailed analyses concerning the following traits in the GABI-WHEAT panel have already been published: Resistance to *Fusarium* head blight (Kollers et al. 2013a), to *Septoria tritici* blotch (Kollers et al. 2013b) and to tan spot (Kollers et al. 2014); association mapping of heading date (Zanke et al. 2014a), plant height (Zanke et al. 2014b) and thousand grain weight (Zanke et al. 2015) and are thus not further discussed here.

As an example for a further trait, we show here the Manhattan plots for grain yield which is the most important trait in wheat (Figure 6).

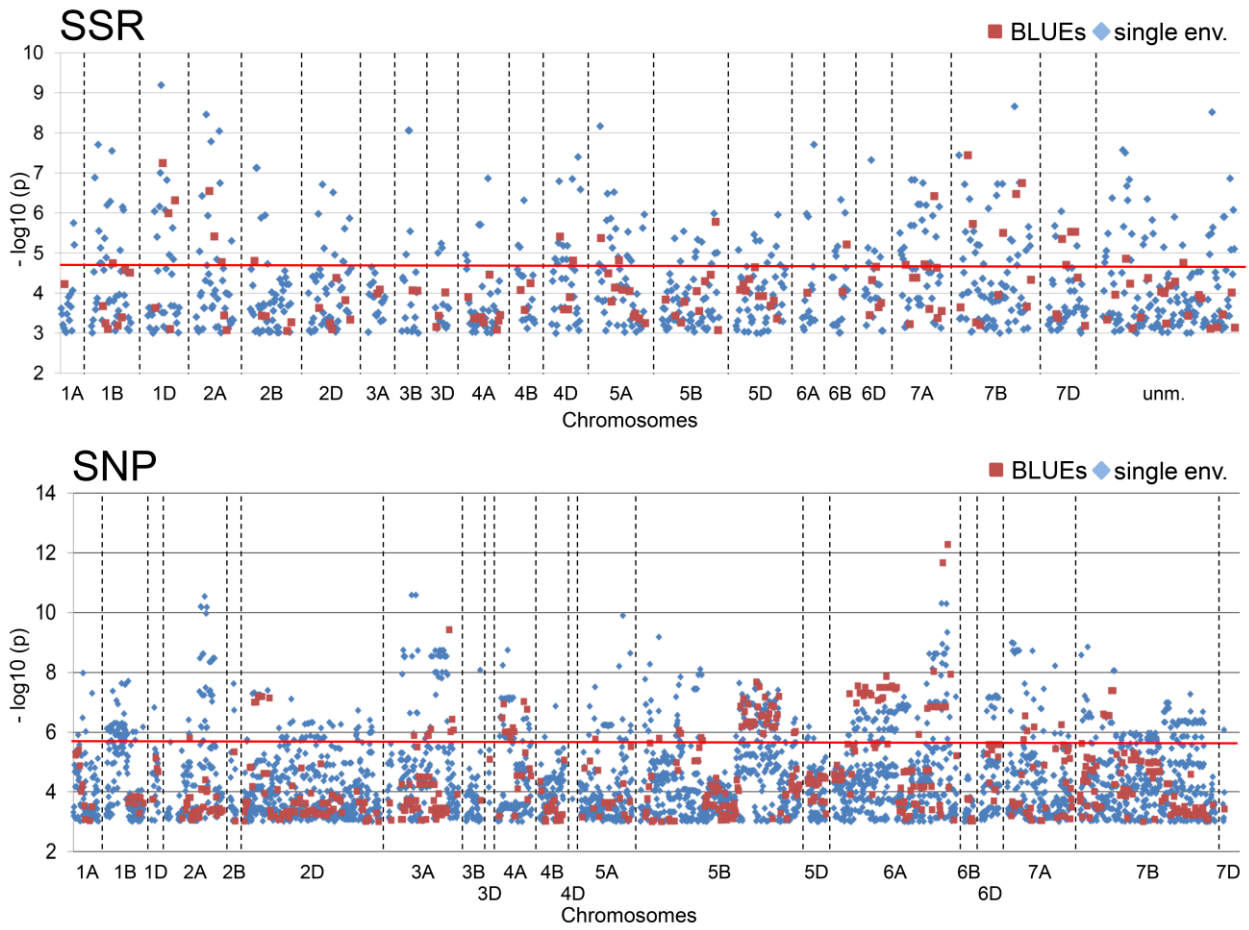


Figure 6: Manhattan plots for grain yield in the GABI-WHEAT panel with SSR markers and 90k iSELECT SNP markers. Blue dots represent single environments, red squares represent BLUEs across eight environments.

One of the main goals of the project was to determine, how many of the MTAs that were found in the GABI-WHEAT panel could be confirmed in a different but genetically related panel of wheat varieties. The VALID panel consisted of varieties that were released after the original GABI-WHEAT panel but more or less in the same geographic regions. For a comprehensive comparison, the VALID material was genotyped with the same SSRs and with the same 90k iSELECT and the 35k Affymetrix SNP-markers. Based on these data the following results were obtained:

- Population structure:

  For a true comparison of the identified MTAs between the two sets of material (GABI-WHEAT and VALID), it was important that the population structure of the two data sets is comparable. Based on a principal component analysis with the combined data sets it could be shown that the VALID material had a

similar population structure as the GABI-WHEAT material. This is reflected by the absence of separate clustering of the two panels (Figure 7).

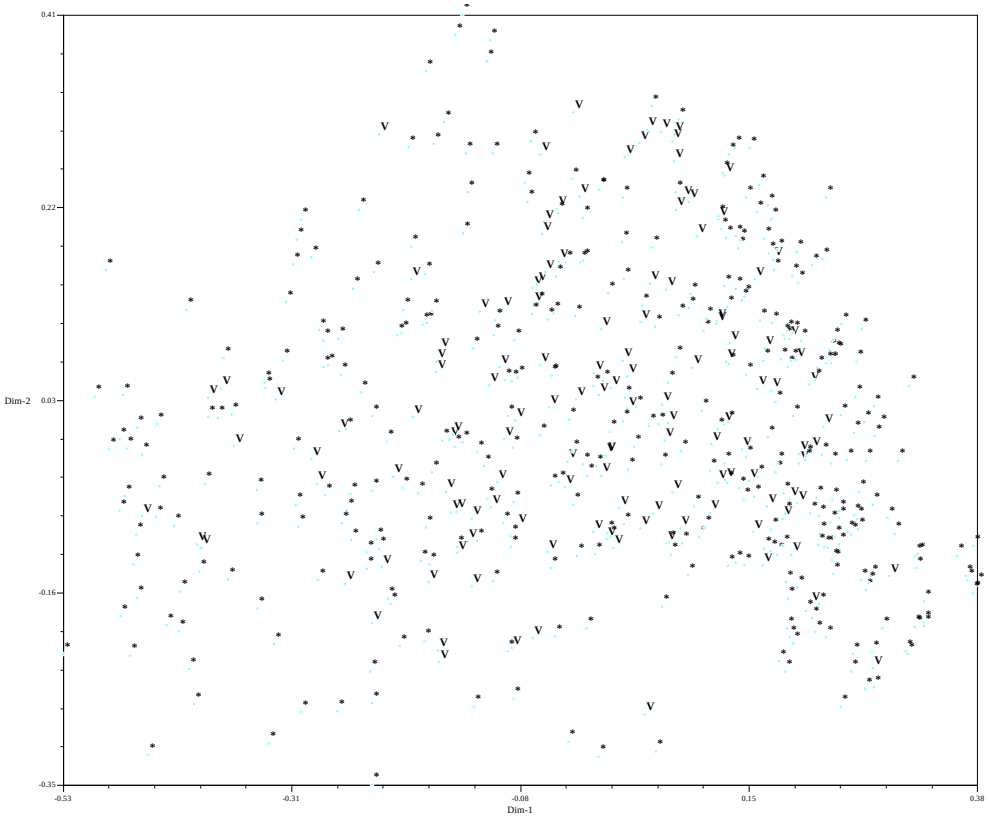


Figure 7: Principal component analysis of the GABI-WHEAT (*) and VALID (V) material. The data clearly demonstrate that the two sets of varieties do not differ significantly in terms of population structure.

- Main phenology genes:

  It is well-known that many agronomic traits in wheat are partly controlled by major phenology genes. One of these major phenology traits is photoperiod sensitivity which is controlled by the *Ppd* genes. It is known that *Ppd-D1* influences strongly the heading date. In the same way, it is known that plant height is controlled by the dwarfing genes *Rht* and especially in European wheat material the gene *Rht-D1*. A meaningful MTA analysis requires that in the GABI-WHEAT and VALID material, these major genes can be identified with high significance because if it would not be possible to identify these MTAs, then the results would not be comparable. Thus, we have analyzed the two data sets for these genes. As shown in Figure 8, in all data sets, the respective genes could be identified with a high LOD score (*ppd* for heading data and *rht* for plant height).

Heading Date in WHEAT

	A	B	C	D
1	Trait	Marker	p	
2	2009.AND	Ppd-D1	5.11E-23	
3	2010.SAU	Ppd-D1	2.69E-20	
4	2010.AND	Ppd-D1	4.06E-20	
5	2010.WOH	Ppd-D1	2.66E-19	
6	2010.JAN	Ppd-D1	3.57E-19	
7	2009.WOH	Ppd-D1	2.76E-18	
8	2010.SEL	Ppd-D1	1.03E-16	
9	2009.SEL	Ppd-D1	8.45E-16	
10	2009.WOH	Ppd-D1	1.49E-05	
11	2010.SEL	GluD1	1.80E-05	
12	2010.WOH	Vrn-D1-D1a-	2.06E-05	
13	2009.AND	Vrn-D1-D1a-	2.09E-05	
14	2010.WOH	Ppd-D1	2.75E-05	
15	2010.AND	Vrn-D1-D1a-	3.19E-05	

Plant Height in WHEAT

	A	B	C	D
1	Trait	Marker	p	
2	2010SEL	RhtD1	2.24E-22	
3	2010AND	RhtD1	3.20E-19	
4	2010JAN	RhtD1	3.36E-19	
5	2009AND	RhtD1	5.87E-17	
6	2009SEL	RhtD1	5.14E-16	
7	2010WOH	RhtD1	3.29E-15	
8	2009WOH	RhtD1	5.15E-15	
9	2010SAU	RhtD1	2.95E-14	
10	2010SEL	VPM	1.57E-06	
11	2010SEL	Ppd-D1	3.79E-06	
12	2010AND	VPM	2.35E-05	
13	2010JAN	VPM	3.81E-05	

Heading Date in TROST (13)

	A	B	C	D
1	Trait	Marker	p	
2	HD-Bir	Ppd-D1	1.78E-11	
3	HD-Bni	Ppd-D1	1.82E-11	
4	HD-Sir	Ppd-D1	2.83E-08	
5	HD-Sni	Ppd-D1	1.32E-07	
6	HD-Bni	Ppd-D1	1.31E-05	
7	HD-Bir	Ppd-D1	1.79E-05	
8	HD-Sni	Ppd-D1	6.77E-05	
9	HD-Sir	Ppd-D1	2.68E-04	

Plant Height in TROST (13)

	A	B	C	D
1	Trait	Marker	p	
2	PH-Bni	RhtD1	2.21E-09	
3	PH-Sni	RhtD1	1.60E-08	
4	PH-Bir	RhtD1	1.14E-07	
5	PH-Sir	RhtD1	9.00E-07	
6	PH-Bni	Ppd-D1	5.61E-04	
7	PH-Sir	Ppd-D1	7.39E-04	
8	PH-Bir	3BS-8	8.40E-04	

Heading Date in VALID (13/14)

	F	G	H	I
1	Trait	Marker(KASP2-Set)	p	
2	AND2014	TaPpdDD001	1.9531E-14	
3	SAU2014	TaPpdDD001	1.1802E-12	
4	WOH2014	TaPpdDD001	6.0745E-12	
5	2013SAU	TaPpdDD001	4.1336E-10	
6	BER2014	TaPpdDD001	4.0228E-08	
7	2013WOH	TaPpdDD001	1.3303E-06	
8	SAU2014	TaPpdDI001	7.1407E-05	
9	2013WOH	TaPpdDI001	7.77E-05	
10	AND2014	TaPpdDI001	0.00010276	
11	2013SAU	TaPpdDI001	0.00018928	
12	WOH2014	TaPpdDI001	0.00023816	
13	2013WOH	RhtD1	0.00056487	
14	WOH2014	RhtD1	0.00324567	
15	AND2014	RhtD1	0.00358771	

Plant Height in VALID (13/14)

	A	B	C	D
1	Trait	Marker(KASP-Set)	p	
2	2014BER	RhtD1-SNP	2.55E-06	
3	2014AND	RhtD1-SNP	1.30E-04	
4	2014WOH	RhtD1-SNP	0.04921945	
5	2013BER	RhtD1-SNP	0.08698384	
6	2013WOH	RhtD1-SNP	0.14037015	
7	2013AND	RhtD1-SNP	0.95907951	

Figure 8: Probability values for the major phenology genes (*Ppd-D1* and *Rht-D1*) in the different data sets

- Comparison of identified MTAs between the GABI-WHEAT and VALID data sets

  The VALID field data were analyzed with the 90k iSELECT and the 35k Affymetrix SNP-markers for the traits FHB resistance, yield, plant height and heading data in comparison to the GABI-WHEAT data (Table 6). Overall a lower level of significant MTAs was observed in the VALID panel compared to GABI-WHEAT. This is most likely caused by lower variety numbers: 137 varieties in VALID versus 372 varieties in GABI-WHEAT which significantly influences the p-values.

Table 6: Comparison of significant MTAs in the GABI-WHEAT and VALID panel

Trait	Markers	log_{10}(P)>3.0 Wheat (W)	log_{10}(P)>3.0 VALID (V)	log_{10}(P)>2.0 VALID (V)	common1 W log_{10}(P)>3 V log_{10}(P)>3	common2 W log_{10}(P)>3 V log_{10}(P)>2
Yield	90k	347	247	436	0	6
Yield	35k	175	64	155	1	3
FHB	90k	520	9	113	1	10
FHB	35k	332	3	47	0	3
Plant height	90k	1417	9	45	1	11
Plant height	35k	863	7	41	3	15
Heading	90k	262	11	135	5	15
Heading	35k	122	7	76	4	7

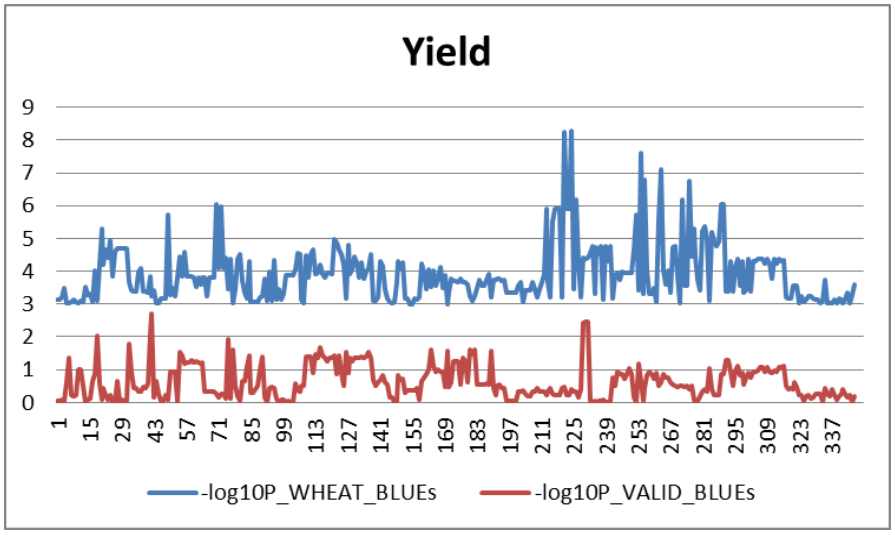


Figure 9: Comparison of log_{10}(P) curves for MTAs for yield with the 90k SNP-markers in the GABI-WHEAT and VALID panels

- Summary

  Comparing the results from the validation of MTAs identified in the GABI-WHEAT data with the VALID data, on average up to 10% of the MTAs were found in both data sets. Factors influencing validation rates could be:

  - Data quality:

Sample mix-up, either in field trials or for genotyping could compromise the data quality, especially since the expected differences for each locus/MTA are typically less than 5% of the total effect. This is however unlikely here since we have repeatedly genotyped the entire field material with a set of SNP markers and corrected the data for all observed problems and eliminated unclear samples.

- Different environments over the various years:

Phenotypes are not only influenced by the genetic make-up of the genotypes, but also by genotype x environment interactions. Those will differ from year to year due to environmental conditions. However, within locations and years of the GABI-WHEAT (Figure 10) and the VALID trials, respectively, there was a relatively good correlation.

- Heritability:

It is known that the heritability of various traits differs. Some traits are of high heritability (plant height, heading date) while others are of lower heritability (e.g. yield). This could lead to a low confirmation rate especially if the genetic component of the trait is small. When looking at the confirmation rate (Table 6), it is however clear that the percentage of confirmed MTAs is dependent on the level of heritability (highest confirmation of MTAs for heading date and plant height).

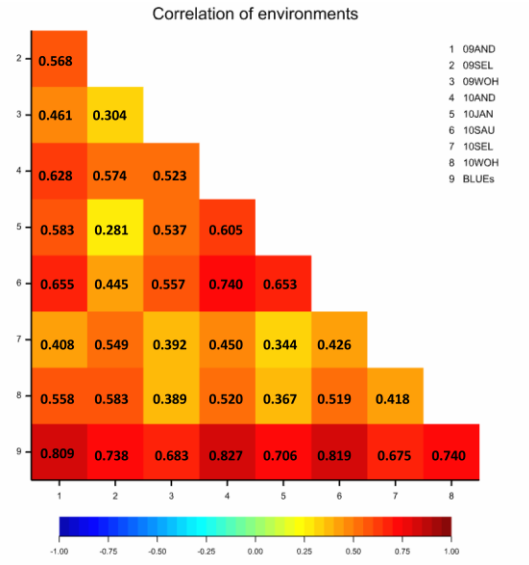


Figure 10: Correlation of environments for the GABI-WHEAT locations for the trait yield. AND=Andelu/F, JAN=Janville/F, SAU= Saultain/F, SEL=Seligenstadt/D, WOH=Wohlde/D, 09= field test in 2009, 10 = fieldtest in 2010, BLUEs = best linear unbiased estimations.

- Significant allele frequency differences between the data sets:

Since in the association analysis an allele frequency threshold of 0.03 (3%) was applied, there was the possibility that certain markers/genomic regions might have been fallen out of the analysis in one of the two data sets and thus could not be confirmed. In order to exclude that, we have performed a genome scan for allele frequencies in the GABI-WHEAT and VALID genotyping data (Figure 11). A genome scan for marker allele frequencies shows that the allele frequencies in the two data sets are almost identical so that this can also be excluded.

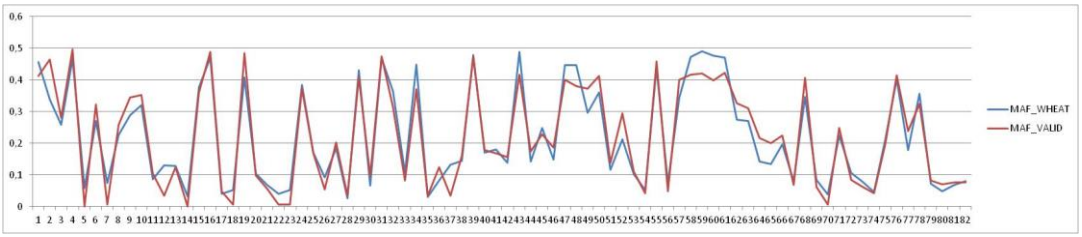


Figure 11: SNP allele frequency scan for the GABI-WHEAT and the VALID material (minimal allele frequency = MAF) for wheat chromosome 1B

- Population structure and statistical approaches:

If the two variety sets (GABI-WHEAT and VALID) would have a different population structure, then the MTAs would probably be not fully comparable. In order to exclude that, we have analyzed the data with two sets of data regarding population structure. In one case, we used 1,000 SNP markers for population stratification and in the other case we used 144 SSR markers for determining the population structure. Since with both approaches, we obtained almost identical data, this explanation can also be excluded.

Another possibility could be that the employed analysis tools have a problem with the two data sets. This was excluded through the analysis at the IPK with the software suite GENSTAT and at TraitGenetics with the software suite TASSEL. In both cases similar data were obtained.

- VALID data set is too small:

The GABI-WHEAT and VALID sets differ significantly in the number of samples (372 versus 137 varieties). This almost three-fold difference has significant influences on the level of significance of the identified MTAs in each data set. As described above VALID MTAs have always a lower significance. In order to determine this effect in more detail, we have subdivided the GABI-WHEAT data set into three sub-data sets and compared the MTAs identified in these sub-data sets with each other. These results clearly indicated that a

comparison of the sub-data sets for a number of traits results also in a quite low confirmation rate between the individual sub-data sets. Thus, it is quite clear that the VALID data set makes it difficult to confirm MTAs identified in the GABI-WHEAT data set due to its small sample size. So it can be expected that the confirmed MTAs between the GABI-WHEAT and the VALID data set are probably truly confirmed MTAs but that many other MTAs have escaped confirmation. As a lesson from that, it is clear now that in the future, the original MTA detection data set should contain approximately the same number of lines/varieties as the validation data set in order to make meaningful statements.

The analyses also confirm observations from association studies performed in animals and humans, where only a relatively small set of MTAs can be confirmed in different data sets (Stranger et al. 2011).

E. Newly analyzed traits

Drought stress in the field

A subset of 184 lines from the GABI-WHEAT panel (= TROST panel) was grown in additional field locations in the seasons 2012/2013 and 2013/2014 at the sites Seligenstadt (SEL) and Biendorf (Bal) in Germany. At each site an irrigated and a non-irrigated field trial was grown. Unfortunately, the field trials for the TROST panel that should be analyzed for drought stress response (comparison between irrigated and non-irrigated conditions) were hampered by the unfavourable weather conditions. Both years were too wet and resulted at maximum in only minimal differences between the two conditions. While in Seligenstadt a small positive effect of irrigation was recorded in both years, in Biendorf the effect was negative or could not be evaluated (Table 7). Because of that, the drought stress experiments in the field could not be fully analyzed.

Table 7: Effects of irrigation in the TROST-panel

		irrigated dt/ha	non irrigated dt/ha	Difference dt/ha	Difference %
2013	**Seligenstadt**	89,1	85,9	+ 3,2	+ 3,7
	Baalberge	73,7	79,7	- 6,0	- 7,5
2014	**Seligenstadt**	120,2	108,6	+ 11,5	+ 10,6
	Baalberge		109,7		

Means over all genotypes

In order to make use of the data from the TROST panel, the irrigated field trials were used as further validation panel for the MTA data that we had observed from the GABI-WHEAT data set. Specifically, we compared the TROST data set to the BLUES of eight environments from the years 2008/2009 and 2009/2010 from the GABI-WHEAT set (Table 8). The overall significance level was much higher for the original data of the complete set of 372 varieties (Figure 12). When the same 174 varieties were selected from both data sets, the significance levels between both sets were comparable (Figure 13), however again only a few matching significant MTAs were detected (Table 8). Thus, these data confirm the results from section D.

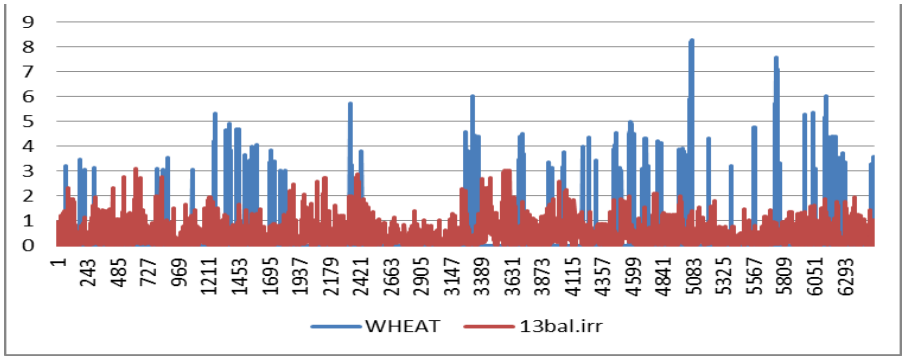


Figure 12: Whole genome association scans of MTAs for yield comparing the original GABI-WHEAT dataset of 372 varieties with the 174 varieties of the TROST set (location 13bal.irr).

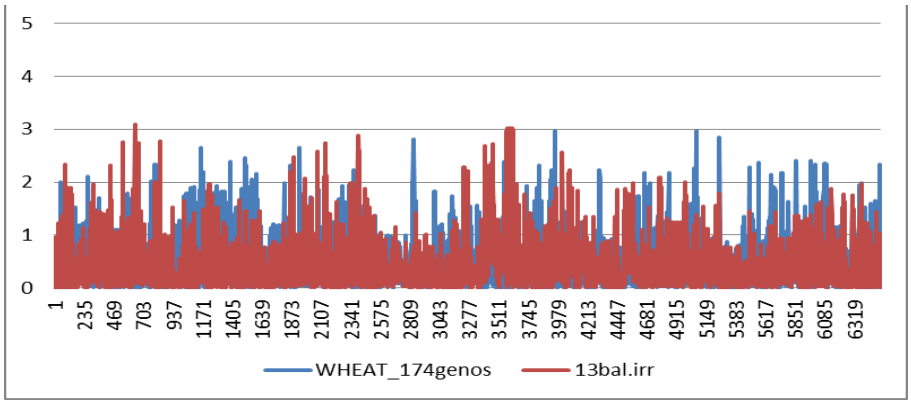
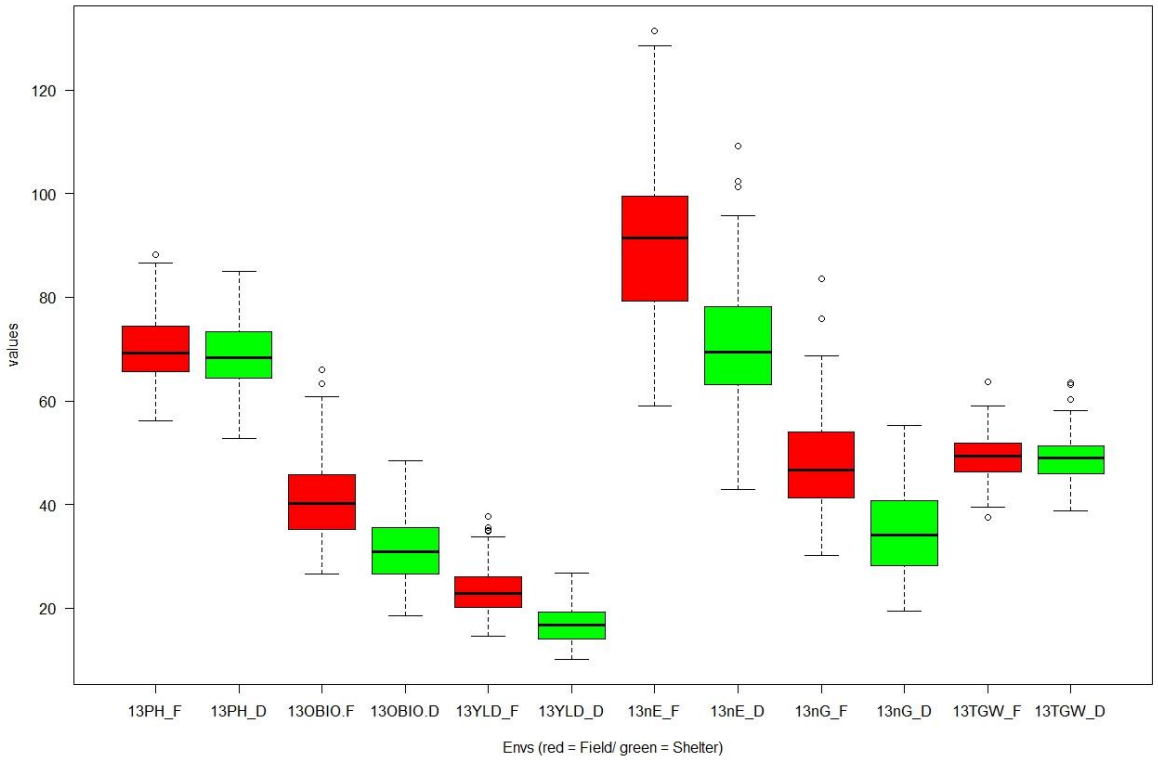


Figure 13: Whole genome association scans of MTAs for yield for a subset of the GABI-WHEAT dataset (174 varieties) to the same 174 varieties evaluated in the TROST set (location 13bal.irr)

Table 8: Comparison between TROST data set and original GABI-WHEAT data set for number of significant MTAs for character yield

Environment	LOD > 2.0	LOD > 2.0 + GABI-WHEAT BLUEs (372 varieties) LOD > 3.0	LOD > 2.0 + GABI-WHEAT BLUEs (174 varieties) LOD > 2.0
13Bal.irr	64	1	6
13Bal.non	76	1	0
13Sel.irr	93	3	1
13Sel.non	73	4	6



LOD = $\log_{10}(P)$

Figure 14: Boxplots for six traits under field (F) and drought (D) conditions in 2013.

PH = Plant height, OBIO = Biomass above ground, YLD = grain yield, nE = Number of ears/plot at harvest, nG = number of grains/plot at harvest, TGW = thousand grain weight.

Analysis of rain-out shelter data

The rain-out shelter experiments performed in Groß-Lüsewitz produced very good results in the season 2012/2013 (see also previous Figure 5). Several traits were strongly affected by drought treatment, such as yield, biomass above ground, ears/plot and grains/plot at harvest (Figure 14).

Numerous QTL were identified for all traits in field and in drought conditions (Table 9). A marker on chromosome 7A was highly significant for yield, biomass and number of grains/plot under drought conditions (Figure 15). Unfortunately, the season 2013/14 could not be fully used for validation since there was severe frost damage in the experiment and several lines were lost.

Table 9: Number of QTL regions (MTAs) for all the traits under field and drought conditions at the threshold of $-\log_{10}(P) >= 3.0$ based on the 90k SNP-data

	No. of QTL regions (MTAs)		
Trait	**Field condition**	**Drought condition**	**Common**
Heading date	5 (15)	4 (17)	1 (5)
Flowering date	3 (3)	4 (6)	-
Ripening date	3 (8)	5 (13)	-
Plant height	4 (14)	3 (12)	2 (8)
Grain yield	6 (18)	1 (1)	-
Thousand grain weight	4 (9)	5 (34)	2 (2)
Biomass above ground	5 (17)	2 (5)	-
Number of Ears/plot at harvest	0	4 (16)	-
Number of Grains/plot at harvest	5 (9)	2 (3)	-
Harvest index	11 (31)	2 (3)	-
Accumulation of free proline	2 (2)	4 (7)	-
Total content of solubale sugars	3 (4)	5 (8)	-
Chlorophyll content	4 (10)	2 (2)	-
Chlorophyll fluorescence	1 (1)	5 (20)	-
Protein content	2 (4)	7 (9)	1 (1)
Starch content	8 (10)	8 (23)	2 (4)
In total	**66 (155)**	**63 (184)**	**8 (20)**

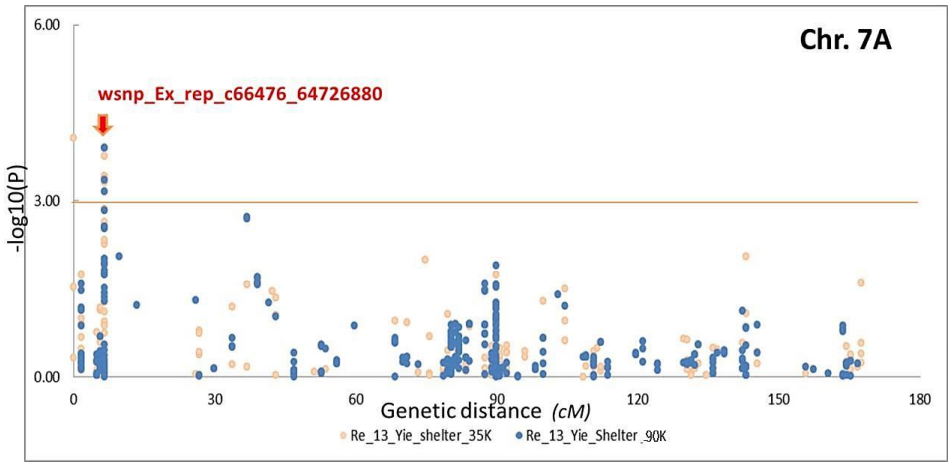


Figure 15: A highly significant MTA on chromosome 7A for yield under drought conditions based on the 90k SNP-data (in blue) and the 35k SNP-data (in pink)

Analysis of resistance to additional fungal pathogens

The data for resistances to fungal pathogens of the JKI Braunschweig are very complex due to the multiple pathogens tested and final analysis is still ongoing. As an already fully analyzed example, we present the results for resistance to eyespot caused by *Oculimacula yallundae* (syn. *Pseudocercosporella herpotrichoides*) in the TROST panel. The genome-wide association analysis revealed significant MTAs mainly on chromosome 7D, which are caused by the known resistance gene *Pch1* which is located on a translocation segment introgressed from the wild species *Aegilops ventricosa* (Figure 16).

A detailed analysis of all markers on chromosome 7D revealed the presence of recombinations within the introgressed fragment of the wild species *Aegilops ventricosa* in some varieties, which eventually break the linkage between the resistance gene *Pch1* and some undesirable traits such as lower yield (Figure 17). Furthermore, a more closely linked marker than the known marker *Ep-D1* (http://maswheat.ucdavis.edu/) which is based on an endopeptidase gene was identified. This marker is of high value for breeding, since it can now be used in material where the linkage between the *Ep-D1* marker and the *Pch1* has been broken.

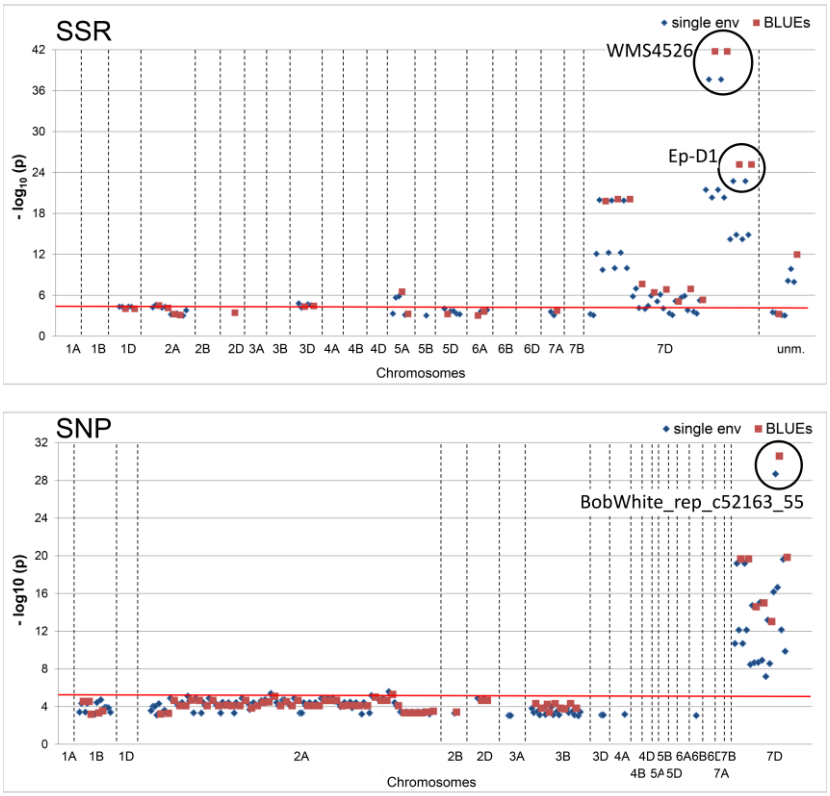


Figure 16: Manhattan plots for resistance to eyespot in the TROST panel. Blue dots represent single environments, red squares represent BLUEs.

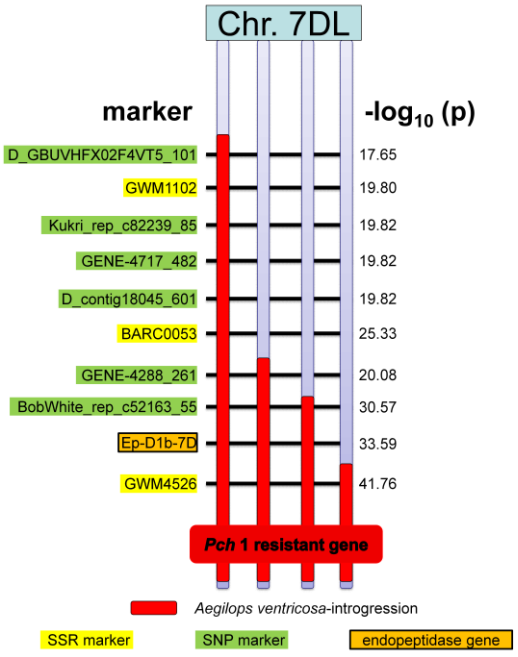


Figure 17: Recombination events in the introgressed segment of *Aegilops ventricosa* on the long arm of chromosome 7D

F. Development of DH- and backcross populations for the validation of MTAs

A number of strong MTAs identified in the GABI-WHEAT project were selected for validation in specifically developed bi-parental populations. For this purpose, the two breeding companies KWS LOCHOW and Syngenta developed three doubled-haploid populations segregating for specific genomic regions, where MTAs for yield had been identified (Table 10). Furthermore, a number of backcross populations were developed by all partners together for 11 MTAs, whereby specific genomic regions associated with either yield, resistance to Fusarium or Septoria were introgressed into another background variety (Table 11). The goal of this procedure was to test these lines for an effect caused by the specifically introgressed regions in order to verify the MTAs obtained in the association panel of varieties. Furthermore, this could directly lead to an improvement of already existing varieties for the respective traits. All backcross-generations were monitored with molecular markers for the specific MTA (foreground selection) and with an Infinium array for the genetic background (background selection). Currently the populations are in stage BC_2 with more than 90% recurrent parent genome.

Table 10: Developed DH-populations

Parents	No. of DH-lines	Trait	Source
Tabasco × Musketeer	160	Yield	Syngenta
Cubus × Lona	141	Yield	KWS LOCHOW
Expert × Mikon	98	Yield	KWS LOCHOW

Table 11: Developed Backcross-lines (BC_2)

Trait	Donor parent	No. of Loci	Source
Fusarium	Ephoros	1	IPK/TG
Fusarium	Mikon	1	IPK/TG
Fusarium	Altigo	1	KWS LOCHOW
Fusarium	Welford	1	KWS LOCHOW
Fusarium	Batis	1	Syngenta

Septoria	Pamier	2	KWS LOCHOW
Septoria	Pamier	2	KWS LOCHOW
Yield	Sogood	2	IPK/TG
Yield	Sogood	2	IPK/TG
Yield	Frument	2	IPK/TG
Yield	Hereford	2	IPK/TG

As already mentioned, the material development in winter wheat is time consuming with not more than 2 generations per year in the greenhouse and the further need of multiplication of the material in the field before testing. Thus, the respective material could not be tested within the actual VALID project. At present, the propagated populations are in the field for the phenotypic evaluation and this project part will be continued by the partners. Preliminary data show that for the DH lines and some introgression lines for Fusarium and Septoria, there appear to be effects after a first round of preliminary disease tests.

G. General conclusions

The results generated and the data analysis performed in the projects GABI-WHEAT and VALID provide a solid fundament for the genetic analysis of main traits in European winter wheat, including yield and yield-related parameters, quality parameters, tolerance to several fungal diseases and preliminary data on drought stress tolerance.

Up-to-date genome-wide genotyping technologies including the application of a 90k iSELECT ILLUMINA SNP-chip and a 35k Affymetrix SNP-chip were combined with solid and repeated phenotyping in the field.

The data analysis already resulted so far in the publication of seven papers in international peer-reviewed journals and several oral presentations at international conferences. Overall the outcome of the two projects GABI-WHEAT and VALID can be regarded as pioneering in the analysis of the genetic architecture of European winter wheat and place the VALID-consortium at the forefront of genetic analysis of winter wheat in Europe and worldwide.

H. Cited Literature

Agrama HA, Eizenga GC and Yan W (2007). Association mapping of yield and its components in rice cultivars. Mol Breeding 19: 341-356.

Atwell S, Huang YS, Vilhjalmsson BJ, Willems G, Horton M, Li Y, Meng DZ, Platt A, Tarone AM, Hu TT, Jiang R, Muliyati NW, Zhang X, Amer MA, Baxter I, Brachi B, Chory J, Dean C, Debieu M, de Meaux J, Ecker JR, Faure N, Kniskern JM, Jones JDG, Michael T, Nemri A, Roux F, Salt DE, Tang CL, Todesco M, Traw MB, Weigel D, Marjoram P, Borevitz JO, Bergelson J and Nordborg M (2010). Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465: 627-631.

Belo A, Zheng PZ, Luck S, Shen B, Meyer DJ, Li BL, Tingey S and Rafalski A (2008). Whole genome scan detects an allelic variant of fad2 associated with increased oleic acid levels in maize. Mol Genet Genomics 279: 1-10.

Breseghello F and Sorrells ME (2006). Association mapping of kernel size and milling quality in wheat (Triticum aestivum L.) cultivars. Genetics 172: 1165-1177.

Buckler ES, Thornsberry JM (2002) Plant molecular diversity and applications to genomics. Curr Op Plant Biol 5: 1007-111.

Chao SM, Zhang WJ, Dubcovsky J and Sorrells M (2007). Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (Triticum aestivum L.) germplasm representing different market classes. Crop Sci 47: 1018-1030

Cockram J, White J, Leigh FJ, Lea VJ, Chiapparino E, Laurie DA, Mackay IJ, Powell W and O'Sullivan DM (2008). Association mapping of partitioning loci in barley. Bmc Genet 9:16.

Crossa J, Burgueno J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH and Ortiz R (2007). Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177: 1889-1913.

Emebiri LC, Oliver JR, Mrva K and Mares D (2010). Association mapping of late maturity alpha-amylase (LMA) activity in a collection of synthetic hexaploid wheat. Mol Breeding 26: 39-49.

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan JB and Buckler ES (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 319: 330-333.

Haseneyer G, Stracke S, Piepho HP, Sauer S, Geiger HH and Graner A (2010). DNA polymorphisms and haplotype patterns of transcription factors involved in barley endosperm development are associated with key agronomic traits. BMC Plant Biol 10:5.

Horvath A, Didier A, Koenig J, Exbrayat F, Charmet G, Balfourier F (2009) Analysis of diversity and linkage disequilibrium along chromosome 3B of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 119: 1523-1537.

Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P and Van Eeuwijk FA (2004). Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168: 435-446.

Maccaferri M, Sanguineti MC, Noli E and Tuberosa R (2005). Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Mol Breeding 15: 271-289.

Mackay I and Powell W (2007). Methods for linkage disequilibrium mapping in crops. Trends Plant Sci 12: 57-63.

Matthies IE, Weise S, Röder MS (2009) Association of haplotype diversity in the α-amylase gene *amy1* with malting quality parameters in barley. Mol Breed 23: 139-152.

Mayor PJ and Bernardo R (2009). Genomewide Selection and Marker-Assisted Recurrent Selection in Doubled Haploid versus F-2 Populations. Crop Sci 49: 1719-1725.

Neumann K, Kobiljski B, Dencic S, Varshney RK, Börner A (2010) Genome-wide association mapping: a case study in bread wheat (Triticum aestivum L.). Mol Breed (online)

Peng JH, Bai Y, Haley SD and Lapitan NLV (2009). Microsatellite-based molecular diversity of bread wheat germplasm and association mapping of wheat resistance to the Russian wheat aphid. Genetica 135: 95-122.

Rafalski JA (2010). Association genetics in crop improvement. Curr Opin Plant Biol 13: 174-180.

Somers DJ, Banks T, DePauw R, Fox S, Clarke J, Pozniak C and McCartney C (2007). Genome-wide linkage disequilibrium analysis in bread wheat and durum wheat. Genome 50: 557-567. doi Doi 10.1139/G07-031

Stich B, Mohring J, Piepho HP, Heckenberger M, Buckler ES and Melchinger AE (2008). Comparison of mixed-model approaches for association mapping. Genetics 178: 1745-1754.

Stranger BE, Stahl EA and Raj T (2011) Progress and promise of genome-wide association studies for human complex traits. Genetics 187: 367-383

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D and Buckler ES (2001). Dwarf8 polymorphisms associate with variation in flowering time. Nat Genet 28: 286-289

Tommasini L, Schnurbusch T, Fossati D, Mascher F and Keller B (2007). Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. Theor Appl Genet 115: 697-708.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B.E., et al. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90,000 SNP array. Plant Biotechnology Journal 12: 787-796.

Waugh R, Jannink JL, Muehlbauer GJ and Ramsay L (2009). The emergence of whole genome association scans in barley. Curr Opin Plant Biol 12: 218-222.

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S and Buckler ES (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38: 203-208.

Zhao KY, Aranzana MJ, Kim S, Lister C, Shindo C, Tang CL, Toomajian C, Zheng HG, Dean C, Marjoram P and Nordborg M (2007). An Arabidopsis example of association mapping in structured samples. Plos Genet 3.

2. Der wichtigsten Positionen des zahlenmäßigen Nachweises

Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK)

Position	**€**
Personal	225.518,71
Verbrauchsmaterial	72.535,23
Reisekosten	3.165,36
Overhead	22.551,87
Unteraufträge	304.850,00
Sonstige Vorhabenkosten	0,00
Gesamt	628.621,17

3. Der Notwendigkeit und Angemessenheit der geleisteten Arbeit

Bei IPK wurden folgende Arbeiten durchgeführt:

- **Genotypisierung des Materials mit SSRs und Kandidatengenen**
  - Die 146 VALID-Sorten wurden mit 136 Mikrosatellitenmarkern (SSR) genotypisiert, da das WHEAT Material auch mit diesen SSRs genotypisiert wurde. Zusätzliche wurden Kandidatengene (vgl. Tabelle 2) auf WHEAT und VALID-Sorten genotypisiert.

- **Entwicklung von Rückkreuzungspopulationen mit assoziierter Markeranalyse**
  - Die Entwicklung von insgesamt sechs Rückkreuzungspopulationen (vgl. Tabelle 11) erfolgte zusammen mit TraitGenetics. Das IPK hat dabei vor allem die Kreuzungsarbeiten und Anzucht und Vermehrung des Pflanzenmateriales übernommen. Daneben wurden ‚Forground'-Analysen mit ausgewählten SSR-Markern durchgeführt um die ‚Loci of interest' im Material zu fixieren. Die ausgewählten BC_2 Linien konnten damit innerhalb des Projektes noch geselbstet werden, so dass homozygote BC_2S_2-Linien zum Projektende vorlagen, welche einen stärkeren Effekt im Feld zeigen sollten als noch aufspaltende BC_3S_1-Linien. Die entwickelten BC_2S_2-Linien sind für eine nachfolgende Feldanalyse deutlich besser geeignet.

- **Phänotypisierungen in Zusammenarbeit mit JKI**

  Die Phänotypisierungen wurden vom JKI in zwei Unteraufträgen für das IPK durchgeführt:

  - Das JKI in Braunschweig führte unter Leitung von Herrn Dr. Bernd Rodemann mehrjährige Resistenztestungen in Feld und Gewächshaus gegen verschiedene pilzliche Pathogene durch (vgl. Abschnitt II.1.C und E.3).
  - Das JKI in Quedlinburg mit Außenstelle in Großlüsewitz führte zweijährige Testungen von Sorten in ‚rain-out-shelters' zur Evaluierung verschiedener Komponenten für Trockenstress-Toleranz durch (vgl. Abschnitt II.1.C und E.2).

- **Datenauswertung**
  - Neben TraitGenetics hat das IPK eine Hauptrolle bei der Datenauswertung gespielt. Die von den anderen Partnern erhaltenen phänotypischen Daten wurden parallel mit TraitGenetics untersucht. Hierzu wurden in einem ersten Schritt die einzelnen Standorte untereinander verglichen und anschließend der gesamte Datensatz für die MTA-Analyse verwendet. Diese Untersuchungen wurden sowohl für die Kandidatengene als auch alle Markerdaten und die gesammelten phänotypischen Daten durchgeführt. Während das IPK diese Untersuchungen mit dem Softwarepaket GenStat durchgeführt hat, hat TraitGenetics die Untersuchungen mit dem Programm TASSEL durchgeführt. Damit konnte geprüft werden, ob die beiden unterschiedlichen Programmpakete ähnliche oder identische Ergebnisse liefern. Sie lieferten ähnliche Ergebnisse aber zeigten bei der Signifikanz und den berechneten Effekten Unterschiede. Im Anschluss wurden die MTAs für die einzelnen Merkmale bestimmt. Die dargestellten populationsgenetischen Untersuchungen waren eine Voraussetzung für die nachfolgenden MTA-Analysen, da beispielsweise die Populationsstruktur einen signifikanten Effekt bei der Analyse hat. Die parallelen Analysen am IPK und bei TraitGenetics waren notwendig um einzelne Fehlerquellen bei der Untersuchung der MTAs auszuschließen und um die Vielzahl der Daten in der Projektlaufzeit auszuwerten.

- **Erstellen von Publikationen, Postern und Präsentationen**
  - Das IPK war für das Schreiben und die Veröffentlichung aller in diesem Projekt entstandenen Publikationen in wissenschaftlichen Zeitschriften verantwortlich. Daneben wurden mehrere Poster und mündliche

Präsentationen auf verschiedenen internationalen Konferenzen, sowie den Statusseminaren in Potsdam präsentiert.

4. Des voraussichtlichen Nutzens, insbesondere der Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Die etablierten Methoden zur MTA-Analyse stellen einen wichtigen Pfeiler für das inzwischen eingereichte Nachfolgeprojekt zu VALID beim BMEL dar und die Publikationen aus dem VALID-Projekt zeigen unsere Kompetenz im Bereich der Datenauswertung von großen Datenmengen.

Die entwickelten Backcross-Populationen sollen noch phänotypisch evaluiert werden und diese Ergebnisse wissenschaftlich ausgewertet werden. Langfristig können Backcrosslinien zur Genklonierung von relevanten QTL-Loci genutzt werden.

Insgesamt haben die bereits veröffentlichten Publikationen, sowie weitere geplante Publikationen zu einer dominierenden Stellung und internationalen Sichtbarkeit des IPK im Bereich von Assoziationsanalysen bei europäischem Winterweizen geführt.

5. Des während der Durchführung des Vorhabens dem ZE bekannt gewordenen Fortschritts auf dem Gebiet des Vorhabens bei anderen Stellen

Während des Vorhabens sind folgende Fortschritte in den dargestellten Bereichen bei Weizen bekannt geworden, welche die Arbeitspakete von IPK betreffen:

- **Genotypisierung:**
  Während der Durchführung des Projektes sind drei SNP-Arrays publiziert worden. Bei dem ersten Array handelt es sich um einen 9K Illumina Infinium Array (Cavanagh et al. 2013). Der zweite Array zur Weizengenotypisierung (90K Array) wurde von einem internationalen Konsortium entwickelt (Wang et al. 2014) und auch intensiv in dem Projekt genutzt. Nach dem Ende des Projektes wurde ein 820K Affymetrix Array publiziert (Winfield et al. 2015). Dieser 820K Array ist auch die Basis für den 35K Affymetrix Array, welcher im Rahmen des VALID-Projektes genutzt wurde und der 35K Array enthält SNPs, welche spezifisch im europäischen Zuchtmaterial polymorph sind. Da der Großteil der Marker auf dem Array aber nur in exotischem Material polymorph ist, war die Nutzung des 820K Arrays für uns nicht interessant.

- **Assoziationsanalysen und genomische Selektion**
  Zu Beginn des Projektes gab es bereits erste Publikationen über genomweite Assoziationskartierung in Weizen (GWAS) (vgl. Abschnitt I.4.). Durch die

Verfügbarkeit der SNP-Arrays hat sich die Zahl der neuen Publikationen jedoch deutlich erhöht. GWAS-Studien in Weizen wurden u.a. für Resistenzen gegen pilzliche Pathogene (Gao et al. 2016, Bajgain et al. 2015, Mirdita et al. 2015, Gurung et al. 2014), sowie Ertrag (Guo et al., 2015a) und ertragsrelevante Faktoren in verschiedenen Umweltregimen (Lopes et al. 2015, Sukumaran et al. 2015, Tadesse et al. 2015, Qurat-ul Ain et al. 2015, Li et al. 2015, Guo et al. 2015b, Edae et al. 2014, Wu et al. 2012) und zur potentiellen Bioethanolproduktion (Bellucci et al. 2015) veröffentlicht. Dabei wurden viele dieser Studien in Sommerweizen und in exotischem Material durchgeführt. Daher kann festgehalten werden, dass die innerhalb des VALID-Projektes publizierten Artikel wesentlich zum Fortschritt des Feldes in europäischem Elite-Material beigetragen haben.

Auch in dem verwandten Feld der genomischen Selektion sind zahlreiche Publikationen für Weizen veröffentlicht worden (He et al. 2016, Bentley et al. 2014, Bassi et al. 2016). Hier wurde innerhalb des Projektes in Zusammenarbeit mit Herrn Prof. Reif eine Publikation über genomische Selektion bei Fusarium-Resistenz veröffentlicht (Jiang et al. 2015, siehe Publikationsliste unter Punkt 6) in der auch die entsprechenden Publikationen von Dritter Seite dargestellt und diskutiert wurden.

Bajgain P, Rouse MN, Bulli P, Bhavani S, Gordon T, Wanyera R Njau PN, Legesse W Anderson JA, Pumphrey MO (2015) Association mapping of North American spring wheat breeding germplasm reveals loci conferring resistance to Ug99 and other African stem rust races. BMC Plant Biology 15:249

Bassi FM, Bentley AR, Charmet G, Ortiz R, Crossa J (2016) Breeding schemes for the implementation of genomic selection in wheat (Triticum spp.) Plant Sci. 242:23-36.

Bellucci A, Torp AM, Bruun S, Magid J, Andersen SB, Rasmussen SK (2015) Association mapping in Skandinavian winter wheat for yield, plant height, and traits important for second-generation bioethanol production. Frontiers Plant Science 6:1046

Bentley AR, Scutari M, Gosman N, Faure S, Bedford F, Howell P, Cockram J, Rose GA, Barber T, Irigoyen J, Horsnell R, Pumfrey C, Winnie E, Schacht J, Beauchene K, Praud S, Greenland A, Balding D, Mackay IJ (2014) Applying association mapping and genomic selection to the dissection of key traits in elite European wheat. Theor Appl Genet 127:2619-2633

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L,

Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci U S A. 110:8057-62.

Edae EA, Byrne PF, Haley SD, Lopes MS, Reynolds MP (2014) genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor Appl Genet 127:791-807

Gao L, Turner MK, Chao S, Kolmer J, Anderson JA (2016) Genome wide association study of seedling and adult plant leaf rust resistance in elite spring wheat breeding lines. Plos ONE 11:e0148671

Guo J, Hao C, Zhang Y, Zhang B, Cheng X, Qin L, Li T, Shi W, Chang K, Jing R, Yang W, Hu W, Zhang X, Cheng S (2015a) Association and validation of yield-favored alleles in Chinese cultivars of common wheat (Triticum aestivum L.). PLoS One 10:e0130029.

Guo J, Zhang Y, Shi W, Zhang B, Zhang J, Xu Y, Cheng X, Cheng K, Zhang X, Hao C, Cheng S (2015b) Association analysis of grain-setting rates in apical and basal spikelets in bread wheat. Frontiers Plant Science 6:1029

Gurung S, Mamidi S, Bonman M, Xiong M, Brown-Guedira G, Adhikari TB (2014) Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. PLOS One 9:e108179

He S, Schulthess AW, Mirdita V, Zhao Y, Korzun V, Bothe R, Ebmeyer E, Reif JC, Jiang Y (2016) Theor Appl Genet, doi:10.1007/s00122-015-2655-1

Li W, Zhang B, Li R, Chang X, Jing R (2015) Favorable alleles for stem water-soluble carbohydrates identified by association analysis contribute to grain weight under drought stress conditions in wheat. PLOS One 10:e0119438

Lopes MS, Dreisigacker S, Pena RJ, Sukumaran S, Reynolds MP (2015) Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits bin spring wheat. Theor Appl Genet 128:453-464

Mirdita V, Liu G, Zhao Y, Miedaner T, Longin CFH, Gowda M, Mette MF, Reif JC (2015) Genetic architecture is more complex for resistance to Septoria tritici blotch than to Fusarium head blight in Central European winter wheat. BMC Genomics 16:430

Qurat-ul Ain, Rasheed A, Anwar A, Mahmood T, Imtiaz M, Mahmood T, Xia X, He Z, Quraishi UM (2015) genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. Frontiers Plant Science 6:743

Sukumaran S, Dreisigacker S, Lopes M, Chavez P, Reynolds MP (2015) Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. Theor Appl Genet 128:253-363

Tadesse W, Ogbonnaya FC, Jighly A, Sanchez-Garcia M, Sohail Q, Rajaram S, Baum M (2015) Genome-wide association mapping of yield and grain quality traits in winter wheat genotypes. PLOS One 10:e0141339

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J; International Wheat Genome Sequencing Consortium, Lillemo M, Mather D, Appels R, Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M, Pozniak C, Luo MC, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C, Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Plant Biotechnol J. 12:787-96.

Winfield MO, Allen AM, Burridge AJ, Barker GL, Benbow HR, Wilkinson PA, Coghill J, Waterfall C, Davassi A, Scopes G, Pirani A, Webster T, Brew F, Bloor C, King J, West C, Griffiths S, King I, Bentley AR, Edwards KJ (2015) High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. Plant Biotechnol J. doi: 10.1111/pbi.12485.

Wu X, Chang X, Jing R (2012) Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments. PLOS One 7:e31249

6. Erfolgten und geplante Veröffentlichungen des Ergebnisses

Publikationen in der Laufzeit des VALID-Projektes (Stand Februar 2016)

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Plieske, J., Kulosa, D., Ganal, M.W., Röder, M.S.: Whole genome association mapping of *Fusarium* head blight resistance in European winter wheat (*Triticum aestivum* L.). Plos One 8: e57500 (2013a)

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Plieske, J., Kulosa, D., Ganal, M.W., Röder, M.S.: Genetic Architecture of resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*) in European winter wheat. Mol Breed 32:411-423 (2013b)

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Plieske, J., Kulosa, D., Ganal, M.W., Röder, M.S.: Genome wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. Mol Breed 34:363-371 (2014)

Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Argillier, O., Stiewe, G., Hinze, M., Beier, S., Ganal, M.W., Röder, M.S.: Genetic architecture of main effect QTL for heading date in European winter wheat. Frontiers in Plant Science 5:217 (2014a)

Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., Argillier, O., Stiewe, G., Hinze, M., Neumann, K., Ganal, M.W., Röder, M.S.: Whole genome association mapping of plant height in winter wheat (*Triticum aestivum* L.). PLOS ONE 9:e113287 (2014b)

Jiang, Y., Zhao, Y., Rodemann, B., Plieske, J., Kollers, S., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Ling, J., Röder, M. S., Ganal, M.W., Mette, M.F., Reif, J.C.: Potential and limits to unravel genetic architecture and predict the variation of Fusarium head blight resistance in European winter wheat (*Triticum aestivum* L.). Heredity 114:318-326 (2015)

Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., Argillier, O., Stiewe, G., Hinze, M., Neumann, F., Eichhorn, A., Polley, A., Jaenecke, C., Ganal, M.W., Röder, M.S.: Analysis of main effect QTL for thousand grain weight in European winter wheat (*Triticum aestivum* L.) by genome-wide association mapping. Frontiers in Plant Science 6:644 (2015)

Vorträge bei internationalen Tagungen (nur Vortragende/r dargestellt)

Röder, M.: Association mapping in European winter wheat. Plant Genetics and Breeding Technologies, Vienna, Austria, 18-20. 2. 2013

ZANKE, C.: Genetic Architecture of Heading Date in European Winter Wheat. Translational Cereal Genomics, Vienna, Austria, 9-12. 2. 2014

Ganal, M.: Association Genetics in European Winter Wheat and Its Use in Wheat Breeding. Plant and Animal Genome Asia, Singapore, Singapore, 19–21. 5. 2014

Röder, M.: Association mapping in European winter wheat. EUCARPIA Cereals Section – ITMI Joint Conference, Wernigerode, Germany, 29.6.–4. 7. 2014

Ling, J.: Association mapping of traits related to drought stress tolerance in European Winter Wheat. Vienna International Conference Series (VISCEA), “Plant abiotic stress tolerance III”, Vienna, Austria, 29. 6.-1. 7. 2015

Ling, J.: Genome-wide association mapping of traits related to drought stress tolerance in European Winter Wheat.18th Genome Research GPZ Conference, “New targets for crops of the future”, Düsseldorf, Germany, 22-24. 9. 2015

Ganal M.: Association Mapping in European Winter Wheat. Wheat Conference, Sydney, Australia, 20-25. 9. 2015

Röder M.: Association Mapping in European Winter Wheat. Plant Genetics and Breeding Technologies II, Vienna, Austria, Feb. 1-2, 2016