Mapping of quantitative trait loci regulating nitrogen stress tolerance and leaf rust seedling resistance in two selected populations derived from crosses between exotic and elite barley

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Chapter 1

General introduction

1.1 Relevance of barley

Barley is one of the most important crops in the world due to its great ecological adaptability. Mainly, it is used for feeding livestock and malting. In 2013, barley was ranked fourth after maize, rice, and wheat in regard to produced amount of crops. Barley was cultivated on 49.8 million hectares worldwide and on 24.6 million hectares in Europe. In Germany, barley was produced on 1.6 million hectares. The total yield worldwide was more than 144.8 million tonnes and in Europe about 85.8 million tonnes were produced in 2013. The main producing country in 2013 was the Russian Federation with about 15.4 million tonnes followed by Germany, France, Canada, and Spain. Each of these countries produced more than 10.0 million tonnes. The worldwide seed production was 7.9 million tonnes. In Europe, 5.1 million tonnes of barley seed were produced whereof Germany had a share of 240,500 tonnes. The main seed producing countries in the growing season 2013 were the Russian Federation with 2.5 million tonnes followed by Turkey and Ukraine where 618,700 tonnes and 470,000 tonnes, respectively were harvested (FAOSTAT 2013).

1.2 Taxonomy, origin and domestication

The species *Hordeum vulgare* is taxonomically classified to the kingdom Plantae, the order Poales, the family Poaceae, the tribe Triticeae and the genus *Hordeum* (ITIS 2014). For further distinction within *H. vulgare*, barley was classified into the subspecies *H. vulgare* ssp. *vulgare* (cultivated barley) and *H. vulgare* ssp. *spontaneum* (wild, exotic barley). In addition, the two-row form *Hordeum vulgare* f. *distichon* and the six-row form *H. vulgare* f. *hexastichon* were introduced. The genus *Hordeum* was divided into three gene pools due to crossability (Harlan and de Wet 1971). The first gene pool contains *H. vulgare* ssp. *vulgare* and *H. vulgare* ssp. *spontaneum*. Between the two subspecies no crossing barrier exists. The only member of the second gene pool is *H. bulbosum*. That species is crossable to *H. vulgare* only with difficulties as haploid progeny occurs. Embryo rescue is needed and new biotechnological methods promise facilitation (Wendler et al. 2014). All further barley species (*e.g. H. murinum*) belong to the tertiary gene pool and are not or rarely crossable to *H. vulgare* (reviewed by Pillen 2001). The genus *Hordeum* has a high genetic variability (von Bothmer et al. 1995; von Bothmer et al. 2003). It is classified into 31 species (in total 45 taxa) and 51 cytotypes at the diploid, tetraploid, and hexaploid level with a basic chromosome number of n = 7 (Taketa et al. 2001). Barley chromosomes are named 1H to 7H, following the relationship with the wheat genome (Linde-Laursen et al. 1997). However, the previous nomenclature following Burnham and Hagberg (1956) is still in use (*e.g.* Poland et al. 2012b; Kindu et al. 2014).

Domestication of barley and other cereals began about 12,000 years ago, mainly in the Fertile Crescent (Glémin and Bataillon 2009). Recent studies indicated two core areas resided in the Karacadag mountain range in south-eastern Turkey and on the Tibetian plateau (Kilian et al. 2010; Dai et al. 2012). Domestication appeared for multiple traits, partly in multiple steps and at different locations. For instance, loss of brittle rachis took place in two independent events about 10,000 years ago and dwarf varieties were developed about 60 years ago (Meyer et al. 2012).

The effect of domestication and modern breeding are detectable on molecular basis. It was observed, that alleles got lost (genetic bottleneck) and allelic diversity decreased (Tanksley and McCouch 1997). However, some lost alleles might be of interest for plant breeding as they might confer, for instance, disease resistance. Exotic germplasm, like wild barley, harbors useful alleles that could be introduced into breeding gene pools in order to increase genetic diversity and enable development of improved varieties.

1.3 Barley breeding, genetics and genomics

Regarding breeding, barley has several advantages compared to other crops. It is easy to cultivate, propagate or evaluate on the field (northern and southern hemisphere) or in the glasshouse. The spring form has a relatively short life cycle of approximately 15 weeks. Barley is an autogamous species and natural cross-pollination is rare (Abdel-Ghani et al. 2004). The crop is easy to cross and the offspring becomes nearly homozygous after about eight selfing cycles. In conventional breeding, two cultivated barley accessions are crossed to generate variation and the resulting progeny is selected after every selfing cycle. Alternatively, time may be saved by double haploid technologies like anthere culture and *H. bulbosum* method, respectively and single seed descent (Bjørnstad et al. 1993; Forster and Thomas 2005). Recently, a hydroponic system was established in academic barley pre-breeding research for detecting quantitative trait loci (QTL) of multiple traits (Hoffmann et al. 2012). Some aims in barley breeding are different in regard to their intended use. The main aims are yield, crop health, tolerance against abiotic stress, enhanced water use and nutrient use efficiency, malting quality (for malting barley) and higher protein content and digestibility (for livestock barley).

There are multiple categories of different barley genes that are important for barley breeding. According to Yan et al. (2015), these genes show essential differences between cultivated and exotic barley. Here is a short overview for some important barley genes. An example are the two tightly linked recessive genes *btr1* and *btr2*, coding for the non-brittle rachis which result in seed shattering prevention before harvest in cultivated barley (Pourkheirandish and Komatsuda 2007). Further examples are multiple row type (*Vrs*) genes. The two-row form and the six-row form of barley are mainly regulated by *Vrs1*. The dominant allele (*Vrs1*) causes the two-row form and the recessive allele causes the six-row form (Pourkheirandish and Komatsuda 2007). An additional category of genes are flowering time genes and vernalization genes (see Maurer et al. 2015) regulating multiple physiological pathways. For instance, the dominant exotic allele of the photoperiod response gene *Ppd-H1* (Turner et al. 2005) resulted in clearly earlier heading compared to the recessive allele in cultivated barley (Wang et al. 2010a; Maurer et al. 2015). Furthermore, resistance genes play an important role for barley health. Genes coding for resistance against the fungus *Puccinia hordei*, *Rph* genes, are described in chapter 1.8.2 in detail. Finally, genes involved in physiological efficiency pathways are of interest for improving barley. For such genes, it is referred to genes involved in nitrogen use efficiency (NUE) pathways which are listed and explained in chapter 1.9.1.

Furthermore, barley is a model crop for plant genetics and genomics re-

search. As reviewed by Varshney et al. (2005), multiple genetic and genomic tools already are implemented for barley (pre-)breeding. The tools combine molecular biological techniques with statistics and bioinformatics. Three of these tools including genetic maps, QTL analysis, and barley genome sequencing are described in the chapters 1.4, 1.5, and 1.6 in detail.

1.4 Molecular markers and genetic maps

Before the development of molecular markers, genetic maps existed for morphological traits, storage proteins and isozymes (Shewry et al. 1983; Kleinhofs et al. 1993; Franckowiak 1997). Since the 1980s, several genetic maps were constructed based on different molecular marker systems. Restriction fragment length polymorphisms (RFLPs) were the first important DNA based class of molecular markers. Restriction enzymes cut DNA at specific locations and DNA fragments are analyzed by gel electrophoresis. Based on RFLPs, genetic maps for different traits of barley were constructed by several research groups (*e.g.* Graner et al. 1991; Laurie et al. 1994, 1995; Borovkova et al. 1997; Backes et al. 2003). A further important molecular marker system is developed on the basis of simple sequence repeats (SSRs), additionally called microsatellites. SSRs are short DNA sequences that are tandemly repeated in the genome. Several SSR maps are available (*e.g.* Ramsay et al. 2000; von Korff et al. 2004; Varshney et al. 2007; Schmalenbach et al. 2008). In recent years, polymorphisms at single base pairs as SNPs were developed and are still under development in barley. Mapping and fine-mapping of barley populations were applied (*e.g.* Rostoks et al. 2005; Close et al. 2009; Sato and Takeda 2009; Schmalenbach et al. 2011), particularly in genome-wide studies (Syvänen 2005).

Consensus maps by joining independent genetic maps are a tool to map and re-map quantitative trait loci (QTL). They integrate different molecular marker systems and phenotypic information from different populations (Wenzl et al. 2006; Szűcs et al. 2009; Schweizer and Stein 2011). Consensus maps offer a great opportunity to better compare genomic regions between QTL and association studies.

After establishment in large genomes as the barley genome, SNPs became the marker system of choice. SNPs are the most abundant form of genetic variation, reproducible, amenable to automation and increasingly cost-effective (Kilian and Graner 2012). For barley, the SNP sets 'Barley Oligonucleotide Pooled Assay 1' (BOPA1) containing 1,536 SNPs (Close et al. 2009; Muñoz-Amatriaín et al. 2011) and 'Illumina iSelect 9K' containing 7,864 SNPs (Comadran et al. 2012), are available. Multiple studies applied the first (Schmalenbach et al. 2011; Pasam et al. 2012; Tondelli et al. 2013; Honsdorf et al. 2014a) and the latter (Sharma 2012; Perovic et al. 2013; Maurer et al. 2015) one.

1.5 Genome sequencing and next-generation sequencing

In 2012, the barley gene space was sequenced by The International Barley Genome Sequencing Consortium, existing of 22 research institutions worldwide. The haploid barley genome size was estimated to be 5.1 gigabases (Gb). Each of these seven barley chromosomes has a size between 622 and 790 megabases (Mb). An integrated and ordered physical, genetic and functional sequence resource that describes the barley gene-space in a structured whole-genome context was described and a physical map of 4.98 Gb, with more than 3.90 Gb anchored to a high-resolution genetic map was developed. The physical map was constructed by 571,000 bacterial artificial chromosome (BAC) clones (14-fold haploid genome coverage) originating from six independent BAC libraries. In total, 79,379 barley genes were identified. For 26,159 of these genes, homology to at least one reference genome was found and 15,719 genes could be directly associated with the genetically anchored physical map (Mayer et al. 2011; The International Barley Genome Sequencing Consortium 2012). It has to be mentioned, that re-sequencing is necessary for highly diverse species as barley to develop SNPs and subsequently detect haplotype differences in reference genomes (Elshire et al. 2011; Poland et al. 2012b). Recently, a sequence-ready physical map was published and SNPs were integrated into this map. These findings underpinned the fine-mapping and cloning of agronomically important genes (Ariyadasa et al. 2014).

For nearly forty years, multiple methods for DNA sequencing were developed. It began with the Sanger (dideoxy) sequencing (Sanger 1975; Sanger and Coulson 1975). However, there are limitations in Sanger sequencing as reviewed by Varshney et al. (2009). Since less than ten years, next-generation sequencing (NGS; also called high-throughput sequencing) methods (Metzker 2010; Quail et al. 2012) became more and more feasible in plant genomics (Kilian and Graner 2012; Edwards et al. 2013; Mascher et al. 2013a, 2013b; Wendler et al. 2014). Applying NGS, DNA is sequenced in parallel and therefore it is less expensive and faster compared to previous methods. The outcome is a large amount of sequence information. According to Edwards et al. (2013) it comprises whole-genome re-sequencing, reduced-representation sequencing, transcriptome sequencing, sequencing and capture for QTL, exomes and target genes. However, in barley NGS approaches are limited by the size of large genomes (Poland et al. 2012b).

Recently, a further NGS method named genotyping-by-sequencing (GBS) was developed (Elshire et al. 2011; Sonah et al. 2013). With GBS large numbers of genome-wide SNP are produced. It uses restriction enzymes to

reduce genome complexity in target regions by excluding of repetitive genome regions. These target regions of specific individuals are regarded to represent complex genomes and sequenced in parallel (Elshire et al. 2011; Poland et al. 2012a; Poland and Rife 2012). GBS was applied in multiple crops (Elshire et al. 2011; Poland et al. 2012b; Sonah et al. 2013) and in barley, for instance, by Mascher et al. (2013a) and Honsdorf et al. (2014b). An increased map resolution was achieved.

1.6 Quantitative trait loci (QTL), QTL analysis and QTL mapping

Most agronomic traits of interest underlay multiple genes, for instance yield, quality parameters, and some diseases resistances. To locate genomic regions where multiple alleles are associated with phenotypic characteristics of traits the term quantitative trait locus (QTL) was defined (Geldermann 1975). The QTL definition became one of the most important genetic terms in quantitative genetics. Essential localization and selection of QTL started in the 1980s with the emergence of molecular marker systems (Collard et al. 2005). Currently, multiple barley QTL studies are published each year.

As reviewed by Collard et al. (2005), QTL mapping (synonyms: genetic mapping, genome mapping) is the process of constructing linkage maps and, subsequently conducting QTL analyses. QTL mapping comprises methods to identify QTL responsible for phenotypic variation (Myles et al. 2009). It is based on the decay of linkage disequilibrium (LD) with the distance between genotyped marker loci. LD is the nonrandom association of alleles at different loci. It is the correlation between loci polymorphisms and recombination (Flint-Garcia et al. 2003). LD can be artificial and high as in populations derived from bi-parental crosses or natural and low as in randomly mating populations.

Multiple mapping strategies with resulting populations have been developed over decades to detect genes responsible for quantitative variation of complex traits of agricultural and evolutionary importance (Zhu et al. 2008). These mapping strategies are linked to different population structures. The population structure, however, is based on the development of each individual mapping population. Although multiple mixed forms exist, genetic mapping can be divided into linkage mapping, which includes using maps on advanced backcross QTL (AB-QTL) analysis, and association mapping (see the following subchapters). Both terms are based on associations between markers and traits by identifying polymorphisms that are linked to functional alleles. Hence, Myles et al. (2009) introduced the terms 'family mapping' for linkage mapping and 'population mapping' for association mapping (synonym: LD mapping).

1.6.1 Advanced backcross (AB)-QTL analysis

The QTL analysis is based on detecting an association between phenotypic and genotypic (haplotype) data. Thereby, markers are used to partition the mapping population into different genotypic (allelic) groups of a particular

marker locus. In regard to the measured trait, it is statistically estimated, if differences exist between the groups (Collard et al. 2005).

Tanksley and Nelson (1996) developed a specific QTL analysis and called it advanced backcross QTL (AB-QTL) analysis. It combines QTL analysis with variety development and was designed to detect and introduce exotic alleles into established breeding material. Backcrossed genotypes are genetically similar to the adapted genotype (the donor). During variety development, unfavorable alleles are replaced by favorable ones introduced from the donor.

Since the beginning of the 21st century the AB-QTL approach was applied to barley research. Multiple barley AB populations were developed where exotic barley (*Hsp*) initially was crossed with cultivars (Pillen 2001; Pillen et al. 2003; von Korff et al. 2004; Li et al. 2005; Yun et al. 2006; Wang and Chee 2010). The power of the AB-QTL approach was demonstrated in detecting multiple QTL for variable traits as yield, agronomic traits, malting parameters and disease resistances (Pillen et al. 2003; Li et al. 2005; von Korff et al. 2005; Li et al. 2006; von Korff et al. 2006; Yun et al. 2006; Gyenis et al. 2007; von Korff et al. 2008). Through this approach, favorable *Hsp* effects were demonstrated in barley. Furthermore, QTL under nitrogen and drought stress were detected in further studies (El Soda et al. 2010; Saal et al. 2011; Sayed et al. 2012).

The cited results illustrate the power of AB-QTL approach where favorable alleles improved barley lines by introducing exotic alleles originating from *Hsp* germplasm. However, AB lines often carry multiple unfavorable exotic introgressions in addition to the favored introgression at the target locus. Such exotic alleles may epistatically influence the target locus (von Korff et al. 2010). Hence, for geneticists it is of interest to develop lines that (ideally) carry only the exotic alleles at the target locus. To achieve this goal, AB lines are further backcrossed with the elite donor. Additionally, improved marker systems and higher marker density are helpful in detecting unintentional exotic alleles. Furthermore, lines developed by the AB-QTL approach are sometimes called pre-introgression lines (Schmalenbach et al. 2008; El Soda et al. 2010) because they are the basis for introgression lines (see chapter 1.7).

1.6.2 Family mapping

Family mapping is conducted in populations derived from controlled biparental crosses. For decades family mapping was the only strategy in plant breeding research. As reviewed by Myles et al. (2009), only the recombination events that have occurred during the development of the population

can be used resulting in large chromosomal regions of 10 to 20 cM which are transmitted in blocks to the next generation (Holland 2007). The controlled cross is the reason that only a small fraction of the phenotypically relevant variation can be detected and that QTL often are not consistent across mapping populations (Myles et al. 2009).

Examples for family mapping populations are populations where two highly diverse parents were chosen and crossed in regard to (at least) one specific trait. In most cases, these populations were derived from double haploid (DH) or F_2 individuals or recombinant inbred lines (RILs). Examples for barley DH, F_2 and RIL populations are given by Poland et al. (2012b), Liu et al. (2011b), and Malosetti et al. (2011). All individuals of the final DH and RIL populations are (theoretically) homozygous at each marker locus with alleles originating from one of the two parents.

1.6.3 Population mapping

Population mapping uses all evolutionary recombination events which almost invariably results in a much higher mapping resolution compared with family mapping (Myles et al. 2009). Additionally, the genetic diversity at each marker locus and across the total genome is higher compared to family mapping populations. A further advantage is that the mapping population does not have to be developed by crossings and subsequent marker assisted selection for multiple generations or by biotechnological methods as DH. Thus, it is cost-effective compared to often expensive processes of establishing mapping families (Myles et al. 2009).

According to Zhu et al. (2008), population mapping itself may be divided into two methods differing in regard to their aim: 1) candidate-gene association mapping relating to polymorphisms in selected candidate genes responsible for trait specific phenotypic variation and 2) genome-wide association mapping. Synonyms are genome scan, whole genome association scan (Waugh et al. 2009), and genome-wide association mapping (GWAS) (Korte and Farlow 2013). The GWAS approach was established for finding genetic variation across the whole genome for various quantitative traits. In population mapping kinship between individuals is rarely known or unknown. The GWAS approach is applied in populations existing of individuals not developed by specific crossings, for instance in sets of varieties or landrace accessions (Pasam et al. 2012; Berger et al. 2013; Tondelli et al. 2013). Individuals of such populations are multi-allelic at each marker locus. Advantages and limitations were reviewed recently (Korte and Farlow 2013). The main advantages are multi-allelic diversity and higher resolution caused by multiple and varying recombination events present in each individual.

Limitations are given in detecting small QTL effects and simulations demonstrated that the most significant SNP was not always the true causative locus (Korte and Farlow 2013).

1.6.4 Mixed forms of family and population mapping

Mixed forms of family and population mapping were established. Such mapping populations were derived from crossings by more than two parents like multiparental admixture mapping populations. A review is given by Rakshit et al. (2012). Two mixed forms are described in the chapters 1.6.4.1 and 1.6.4.2.

1.6.4.1 Mapping in multiparent advanced generation intercross (MAGIC) populations

One example are multiparent advanced generation intercross (MAGIC) populations existing of RILs or DHs which are derived from crossing diverse parents and their progeny among each other (Cavanagh et al. 2008). MAGIC populations were established in multiple crops including wheat (Huang et al. 2012; Cavanagh et al. 2013; Mackay et al. 2014; Scutari et al. 2014; Thépot et al. 2015), triticale (Würschum et al. 2014), barley (Sannemann et al. 2015) and rice (Bandillo et al. 2013) for instance. In Huang et al. (2015) a general review explaining the MAGIC approach and, additionally, listing mating designs, population structures, mapping approaches for each individual crop, is given. However, it shall be mentioned that MAGIC mainly relies on population development and mapping follows conventional strategies. The only barley MAGIC population that was developed so far consists of 533 DH lines that were derived from crossing eight parents and their progeny, respectively among each other. A total of 17 QTL was detected in genomic regions, mainly where known flowering time genes are located (Sannemann et al. 2015).

1.6.4.2 Nested association mapping (NAM)

Recently, a pathbreaking mapping strategy was developed: the nested association mapping (NAM) strategy. Following this approach, multiple diverse parents (sometimes called founders) are crossed and backcrossed with the same recipient. The final population called NAM population is composed of subpopulations sometimes called NAM families (Yu et al. 2008). The main advantage of the NAM strategy is the combination of linkage analysis with high-resolution association mapping (Yu et al. 2008). The NAM strategy

was applied in crops like maize (McMullen et al. 2009), sorghum (Jordan et al. 2011), and barley (Schnaithmann et al. 2014; Maurer et al. 2015).

The maize NAM population consists of 25 founders that were backcrossed with one recipient. Each of these maize NAM families has 200 NAM lines, 5,000 NAM lines in total (McMullen et al. 2009). Buckler et al. (2009) detected, that numerous small-effect QTL were responsible for differences in flowering time. No QTL responsible for large flowering time effects were found. These two findings were confirmed for mapping quantitative resistance to northern leaf blight (Poland et al. 2011) and southern leaf blight (Kump et al. 2011), leaf angel and size (Tian et al. 2011), kernel starch, protein, and oil (Cook et al. 2012), and stalk strength (Peiffer et al. 2013) in maize. In addition to QTL mapping of the whole maize NAM population individual lines or NAM families were investigated in multiple scientific fields, for instance in chromosomal karyotyping (Albert et al. 2010), ionomics (Baxter 2010), SNP development (Guo et al. 2010; Guo and Beavis 2011), and studying the evolutionary history of maize (Chia et al. 2012). Additionally, the maize NAM population was used for comparison of multiple statistical prediction models (Guo et al. 2012; Guo et al. 2013).

The first sorghum NAM population was developed for hybrid combination performance consisting of 56 NAM families with 30-90 NAM lines per family. There, the main focus was set on locating multiple QTL within NAM families (Jordan et al. 2011). The QTL for nodal root angle and flowering time were located (Jordan et al. 2011; Mace et al. 2012; Mace et al. 2013) in subsets of the sorghum NAM population. Recently, a second sorghum NAM population derived from ten founders was reported. The final population consists of 2,400 RILs. As such, new QTL for flowering time and plant height were mapped, and known QTL were verified (Bouchet et al. 2015).

Recently, the barley NAM population named 'Halle Exotic Barley 25' (HEB-25) was developed from crossing and backcrossing 25 exotic barley donors with the elite barley cultivar 'Barke' until BC_1S_3 (Maurer et al. 2015). In total, HEB-25 consists of 1,420 NAM lines, distributed across 25 NAM families with up to 75 NAM lines per family. QTL where exotic alleles were associated with flowering time were detected and verified, respectively. Eight major QTL were identified and the QTL accounted for 64 % of the cross validated (Utz et al. 2000) proportion of explained genotypic variance (Maurer et al. 2015). During the development of the population HEB-25 a subset of five NAM families (HEB-5) in BC_1S_1 were screened for leaf rust (*Puccinia hordei*) seedling resistance. The QTL across the population HEB-5 and within one individual NAM family were mapped where exotic alleles were associated with decrease and increase, respectively of leaf rust resistance (Chapter 2.2).

1.7 Introgression lines (ILs)

Introgression lines (ILs) are genotypes carrying exotic chromosomal segments in a common genomic background. In barley, the term is usually used for alleles introduced from *H. vulgare* ssp. *spontaneum* or *H. bulbosum* in the background of *H. vulgare* ssp. *vulgare* (Schmalenbach et al. 2008; Johnston et al. 2009; Lakew et al. 2011, 2013).

A set of ILs covering the complete exotic genome is named exotic library or IL library (Zamir 2001). By definition, each IL only carries one exotic introgression verified by molecular markers (Zamir 2001). In reality, ILs often carry more than one exotic genomic region and, additionally, overlapping regions between harboring ILs (Schmalenbach et al. 2008). Using improved molecular marker systems these findings were extended (Schmalenbach et al. 2011).

Schmalenbach et al. (2008) developed the wild barley introgression line library S42IL which originates from a cross between *H. vulgare* ssp. *vulgare* ('Scarlett') and *H. vulgare* ssp. *spontaneum* ('ISR42-8'). The power of the S42IL library to detect QTL was demonstrated in several studies where *Hsp* alleles were associated with disease resistances, agronomic traits and malting quality traits (Schmalenbach et al. 2008; Schmalenbach et al. 2009; Schmalenbach and Pillen 2009). Further QTL for multiple traits were detected under variable conditions in different experimental systems. The QTL detected in a hydroponic system where different N levels were applied corresponded to the QTL detected in field trials (Hoffmann et al. 2012). Furthermore, different QTL were detected which controlled growth rate and water use efficiency (Honsdorf et al. 2014a) in a high-throughput genotyping experiment. In a further drought stress experiment S42ILs were evaluated with genotyping by sequencing (Honsdorf et al. 2014b). Naz et al. (2012) showed that exotic alleles were associated with multiple root length parameters in two S42ILs. In a subset of three S42ILs March et al. (2012) identified proteins that were associated with malting quality. In a set of 28 S42ILs Schnaithmann and Pillen (2013) detected exotic QTLs controlling nitrogen stress tolerance among wild barley introgression lines (chapter 2.1).

In addition to the cited QTL analyses an individual S42IL was used for expression analysis of genes regulation flowering time (Campoli et al. 2012) and the effect of flowering time genes on osmotic stress (Haile 2013).

1.8 Leaf rust and its pathogen *Puccinia hordei* and genes as well as QTL for qualitative and quantitative resistance

Yield, yield stability, and quality are important in crop production. These parameters are influenced by factors like climate, soil type, cropping system, crop species, and crop health. Crop health is mainly reached by pesticide treatment and cultivation of disease resistant plants (Das et al. 2007). Resistance of crops against pathogens reduces pesticide treatment and provides a great advantage both economically and ecologically. Therefore, it is of interest that crops carry favorite alleles of qualitative resistance genes (R genes) as well as quantitative resistances (QTL). Plant breeders introduce these favorite alleles into their gene pools.

1.8.1 Leaf rust and its pathogen *Puccinia hordei*

One economically important foliar disease in barley is named leaf rust. It is caused by the biotrophic fungus *Puccinia hordei*. This fungus affects barley worldwide (Clifford 1985) which leads to up to 32 % yield loss (Griffey et al. 1994). Therefore, scientists of multiple disciplines as breeding, botany, and phytopathology focus on improving barley in regard to leaf rust resistance since the beginning of the 20$^{\text{th}}$ century (Raines 1922).

1.8.2 Genes as well as QTL for qualitative and quantitative resistance against *P. hordei*

There is a main difference between qualitative (full) and quantitative (partial) resistance. Qualitative resistance is controlled by single major genes and quantitative resistance is controlled by multiple genes.

Qualitative resistance against *P. hordei* was detected in cultivated barley, landraces and exotic barley like *H. vulgare* ssp. *spontaneum* (Manisterski and Anikster 1994; Ivandic et al. 1998; Perovic et al. 2013). Decades before present, R genes against *P. hordei* were named *Pa* genes after *Puccinia anomala*. In 1972, *Pa* genes were re-named to *Rph* genes (resistance against *P. hordei*), because the pathogen *P. anomala* was re-classified to *P. hordei* (Moseman 1972; Ramage 1972). The *Rph* genes were numbered consecutively as *Rph1*, *Rph2*, and so on. Specific allele symbols following the *Rph* number (*cf.* Franckowiak et al. 1997) were partly established (Weerasena et al. 2004). Currently, at least 23 *Rph* genes with qualitative resistance against *P. hordei* are known (Golegaonkar et al. 2009a; Hickey et al. 2011; König

et al. 2012; Sandhu et al. 2012; Johnston et al. 2013; Sandhu et al. 2014). Resistance against *P. hordei* is known for seedling stage and for adult plant stage (Golegaonkar et al. 2009a; Golegaonkar et al. 2010). All known *Rph* genes are effective during the seedling stage but adult plant resistance is reported only for a few R genes (Hickey et al. 2011; Derevnina et al. 2013; Singh et al. 2013; Sandhu et al. 2014). The already mapped *Rph* genes were located on all barley chromosomes by multiple strategies (*e.g.* trisomic, morphological or molecular analyses) as cited or detected recently (Chełkowski et al. 2003; Weerasena et al. 2004; Hickey et al. 2011; Sandhu et al. 2012; Johnston et al. 2013). In this context it shall be mentioned that the term 'adult plant resistance' normally is used for race-specific qualitative *Rph* genes (Ziems et al. 2014) and not for QTL detected during adult plant stage. Currently, no *Rph* genes were cloned like for near relatives of *P. hordei*. For instance multiple resistance genes against the disease barley stem rust (*Rpg* genes), caused by *Puccinia graminis*, were cloned (Brueggeman et al. 2002; 2008). However, Dracatos et al. (2014) identified possible CGs for further gene cloning at the *Rph9/Rph12* locus. A step toward understanding the molecular and biochemical mechanisms responsible for *Rph* genes was made by Bernardo et al. (2012). These authors subjected defense genes coding for oxalate oxidase and callose synthase activation of barley leaves to a proteomic analyses. They identified proteins that can be related to modulation of the photosynthetic apparatus components, re-direction of the metabolism to sustain defence responses and deployment of defence proteins of *Rph15* (Bernardo et al. 2012).

It is assumed that qualitative resistance is not as durable as quantitative resistance (Parlevliet 2002). Hence, the introduction of quantitative resistance is one further objective in plant breeding. However, the molecular mechanisms that control quantitative disease resistance are poorly understood as the behavior of the host is inconsistent due to multiple small QTL effects (Poland et al. 2009). Multiple genes cause partial resistance against *P. hordei*. Therefore, multiple QTL studies were carried out using variable barley accessions and *P. hordei* isolates. QTL were detected during seedling and adult plant stage. Some QTL were detected for both plant stages, some either for seedling or adult plant stage. Mapping populations were created by specific crossings between varieties or varieties and breeding lines or varieties and exotic barley. Furthermore, variety panels were tested (Qi et al. 1998; Qi et al. 1999; Kicherer et al. 2000; Qi et al. 2000; van Berloo et al. 2001; Backes et al. 2003; Kopahnke et al. 2004; von Korff et al. 2005; Kraakman et al. 2006; Marcel et al. 2007a; Marcel et al. 2007b; Schmalenbach et al. 2008; Cakir et al. 2011; Liu et al. 2011a; Castro et al. 2012; González et al. 2012; Li et al. 2013; Ziems et al. 2014).

Co-location of leaf rust QTL with *Rph* genes was detected (Kicherer et al. 2000; Ziems et al. 2014). However, it is assumed that qualitative and quantitative resistances against *P. hordei* underlie different evolutionary origins due to different physiological mechanisms (Qi et al. 1998; Backes et al. 2003).

Biotrophic fungi normally are very host specific. For instance, barley is not (or marginal) susceptible against rust species that may infect other cereals (Niks and Marcel 2009). Therefore, the term 'nonhost' was introduced. It shall be mentioned that *Puccinia* nonhost resistance (Niks and Marcel 2009) which is sometimes called immunity (Jafary et al. 2006) and near-nonhost status (Niks 1987; Niks et al. 2011 cited in Yeo et al. 2014) play an important role in basic resistance breeding research of barley (Zellerhoff et al. 2010; Niks 2014; Yeo et al. 2014). Barley appears to be a near-nonhost to several non-adapted rust fungal species, such as *P. triticina* and *P. hordei-murini* for instance (Yeo et al. 2014). On the contrary, it has been demonstrated that *P. hordei* may infect rice (Ayliffe et al. 2011).

Due to different marker systems, mapping populations, and isolates, most genomic regions are hardly comparable. Nevertheless, results from previous studies were integrated into consensus maps (Schweizer and Stein 2011; Ziems et al. 2014). Hot spot (Meta-QTL) regions for resistances against multiple diseases including leaf rust were detected (Chen et al. 2010; Schweizer and Stein 2011; Chen and Duan 2014). It is assumed that nonhost resistance in barley against rust and powdery mildew fungi of related *Gramineae* is not due to R genes, but to pathogen species-specific quantitative resistances (Jafary et al. 2008; Chen and Duan 2014).

1.9 Nitrogen use efficiency (NUE)

Nitrogen (N) is a macro plant nutrient. For instance, it is part of multiple compounds as proteins, protein complexes, nucleic acids, nucleotide phosphates, vitamin B complexes, chlorophylls and cytochromes. The main N sources are nitrate (NO_3^-), ammonium (NH_4^+) and urea ($CO(NH_2)_2$) (Schilling 2000). In regard to high fertilizer costs and avoidance of ground water pollution, nitrogen use efficiency (NUE) is an important breeding goal. Following Kant et al. (2011), the most simple definition for NUE is yield per unit of N available in the soil. Plant scientists of multiple disciplines try to identify genes that improve NUE. Ideally, NUE genes coding for pathways relating to N uptake, assimilation, amino acid biosynthesis, C/N storage and metabolism, signaling and regulation of N metabolism and translocation, remobilization, and senescence are detected (McAllister et al. 2012). Kant et al. (2011) review that there are two general stages for N use in the plant life cycle. First, during biomass formation (amount of N uptake, storage, and assimilation into amino acids and other important nitrogenous compounds) and second the proportion of N that is partitioned to the seed, resulting in final yield. Plant roots have uptake systems for both nitrate and ammonium which differ in their affinities to these substrates: high and low-affinity nitrate and ammonium transport systems (Xu et al. 2012). It has been observed that uptake of nitrate and ammonium efficiency and utilization efficiency is higher at low N levels compared to standard N levels for a set of two row barley cultivars (Le Gouis et al. 1999). NUE under low N nutrition was demonstrated for cultivated and exotic barley (Górny 2001). Thus, it is recommended for breeding programs to integrate the two breeding goals yield and NUE (Anbessa et al. 2009) as barley accessions have different NUE (Abeledo et al. 2008; Anbessa and Juskiw 2012). However, it is further complicated by the fact that breeding for high NUE is different from breeding for low input compared to moderate and high input systems (Anbessa and Juskiw 2012). The response of plants to high and low N regimes is essential for investigating genes involved in corresponding N pathways (Beatty et al. 2010; Kant et al. 2011).

1.9.1 Genes and QTL involved in NUE pathways

Genes and QTL involved in multiple NUE pathways as in N uptake, translocation, assimilation and remobilization (Xu et al. 2012) are of interest for breeding of cultivars for sustainable cropping (Hirel et al. 2011). Nitrogen uptake and partitioning is achieved by transporters, differing in their specificity, affinity (high or low) and capacity. Transporter activity depends on

plant stage and environmental conditions. The transport systems involved in N physiology are classified as: NO_3^- transporters, NH_4^+ transporters, amino acid transporters, peptide transporters, and purine and purine-derivate transporters (*cf.* Williams and Miller 2001). The N metabolism is divided into the different phases of uptake, assimilation, mobilization, and remobilization (McAllister et al. 2012). Multiple enzymes are involved in the listed pathways of each phase. A huge amount of experiments cited in the review of McAllister et al. (2012) indicate that some enzymes are related to NUE. These are glutamine synthetase (GS) 1 and 2, glutamate synthase (GOGAT), asparaginase, rubisco, Dof1 (a transcription factor that is over-expressed under low N conditions in maize), the specific haeme activator protein 3 (which is involved in flowering time regulation) and the membrane protein amino acid permease 1. Furthermore, the two aminotransferase enzymes aspartate aminotransferase (AspAT) and alanine aminotransferase (AlaAT) can serve as markers of NUE (Cañas et al. (2010) cited in McAllister et al. 2012). Enhanced NUE was observed in multiple crops where AlaAT was over-expressed (McAllister et al. 2012). Recently, five transcription factors responsible for the N remobilization during flag leaf senescence were detected (Christiansen and Gregersen 2014; Hollmann et al. 2014). However, their relation on NUE is unknown thus far.

Multiple QTL involved in the N pathways along with the N remobilization were mapped in barley. Thus, genomic regions where alleles were associated with eight traits, for instance leaf nitrogen content at multiple plant stages and soluble α-NH_2 nitrogen content were detected (Mickelson et al. 2003). These traits were mapped on all barley chromosomes. For instance, QTL for grain protein concentration were mapped on chromosomes 2H and 6H (Mickelson et al. 2003; Jukanti and Fischer 2008; Jukanti et al. 2008; Laccerenza et al. 2010; Goodall et al. 2013). Recently, Matthies et al. (2013) mapped genes, that code for enzymes involved in NUE pathways, to multiple barley chromosomes as 2H (asparaginase, GOGAT, GS2), 5H (AspAT) and 6H (nitrate reductase). A specific GS isoform was located on chromosome 6H (Goodall et al. 2013). Bus and Pillen (unpublished) mapped candidate genes for the regulation of grain protein content in S42ILs. Further, the candidate genes GOGAT, NAM2 (encoding a NAC (Uauy et al. 2006) transcription factor), and CNX-1 (a cofactor for nitrate reductase and xanthine dehydrogenase) are located on chromosome 2H and NAM-1 is located on chromosome 6H. In a genomic region corresponding to the grain protein content region GPC1 on chromosome 6H, a further major region for GPC was recently detected (Falcon et al. 2014). In barley introgression lines, Schnaithmann and Pillen (2013) detected multiple QTL where exotic alleles were associated with N stress tolerance. For instance, exotic alleles on chro-

mosome 4H of S42IL-119 were associated with a 20.0 % increase of thousand grain weight increase under N stress (chapter 2.1).

1.10 Objectives of the present PhD thesis

The main objective of this PhD thesis was the development of the barley pre-nested association mapping (NAM) population HEB-5 and the detection of quantitative trait loci (QTL) based on exotic alleles in the two different barley populations S42IL and HEB-5, each derived from crosses between exotic and elite barley. The ascertained QTL will be genetically mapped and compared with QTL and potentially responsible genes, respectively that were already detected in other research. For this purpose, the objective was divided into the following three goals, where goal 1 corresponds to chapter 2.1 and goals 2 and 3 correspond to chapter 2.2:

1. Detection of exotic QTL controlling nitrogen stress tolerance among wild barley introgression lines:

The aim was to detect and map QTL in wild barley introgression lines (S42ILs) under two different nitrogen levels in a glasshouse experiment. The QTL mapping will comprise multiple agronomic traits and grain parameters as well as carbon and nitrogen content and ratio, respectively. The mapped positions of the detected QTL for each trait will be compared with already known regions where alleles code for characteristics of the specific traits, particularly under N deficiency.

A first step toward the development of a barley NAM population and its utilization to detect QTL conferring leaf rust seedling resistance:

2. Development of a barley pre-nested association mapping (NAM) population (HEB-5):

Five diverse exotic barley donors will be crossed with the elite barley cultivar 'Barke'. These offspring will be backcrossed with 'Barke' until the backcross level BC_1S_1 is reached. The developed pre-NAM population HEB-5 will consist of sufficient lines for a subsequent QTL study.

3. A proof of concept by testing the genetic regulation of leaf rust in the multiparental pre-NAM population HEB-5 includes the following items:

Leaf rust QTL will be mapped in the multiparental barley pre-NAM population HEB-5. Coexistence between the QTL detected in HEB-5 and in the individual bi-parental pre-NAM families will be verified. The genomic regions where QTL were mapped will be compared to genomic regions which are known for their resistance and susceptibility, respectively against leaf rust or other diseases.

Chapter 2

Original papers

2.1 Detection of exotic QTLs controlling nitrogen stress tolerance among wild barley introgression lines

Published in:
Euphytica (2013) 189:67-88
DOI: 10.1007/s10681-012-0711-3
Authors: Florian Schnaithmann • Klaus Pillen

2.2 A first step toward the development of a barley NAM population and its utilization to detect QTLs conferring leaf rust seedling resistance

Published in:
Theor Appl Genet (2014) 127:1513-1525
DOI: 10.1007/s00122-014-2315-x
Authors: Florian Schnaithmann • Doris Kopahnke • Klaus Pillen

Chapter 3

General discussion and future prospects

The present PhD thesis comprises two original papers (see chapter 2). Both publications describe QTL mapping studies based on barley populations derived from crosses between elite and exotic barley accessions. However, the two mapping populations (S42IL and HEB-5) differ in multiple characteristics of their genetic architecture. Furthermore, different traits were phenotyped, mapped and compared with previous studies. These differences are pointed out in the following section. Subsequently, each of the two papers is discussed in regard to the objectives (Chapter 3.1). Thereupon, the two original papers are compared with the literature (Chapter 3.2) in a broader context. Finally, a short future prospect of QTL mapping (Chapter 3.3) is given.

The S42ILs chosen for the NUE QTL study were already available at the beginning of the scientific work for the present PhD study. They were selected from fifty-nine lines of the S42IL library developed by Schmalenbach et al. (2008). The S42ILs were developed from a cross between two parents that resulted in a bi-parental population. They were nearly homozygous as they were developed until BC_3S_4. The heterozygous genotype (*HvHsp*) frequency of each individual S42IL had an expected value of 6.25 %. On the contrary to S42IL, HEB-5 was developed from the initial cross until BC_1S_1 which was one objective of the PhD thesis (chapter 3.1.2). Remarkably, HEB-5 lines still had an expected *HvHsp* frequency of 25.0 % and further backcrossings are needed to achieve the same low heterozygousity as S42ILs.

The main difference between the two mapping populations S42IL and HEB-5 is based on the number of parents, each population was derived of. The S42ILs were derived from crossing two highly diverse barley accessions,

the cultivar 'Scarlett' and the exotic line 'ISR-42-8' (Schmalenbach et al. 2008). Thus, S42ILs only carry alleles from either 'Scarlett' or 'ISR-42-8' at each SNP locus. On the contrary, HEB-5 lines potentially carry one out of six different alleles at each SNP locus, because HEB-5 lines were derived from crossings (and backcrossings) between the cultivar 'Barke' and five diverse exotic barley accessions. Thus, 'allele richness' is given for the pre-NAM population HEB-5 but not for S42IL (and additionally not for individual bi-parental NAM families).

Both barley populations, S42IL and HEB-5, were developed for QTL mapping. However, none of the donors was chosen based on a specific trait. On the contrary, 'classical' bi-parental mapping populations often were developed for investigating specific traits, notably plant diseases, where one donor is highly resistant and the other donor is highly susceptible against the investigated pathogen (Wang et al. 2010b; Castro et al. 2012; Kelm et al. 2012). For both investigated mapping populations, no behavior in regard to any trait was presumed. However, it was expected that exotic alleles in general make a contribution to barley improvement. The power of S42ILs was demonstrated in multiple studies for variable traits before conducting the N stress experiment (*cf.* chapter 1.7). The mapping of alleles that were associated with leaf rust seedling resistance in the pre-NAM population HEB-5 (and individual pre-NAM families), however, was regarded as a proof of concept toward the final barley NAM population HEB-25 (Maurer et al. 2015).

3.1 Results and discussion of the present study in regard to the objectives

3.1.1 Nitrogen use efficiency (NUE)

The first part of the objective for this PhD study was the detection and mapping of QTL in wild barley introgression lines (S42ILs) under two different nitrogen (N) levels in a glasshouse experiment. Differences between the two N levels N0 (N deficient) and N1 (sufficient N) were observed for all investigated traits except grain width, as calculated by the mixed model analysis. N application effects were observed for all traits except for C/N ratio of grains. Such N application effects were expected and demonstrated for many crops (Raun and Johnson 1999) in particular for the barley pre-IL population S42 (Saal et al. 2011). The effect of the two selection environments N0 and N1 on QTL detection is discussed in chapter 3.2.1.

A total of 15 traits were investigated and resulted in 112 significant SNP × trait associations that were summarized to 65 QTL. These QTL were distributed over the two N levels. The QTL detected for each trait were genetically mapped and these positions were compared with corresponding QTL effects in S42-derived lines and with CGs. Each investigated trait was discussed individually. A detailed discussion is given in the original publication of Schnaithmann and Pillen (2013). In addition to the discussion in the mentioned publication, specific QTL of the traits days until heading (HEA), straw per plant (STR) and thousand grain weight (TGW) are discussed here. For STR and TGW, more actual research findings are available.

As an example for the morphological traits, the HEA QTL effect of S42IL-128 is discussed. For this line, the exotic alleles reduced HEA by 2.7 days compared to 'Scarlett'. In pre-ILs S42IL-128 was derived of, corresponding decreasing effects of exotic alleles were observed by von Korff et al. (2006) and Saal et al. (2011). Two candidates potentially responsible for the decreasing QTL effect on chromosome 6H are the 'earliness per se' gene *eps6L.1* which is expressed independently from photoperiod and vernalization genes (Laurie et al. 1995) and *HvCO2*, a *CONSTANS*-like photoperiod response gene (Griffiths et al. 2003) detected in S42IL-128 (Wang et al. 2010a).

For STR and the grain parameter TGW multiple QTL were detected. STR QTL were distributed on all chromosomes except 7H and TWG QTL were located on the chromosomes 3H, 4H, 6H and 7H. Corresponding effects were detected in von Korff et al. (2006), Schmalenbach et al. (2009) and Saal et al. (2011). For instance, two STR QTL on the chromosomes 2H and 3H,

respectively, were in accordance with STR QTL detected by von Korff et al. (2006). Recently, a further QTL study comprising multiple traits including STR and TGW in the population S42 was published (Arifuzzaman et al. 2014). There, QTL were mapped with SSR and diversity array technology (DArT) markers. Therefore, precise chromosomal positions of these QTL are hardly comparable to the SNP regions of the present study. However, coincidence (the same direction of the QTL effect on the same chromosome) was found on the chromosomes 4H and 6H for TGW and on chromosome 2H for STR. Thus, QStr.S42IL-2H could show coincidence with QSdw.S42.2H.a, QSdw.S42.2H.b, or QSdw.S42.2H.c. Furthermore, QTgw.S42IL-4H could be mapped to the same region as QTkw.S42.4H.a and, finally, QTgw.S42IL-6H to QTkw.S42.6H.a or QTkw.S42.6H.b. All the potentially corresponding QTL are listed in Arifuzzaman et al. (2014).

Specific S42ILs are of interest for further investigations in regard to NUE, in particular under N deficiency. These lines carry exotic (*Hsp*) alleles that result in 'improved' phenotypic behavior compared to *Hv* alleles under N deficiency. For some traits like grain parameters it is obvious what 'improved' means. The S42ILs showing a higher TGW are generally preferable, if further parameters, as starch content for instance, are on a constant level. Thus, the two introgression lines S42IL-111 and S42IL-130 are of specific interest as they showed an increase in TGW of 15.9 % and 19.3 %, respectively under N0. These improving QTL effects and its causative S42ILs were observed only under N stress (*cf.* chapter 3.2.1). The traits grain width (G_WIDTH) and grain area (G_AREA) which were highly correlated with TGW showed the same tendency in S42IL-111, S42IL-130 and further lines.

Due to potential heat stress during flowering time and ripening, an early occurrence of these two traits is often considered to be favorable. However, phenotyping of these two traits is very extensive. On the contrary, HEA is very easy to phenotype and it is highly correlated with flowering time (von Korff et al. 2010). Therefore, flowering time and HEA are evaluated and discussed as equivalent substitutes among scientists and breeders. Consequently, breeding for early HEA became continuously more important. Multiple S42ILs showed earlier HEA than the recurrent parent 'Scarlett', mainly under N1. For instance, S42IL-108 showed a 28.7 % earlier HEA than 'Scarlett'.

For the remaining traits, the QTL detected under N0, N1, or across both N levels are of interest for breeding science in general and QTL mapping in particular. However, these exotic effects are favorable only under conventional N supply, or it is not possible to evaluate them as 'favorable'. For instance, S42IL-115 showed a 13.7 % increase in grains per ear (GEA) only

under N1. The evaluation of exotic effects for traits as N content of grains (N_CONT_G) and straw (N_CONT_STR) depends on the individual question or goal of the scientists and breeders.

3.1.2 Development of a pre-NAM population (HEB-5)

The second part of the objective for this PhD study was the development of a multi-parental barley pre-NAM population. It was named 'HEB-5'. HEB-5 is an acronym for 'Halle Exotic Barley' consisting of five pre-NAM families. The prefix 'pre' was chosen because HEB-5 was developed by 'merely' five exotic donors and it was developed 'merely' until the generation BC_1S_1. In contrast, the final barley NAM population HEB-25 (Maurer et al. 2015) was developed with 25 exotic donors until BC_1S_3.

The development of HEB-5 was conducted in order to introduce exotic alleles into the genetic background of cultivated barley. Therefore, five highly diverse donors from the Fertile Crescent, in particular from the countries Israel, Syria, Turkey, Iraq and Iran, were originally crossed with the German elite cultivar 'Barke'. The F_1 plants were genetically verified with SSR markers and subsequently backcrossed once with 'Barke' as the female parent. Subsequently, BC_1 plants were selfed once, using the single seed descent (SSD) method and once again genetically verified with SSR markers. Finally, the pre-NAM lines were genetically verified with SNP markers to ensure that the backcross and selfing steps were successful and furthermore, that the progeny was correctly assigned to the according NAM family. In total, the pre-NAM population HEB-5 resulted in 295 pre-NAM lines. During HEB-5 development, no artificial selection was carried out.

The used five donors are a subset of the exotic barley accession provided by Badr et al. (2000). The genetic distance among these donors was estimated with amplified fragment length polymorphisms (AFLP) and resulted in geographic classes (Badr et al. 2000). According to the five different geographic classes 'Israel', 'Syria', 'Turkey', 'Iraq' and 'Iran', it can be assumed that the five donors that were chosen for pre-NAM development were 'sufficiently' diverse. On the contrary, it can be assumed that donors from less diverse geographic regions would have led to lower genetic diversity among the exotic donors. All exotic donors were classified to *H. vulgare* ssp. *spontaneum* and did not show any *H. vulgare* ssp. *vulgare* introgressions based on natural outcrossing in the Fertile Crescent (Badr et al. 2000). Genetic similarity among the five exotic donors and among 'Barke' was estimated. The exotic donors showed a genetic similarity between 71.2 % among each other. The genetic similarity between 'Barke' and the five exotic donors ranged from 33.0 % to 49.7 %.

Thus, there is evidence that 'sufficiently' diverse exotic alleles were provided for antagonizing against allelic loss during domestication in the genetic background of the elite cultivar 'Barke'. Furthermore, a lack of significant natural or unintentional artificial selection during development of the pre-NAM population HEB-5 was indicated due to the low genetic similarity between 'Barke' and the five exotic donors.

In the following chapter (3.1.3) a proof of concept for the pre-NAM population HEB-5 is given by testing the genetic regulation of leaf rust during seedling stage.

3.1.3 Proof of concept by testing the genetic regulation of leaf rust in the pre-NAM population HEB-5

The third part of the objective for this PhD study was a proof of concept (PoC) by testing the genetic regulation of seedling leaf rust in the pre-NAM population HEB-5. Therefore, leaf rust QTL should be mapped in HEB-5 and in each individual NAM family. A further part of the PoC was the investigation, if the leaf rust QTL detected in HEB-5 coexisted in individual pre-NAM families. Finally, it should be discovered if the genomic regions where the leaf rust QTL were mapped were already known and, if so, these regions lay in hot spot regions for multiple diseases.

Eight QTL were detected where exotic alleles were associated with leaf rust symptoms during seedling stage. According to Muñoz-Amatriaín et al. (2011) each of these QTL consisted of one to nine SNP positions with the total of 27 significant SNP × leaf rust associations. The QTL were mapped on all barley chromosomes except of chromosome 6H.

The cited QTL were detected only across HEB-5 and in one individual pre-NAM family, the family HEB-F23. For the remaining four pre-NAM families (HEB-F06, -F08, -F14, -F15), no QTL where exotic alleles were associated with leaf rust symptoms were found. Two QTL, one detected in HEB-5 (QLr.HEB-5-4H) and the other detected in HEB-F23 (QLr.HEB-F23-4H) corresponded to each other. On chromosome 4H, they overlapped at 61.56 cM. Thus, one genomic region where a QTL for leaf rust during seedling stage coexisted in HEB-5 and one individual pre-NAM family was found in the present experiment.

Comparison to known positions of R genes and QTL showed that the majority of the leaf rust QTL lay in hot spot regions where Schweizer and Stein

(2011) located Meta-QTL for multiple diseases. Furthermore, *Rph* genes are located on the same chromosome or chromosome arm as the detected QTL. Additionally, one QTL (QLr.HEB-5-1H) was detected at the end of the log arm of chromosome 1H where so far no QTL for seedling resistance was reported. In this genomic region, *Hsp* alleles reduced leaf rust symptoms by 33.3 %. If QLr.HEB-5-1H can be verified in field experiments across years, the exotic alleles of this QTL would provide a valuable source for leaf rust resistance breeding. Furthermore, exotic alleles on chromosome 7H were associated with a leaf rust decrease of 36.2 % (QLr.HEB-F23-7H). This favorable QTL was detected in the pre-NAM family HEB-F23 and could additionally be valuable for resistance breeding. QLr.HEB-F23-7H was mapped to the hot spot region MQTL18 for *P. hordei* and *B. graminis* (Schweizer and Stein 2011) and, potentially, to the same region where the resistance gene *Rph19* (Park and Karakousis 2002) is located. However, fine-mapping needs to be conducted for an ultimate confirmation. A detailed description of further individual QTL is given in Schnaithmann et al. (2014).

It finally can be concluded that HEB-5 provides a valuable genetic resource based on exotic alleles introgressed by five diverse donors. In the chapters 3.2.2 and 3.2.3 a comparison with the literature in regard to multiple aspects as isolate specificity and other multiparental mapping populations is given.

3.2 Comparison with the literature in regard to strategies and populations

3.2.1 Detecting and improving barley NUE

Barley is a highly diverse crop for which genetic NUE variability was observed (Sinebo et al. 2004; Anbessa et al. 2009; Beatty et al. 2010; Falcon et al. 2014). Therefore, Anbessa and Juskiw (2012) refer that there is a significant margin to improve NUE in barley through breeding. These authors review, that new sources of NUE related genes from across modern germplasm and landraces of barley are needed to be identified and used. The introduction of exotic alleles into the genetic background of cultivated barley is a general target of the AB-QTL approach and derived ILs. Here, it was specified in regard to detect NUE related QTL according to Anbessa et al. (2009), who suggested the identification and introgression of low N adaptive genes from across cultivated or exotic barley into high yielding genetic backgrounds. So far, barley ILs were not proposed for NUE improvement. However, barley landraces that have evolved under N stress conditions were considered as donors for improvement in NUE as recommended by Muurinen et al. (2006) and Anbessa et al. (2010).

In total, 25 QTL showing the same direction (increasing or decreasing) were detected across both N levels. Additionally, 18 QTL were detected only under N0 and 22 QTL only under N1. These results indicate that direct selection under N deficiency and moderate N, respectively is reasonable. Indirect selection in only one of the two N levels would have resulted in losing QTL in the other N level. These findings are in accordance with multiple cited studies where yield improvement under N stress was achieved (Anbessa et al. 2010). On the contrary, Anbessa et al. (2010) recommended indirect selection under 'moderate low N levels', which is comparable to local practice in growing malting barley, because it could be performed as part of the selection strategy for broad adaptation in barley. Similar results for grain yield were observed by Sinebo et al. (2002) who detected a high correlation for barley genotypes in high and low input systems. Particularly for yield QTL, the presented results were contrarily as more than half of the QTL were either found under N stress or conventional N supply. Generally, selection environments depend on breeding goals. For instance, selection for high average yield across multiple high N environments should be suitable for simultaneous improvement under high and moderately low N conditions (Anbessa et al. 2010). In the following, one example for the advantage of direct NUE

QTL selection under N stress is given. For instance, S42IL-130 showed a 19.3 % increase in TGW only under low N. Anbessa and Juskiw (2012) assumed that the greatest improvement in NUE can be made for 'moderate low' N conditions and recommended that NUE research should focus on this practice. Applying this strategy, the QTL detected only under low N would not have been detected. The advantage of multiple, at least two N levels (including N stress) is obvious. QTL under each N level are detectable separately. Additionally, the average across the two N levels simulates a 'third N level'. The S42ILs for which QTL across both N levels were detected showed the tendency for the same behavior under each of both N levels, although significance was not given. For instance, S42IL-134 showed a reduction in HEA under moderate N and across both N levels. This S42IL also showed a reduction in HEA under low N, however, not significantly. Such specifically and generally better-behaving S42ILs only were detected because they were tested under two N levels including the average across both N levels.

The question, how many selection environments shall be used for genetic NUE improvement in general, is ambiguous to answer. For a more general QTL detection of NUE related traits, a low N plus a moderate N level complement each other as demonstrated. For specific QTL detection in low-input systems (*cf.* Sinebo et al. 2004), direct selection under a N stress level only is meaningful (Brancourt-Hulmel et al. 2005).

Plant scientists cultivate and phenotype plants in different environments. Experiments are set up in growth chambers, glasshouses, and field trials. Each of these environments has advantages and disadvantages. In growth chamber and glasshouse experiments, environmental conditions as soil type, irrigation, temperature and illumination are automatable and, are thus, largely controllable compared to field experiments. The experience shows that QTL detected in growth chambers and glasshouses are partly environment-specific and not completely reproducible in the field, where barley finally is grown. However, Beatty et al. (2010) demonstrated a general consistence of NUE characteristics between growth chamber and field experiments.

Multiple NUE related QTL were detected in the present glasshouse experiment. These QTL were compared with QTL of previous studies for some traits that were found with S42-derived lines. Multiple corresponding QTL in S42-derived lines were found. The corresponding QTL were detected under field conditions (von Korff et al. 2006; Schmalenbach et al. 2009; Saal et al. 2011) and in a growth chamber experiment (Wang et al. 2010a). The field experiment conducted by Saal et al. (2011) had two N levels. Stability across environments may be assumed for all QTL for which corresponding QTL were found in the experiments cited above. For the remaining QTL,

glasshouse specific effects as substrate, water and nutrient status, temperature or illumination seem to be crucial.

Genetic improvement in NUE may be achieved by improving morpho-physiological traits, which directly or indirectly contribute to its superior ability to take up and/or utilize available N (Anbessa and Juskiw 2012). Two traits are regarded to be the two most effective ones for increasing NUE in barley: an improved root system and a high above-ground biomass yield (Anbessa and Juskiw 2012). However, conducting field and pot experiments for root system analysis require a high amount of work (*e.g.* Sayed 2011; Arifuzzaman et al. 2014). Hydroponic systems (Beatty et al. 2010; Hoffmann et al. 2012) where roots do not have to be cleaned of soil before biomass determination is generally labor-intensive.

Therefore, above-ground biomass yield which corresponds to straw per plant (STR) plus grain yield per plant (YDP) in the present PhD study was taken as a trait highly correlated with NUE. Lemaire et al. (2007) and Abeledo et al. (2008) refer that high biomass is the result of N utilization efficiency (a part of NUE) for dry matter production including grain yield. Furthermore, Lemaire et al. (2007) consider a co-regulation of N uptake efficiency (additionally a part of NUE) induced by well-developed root systems and above ground biomass. Regarding the total of seven favorite QTL detected for STR and YDP, it may be concluded that the corresponding S42ILs, for instance S42IL-137 accounting for an increase of 20.1 % in STR, provide a good genetic resource for NUE improvement.

A second trait for which multiple QTL were detected, will be discussed in the following. Phenotyping of chlorophyll content during heading (CC_HEA) was conducted with the optical sensor SPAD-502 meter (Minolta Camera Co., Osaka, Japan). The tool is well-established for estimating N demand of plants in applied plant production (*e.g.* Follett et al. 1992; Montemurro et al. 2006). As Emebiri (2013) reviews, it measures the amount of chlorophyll in the leaf, and the results correlate linearly with extractable chlorophyll concentrations (Arunyanark et al. 2008). It shall be mentioned that values measured with the SPAD-502 depend on factors as genotype, plant growth stage, leaf age, measurement position, leaf water status and measurement time for instance (Giunta et al. 2002; Martínez and Guiamet 2004; Spaner et al. 2005). Nevertheless, the dimension-free CC_HEA values are recommended for barley breeding as indirect selection criterion (Giunta et al. 2002). In barley genetics research, the SPAD-502 meter was recently applied for detecting N deficiency and drought stress QTL, respectively (Hoffmann et al. 2012; Honsdorf et al. 2014b). However, only in the latter study exotic QTL alleles were associated with chlorophyll content were detected.

The QTL QCc_hea.S42IL-4H.b detected in the present study under N stress in S42IL-123 was coincident with QSpad.S42IL-4H under drought stress as Honsdorf et al. (2014b) determined.

According to Anbessa and Juskiw (2012), it finally can be concluded that the traits investigated in the present PhD study, in particular STR, YDP and CC_HEA, were meaningful in regard to NUE improvement in barley.

3.2.2 Isolate specificity and genomic regions for quantitative seedling resistance against leaf rust

There are about 350 *P. hordei* isolates available worldwide (Bernardo et al. 2012). However, for QTL studies usually only one to five virulent isolates are chosen as a panel for representing disease pressure. It was demonstrated that molecular diversity in *P. hordei* can be associated with virulence, but not well with the geographic origin (Sun et al. 2007). Therefore, isolates with potentially high virulence are chosen. Most studies (including QTL analyses) were conducted with the isolates '1.2.1' (Marcel et al. 2007a; Shtaya et al. 2007; Silvar et al. 2010; Wang et al. 2010b; Aghnoum and Niks 2012) or 'I-80' due to its powerful virulence. With 'I-80', new qualitative (*Rph*) and quantitative (QTL) resistances against *P. hordei* were detected in multiple studies (Ivandic et al. 1998; Kicherer et al. 2000; Backes et al. 2003; Kopahnke et al. 2004; Silvar et al. 2010; König et al. 2012; Perovic et al. 2013).

The leaf rust QTL detected in the pre-NAM population HEB-5 were found with the *P. hordei* isolate 'I-80' which is a standard isolate avirulent against multiple *Rph* genes (Kopahnke et al. 2004; König et al. 2012) that additionally was successfully applied to detect leaf rust QTL for about two decades (see references above).

The general view is that partial, quantitative resistance is largely race-nonspecific. However, some small cultivar × isolate interactions have been reported (Marcel et al. 2008; González et al. 2012). Thus, the QTL detected in the present PhD study are potentially race-nonspecific or race-specific to 'I-80'. Leaf rust QTL were compared to genomic regions of previous studies, where QTL were detected with different specific isolates in controlled experiments and under natural inoculation. Following the Meta-QTL study of Schweizer and Stein (2011), leaf rust QTL (that were detected with the isolate '1.2.1') cluster in hot spot regions for multiple diseases. As six of eight detected QTL corresponded to the cited QTL and Meta-QTL regions, respectively, it can be assumed that these QTL are race-nonspecific as they

were based on different isolates. Additionally, one QTL (QLr.HEB-5-1H) was detected at the end of the long arm of chromosome 1H at 142.6 cM, where so far no QTL for seedling resistance was known. Findings for isolate specificity were made in multiple QTL studies for leaf rust resistance. Qi et al. (1998, 1999) found that the three QTL with the largest effects were consistently effective against both applied isolates, but that seven QTL with small effects were only effective against one of the two isolates, suggesting an isolate-specific effect (Marcel et al. 2008). On the contrary, Niks et al. (2000) did not find any isolate specificity in detecting leaf rust QTL with four different *P. hordei* isolates.

A leaf rust resistance QTL for adult plant growth stage was recently detected under natural inoculation. It was mapped approximately four cM apart from QLr.HEB-5-1H (Gutiérrez et al. 2015). Gutiérrez et al. (2015) detected the cited QTL across multiple environments and years. A similar coincidence between leaf rust QTL for seedling resistance and resistance during adult plant growth stage was detected by Qi et al. (1998). However, such coincidences between quantitative seedling resistance and QTL for adult plant growth stage are rare and, additionally, may depend on QTL × location and QTL × year interactions (von Korff et al. 2005; Castro et al. 2012; Ziems et al. 2014).

The mapping results suggest a combination of race-nonspecificity and race-specificity of the genes underlying barley seedling leaf rust resistance. However, this hypothesis has to be confirmed or rejected by the final NAM population HEB-25 (Maurer et al. 2015) with different leaf rust isolates and natural inoculation, respectively across locations and years.

Furthermore, it is known that QTL may depend on the mapping population (Ziems et al. 2014). It shall be pointed out that these QTL × population interactions are (theoretically) lower for multiparental populations like HEB-5 compared to bi-parental populations, because in the first one, (theoretically) more than two segregating alleles at each locus provide higher diversity.

In addition, corresponding genomic regions where qualitative, mainly as 'race-specific' regarded *Rph* genes are located were compared to the presented QTL regions. It recently was assumed that *Rph20* conferred partial resistance due to a combination with QTL (Ziems et al. 2014). The four leaf rust resistance genes *Rph14* (Golegaonkar et al. 2009a), *Rph15* (Weerasena et al. 2004), *Rph16* (Ivandic et al. 1998) and *Rph17* (Pickering et al. 1998) on chromosome 2H and *Rph19* (Park and Karakousis 2002) on chromosome 7H were located on the same chromosome or chromosome arm as the detected QTL. Of these, *Rph16* was detected with the isolate 'I-80'. It could be that

the cited *Rph* genes map to the same positions as the detected QTL. However, for confirmation of coincident map positions fine-mapping of the five cited *Rph* genes is necessary. Eventually, the *Rph* genes on the same chromosomes where QTL were found conferred partial resistance like *Rph20*. Backes et al. (2003) reviewed potentially causing factors for the co-location of *Rph* genes and resistance QTL. These factors may be understood as a relativization of the strict separation between *Rph* genes and QTL. They are an explanation approach for the complexity of co-localizations that can be assigned to the present results. For instance, a QTL may result in complete susceptibility or resistance against the inoculum. However, an inoculum often consists of isolate mixtures and, thus, suggests partial resistance. Furthermore, combinations of *Rph* genes can result in higher resistance levels than the individual genes. The third and fourth factors listed in Backes et al. (2003) are that *Rph* genes that were overcome by the pathogen can afterward act as QTL, or that it is possible for *Rph* loci to represent 'extreme' alleles of QTL. A further assumption is that *Rph* genes and QTL could belong to the same or closely related gene family. Finally, Backes et al. (2003) review that regulatory elements and genes necessary for complementary factors in resistance reaction can be located in close linkage to *Rph* genes and QTL.

3.2.3 Comparison of the pre-NAM population HEB-5 with other multiparental populations

This subchapter comprises the discussions for the SNP saturation and the population design of the pre-NAM population HEB-5. Subsequently, multiple statistical models are compared with the model chosen in the present PhD thesis.

The pre-NAM population HEB-5 was genotyped with 1,211 informative BOPA1 SNPs (Close et al. 2009) following the revised SNP order of Muñoz-Amatriaín et al. (2011). The mean genome coverage between two single adjacent SNPs was 1.0 cM, which was in the range of marker saturation described in other high-density maps (Sato and Takeda 2009; Li et al. 2010; Sato et al. 2011; Schmalenbach et al. 2011). The final NAM population HEB-25 was genotyped with 5,709 informative Infinium iSELECT 9 k barley SNPs described by Comadran et al. (2012). This genotyping resulted in an average genetic distance of 0.17 cM between adjacent SNPs (Maurer et al. 2015). However, the maximum genetic distance between two adjacent SNPs of HEB-5 was in the same range as of HEB-25. For HEB-5 the distance was estimated with 11.2 cM and for HEB-25 with 11.1 cM (Maurer et al. 2015).

The NAM approach was originally applied in maize. There, an average marker saturation of 1.3 cM based on 1,106 informative SNPs was achieved (McMullan et al. 2009). For the sorghum NAM population (Jordan et al. 2011) it was referred to the consensus map developed by Mace et al. (2009) with an average marker saturation of 0.79 cM. The average marker distance in the barley MAGIC population using the Infinium iSELECT 9 k chip (Sannemann et al. 2015) resulted in 0.22 cM. For hexaploid wheat, different marker density for each genome is reported in MAGIC populations. For instance, Huang et al. (2012) achieved 2.4 to 8.7 cM with a chip consisting of 1,162 SNP, SSR, and DArT markers, and Cavanagh et al. (2013) referred to a consensus map with a marker saturation of 0.9 to 2.9 cM, achieved with the '9K iSelect Beadchip Assay' (7,733 SNPs).

Thus, the marker densities depend on the crop, the marker system and the individual type of chip (number of markers). For instance, the SNP density was increased six times from HEB-5 (BOPA1) to HEB-25 (Infinium iSELECT 9 k). The mapping population plays a minor role as HEB-25 and the barley MAGIC population both were genotyped with the Infinium iSELECT 9 k chip and genotyping resulted in the same SNP saturation range for both multiparental populations.

The barley pre-NAM population HEB-5 consisted of 295 pre-NAM lines distributed among five pre-NAM families and it was demonstrated that it is possible to detect exotic QTL alleles that were associated with leaf rust scores with this number of pre-NAM lines. The final barley NAM population HEB-25 consists of 1,420 barley NAM lines distributed among 25 NAM families (Maurer et al. 2015). Recently, the power of HEB-25 was demonstrated in detecting eight major QTL where exotic alleles were responsible for a genotypic variance of 64 % in flowering time (Maurer et al. 2015).

The population design of the multiparental barley MAGIC population developed by Sannemann et al. (2015) differs in regard to multiple aspects with the population design of the pre-NAM population HEB-5. The MAGIC population consists of approximately 5000 barley DH lines, whereof 533 were selected for QTL analysis. The development of the MAGIC lines is described in Sannemann et al. (2015) in detail. The constitutive difference compared to the pre-NAM lines is that exclusively cultivars (and no exotic barley accessions) and, subsequently, the progeny of each generation was crossed among each other. Such intercrosses finally result in individual lines that theoretically carry alleles of each parent across their genome. On the contrary, each individual NAM line only carries alleles from either the exotic founder or the line that was chosen for initial cross and backcross, respectively. Additionally, a specific difference between the two multiparent barley populations

named pre-NAM and MAGIC shall be referred. The pre-NAM lines had a theoretical heterozygosity of 25.0 %, whereas DH lines are theoretically completely homozygous.

According to the NAM population design, each NAM family may be handled as an individual bi-parental population for 'classical' QTL mapping. Furthermore, multiple bi-parental populations can be combined to a subset of the complete NAM population. This could be interesting for splitting the whole NAM population into subsets based on geographic regions of the individual exotic founders. Such splitting is not possible in MAGIC populations, although final MAGIC lines can be traced back to (sub-)subfamilies of earlier generations (Sannemann et al. 2015).

General linear models use the least square means method of observed values (here: measured traits) for multiple statistical models including analysis of variance and multiple linear regression (SAS Institute Inc. 2009). For QTL detection, a mixed (linear) model analysis (of variance) with a subsequent line × trait association study was conducted with the statistical software SAS® Enterprise Guide® 4.2 (SAS Institute Inc. 2008). Currently, mainly the mixed model and linear regression approaches are recommended and applied in the scientific community (Würschum et al. 2012; Korte and Farlow 2013). In addition to the general linear model, the mixed model allows the extension by variances and covariances of means (SAS Institute Inc. 2009). Recent examples are Arifuzzaman et al. (2014) and Honsdorf et al. (2014a, 2014b).

The NAM approach was classified to mixed forms of family mapping and population mapping. For the latter type of approach it is common to correct the statistical model for population structure and genetic relatedness by cofactors (Kang et al. 2008). The reason is to reduce the amount of false positive marker × trait associations. According to Price et al. (2006), a principal component (PCo) analysis was conducted. The PCos were estimated by relatedness of individuals based on SNPs and included as cofactors into the statistical model for GWAS. These cofactors reduce the detection of false-positive associations. Subsequently, a false discovery rate (FDR) correction for multiple testing according to Benjamini and Hochberg (1995) was conducted. This is an additional approach to reduce false-positive positive marker × trait associations at a defined level of significance (here: $P(\text{FDR}) < 0.05$), that was applied in NAM populations of other crops (Mace et al. 2013).

The accuracy and robustness of the detected QTL is profoundly based on the chosen statistical model. Different statistical models including mixed model and multiple regression approaches correct for population structure

and kinship in GWAS (*cf.* Cockram et al. 2008; Stich et al. 2008; Stich and Melchinger 2009; Würschum et al. 2012). However, model comparisons are suggested to detect the best fitting statistical model (Liu et al. 2011b; Würschum et al. 2012). Potentially, an improved statistical model could have been found for leaf rust QTL detection in the pre-NAM population HEB-5, if a model comparison would have been conducted. For instance, the statistical model proposed by Liu et al. (2011b), originally developed for GWAS in maize, was found to be most suitable for populations consisting of 'multiple' related families (Würschum et al. 2012) and it was applied in the barley NAM population HEB-25 (Maurer et al. 2015). The model is based on multiple linear regression and contains cofactors which control for population structure and genetic background, a SNP effect and a family effect (Würschum et al. 2012). Such statistical models would verify or reject specific leaf rust QTL of HEB-5. Therefore, it is suggested to compare the QTL mapping results with different statistic models in general to ensure a higher grade of QTL robustness.

According to Pasam et al. (2012), it reminds that correcting for population structure not only reduces the number of false-positives but also may reduce false-negative marker × trait associations.

3.3 Future prospects of QTL mapping

The prerequisite for a successful application of genetic and genomic tools is the best possible phenotyping (Varshney et al. 2005). In the present study, QTL of multiple traits were detected for several S42ILs and pre-NAM lines. However, all traits were phenotyped under glasshouse conditions. Thus, phenotyping should additionally be conducted in field experiments. The QTL confirmed under natural cultivation conditions would be of further interest for breeders and plant scientists. They could be implemented into breeding programs and scientists would be able to give a more generalized evidence of QTL regions.

Currently, phenotyping is still mainly conducted by humans whose perception is partly subjective. Potentially, future technologies supporting and replacing human evaluation of plants will take place. The sensing tool 'SPAD meter' which measures the leaf greenness ('chlorophyll content') already provides a basis for specific N fertilization (Huang et al. 2011; Winterhalter et al. 2012). Furthermore, high-throughput phenotyping provides a great potential for multiple traits and it was already applied in the glasshouse. 'The Plant Accelerator®', for instance, takes images of plants and computes quantitative measures based on algorithms. Growth curves and growth rates, plant health status and further traits are calculated (Honsdorf et al. 2014a). For pathogens like *P. hordei* proximal sensing of plant-pathogen interactions based on chlorophyll fluorescence was already applied and provides an insight into plant-physiological alterations due to pathogen infections (Leufen et al. 2014). Such sensing data potentially provides a novel category for association mapping of resistance genes. Apart from automated phenotyping for QTL detection and applied plant breeding, these phenotyping techniques are discussed in precision farming where optical sensors detect the nutrient demand of crops in real-time.

For leaf rust QTL detection, further isolates are an option for generalizing the present results. Potentially, hot spot regions for multiple diseases including leaf rust during seedling and adult plant stage will be confirmed or individual QTL defined as race-specific. Furthermore, coincidences with *Rph* genes for which virulence is given with I-80 could be investigated. For the final NAM population HEB-25, QTL mapping of traits including morphological traits, multiple disease resistances and response to stresses like N deficiency are planned (Pillen pers. comm.).

High-resolution mapping provides the basis for map-based cloning of genes. Specific S42ILs and HEB-25 lines are valuable sources for fine-mapping of QTL toward map-based cloning of CGs. Currently, it is panned to clone

the *thresh-1* locus detected in S42IL-HR (Schmalenbach et al. 2011) and the powdery mildew resistance genes out of lines from the two barley populations S42IL-HR and HEB-25 (Pillen pers. comm.). Furthermore, exome capture based re-sequencing (Mascher et al. 2013b) could be applied in both populations. This approach would give an insight to exotic and cultivated allele effects.

The performance of statistical models will likely play a more important role in the near future. Therefore, cross-validation was recently applied in QTL mapping. It is a statistical method of splitting data into training and validation sets by using the validation set to evaluate the prediction ability of the trained model (Guo et al. 2013). For the final barley NAM population HEB-25 (Maurer et al. 2015) and the barley MAGIC population (Sannemann et al. 2015) models that best describe the genotypic variance were estimated bases on cross-validation. Finally, the SNP that best explains specific QTL and CGs, respectively may be detected.

Chapter 4

Summary

One aim of modern plant breeding research is to introduce favorable exotic alleles from wild barley into cultivated barley, as this provides multiple desired characteristics. Most of these traits (*e.g.* yield) are complex and determined through multiple genes. Such genes are represented through quantitative trait loci (QTL) that are mapped molecularly.

Under this theme multiple traits were mapped in barley in the present study. The first mapping population was the introgression line population 'S42IL'. It was originally developed out of the bi-parental crossing between the German elite variety 'Scarlett' and the Israeli exotic form 'ISR42-8'. The second mapping population was 'HEB-5' that was developed in the framework of the present study. It consisted of progeny originating from the crossing between the German elite variety 'Barke' and five highly diverse exotic barley accessions from the Fertile Crescent. The development and the QTL mapping followed the principle of nested association mapping (NAM) and should be regarded as a precursor (pre-NAM) for the final NAM population 'HEB-25' (Maurer et al. 2015).

Nitrogen (N) is a macro nutrient of plants. For economic and ecological reasons QTL should be detected and selected under N deficiency and conventional conditions to finally develop more efficient barley cultivars. The QTL mapping in S42IL occurred in a glasshouse experiment. Therefore, 28 S42ILs were tested under two N levels (stress and conventional condition). In total, 15 morphological traits, grain parameters as well as carbon (C) and N traits were investigated.

Across all barley chromosomes, 65 QTL were detected that were distributed across both N levels as follows: Twenty-two times only under conventional condition, 18 times only under N deficiency and 32 times under

both N levels. The number of QTL varied depending on the trait. For chlorophyll content during heading ten QTL and for C/N ratio in the straw, only one QTL was found. The number for the remaining traits lay in between (whereas for C/N ratio in seeds no QTL was detected). The number of QTL varied depending on the S42IL and, thus, on the exotic chromosome segment. The exotic segment of S42IL-108 resulted in eight QTL. In contrast to this no QTL was detected with S42IL-145. The QTL regions were compared with results of previous studies. Here, corresponding QTL and potentially responsible candidate genes were detected concerning multiple traits. Specific S42ILs were advantageous compared to 'Scarlett'. For instance, S42IL-119 showed a 20 % higher thousand grain weight under N deficiency conditions as 'Scarlett'. In general, the results showed that breeding under N deficiency is meaningful and that specific S42ILs may be implemented into breeding programs.

Leaf rust caused by the fungal pathogen *Puccinia hordei* is an important foliar disease of barley, for which qualitative and quantitative resistances are known. The pre-NAM population HEB-5 was investigated in regard to leaf rust resistance during seedling stage in a glasshouse experiment. The main aim was to test if the NAM approach can be successfully transfered from maize to barley. A QTL analysis concerning *P. hordei* resistance was conducted for HEB-5 and for each of the five pre-NAM families.

Thereby it was demonstrated that QTL were detected across HEB-5 (four QTL) as well as within family HEB-F23 (four QTL), however, they were not detected within the remaining four NAM families. On chromosome 4H at 61.6 cM a QTL of HEB-5 and a QTL of HEB-F23 overlapped. Most QTL were found in hot spot regions where alleles code for leaf rust resistance and other diseases resistances. Furthermore, potential chromosomal coincidences with qualitative resistance genes were found. A novel QTL was detected in HEB-5 on chromosome 1H which showed a 33 % decrease in leaf rust symptoms compared to 'Barke'. Additionally, a leaf rust QTL was detected within HEB-F23 on chromosome 7H, where the exotic alleles reduced the symptoms by 36 %. Both genomic regions are potentially favorable sources for leaf rust resistance breeding. These findings demonstrate the high potential of using the NAM approach for barley breeding research.

Chapter 5

Zusammenfassung

Ein Ziel der modernen Pflanzenzüchtungsforschung ist es, vorteilhafte exotische Allele aus Wildgerste in Kulturgerste zu übertragen, da diese mehrere gewünschte Eigenschaften bereitstellt. Die meisten dieser Merkmale (z.B. Ertrag) sind komplex und durch viele Gene bestimmt. Solche Gene werden durch quantitative Merkmalsloci (engl.: *quantitative trait loci*, QTL) repräsentiert, die molekular kartiert werden.

Basierend darauf wurden in der vorliegenden Arbeit QTL für verschiedene Merkmale in Gerste kartiert. Die erste Kartierungspopulation war die bereits zur Verfügung stehende Introgressionslinienpopulation 'S42IL'. Sie wurde ursprünglich aus der bi-parentalen Kreuzung zwischen der deutschen Elitesorte 'Scarlett' und der israelischen exotischen Form 'ISR42-8' erstellt. Die zweite Kartierungspopulation war 'HEB-5' welche im Rahmen dieser Arbeit erstellt wurde. Sie bestand aus Nachkommen der Ursprungs-Kreuzung zwischen der deutschen Elitesorte 'Barke' und fünf hoch-diversen exotischen Gerstenakzessionen des Fruchtbaren Halbmonds. Die Erstellung und QTL-Kartierung erfolgte nach dem Prinzip der genesteten Assoziationskartierung (engl.: *nested association mapping*, NAM) und sollte als Vorläufer (prä-NAM) für die finale NAM-Population 'HEB-25' (Maurer et al. 2015) geprüft werden.

Stickstoff (N) ist ein Makronährstoff der Pflanzen. Aus ökonomischen und ökologischen Gründen sollten QTL unter N-Mangel und -Normalbedingungen detektiert und selektiert werden, um letztendlich N-effizientere Gerstensorten zu entwickeln. Die QTL-Kartierung in S42IL erfolgte in einem Gewächshausversuch. Hierzu wurden 28 S42ILs unter zwei N-Stufen (Stress und Normalbedingung) getestet. Insgesamt 15 morphologische Merkmale, Kornparameter sowie Kohlenstoff (C)- bzw. N-Merkmale wurden untersucht.

Es wurden 65 QTL auf allen Gerstenchromosomen detektiert, die wie

folgt über beide N-Stufen verteilt waren: Zweiundzwanzig mal nur unter Normalbedingungen, 18 mal nur unter N-Mangel und 32 mal in beiden N-Stufen. Die Anzahl der QTL variierte je nach Merkmal. Für den Chlorophyllgehalt während der Blüte wurden zehn QTL und für das C/N-Verhältnis im Stroh nur ein QTL gefunden. Die Anzahl für die verbleibenden Merkmale lag dazwischen (wobei für das C/N-Verhältnis im Korn kein QTL detektiert wurde). Die Anzahl der QTL variierte je nach S42IL und somit je nach exotischem Chromosomensegment. Das exotische Segment von S42IL-108 resultierte in acht QTL. Im Gegensatz dazu wurden mit S42IL-145 keine QTL detektiert. Die QTL-Regionen wurden mit Ergebnissen aus vorherigen Studien verglichen. Hierbei wurden Übereinstimmungen mit QTL und potentiell verantwortlichen Kandidatengenen hinsichtlich mehrerer Merkmale entdeckt. Spezifische S42ILs waren vorteilhaft gegenüber 'Scarlett'. Zum Beispiel zeigte S42IL-119 unter N-Mangel ein 20 % höheres Tausendkorngewicht als 'Scarlett'. Generell zeigten die Ergebnisse, daß Züchtung unter N-Mangel-Bedingungen sinnvoll sein kann und spezifische S42ILs in Züchtungsprogramme integriert werden könnten.

Zwergrost, hervorgerufen durch den pilzlichen Erreger *Puccinia hordei*, ist eine bedeutende Blattkrankheit der Gerste, für welche qualitative und quantitative Resistenzen bekannt sind. Die prä-NAM-Population HEB-5 wurde in einem Gewächshausversuch hinsichtlich Zwergrostresistenz im Sämlingsstadium untersucht. Das Hauptziel dabei war, zu testen, ob man den NAM-Ansatz von anderen Kulturarten auf Gerste übertragen kann. Eine QTL-Analyse hinsichtlich *P. hordei*-Resistenz wurde für HEB-5 und für jede der fünf prä-NAM-Familien durchgeführt.

Hierbei zeigte sich, daß QTL sowohl in HEB-5 (vier QTL) als auch in der Familie HEB-F23 (vier QTL), nicht jedoch in den verbleibenden vier Familien gefunden wurden. Auf Chromosom 4H bei 61.6 cM überlappten ein QTL von HEB-5 und HEB-F23. Die meisten QTL wurden in Hotspot-Regionen gefunden, wo Allele für Zwergrost-Resistenz und andere Krankheitsresistenzen kodieren. Des Weiteren wurden potentiell chromosomale Übereinstimmungen mit verschiedenen qualitativen Resistenzgenen gefunden. Auf Chromosom 1H wurde ein neuer QTL in HEB-5 detektiert, der eine um 33 % verringerte Zwergrostausprägung verglichen mit 'Barke' zeigte. Zudem wurde in HEB-F23 auf Chromosom 7H ein Zwergrost-QTL detektiert, bei dem die exotischen Allele die Merkmalsausprägung um 36 % verringerten. Beide Genomregionen sind potentiell vorteilhafte exotische Quellen für die Zwergrost-Resistenzzüchtung. Die vorliegenden Ergebnisse belegen anschaulich das Potential des NAM-Ansatzes für die Gersten-Züchtungsforschung.

Chapter 6

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Chapter 7

Abbreviations

Table 1 List of abbreviations

Abbreviation	Explanation
AB-QTL	Advanced Backcross QTL
AFLP	Amplified fragment length polymorphism
AspAT	Aspartate aminotransferase
BAC	Bacterial artificial chromosome
BC_2	Backcross two
BOPA1	Barley oligonucleotide pool assay 1
C	Carbon
C_CONT_G	C content of grains
C_CONT_STR	C content of straw
CC_HEA	Chlorophyll content at heading
cf.	*confer* (from Latin): 'compare'
CG	Candidate gene
cM	Centimorgan
CN_G	C/N ratio of grains
CN_STR	C/N ratio of straw
CNX-1	Cofactor for nitrate reductase and xanthine dehydrogenase
$CO(NH_2)_2$	Urea
DArT	Diversity array technology
DH	Doubled haploid
DNA	Desoxyribonucleic acid
e.g.	*exempli gratia* (from Latin): abbr. for 'for example'
F_x	Filial generation x

Table 1 continued

Abbreviation	Explanation
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDR	False discovery rate
G_AREA	Grain area
G_LENGTH	Grain length
G_WIDTH	Grain width
Gb	Gigabase (pairs)
GBS	Genotyping-by-sequencing
GEA	Grains per ear
GOGAT	Glutamate synthase
GPC1	Grain protein content region 1
GS	Glutamine synthetase
GWAS	Genome-wide association study
HEA	Days until heading
HEB-25	Halle Exotic Barley 25
HEB-5	Halle Exotic Barley 5
HR	High resolution
Hsp	*Hordeum vulgare* ssp. *spontaneum*
Hv	*Hordeum vulgare* ssp. *vulgare*
IL	Introgression line
ITIS	Integrated Taxonomic Information System
Kb	Kilo base (pairs)
LD	Linkage disequilibrium
MAGIC	Multiparent advanced generation intercross
Mb	Mega base (pairs)
N	Nitrogen
N_CONT_G	N content of grains
N_CONT_STR	N content of straw
NAM	Nested association mapping
NGS	Next-generation sequencing
NH_4^+	Ammonium
NO_3^-	Nitrate
NUE	Nitrogen use efficiency
P. anomala	*Puccinia anomala*

Table 1 continued

Abbreviation	Explanation
P. graminis	*Puccinia graminis*
P. hordei	*Puccinia hordei*
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pre-NAM	Pre-nested association mapping
QTL	Quantitative trait locus
R gene	Resistance gene
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
Rpg	Resistance against *P. graminis*
Rph	Resistance against *P. hordei*
S42	Name of barley population derived from the cross 'Scarlett' × S42
S42IL	Population S42IL / Introgression line of the population S42IL
SNP	Single nucleotide polymorphism
SPAD	Soil-Plant Analysis Development
SSR	Simple sequence repeat
STR	Straw per plant (excluding ears) at maturity
TGW	Thousand grain weight
YDP	Grain yield per plant

Chapter 8

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Chapter 9

Curriculum vitae

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2007-11 - 2008-03	Research assistant at the Max Planck Institute for Plant Breeding Research in Cologne, Germany
2007-03 - 2007-10	Assistant breeder at the breeding company SW Seed Hadmersleben GmbH in Hadmersleben, Germany
2003-10 - 2007-02	Assistant breeder at the breeding company Saatzucht Josef Breun GdbR in Herzogenaurach, Germany
2000-07 - 2000-09	Trainee at the breeding company Südwestsaat GbR in Rastatt, Germany

Education

Since 2008-04	PhD student at the Chair of Plant Breeding at the Martin Luther University Halle-Wittenberg in Halle (Saale), Germany
2009-03	Participant at the course 'Quantitative Methods in Plant Breeding' at the National Institute of Agricultural Botany in Cambridge, Great Britain
1998-10 - 2003-09	Student of Agricultural Science (with focus on Plant Breeding) at the University of Hohenheim in Stuttgart, Germany

Chapter 10

List of publications

Maurer A, Draba V, Jiang Y, **Schnaithmann F**, Sharma R, Schumann E, Kilian B, Reif JC, Pillen K (2015) Modelling the Genetic Architecture of Flowering Time Control in Barley through Nested Association Mapping. BMC Genomics 16:290
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Pillen K, Draba V, Maurer A, **Schnaithmann F**, Pencs S, Kopahnke D, Buettner B, Schweizer G, Ordon F, Schumann E (2014) Allele Mining Using the Barley Nested Association Mapping Population HEB-25. Plant & Animal Genome (PAG) XXII, W084, San Diego, CA, USA

Flavell AJ, Xu X, Comadran J, Thomas WTB, Marshall DF, Russell J, Waugh R, Tondelli A, Cattivelli L, **Schnaithmann F**, Pillen K, Schulman AH, Ingvardsen C, Rasmussen SK, Sharma R, Kilian B: An incremental approach for association mapping of useful traits in barley - The EXBARDIV Project. 2nd International Symposium on Genomics of Plant Genetic Resources 24-27.04-2010 Bologna, Italy

Chapter 13

Declaration under oath

Eidesstattliche Erklärung / Declaration under Oath

Ich erkläre an Eides statt, daß ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

Datum / Date Unterschrift des Antragstellers / Signature of the applicant