Aus dem Institut für Agrar- und Ernährungswissenschaften
(Geschäftsführender Direktor: Prof. Dr. Reinhold Jahn)

der Naturwissenschaftlichen Fakultät III
(Dekan: Prof. Dr. Peter Wycisk)

der Martin-Luther-Universität Halle-Wittenberg

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Selection and Phenotypic Evaluation of a Wild Barley Introgression Library

Dissertation

zur Erlangung des akademischen Grades
doctor agriculturarum (Dr. agr.)

von

Diplom-Agraringenieurin Inga Schmalenbach

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vorgelegt von
Diplom-Agraringenieurin Inga Schmalenbach
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1 General introduction

Since ancient times, barley (*Hordeum vulgare* L.) has been used with versatility by man. Nowadays, it is one of the major cereal crops worldwide and also an important model crop in modern plant genetic research. A multitude of valuable barley genetic resources and tools, also useful for comparative genome research in other cereal species, is now available. Current efforts for sequencing the whole barley genome will, in the future, provide deep insights into biological mechanisms such as gene regulation and genome organization. Nevertheless, molecular markers which revolutionized the genetic research and molecular biology sector since their advent in the early 1980s, remain as a genomic tool with outstanding value. For instance, molecular markers are essential for decoding the genetic determinants of complex traits which is still a great challenge in modern plant breeding. In order to identify and localize the numerous genes underlying complex traits, diverse studies, mostly using elite cultivars, have been conducted in barley and other crop species. The success of these studies has been limited by the low genetic diversity present in the elite gene pool. To overcome this limitation unadapted germplasm offering a higher rate of genetic diversity have been established as an alternative. For instance, the wild ancestor of barley (*H. vulgare* ssp. *spontaneum*) has proved to be a valuable source of novel alleles resulting in enhanced disease resistance, abiotic stress tolerance or yield performance. Because these alleles are of value for plant breeding only if transferred into the elite gene pool, new genetic resources such as advanced backcross populations or introgression lines have been developed in recent years. Due to the low portion of exotic germplasm present in these resources, they provide a great basis in order to detect and utilize favorable exotic alleles almost simultaneously. The present study exemplifies the usefulness of wild barley introgression lines for revealing the genetic basis of complex traits and for identifying new favorable exotic alleles.

1.1 Barley - relevance, taxonomy, origin and domestication

With the production of more than 138 million tons in 2006 (FAOSTAT), barley ranks number four among the world cereals after wheat, rice and maize. In industrialized countries, including Germany which ranks among the three countries with the highest production (FAOSTAT), barley is mainly used for livestock feeding and as a substrate for brewing beer and distilling whisky.

Due to its great ecological adaptability, barley furthermore plays a major role for human nutrition in regions with extreme climates or salinity of soil in, for instance in North Africa or the mountainous regions of Central Asia (von Bothmer et al. 2003a).

Barley belongs to the tribe *Triticeae* and the genus *Hordeum* which comprises 32 species and 45 taxa including diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) cytotypes. Common to all *Hordeum* species is the existence of three, one-flower spikelets at each rachis node, among which the two lateral florets are either sterile as in two-rowed forms or fertile as in six-rowed forms (von Bothmer et al. 2003b). The high genetic variability existent in the genus *Hordeum* is for instance demonstrated by spring and winter types and forms with hulled or naked caryopsis (von Bothmer et al. 1995). According to the gene pool concept developed by Harlan and de Wet (1971), the genus *Hordeum* has been divided into three different gene pools. Since there exist no crossing barriers between them, cultivated barley (*Hordeum vulgare* ssp. *vulgare* L., hereafter abbreviated with *Hv*) and its wild progenitor (*Hordeum vulgare* ssp. *spontaneum* C. Koch, hereafter abbreviated with *Hsp*) build the primary gene pool. Common characteristics of both subspecies are the diploid level of ploidy, the annual character and a relatively strong self-pollination (Zohary and Hopf 2000). The secondary gene pool includes solely the wild species *H. bulbosum* which shows diploid as well as tetraploid cytotypes (Zeller 1998). This species can be crossed with *Hv*, but, due to the elimination of the *H. bulbosum* chromosomes after the first cell divisions of the embryo, the hybrids are generally sterile. However, this phenomenon is utilised for the production of double haploids (Becker 1993). The tertiary gene pool comprises the remaining *Hordeum* wild species, such as different subspecies of *H. murinum*, which possess different levels of ploidy and exhibit sterility barriers with *Hv*.

Based on archaeological remains of barley grains, the Fertile Crescent in the Middle East has been defined as the origin and domestication site of the oldest crops of human like barley, einkorn, emmer, and lentils. About 10,000 years ago, domesticated barley most likely arose in the Israel-Jordan area (Badr et al. 2000). In general, wild species were modified during domestication to meet human needs resulting in a common group of traits distinguishing most food crops from their progenitors. The modification of these traits during domestication is known as ‘domestication syndrome’ (Hammer 1984, Doebley et al. 2006). Examples of modified traits

which are distinctive for cultivated crops include larger but fewer fruits or grains, loss of natural seed dispersal and seed dormancy, and changes in photoperiod sensitivity.

As underlined by Salamini et al. (2002), three key traits - non-brittle rachis, six-rowed spike and naked caryopsis - are associated with the transition of wild to cultivated barley. These new emerged phenotypes were associated with a dramatic change in productivity. The development of a non-brittle rachis enabled an efficient harvest without loss of grains as the basic prerequisite for barley cultivation. The selection for the six-rowed spike character resulted in a considerable increase in yield, whereas the naked caryopsis phenotype is directly linked to dietary use (Pourkheirandish and Komatsuda 2007). All three phenotypes are determined by mutation of single or few genes during domestication and show recessive inheritance. The most important non-brittle rachis genes *btr1* and *btr2* are tightly linked and map to the short arm of barley chromosome 3H (Komatsuda and Mano 2002). The six-rowed spike character is controlled by at least five independent *vrs* loci located on chromosome arms 1HL, 2HL, 3HL, 4HS and 5HL, among which *vrs1* is probably the most important gene (Lundqvist et al. 1997). The naked caryopsis phenotype is controlled by *nud*, a single recessive gene which maps to chromosome arm 7HL and affects agronomic traits, like decreased yield and lower seed weight (Choo et al. 2001). Moreover, in comparison to wild barley, cultivated barley is characterized by reduced dormancy and reduced vernalization requirement (Pourkheirandish and Komatsuda 2007). Both features are essential for a widespread agricultural use of barley.

1.2 Barley genomics

In addition to its high economic relevance, barley is an important model crop for plant genetic research. Due to extensive chromosomal synteny, barley is especially useful for comparative analyses within the tribe *Triticeae* including wheat. The advantages of barley compared to other crop species are as follows: It is a diploid, self-pollinating organism, it possesses a low number (n = 7) of large sized chromosomes (6-8 μm), it is easy to cross, it has a relatively short life cycle of approximately 15 weeks, it provides a wide range of physiological and morphological variation, and it offers a great adaptability to extreme environments (Sato et al. 2003).

Moreover, several tools for modern genome research are available for barley. DHs (doubled haploids) can be easily generated via anther culture or crossing with *H. bulbosum*. Since they are completely homozygous, DH lines constitute an immortal resource for the production of mapping populations (Hearnden et al. 2007, Karsai et al. 2007), and are routinely used in practical barley breeding (Tuvesson et al. 2007).

One essential genomics tool, useful particularly for structural but also for functional genomics, are molecular markers. The first generation of molecular marker systems, developed in the early 1980s, are RFLP (restriction fragment length polymorphism) markers which have been used for constructing several genetic linkage maps of barley (Graner et al. 1991, Heun et al. 1991). PCR (polymerase chain reaction)-based DNA markers, relying on sequence variation in annealing sites or DNA length differences between amplified products, are referred to as second generation molecular markers (Somers 2004). Here, the most important category are SSR (simple sequence repeat) or microsatellite markers (Li et al. 2002). Microsatellites are iterations of short tandemly organized DNA sequences such as mono-, di-, tri-, tetra- or penta-nucleotide repeats. Multiple alleles of a plant species are distinguished based on a variation in the number of repeat units (Powell et al. 1996). SSR markers are abundant with a uniform genome coverage, co-dominantly inherited, multi-allelic, highly informative, robust, reproducible, and easily detectable (Gupta and Varshney 2000). Thus, they have been extensively applied in crop species for different purposes. In barley, SSR markers have been used, for instance for constructing linkage maps (Liu et al. 1996, Ramsay et al. 2000, Li et al. 2003a), DNA fingerprinting and variety identification (Russell et al. 1997, Lin et al. 2007), genetic diversity studies (Brantestam et al. 2007, Eleuch et al. 2008, Yahiaoui et al. 2008), and QTL (quantitative trait locus) studies (e.g. Karakousis et al. 2003, Li et al. 2008, von Korff et al. 2006).

In recent years, available sequence data generated from EST (expressed sequence tag) sequencing projects in combination with new bioinformatic tools have facilitated and accelerated the development of SSR markers and microsatellite maps in barley (Pillen et al. 2000, Varshney et al. 2007a, Hearnden et al. 2007). ESTs are 200-500 bp long single sequence reads from individual clones which are randomly chosen from a cDNA (complementary DNA) library. Since ESTs originate from a sequence transcribed in a certain tissue at a particular stage of development, they only represent transcribed gene regions (Adams et al. 1991). To date, the NCBI (National Center

for Biotechnology Information) provides ~ 501,000 barley ESTs for general use (http://www.ncbi.nlm.nih.gov/dbEST/dbESTsummary.html). ESTs have also been used for developing and mapping SNP (single nucleotide polymorphism) and InDel (insertion/deletion) markers in barley (Stein et al. 2007; Kota et al. 2003, 2008). Due to their high abundance and uniform distribution throughout the genome, SNPs are nowadays the markers of choice and have been extensively applied in plant genetics and breeding (reviewed by Rafalski 2002a, Jehan and Lakhanpaul 2006). In barley, SNPs have been used for genetic diversity studies (Russell et al. 2004, Varshney et al. 2007b) and haplotype analysis (Cockram et al. 2007). More than 30 diverse SNP genotyping methods based on different reaction principles have been developed (Syvänen 2001, Kim and Misra 2007). One method applied in barley is the analysis of CAPS (cleaved amplified polymorphic sequence) markers, which are inexpensive and easily performed, , but are not suitable for high throughput genotyping (Zhang et al. 2007, Varshney et al. 2008). In contrast, microarray technology like the Illumina GoldenGate or the Affymetrix GeneChip assay, based on hybridization with allele-specific probes, provides an opportunity to analyse up to thousands of SNPs in parallel (Gupta et al. 2008). In barley, an Illumina assay for detection of SNPs in more than 1,500 unigenes was used for a whole-genome association study (Rostoks et al. 2006). More recently, the multiplexing Illumina technology has also proven useful for ultra-high-throughput genotyping in more complex and so far poorly known plant genomes, like soybean and spruce (Hyten et al. 2008, Pavy et al. 2008). Another high-density platform for molecular marker analysis is the Diversity arrays technology (DArT) which is also based on microarray hybridization. This technology has been extensively used, for instance for the construction and application of high-density genetic maps in barley (Wenzl et al. 2004, 2006; Hearnden et al. 2007; Comadran et al. 2008; Pswarayi et al. 2008).

To date, whole genome sequence information is available for a number of microbial and higher eukaryotic genomes. This provides a wealth of data for advanced plant biology research. Due to their small size, the genomes of *Arabidopsis thaliana*, rice, poplar, grapevine, and *Medicago truncatula* have been completely sequenced either following the clone-by-clone or the whole genome shotgun sequencing strategies (e.g. The *Arabidopsis* Genome Initiative 2000, International Rice Genome Sequencing Project 2005). These species, thus, became important as model plants. For *Brachypodium distachyon*, a model organism for temperate grasses, a 4x draft whole genome shotgun sequence has been completed recently (http://www.brachypodium.org/).

In contrast, the genomes of barley and wheat are relatively large with genome sizes of 5,500 and 17,000 Mb, respectively; hence, whole genome sequencing using currently available techniques is very cost-intensive. Nevertheless, several international consortia are currently working on sequencing the *Triticeae* genomes (e.g. International Barley Sequencing Consortium, IBSC, http://barleygenome.org; International Wheat Genome Sequencing Consortium, IWSC, http://www.wheatgenome.org/). Furthermore, large-scale sequencing projects for other crop species such as tomato (http://www.sgn.cornell.edu/about/tomatosequencing.pl) and maize (http://www.maizesequence.org) are also currently in progress. Until the barley genome sequence is accomplished, the rice genome sequence information can be used for comparative analyses (Devos 2005, Laurie and Devos 2002). Based on the synteny and gene homology between the genomes of rice and other cereal crops, the comparative genomics approach has proven very useful as illustrated by diverse comparative mapping (Huang et al. 2008, March et al. 2008) and gene evolution studies (Ramakrishna et al. 2002, Chantret et al. 2008). Useful resources for these purposes are 'Gramene', an online available database for plant comparative genomics (Liang et al. 2007, http://www.gramene.org/), and the HarvEST:Barley software (http://harvest.ucr.edu/).

As illustrated by Stein (2007), alternative approaches for accessing the genome sequence of a species include the generation of large EST datasets or the sequencing of individual BACs (bacterial artificial chromosomes). Since one expressed gene is represented by several ESTs, different bioinformatic tools are used for building groups of ESTs. This is done using an EST clustering and assembly process, resulting in the prediction of so-called unigenes, where each unigene contains sequences that represent a unique gene (Pontius et al. 2003, Matthews et al. 2004). To date, the NCBI UniGene data base provides ~23,000 and ~41,200 unigenes entries for barley and wheat, respectively (http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene). The EST and unigene resources have been widely applied for large-scale gene expression studies, and, hence, are a valuable tool for functional genomics. They either have been directly used in order to identify differentially expressed genes (Zhang et al. 2004), or they have been applied to perform microarray expression studies (reviewed by Rensink and Buell 2005). Recently, Potokina et al. (2008) used a 22K barley GeneChip (Close et al. 2004) for constructing a genetic map and for a subsequent genome-wide expression analysis of about 16,000 genes.

Sequencing of selected BACs provides an insight into the arrangement and organization of genes and other DNA elements within a genome. BACs contain large segments (of on average 80-120 kb) of foreign DNA and are used to produce large insert genomic libraries, which may cover the genome several fold (Somers 2004). BAC libraries are an essential tool for genome analysis and are used for physical mapping, map-based gene cloning, comparative genomic analyses, and whole genome sequencing. In barley, BAC libraries have been constructed from the cultivars 'Morex' (Yu et al. 2000), 'Cebada Capa' (Isidore 2005), and 'Haruna Nijo' (Saisho et al. 2007). To date, several databases and bioinformatic tools for exploiting BAC libraries are available, for instance the barley BAC physical mapping database developed by the group of A. Kleinhofs at the Washington State University (http://phymap.ucdavis.edu:8080/barley/index.jsp). Another type of large insert libraries, for instance constructed from the winter barley cultivar 'Franka' (Kleine et al. 1997, Schmidt et al. 2001), are YAC (yeast artificial chromosome) libraries. Compared to BAC libraries, they offer larger insert sizes but are more difficult to handle and possess a higher sensitivity to recombination events and chimaerism (Zhang et al. 1996). Due to this, BAC clones are preferred for constructing physical maps, which are composed of overlapping BACs in linear order, and for performing map-based cloning (Rafalski 2002b). Nevertheless, BAC as well as YAC clones contributed to the cloning of several barley genes, like *Ppd-H1*, the major photoperiod response gene (Turner et al. 2005), and diverse resistance genes like *mlo* (Büschges et al. 1997), *Mla1* (Wei et al. 1999), *Rar1* (Shirasu et al. 1999), and *Rpg1* (Brüggeman et al. 2002).

Map-based cloning (also called positional cloning) is a forward genetics approach of gene isolation based solely on the gene's map location. It can be applied to any gene whose effect on phenotype can be followed in a segregating population (Yano 2001, Peters et al. 2003). Once a candidate gene has been identified and isolated, it is required to prove it's biological function via transformation or mutant analysis. As described by Dahleen and Manoharan (2007), barley is a suitable organism for genetic transformation. Several barley transformation methods have been developed involving gene transfer via *Agrobacterium tumefaciens* (Shrawat et al. 2007), microprojectile bombardment (Obert et al. 2008), and protoplast transformation (Nobre et al. 2000). Another proof of function strategy is the mutagenesis of the target gene by different techniques like chemical mutagens, mutagenic irradiation, T-DNA (transfer DNA) insertion or transposon insertion (Stein and Graner 2004). The resulting knockout or overexpression of the

target gene in mutants gives information about the function of the gene product. Initiated by Caldwell et al. (2004), a database of barley mutants consisting of 5376 EMS (ethyl methanesulfonate) mutagenised lines of the cultivar 'Optic' is publicly available at the SCRI (Scottish Crop Research Institute, http://germinate.scri.ac.uk/cgi-bin/mutantsdatabase/index.pl). The SCRI also provides facilities for performing TILLING (Targeting Induced Local Lesions in Genomes) using that mutant collection. As described by McCallum et al. (2000), TILLING is a genome-wide, non-transgenic, forward and reverse genetics approach for the targeted screening of induced mutations in a genome, which has been applied in *Arabidopsis* (Till et al. 2003) and several crop species (Slade et al. 2005, Till et al. 2007, Weil and Monde 2007). In barley, Talame et al. (2008) developed a mutant collection for the cultivar 'Morex' which was subsequently screened for mutations at four agronomically important genes including the barley photoperiod response gene *HvCO1*. A similiar strategy is EcoTILLING, a technique that aims to uncover naturally occuring genetic variation in contrast to induced mutations (Comai et al. 2004, Barkley and Wang 2008). In barley, Mejlhede et al. (2006) have demonstrated that this technique can also be used as a genetic marker system.

1.3 QTL and AB-QTL analyses in barley

Most agronomically important traits such as yield, quality parameters and some disease resistances are quantitatively inherited. They are conditioned by a few or many genes, so called polygenes or QTLs (quantitative trait loci), and show a continuous variation predominantly depending on gene by gene and genotype by environment interactions (Becker 1993). The quantitative genetics approach focuses on unraveling the complex genetic architecture of quantitative traits which is governed by different phenomena like epistasis, pleiotropy and heterosis (Mackay 2001, Holland 2007). With the advent of molecular markers in the early 1980s, extensive QTL analyses focusing on QTL localization and characterization became feasible. QTL mapping is based upon the linkage of a QTL to a marker gene which is given if an association between a certain phenotype and the genotype of a marker is detected (Collard et al. 2005). There are mainly two prerequisites for localizing QTLs, namely a population which segregates in the trait of interest and a dense and homogeneous marker map (Paterson 1996).

Since the early 1990s, numerous barley QTL analyses have been conducted focussing on different trait complexes such as yield related traits (Ayoub et al. 2002, Peighambari et al. 2005, Xu et al. 2007), disease resistances (Cheong et al. 2006, Lehmensiek et al. 2007, Grewal et al. 2008), abiotic stress tolerances (Reinheimer et al. 2004, Guo et al. 2008, Li et al. 2008), and malting quality (Mather et al. 1997, Barr et al. 2003, Panozzo et al. 2007). P. Hayes and colleagues from the Oregon State University provide a comprehensive review on QTLs detected in a number of barley studies (http://barleyworld.org/northamericanbarley/qtlsummary.php). Besides the different traits investigated, QTL studies differ considerably in the material and methods applied for detecting QTLs. Differences can be found regarding the initial crossing parents, the type and size of the segregating population (e.g. F_2, BC_1, DH or RI), the number of locations and replications applied for collecting phenotype data, the molecular marker system used for genotyping, and the statistical methods for assessing QTL effects (e.g. single marker analysis, simple interval mapping, composite interval mapping). As reviewed by Tanksley (1993), all these aspects have a considerable impact on the accuracy of QTL localization.

Tanksley and Nelson (1996) pointed to the disadvantages of classical QTL analyses and proposed the AB (advanced backcross)-QTL analysis as a strategy to overcome these problems. In classical QTL studies, the reduced level of genetic variation present in the used elite gene pool (1) hampers the detection of polymorphisms with molecular markers, and (2) reduces the chance to find new valuable QTL alleles for breeding improved cultivars. In general, the depletion of genetic variation in elite germplasm started with the onset of crop domestication. In many cases, it accelerated dramatically during the onset of modern breeding (Tanksley and McCouch 1997). In contrast, several studies have proven that wild progenitors of crop species harbor a multitude of alleles causing improved disease resistance (Fetch et al. 2003, Repkova et al. 2006) and enhanced tolerance to abiotic stress such as drought (Gorny 2001, Gunasekera et al. 1994), cold (Crosatti et al. 1996), and salt tolerance (Forster et al. 1997). Accordingly, the genetic potential of wild crop relatives has been used in crop improvement programs for more than 60 years in order to broaden the genetic basis of the elite gene pool (Hajjar and Hodgkin 2007). First of all, a number of major genes from wild species and regionally adapted landraces, conveying qualitative resistance to pests and pathogens, have been introgressed into the elite crop gene pool. For instance, the *mlo-11* allele introgressed from an Ethiopian landrace into the European spring barley gene pool provided durable broad spectrum resistance against powdery mildew for more

than 30 years (Jørgensen 1992). In contrast, only a few examples have been reported for using unadapted germplasm in order to breed elite cultivars with improved abiotic stress tolerance, such as barley cultivars with increased drought tolerance derived from *Hsp* (Hajjar and Hodgkin 2007) or improved quality traits, such as enhanced fruit color and soluble solids content introgressed from the wild tomato species *Solanum hirsutum* (Tanksley and McCouch 1997).

The use of wild accessions for the improvement of yield or other complex traits is mainly hampered by difficulties to identify accessions with phenotypes superior to elite cultivars. This might be attributed either to unfavorable epistatic interactions between non-linked exotic alleles or the linkage of the target gene and unfavorable attributes. The latter phenomenon is referred to as 'linkage drag'. To overcome this obstacle, the AB-QTL analysis is conducted in advanced backcross populations where each line harbors a reduced portion of exotic germplasm (Tanksley and Nelson 1996). Due to the reduced frequency of deleterious exotic alleles, the investigation of traits which otherwise cannot be detected becomes feasible. In contrast, in balanced populations which are utilised in classical QTL analyses, sterility or seed shattering often renders it impossible to measure yield and yield related traits (Swamy and Sarla 2008).

Using the AB-QTL analysis, a multitude of favorable exotic QTLs affecting diverse traits have been identified in several crop species. Initially, this method was extensively applied in tomato (Tanksley et al. 1996, Fulton et al. 1997a) and rice (Xiao et al. 1996, Moncada et al. 2001), recently followed by studies conducted in wheat (Huang et al. 2003, 2004; Kunert et al. 2007; Naz et al. 2008), maize (Ho et al. 2002, Li et al. 2007), common bean (Blair et al. 2006), soybean (Wang et al. 2004), and pepper (Rao et al. 2003).

In barley, *Hsp* has been extensively used to localize QTLs for agronomic traits and yield components (Hori et al. 2005, Li et al. 2006), disease resistances (Yun et al. 2005, 2006), malting quality (Li et al. 2005a; Pillen et al. 2003, 2004), abiotic stress tolerance (Baum et al. 2003, Talame et al. 2004), seed dormancy (Hori et al. 2007), and morphological traits (Gyenis et al. 2007). A BC_2DH population developed from a cross between the spring barley cultivar 'Scarlett' (*Hv*) and the Israeli wild barley accession 'ISR 42-8' (*Hsp*) was subjected to extensive field tests in order to detect QTLs and to identify favorable wild barley alleles. For nine agronomic traits, such as plant height, days until heading and grain yield, altogether 86 putative QTLs were

detected among which 36.0 % of all QTLs exhibited a favorable *Hsp* effect on trait performance (von Korff et al. 2006). In addition, von Korff et al. (2005, 2008) identified a total of 18 QTLs for resistance to powdery mildew, leaf rust and scald, as well as 48 QTLs for several malting quality parameters. For these trait complexes, the trait value was improved by the exotic allele at 61.1 % and 37.5 % of all identified QTLs, respectively.

According to Tanksley and Nelson (1996), an important aim of the AB-QTL strategy is to integrate the process of QTL detection with the development of new cultivars. For this, advanced backcross populations provide a great basis since, due to the relatively small portion of exotic germplasm present in each line, only a few additional backcrosses are needed to generate so called QTL-NILs (near isogenic lines). These NILs carry a single chromosomal fragment of the exotic parent within the genetic background of the elite parent. They can be used directly for developing cultivars with improved trait performance. As demonstrated by Tanksley et al. (1996) and Bernacchi et al. (1998b), before applying favorable QTL alleles to breeding, it is required to verify the favorable exotic QTL effect by testing the NILs in diverse environments. As described in detail in chapter 1.4, QTL-NILs are furthermore a valuable genetic resource for QTL fine mapping and QTL cloning.

One limitation of the AB-QTL method is the fact that effects of exotic alleles are in general tested in only one elite genetic background (Salvi and Tuberosa 2005). To evaluate the consistency of QTL effects across different genetic backgrounds, Swamy and Sarla (2008) compared QTLs for yield and yield related traits detected between different rice populations. These populations were generated using one exotic rice accession along with four different elite varieties. Although few QTLs exhibited opposed effects in different populations, most of the exotic alleles generally produced the same effect in all tested elite backgrounds (Swamy and Sarla 2008). In spring barley, Pillen et al. (2004) conducted a similar comparative AB-QTL analysis in order to test the stability of QTL effects identified in two different BC_2F_2 populations developed from the same *Hsp* donor. Altogether, 66.0 % of all QTL effects did not coincide in both of the populations compared. This inconsistency can be attributed to strong epistatic interactions between the detected QTLs and the elite genetic background (Pillen et al. 2004).

1.4 Introgression lines – a multifunctional plant genomic tool

Introgression lines are a relatively new tool for genome research as well as for breeding. A complete set of introgression lines - also termed an 'exotic library' – is supposed to represent the complete genome of an exotic donor parent through overlapping introgressions. Each IL harbors a single, marker-defined homozygous exotic segment in the uniform genetic background of an elite parent (Zamir 2001). This stringent definition of ILs does often not apply to lines referred to as introgression lines in literature, since in many cases, these lines are not 'clean', but carry several homo- or heterozygous introgressions, simultaneously. Nevertheless, the attributes and possible applications of ILs described in the following are more or less also valid for those lines. Moreover, the term near isogenic line (NIL) can be considered as conform to IL, although ILs focus rather on the genome-wide coverage of the donor genome.

ILs are widely applied in plant biology. Analogous approaches have been used in animal research where different populations of congenics or chromosome substitution strains (CSS) have been generated for mice (Lhote et al. 2007, Singer et al. 2004). As recommended by Zamir (2001), introgression lines (ILs) provide a promising opportunity for a straightforward, efficient and manifold exploitation of exotic plant germplasm, and, thus, contribute to the enrichment of the elite gene pool.

The construction of introgression libraries is carried out by several rounds of recurrent backcrossing (to reduce the portion of donor genome), repeated selfing (to achieve complete homozygous lines), and, in parallel, marker-assisted selection (MAS) of recombinants. As documented by different IL studies (e.g. Eshed and Zamir 1994, Tian et al. 2006), the construction of an IL set takes a relatively long time of, sometimes, ten generations. This high expenditure of time can be partially overcome by a higher number of backcrosses and one or several pre-selection steps in earlier generations. However, the advantages of introgression libraries compared to commonly used QTL mapping populations, like F_2, BC_1, recombinant inbred line (RIL), and advanced backcross populations, might outweigh the time-consuming IL development. The low proportion of exotic germplasm present in each IL can be considered as the greatest benefit since it enables the statistical detection of small phenotypic effects. Due to linkage drag between linked exotic loci or masking effects of major QTLs, these effects are

otherwise not or with more difficulties to detect (Zamir 2001). Furthermore, any phenotypic difference between an IL and the elite control parent can be clearly attributed to one donor segment present in the exotic introgression. One further benefit, for instance in comparison to AB-QTL analyses, is the nearly simultaneous identification and transfer of QTLs into elite cultivars since no further backcrossing or selfing is required between both steps. Due to their homozygosity, ILs can be maintained by selfing and, thus, are a stable and immortal genetic resource which can be tested repeatedly by independent research groups, simultaneously.

To date, introgression libraries have been developed and well studied in several crop species among which tomato, offering IL sets for several wild species (e.g. Fulton et al. 1997b, Monforte and Tanksley 2000, Canady et al. 2005, Finkers et al. 2007), is the outrider. Here and in general, the *Solanum pennellii* IL library, initiated by Eshed and Zamir (1994) which currently includes 76 ILs (Lippman et al. 2007), is to date the best studied population. For instance, it was used to detect nearly 3,000 putative QTLs affecting yield associated traits (Eshed and Zamir 1995, Hanson et al. 2007) as well as morphological traits (Holtan and Hake 2003) and metabolites (Baxter et al. 2005, Overy et al. 2005, Schauer et al. 2006). Focusing on agronomic performance, end use quality, disease resistance, and abiotic stress tolerance, ILs were also used to explore the genetic basis of quantitative traits in cereals - first of all in rice (e.g. Mei et al. 2006, Zheng et al. 2007, Siangliw et al. 2007, Zhao et al. 2008), but also in wheat (Liu et al. 2006, Pestsova et al. 2006, Kaur et al. 2007), rye (Falke et al. 2008), and maize (Szalma et al. 2007, Wang et al. 2008). In melon, a collection of NILs has been intensively used to map QTLs and candidate genes for fruit quality (Eduardo et al. 2007, Fernandez-Trujillo et al. 2007, Moreno et al. 2008, Obando et al. 2008). Jeuken and Lindhout (2004) developed a set of 28 backcross inbred lines (BILs) for lettuce which altogether represent 96.0 % of the wild lettuce donor genome. The set was subsequently used for mapping morphological and amplified fragment length polymorphic (AFLP) markers. The advantages of ILs for the identification of small QTL effects were exemplified in the model plant *Arabidopsis thaliana* (Keurentjes et al. 2007, Lisec et al. 2008), where RIL populations exhibiting the aforementioned limitations are commonly used for QTL studies.

Several studies have illustrated that ILs enable the uncovering of favorable phenotypes which cannot be observed in the parents (Eshed and Zamir 1996, Semel et al. 2006). The occurrence of

transgressive segregation can be attributed to new interactions between the elite genome and the exotic segments (deVincente and Tanksley 1993). In spring barley, Matus et al. (2003) exemplified the usefulness of ILs to identify trait enhancing exotic alleles. They conducted an association study using a set of 140 recombinant chromosome substitution lines (RCSLs) which possessed a similar genetic structure to ILs. Based on phenotype data collected in the field, RCSLs were identified which revealed a superior phenotype, compared to the elite parent, for the traits number of grains per ear, ear length, and thousand grain weight.

Furthermore, introgression lines have proven highly valuable for QTL fine mapping as an essential step towards the map-based cloning of the gene or genes underlying a quantitative trait. ILs provide the basis for constructing so-called sub-ILs which contain very small exotic fragments within the interesting QTL interval, and, thus, enable the high resolution mapping of QTL to an interval <1 cM (Lippman et al. 2007). In tomato, Several QTLs affecting yield, fruit quality, and other parameters have been fine mapped using ILs or sub-ILs (e.g. Eshed and Zamir 1995, Alpert and Tanksley 1996, Yates et al. 2004, Xu et al. 2008). Finally, high-resolution maps, constructed using *S. pennellii* ILs, were utilised for the cloning of QTLs affecting tomato fruit quality (Frary et al. 2000, Fridman et al. 2004). In barley, Marcel et al. (2007) dissected a region containing QTLs for leaf rust resistance on the long arm of chromosome 2H using a set of 38 Sub-NILs constructed from population 'L94' x 'Vada'. To enable the subsequent map-based cloning of the most promising QTL, Marcel et al. (2007) is currently constructing a new BAC library from the cultivar 'Vada'.

ILs were also applied to explore the genetic and molecular basis of epistasis and heterosis, two important phenomenons of quantitative genetics. Phenotypic effects of QTL interactions can be examined using crosses of ILs carrying different introgressed segments, as exemplified for tomato by Eshed and Zamir (1996). IL crosses could also be used for QTL pyramiding, i.e. accumulating several independent introgressions, each showing a favorable QTL effect on the same or different traits, into a single genotype. This genotype is assumed to considerably outclass the recurrent parent for the target trait, and, hence, can be used directly for breeding improved cultivars (Ashikari and Matsuoka 2006). The pyramiding approach was applied in rice, where two QTLs affecting plant height and grain number have been combined in one IL (Ashikari et al. 2005). Furthermore, Gur and Zamir (2004) report on the construction of a single tomato IL which

harbors three independent yield-associated QTLs simultaneously, and which was subsequently used to generate outperforming hybrids. The ability of hybrids to outclass both parents for traits such as yield or biomass is named heterosis or hybrid vigor. Three different genetic models - the dominance, the overdominance and the pseudooverdominance model - possibly underlying heterosis have been proposed (Birchler et al. 2006). As demonstrated in tomato, ILs are highly suitable to investigate the genetic basis of heterosis since, due to the small portion of exotic genome present in each IL, the effects of single loci are masked to a lesser extent by epistasis (Eshed et al. 1996). For this approach, single ILs or a complete IL library are crossed with one or several tester lines and the resulting heterozygous F_1 plants are subsequently phenotyped. This enables, for instance, to assess the contribution of overdominant genomic regions to heterosis (Semel et al. 2006).

Finally, ILs can be directly used for breeding improved cultivars. So far, this has been demonstrated in tomato where *S. pennellii* ILs were used for developing the hybrid 'AB2', which is the presently leading tomato cultivar in California (Lippman et al. 2007).

1.5 Objectives of the study

The principal aims of the present study are the development and the phenotypic evaluation of an introgression library in spring barley. Described in detail, this comprises:

1. The selection and genetic characterization of a genome-wide set of wild barley introgression lines (ILs) starting with 40 BC_2DH lines of the spring barley population S42 (von Korff et al. 2004). In order to generate the ILs, an approach combining recurrent backcrossing, selfing, and MAS is carried out. It is intended that the IL set represents as much of the exotic donor genome as possible through overlapping introgressions, whereas each IL contains a single and preferably small exotic genome segment.

2. The evaluation of the selected ILs with regard to different trait complexes in order to verify QTL effects previously localized in population S42 (von Korff et al. 2005, 2006, 2008). In addition, it is intended to identify new QTLs as well as new favorable exotic QTL alleles. To

do that, phenotype data from field tests with the ILs in three different environments are collected for agronomic traits, disease resistances and malting quality parameters. With the objective to assess QTLs affecting these traits, the genotype and phenotype data are analysed within a line x phenotype association study.

3. The identification of ILs exhibiting multiple QTL effects simultaneously. Those ILs carrying favorable exotic QTL alleles for several traits in parallel are valuable starting material for breeding improved cultivars.

2 Original papers

The present thesis which comprises three original papers illustrates the generation and application of an introgression library in spring barley. In chapter 2.1 (Schmalenbach et al. 2008) the development of the introgression library, including the selection strategy and the genetic characterization of the constructed lines, is described. Furthermore, a first application of the library is given focusing on the detection and verification of QTLs for powdery mildew and leaf rust resistance. As illustrated in chapters 2.2 and 2.3 (Schmalenbach et al. 2009, Schmalenbach and Pillen 2009, respectively), the introgression lines were furthermore used in order to identify and validate QTLs affecting agronomic traits and malting quality parameters, respectively.

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ORIGINAL PAPER

Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust

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Abstract A set of 59 spring barley introgression lines (ILs) was developed from the advanced backcross population S42. The ILs were generated by three rounds of backcrossing, two to four subsequent selfings, and, in parallel, marker-assisted selection. Each line includes a single marker-defined chromosomal segment of the wild barley accession ISR42-8 *(Hordeum vulgare* ssp. *spontaneum)*, whereas the remaining part of the genome is derived from the elite barley cultivar Scarlett (*H. vulgare* ssp. *vulgare)*. Based on a map containing 98 SSR markers, the IL set covers so far 86.6% (1041.5 cM) of the donor genome. Each single line contains an average exotic introgression of 39.2 cM, representing 3.2% of the exotic genome. The utility of the developed IL set is illustrated by verification of QTLs controlling resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei* L.) and leaf rust (*Puccinia hordei* L.) which were previously identified in the advanced backcross population S42. Altogether 57.1 and 75.0% of QTLs conferring resistance to powdery mildew and leaf rust, respectively, were verified by ILs. The strongest favorable effects were mapped to regions 1H, 0–85 cM and 4H, 125–170 cM, where susceptibility to powdery mildew and leaf rust was decreased by 66.1 and 34.7%, respectively, compared to the recurrent parent. In addition, three and one new QTLs were localized, respectively. A co-localization of two favorable QTLs was identified for line S42IL-138, which holds an introgressed segment in region 7H, 166–181. Here, a reduction effect was revealed for powdery mildew as well as for leaf rust severity. This line might be a valuable resource for transferring new resistance alleles into elite cultivars. In future, we aim to cover the complete exotic genome by selecting additional ILs. We intend to conduct further phenotype studies with the IL set in regard to the trait complexes agronomic performance, malting quality, biotic stress, and abiotic stress.

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Introduction

Cultivated barley (*Hordeum vulgare* ssp. *vulgare*, hereafter abbreviated with *Hv*) is one of the four most important cereals worldwide and is mainly used as feed grain and for malt production. The domestication of barley took place approximately 10,000 years ago in the Fertile Crescent from its wild relative *Hordeum vulgare* ssp. *spontaneum* (hereafter abbreviated with *Hsp,* Badr et al. 2000). As illustrated by Tanksley and McCouch (1997), the domestication of crops has led to a dramatic loss of allelic diversity. As a result, exotic alleles from related wild species become more and more valuable for breeding of improved crop varieties, in regard to qualitative as well as quantitative traits like disease resistances and yield-related traits, respectively. The development of introgression lines (ILs) provides a promising opportunity to efficiently use the genetic potential of wild species. As described by Zamir (2001), a complete set of introgression lines is supposed to

represent the entirety of the exotic genome, while each line contains only a single marker-defined chromosomal segment of the exotic parent and the remaining genome is consistently derived from the elite parent. First IL sets in plants were developed for tomato (Eshed et al. 1992; Eshed and Zamir 1994; Fulton et al. 1997) and rice (Jena et al. 1992; Aida et al. 1997). In recent years, complete or nearly complete IL sets were developed for further tomato (Monforte and Tanksley 2000; Canady et al. 2005; Finkers et al. 2007) and rice wild species (Li et al. 2005; Tian et al. 2006a; Tan et al. 2007), as well as *Arabidopsis thaliana* (Keurentjes et al. 2007), the D-genome of wheat (Pestsova et al. 2006), maize (Szalma et al. 2007), and melon (Eduardo et al. 2005). Also in other crop species, lines containing introgressed segments from wild species (e.g., near-isogenic lines, recombinant, or backcross inbred lines) were generated and used for genetic studies. But here, the coverage of the donor genome was not complete and/or the lines contained multiple exotic introgressions simultaneously. Examples for these lines can be found for instance for cabbage (Ramsay et al. 1996), coffee (Prakash et al. 2002), cotton (Percy et al. 2006), lettuce (Jeuken and Lindhout 2004), peanut (Garcia et al. 1995), pepper (Ben Chaim et al. 2003), rapeseed (Howell et al. 1996), and soybean (Concibido et al. 2003). For spring barley, Brown et al. (1988) primarily generated a set of 84 backcross lines, each carrying a single homozygous isozyme marker-defined segment of an *Hsp* accession. Matus et al. (2003) developed 140 recombinant chromosome substitution lines (RCSLs) for spring barley by introgression of *Hsp* alleles. The lines, selected on the basis of genotype data from 47 SSR markers, contained on average 12.6% *Hsp* genome, and mostly carried several *Hsp* introgressions simultaneously. A similar population was generated by Hori et al. (2005). Here, altogether 134 RCSLs with an averaged proportion of *Hsp* genome of 12.9% were genotyped with 25 SSR and 60 EST markers and subsequently applied to QTL analysis. A core-set of 19 RCSLs represented the complete *Hsp* genome, where each line included one or several exotic introgressions. Valuable plant material for the development of a complete IL set in barley was also generated by von Korff et al. (2004). Two sets of candidate introgression lines (pre-ILs) were selected from two BC_2DH populations, using the *Hsp* accession 'ISR42-8' as donor and the spring barley cultivars 'Scarlett' (S) and 'Thuringia' (T) as recurrent parents. The selection of 49 (S42) and 43 (T42) pre-ILs was carried out on the basis of genotype data from 98 SSRs. The sets of selected lines carried 98.1 and 93.0% of the exotic genome, and contained on average 3.0 and 2.5 introgressions (S42 and T42, respectively).

The numerous advantages of ILs are described in detail by Zamir (2001). Due to the very small portion of exotic genome, epistatic and linkage drag effects are reduced, and the phenotypic variation between the ILs can be attributed with high accuracy to the particular introduced segment. Furthermore, once a complete homozygous IL set is developed, it is a reliable and stable genetic resource, and each IL can be used directly for breeding. To date, ILs are applied to a multitude of genetic studies. The *Solanum pennellii* IL population, established by Eshed and Zamir (1994), is so far the best characterized. Here, almost 3,000 putative QTLs for different trait complexes like yield-associated traits, (Eshed and Zamir 1995; Hanson et al. 2007); morphological traits (Holtan and Hake 2003; Semel et al. 2006), and metabolites (Baxter et al. 2005; Overy et al. 2005; Schauer et al. 2006) were identified. For yield-related traits, several QTL studies were also conducted in rice (e.g. Mei et al. 2006; Tan et al. 2007) and wheat (Liu et al. 2006; Pestsova et al. 2006). As it was reported by several publications, ILs were also applied to the analysis of quality parameters like amount of antioxidants in tomato fruits (Rousseaux et al. 2005); milling quality of rice grains (Zheng et al. 2007), amount of high molecular weight glutenin subunits in wheat grains (Liu et al. 2007) and post-harvest decay and quality of melon fruits (Eduardo et al. 2007; Fernandez-Trujillo et al. 2007; Obando et al. 2008). ILs were also tested under abiotic stress conditions to detect QTLs for drought tolerance (Xu et al. 2005; Zhang et al. 2006; Zhou et al. 2006; Siangliw et al. 2007), and tolerance to salinity and phosphorus deficiency in rice (Li et al. 2005). Not only quantitative traits were dissected by ILs, but also qualitative traits, which are affected by a single or a few loci. Examples for investigation of disease resistances are described for wheat (Leonova et al. 2007; Simón et al. 2007; Song et al. 2007), but also for tomato (Finkers et al. 2007). The above-mentioned barley S42 pre-ILs were evaluated with regard to disease resistances, agronomic performance, and malting quality (von Korff et al. 2005, 2006, 2008).

In the present paper, we report on the first development of an IL set for barley. Each IL carries a single introgression of the exotic *Hsp* accession 'ISR42-8' in the genetic background of the elite spring barley cultivar 'Scarlett'. The set is generated by backcrossing, selfing, and marker-assisted selection. In order to illustrate the applicability of the spring barley ILs, the lines were used for verification of QTLs for field resistance against powdery mildew (*Blumeria graminis* f. sp. *hordei* L.) and leaf rust (*Puccinia hordei* L.).

Materials and methods

Development and molecular characterization of introgression lines

The project started with 40 pre-ILs (candidate introgression lines), which were selected from 301 BC_2DH lines of the

spring barley population S42 (von Korff et al. 2004). The lines were generated from a primary cross between the spring barley cultivar 'Scarlett' (*Hordeum vulgare* ssp. *vulgare*) and the wild barley accession ISR42-8 from Israel (*Hordeum vulgare* ssp. *spontaneum*).

For the development of the final introgression lines, the selected 40 pre-ILs were backcrossed once again with 'Scarlett' (BC_3) in order to further reduce the portion of the *Hsp* genome and to minimize the target introgression (Fig. 1). The BC_3 plants were subsequently selfed twice to achieve recombined homozygous lines. In BC_3S_2, all plants were genotyped with a total of 98 SSR markers as described by von Korff et al. (2004). On average, 140 plants per line were investigated with, on average, 12 informative SSRs, which revealed the *Hsp* genotype in the appropriate pre-IL. Based on these genotype data, useful plants were selected as introgression lines. The criteria for selection were as follows: (1) a line contains a single continuous *Hsp* introgression and carried the *Hv* allele at all remaining SSR loci. (2) Within the IL set, the introgressions should overlap to ensure that the complete exotic genome is represented. All finished ILs were verified again with SSR markers in BC_3S_4. Here, the two outer loci of the

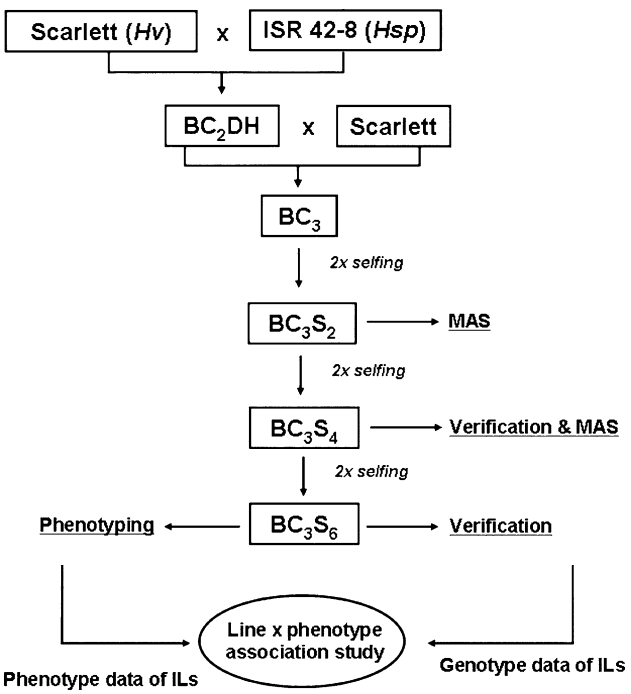

Fig. 1 Strategy for development and evaluation of introgression lines from population S42. After several rounds of backcrossing and selfing, a set of introgression lines, originating from the cross 'Scarlett' × 'ISR42-8', was selected by marker-assisted selection (MAS). The genotypes of the ILs were verified in subsequent generations. Afterward, the lines were evaluated with regard to disease resistances. Finally, the genotype and phenotype data of the ILs were used for line × phenotype association studies to localize QTL effects and favorable *Hsp* introgressions

Hsp introgression and one random locus, which revealed the exotic allele in the corresponding pre-IL, were investigated again to confirm the homozygous exotic genotype of each line. The ILs were subsequently propagated until BC_3S_6 to obtain a sufficient number of seeds for phenotype studies in 2007. In this generation, the ILs were verified again on the basis of 30 random SSR loci, which were not investigated before, to confirm the pure genetic *Hv* background. The size of the *Hsp* introgression was calculated for each finished IL. Here, the half-intervals flanking a marker locus were considered to be of the same genotype. For calculation of the portion of exotic germplasm per IL, a total genome size of 1,202 cM was assumed on the basis of the applied SSR map (von Korff et al. 2004).

Evaluation of powdery mildew and leaf rust

In order to evaluate the selected S42ILs in regard to their resistance to powdery mildew and leaf rust, field tests with 39 lines were conducted at two different locations in Germany in 2007. The locations were the experimental research station Dikopshof of the University of Bonn (D07, West Germany), and the breeder's experimental field station Gudow (G07, Nordsaat Saatzucht, North Germany). The ILs were cultivated in three replications of two rows per line. As a control, the recurrent parent 'Scarlett' was tested in 12 replications of two rows. In addition, the spring barley cultivar 'Barke' was tested in nine replications to compare the performance of the S42ILs with a current elite cultivar. The field management was in accordance with local practice, but without applying fungicides. No artificial infection with pathogens was carried out. The severity of disease symptoms was surveyed on leaves at the maximum stage of disease development on a scale from one (no symptoms) to nine (completely covered with symptoms).

Genetic correlations

Pearson's correlation coefficient (r) was calculated with the CORR procedure, implemented in the software package SAS Enterprise Guide 4.1 (SAS Institute 2006). The genetic correlations between leaf rust and powdery mildew were calculated based on least squares means (LSMEANS) for each of the 39 S42ILs averaged across replications and environments.

Line × phenotype association study

The data for powdery mildew and leaf rust were analyzed with SAS Enterprise Guide 4.1 (SAS Institute 2006). For detection of QTLs and identification of favorable *Hsp* introgressions, a two-factorial mixed model analysis of

variance (ANOVA) was carried out with the general linear model (GLM) procedure. The GLM model is stated below:

$$Y_{ijk} = \mu + L_i + E_j + L \times E_{ij} + \varepsilon_{k(ij)},$$

where μ is the general mean, L_i is the fixed effect of the ith line, E_j is the random effect of the jth environment, $L \times E_{ij}$ is the random interaction effect of the ith line and the jth environment and $\varepsilon_{k(ij)}$ is the error term of the phenotype Y_{ijk}, calculated from k replications. When the ANOVA revealed significant differences with $P < 0.05$ between lines or line × environment interactions, a Dunnett multiple comparison test of LSMEANS differences between the ILs was conducted with the recurrent parent 'Scarlett' as the control (Dunnett 1955). A QTL main effect for powdery mildew or leaf rust resistance was accepted, when the disease score of a particular IL was significantly different from 'Scarlett' across all environments with $P < 0.05$. A QTL × environment interaction effect was accepted, when the disease score of a particular IL was significantly different from 'Scarlett' in at least one of the environments with $P < 0.05$. If several ILs with overlapping or flanking introgressions showed significant effects of the same direction, a single QTL was assumed to cause the effect. Overlapping introgressions contained at least one common SSR locus, whereas flanking introgressions covered adjacent loci. The relative performance of an introgression line [RP(IL)] was calculated as follows: RP(IL) = [LSMEANS(IL) − LSMEANS(Scarlett)] × 100/LSMEANS(Scarlett). For each line the LSMEANS were calculated across replications and environments as stated above.

Results

Selection and characterization of introgression lines

As a result of the marker-assisted selection in BC_3S_2 and BC_3S_4, a set of 59 S42ILs was developed (Table 1). The ILs carry single exotic introgressions from the *Hsp* accession 'ISR42-8'. Four of these lines, S42IL-102, -114, -123, and S42IL-129, revealed a second, non-target, introgression on chromosomes 2H, 5H, 4H, and 1H, respectively (Fig. 2). Furthermore, two target introgressions on chromosome arm 3HL and 4HS were each represented by two lines, S42IL-114 and S42IL-140 and S42IL-118 and S42IL-120, respectively. Both pairs remain in the final IL set since it is expected that future high-resolution genotyping will reveal differences in introgression size between the independent sister lines.

Based on the SSR map published by von Korff et al. (2004), 1,041.5 cM (86.6% of 1,202 cM) of the exotic *Hsp* genome is covered by S42ILs. A similar estimate can be found by counting exotic SSR alleles. Altogether 87 (88.8%) *Hsp* alleles at the 98 SSR loci are represented by at least one IL, whereas 11 exotic SSR alleles are not yet covered by S42ILs. Chromosome 6H of the exotic parent is completely represented by ten different S42ILs. Chromosomes 4H and 7H are largely covered by ILs (92.1 and 85.9% genome coverage per chromosome, respectively). The selected S42ILs contain on average exotic introgressions of 39.2 cM (range 9.0–134.0 cM), representing 3.2% of the exotic genome (range 0.7–11.1%). Thirteen lines carry the *Hsp* allele only at a single SSR locus, whereas in 27 ILs the exotic segment includes more than three linked marker loci. In all 59 selected ILs, the introgression covers on average 3.4 SSR loci. Twenty-five S42ILs are unique. They represent the minimum number of S42ILs to cover the *Hsp* genome. The 34 non-unique lines are partial duplicates with shorter introgressions, which are useful for fine-mapping of markers and QTLs. Examples are S42ILs -106, -107 and -108, possessing introgressions on the top of chromosome 2H. S42IL-108 represents a unique introgression, extending from 17 to 92 cM. In contrast, the non-unique introgressions of the remaining two ILs extend from 17 to 27 cM and from 17 to 42 cM, respectively, and are thus already represented in the first IL.

Field experiment for powdery mildew and leaf rust resistance

In season 2007, 39 S42ILs were tested for reactions to powdery mildew and leaf rust infection in the field at two different locations. Across both environments, the mean score of powdery mildew symptoms per S42IL ranged from 1.7 to 7.0, whereas 'Scarlett' exhibited a mean disease score of 4.9 (Table 2). The check 'Barke' showed a mean disease score of 1.1. For leaf rust, the average disease severity for the S42ILs ranged from 2.7 to 5.7 scores across both environments. For 'Scarlett', a mean value of 4.1 scores was recorded, and the check 'Barke' exhibited a mean disease score of 2.8. The field experiment revealed only a weak correlation ($r = 0.25$ with $P = 0.02$) between scores for powdery mildew and leaf rust diseases based on LSMEANS for 39 S42ILs calculated across two environments and three replications.

Identification and verification of QTLs for powdery mildew resistance

The 39 S42ILs were subjected to a line × phenotype association study in order to verify QTL effects, which were previously detected with the advanced backcross population S42, as well as to identify new QTLs. The mixed model ANOVA for powdery mildew resistance revealed significant line effects ($P < 0.001$) and

Table 1 List of 59 *Hsp* introgression lines from population S42

Chr.[a]	Intro. (in cM)[b]	Name of IL	SSR interval[c]	No. of SSRs[d]	Size (in cM)[e]	Percent. *Hsp*[f]	Unique IL[g]	No. of matching ILs[h]
1H	0–14	S42IL-101	1–2	2	17.0	1.4		1
	0–85	S42IL-102[i]	1–12	12	95.0	6.7	Unique	5
	39–70	S42IL-103	5–10	6	39.0	3.2		4
	52–85	S42IL-157	6–12	7	49.5	4.1		4
	52–70	S42IL-104	6–10	5	27.0	2.2		4
	70–85	S42IL-105	10–12	4	26.0	2.2		4
	105	S42IL-141	13	1	15.0	1.2	Unique	0
	115	S42IL-142	14	1	12.5	1.0	Unique	0
	144	S42IL-143	16	1	16.0	1.3	Unique	0
2H	17–27	S42IL-106	18–19	2	17.5	1.5		2
	17–42	S42IL-107	18–20	3	37.5	3.1		2
	17–92	S42IL-108	18–24	7	82.5	6.9	Unique	6
	67	S42IL-144	21	1	19.0	1.6		2
	67–92	S42IL-109	21–24	4	45.0	3.7		4
	80–86	S42IL-110	22–23	2	15.5	1.3		2
	92–107	S42IL-153	24–25	2	25.5	2.1	Unique	2
	139–159	S42IL-175	27–30	4	28.5	2.4	Unique	0
3H	65–70	S42IL-111	34–35	2	25.0	2.1		1
	65–110	S42IL-154	34–38	5	63.0	5.2	Unique	3
	94–110	S42IL-155	36–38	3	38.0	3.2		2
	100–130	S42IL-112	37–39	3	45.5	3.8	Unique	4
	130–175	S42IL-114[i]	39–42	4	62.5	5.2		4
	130–175	S42IL-140	39–42	4	62.5	5.2	Unique	4
	155–175	S42IL-113	40–42	3	40.0	3.3		3
	155–190	S42IL-115	40–43	4	47.5	4.0	Unique	3
4H	14–31	S42IL-116	44–46	3	23.5	2.0		4
	14–44	S42IL-117	44–48	5	35.5	3.0	Unique	5
	31–95	S42IL-119	47–52	6	82.0	6.8	Unique	7
	31–57	S42IL-118	47–50	4	40.5	3.4		5
	31–57	S42IL-120	47–50	4	40.5	3.4		5
	44	S42IL-145	48	1	12.0	1.0		4
	80–95	S42IL-121	51–52	2	41.5	3.5		2
	95	S42IL-146	52	1	22.5	1.9		2
	125–132	S42IL-122	53–56	4	31.0	2.6		1
	125–170	S42IL-123[i]	53–58	6	65.0	5.4	Unique	2
	170–190	S42IL-124	58–61	4	30.0	2.5	Unique	1
5H	43–69	S42IL-125	65–67	3	43.5	3.6	Unique	3
	69	S42IL-147	67	1	18.5	1.5		3
	69–85	S42IL-126	67–68	2	47.0	3.9		3
	69–137	S42IL-176	67–70	4	91.0	7.6	Unique	3
	162–165	S42IL-127	71–72	2	15.5	1.3	Unique	0
6H	6	S42IL-148	73	1	17.0	1.4		1
	6–135	S42IL-156	73–79	7	134.0	11.1	Unique	8
	40	S42IL-149	74	1	45.0	3.7		2
	40–112	S42IL-128	74–78	5	100.5	8.4		6
	96	S42IL-150	75	1	31.5	2.6		3
	96–112	S42IL-129[i]	75–78	4	55.5	4.6		5
	112	S42IL-151	78	1	14.0	1.2		4
	112–155	S42IL-130	78–81	4	45.5	3.8	Unique	6
	135–155	S42IL-131	79–81	3	31.5	2.6		3
	145–155	S42IL-132	80–81	2	15.0	1.2		2

Table 1 continued

Chr.[a]	Intro. (in cM)[b]	Name of IL	SSR interval[c]	No. of SSRs[d]	Size (in cM)[e]	Percent. *Hsp*[f]	Unique IL[g]	No. of matching ILs[h]
7H	50	S42IL-133	84	1	17.5	1.5	Unique	0
	62–75	S42IL-134	85–86	2	28.0	2.3	Unique	1
	75–155	S42IL-135	86–94	9	92.0	7.7	Unique	4
	133	S42IL-152	91	1	13.0	1.1		1
	146–155	S42IL-136	92–94	3	21.0	1.7		2
	146–166	S42IL-137	92–95	4	32.5	2.7	Unique	3
	166–181	S42IL-138	95–98	4	20.5	1.7	Unique	2
	178–181	S42IL-139	96–98	3	9.0	0.7		1
		Average		3.4	39.2	3.2		3

[a] Chromosomal location of the target introgression

[b] Estimated extent of the target introgression in cM, based on von Korff et al. (2004)

[c] SSR marker interval: see von Korff et al. (2004) for numeric code of SSR markers

[d] Total number of SSR loci revealing the exotic genotype in each IL

[e] Estimated size of the target introgression in cM (see: "Materials and methods")

[f] Percentage of exotic genome per IL

[g] Indication, if IL is unique or already represented in a larger introgression

[h] Number of ILs partly or completely matching the *Hsp* genotype in the given SSR interval (see Fig. 2)

[i] The S42ILs -102, -114, -123, and -129 contain an additional introgression on 2H, 80–92 cM, 5H, 43 cM, 4H, 31–55 cM, and 1H, 0 cM, respectively (see Fig. 2)

line × environment interaction effects ($P = 0.003$, data not shown). Ten introgression lines revealed powdery mildew scores significantly different from the control 'Scarlett'. As illustrated in Fig. 3, the *Hsp* introgressions of these lines are located on chromosomes 1H, 2H, 4H, and 7H. Taking into account that some introgressions overlapped or were flanked, a total of seven QTLs for powdery mildew resistance could be assumed. Two QTLs are presumably located on chromosome 1H since the S42ILs-101 and -104 do not overlap. One QTL, represented by S42IL-110, is located on chromosome 2H. Three further QTLs are located on chromosome 4H. Although the two corresponding S42ILs -123 and -124 do overlap, their effects are contrasting (Table 3). Finally, at least one QTL is located on chromosome 7H. Although the two introgressions present in S42ILs -135 and -138 do not share a common SSR allele, their *Hsp* introgressions might potentially overlap in the border interval 155–166 cM. Six QTLs could be detected both as line effect and as L × E interaction effect (Table 3), whereas one QTL, present in S42IL-118, was only detected as line L × E interaction effect. The strongest favorable effect was measured for QPm.S42IL-1H.a, which was identified by S42ILs -101 and -102. Here, the *Hsp* introgression located on chromosome 1H, 0–14 cM and 1H, 0–85 cM, respectively, was associated with a reduction of disease severity by 52.5% (2.6 scores) and 66.1% (3.3 scores), respectively, relative to the control 'Scarlett'. For both lines, the QTL effect was highly significant ($P < 0.001$) across both environments and also in each single environment (Table 2). As shown in Table 3, QPm.S42IL-1H.a verified the favorable QTL

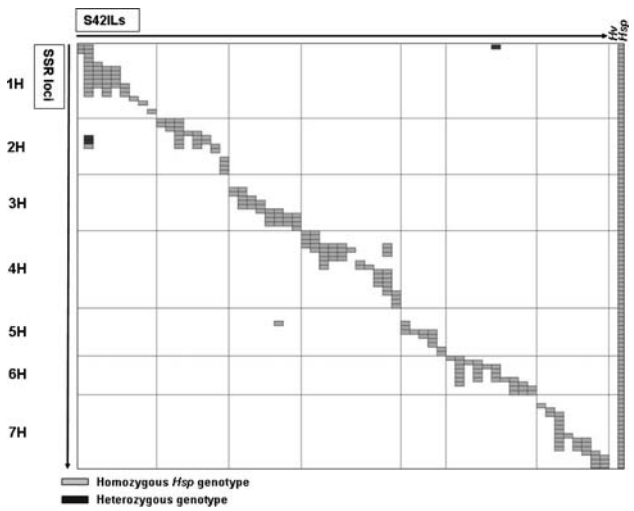

Fig. 2 Graphical genotypes of 59 *Hsp* introgression lines from population S42. The *horizontal lines* represent the seven barley chromosomes with 98 SSR loci. The order of SSRs is in agreement with von Korff et al. (2004). The 59 S42ILs, the recurrent parent 'Scarlett' (*Hv*) and the donor parent 'ISR 42-8' (*Hsp*) are illustrated vertically. The order of the S42ILs is in agreement with Table 1. Loci, which carry the homozygous *Hsp* genotype are depicted in *grey*, and heterozygous loci are illustrated in *black*. The remaining genome is derived from the recurrent parent 'Scarlett' (indicated in *white*)

effect QPm.S42-1H.a, which was detected in the S42 population by von Korff et al. (2005). It was located in the chromosomal region 1H, 0–28 cM and caused a reduced powdery mildew susceptibility of 51.5%. One additional favorable QTL effect was localized in S42ILs as well as in the S42 population on chromosome 7H (QPm.S42IL-7H.a

Table 2 LSMEANS of disease scores for powdery mildew (PM) and leaf rust (LR) across environments and per environment for 39 tested S42ILs and controls 'Scarlett' and 'Barke'

Chr.[a]	Intro. (in cM)[b]	Name of IL	PM[c]						LR[c]					
			ALL	Sig.	D07	Sig.	G07	Sig.	ALL	Sig.	D07	Sig.	G07	Sig.
1H	0–14	S42IL-101	2.3	***	1.7	***	3.0	***	3.5		3.0		4.0	
	0–85	S42IL-102	1.7	***	2.0	***	1.3	***	3.8		3.0		4.7	
	39–70	S42IL-103	5.2		5.0		5.3		4.2		3.0		5.3	
	52–70	S42IL-104	2.7	***	3.7		1.7	***	4.2		3.3		5.0	
	70–85	S42IL-105	5.3		5.0		5.7		4.0		3.0		5.0	
2H	17–27	S42IL-106	4.8		5.0		4.7		4.0		2.7		5.3	
	17–42	S42IL-107	4.7		4.3		5.0		5.7	***	4.7	**	6.7	
	17–92	S42IL-108	5.3		4.7		6.0		5.0		4.0		6.0	
	67–92	S42IL-109	5.3		5.7		5.0		4.8		4.0		5.7	
	80–86	S42IL-110	6.2	**	6.7	***	5.7		5.3	*	4.7	**	6.0	
3H	65–70	S42IL-111	4.3		4.0		4.7		4.0		3.0		5.0	
	100–130	S42IL-112	5.3		5.0		5.7		4.2		3.3		5.0	
	130–175	S42IL-114	5.3		5.0		5.7		4.5		3.7		5.3	
	155–175	S42IL-113	5.7		5.7		5.7		4.2		3.3		5.0	
	155–190	S42IL-115	5.5		5.0		6.0		4.8		4.0		5.7	
4H	14–31	S42IL-116	5.7		5.3		6.0		3.8		3.0		4.7	
	14–44	S42IL-117	5.3		5.0		5.7		4.5		3.7		5.3	
	31–57	S42IL-118	5.7		6.0	*	5.3		3.8		3.0		4.7	
	31–57	S42IL-120	5.0		5.0		5.0		3.8		2.7		5.0	
	31–95	S42IL-119	5.3		4.7		6.0		3.8		3.3		4.3	
	80–95	S42IL-121	4.8		4.0		5.7		4.7		4.3		5.0	
	125–132	S42IL-122	4.0		4.0		4.0		2.7	**	2.3	*	3.0	
4H	125–170	S42IL-123	7.0	***	7.7	***	6.3		2.7	**	2.0	***	3.3	
	170–190	S42IL-124	3.7	**	3.0	**	4.3		3.3		3.0		3.7	
5H	43–69	S42IL-125	5.2		5.0		5.3		3.5		3.3		3.7	
	69–85	S42IL-126	5.2		5.3		5.0		3.8		3.3		4.3	
	162–165	S42IL-127	4.7		4.0		5.3		3.7		3.3		4.0	
6H	40–112	S42IL-128	5.7		5.3		6.0		4.3		3.3		5.3	
	96–112	S42IL-129	5.5		5.3		5.7		4.7		3.3		6.0	
	112–155	S42IL-130	5.2		5.0		5.3		3.7		3.3		4.0	
	135–155	S42IL-131	5.7		5.7		5.7		3.5		3.0		4.0	
	145–155	S42IL-132	5.8		5.7		6.0		4.0		3.3		4.7	
7H	50	S42IL-133	4.2		3.7		4.7		3.8		3.7		4.0	
	62–75	S42IL-134	4.3		4.0		4.7		3.7		3.0		4.3	
	75–155	S42IL-135	3.8	*	3.7		4.0		3.8		3.0		4.7	
	146–155	S42IL-136	4.7		4.3		5.0		3.8		3.0		4.7	
	146–166	S42IL-137	3.3	***	3.0	**	3.7		5.0		3.7		6.3	
	166–181	S42IL-138	2.7	***	2.0	***	3.3	**	3.2		2.0	***	4.3	
	178–181	S42IL-139	4.3		3.7		5.0		3.3		3.0		3.7	
		Barke	1.1	***	1.0	***	1.1	***	2.8	***	2.2	***	3.3	
		Scarlett	4.9		4.7		5.2		4.1		3.4		4.8	

[a] Chromosomal location of the target introgression

[b] Estimated extent of the target introgression in cM, based on von Korff et al. (2004)

[c] The LSMEANS are indicated across both environments (ALL) and per environment (D07: Dikopshof 2007; G07: Gudow 2007). Significant differences between introgression lines and the control 'Scarlett' are indicated with *** $P < 0.001$, ** $P < 0.01$, or *$P < 0.05$

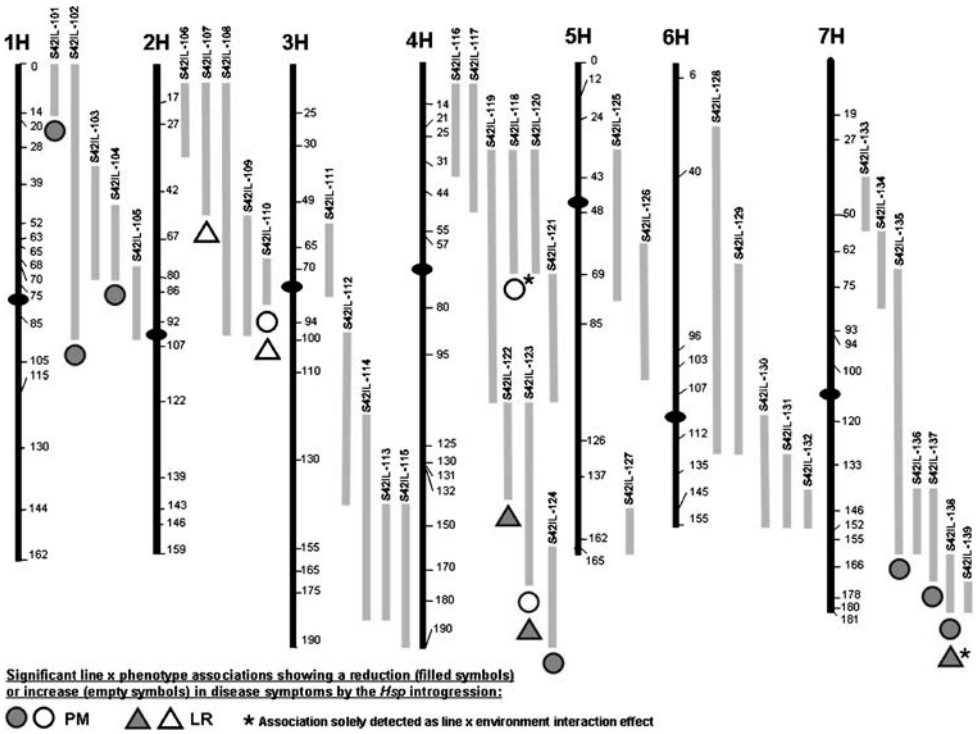

Fig. 3 SSR map showing the *Hsp* introgressions of 39 S42ILs and illustrating significant line × phenotype associations for resistance to powdery mildew and leaf rust. Chromosomes are shown as *black bars* with cM values for SSR loci following the order of von Korff et al. (2004). The extent of *Hsp* introgressions are given in *grey bars* right to the chromosomes. Significant associations are illustrated as *symbols* below the S42ILs. They either exhibit a reduction (*filled symbols*) or increase (*empty symbols*) in disease symptoms caused by the *Hsp* introgression. Associations solely detected as line × environment interaction effects are highlighted by an *asterisk right* to the *symbol*. S42ILs -110, -123 and -138 on chromosomes 2H, 4H and 7H, respectively, revealed two QTL effects each

Table 3 List of 15 significant line x phenotype associations for resistance to powdery mildew (PM) and leaf rust (LR) detected among 39 S42ILs

Trait	QTL in S42IL	Name of IL	Chr.[a]	Intro. (in cM)[b]	Eff.[c]	Diff.[d]	RP(IL) (in %)[e]	QTL in BC_2DH[f]	CGs[g]
PM	QPm.S42IL-1H.a	S42IL-101	1H	0–14	L + I	−2.6	−52.5	QPm.S42-1H.a	*Mla*[1]
		S42IL-102	1H	0–85	L + I	−3.3	−66.1		
	QPm.S42IL-1H.b	S42IL-104	1H	52–70	L + I	−2.3	−45.8		
	QPm.S42IL-2H.a	S42IL-110	2H	80–86	L + I	1.3	25.4	QPm.S42-2H.b	
	QPm.S42IL-4H.a	S42IL-118	4H	31–57	I	1.3	28.6^{D07}		
	QPm.S42IL-4H.b	S42IL-123	4H	125–170	L + I	2.1	42.4	QPm.S42-4H.a	*Mlg*[2]
	QPm.S42IL-4H.c	S42IL-124	4H	170–190	L + I	−1.3	−25.4		
	QPm.S42IL-7H.a	S42IL-135	7H	75–155	L	−1.1	−22.0	QPm.S42-7H.a	*Mlf*[3]
		S42IL-137	7H	146–166	L + I	−1.6	−32.2		
		S42IL-138	7H	166–181	L + I	−2.3	−45.8		
LR	QLr.S42IL-2H.a	S42IL-107	2H	17–42	L + I	1.6	38.8		
	QLr.S42IL-2H.b	S42IL-110	2H	80–86	L + I	1.3	30.6	QLr.S42-2H.a	
	QLr.S42IL-4H.a	S42IL-122	4H	125–132	L + I	−1.4	−34.7	QLr.S42-4H.a	
		S42IL-123	4H	125-170	L + I	−1.4	−34.7		
	QLr.S42IL-7H.a	S42IL-138	7H	166–181	I	−1.4	-41.5^{D07}	QLr.S42-7H.a	*Rph19*[4]

[a] Chromosomal location of the target introgression

[b] Extent of the target introgression in centiMorgans

[c] Significant line × phenotype associations are detected as line main effect (L) or line × environment interaction effect (I) with $P < 0.05$

[d] Score difference = LSMEANS(IL) − LSMEANS(Scarlett)

[e] Relative performance of IL: RP(IL) = [LSMEANS(IL) − LSMEANS(Scarlett)] × 100/LSMEANS(Scarlett). If line main effects (L), or line main effects and a line x environment interaction effects (L + I) were detected, the RP(IL) across all environments is listed. If solely a line × environment interaction (I) was identified, the RP(IL) in the particular environment is given

[f] Reference von Korff et al. (2005)

[g] References for candidate genes: [1]Zhou et al. (2001), [2]Kürth et al. (2001), [3]Schönfeld et al. (1996), [4]Park and Karakousis (2002)

and QPm.S42-7H.a, respectively). Here, the disease severity was reduced by 22.0 and 31.9%, respectively, in comparison to 'Scarlett'. The three lines S42IL-110, S42IL-118, and S42IL-123, which carry *Hsp* introgressions on chromosome 2H, 80–86 cM; 4H, 31–57 cM; and 4H, 125–170 cM, respectively, revealed a significant higher susceptibility against powdery mildew in comparison to 'Scarlett'. As illustrated in Table 3, the disease severity was increased by 25.4% (1.3 scores), 28.6% (1.3 scores) and 42.4% (2.1 scores), respectively. Two of these effects (QPm.S42IL-2H.a and QPm.S42IL-4H.b) correspond to QPm.S42-2H.b and QPm.S42-4H.a, which were mapped by von Korff et al. (2005) to the regions 2H, 42–86 cM and 4H, 125–132 cM. They revealed an unfavorable *Hsp* effect, increasing powdery mildew symptoms by 17.0 and 16.1%, respectively.

Identification and verification of QTLs for leaf rust resistance

The mixed model ANOVA for leaf rust resistance revealed significant line effects ($P < 0.001$), but no significant line × environment interaction effect ($P = 0.1855$, data not shown). Altogether, four QTL effects, present in five S42ILs, were detected for leaf rust resistance (Table 3; Fig. 3). Two QTLs were located on chromosome 2H and one QTL each on chromosome 4H and 7H. Three QTLs were simultaneously detected as line effect and L × E interaction effect. One QTL was solely detected as L × E interaction effect. The strongest favorable effect of an *Hsp* introgression was measured in S42IL-138 (QLr.S42IL-7H.a). Here, the disease severity was reduced in environment D07 by 41.5% (1.4 scores). As illustrated in Table 3, this QTL effect confirmed QLr.S42-7H.a, which was identified in the S42 population by von Korff et al. (2005). Here, a significant marker main effect was detected in region 7H, 166–181. The disease symptoms were reduced by 28.9%, relative to 'Scarlett'. The overlapping S42ILs -122 and -123 with *Hsp* introgressions located on chromosome 4H, 125–132 cM and 4H, 125–170 cM, respectively, revealed reductions in disease severity by 34.7% (-1.4 scores) each. In population S42, QLr.S42-4H.a was also mapped to the region 4H, 125–132 and showed a significant reduction of leaf rust symptoms by 37.6% (von Korff et al. 2005). The strongest unfavorable *Hsp* effect was measured in S42IL-107 on chromosome 2H, 17–42 cM (QLr.S42IL-2H.b). In this region, an increase of 38.8% (1.6 scores) in leaf rust susceptibility was detected. S42IL-110, which also carries an introgression on 2H from 80–86 cM, showed an increase in leaf rust symptoms by 30.6% (1.3 scores) in comparison to 'Scarlett'. This effect corresponds to QLr.S42-2H.a, which was detected in the S42 population, and showed a significantly reduced susceptibility of −10.1% at the locus 2H, 86 cM (von Korff et al. 2005).

Discussion

Selection and characterization of introgression lines

In the present study, a set of 59 introgression lines of the population S42 was generated by three rounds of backcrossing, each theoretically reducing the portion of the exotic genome by 50% in the followed generation. Two or four selfings were subsequently performed to derive completely homozygous lines, which are a stable genetic resource for further evaluation. As described by von Korff et al. (2004), a pre-selection step, based on genotype data of 98 SSR markers, was carried out in the BC_2DH generation. Here, a set of 40 candidate introgression lines were selected from a population consisting of 301 lines. The set presented the complete *Hsp* genome by a minimum number of lines, each containing a portion of the exotic genome as small as possible. Pure S42ILs were finally identified after a second selection step in the BC_3S_2 or BC_3S_4 generation. One or several pre-selection steps are generally advisable to reduce the required population size. Therefore, the propagation and genotyping of a large number of unusable recombinant plants can be avoided. A pre-selection in previous backcross generations, either based on genotype or phenotype data, has also proved to be helpful for the selection of IL sets in other crop species. For instance, Eshed and Zamir (1994) conducted two phenotypic selection steps in BC_1 to BC_1S_5 generation based on horticultural characteristics, followed by two rounds of genotypic selection in BC_1S_6 to BC_4S_1 generation. For melon, Eduardo et al. (2005) pursued a selection strategy similar to those of the S42ILs. They initially performed a selection of 25 double haploid lines, based on the proportion of the donor genome. Several selection steps in subsequent backcross generations were followed, and finally a total of 57 near-isogenic lines (NILs) were predominantly selected in the BC_3S_1 or BC_3S_2 generation. In general, most IL sets were finally selected after up to ten generations, including three or four rounds of backcrossing (e.g. Szalma et al. 2007; Tian et al. 2006a).

So far, 86.6% (1,041.5 cM) of the *Hsp* genome is covered by pure S42ILs. Introgression lines, covering the missing chromosomal regions, will be selected in future within BC_3 or BC_4 progenies. For this purpose, a pool of 45 potential ILs, which are still under selection, is available. The selection of more ILs with smaller exotic introgressions is also advisable, since there are several S42ILs which possess very large introgressions. Therefore, all pure S42ILs will be backcrossed again with 'Scarlett', and

appropriate recombinants will be identified and enhanced as so called Sub-ILs. Subsequently, selected Sub-ILs containing very small introgressions and carrying interesting QTLs, will be directly used for fine-mapping and, eventually, map-based cloning. Several reports for different crop species underline the usefulness of ILs for these purposes. By means of ILs, yield-associated QTLs were fine-mapped in tomato (Eshed and Zamir 1995), rice (Tian et al. 2006b), and wheat (Röder et al. 2008). Two studies in tomato focused on the dissection of regions containing QTLs for fruit quality and shape (Yates et al. 2004; van der Knaap et al. 2004, respectively). Furthermore, the tomato fruit color gene *Beta*, the fruit weight QTL *fw2.2*, and the sugar yield QTL *Brix9-2-5* were cloned based on IL mapping and fine-mapping (Ronen et al. 2000; Frary et al. 2000; Fridman et al. 2004, respectively).

In future, the genotyping of the S42ILs with Illumina SNP chip markers (Rostoks et al. 2006) or Diversity Arrays Technology (DArT) markers (Wenzl et al. 2006) will foster the fine-mapping of the introgressions. So far, the average genomic resolution of the S42IL set is 12.5 cM/SSR. The least marker density of 21.4 cM/SSR was detected for the long arm of chromosome 6H. An increased genomic resolution is also necessary to confirm that the S42ILs do not possess additional non-target introgressions. Despite these prospective demands, the described set of 59 S42ILs is comparable to IL sets developed for other crop species, with regard to the 'quality' of introgression lines, e.g., exotic genome coverage and proportion of exotic genome present in each line. Among so far generated IL sets, the IL library of *Solanum pennellii* in *S. esculentum*, is the furthest developed set. Eshed and Zamir (1994) selected 50 ILs, which cover the whole genome of the exotic species *S. pennellii*. Based on the analysis with 350 RFLP markers, all ILs were verified as pure lines, each carrying single exotic segments of 33 cM in average. Currently, the *S. pennellii* IL set consists of 76 different lines covering the complete tomato genome (Lippman et al. 2007). In *Arabidopsis thaliana*, a set of 92 near-isogenic lines (NILs) containing one to four introgressions of the Cape Verde Islands (Cvi) accession within the genetic background of Landsberg *erecta* (L*er*), was developed by Keurentjes et al. (2007). The target introgressions included on average 31.7 cM. Twenty-five lines, selected as a core-set, covered >90% of the donor genome. Eduardo et al. (2005) reported on the development of 57 NILs for melon, which altogether represented 85% of the introgressed exotic genome. The single lines exhibited an average introgression size of 41 cM and an average proportion of exotic genome of 3.4%. Also the genomic resolution of 15.7 cM/marker, provided by a map with 62 SSR markers, was similar to our results for the S42ILs. In comparison to IL sets of several other crop species, the S42ILs so far exhibited a high grade of pureness since 55 lines possess single exotic introgressions. These lines allow a precise localization of the chromosomal region, when the exotic introgression causes a phenotypic effect relative to the recurrent parent 'Scarlett'.

Identification and verification of QTLs for powdery mildew and leaf rust resistance

As a first example for applications, the S42ILs were evaluated with regard to powdery mildew and leaf rust resistance. The aims were (1) to verify QTLs, previously detected with 301 double haploid lines of the advanced backcross population S42 and (2) to identify new QTLs. Von Korff et al. (2005) detected a total of nine QTLs for powdery mildew and altogether six QTLs for leaf rust resistance. Seven and four QTLs are localized in genomic regions, which are already represented by *Hsp* introgressions of the evaluated 39 S42ILs. As listed in Table 3, four out of these seven QTLs (57.1%) and three out of these four QTLs (75.0%) were verified with the S42ILs for powdery mildew and leaf rust, respectively. In the S42ILs, the strongest effect reducing the susceptibility to powdery mildew was measured for QPm.S42IL-1H.a on top of chromosome 1H. This effect could be induced through an allele of the *Mla* resistance locus, which is localized within the same region (Zhou et al. 2001). The QTL QPm.S42IL-4H.b on chromosome 4H, 125-170 cM, which is represented by S42IL-123, showed a significant higher susceptibility than 'Scarlett' (42.4%). The region possibly corresponds to the *Mlg* locus, mapped by Kürth et al. (2001). The *Mlg* gene is known to be present in 'Scarlett', instead of the *mlo* gene (von Korff et al. 2005). Schönfeld et al. (1996) reported on the *Mlf* powdery mildew resistance gene, which is located on the short arm of chromosome 7H, close to the QTL QPm.S42IL-7H.a. Here, strong effects reducing the susceptibility to powdery mildew were detected for the S42ILs -135, -137, and -138 (Table 3). The strongest favorable QTL effect for leaf rust (QLr.S42IL-7H.a) was detected for S42IL-138 within the region 7H, 166-181 cM. This effect could possibly be caused by *Rph19*, a major resistance gene, which was mapped by Park and Karakousis (2002) to a locus, which is only 2 cM distant to QLr.S42IL-7H.a. In future, it is intended to fine-map or sequence the mentioned resistance genes in the corresponding S42ILs to verify the assumption that they indeed are causal for the detected QTL effects.

Bernacchi et al. (1998b) conducted a comprehensive NIL study for tomato to verify QTLs, which were previously detected in two advanced backcross populations (Bernacchi et al. 1998a). They developed a total of 23 NILs, carrying single exotic introgressions either from *S. hirsutum* or *S. pimpinellifolium*. The NILs were generated

for regions, which contained QTLs with a favorable effect on one or several traits. They covered 15 genomic regions with 25 QTLs for seven agronomic traits. Altogether, 22 QTLs (88%) were confirmed by the NILs. So far, no report is available on the verification of QTL data by a complete or nearly complete barley IL set. However, there are several studies that confirmed QTL effects for powdery mildew resistance genes (Czembor and Czembor 2004) and leaf rust resistance QTLs (van Berloo et al. 2001; Marcel et al. 2007, 2008) by NILs.

Bernacchi et al. (1998b) pointed out that the verification of QTLs with NILs can be characterized with regard to the stability of the QTL effect across environments and the magnitude of the effect. In the present QTL verification study, all four corresponding QTLs for powdery mildew and two out of three corresponding QTLs for leaf rust were significant ($P < 0.05$) across all environments in the S42 population as well as in the S42ILs. In addition, three and one QTL, respectively, were detected as line × environment interaction effects in the S42ILs. Furthermore, nearly all verified QTLs showed the same direction of the effect in the S42ILs and the S42 population. One exception was the QTL QLr.S42IL-2H.b/QLr.S42-2H.a, which revealed an increased susceptibility to leaf rust in the S42ILs, but a reduction effect in the S42 population. For all QTL effects with the same direction, except QLr.S42IL-4H.a/QLr.S42-4H.a, the magnitude of effect was higher in the S42ILs than in the S42 population. The strongest boost was measured for QPm.S42IL-4H.b which showed an increased effect on powdery mildew susceptibility of 42.4% in S42IL-123, whereas the corresponding QTL QPm.S42-4H.a revealed an effect of 16.1%, relative to 'Scarlett' in the S42 population. These discrepancies could be caused by environmental effects since both populations have been tested in different locations and years. In addition, the presence of different strains of powdery mildew and leaf rust in the two experiments could also result in deviating effects. Finally, a reduction in the number of epistatic effects between *Hsp* alleles, which are present with lower frequency in the S42ILs, can be assumed. Whereas the 301 BC_2DH lines of population S42 carry on average four independent exotic introgressions (von Korff et al. 2004), 55 out of the 59 S42ILs contain only single introgressed *Hsp* segments. To test the epistasis hypothesis we are currently crossing the S42ILs systematically with each other. In future, it is intended to search for epistatic interactions in the resulting plants, each harboring zero, one or two independent *Hsp* introgressions. With this material, the performance of all four possible allele combinations (*Hv*/*Hv*, *Hsp*/*Hsp*, *Hv*/*Hsp*, and *Hsp*/*Hv*) will be compared.

Altogether, three and one new QTL were detected by S42ILs for powdery mildew and leaf rust resistance, respectively. At two QTLs for powdery mildew (QPm.S42IL-1H.b and QPm.S42IL-4H.c), the *Hsp* introgression caused a reduced susceptibility in comparison to 'Scarlett' (Table 3). Three co-localizations of QTL effects for both diseases were exposed by S42ILs on chromosomes 2H, 4H, and 7H (Fig. 3). As illustrated in Table 3, one favorable effect on both diseases was identified by S42IL-138 in the region 7H, 166–181. Due to this, S42IL-138 could be used to improve resistance of current spring barley cultivars to both diseases simultaneously by transfer of *Hsp* resistance genes. With most of the S42ILs, the QTL effects could be precisely localized to one chromosomal region, since they only carry one target introgression. In contrast, the S42ILs -102, -114, -123, and -129 each contain one additional non-target *Hsp* segment (Table 1). Due to this, a precise QTL mapping with these lines is not possible so far. It can be assumed that (1) only one introgression, either the target or the non-target one, causes a significant QTL effect or that (2) there are two independent QTL effects, localized in both the target and the non-target introgressions.

There are several instances where a S42IL revealed a significant QTL effect, whereas overlapping sister lines containing *Hsp* alleles in the same chromosomal region do not show this effect. These discrepancies can be attributed to the following three reasons: (1) the true size of an exotic introgression might slightly differ from our estimation which is based on genotype data from a limited number of 98 SSR markers (see Fig. 3). Thus, it might be possible that *Hsp* alleles, present in an extended introgression, cause a QTL effect, which is missing in the apparent sister S42IL. For example, S42IL-108, revealing no QTL effect, might actually possess the estimated introgression 2H, 17-92 cM. However, the sister line S42IL-107 (2H, 17–42 cM), harboring a QTL for powdery mildew, might eventually contain an introgression which begins further north of the first chromosomal SSR marker (2H, 17 cM), resulting in a unique QTL effect which is not detectable in the sister line S42IL-108. (2) As an alternative explanation, the IL with the larger introgression could include additional *Hsp* alleles which interact with the QTL present in the small introgression. This interaction could result in a loss of significant QTL effects. Under this light, a second *Hsp* allele could be present in S42IL-108, located south of the small introgression in S42IL-107, which counter-acts or turns down the original QTL effect. (3) Some S42ILs might also contain a so far hidden non-target introgression, carrying a *Hsp* allele which exhibit the significant QTL effect. This explanation could eventually be the case for S42IL-110 on chromosome 2H, 80-86 cM, where two QTL effects are present. In contrast, no QTL was detected in the sister lines S42IL-108 and -109 although the latter two ILs completely cover the introgression of the former IL. To test the three hypotheses presented, it is required to

characterize the introgressions more precisely with additional DNA markers in order (1) to define the extensions of the introgressions with a higher resolution, (2) to discover and then eliminate potential extra non-target introgressions, and (3) to fine-map and possibly dissect the detected QTL effects in sub-ILs carrying smaller *Hsp* segments.

In future, there are several demands concerning the further development and evaluation of the S42IL set. The first aim will be the completion of the IL set by selecting lines for the chromosomal *Hsp* regions, which are so far not represented. As mentioned above, it is also intended to increase the marker density by genotyping new DNA markers. In addition, Sub-ILs will be selected and used for high-resolution mapping as a first step toward map-based cloning of interesting QTL. The S42ILs will also be extensively characterized on phenotype level. Here, the focus will be laid on agronomic traits, malting quality, biotic, and abiotic stress. All genotype and phenotype data of the S42ILs will be archived for general use in the public IL data base 'Phenom Networks' (http://phn.huji.ac.il/RTQ/). Our first application demonstrates that the S42ILs are a powerful genetic tool to unravel the genetic architecture of agriculturally relevant traits, as well as for providing cereal breeders with newly selected exotic germplasm.

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ORIGINAL PAPER

Identification and verification of QTLs for agronomic traits using wild barley introgression lines

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Abstract A set of 39 wild barley introgression lines (hereafter abbreviated with S42ILs) was subjected to a QTL study to verify genetic effects for agronomic traits, previously detected in the BC_2DH population S42 (von Korff et al. 2006 in Theor Appl Genet 112:1221–1231) and, in addition, to identify new QTLs and favorable wild barley alleles. Each line within the S42IL set contains a single marker-defined chromosomal introgression from wild barley (*Hordeum vulgare* ssp. *spontaneum*), whereas the remaining part of the genome is exclusively derived from elite spring barley (*H. vulgare* ssp. *vulgare*). Agronomic field data of the S42ILs were collected for seven traits from three different environments during the 2007 growing season. For detection of putative QTLs, a two-factorial mixed model ANOVA and, subsequently, a Dunnett test with the recurrent parent as a control were conducted. The presence of a QTL effect on a wild barley introgression was accepted, if the trait value of a particular S42IL was significantly ($P < 0.05$) different from the control, either across all environments and/or in a particular environment. A total of 47 QTLs were localized in the S42IL set, among which 39 QTLs were significant across all tested environments. For 19 QTLs (40.4%), the wild barley introgression was associated with a favorable effect on trait performance. Von Korff et al. (2006 in Theor Appl Genet 112:1221–1231) mapped altogether 44 QTLs for six agronomic traits to genomic regions, which are represented by wild barley introgressions of the S42IL set. Here, 18 QTLs (40.9%) revealed a favorable wild barley effect on the trait performance. By means of the S42ILs, 20 out of the 44 QTLs (45.5%) and ten out of the 18 favorable effects (55.6%) were verified. Most QTL effects were confirmed for the traits days until heading and plant height. For the six corresponding traits, a total of 17 new QTLs were identified, where at six QTLs (35.3%) the exotic introgression caused an improved trait performance. In addition, eight QTLs for the newly studied trait grains per ear were detected. Here, no QTL from wild barley exhibited a favorable effect. The introgression line S42IL-107, which carries an introgression on chromosome 2H, 17–42 cM is an example for S42ILs carrying several QTL effects simultaneously. This line exhibited improved performance across all tested environments for the traits days until heading, plant height and thousand grain weight. The line can be directly used to transfer valuable *Hsp* alleles into modern elite cultivars, and, thus, for breeding of improved varieties.

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Introduction

Most agronomically important traits, such as yield and quality parameters, are quantitatively inherited. They are not conditioned by single genes, but polygenes or so called

"quantitative trait loci" (QTLs, Collard et al. 2005). Based on molecular linkage maps, many QTL studies were conducted since the late 1980s to identify and map the loci, which affect complex traits. In barley, such QTL studies focused on yield associated traits, as well as malting quality, abiotic stress tolerance, and disease resistance (e.g. Tinker et al. 1996; Thomas et al. 1996; Jefferies et al. 1999; Kicherer et al. 2000).

Tanksley and Nelson (1996) proposed the advanced backcross (AB)-QTL analysis, which has several advantages over classical QTL analyses. These are: (1) the detection of QTLs and the development of improved varieties are conducted simultaneously. (2) Unadapted germplasm from wild species or land races is used to detect exotic alleles superior to the elite alleles which might ultimately broaden the genetic variation of modern crop cultivars (Tanksley and McCouch 1997). (3) Since the AB-QTL strategy is based on BC_2 or BC_3 generations, the portion of the exotic donor genome, and thus linkage drag, is considerably reduced. The fact that wild species contain favorable alleles, which could be useful for the enhancement of quantitative traits, was proven by many AB-QTL studies for several crop species, such as tomato (e.g. deVicente and Tanksley 1993; Fulton et al. 2002; Frary et al. 2004) and rice (Xiao et al. 1996; Li et al. 2002; Thomson et al. 2003; McCouch et al. 2007). In barley, several AB-QTL studies were conducted with regard to a multitude of characters, like agronomic traits, morphological traits, yield components, disease resistances, tolerance to abiotic stress and malting quality (Pillen et al. 2003, 2004; Talame et al. 2004; Li et al. 2005, 2006; Yun et al. 2006; Gyenis et al. 2007). Comprehensive studies were also performed by von Korff et al. (2005, 2006, 2008), who generated a BC_2DH population consisting of 301 lines based on an initial cross between the malting barley cultivar 'Scarlett' (*Hordeum vulgare* ssp. *vulgare*, hereafter abbreviated with *Hv*) and the Israeli wild barley accession 'ISR 42-8' (*H. vulgare* ssp. *spontaneum,* hereafter abbreviated with *Hsp*, von Korff et al. 2004). The lines were genotyped with 98 SSR markers, and subsequently evaluated under field conditions in up to eight environments. By a three-factorial mixed model analysis of variance (ANOVA), altogether 86 putative QTLs were identified for nine agronomic traits, e.g. days until heading, plant height, thousand grain weight and grain yield, where 31 QTLs (36.0%) revealed a favorable effect of the exotic allele (von Korff et al. 2006). In addition, 18 QTLs conferring resistance to diseases (von Korff et al. 2005), and 48 QTLs for seven malting parameters (von Korff et al. 2008), were detected. For these trait complexes, the performance was improved by the *Hsp* allele at 11 QTLs (61.1%) and 18 QTLs (37.5%), respectively.

As recommended by Zamir (2001), one step further towards the nearly simultaneous identification and transfer of exotic QTL alleles associated with an improved trait performance, is the development and evaluation of introgression lines (ILs). An IL set represents the complete genome of a wild species in the uniform background of an elite cultivar, where each single line solely contains a single marker-defined segment of the exotic parent. Due to the homozygosity of the ILs, they are a stable, and, thus, immortal, genetic resource which can be used with versatility. Furthermore, the low proportion of exotic germplasm, present in each IL, is a great advantage compared to commonly used QTL mapping populations, especially F_2, BC_1 and RI, but also AB populations. It allows the statistical verification of small phenotypic effects, which are otherwise more difficult to detect (Zamir 2001). As an advancement compared to the AB-QTL analysis, the detection of QTLs and their simultaneous transfer into elite cultivars is carried out with ILs in one step. It does not require further steps of backcrossing or selfing. The usefulness of ILs for the detection of QTL effects and the identification of favorable exotic QTL alleles was demonstrated by numerous QTL studies for several different crops, first of all for tomato. The *Solanum pennellii* IL library, which was initiated by Eshed and Zamir (1994) and currently consists of 76 ILs (Lippman et al. 2007), was used to identify nearly 3,000 putative QTLs for yield associated traits (Eshed and Zamir 1995; Hanson et al. 2007), morphological traits (Holtan and Hake 2003; Semel et al. 2006) and metabolites (Baxter et al. 2005; Overy et al. 2005; Schauer et al. 2006). With regard to quantitative traits, QTL studies using ILs were also conducted for rice (e.g. Mei et al. 2006; Zheng et al. 2007; Siangliw et al. 2007), wheat (Liu et al. 2006, 2007; Pestsova et al. 2006), maize (Szalma et al. 2007), melon (Eduardo et al. 2007; Fernandez-Trujillo et al. 2007; Moreno et al. 2008; Obando et al. 2008) and *Arabidopsis* (Keurentjes et al. 2007). In spring barley, Matus et al. (2003) conducted an association analysis to identify recombinant chromosome substitution lines (RCSLs) showing positive transgressive segregation with respect to the elite parent for agronomic traits, domestication-related traits, and malting quality traits. Here, altogether 140 RCSLs, with a similar genetic structure as ILs, were evaluated in the field. A superior phenotype, compared to the elite parent, was exhibited by RCSLs for the traits number of grains per ear, ear length and thousand grain weight. A further barley QTL study using RCSLs was conducted by Hori et al. (2005). They developed a total of 134 RCSLs, each line carrying one or several *Hsp* introgressions, and containing on average 12.9% of the *Hsp* genome. By means of a core-set of 19 RCSLs and, in addition, 93 double-haploid lines (DHLs) originating from the same cross, altogether 18 and 24 QTLs could be identified, respectively. Several QTLs revealed a coincident or very close localization in both populations (Hori et al. 2005).

In the present study, we demonstrate the usefulness of wild barley introgression lines (S42ILs) for the detection of QTLs for important agronomic traits. The performance of a set of 39 S42ILs, each containing a single marker-defined *Hsp* introgression in the uniform genetic background of *Hv*, was evaluated with regard to seven traits in three different field environments during 2007 growing season. Subsequently, a line × phenotype association study was conducted to verify QTL effects, which were previously identified in the BC_2DH population S42 (von Korff et al. 2006), and, in addition, to detect new QTLs. A further aim was to identify S42ILs, which exhibited an enhanced trait performance compared to the recurrent parent. These lines are a potentially valuable resource for the development of new elite cultivars.

Materials and methods

Plant material

A set of 39 S42ILs (S42IL-101 to S42IL-139) were evaluated with regard to seven agronomic traits. The lines differ among each other in only one single marker-defined chromosomal segment, introgressed from the Israeli wild barley accession 'ISR 42-8' (*Hsp*), whereas the remaining genome is consistently derived from the German spring barley cultivar 'Scarlett' (*Hv*). Based on 40 BC_2DH lines, previously selected from spring barley population S42 (von Korff et al. 2004), the S42ILs were developed by a further round of backcrossing with the recurrent parent 'Scarlett', several rounds of selfing and in parallel SSR marker-assisted selection. Finally, the 39 S42ILs were selected in BC_3S_2 or BC_3S_4 generation. The approach and the genetic characterization of the 39 S42ILs, as well as the verification of QTLs for powdery mildew and leaf rust, are described in detail in Schmalenbach et al. (2008).

Phenotypic evaluation of agronomic traits

In order to evaluate the agronomic performance of the 39 S42ILs with regard to seven traits, field tests at three different locations in Germany were conducted in the 2007 growing season. The locations were the experimental field station Dikopshof (D07, University of Bonn, West Germany), and the breeders' experimental field stations in Gudow (G07, Nordsaat Saatzucht, North Germany) and Herzogenaurach (H07, Saatzucht Josef Breun, South East Germany). The field tests were designed in three randomized complete blocks (i.e. replications). The recurrent parent 'Scarlett' was tested as a control in four replications per block. In addition, the spring barley malting cultivar 'Barke' was tested in three replications per block to compare the performance of the S42ILs with a current cultivar. Net plot sizes (4.5–6.0 m^2), seed density (300–390 kernels/m^2), nitrogen fertilization (30–80 kg N/ha) taking into account the N_{min} content of the soil, and field management were in accordance with the local practice. The grain was harvested with a plot harvester at total maturity (EC 92). The investigated traits and methods of measurement are listed in Table 1.

Statistical analyses

Statistical analyses were carried out with SAS Enterprise Guide 4.1 (SAS Institute 2006). Genetic correlations between trait values were calculated with the least squares means (LSMEANS) for each of the 39 S42ILs averaged across all replications and environments. The LSMEANS were computed with the GLM procedure and used for calculating Pearson's correlation coefficient (r) with the CORR procedure.

For the detection of QTLs and identification of favorable *Hsp* introgressions, a two-factorial mixed model ANOVA was carried out. The GLM model includes the line as a fixed factor, and the environment and line × environment interaction as random factors: $Y_{ijk} = \mu + L_i + E_j + LxE_{ij} + \varepsilon_{k(ij)}$, where μ is the general mean, L_i the fixed effect of the ith line, E_j the random effect of the jth environment, $L \times E_{ij}$ is the random interaction effect of the ith line and the jth environment, $\varepsilon_{k(ij)}$ is the error of Y_{ijk}. When the analysis revealed significant differences between lines or line × environment interactions, a Dunnett multiple comparison of LSMEANS differences between the ILs with the recurrent parent 'Scarlett' as the control was conducted (Dunnett 1955). The presence of a QTL in an *Hsp* introgression was accepted, when the trait value of a particular IL was significantly ($P < 0.05$) different from 'Scarlett' across all environments (line main effect) and/or in a particular environment (line × environment interaction effect). If several lines, which contained overlapping or flanking introgressions, showed a significant effect of the same direction (increase or reduction of trait performance), it was assumed that these ILs carried the same QTL. Introgressions do overlap, if they possess at least one common *Hsp* allele. They flank each other, if they have adjacent *Hsp* alleles. The relative performance of an introgression line [RP (IL)] was calculated as follows: RP (IL) = [LSMEANS (IL) − LSMEANS ('Scarlett')] × 100/LSMEANS ('Scarlett'), where for each trait the LSMEANS were calculated across all replications and environments.

Results

Trait performances of 'Scarlett' compared to the S42IL set

In Table 2, the trait performances of the recurrent parent 'Scarlett' and the S42ILs are described per environment

Table 1 List of seven agronomic traits evaluated for 39 S42ILs in up to three environments

Abbr.	Trait	Units	Method of measurement	Breeding goal[a]	Environment tested[b]
EAR	Ears per square meter	No. of ears/m^2	Number of ears counted from a row of 50 cm (D07) or 100 cm (H07)	+	D07, H07
GEA	Grains per ear	No. of grains/ear	Number of grains per ear calculated from a row of 50 cm (D07) or 20 average ears (G07 and H07)	+	D07, G07, H07
HEA	Days until heading	d	Number of days from sowing until emergence of 50% of ears on main tillers	–	D07, G07
HEI	Plant height	cm	Average plant height (in cm) measured from soil surface to tip of spike (including awns) 2 weeks after flowering	–	D07, G07, H07
LAH	Lodging at harvest	Scores 1–9	Visual rating of the severity of lodging at harvest, where one represents no lodging and nine represents total lodging of plot	–	D07, G07, H07
TGW	Thousand grain weight	g/1,000 grains	Average weight (in g) of 1,000 grains calculated from two samples of 250 grains	+	D07, G07, H07
YLD	Grain yield	dt/ha	Weight of barley grain (in dt/ha), harvested from 6.0 m^2 (D07), 4.5 m^2 (G07), 5.0/5.5 m^2 (H07), after drying for 1–2 days	+	D07, G07, H07

[a] The breeding goals for the evaluated traits were defined according to breeding programs for spring malting barley, where (–) indicates that a reduction, and (+) that an increase of the trait values is desirable

[b] The environment names are combinations of the location [Dikopshof (D), Gudow (G), Herzogenaurach (H)] and the year 2007 (07)

and across all tested environments by the parameters mean, minimum and maximum, and coefficient of variation. Across all three environments, both 'Scarlett' and the S42IL set revealed the highest coefficient of variation for the traits lodging at harvest (73.7 and 82.3%, respectively) and grain yield (34.8 and 35.0%, respectively). In contrast, a low variation of trait performance was measured for the traits grains per ear, days until heading, and plant height. As expected, 'Scarlett' exhibited a lower coefficient of variation than the S42ILs for all traits and environments. Averaged across all environments and in G07, 'Scarlett' showed a slightly higher performance in grain yield than the S42IL set (50.8 and 60.0 dt/ha, compared to 50.3 and 57.7 dt/ha, respectively). In D07 and H07, an opposite effect was measured (Table 2).

Trait correlations

A total of 17 significant correlations were detected between the seven investigated traits (Table 3). The strongest positive correlations were found between days until heading and the traits grains per ear ($r = 0.74$) and thousand grain weight ($r = 0.73$), as well as between plant height and lodging at harvest ($r = 0.73$). In addition, days until heading exhibited strong negative correlations with lodging at harvest ($r = -0.84$) and plant height ($r = -0.61$). Grain yield revealed significant correlations with all traits. Whereas negative correlations were found with days until heading and grains per ear ($r = -0.52$ and $r = -0.27$, respectively), the other four traits showed positive correlations with grain yield ranging from 0.37 for plant height to 0.68 for thousand grain weight.

QTL detection

In order to verify QTL effects, which were previously detected within the BC_2DH population S42, and to identify new QTLs, the 39 S42ILs were subjected to a line × phenotype association study. The initial two-factorial mixed model ANOVA exhibited significant line effects ($P < 0.05$) for all seven evaluated traits except ears per square meter. Significant line × environment interaction effects ($P < 0.05$) were detected for all traits (data not shown).

The subsequent Dunnett test revealed altogether 65 significant line × phenotype associations with four line main effects and 18 line × environment interaction effects. For 43 associations, the line main effect and the line × environment interaction effect, were significant, simultaneously (Table 4). For 29 associations (44.6%), the respective IL showed an improved trait performance in comparison to the recurrent parent 'Scarlett'. Taking into account that several ILs do overlap or are flanked, a

Table 2 Parameters describing the trait performance of the recurrent parent 'Scarlett' and the S42ILs per environment and across all tested environments

Trait and environment[a]	'Scarlett'					S42ILs				
	N[b]	Mean[c]	Min[d]	Max[d]	CV (in %)[e]	*N*[b]	Mean[c]	Min[d]	Max[d]	CV (in %)[e]
EAR										
D07	12	968.0	673.1	1250.0	18.0	117	1033.7	576.9	1634.6	22.2
H07	12	946.7	840.0	1088.0	10.2	117	903.9	616.0	1216.0	14.3
Across all env.	24	957.3	673.1	1250.0	14.4	234	968.8	576.9	1634.6	20.3
GEA										
D07	12	22.3	19.0	24.0	6.4	117	19.9	13.0	23.0	11.4
G07	4	23.0	22.0	24.0	3.5	39	22.5	17.0	26.0	7.8
H07	12	23.5	20.0	27.0	8.4	117	22.2	13.0	26.0	10.0
Across all env.	28	2.9	19.0	27.0	7.4	273	21.2	13.0	26.0	11.7
HEA										
D07	12	60.3	60.0	61.0	0.8	117	59.1	47.0	64.0	5.4
G07	12	72.3	70.0	75.0	2.0	117	71.0	62.0	76.0	4.7
Across all env.	24	66.3	60.0	75.0	9.4	234	65.1	47.0	76.0	10.4
HEI										
D07	12	90.9	83.0	101.0	5.7	117	89.3	70.0	100.0	6.3
G07	12	75.4	72.0	80.0	3.6	117	78.6	63.0	103.0	12.3
H07	8	76.0	70.0	82.0	5.3	78	77.5	64.0	98.0	8.6
Across all env.	32	81.4	70.0	101.0	10.4	312	82.3	63.0	103.0	11.3
LAH										
D07	12	7.4	5.0	8.0	12.1	117	7.3	3.0	9.0	16.5
G07	12	3.2	1.0	5.0	50.1	117	2.6	1.0	9.0	86.4
H07	12	1.1	1.0	2.0	26.6	117	1.3	1.0	8.0	92.4
Across all env.	36	3.9	1.0	8.0	73.7	351	3.7	1.0	9.0	82.3
TGW										
D07	12	39.7	37.1	43.0	4.8	117	39.3	34.6	47.0	5.8
G07	12	44.2	38.6	47.8	5.8	117	44.9	36.4	52.0	6.9
H07	12	33.7	32.2	36.2	4.2	117	33.5	26.9	41.8	8.7
Across all env.	36	39.2	32.2	47.8	12.3	351	39.2	26.9	52.0	13.8
YLD										
D07	12	65.7	61.8	72.4	5.0	117	66.2	52.4	78.7	6.9
G07	12	60.0	54.7	64.9	5.0	117	57.7	38.0	67.3	9.4
H07	12	26.8	20.4	30.4	11.9	117	26.9	13.9	35.9	16.6
Across all env.	36	50.8	20.4	72.4	34.8	351	50.3	13.9	78.7	35.0

[a] The trait and environment abbreviations are given in Table 1

[b] Number of observations

[c] Average trait performance

[d] Minimum and maximum trait performance

[e] Coefficient of variation (in %) = SD/mean

total of 47 putative QTLs were identified. Here, one QTL (QTgw.S42IL-2H.b) was detected solely as line main effect and eight QTLs solely as line × environment interaction effect. For the remaining 38 QTLs both the line main effect and the line × environment interaction effect, were significant. Nineteen QTLs (40.4%) showed a favorable effect of the *Hsp* introgression. In the following paragraphs, the QTL effects are described for each trait separately.

Ears per square meter (EAR)

For EAR, altogether four S42ILs revealed a significant line × phenotype association. Taking into account that

Table 3 Pearsons correlation coefficient (r) between seven agronomic traits in 39 S42ILs

Traits	GEA	HEA	HEI	LAH	TGW	YLD
EAR	−0.51***	−0.36*	0.03	0.39***	0.25*	0.47***
GEA		0.74***	−0.19*	−0.46***	−0.02	−0.27**
HEA			−0.61***	−0.84***	0.73***	−0.52***
HEI				0.73***	0.03	0.37***
LAH					0.13	0.67***
TGW						0.68***

The agronomic traits are defined in Table 1. For calculating correlation coefficients, the least squares means of the trait performance of each IL were averaged across replications and environments. The r values are significant with * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$

several lines contain overlapping or flanking introgressions, these associations were summarized to two QTLs on chromosome 2H and 6H, respectively. At QEar.S42IL-2H.a, the line main effect and the line × environment interaction effect were significant, whereas for QEar.S42IL-6H.a a significant effect was only found in environment D07. For both QTLs, the *Hsp* introgression was associated with an increased number of ears per square meter relative to 'Scarlett'. The strongest effect was exhibited by S42IL-107, containing an *Hsp* segment in the region 2H, 17-42 cM. This line showed a significantly higher trait performance than the recurrent parent only in the environment D07. Here, the number of ears per square meter was increased by 397.4 (41.1%) relative to 'Scarlett'.

Grains per ear (GEA)

A total of 14 significant line × phenotype associations were identified for the trait GEA on all chromosomes except 5H and 6H. These associations were summarized to eight QTLs, where five QTLs showed both a significant line main effect and a line × environment interaction effect. Two QTLs each were localized on chromosomes 2H, 4H and 7H. At all eight QTLs, the *Hsp* introgressions were associated with a reduced number of GEA by up to −6.1 (−26.8%) for S42IL-107 at QGea.S42IL-2H.a.

Days until heading (HEA)

Altogether 19 significant line × phenotype associations, summarized to 14 putative QTLs, were identified for HEA on all seven barley chromosomes. For 12 QTLs both the line main effect and the line × environment interaction effect, were significant, whereas two QTLs on chromosome 6H revealed an interaction effect solely for D07. For 13 line × phenotype associations, and according to this, for nine QTLs, the exotic introgression was associated with a reduced number of days until heading relative to 'Scarlett'. The strongest favorable effect was mapped to the region 2H, 17–42 cM by S42ILs −107 and −108. Here, the number of days until heading was reduced by 10.4 (15.7%) and 10.3 (15.5%), respectively. Strong reduction effects of −6.6 days (−9.9%) and −6.1 days (−9.2%) caused by *Hsp* introgressions were mapped to the regions 4H, 80–95 cM and 7H, 146–166 cM by the S42ILs −121 and −137, respectively.

Plant height (HEI)

For HEI, eight significant line × phenotype associations were localized on all chromosomes except 6H. Due to the flanking introgressions of S42ILs −107 and −109, a total of seven QTLs were detected. At all loci, the line main effect and the line × environment interaction effect were significant. At QHei.S42IL-2H.a, the *Hsp* segment, located within the region 2H, 17–42 cM, caused a plant height reduced by 11.8 cm (14.6%) relative to the control 'Scarlett'. At the remaining six QTLs, the *Hsp* introgression increased the plant height by up to 15.9 cm (19.7%) at QHei.S42IL-7H.b.

Lodging at harvest (LAH)

Seven significant line × phenotype associations were localized for LAH on chromosomes 1H, 2H, 4H and 7H. Taking into account that two S42ILs on chromosome 2H do overlap, altogether six QTLs were detected. For five QTLs both the line main effect and the line × environment interaction effect, were significant, whereas for one QTL solely an interaction effect was significant in environment G07. At QLah.S42IL-2H.a, the exotic introgression caused a maximum reduction of lodging severity at harvest by 1.6 scores (40.0%) relative to 'Scarlett' within the region 2H, 67–92 cM. In addition, S42IL-138 containing an exotic introgression on chromosome 7H, 166–181 cM, revealed a LAH reduced by 1.4 scores (37.1%) at QLah.S42IL-7H.b.

Thousand grain weight (TGW)

For TGW, eight significant line × phenotype associations were localized on chromosomes 2H, 4H and 6H. These effects were summarized to six QTLs. For one QTL the line

Table 4 List of 65 significant line × phenotype associations for seven agronomic traits detected with 39 S42ILs

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Introgression line	Effect[d]	LSMEANS (IL)[e]	Diff.[f]	RP (IL) %[g]	QTL in BC2DH (von Korff et al. 2006)	Candidate genes[h]
EAR	QEar.S42IL-2H.a	2H	17–42	S42IL-107	I	1365.4^{D07}	397.4	41.1	QEar.S42-2H.a	*Ppd-H1*[1]
		2H	17–92	S42IL-108	L + I	1243.4	286.1	29.9	QEar.S42-2H.a, QEar.S42-2H.b	
		2H	67–92	S42IL-109	L + I	1237.9	280.6	29.3	QEar.S42-2H.b	
	QEar.S42IL-6H.a	6H	145–155	S42IL-132	I	1359.0^{D07}	391.0	40.4	QEar.S42-6H.a	
GEA	QGea.S42IL-1H.a	1H	39–70	S42IL-103	I	19.7^{H07}	−3.8	−16.3		
	QGea.S42IL-2H.a	2H	17–42	S42IL-107	L + I	16.8	−6.1	−26.8		*Ppd-H1*[1]
		2H	17–92	S42IL-108	L + I	19.7	−3.3	−14.2		
	QGea.S42IL-2H.b	2H	67–92	S42IL-109	L + I	18.4	−4.5	−19.5		
		2H	80–86	S42IL-110	L + I	18.2	−4.7	−20.5		
	QGea.S42IL-3H.a	3H	65–70	S42IL-111	L + I	19.1	−3.8	−16.6		
	QGea.S42IL-4H.a	4H	80–95	S42IL-121	L + I	20.4	−2.5	−10.8		
		4H	125–132	S42IL-122	I	19.0^{D07}	−3.3	−14.6		
		4H	125–170	S42IL-123	L + I	20.6	−2.4	−10.3		
	QGea.S42IL-4H.b	4H	170–190	S42IL-124	I	18.3^{D07}	−3.3	−17.6		
	QGea.S42IL-7H.a	7H	50	S42IL-133	I	18.3^{D07}	−3.9	−17.6		
		7H	62–75	S42IL-134	L + I	20.7	−2.3	−9.8		
	QGea.S42IL-7H.b	7H	146–155	S42IL-136	I	19.0^{D07}	−3.3	−14.6		
		7H	146–166	S42IL-137	I	17.7^{D07}	−4.6	−20.6		
HEA	QHea.S42IL-1H.a	1H	0–85	S42IL-102	L + I	68.2	1.9	2.9		
		1H	39–70	S42IL-103	L + I	68.3	2.1	3.1		
	QHea.S42IL-1H.b	1H	70–85	S42IL-105	L + I	62.0	−4.2	−6.4		
	QHea.S42IL-2H.a	2H	17–42	S42IL-107	L + I	55.8	−10.4	−15.7	QHea.S42-2H.a	*Ppd-H1*[1]
		2H	17–92	S42IL-108	L + I	56.0	−10.3	−15.5		
	QHea.S42IL-2H.b	2H	67–92	S42IL-109	I	59.0^{D07}	−1.3	−2.1		
		2H	80–86	S42IL-110	L + I	62.3	−3.9	−5.9		
	QHea.S42IL-3H.a	3H	130–175	S42IL-114	L + I	62.8	−3.4	−5.2	QHea.S42-3H.b	*sdw1(denso)*[2]
		3H	155–190	S42IL-115	I	59.0^{D07}	−1.3	−2.1		
	QHea.S42IL-4H.a	4H	31–57	S42IL-120	L + I	68.0	1.8	2.6		
	QHea.S42IL-4H.b	4H	80–95	S42IL-121	L + I	59.7	−6.6	−9.9		
	QHea.S42IL-4H.c	4H	170–190	S42IL-124	L + I	68.2	1.9	2.9	QHea.S42-4H.a	*Vrn-H2*[3]
	QHea.S42IL-5H.a	5H	43–69	S42IL-125	L + I	62.2	−4.1	−6.2		
	QHea.S42IL-6H.a	6H	96–112	S42IL-129	I	59.0^{D07}	−1.3	−2.1	QHea.S42-6H.b	*eps6L.1*[2]
	QHea.S42IL-6H.b	6H	145–155	S42IL-132	I	61.7^{D07}	1.4	2.4		
	QHea.S42IL-7H.a	7H	50	S42IL-133	L + I	69.0	2.8	4.2		*Vrn-H3*[4]
	QHea.S42IL-7H.b	7H	62–75	S42IL-134	L + I	63.2	−3.1	−4.7		*HvCO1*[5]
		7H	75–155	S42IL-135	I	59.0^{D07}	−1.3	−2.1	QHea.S42-7H.b	*HvCO1*[5], *eps7L*[2]
	QHea.S42IL-7H.c	7H	146–166	S42IL-137	L + I	60.2	−6.1	−9.2		*eps7L*[2]
HEI	QHei.S42IL-1H.a	1H	70–85	S42IL-105	L + I	89.9	9.1	11.3		
	QHei.S42IL-2H.a	2H	17–42	S42IL-107	L + I	69.0	−11.8	−14.6	QHei.S42-2H.a	*Ppd-H1*[1]
		2H	67–92	S42IL-109	L + I	73.1	−7.7	−9.6	QHei.S42-2H.b	*sdw3*[6]
	QHei.S42IL-3H.a	3H	130–175	S42IL-114	L + I	94.9	14.1	17.5	QHei.S42-3H.b	*sdw1 (denso)*[2]
	QHei.S42IL-4H.a	4H	80–95	S42IL-121	L + I	95.4	14.6	18.1		
	QHei.S42IL-5H.a	5H	43–69	S42IL-125	L + I	92.9	12.2	15.1	QHei.S42-5H.a	
	QHei.S42IL-7H.a	7H	62–75	S42IL-134	L + I	90.0	9.2	11.4	QHei.S42-7H.b	*HvCO1*[5]
	QHei.S42IL-7H.b	7H	146–166	S42IL-137	L + I	96.7	15.9	19.7	QHei.S42-7H.c	

Table 4 continued

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Introgression line	Effect[d]	LSMEANS (IL)[e]	Diff.[f]	RP (IL) %[g]	QTL in BC2DH (von Korff et al. 2006)	Candidate genes[h]
LAH	QLah.S42IL-1H.a	1H	70–85	S42IL-105	I	6.3^{G07}	3.2	100.0		
	QLah.S42IL-2H.a	2H	67–92	S42IL-109	L + I	2.3	−1.6	−40.0	QLof.S42-2H.a	*sdw3*[6]
		2H	80–86	S42IL-110	L	2.6	−1.3	−34.3		
	QLah.S42IL-4H.a	4H	80–95	S42IL-121	L + I	6.8	2.9	74.3		
	QLah.S42IL-4H.b	4H	125–170	S42IL-123	L + I	5.4	1.6	40.0		
	QLah.S42IL-7H.a	7H	146–166	S42IL-137	L + I	7.3	3.4	88.6	QLof.S42-7H.b	
	QLah.S42IL-7H.b	7H	166–181	S42IL-138	L + I	2.4	−1.4	−37.1		
TGW	QTgw.S42IL-2H.a	2H	17–42	S42IL-107	L + I	42.3	3.1	7.9	QTgw.S42-2H.a	*Ppd-H1*[1]
		2H	17–92	S42IL-108	I	38.1^{H07}	4.4	13.1		
	QTgw.S42IL-2H.b	2H	80–86	S42IL-110	L	41.9	2.7	7.0	QTgw.S42-2H.a	
	QTgw.S42IL-4H.a	4H	31–95	S42IL-119	L	42.6	3.4	8.7	QTgw.S42-4H.a	
		4H	80–95	S42IL-121	I	44.6^{D07}	4.9	12.3		
	QTgw.S42IL-4H.b	4H	125–132	S42IL-122	L + I	35.0	−4.2	−10.8	QTgw.S42-4H.b	
	QTgw.S42IL-4H.c	4H	170–190	S42IL-124	L + I	35.7	−3.5	−8.8		
	QTgw.S42IL-6H.a	6H	112–155	S42IL-130	L + I	42.2	3.0	7.7		
YLD	QYld.S42IL-4H.a	4H	31–57	S42IL-118	I	73.5^{D07}	7.8	11.8		
	QYld.S42IL-4H.b	4H	80–95	S42IL-121	L + I	41.9	−9.0	−17.7		
	QYld.S42IL-7H.a	7H	50	S42IL-133	L	45.2	−5.6	−11.1		
		7H	62–75	S42IL-134	I	49.2^{G07}	−10.8	−18.0		
	QYld.S42IL-7H.b	7H	146–166	S42IL-137	L + I	41.8	−9.1	−17.9	QYld.S42-7H.b	

[a] Abbreviations of traits see Table 1

[b] Chromosomal location of the target introgression

[c] Chromosomal extent of the target introgression in centiMorgans

[d] In the two-factorial ANOVA, significant line × phenotype associations ($P < 0.05$) were detected as line main effect (L) or line × environment interaction effect (I)

[e] If line main effects (L), or line main effects and a line × environment interaction effects (L + I) were detected, the LSMEANS[IL] across all environments are listed. If solely a line × environment interaction (I) was identified, the LSMEANS[IL] in the particular environment (D07, G07 or H07) is given

[f] Score difference = LSMEANS[IL] − LSMEANS [Scarlett]

[g] Relative performance: RP[IL] = (LSMEANS[IL] – LSMEANS [Scarlett]) × 100/LSMEANS[Scarlett]

[h] References: [1]Turner et al. (2005), [2]Laurie et al. (1995), [3]Yan et al. (2004), [4]Yan et al. (2006), [5]Griffiths et al. (2003), [6]Gottwald et al. (2004)

main effect and for three QTLs the line main effect plus the line × environment interaction effect were significant. At the remaining QTLs a line plus a line × environment interaction and a sole interaction effect was detected for the two linked ILs −107 and −108 whereas a line and a sole interaction effect was detected for the two linked ILs −119 and −121. Four QTLs revealed a favorable effect of the exotic introgression on TGW. The highest increase in trait performance was measured at QTgw.S42IL−2H.a in S42IL-108, which harbors a *Hsp* segment in the region 2H, 17–92 cM. Here, the thousand grain weight was increased by 4.4 g (13.1%) relative to 'Scarlett'. This effect was solely significant in environment H07. The strongest favorable *Hsp* effect, which was significant across all three environments, was mapped to 4H, 31–95 cM. Here, S42IL-119 revealed a TGW increased by 3.4 g (8.7%) relative to 'Scarlett'.

Grain yield (YLD)

Altogether five significant line × phenotype associations were identified for YLD. Due to two flanking S42ILs on chromosome 7H, these effects were summarized to four QTLs on chromosomes 4H and 7H. Three QTLs were detected both as line main effect and line × environment interaction effect. At all three loci, the exotic segment was associated with a grain yield reduced by up to 10.8 dt/ha (18.0%) in S42IL-134 at QYld.S42IL-7H.a. The QTL QYld.S42IL-4H.a was assessed solely as line × environment interaction effect, and revealed an improved trait performance compared to the recurrent parent for S42IL-118. Here, the introgression within region 4H, 31–57 cM caused an increase in grain yield by 7.8 dt/ha (11.8%) relative to 'Scarlett' in environment D07.

Discussion

Identification and verification of QTLs

During the 2007 growing season, a set of 39 wild barley introgression lines (S42ILs) were evaluated in the field with regard to seven agronomic traits, and subsequently subjected to a line × phenotype association study. The aim of the experiment was to verify QTL effects, previously detected with 301 double haploid lines of the population S42, and to identify new QTLs. It was furthermore intended to identify *Hsp* introgressions which cause an improvement of several traits simultaneously. The S42ILs carrying these introgressions could be used directly for the development of new elite cultivars. For the five traits EAR, HEA, HEI, TGW and YLD, both the S42ILs and the population S42, were evaluated. In addition, lodging was investigated at harvest (LAH) in the S42IL set, but at flowering (LOF) in the S42 population. The QTLs for LAH and LOF are directly comparable since both traits are closely linked. Von Korff et al. (2006) identified altogether 63 putative QTLs for the six traits in the S42 population, where 23 QTLs (36.5%) showed a favorable effect of the *Hsp* allele. Forty-four QTLs, among which 18 QTLs revealed a favorable *Hsp* effect, are located in chromosomal regions which are already represented by *Hsp* introgressions in S42ILs, and are therefore comparable with the QTLs detected in the S42ILs. As illustrated in Table 4, 20 out of 44 QTLs (45.5%) and ten out of 18 QTLs with a favorable *Hsp* effect (55.6%) were verified by the evaluated S42ILs. Altogether, 18 out of 20 corresponding QTLs (90.0%) showed an effect of the same direction in both populations.

As underlined by Bernacchi et al. (1998b), the verification of a QTL can be characterized by the stability of its effect across environments. In the present study, 18 QTLs were stable across all tested environments in the S42 population as well as in the S42ILs. A further important criterion for the evaluation of QTL verification results is the magnitude of the effect measured in both populations. For all corresponding QTLs, with the exception of QHei.S42IL-7H.a and QHei.S42-7H.b, at least one S42IL exhibited a higher relative performance in comparison to 'Scarlett' than measured for the corresponding QTL in the S42 population. These differences as well as the non-confirmation of QTL effects from population S42 might be explained by the reduction of epistatic *Hsp* effects which are still active in the S42ILs. Since the S42ILs contain single exotic introgressions, whereas the BC_2DH lines in the S42 population carry multiple independent introgressions, potential interactions between non-linked *Hsp* alleles are expected to be reduced in the S42IL set. Furthermore, the QTL data of the S42 population were based on field tests at up to eight different environments, and thus offered a high reliability, whereas the S42ILs were only tested in three environments.

For the six traits analyzed in both populations, altogether 17 new QTLs, previously not identified in the S42 population, were detected in the S42IL set. Here, six QTLs (35.3%) revealed a favorable *Hsp* effect. Furthermore, eight QTLs for the newly identified trait GEA were mapped by S42ILs, among which no QTL showed a favorable *Hsp* effect.

A similar approach to verify QTLs was conducted in tomato by Bernacchi et al. (1998b). The authors generated a set of 23 near isogenic lines (NILs), which harbored QTLs with a favorable effect on one or several agronomic traits. Each NIL contained a single introgression of the wild species *S. hirsutum* or *S. pimpinellifolium*, whereas the remaining part of the genetic background was consistently derived from the elite parent *S. esculentum*. The aim of the study was to verify 25 QTLs for seven traits, previously mapped to 15 different genomic regions in two AB populations (Bernacchi et al. 1998a). The agronomic performance of the NILs was tested in five different environments, and a total of 22 QTLs (88%) could be confirmed by the NILs in at least one environment.

In the following paragraphs, the QTL results of the present study are discussed for each trait or trait complex separately. They are compared to the QTL effects, previously identified in population S42 (von Korff et al. 2006), and to different candidate genes which map to the same chromosomal region.

Days until heading

For HEA, von Korff et al. (2006) detected a total of ten QTLs, half of them were located in genomic regions, which are already represented by *Hsp* introgressions of S42ILs. These five QTLs were verified by QTL effects detected in the S42IL set, and exhibited an effect with the same direction in both populations (Table 4). Four QTLs were significant across all environments in the S42 population as well as in the S42ILs. In both populations, the strongest favorable *Hsp* effect, associated with a reduction of HEA, was mapped to the top of chromosome 2H (QHea.S42-2H.a and QHea.S42IL-2H.a, respectively). Here, the number of days until heading was reduced by up to 10.4 (15.7%) in S42IL-107, compared to 'Scarlett'. In the same region, *Ppd-H1*, the major photoperiod response gene of barley, whose day-length sensitive alleles promote flowering under long day conditions, is located (Turner et al. 2005). *HvCO1*, another barley photoperiod response gene of the circadian clock (Griffiths et al. 2003), coincides with the QTL QHea.S42IL-7H.b, which was detected in the S42ILs −134 and −135 on chromosome 7H. The barley vernalization response genes *Vrn-H2* and *Vrn-H3*, which regulate flower-

ing depending on low temperature (Laurie et al. 1995; Yan et al. 2004, 2006), are located on chromosomes 4H and 7H. These loci correspond to the QTLs QHea.S42IL-4H.c and QHea.S42-4H.a, and the newly identified QTL QHea.S42IL-7H.a, respectively (Table 4). All three QTLs exhibited an increased *Hsp* effect on number of days until heading. This is consistent with the fact that wild barley requires a period of low temperature as impulse for flowering (Laurie 1997). Several 'earliness per se' genes, which are relatively independent from photoperiod and vernalization conditions, were mapped by Laurie et al. (1995). The genes *eps6L.1* and *eps7L* coincide with the QTLs QHea.S42IL-6H.a and QHea.S42-6H.b, and QHea.S42IL-7H.c and QHea.S42-7H.b on chromosome 6H and 7H, respectively. Laurie et al. (1995) demonstrated a delay in flowering time in barley caused by the dwarfing gene *sdw1* (previously named *denso*). The gene location on chromosome 3H corresponds to the localization of the QTLs QHea.S42IL-3H.a and QHea.S42-3H.b, which both exhibited an enhanced *Hsp* effect on HEA. Altogether eight new QTLs, which were not detected in the S42 population, were revealed by S42ILs. Half of them showed a reducing *Hsp* effect on HEA.

Plant height and lodging at harvest

In population S42, altogether 11 QTLs were detected for HEI (von Korff et al. 2006), at which seven QTLs were localized in regions which are so far represented by S42ILs. Six out of these seven QTLs (85.7%) were confirmed by QTL effects detected in S42ILs, exhibiting effects with the same direction in both populations (Table 4). Furthermore, five verified QTLs revealed significant effects across all environments, both in S42 population and in the S42ILs. In both populations, the strongest favorable *Hsp* effect on HEI was mapped to region 2H, 17–42 cM. Here, plant height was reduced in S42IL-107 by 14.6%, relative to 'Scarlett', whereas the *Hsp* allele in the S42 population caused a reduction effect of 11.7%. As described by Laurie et al. (1994), this localization corresponds to the *Ppd-H1* locus which, in addition to flowering regulation, exerts a strong effect on plant height (Turner et al. 2005). A possible candidate gene for QHei.S42-2H.b and the effect of QHei.S42IL-2H.a in S42IL-109 is the gibberellic-acid insensitive dwarfing gene *sdw3* of barley (Gottwald et al. 2004). It might also be causal for the strong reduction of LAH in S42ILs −109 and −110 at QLah.S42IL-2H.a. As illustrated in Fig. 1, further co-localizations of QTLs for HEI and LAH are present in the chromosomal regions 1H, 70–85 cM, 4H, 80–95 cM, and 7H, 146–166 cM. For all three QTLs, the *Hsp* introgression caused an increasing effect on both traits, which coincides with their strong positive correlation of 0.73 (Table 3). The dwarfing gene *sdw1*, which reduces plant height and lodging (Bezant et al. 1996), maps to the same genomic region as QHei.S42IL-3H.a and QHei.S42-3H.b. However, no QTL effect on LAH was detected by S42ILs in this region. The QTLs QHei.S42IL-7H.a and QHei.S42-7H.b, which both revealed an increasing *Hsp* effect on HEI, coincide with *HvCO1* (Griffiths et al. 2003). As described above, the number of days until heading is reduced by *Hsp* introgressions in the same region. These opposed effects on HEA and HEI correspond to their negative correlation ($r = -0.61$). In addition to the verified QTLs, two new unfavorable *Hsp* effects on HEI were identified in S42ILs on chromosome 1H and 4H.

Altogether seven QTLs for lodging at flowering which mapped to regions covered by exotic introgressions in the S42IL set, were identified by von Korff et al. (2006). As shown in Table 4, the QTLs QLof.S42-2H.a and QLof.S42-7H.b were verified by QLah.S42IL-2H.a and QLah.S42IL-7H.a, detected by the S42ILs -109 and −110, and −137, respectively. In the S42 population as well as in the S42ILs, both QTLs were detected as significant effects of the same direction across all environments. In the present study, four new QTLs for LAH were localized on chromosomes 1H, 4H and 7H. Here, QLah.S42IL-7H.b exhibited a favorable *Hsp* effect.

Yield components

In the present study, the S42IL set was evaluated with regard to the three yield components EAR, GEA and TGW. Von Korff et al. (2006) detected altogether 11 and nine QTLs for EAR and TGW, respectively. Nine and eight QTLs are localized in chromosomal regions which are also represented by S42ILs. For both traits, three QTLs could be confirmed in the S42IL study (Table 4). For EAR as well as for TGW, one QTL revealed an unfavorable *Hsp* effect in the S42 population, but a favorable exotic effect in the S42ILs. Furthermore, one new QTL effect for TGW with an enhanced trait performance of S42IL-130 was mapped to the genomic region 6H, 112–155 cM. For EAR and GEA, the S42ILs −107, −108 and −109 revealed co-localizations of significant line × phenotype associations in the region 2H, 17–92 cM. All three S42ILs showed an increased EAR and simultaneously a reduced GEA, compared to 'Scarlett' (Fig. 1). These effects with an opposed direction coincide with a negative correlation of −0.51 between both traits. For all three yield components, QTL effects were detected by the S42ILs −107 and −109 on the top of chromosome 2H. As described by Laurie et al. (1994), the *Ppd-H1* locus, which mapped to the same region, also has an effect on yield components, probably as a direct result of the effect on flowering time. Also on chromosome 2H, but not directly at the *Ppd-H1* locus, Li et al. (2005, 2006)

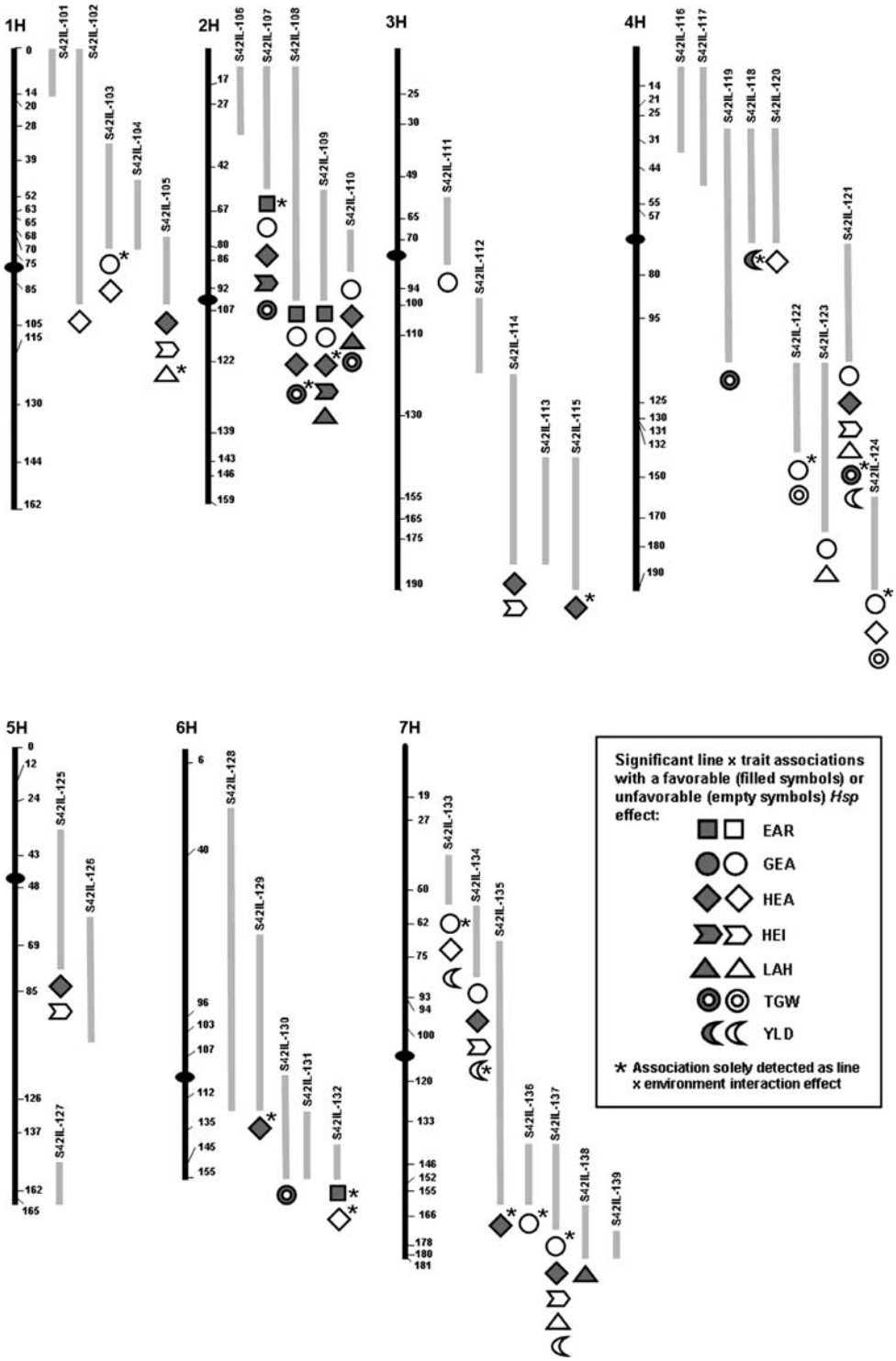

Fig. 1 SSR map with 65 significant ($P < 0.05$) line × phenotype associations for 39 S42ILs and seven agronomic traits. The chromosomes are shown as *black bars* with centiMorgan values for SSR loci following the order of von Korff et al. (2004). The extent of *Hsp* introgressions are given in *grey bars* right to the chromosomes. The associations are illustrated as *symbols* below the S42ILs. They either reveal a favorable (*filled symbols*) or unfavorable (*empty symbols*) *Hsp* effect. Associations solely detected as line × environment interaction effect are marked by an *asterisk* right to the symbol

detected altogether eight QTLs for the five yield components ear length, spikelet number per spike, grain number per spike, spike number per plant, and thousand grain mass. At one QTL each for spike number per plant and thousand grain mass, the *Hsp* allele was associated with an improved trait performance.

Grain yield

For YLD, von Korff et al. (2006) detected 13 QTLs on all chromosomes. At three QTLs the *Hsp* allele caused an increased grain yield compared to the recurrent parent. Nine QTLs could theoretically be verified by the 39

S42ILs since they are located in genomic regions which are covered by *Hsp* introgressions. Solely the QTL QYld.S42-7H.b, showing an unfavorable *Hsp* effect in the S42 population, was verified by QYld.S42IL-7H.b. The latter revealed a reduced grain yield in S42IL-137, compared to 'Scarlett'. In addition, three new QTLs for YLD were mapped to chromosomes 4H and 7H present in four different S42ILs (Table 4). A potentially valuable introgression line for breeding of cultivars with enhanced grain yield might be S42IL-118 where yield was increased by 11.8% relative to 'Scarlett'. However, the stability of this favorable effect has to be verified since the YLD effect was only detected as a line × environment interaction active in D07.

Several times, co-localizations of QTLs for YLD and yield associated traits were detected which were consistent with the genetic correlations, for example, for HEA and TGW (Fig. 1). In the region 4H, 31–57 cM, a QTL effect for YLD as well as for TGW was detected. For both traits, the *Hsp* introgression caused an enhanced performance compared to the recurrent parent. This coincides with the strong positive correlation ($r = 0.68$) between YLD and TGW (Table 3). One coincidence of QTLs for YLD and HEA was identified in the genomic region 7H, 50 cM. Here, an opposite *Hsp* effect was in accordance with the negative trait correlation of −0.52. For YLD and LAH, two QTL co-localizations were detected in the regions 4H, 80–95 cM and 7H, 146–166 cM. On both QTLs the exotic introgression was associated with a reducing effect on YLD and an increasing effect on LAH. This is contrary to the positive genetic correlation between both traits. Due to the fact that a strong severity of lodging is assumed to be associated with a loss of grains, and, thus, a reduced grain yield, a negative trait correlation would be expected. For instance, von Korff et al. (2006) measured a genetic correlation of −0.55 between the traits grain yield and lodging at flowering. In the present study, the unexpected positive correlation between YLD and LAH might be due to the fact, that both traits differed substantially between the three examined environments. Whereas LAH was extremely high in D07, YLD was extremely low in H07 (Table 2). We assume that the observed positive correlation might be artificial since it disappeared when the calculation was based on single plot entries per environment (data not shown). Despite a positive genetic correlation ($r = 0.47$) between YLD and EAR, no co-localizations of QTLs could be revealed. In contrast, three co-localizations for YLD and GEA were detected on chromosomes 4H and 7H (Fig. 1). In all cases, an unfavorable *Hsp* effect on both traits was observed. Here, the negative correlation of −0.27 was contradictory.

Examples of S42ILs harboring QTL effects for several traits simultaneously

As illustrated in Fig. 1, several chromosomal regions revealed clusters of QTLs for different traits. Among the set of 39 S42ILs, altogether seven lines on chromosomes 2H, 4H and 7H exhibited at least four QTLs simultaneously. Four of these lines carry introgressions on chromosome 2H, among which the S42ILs −107 and −109 exhibited a favorable *Hsp* effect on four different traits. S42IL-107, carrying a *Hsp* segment in the region 2H, 17–42 cM, showed a significant improved performance relative to the recurrent parent for the traits HEA, HEI and TGW across all tested environments. In addition, one favorable *Hsp* effect on EAR, only significant in environment D07 was measured (Table 4). In S42IL-109, the exotic introgression in the region 2H, 67–92 cM was associated with an improved performance for EAR, HEI and LAH. All three effects were stable across environments, whereas an additional favorable effect on HEA was only significant in environment D07 (Fig. 1). Amongst others, S42ILs −107 and −109 represent valuable genetic resources for the development of new elite cultivars with enhanced agronomic performance. The relatively small portion of the *Hsp* genome present in most of the 39 S42ILs facilitates and accelerates the direct transfer of favorable exotic alleles into modern cultivars through backcrossing and marker-assisted selection. However, during the selection of favorable introgressions for a particular trait, it is worthwhile to assure the complete absence of unfavorable effects on other traits. ILs, exhibiting negative side effects, should not be transferred directly into new cultivars without selection of recombination events. It is, thus, advisable to select ILs with small *Hsp* introgressions, only exhibiting favorable exotic QTL effects.

Future prospects

The present study describes the application of wild barley introgression lines for the identification and verification of QTL effects. Altogether, 45.5% of the QTLs for six agronomic traits, previously identified in population S42 by von Korff et al. (2006), were verified by S42ILs. In future, promising *Hsp* effects will be subjected to map-based cloning. These effort will focus on effects chosen by the following criteria: the QTL effects are (1) verified, (2) significant across all environments and (3) strong, i.e. the elite and the exotic allele differ substantially in their phenotypic performance. Appropriate S42ILs for map-based cloning projects are the S42ILs −110 and −137 carrying an exotic segment in the region 2H, 80−86 cM and 7H, 146–166 cM, respectively. Both S42ILs exhibit significant and verified QTL

effects for multiple traits. A high-resolution mapping approach will eventually help to distinguish between gene linkage and true pleiotropy as the alternative hypotheses to explain the effects on multiple traits which are observed in these S42ILs. The required high-resolution mapping populations for chosen S42ILs are currently under construction. For this reason, all 39 S42ILs are currently backcrossed again with 'Scarlett'. In subsequent generations, recombination events and SUB-ILs with smaller introgressions will be selected. This material can serve as a starting point for both high-resolution mapping and molecular breeding.

In future, the S42IL set will also be characterized in detail through genotyping with Illumina SNPs (Rostoks et al. 2006) or Diversity Arrays Technology (DArT) markers (Wenzl et al. 2006), which will facilitate the fine-mapping of QTL effects. Currently, the S42ILs are also extensively tested with regard to malting quality and abiotic stress tolerance. All genotype and phenotype data of the S42ILs will be archived in the public IL data base 'Phenom Networks' (http://phn.huji.ac.il/RTQ/).

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ORIGINAL PAPER

Detection and verification of malting quality QTLs using wild barley introgression lines

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Abstract A malting quality quantitative trait locus (QTL) study was conducted using a set of 39 wild barley introgression lines (hereafter abbreviated with S42ILs). Each S42IL harbors a single marker-defined chromosomal segment from the wild barley accession 'ISR 42-8' (*Hordeum vulgare* ssp. *spontaneum*) within the genetic background of the elite spring barley cultivar 'Scarlett' (*Hordeum vulgare* ssp. *vulgare*). The aim of the study was (1) to verify genetic effects previously identified in the advanced backcross population S42, (2) to detect new QTLs, and (3) to identify S42ILs exhibiting multiple QTL effects. For this, grain samples from field tests in three different environments were subjected to micro malting. Subsequently, a line × phenotype association study was performed with the S42ILs in order to localize putative QTL effects. A QTL was accepted if the trait value of a particular S42IL was significantly ($P < 0.05$) different from the recurrent parent as a control, either across all tested environments or in a particular environment. For eight malting quality traits, altogether 40 QTLs were localized, among which 35 QTLs (87.5%) were stable across all environments. Six QTLs (15.0%) revealed a trait improving wild barley effect. Out of 36 QTLs detected in a previous advanced backcross QTL study with the parent BC_2DH population S42, 18 QTLs (50.0%) could be verified with the S42IL set. For the quality parameters α-amylase activity and Hartong 45°C, all QTLs assessed in population S42 were verified by S42ILs. In addition, eight new QTL effects and 17 QTLs affecting two newly investigated traits were localized. Two QTL clusters harboring simultaneous effects on eight and six traits, respectively, were mapped to chromosomes 1H and 4H. In future, fine-mapping of these QTL regions will be conducted in order to shed further light on the genetic basis of the most interesting QTLs.

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Introduction

The most important end use of spring barley is the production of malt as substrate for brewing beer and distilling whisky. Malting quality is composed of numerous interacting traits with a high complexity concerning their biochemical and genetic basis (Fox et al. 2003). In most instances, the traits are conditioned by interaction of polygenes, so called quantitative trait loci (QTLs). To accelerate the breeding of cultivars with improved malting quality, molecular markers and genetic linkage maps were used to localize these loci. Thus, in recent years, the elite barley gene pool was extensively used for numerous QTL studies on malting quality (e.g. Marquez-Cedillo et al. 2000; See et al. 2002; Barr et al. 2003a, b; Collins et al. 2003; Emebiri et al. 2003, 2004, 2005; Hayes et al. 2003; Edney and Mather 2004; Rae et al. 2007; Panozzo et al. 2007).

The value of exotic barley germplasm as a source for trait improving alleles was proven by studies targeting

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quantitative traits. Although potentially useful allelic variation regarding important malting quality characteristics was identified in wild barley (Ahokas and Naskali 1990a, b; Zhang et al. 2007), the utilization of unadapted germplasm is, so far, restricted to a limited number of malting quality QTL studies. By means of "advanced backcross" (AB)-QTL studies with germplasm of the wild barley form *Hordeum vulgare* ssp. *spontaneum*, hereafter abbreviated with *Hsp* Pillen et al. (2003, 2004) and Li et al. (2005) detected putative QTLs affecting the parameters friability, protein content, water absorption and malt extract. A further AB-QTL study involving the same *Hordeum* subspecies as exotic parent was performed by von Korff et al. (2008). Here, 301 BC_2DH lines of the population S42 ('Scarlett' × 'ISR 42-8') were genotyped with 98 SSR markers, and, in addition, subjected to a micro malting experiment. Subsequently, the performance of the lines regarding seven malting quality characteristics (e.g. α-amylase activity, fermentability, grain protein content and viscosity) was evaluated. By means of a mixed model analysis of variance, altogether 48 putative QTLs were assessed. At 18 QTLs (37.5%), the exotic allele caused an improved trait performance.

Once QTLs influencing important traits are localized, this information can be exerted for further studies, for instance to investigate gene by gene and gene by environment interactions, pleiotropic effects and to map-based clone strong QTL effects. It is furthermore advisable to transfer favorable exotic QTL alleles into existing varieties, and, thus, to breed improved cultivars. Zamir (2001) recommended the use of introgression libraries for those applications. Each introgression line (IL) within such a library contains a single marker-defined chromosomal fragment of an exotic species, whereas the remaining part of the genome originates from an elite variety. A complete IL library, developed by several rounds of backcrossing and selfing and in parallel marker-assisted selection (MAS), represents the whole donor genome in overlapping introgressions. The great advantage of such an IL set compared to commonly used QTL mapping populations is the low proportion of exotic germplasm present in each line. Thereby, negative effects of donor alleles, i.e. linkage drag, are reduced and the phenotypic variation between the ILs can be attributed with high accuracy to the particular introduced segment. Due to their homozygosity, ILs are a stable resource which can be exerted for a multitude of purposes. Regarding end use quality, introgression libraries were so far evaluated in tomato (Rousseaux et al. 2005), rice (Zheng et al. 2007), wheat (Liu et al. 2007), melon (Eduardo et al. 2007; Fernandez-Trujillo et al. 2007; Obando et al. 2008), and rye (Falke et al. 2008). In barley, Matus et al. (2003) developed a set of 140 recombinant chromosome substitution lines (RCSLs) which contained in most instances multiple exotic segments of the exotic accession 'Caesarea 26-24'. By association analysis, the trait performance of the RCSLs was evaluated. Matus et al. (2003) observed significant transgressive segregation, and identified several QTL regions for the malting quality parameters α-amylase activity, diastatic power, grain protein content, malt extract and wort β-glucan content. Furthermore, a set of 59 ILs, each harboring a single wild barley introgression in the uniform genetic background of elite barley, was selected and subsequently used for the verification of QTLs affecting disease resistance and agronomic performance (Schmalenbach et al. 2008, 2009).

The present paper reports on the application of a set of 39 wild barley introgression lines (S24ILs), taken from Schmalenbach et al. (2008), to conduct a malting quality QTL analysis. Each line harbors one single introgression of the exotic barley accession 'ISR 42-8' (*Hsp*) in the genetic background of the elite barley cultivar 'Scarlett' (*Hordeum vulgare* ssp. *vulgare*, hereafter abbreviated with *Hv*). For evaluating the malting quality performance of the S42ILs, a micro malting experiment with grain samples from field tests in three different environments was conducted. Subsequently, the resulting data for eight malting quality parameters were analyzed within a line × phenotype association study in order (1) to verify QTL effects previously localized in the population S42 which consists of 301 BC_2DH lines (von Korff et al. 2008), (2) to detect new QTLs, and (3) to identify S42ILs exhibiting significant effects on several traits simultaneously.

Materials and methods

Plant material

Altogether 39 selected wild barley introgression lines (named S42IL-101 to S42IL-139) were subjected to a malting quality analysis. The S42ILs were developed based on an initial cross between the German spring barley cultivar 'Scarlett' and the Israeli wild barley accession 'ISR 42-8'. Subsequently, three rounds of backcrossing with the recurrent parent, repeated selfing and, in parallel, marker-assisted selection (MAS) were conducted. Finally, the S42ILs were selected in BC_3S_2 or BC_3S_4 generation. Each S42IL harbors a single SSR marker-defined chromosomal segment of the exotic parent, whereas the remaining genome is derived from the elite parent. The construction and genotypic characterization of the lines, as well as their application to verify QTL effects for disease resistances, are described in detail in Schmalenbach et al. (2008).

Field cultivation of the introgression lines

The 39 S42ILs were cultivated at three different locations in Germany during the season 2007 to evaluate agronomic performance (Schmalenbach et al. 2009) and to obtain seed material for micro malting. The locations were the experimental research station Dikopshof (D07, University of Bonn, western Germany), and the breeders' experimental field stations in Gudow (G07, Nordsaat Saatzucht, northern Germany) and Herzogenaurach (H07, Saatzucht Josef Breun, southeastern Germany). The introgression lines were grown in three replications (blocks) per location. As a control, the elite parent 'Scarlett' was tested in four replications per block. Growing conditions such as net plot sizes (4.5–6.0 m^2), seed density (300–390 kernels/m^2), nitrogen fertilization (30–80 kg N/ha) and field management followed local practice. Further details are given in Schmalenbach et al. (2009).

Malting quality analysis

Micro malting and malting quality analysis were conducted in the laboratory of Nordsaat Saatzucht (Böhnshausen, Germany), using 100 g of grain of the sieving fraction > 2.5 mm. The malting process and the determination of α-amylase activity and raw protein content were performed as described in von Korff et al. (2008). Both traits as well as the quality characters fine-grind extract of malt, friability of malt, and viscosity of wort were measured using the methods recommended by the European Brewery Convention (EBC). The traits Kolbach index and Hartong 45°C were determined according to the methods of the Mitteleuropäische Brautechnische Analysenkommission (MEBAK). The investigated traits are explained in Table 1. The exotic parent 'ISR 42-8' was not included in the experiment since, in general, wild barley exhibits no malting quality, and, in addition, sufficient number of seeds for micro malting was not available.

Statistical analyzes

Statistical analyzes were performed with SAS Enterprise Guide 4.1 (SAS Institute 2006). Genetic correlations between trait values were determined with the least squares means (LSMEANS) for each of the 39 S42ILs averaged across all replications and environments. The LSMEANS were computed with the general linear model (GLM) procedure and used for calculating Pearson's correlation coefficient (r) with the CORR procedure.

For identification and verification of QTL effects, the phenotype data of the S42ILs was subjected to a line x phenotype association study. First, the following two-factorial mixed model analysis of variance (ANOVA) was carried out with the GLM procedure:

$$Y_{ijk} = \mu + L_i + E_j + L \times E_{ij} + \varepsilon_{k(ij)},$$

where μ is the general mean, L_i is the fixed effect of the ith line, E_j is the random effect of the jth environment, $L \times E_{ij}$ is the random interaction effect of the ith line and the jth environment, and $\varepsilon_{k(ij)}$ is the error of Y_{ijk}.

When the analysis revealed significant ($P < 0.05$) differences between lines or line x environment interactions, a Dunnett test was conducted (Dunnett 1955). Here, the LSMEANS of each S42IL and of 'Scarlett' as a control were tested for significant differences. When the LSMEANS of a particular S42IL was significantly ($P < 0.05$) different from 'Scarlett' across all three environments and/or in a particular environment, the presence of a QTL was assumed. It was detected either as line main effect or as line × environment interaction effect or as

Table 1 List of eight malting quality traits evaluated for 39 S42ILs in three environments

Abbr.	Trait	Units	Reference/method of measurement[a]	Breeding goal[b]
AA	α-amylase activity of malt	Dextrinizing units (DU)	EBC 4.13	+
FGE	Fine-grind extract of malt	% dry mass	EBC 4.5.1	+
FRI	Friability of malt	%	EBC 4.15	+
GPC	Grain protein content	% dry mass	EBC 3.3.1	−
GSF	Grain sieving fraction > 2.5 mm	%	MEBAK 2.3.1	+
KOL	Kolbach index (soluble protein/ total protein ratio)	%	MEBAK I 4.1.4.5.2 (soluble protein), MEBAK I 4.1.4.5.3 (Kolbach index)	+
VIS	Viscosity of wort	mPas	EBC 4.8	−
VZ45	Hartong 45°C, extract at 45°C	%	MEBAK I 4.1.4.11	+

[a] EBC and MEBAK: see reference list

[b] The breeding goals are defined in order to select improved malting barley, where (−) indicates that a reduction, and (+) that an increase of the trait is desired

both effects simultaneously. If several lines, which carry overlapping or flanking introgressions, revealed a significant effect of the same direction (increase or reduction of trait value), it was assumed that these lines harbored the same QTL. Introgressions do overlap, if they possess at least one common *Hsp* allele. They flank each other, if they have adjacent *Hsp* alleles. The relative performance of a particular S42IL was calculated as follows: RP [S42IL] = (LSMEANS [S42IL] − LSMEANS ['Scarlett'])*100/LSMEANS ['Scarlett'], where for each trait the LSMEANS were calculated across all replications and environments.

Data storage

For general use, all genotype and phenotype data of the S42IL set will be archived in the public IL data base 'Phenom Networks' (http://phn.huji.ac.il/RTQ/).

Results

Trait performances of 'Scarlett' and the S42IL set

In Table 2, the parameters mean, minimum, maximum, and coefficient of variation specify the performance of the recurrent parent 'Scarlett' and the S42IL set for each trait. The values are indicated per environment and across all three environments. For all traits and environments, except for grain protein content in environment D07, the S42ILs revealed a higher coefficient of variation than 'Scarlett'. Across all environments, the recurrent parent as well as the S42ILs, exhibited the highest coefficient of variation for the traits α-amylase activity (22.0 and 22.1%, respectively) and grain sieving fraction > 2.5 mm (13.6 and 14.9%, respectively). Low variation in performance was assessed for fine-grind extract, viscosity and grain protein content. For almost all traits and environments 'Scarlett' showed a higher average performance than the S42IL set. As listed in Table 2, an exception is grain protein content where the mean is slightly lower for the recurrent parent than the S42ILs in each environment. The same observation was detected for viscosity in environments D07 and G07.

Genetic correlations

Altogether 27 significant correlations were assessed between the eight investigated parameters (Table 3). Solely α-amylase activity and fine-grind extract showed no significant correlation. Twenty-two genetic correlations were highly significant ($P < 0.001$). Strong positive correlations were detected between VZ45 and the traits Kolbach index, α-amylase activity and friability ($r = 0.92$, 0.83 and 0.73, respectively). The latter two traits were also highly positively correlated with Kolbach index ($r = 0.86$ and 0.82, respectively) and among each other ($r = 0.69$). Finally, a strong positive correlation of 0.73 was measured between grain sieving fraction > 2.5 mm and viscosity. Grain protein content revealed negative correlations with friability, Kolbach index, Hartong 45°C, fine-grind extract and α-amylase activity ($r = -0.69$, -0.69, -0.67, -0.52 and -0.44, respectively). In addition, friability and Kolbach index exhibited negative correlations of -0.64 and -0.59, respectively, with viscosity which was also negatively correlated with α-amylase activity ($r = -0.55$). The trait grain sieving fraction > 2.5 mm correlated negatively to Kolbach index, α-amylase activity, friability and Hartong 45°C ($r = -0.60$, -0.55, -0.48 and -0.46 respectively), but positively to fine-grind extract and grain protein content ($r = 0.46$ and 0.22, respectively).

Detection of QTL effects

In order to assess QTL effects on malting quality, the 39 S42ILs were subjected to a line x phenotype association study. For all eight investigated traits, the two-factorial mixed model ANOVA revealed significant line effects ($P < 0.05$). Significant line × environment interactions ($P < 0.05$) were detected for all traits with the exception of fine-grind extract and viscosity (data not shown).

As presented in Table 4, altogether 68 significant line × phenotype associations were exhibited by the subsequently performed Dunnett test. For 45 associations, both, the line main effect and the line × environment interaction effect were significant, whereas for eleven associations solely the line main effect was significant. The remaining 12 associations were detected solely as line × environment interaction effect. Due to the overlapping or flanking of several introgressions, the associations were summarized to a total of 40 QTLs. At 30 QTLs the line main as well as the line x environment interaction effect was significant. At six out of the 40 QTLs (15.0%), the exotic introgression was associated with an improved trait performance compared to the control 'Scarlett'. Those favorable *Hsp* effects were detected for the traits α-amylase activity, grain sieving fraction > 2.5 mm and Kolbach index. In the following, the detected QTL effects are specified for each trait separately (see Table 4).

α-amylase activity (AA)

For AA, altogether ten S42ILs on all chromosomes except 2H and 3H exhibited a significant line x phenotype association, among which four lines harbor an exotic segment on chromosome 1H. Taking into account that some lines carry

Table 2 Parameters describing the trait performance of the recurrent parent 'Scarlett' and the S42ILs per environment and across all tested environments

Trait and environment[a]	'Scarlett'					S42ILs				
	N[b]	Mean[c]	Min[d]	Max[d]	CV[e]	N[b]	Mean[c]	Min[d]	Max[d]	CV[e]
AA										
D07	12	382.8	355.0	439.0	7.8	117	377.4	203.0	513.0	17.4
G07	12	411.5	338.0	522.0	14.1	117	394.9	258.0	561.0	16.9
H07	12	572.3	481.0	691.0	12.1	117	527.0	361.0	734.0	13.9
Across all env.	36	455.6	338.0	691.0	22.0	351	433.1	203.0	734.0	22.1
FGE										
D07	12	81.0	79.8	82.3	0.9	117	80.4	77.8	82.5	1.1
G07	12	82.4	80.7	84.2	1.2	117	81.6	78.9	85.3	1.5
H07	12	81.8	80.2	84.3	1.5	117	80.8	77.7	83.4	1.6
Across all env.	36	81.7	79.8	84.3	1.4	351	80.9	77.7	85.3	1.6
FRI										
D07	12	71.6	61.0	83.0	8.9	117	67.9	48.0	86.0	11.7
G07	12	70.0	62.0	83.0	8.3	117	68.0	45.0	86.0	11.0
H07	12	81.0	73.0	91.0	6.3	117	80.2	59.0	94.0	8.6
Across all env.	36	74.2	61.0	91.0	10.1	351	72.0	45.0	94.0	13.1
GPC										
D07	12	12.4	11.7	13.1	3.9	117	12.5	11.7	13.7	3.4
G07	12	11.9	11.4	12.5	2.6	117	12.1	10.8	13.7	5.0
H07	12	11.5	10.7	12.5	5.2	117	11.6	10.0	13.0	5.4
Across all env.	36	11.9	10.7	13.1	4.9	351	12.1	10.0	13.7	5.6
GSF										
D07	12	81.1	72.3	87.4	4.6	117	77.7	64.5	89.9	7.3
G07	12	91.3	88.5	93.0	1.6	117	89.9	73.1	96.4	4.8
H07	12	66.6	60.6	77.2	6.7	117	65.6	47.9	86.1	11.5
Across all env.	36	79.7	60.6	93.0	13.6	351	77.7	47.9	96.4	14.9
KOL										
D07	12	41.7	40.0	43.3	2.3	117	40.2	34.0	45.0	5.4
G07	12	42.0	39.5	43.7	3.0	117	41.2	35.7	46.8	5.2
H07	12	48.7	45.6	51.6	3.0	117	48.1	40.7	54.1	5.9
Across all env.	36	44.1	39.5	51.6	7.9	351	43.2	34.0	54.1	9.8
VIS										
D07	12	1.46	1.43	1.50	1.6	117	1.47	1.41	1.61	2.5
G07	12	1.54	1.51	1.59	1.6	117	1.55	1.48	1.72	3.0
H07	12	1.44	1.41	1.47	1.6	117	1.43	1.39	1.53	1.9
Across all env.	36	1.48	1.41	1.59	3.4	351	1.48	1.39	1.72	4.2
VZ45										
D07	12	42.0	38.6	43.8	3.7	117	40.5	34.7	46.8	6.8
G07	12	46.1	43.4	49.4	3.5	117	46.0	39.5	54.3	6.6
H07	12	55.3	51.8	58.5	3.1	117	54.0	45.6	62.0	6.9
Across all env.	36	47.8	38.6	58.5	12.2	351	46.8	34.7	62.0	13.7

[a] Trait and environment abbreviations are listed in Table 1 and "Materials and methods", respectively

[b] Number of observations

[c] Average trait performance

[d] Minimum and maximum trait performance

[e] Coefficient of variation

Table 3 Pearson correlation coefficients (*r*) between eight malting quality traits in 39 S42ILs

Traits	FGE	FRI	GPC	GSF	KOL	VIS	VZ45
AA	0.06	0.69***	−0.44***	−0.55***	0.86***	−0.55***	0.83***
FGE		0.30***	−0.52***	0.46***	0.18*	0.22*	0.25**
FRI			−0.69***	−0.48***	0.82***	−0.64***	0.73***
GPC				0.22*	−0.69***	0.18*	−0.67***
GSF					−0.60***	0.73***	−0.46***
KOL						−0.59***	0.92***
VIS							−0.37***

The malting quality traits are defined in Table 1. For calculating correlation coefficients, the least squares means of the trait performance of each IL were averaged across replications and environments. The *r* values are significant with * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$

overlapping or flanking *Hsp* segments, the associations were summarized to seven putative QTLs, among which five QTLs revealed a significant line main and line × environment interaction effect simultaneously. At two QTLs on chromosome 4H, QAa.S42IL-4H.a and QAa.S42IL-4H.b, a favorable *Hsp* effect was detected. Here, the α-amylase activity of the S42ILs -116 and -124 was enhanced by 20.0 and 16.2%, respectively, relative to the recurrent parent 'Scarlett'. A maximum reduction of trait performance of −32.5% was measured at QAa.S42IL-5H.a for S42IL-125 containing an exotic introgression in the region 5H, 43–69 cM (Table 4).

Fine-grind extract (FGE)

A total of seven significant line × phenotype associations on chromosomes 1H, 4H, 6H and 7H, summarized to four QTL effects, were detected for FGE. At all QTLs, both, the line main and the line × environment interaction effect were significant, and the *Hsp* introgression was associated with a decreased FGE compared to 'Scarlett'. The highest differences between the performance of an IL and the control were exhibited at QFge.S42IL-1H.a for S42IL-102 and -103, containing *Hsp* introgressions within the region 1H, 0–85 cM and 1H, 39–70 cM, respectively (−3.2 and −2.9%, respectively).

Friability (FRI)

For FRI, six significant line × phenotype associations were detected on chromosomes 1H and 4H. In two cases, represented by S42ILs -118 and -119, the effect was solely significant in environment D07 (Table 4). The six significant associations could be summarized to two QTL effects. The exotic introgression reduced the trait value by a maximum of −20.0% in S42IL-102, carrying a *Hsp* segment in the chromosomal region 1H, 0–85 cM.

Grain protein content (GPC)

For GPC, six significant line × phenotype associations, summarized to four putative QTLs, were identified on chromosomes 1H, 4H, 6H and 7H. Five associations showed both, a significant line main and a line × environment interaction effect, whereas S42IL-128 exhibited solely a significant line main effect. At QGpc.S42IL-6H.a the *Hsp* segment of S42IL-129, present in the region 6H, 96–112 cM, caused a maximum increase of GPC of 8.9%, relative to the control. Other strong exotic increasing effects were measured at QGpc.S42IL-4H.a and QGpc.S42IL-7H.a (7.7 and 7.4%, respectively).

Grain sieving fraction > 2.5 mm (GSF)

Seventeen significant line × phenotype associations were identified for GSF on all chromosomes with the exception of 5H and 6H. Due to the overlapping or flanking of several introgressions, a total of ten putative QTLs were identified, among which three QTLs each were mapped to chromosomes 2H and 4H. At seven loci the line main and the line x environment interaction effect were significant. In contrast, two QTLs were solely detected as line main effects, and one QTL (QGsf.S42IL-3H.a) only as interaction effect in environment D07. At QGsf.S42IL-2H.a, QGsf.S42IL-2H.c and QGsf.S42IL-4H.a, the exotic introgression caused a favorable increase of GSF by 8.4, 6.7 and 6.2%, respectively. The strongest unfavorable effect on GSF was exhibited by S42IL-122, carrying an *Hsp* segment on chromosome 4H, 125–132 cM. Here, the trait performance was reduced by 14.1%.

Kolbach index (KOL)

Altogether eleven S42ILs on all chromosomes except 2H revealed a significant line x phenotype association for KOL. Due to the overlapping or flanking of several

Table 4 List of 68 significant line × phenotype associations for eight malting quality traits detected with 39 S42ILs

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Bin range[d]	Introgression line	Effect[e]	LSMEANS (IL)[f]	Diff.[g]	RP [S42IL] (%)[h]	QTL in parent population S42[i]	Candidate genes[j]
AA	QAa.S42IL-1H.a	1H	0–14	1–2	S42IL-101	L	373.0	−82.6	−18.1	QAa.S42-1H.a	
		1H	0–85	1–9	S24IL-102	L + I	332.0	−123.6	−27.1	QAa.S42-1H.a	
	QAa.S42IL-1H.b	1H	39–70	6–7	S42IL-103	L + I	370.3	−85.2	−18.7		
		1H	70–85	7–9	S42IL-105	L + I	386.8	−68.8	−15.1		
	QAa.S42IL-4H.a	**4H**	**14–31**	**2–4**	**S42IL-116**	**L + I**	**546.9**	**91.3**	**20.0**	QAa.S42-4H.a	
	QAa.S42IL-4H.b	**4H**	**170–190**	**12–13**	**S42IL-124**	**L + I**	**529.4**	**73.9**	**16.2**	QAa.S42-4H.c	*Bmy1*[1]
	QAa.S42IL-5H.a	5H	43–69	5–7	S42IL-125	L + I	307.7	−147.9	−32.5	QAa.S42-5H.b	*Dhn1/Dhn2*[2]
		5H	69–85	7–8	S42IL-126	L	361.6	−94.0	−20.6	QAa.S42-5H.b	*Dhn1/Dhn2*[2], *HvCbf3*[3]
	QAa.S42IL-6H.a	6H	112–135	9–10	S42IL-130	L	387.7	−67.9	−14.9	QAa.S42-6H.a	*Amy1*[4]
	QAa.S42IL-7H.a	7H	50	3	S42IL-133	I	293.7[G07]	−117.8	−28.6		
FGE	QFge.S42IL-1H.a	1H	0–85	1–9	S42IL-102	L + I	79.1	−2.6	−3.2	QFge.S42-1H.a, QFge.S42-1H.b	*Hor* genes[4]
		1H	39–70	6–7	S42IL-103	L + I	79.4	−2.3	−2.9	QFge.S42-1H.b	
		1H	70–85	7–9	S42IL-105	L	80.5	−1.3	−1.6	QFge.S42-1H.b	
	QFge.S42IL-4H.a	4H	80–95	7–8	S42IL-121	L + I	79.6	−2.1	−2.6		
	QFge.S42IL-6H.a	6H	40–112	3–9	S42IL-128	L + I	79.8	−1.9	−2.3		*Amy1*[4]
		6H	96–112	5–9	S42IL-129	L + I	79.9	−1.8	−2.2		*Amy1*[4]
	QFge.S42IL-7H.a	7H	146–166	8–11	S42IL-137	L + I	79.8	−1.9	−2.3		*Amy2*[4]
FRI	QFri.S42IL-1H.a	1H	0–85	1–9	S42IL-102	L + I	59.3	−14.9	−20.0	QFri.S42-1H.a	*Glb1*[10]
		1H	39–70	6–7	S42IL-103	L + I	62.6	−11.6	−15.7	QFri.S42-1H.a	
		1H	70–85	7–9	S42IL-105	L + I	62.1	−12.1	−16.3	QFri.S42-1H.a	
	QFri.S42IL-4H.a	4H	31–57	4–6	S42IL-118	I	58.7[D07]	−12.9	−18.0		
		4H	31–95	4–8	S42IL-119	I	58.0[D07]	−13.6	−19.0	QFri.S42-4H.a	
		4H	80–95	7–8	S42IL-121	L	65.6	−8.6	−11.6	QFri.S42-4H.a	
GPC	QGpc.S42IL-1H.a	1H	0–85	1–9	S42IL-102	L + I	12.6	0.7	5.9	QPro.S42-1H.a, QPro.S42-1H.b	*Hor* genes[4]
		1H	70–85	7–9	S42IL-105	L + I	12.7	0.8	6.6	QPro.S42-1H.b	
	QGpc.S42IL-4H.a	4H	80–95	7–8	S42IL-121	L + I	12.8	0.9	7.7	QPro.S42-4H.b	
	QGpc.S42IL-6H.a	6H	40–112	3–9	S42IL-128	L	12.5	0.6	4.7	QPro.S42-6H.a, QPro.S42-6H.b	*HvNAM-1*[5]
		6H	96–112	5–9	S42IL-129	L + I	13.0	1.1	8.9	QPro.S42-6H.b	*HvNAM-1*[5]
	QGpc.S42IL-7H.a	7H	146–166	8–11	S42IL-137	L + I	12.8	0.9	7.4		

Table 4 continued

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Bin range[d]	Introgression line	Effect[e]	LSMEANS (IL)[f]	Diff.[g]	RP [S42IL] (%)[h]	QTL in parent population S42[i]	Candidate genes[j]
GSF	QGsf.S42IL-1H.a	1H	0–85	1–9	S42IL-102	L + I	69.5	−10.1	−12.7		*Hor* genes[4]
		1H	39–70	6–7	S42IL-103	L + I	74.6	−5.1	−6.4		
	QGsf.S42IL-2H.a	**2H**	**17–42**	**2–4**	**S42IL-107**	**L + I**	**86.4**	**6.7**	**8.4**		*Ppd-H1*[6]
	QGsf.S42IL-2H.b	2H	17–92	2–8	S42IL-108	I	85.7^{G07}	−5.6	−6.1		*Ppd-H1*[6]
		2H	67–92	6–8	S42IL-109	L + I	70.1	−9.6	−12.0		
	QGsf.S42IL-2H.c	**2H**	**80–86**	**7–8**	**S42IL-110**	**L**	**85.0**	**5.3**	**6.7**		
	QGsf.S42IL-3H.a	3H	130–175	10–15	S42IL-114	I	72.8^{D07}	−8.3	−10.2		*sdw1 (denso)* [7]
		3H	155–190	13–16	S42IL-115	I	72.9^{D07}	−8.1	−10.0		*sdw1 (denso)*[7]
	QGsf.S42IL-4H.a	**4H**	**31–95**	**4–8**	**S42IL-119**	**L**	**84.6**	**4.9**	**6.2**		
	QGsf.S42IL-4H.b	4H	80–95	7–8	S42IL-121	I	80.5^{G07}	−10.9	−11.9		
		4H	125–132	9–10	S42IL-122	L + I	68.5	−11.2	−14.1		
		4H	125–170	9–12	S42IL-123	L + I	73.3	−6.4	−8.0		
	QGsf.S42IL-4H.c	4H	170–190	12–13	S42IL-124	L + I	73.9	−5.7	−7.2		*Vrn-H2*[7]
GSF	QGsf.S42IL-7H.a	7H	62–75	5	S42IL-134	L + I	73.7	−5.9	−7.5		*HvCO1*[8]
		7H	75–155	5–10	S42IL-135	L	74.4	−5.3	−6.6		*HvCO1*[8], *eps7L*[7]
	QGsf.S42IL-7H.b	7H	146–166	8–11	S42IL-137	L + I	71.5	−8.2	−10.3		*eps7L*[7]
		7H	166–181	11–12	S42IL-138	L + I	73.6	−6.1	−7.7		
KOL	QKol.S42IL-1H.a	1H	0–85	1–9	S24IL-102	L + I	39.1	−5.1	−11.5		*Hor* genes[4]
		1H	39–70	6–7	S42IL-103	L + I	41.1	−3.1	−7.0		
		1H	70–85	7–9	S42IL-105	L + I	40.5	−3.6	−8.2		
	QKol.S42IL-3H.a	3H	155–190	13–16	S42IL-115	I	38.8^{D07}	−2.9	−7.0		*CepB*[9]
	QKol.S42IL-4H.a	**4H**	**14–31**	**2–4**	**S42IL-116**	**L**	**46.4**	**2.2**	**5.1**		
	QKol.S42IL-4H.b	4H	80–95	7–8	S42IL-121	L + I	39.7	−4.5	−10.1		
	QKol.S42IL-5H.a	5H	43–69	5–7	S42IL-125	L + I	40.9	−3.2	−7.3		
		5H	69–85	7–8	S42IL-126	L + I	41.0	−3.1	−7.1		
	QKol.S42IL-6H.a	6H	96–112	5–9	S42IL-129	L + I	41.3	−2.8	−6.4		
		6H	112–135	9–10	S42IL-130	I	38.3^{G07}	-3.7	-8.8		
	QKol.S42IL-7H.a	7H	50	3	S42IL-133	I	37.6^{G07}	−4.5	−10.6		
VIS	QVis.S42IL-1H.a	1H	0–85	1–9	S42IL-102	L + I	1.55	0.07	5.0	QVis.S42-1H.a	*Glb1*[10]
		1H	39–70	6–7	S42IL-103	L + I	1.56	0.08	5.6	QVis.S42-1H.a	
		1H	70–85	7–9	S42IL-105	I	1.52^{D07}	0.07	4.5	QVis.S42-1H.a	
	QVis.S42IL-6H.a	6H	112–155	9–10	S42IL-130	I	1.62^{G07}	0.09	5.6	QVis.S42-6H.a	*Amy1*[4]

Table 4 continued

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Bin range[d]	Introgression line	Effect[e]	LSMEANS (IL)[f]	Diff.[g]	RP [S42IL] (%)[h]	QTL in parent population S42[i]	Candidate genes[j]
VZ45	QVZ45.S42IL-1H.a	1H	0–85	1–9	S42IL-102	L + I	43.1	−4.7	−9.8	QVZ45.S42-1H.a	*Hor* genes[4]
		1H	39–70	6–7	S42IL-103	L + I	42.8	−5.0	−10.5	QVZ45.S42-1H.a	
		1H	70–85	7–9	S42IL-105	L + I	44.2	−3.7	−7.6	QVZ45.S42-1H.a	
	QVZ45.S42IL-4H.a	4H	80–95	7–8	S42IL-121	L + I	44.5	−3.3	−6.9		
	QVZ45.S42IL-5H.a	5H	43–69	5–7	S42IL-125	L + I	43.0	−4.9	−10.1	QVZ45.S42-5H.b	
		5H	69–85	7–8	S42IL-126	L + I	43.8	−4.0	−8.4	QVZ45.S42-5H.b	
	QVZ45.S42IL-6H.a	6H	40–112	3–9	S42IL-128	L	44.6	−3.3	−6.8		*Amy1*[4]

Favorable QTL effects of exotic introgressions are given in bold

[a] Abbreviations of traits, see Table 1

[b] Chromosomal location of the target introgression

[c] Chromosomal extent of the target introgression in centiMorgans

[d] Bin range of the exotic introgression of the respective S42IL according to information by Kleinhofs and Graner (2001) and by the OWB population (Costa et al. 2001, http://barleyworld.org)

[e] In the two-factorial ANOVA, significant line × phenotype associations ($P < 0.05$) were detected as line main effect (L) or line × environment interaction effect (I)

[f] If a line main effect (L) was detected, the LSMEANS [IL] across all environments is listed. If solely a line × environment interaction effect (I) was identified, the LSMEANS [IL] in the particular environment (D07, G07 or H07) is assigned

[g] Score difference = LSMEANS [IL] − LSMEANS [Scarlett]

[h] Relative performance: RP [S42IL] = (LSMEANS [S42IL] − LSMEANS [Scarlett]) × 100/LSMEANS [Scarlett]

[i] Reference: von Korff et al. (2008); The traits GSF and KOL were not tested in von Korff et al. (2008)

[j] References: [1]Clark et al. (2003); [2]Choi et al. (2000); [3]Choi et al. (2002); [4]Rostoks et al. (2005); [5]Distelfeld et al. (2008); [6]Turner et al. (2005); [7]Laurie et al. (1995); [8]Griffiths et al. (2003); [9]Guerin et al. (1994); [10]MacLeod et al. (1991)

introgressions, they were summarized to seven QTL effects, among which four effects were detected as significant line main and line x environment interaction effect, simultaneously. In contrast, at QKol.S42IL-3H.a and QKol.S42IL-7H.a solely the line × environment interaction effect was significant, whereas at QKol.S42IL-4H.a only the line main effect was significant. Here, the exotic introgression, located within the region 4H, 14–31 cM, increased KOL by 5.1%, relative to the control. The strongest reduction effects were mapped to the regions 1H, 0–85 cM and 7H, 50 cM by S42IL-102 and -133, respectively. Here, the trait value was decreased by 11.5 and 10.6%, respectively, relative to 'Scarlett'.

Viscosity (VIS)

Four significant line × phenotype associations, summarized to two QTL effects, were identified for VIS on chromosomes 1H and 6H. The strongest *Hsp* effect was mapped to the chromosomal regions 1H, 39–70 cM and 6H, 112–155 cM by the S42ILs-103 and -130, respectively. Both lines revealed a viscosity increased by 5.6%, relative to 'Scarlett' (Table 4). At QVis.S42IL-6H.a, this effect was only significant in environment G07.

Hartong 45°C (VZ45)

A total of seven significant line × phenotype associations were detected for VZ45 on chromosomes 1H, 4H, 5H and 6H. These associations were summarized to four QTL effects. At three QTLs, the line main effect as well as the line × environment interaction effect was significant, whereas QVZ45.S42IL-6H.a was solely detected as line main effect. At QVZ45.S42IL-1H.a, the exotic introgression, localized in the region 1H, 39–70 cM, was associated with a trait performance decreased by 10.5%, relative to the recurrent parent. Further strong reduction effects were mapped to the regions 5H, 43–69 cM and 1H, 0–85 cM by S42IL-125 and -102, respectively (−10.1 and −9.8%, respectively).

Discussion

Detection and verification of QTL effects

The objectives of this study were (1) to verify QTLs affecting malting quality parameters which were previously detected within the parent population S42 (von Korff et al. 2008), (2) to identify new QTLs, and (3) to assess S42ILs revealing multiple QTL effects.

By association analysis, altogether 68 significant line × phenotype associations, summarized to 40 putative QTLs, were identified using the S42IL set. Since only at six out of 40 QTLs (15.0%) the exotic introgression was associated with an improved trait performance, a strong selection on favorable malting quality alleles during the breeding process can be assumed. This observation is in contrast to a previous QTL study where the agronomic performance of the S42IL set was evaluated. In the latter case, a high portion of the detected QTLs (40.4%) for yield related traits revealed a favorable *Hsp* effect (Schmalenbach et al. 2009). This discrepancy possibly indicates that the selective breeding for malting quality might have been stronger than for agronomic performance. Alternatively, the genetic diversity in exotic barley might be higher for agronomic parameters than for malting quality.

The six traits AA, FGE, FRI, GPC, VIS and VZ45 were investigated within the S42ILs as well as in the parent population S42. In addition, the S42ILs were evaluated with regard to GSF and KOL. In population S42, von Korff et al. (2008) identified a total of 45 putative QTLs for the six corresponding traits, among which one-third showed a favorable effect of the exotic allele. Thirty-six out of the 45 QTLs (80.0%) are located in chromosomal regions which are represented by *Hsp* segments in S42ILs. Hence, these 36 QTL effects are comparable with QTLs detected in the S42IL set. To enable the verification of the remaining QTLs of population S42, introgression lines carrying the donor allele in these regions are currently under construction and will in future be subjected to malting quality analysis. As listed in Table 4, half of the 36 QTLs of the parent population S42 were confirmed by the evaluated S42ILs. For the traits AA and VZ45 all QTLs except one were verified, whereas for VIS and FRI two out of seven QTLs (28.6%) were confirmed. At 15 out of the 18 verified QTLs (83.3%), an *Hsp* effect of the same direction, i.e. either an increase or a reduction of the trait value caused by the exotic genotype, was measured in both populations. For 12 out of these 15 consistent QTLs (80.0%), at least one S42IL revealed a higher relative trait performance in comparison to the control 'Scarlett' than assessed for the corresponding QTL in population S42 (see also von Korff et al. 2008).

For traits AA, FGE, GPC and VZ45, altogether eight new QTL effects, previously not assessed in population S42, were identified in the S42IL set. All these QTLs exhibited an unfavorable *Hsp* effect. In addition, a total of 17 QTLs affecting the newly investigated traits GSF or KOL were mapped by S42ILs. At four QTLs (23.5%), the exotic introgression was associated with a favorable increase of the trait value compared to 'Scarlett'.

Inconsistent results of the two compared studies might be explained by different hypotheses. It could be that interactions between non-linked *Hsp* alleles, present in the S42 population, are no more existent in the S42ILs. Since

the S42ILs carry single exotic segments, whereas the BC_2DH lines of population S42 contain several independent introgressions, potential epistatic effects are expected to be reduced in the S42ILs. Furthermore, contradictory results of the two compared studies might be attributed to genotype x environment interactions.

In the following, the QTL results of the present study are discussed in detail. They are compared to significant effects, detected in population S42 (von Korff et al. 2008) and in other malting quality QTL studies, and are related to possible candidate genes which are located in the same chromosomal region.

α-amylase activity

In population S42 von Korff et al. (2008) detected six putative QTLs which map to chromosomal regions represented by exotic introgressions of S42ILs. All these QTLs, with the exception of QAa.S42-4H.b, were confirmed by S42ILs, and at four out of the five verified QTLs an effect with the same direction was assessed in both populations. In contrast, at QAa.S42IL-6H.a/QAa.S42-6H.a the *Hsp* genotype caused a decrease of AA in S42IL-130, but a favorable increase in population S42 (−14.9 and 16.0% relative to 'Scarlett', respectively). Two favorable exotic QTL effects, stable across all tested environments in both populations, were localized on chromosome 4H (Table 4). Here, α-amylase activity was enhanced by a maximum of 20.0% at QAa.S42IL-4H.a in S42IL-116, and by 16.3% at QAa.S42-4H.c in population S42. The region 4H, 170–190 cM, harboring QAa.S42IL-4H.b and QAa.S42-4H.c, corresponds to the locus of *Bmy1*, coding for *β*-amylase (Clark et al. 2003). Different hypotheses, possibly explaining the association between QTLs for α-amylase activity and *Bmy1* are discussed by von Korff et al. (2008). For instance, it could be assumed that this gene might have a pleiotropic effect. In both studies compared, the strongest unfavorable *Hsp* effect, stable across all environments, was mapped to the region 5H, 43–69 cM (QAa.S42IL-5H.a and QAa.S42-5H.b, respectively). Here, the exotic genotype was associated with an enzyme activity reduced by −32.5% in S42IL-125 and −21.5% in population S42. Several stress related genes like *Dhn1/Dhn2* (Choi et al. 2000) and *HvCbf3* (Choi et al. 2002) also map to this region on chromosome 5H. They might be associated with a high thermostability of α-amylase, and, thus, with a preserved enzyme activity during the mashing process. As shown in Table 4, a further decreasing *Hsp* effect on AA was identified in both populations in the chromosomal region 6H, 112–135 cM, which coincides with *Amy1* encoding for one of the two known forms of α-amylase (Rostoks et al. 2005).

As shown in Table 3, a strong positive genetic correlation of 0.86 was assessed between AA and KOL. This coincides with the co-localization of five QTLs on all chromosomes except 2H and 3H, all revealing an *Hsp* effect of the same direction on both traits. Furthermore, two QTLs on chromosome 1H and 5H were associated with a reducing *Hsp* effect on AA as well as on VZ45. These consistent effects correspond to a positive correlation of 0.83 between both traits.

Fine-grind extract and Hartong 45°C

For FGE, altogether four QTL effects were localized in population S42 on chromosomes 1H, 3H and 7H. All QTLs are located within genomic regions which are covered by exotic introgressions of S42ILs. The two QTLs QFge.S42-1H.a and QFge.S42-1H.b were confirmed by QFge.S42IL-1H.a, which showed significant line × phenotype associations for S42ILs-102, -103 and -105 (Table 4). In this QTL region, a maximum reduction of trait performance caused by the *Hsp* genotype was assessed in population S42 as well as in the S42ILs (−2.2 and −3.2%, respectively). Furthermore, the QTLs were stable across all environments in both populations. In the same region, Thomas (2003) revealed a QTL "hot-spot" harboring QTLs for the malting parameter hot water extract from five different barley crosses.

In addition to the confirmed QTLs, altogether three new significant effects on FGE were identified on chromosomes 4H, 6H and 7H by S42ILs. The location of QFge.S42IL-6H.a coincides with the *Amy1* locus (Rostoks et al. 2005) as well as with a second QTL cluster for hot water extract which was mapped on the 'Steptoe' × 'Morex' map (Thomas 2003). The locus of *Amy2*, coding for the second form of α-amylase, corresponds to the newly identified QTL QFge.S42IL-7H.a (Rostoks et al. 2005).

In population S42, three putative QTLs affecting VZ45 were mapped to chromosomal regions which are already represented by S42ILs. Two QTL effects (66.7%) were confirmed within the present study. In both populations, the exotic genotype caused a maximum decrease of VZ45 on the top of chromosome 1H (−9.1% at QVZ45.S42-1H.a and −10.5% at QVZ45.S42IL-1H.a). Within the S42IL set, two new QTLs were localized in the regions 4H, 80–95 cM and 6H, 40–112 cM. Both effects were stable across all three tested environments and exhibited an unfavorable *Hsp* effect.

In the present study, a low positive correlation between FGE and VZ45 was measured ($r = 0.25$). In contrast, the traits exhibited a strong positive correlation ($r = 0.58$) in population S42 (von Korff et al. 2008) which was expected since both parameters characterize the activity of cytolytic and proteolytic malt enzymes, and, thus, are closely linked.

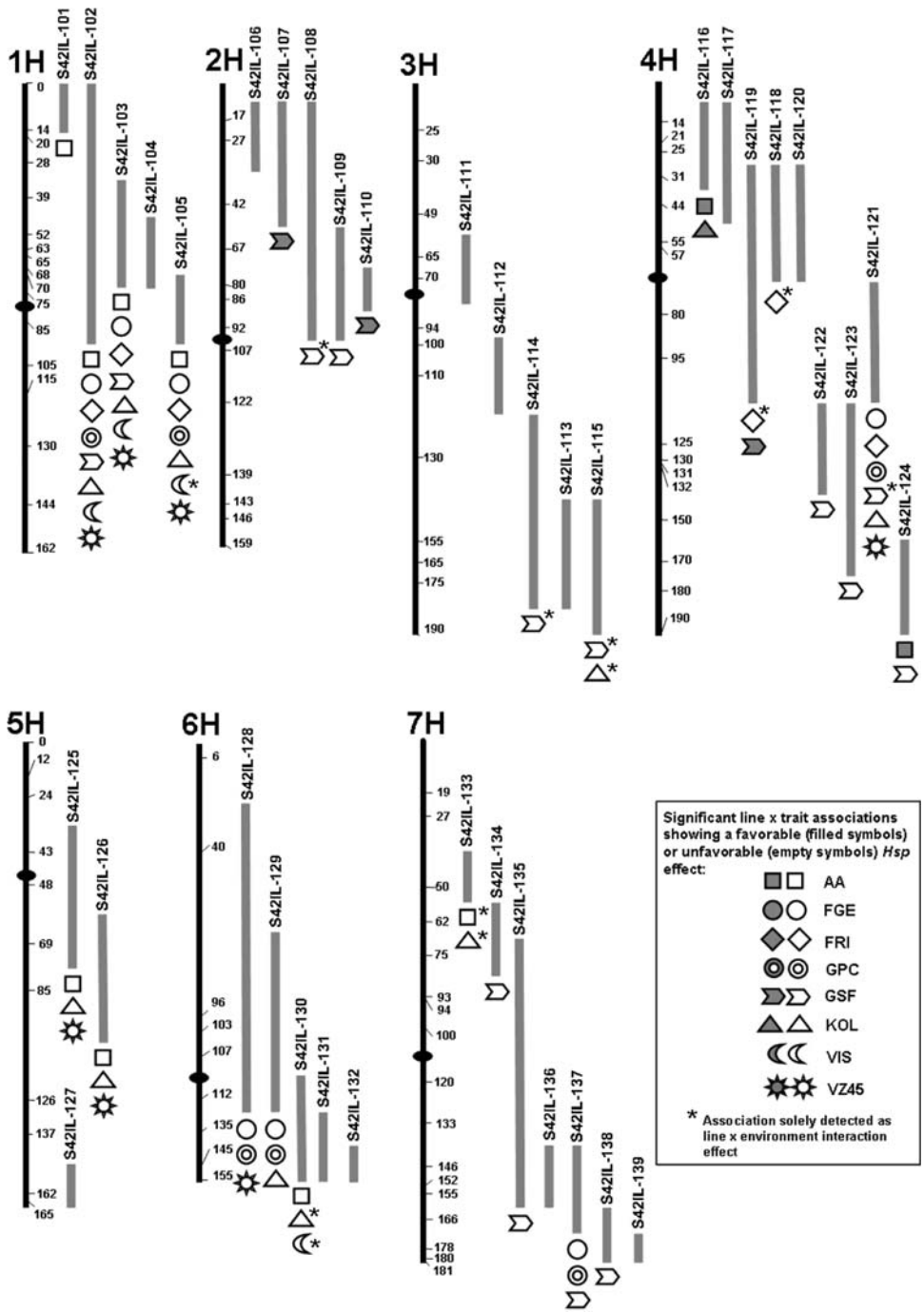

Fig. 1 SSR map with 68 significant ($P < 0.05$) line × trait associations for 39 S42ILs and eight malting quality traits. The chromosomes are shown as *black bars* with cM values for SSR loci following the order of von Korff et al. (2004). The extent of *Hsp* introgressions are given in grey bars right to the chromosomes. The associations are illustrated as *symbols* below the S42ILs. They either reveal a favorable (*filled symbols*) or unfavorable (*empty symbols*) *Hsp* effect. Associations solely detected as line x environment interaction effect are marked by an *asterisk* right to the symbol

As shown in Table 3, VZ45 indeed revealed high positive correlations with KOL, AA and FRI in the S42IL study ($r = 0.92$, 0.83 and 0.73, respectively). This coincides with multiple QTL effects on these traits, which are predominantly located on chromosomes 1H and 5H (Fig. 1). One QTL cluster, localized in the region 1H, 0–85 cM, corresponds to the map location of several *Hor* genes coding for different forms of hordein, the major storage protein in barley (Rostoks et al. 2005).

Friability and viscosity

Von Korff et al. (2008) assessed seven QTLs for FRI which are located in chromosomal regions represented by S42ILs. As listed in Table 4, two out of these seven QTLs (28.6%) were confirmed in the present study and revealed a reducing *Hsp* effect on trait performance in both populations. At QFri.S42-1H.a/QFri.S42IL-1H.a, the exotic genotype decreased FRI by a maximum of 19.4 and 20.0%

in population S42 and S42IL-102, respectively. In both verified QTL regions on chromosomes 1H and 4H, Li et al. (2005) identified a QTL effect on friability. Here, the allele of the *Hsp* accession 'HS213' was associated with an increased trait value.

In population S42, seven QTLs affecting VIS were mapped to chromosomal regions, which are already represented by the S42IL set. Two out of these seven QTLs (28.6%) were confirmed by S42ILs. QVis.S42-1H.a and QVis.S42IL-1H.a are located within the chromosomal region 1H, 0–85 cM, which exhibited multiple QTL effects on several traits simultaneously. As described above, one QTL for FRI, associated with a reducing *Hsp* effect, also maps to this region. This coincides with the strong negative correlation between FRI and VIS ($r = -0.64$, Table 3). Both traits, FRI and VIS, are cytolytic parameters which are mainly affected by the breakdown of β-glucan, the major constituent of barley endosperm cell walls. Indeed, Han et al. (1995) and Zwickert-Menteur et al. (1996) mapped several QTLs for β-glucan content and β-glucanase activity in the 'Steptoe'/'Morex' population to the described region on chromosome 1H. These findings correspond to the locus of *Glb1* encoding for (1→3, 1→4)-β-glucan 4-glucanhydrolase (MacLeod et al. 1991). The second verified QTL for VIS was mapped to the genomic region 6H, 112–155 cM, and revealed an *Hsp* effect with an opposed direction in the two compared populations. This could be due to the fact, that in the present study this effect was significant solely in environment G07.

Grain protein content and Kolbach index

Grain protein content is one of the major determinants of malting quality. For GPC, von Korff et al. (2008) identified nine QTLs which were located within chromosomal regions already covered by S42ILs. Indeed, five out of these nine QTLs (55.6%) were confirmed by the present study, all exhibiting an increasing *Hsp* effect. As mentioned above, the region 1H, 0–85 cM, harboring QPro.S42-1H.b and QGpc.S42IL-1H.a, corresponds to the map position of several *Hor* genes (Rostoks et al. 2005). A different organization of these genes could possibly explain the variation in GPC between the exotic and the elite genotype. Pelger et al. (1993) detected differences regarding the number of segregating *Hor* loci both between two different wild barley species and between them and several forms of *H. vulgare*.

In the S42ILs as well as in population S42, QTL effects on GPC were localized on chromosome arm 6HS. These findings coincide with the localization of QTLs for GPC, nitrogen storage, and nitrogen remobilization detected in a RIL population which was derived from a cross between high- and low-GPC barley cultivars (See et al. 2002; Mickelson et al. 2003). As See et al. (2002) reported, a major QTL for GPC was localized near SSR marker *HVM74*. In our present study, this marker maps to the chromosomal region showing the strongest QTL effect on this trait (QGpc.S42IL-6H.a). Based on different studies conducted in wheat and barley, Distelfeld et al. (2008) assumed that sequence polymorphism in the barley NAC transcription factor *HvNAM-1* could be a possible explanation for this QTL. The wheat ortholog of *HvNAM-1* was cloned by Uauy et al. (2006) and proved to be responsible for the regulation of grain protein content in wheat. The exotic *HvNAM-1* gene, which was mapped onto the S42ILs -128 and -129 (data not shown) is thus a strong candidate to explain the observed effect on GPC in our study. Further transformation experiments will be conducted to test for this hypothesis.

In addition to the verified QTLs, one new QTL for GPC, revealing an unfavorable exotic effect, was mapped to chromosome 7H (QGpc.S42IL-7H.a). In this region, a further QTL for protein content, also exhibiting an unfavorable *Hsp* effect, was assessed by Li et al. (2005).

For the newly investigated parameter KOL, seven QTL effects, among which one was associated with a favorable increasing effect of the exotic introgression, were mapped to all chromosomes except 2H. The four QTLs QKol.S42IL-1H.a, QKol.S42IL-3H.a, QKol.S42IL-4H.a and QKol.S42IL-5H.a were localized within the same genomic regions as QTLs for KOL identified in the populations 'Dicktoo'/'Morex' and 'Harrington'/'Morex' (Oziel et al. 1996; Marquez-Cedillo et al. 2000). The parameter Kolbach index is conform to the soluble protein/total protein ratio and provides an indication of the proteolytic enzyme content of malt. In fact, QKol.S42IL-3H.a maps to the same chromosome arm as *CepB*, a gene encoding for malt endopeptidase 1 which hydrolyses hordein (Guerin et al. 1992, 1994).

Grain sieving fraction > 2.5 mm

For the newly investigated trait GSF, altogether ten putative QTLs were identified by S42ILs, where three QTLs exhibited a favorable exotic effect. For nearly all these regions coincident QTLs can be found in the literature. In region 1H, 0–85 cM containing QGsf.S42IL-1H.a, several QTLs for grain size were identified in the populations 'Blenheim'/'E224/3' and 'Harrington'/'Morex' (Thomas et al. 1995; Powell et al. 1997; Marquez-Cedillo et al. 2000). Since grain size is strongly influenced by the accumulation of storage material, like carbohydrates and proteins, during the grain-filling phase of the post-anthesis period (Coventry et al. 2003), different *Hor* loci, located within the same region, could be assumed to affect GSF. Furthermore, grain size is indirectly influenced by the

pre-anthesis period which length is affected by responses to photoperiod and temperature. Thus, as Coventry et al. (2003) underlined, different response genes might be involved in the determination of grain size. Indeed, the major photoperiod response gene of barley, *Ppd-H1* (Turner et al. 2005), maps to the same genomic region as QGsf.S42IL-2H.a and QGsf.S42IL-2H.b (Table 4). Moreover, the location of *Vrn-H2*, one of the barley vernalization response genes (Laurie et al. 1995), corresponds to QGsf.S42IL-4H.c. On chromosome 4H, two further QTL effects (QGsf.S42IL-4H.a and QGsf.S42IL-4H.b) were identified in the present study, and are consistent with QTLs for kernel plumpness assessed in the populations 'Harrington'/'Morex' and 'Harrington'/'TR306' (Marquez-Cedillo et al. 2000; Mather et al. 1997). The barley photoperiod response gene *HvCO1* (Griffiths et al. 2003) and the 'earliness per se' gene *eps7L* (Laurie et al. 1995) could be assumed to have an effect on GSF at QGsf.S42IL-7H.a and/or QGsf.S42IL-7H.b. Laurie et al. (1995) demonstrated a delay in flowering time in barley caused by the dwarfing gene *sdw1* (previously named *denso*), which maps to the long arm of chromosome 3H and, thus, could be a candidate gene for GSF at QGsf.S42IL-3H.a. Here, a QTL effect on GSF was also detected by Thomas et al. (1995) and Powell et al. (1997).

Identification of malting quality QTL clusters

As illustrated in Fig. 1, several chromosomal regions exhibited multiple QTL effects. Two QTL clusters mapping to region 1H, 0–85 cM and 4H, 80–95 cM are evidently of particular interest as they harbor QTL effects for eight and six malting quality parameters simultaneously. In previous studies using the S42IL set, additional significant effects on the traits grains per ear, days until heading, plant height, lodging at harvest, and resistance to powdery mildew were also identified in both regions (Schmalenbach et al. 2008, 2009). In the present study, QTLs affecting seven or eight malting quality traits in region 1H, 0–85 cM were assessed in S42ILs-102, -103 and -105 (Fig. 1). In contrast, S42ILs-101 and -104 whose introgressions (1H, 0–14 cM and 1H, 52–70 cM, respectively) are completely represented by S42IL-102 revealed one and no significant effect, respectively. Due to these facts, it could be assumed that the exotic segment present in S42IL-103 is further extended towards the centromere than expected on the basis of SSR genotype data, whereas the introgression of S42IL-104 has the expected size. Thus, introgressions of S42ILs-102, -103 and -105 would cover the same genomic region harboring the QTL cluster. However, it is required to verify this hypothesis by genotyping the S42ILs with additional markers, and thus to define the extension of exotic introgressions more precisely.

One hypothesis explaining the occurrence of QTL clusters might be the presence of relatively few genes with pleiotropic effects. Several studies investigating the 'domestication syndrome' in different crop species have supported this assumption by identifying major QTLs as well as major genes directly associated with the domestication process (Bomblies and Doebley 2006; Koinange et al. 1996; Weeden 2007). In contrast, it could be proposed that numerous genes affecting different traits are closely linked within QTL complexes and were fixed during the domestication and breeding process (Xiong et al. 1999). It is advisable to dissect the malting quality QTL clusters identified in the present study and thus to unravel their genetic basis. So far, two malting quality QTL complexes located near the chromosome 4H telomere and in the chromosome 7H centromere region were fine-mapped using two sets of barley isolines (Gao et al. 2004; Han et al. 1997, 2004). By means of these lines, which carried either 'Steptoe' or 'Morex' segments of different size in the target region, the QTL complexes on chromosome 4H and 7H were resolved to intervals of 0.7–27.9 cM and 2.0–6.4 cM, respectively. A similar study could be conducted using the described S42ILs on chromosomes 1H and 4H as initial material for the development of so called SUB-ILs which possess smaller exotic introgressions.

Future prospects

Exemplified by malting quality, the present study indicates the usefulness of wild barley introgression lines for the localization and verification of QTLs. In future, additional S42ILs harboring exotic introgressions in so far missing genomic regions will be constructed. They could enable the detection of further QTL effects for malting quality as well as for agronomic parameters and disease resistances. In addition, the complete S42IL set will be extensively evaluated with regard to abiotic stress tolerance, i.e. tolerance to drought stress and nutrient deficiency. Through genotyping with Illumina SNPs (Rostoks et al. 2006) or Diversity Arrays Technology (DArT) markers (Wenzl et al. 2006), a more precise characterization of the lines on the genome level is also intended. This will facilitate the selection of SUB-ILs with smaller *Hsp* introgressions, which could be a valuable resource for fine-mapping of QTL clusters and map-based cloning of promising QTL effects. As described above, clustered QTL effects for malting quality which were detected in S42ILs-102, -103, -105, and -121 on chromosomes 1H and 4H, respectively, would be of special interest for such high-resolution mapping approaches. Other studies will focus on the investigation of epistatic effects by constructing S42ILs carrying two or several exotic introgressions simultaneously. The first step towards such a set of lines has

already been done by crossing selected S42ILs. These lines could also be used for QTL pyramiding.

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3 General discussion and future prospects

The results of the S42IL study presented in the three original papers are discussed in the following subchapters. In chapter 3.1, the strategy applied to select the wild barley introgression lines as well as their genetic constitution are evaluated and compared to IL sets developed in other plant species. Subsequently, the verification of QTL effects from parent population S42 using a set of 39 introgression lines are discussed for all trait complexes (chapter 3.2.1). In chapters 3.2.2 and 3.2.3, the consistency of QTL results across overlapping S42ILs and the identification of QTL clusters are discussed, respectively. Finally, in chapter 3.3, possible future applications of the S42ILs are presented.

3.1 Selection and genetic characterization of the wild barley introgression library

A set of 59 wild barley introgression lines (hereafter abbreviated with S42ILs) has been constructed from the BC_2DH population S42. The parent population has been generated from a primary cross between the spring barley cultivar 'Scarlett' (*Hv*) and the Israeli wild barley accession 'ISR 42-8' (*Hsp*, von Korff et al. 2004). According to the selection strategy recommended by Zamir (2001), the S42IL set has been developed by three rounds of recurrent backcrossing, two or four selfings, and, in parallel, MAS. Recurrent backcrossings were required in order to reduce the portion of exotic genome present in each line by theoretically 50 % per generation. Subsequent selfings resulted in completely homozygous lines which constitute a stable genetic resource for further evaluation. For developing pure S42ILs, a pre-selection step was initially carried out in BC_2DH generation (von Korff et al. 2004). Based on genotype data derived by analysis of 98 SSRs, a set of 40 candidate introgression lines, referred to as pre-ILs, was selected from 301 lines of population S42. The pre-IL set represented the entire *Hsp* genome by a minimum number of lines, each containing a small as possible portion of the donor genome. In addition to the target introgression they were selected for, the pre-ILs harbored on average three independent introgressions (von Korff et al. 2004). The present S42IL study aimed to eliminate these additional introgressions and to preserve solely the

target segment. To do so, the strategy mentioned above was applied. MAS of pure S42ILs, based on genotyping with a genome wide panel of 98 SSR markers, was carried out in the BC_3S_2 generation. In the BC_3S_4 and BC_3S_6 generations, the selected lines were verified in order to validate both the presence and extent of the target *Hsp* introgression and the pure genetic *Hv* background. The present study as well as IL studies performed in other crop species demonstrates that one or several pre-selection steps in early backcross generations are generally advantageous for an efficient development of introgression libraries. Pre-selection, either based on genotype or phenotype data, helps to avoid the propagation and genotyping of a large number of unusable recombinant plants. In tomato, Eshed and Zamir (1994) combined several steps of phenotypic selection carried out in BC_1 to BC_1S_5 generation and genotypic selection in BC_1S_6 to BC_4S_1 generation. Similar to the selection strategy pursued in the present S42IL study, Eduardo et al. (2005) developed a set of NILs in melon. Based on the portion of donor genome, twenty-five DH lines were initially selected. Subsequently, several selection steps were performed in different backcross generations, and finally altogether 57 NILs were selected predominantly in BC_3S_1 or BC_3S_2 generation. As demonstrated by several studies, most IL sets were finally selected after up to ten generations, including three or four rounds of recurrent backcrossing (e.g. Ali et al. 2005, Szalma et al. 2007).

Based on the SSR map developed by von Korff et al. (2004), the S42IL set covers altogether 86.6 % of the wild barley genome (1,041.5 cM out of 1,202 cM). Within an uniform elite genetic background, each individual line contains a single, marker-defined exotic introgression with an average size of 39.2 cM (range 9.0-134.0 cM). Thus, each S42IL represents on average 3.2 % of the *Hsp* genome (range 0.7-11.1 %). Altogether 87 out of 98 SSR loci (88.8 %) exhibit the donor allele present in at least one S42IL. Regarding the complete introgression library, the donor segment comprises on average 3.4 SSR loci. Chromosome 6H is completely covered by the introgressions of ten different S42ILs, whereas chromosomes 4H and 7H are largely represented (92.1 and 85.9 % genome coverage per chromosome, respectively). In future, it is intended to complete the S42IL library by selecting lines for the so far missing parts of the donor genome in BC_3 or BC_4 generations. For this purpose, a pool of potential introgression lines which to date contain several homozygous or heterozygous introgressions is

available. By recurrent backcrossing and/or selfing, complete homozygous lines carrying only the target introgression will be developed. In addition, all pure S42ILs will be backcrossed again with 'Scarlett' in order to enable the selection of sub-ILs carrying very small introgressions. So far, the S42IL set includes thirteen lines which harbor the donor allele only at a single SSR locus. In contrast, 27 S42ILs contain an *Hsp* segment of more than three linked marker loci.

Regarding diverse characteristics such as exotic genome coverage and average size of the introgressed segment, the S42IL set bears resemblance to different IL sets constructed for other crop species. For instance, a set of 57 NILs generated for melon represented altogether 85 % of the introgressed donor genome, whereas each line on average harbored 3.4 % (41 cM) of the exotic genome (Eduardo et al. 2005). Furthermore, the genomic resolution of 15.7 cM/marker, provided by a map with 62 SSRs, was similar to those in the present S42IL study (12.5 cM). For lettuce, Jeuken and Lindhout (2004) constructed a set of 28 BILs among which 20 lines contained a single homozygous segment of *Lactuca saligna* (wild lettuce) within the genetic background of *L. sativa*. Based on a map with more than 700 DNA markers (AFLPs, ESTs and SSRs), the BILs covered altogether 96 % and indvidually on average 4.0 % (33 cM) of the donor genome. Overall, the IL library of *Solanum pennellii* in *S. esculentum* is to date the furthest developed population. Eshed and Zamir (1994) initially selected a set of 50 ILs which offered whole genome coverage for the tomato wild species *S. pennellii*. Based on the analysis with 350 RFLP markers, all lines have been validated as pure ILs, each harboring a single donor segment of 33 cM on average. Presently, the *S. pennellii* library includes 76 different lines (Lippman et al. 2007). In contrast to the described IL libraries and the S42IL set, a number of IL sets constructed for other crop species such as rye, maize, and rice do not include solely pure lines but contain one or several non-target introgressions (Falke et al. 2008, Szalma et al. 2007, Tian et al. 2006). Nevertheless, a high 'grade of pureness' is generally worthwile for further IL applications since it enables a precise association between the introgressed segment and a phenotypic effect measured in a particular IL. In this respect, the S42IL set constitutes a valuable genetic resource since 55 out of 59 lines (93.2 %) contain a single donor segment.

3.2 Localization of QTLs using wild barley introgression lines

Numerous studies in diverse crop species have demonstrated that ILs are a valuable genetic resource for assessing major but also minor QTL effects (e.g. Li et al. 2005b, Lippman et al. 2007). The outstanding value of ILs for this purpose can be attributed to their fixed genotype and the low portion of exotic germplasm present in each line. The S42IL study described here illustrates the usefulness of the present introgression library for detecting QTLs affecting complex traits in spring barley. By means of field tests in three different environments in Germany, a sub-set of 39 S42ILs was evaluated with regard to disease resistances, agronomic performance and malting quality. The collected phenotype data were used for performing a line x phenotype association study in order to verify QTL effects previously localized in the parent population S42, and to identify new QTLs as well as favorable exotic QTL alleles. The QTL results as well as arising future prospects are discussed in the following chapters.

3.2.1 Verification of QTL effects from population S42

Altogether, 14 different traits were investigated in the parent population S42 as well as in the S42ILs, and, thus, the QTLs affecting these traits are directly comparable between both populations. In population S42, a total of 91 QTLs, among which 38 QTLs (41.8 %) exhibited a favorable exotic effect, were mapped to genomic regions which are already covered by S42ILs (see also von Korff et al. 2005, 2006, 2008), and, thus, could be verified theoretically within the S42IL study. In fact, 45 out of these 91 QTLs (49.5 %) and 16 out of the 38 QTLs (42.1 %) with a favorable exotic effect were confirmed by S42ILs. Most QTL effects were verified for the agronomic parameters days until heading and plant height, and the malting quality trait α-amylase activity (100 %, 85.7 %, and 83.3 %, respectively). In contrast, the fewest number of QTLs was confirmed for grain yield (11.1 %). For lodging at harvest and the malting quality traits friability and viscosity 28.6 % of the QTL effects detected in the S42 population were validated in S42ILs. In addition to the verified QTLs, altogether 29 new QTLs, previously not assessed in population S42, were identified by S42ILs. Here, eight out of 29 QTLs

(27.6 %) revealed a favorable *Hsp* effect. Furthermore, a total of 25 QTLs affecting the newly investigated traits grains per ear, grain sieving fraction > 2.5 mm, and Kolbach index were localized on all seven barley chromosomes.

Using different population types, QTL validation studies have been conducted in several crop species. By means of two tomato NIL sets developed for 15 selected genomic regions, Bernacchi et al. (1998b) confirmed altogether 22 out of 25 (88.0 %) favorable exotic QTL effects which were previously localized in two advanced backcross populations (Bernacchi et al.1998a). Hori et al. (2005) developed a barley RCSL set consisting of 134 lines, where each line contained one or several wild barley introgressions and harbored on average 12.9 % of the exotic genome. By means of a QTL study using a core set of 19 RCSLs and, in addition, 93 DH lines originating from the same cross, a total of 18 and 24 QTLs were assessed, respectively. The coincident or very close localization of several QTLs in both populations has demonstrated the benefit of introgression libraries for verifying QTLs. The QTL mapping power of different population types has been furthermore studied by Keurentjes et al. (2007). By comparing the mapping power of an *Arabidopsis* RIL and NIL population generated for the same parents, they demonstrated that NILs are more advantageous for detecting QTLs with smaller effects whereas unique epistatic effects has been exhibited by RILs. The latter also showed a higher localization resolution. In lettuce, Jeuken et al. (2008) showed that the chances for detecting QTLs for downy mildew resistance were higher in a set of BILs than in the compared F_2 population. Using the BILs, four additional QTLs were identified. Based on a simulation study, two out of these QTLs have been attributed to the skewness of the recessive donor allele in the F_2 population possibly caused by the high genomic divergence between the parental species. Jeuken et al. (2008) argued that, due to its advantageous segregation ratio compared to F_2 or RILs, a BIL set exhibits a higher power to assess especially recessive QTLs.

According to Bernacchi et al. (1998b), QTLs detected in ILs and previous generations can be compared with regard to the stability of the QTL effects across environments and the magnitude of the effects. In the S42IL study, altogether 40 out of all 45 validated QTLs (88.9 %) were significant ($P<0.05$) across all tested environments in both, the S42

population and the S42ILs (see also von Korff et al. 2005, 2006, 2008). Moreover, a total of 39 out of 45 QTLs (86.7 %) revealed an effect with the same direction on trait performance in both populations. For 33 out of these 39 consistent QTL effects (84.6 %), at least one S42IL exhibited a higher trait performance relative to the elite parent 'Scarlett' than measured for the corresponding QTL in population S42. This indicates that effects measured are stronger if they are present in refined ILs.

Inconsistent QTL results across both the populations compared might be attributed to a reduction of epistatic effects between wild barley alleles in the S42ILs. Whereas the 301 BC_2DH lines of the population S42 harbor on average four independent wild barley introgressions, the evaluated 39 S42ILs contain predominantly only one donor segment. Due to this, it can be assumed that favorable as well as unfavorable interaction effects between non-linked exotic QTL alleles are reduced in the S42ILs. In the parent population S42, von Korff Schmising (2005) exemplified the importance of epistatic interactions based on the agronomic traits days until heading, plant height, and grain yield. Altogether 54 markers were involved in significant digenic interactions between non-linked marker loci for these traits. Here, 46.3 % of the markers were not associated with any QTL detected in the single marker analysis, whereas 31.5 % of the markers were also significant in the single marker analysis. Furthermore, von Korff Schmising (2005) assessed that for 88.9 % of all significant digenic interactions, the LSMEANS of the *HspHsp* genotype differed highly significantly from the LSMEANS of the *HvHv* genotype. In order to verify the findings of von Korff (2005) as well as to investigate the importance of epistasis for other traits, crossings between all 39 S42ILs has been initiated. Thus, epistatic interactions between independent introgressions can be tested systematically in future. Similar strategies were applied in tomato, where IL crosses were used for investigating the role of epistasis in the determination of four different fruit quality traits (Eshed and Zamir 1996). Here, epistatic effects, frequently detected for homozygous as well as for heterozygous linked QTLs, were generally less-than-additive. Discrepancies of QTLs identified in population S42 and the S42ILs could be furthermore attributed to environmental effects since both populations were evaluated in different environments. In order to further increase the reliability of field data, it is intended to perform field tests with the 39 S42ILs in additional environments. Moreover, the 20

residual lines of the developed S42IL set (see chapter 2.1) plus new lines, which will be selected in future for the so far missing parts of the wild barley genome, will be subjected to phenotypic analysis. Accordingly, a genome-wide QTL validation of all effects measured in population S42 will become feasible.

Testing ILs in numerous replications as well as environments is in general required to obtain reliable QTL data. Since the lines of one introgression library differ only in one chromosomal segment, the main part of their genome is identical. Thus, the detection of an exotic effect in a particular marker interval, which is only present in a single IL necessitates a high number of replicated measurements. Keurentjes et al. (2007) suggested to test NILs in at least five replications in order to assure a high QTL mapping power. In commonly used QTL mapping populations such as advanced backcross lines or RILs, the different genotype classes at one specific marker locus are represented in contrast by many different lines. Here, especially the number of different lines tested, i.e. the population size, has a considerable impact on the power of QTL detection (Vales et al. 2005, Jeuken et al. 2008). Due to their fixed genotype, ILs as well as RILs and DH populations can be easily tested in diverse environments for producing reliable QTL data (Mackay 2001). Testing ILs and other QTL mapping populations in multiple environments enables to investigate environmental influences on QTLs and, accordingly, to assess QTL effects which are stable across environments (Li et al. 2003b, Wan et al. 2005, Maccaferri et al. 2008). These stable effects are especially valuable for developing improved cultivars since new cultivars should in general exhibit high trait performance in a broad spectrum of environments. In contrast, those effects which are only significant in a particular environment are of interest for adaptive breeding.

3.2.2 Comparison of QTL effects across overlapping introgression lines

Within the S42IL set, numerous chromosomal regions are represented by diverse overlapping lines which contain at least one common SSR locus. Especially if some lines carry relatively large exotic segements, testing different sister lines with overlapping *Hsp* fragments enables a more precise localization of QTLs than by investigating only one line

per region. For instance, the region 4H, 125-132 cM is represented by both S42IL-122 and -123 where the latter line possesses an introgression further extended to 4H, 170 cM. Since both S42ILs revealed a QTL with a favorable *Hsp* effect on leaf rust resistance, one can assume that the QTL maps to the region common to both lines (see chapter 2.1). Similar assumptions can be made for S42ILs -125 and -126 which overlap only at marker locus 5H, 69 and both display QTLs for α-amylase activity, Kolbach index, and Hartong 45°C in parallel (see chapter 2.3). These results implicate that one or several genes affecting these parameters are located within the overlapping region.

Contrary to the examples described above, some overlapping S42ILs exhibit inconsistent QTL results. For instance, although the introgression of S42IL-107 covers a region (2H, 17-42 cM) which is completely represented by S42IL-108, only the first line exhibited a significant exotic effect on powdery mildew resistance. As exemplified in chapter 2.1, different explanations can be given for these discrepancies: (1) Due to the limited number of 98 SSR markers analysed, the true extent of an exotic fragment might slightly differ from its estimated size. Accordingly, the introgression of S42IL-107 might be further extended to the top of chromosome 2H, whereas S42IL-108 possesses the assumed segment size. Thus, *Hsp* alleles present in the extended introgression might cause a QTL effect which is missing in the apparent sister line. (2) As an alternative explanation, wild barley alleles simultaneously present in the longer target introgression of S42IL-108 might show negative epistatic interactions which impede QTL detection. Due to its shorter introgression, these interactions might be missing in S42IL-107. (3) As a third explanation, S42IL-107 possibly harbors one or several to date hidden non-target introgressions which contain an *Hsp* allele causing a significant QTL effect, maybe interacting with other exotic alleles present in the target introgression. The first two possible explanations correspond to assumptions made by Bernacchi et al. (1998b) who compared the QTL results of different independent tomato NILs for the same targeted region. Nevertheless, in order to verify the three hypotheses, it is required to characterize the S42ILs more precisely with new DNA markers. Doing that, the precise extent of the target introgressions as well as possible additional non-target segments might become visible. The S42ILs will therefore be genotyped again using the Illumina 1.5 k barley SNP chip (Rostoks et al. 2006) or DArT markers (Wenzl et al. 2006).

3.2.3 Identification of QTL clusters

For all three analysed trait complexes, S42ILs exhibiting QTL effects on several traits simultaneously have been identified. Lines exhibiting QTL clusters are for instance S42IL-107 and -109, both harboring an exotic introgression on chromosome 2H and revealing a significantly enhanced trait performance for five and four different traits compared to 'Scarlett', respectively. Both S42ILs revealed an improved performance for the parameters ears per m^2, days until heading and plant height. In addition, the traits thousand grain weight and grain sieving fraction >2.5 mm were significantly increased in S42IL-107, whereas lodging at harvest was significantly reduced in S42IL-109. Four and three of the QTL effects were stable across all tested environments, respectively (see chapters 2.2 and 2.3). For both S42ILs, all QTLs exhibited strong phenotypic effects and all, except the newly identified QTL QGsf.S42IL-2H.a, were confirmed by QTL effects identified in population S42 (von Korff et al. 2006). Thus, these and similar S42ILs represent valuable starting material for breeding improved cultivars. Due to the small portion of exotic germplasm present in each S42IL, they allow a fast and efficient transfer of favorable exotic alleles into elite cultivars using crossing and MAS. To begin with, it is required to assure the absence of unfavorable exotic effects on other traits which might be possibly present in the original S42IL. To date, the applicability of ILs in plant breeding has been demonstrated in tomato, where *S. pennellii* ILs were used for developing the tomato hybrid 'AB2', the current leading variety in California (Lippman et al. 2007). In rice, three comprehensive sets of ILs have been constructed in the context of a large backcross breeding program initiated at the International Rice Research Institute (IRRI). The program focused on the development of new cultivars with high tolerance to abiotic and biotic stresses (Ali et al. 2005, Lafitte et al. 2005, Li et al. 2005b).

In general, S42ILs with multiple QTL effects could be subjected to further analysis in order to unravel the genetic nature of QTL clusters. As described in chapter 2.3, two malting quality QTL clusters which have been mapped to chromosome 1H and 4H are of special interest regarding this approach. These clusters include QTLs affecting eight and six malting quality parameters in parallel, respectively, and revealed strong phenotypic effects. Furthermore, all QTLs except one were also detected in parent population S42

(von Korff et al. 2008), and exhibit coincidence with several malting quality QTLs detected in other QTL studies (e.g. Thomas 2003, Li et al. 2005a). The corresponding S42ILs could provide a basis for dissecting these QTL clusters in order to elucidate if they are caused either by a few genes with pleiotropic effects or numerous closely linked genes affecting different traits. To do so, it is intended to carry out a fine-mapping approach in the future. As a first step towards this, the development of high-resolution mapping populations for the interesting QTL regions is currently in progress. This is accomplished by one further round of backcrossing of all S42ILs and two subsequent rounds of selfing. The regions of interest will be enriched with new molecular markers in order to facilitate the selection of informative recombinants.

The availability of new marker data will also enable the selection of so called sub-ILs harboring preferably small exotic introgressions. They could be applied in order to localize QTLs more precisely than with the original S42ILs. A limitation of sub-ILs is that the smaller the exotic introgressions are the more lines have to be tested for a genome-wide QTL screening. Thus, as recommended by Keurentjes et al. (2007), it would be advisable to initially select a core set of relatively few lines which offer complete genome coverage. Within a set of 92 *Arabidopsis* NILs, Keurentjes et al. (2007) identified a core set of 25 lines representing >90% of the donor genome. These NILs could be used for a first round of QTL analysis, whereas additional sub-NILs would be valuable for a subsequent fine-mapping of previously identified QTLs. In the present S42IL study, a set of 25 unique lines was identified. This set represents the minimum number of S42ILs required to achieve the *Hsp* genome coverage of the complete S42IL library (86.6%). Sixteen out of these lines have been already evaluated phenotypically and used for QTL detection. For the remaining 11 S42ILs a sufficient number of seeds for field tests was so far not available. Therefore where possible, lines containing smaller introgressions in the same chromosomal regions have been tested instead. In future, all unique S42ILs will be applied to QTL analysis in order to enable a near genome-wide detection of QTL effects. Based on the corresponding S42ILs, high-resolution mapping populations are currently under development (see above). They will provide the basis for dissecting QTL regions of special interest, such as the described QTL clusters on chromosomes 1H and 4H.

To date, several fine-mapping studies using ILs have been conducted in different crop species such as rice (Tian et al. 2006, Xie et al. 2008), wheat (Röder et al. 2008), and *Arabidopsis* (Törjék et al. 2008). In tomato, QTL fine-mapping with ILs resulted in the cloning of the fruit color gene *Beta*, the fruit weight QTL *fw2.2*, and the sugar yield QTL *Brix9-2-5* (Ronen et al. 2000, Frary et al. 2000, Fridman et al. 2004, respectively). Accordingly, S42ILs corresponding to interesting QTL regions might be applied in future to different map-based QTL cloning projects. Here, the criteria for selecting promising QTLs will be that the QTL exhibited a strong phenotypic effect, and that it is verified and, in addition, stable across all tested environments.

One strategy to identify the genes underlying QTLs is to propose candidate genes of known function that might, either based on their physiological function or genetic map position, correspond to the QTLs (Pflieger et al. 2001). With the aim to clone interesting QTLs, this candidate gene approach could also be applied in the present S42IL study. For all three trait complexes, different candidate genes have been identified. For instance, the major QTL detected for powdery mildew resistance which has been identified in S42IL -101 and -102 (QPm.S42IL-1H.a) might be attributed to the *Mla* gene which is present in the same chromosomal region (Zhou et al. 2001, see chapter 2.1). As described in chapter 2.2, the map locations of several genes controlling flowering time such as *Ppd-H1* (Turner et al. 2005), *HvCO1* (Griffiths et al. 2003), *Vrn-H2*, and *Vrn-H3* (Yan et al. 2004, 2006) coincide with QTLs for days until heading and plant height on chromosomes 2H, 4H and 7H, respectively. Due to a consistent map position on the top of chromosome 1H, it might be assumed that QTLs affecting diverse malting quality parameters such as fine-grind extract, grain protein content and Kolbach index are influenced by several *Hor* genes coding for different forms of hordein, the main storage protein in barley (Rostoks et al. 2005). In order to validate the assumption that they actually account for the measured QTL effects, it is required to fine-map or sequence these and other identified candidate genes. So far, the barley NAC transcription factor *HvNAM-1* (Distelfeld et al. 2008) which is a candidate gene for the major grain protein content QTL QGpc.S42IL-6H.a was further analysed (see chapter 2.3). The exotic *HvNAM-1* allele is present in S42IL-128 and -129 (see chapter 2.3), confirming its assumed co-localization with

QGpc.S42IL-6H.a. Future transformation experiments will be performed to prove the hypothesis that *HvNAM-1* accounts for this QTL.

3.3 Future prospects

In addition to the prospective applications described before, the S42ILs will be evaluated extensively on the transcriptome, proteome, and metabolome level. Furthermore, new phenotype studies will be conducted in order to evaluate the S42IL set regarding a broader spectrum of complex traits. Here, in conjunction with current global problems such as climate change and lack of food in the developing countries, exploring the genetic basis and physiological pathways of abiotic stress tolerance will be of special interest. Projects focusing on tolerance to drought stress, nutrient deficiency and contamination of soil with heavy metals are currently being carried out using the S42ILs. Finally, all phenotype and genotype data of the S42ILs will be archived in the IL database 'Phenom Networks' (http://phn.huji.ac.il/RTQ/). This database has been initiated at the Hebrew University of Jerusalem in order to enable general access to data collected for ILs. To date, it provides information for IL libraries constructed in tomato, *Arabidopsis*, melon, rose, barley, and mouse, and, thus, facilitates the comprehensive use of these genetic resources by different research groups and for different purposes.

The present thesis has demonstrated that the developed S42ILs are a powerful and versatile genetic tool for identifying QTLs and favorable wild barley alleles for complex traits. Future challenges are the further application of this tool in both plant genome research and plant breeding. Map-based cloning using the S42ILs will be a promising approach to zoom in on the genetic basis of the localized QTLs. After all, the knowledge acquired can be applied for breeding improved cultivars by transfering favorable *Hsp* alleles into the elite gene pool of modern barley cultivars.

4 Summary

The objectives of the present study were the construction and phenotypic evaluation of a set of wild barley introgression lines. Originating from 40 BC_2DH lines of the spring barley population S42 (von Korff et al. 2004), a set of 59 introgression lines, referred to as S42ILs, has been developed by three rounds of recurrent backcrossing, two to four subsequent selfings, and, in parallel, marker-assisted selection (Schmalenbach et al. 2008). Each S42IL harbors a single marker-defined chromosomal segment of the wild barley accession 'ISR 42-8' (*Hordeum vulgare* ssp. *spontaneum*) within the uniform genetic background of the spring barley cultivar 'Scarlett' (*H. vulgare* ssp. *vulgare*). Based on a genetic map including 98 SSR markers, the S42IL set covers in total 86.8 % (1,041.5 cM) of the wild barley genome through overlapping introgressions. Each single line contains an average exotic segment of 39.2 cM, representing 3.2 % of the exotic genome. In addition, 45 potential ILs which, so far, include multiple exotic introgressions are under selection. In future, these lines will be used for selecting pure introgression lines covering the so far missing regions of the donor genome. To date, wild barley chromosome 6H is completely covered and chromosomes 4H and 7H are largely covered by S42ILs. A core set of 25 unique S42ILs represents the minimum number of lines required to achieve the maximum exotic genome coverage of the complete set.

In order to verify QTL effects previously detected in parent population S42 (von Korff et al. 2005, 2006, 2008) and, in addition, to identify new QTLs as well as favorable wild barley alleles, altogether 39 S42ILs were phenotyped in field tests during the 2007 growing season. Phenotype data for seven agronomic traits and two disease resistance traits were collected in up to three different environments in Germany. For evaluating the S42ILs regarding eight malting quality parameters, a micro malting experiment was additionally conducted. Subsequently, all genotype and phenotype data of the 39 S42ILs were used to perform a line x phenotype association study which comprised a two-factorial mixed model analysis of variance and a subsequent Dunnett test with the recurrent parent 'Scarlett' as control. The presence of a QTL effect on a wild barley introgression was accepted if the trait value of a particular S42IL was significantly ($P<0.05$) different from 'Scarlett', either across all tested environments and/or in a

particular environment. The S42ILs exhibited a total of 148 significant line x phenotype associations for all investigated traits. Taking into account that several lines contain overlapping or flanking introgressions, the associations were summarized to a total of 98 QTLs, where at 31.6 % of the QTLs the exotic introgression was associated with an improved trait performance. At 77 QTLs both the line main effect and the line x environment interaction effect were significant, whereas at six QTLs solely the line main effect and at 15 QTLs solely the interaction effect was significant. For resistance to powdery mildew and leaf rust as well as for all agronomic traits, except grains per ear, wild barley introgressions were identified which are associated with an improved trait performance compared to 'Scarlett' (Schmalenbach et al. 2008, 2009). In addition, favorable exotic QTL effects were assessed for the malting quality parameters α-amylase activity, grain sieving fraction > 2.5 mm and Kolbach index (Schmalenbach and Pillen 2009).

Altogether, 14 different traits were analysed in the parent population S42 as well as in the S42ILs, and, hence, QTLs affecting these traits are comparable between both populations. For all corresponding traits, altogether 49.5 % of all QTLs and 42.1 % of the QTLs with a favorable exotic effect localized in population S42 were verified by S42ILs. A total of 29 new QTLs, which were previously not identified in the S42 population, were detected in the S42IL study. Furthermore, altogether 25 QTLs affecting the newly analysed traits grains per ear, grain sieving fraction > 2.5 mm, and Kolbach index were mapped. Inconsistency of QTL effects across both populations might be attributed to a reduction of epistatic effects between wild barley alleles in the S42ILs since the S42ILs harbored predominantly one single donor segment whereas each line of the parent population S42 contained several introgressions simultaneously. Alternatively, inconsistent QTL results might be explained by environmental effects as both populations were tested in different environments. The comparison of QTL effects across overlapping S42ILs exhibited consistent as well as contrasting results. Some lines harboring overlapping wild barley introgressions revealed the same QTL effects. This may indicate that one or several genes controlling the trait are located within the overlapping region. In contrast, inconsistent QTL effects in overlapping S42ILs might be attributed to genes present in the non-overlapping regions. Characterizing the S42ILs more precisely by analyzing new DNA

markers in future, e.g. Illumina SNPs (Rostoks et al. 2006), will shed light on the hypothesis that some introgression lines might contain so far undetected additional non-target introgressions. In addition, epistatic interactions between wild barley alleles present in the same or different introgressions might be responsible for inconsistent QTL results across overlapping S42ILs.

Within the present study, several QTL clusters have been localized on different chromosomes. Major QTL clusters for diverse agronomic traits and malting quality parameters have been assessed on chromosomes 2H and 1H, respectively. The QTL effects detected here were predominantly stable across all environments and have been validated by QTLs mapped in parent population S42. Furthermore, S42ILs with multiple QTL effects could be subjected to further studies in order to elucidate the genetic nature of QTL clusters. To do that, fine-mapping of the interesting QTL regions followed by map-based cloning of the underlying genes is intended in future. Analyzing so called sub-ILs containing reduced introgressions and the enrichment of the introgressions with additional molecular markers will enable the further dissection of the QTL regions. Applying a candidate gene approach would also be useful for map-based QTL cloning. Within the present S42IL study, different candidate genes possibly underlying the QTLs detected have been identified for different traits. Future fine-mapping and transformation experiments will test the hypothesis that these genes actually account for the corresponding QTLs.

The present study has demonstrated the usefulness of introgression lines for localizing QTLs for complex traits in spring barley. Due to the low portion of the wild barley genome present in each line combined with complete homozygosity, the S42ILs are a stable genetic resource which will be used with versatility in future. A more precise characterization of the S42ILs on the levels of genotype, phenotype, transcriptome, proteome, and metabolome is intended. Exploring the genetic basis and physiological pathways of abiotic stress tolerance will be especially focused on. Finally, S42ILs exhibiting favorable exotic alleles are considered as a valuable resource for breeding barley cultivars with enhanced trait performance.

5 Zusammenfassung

Die Ziele der vorliegenden Arbeit waren die Erstellung und phänotypische Bewertung eines Sets von Wildgerste-Introgressionslinien. Ausgehend von 40 BC_2DH-Linien der Sommergerstenpopulation S42 (von Korff et al. 2004) wurde ein Set von 59 Introgressionslinien, nachfolgend als S42ILs bezeichnet, mittels rekurrenter Rückkreuzung, mehrfacher Selbstung und marker-gestützter Selektion entwickelt (Schmalenbach et al. 2008). Jede S42IL trägt ein einzelnes marker-definiertes Chromosomensegment der Wildgerstenakzession ‚ISR42-8' (*Hordeum vulgare* ssp. *spontaneum*) vor dem einheitlichen genetischen Hintergrund der Sommergerstensorte ‚Scarlett' (*H. vulgare* ssp. *vulgare*). Basierend auf einer genetischen Karte mit 98 SSR-Markern deckt das S42IL-Set insgesamt 86,8 % (1.041,5 cM) des Wildgerstengenoms in Form von sich überlappenden Introgressionen ab. Jede einzelne Linie beinhaltet eine Introgression von durchschnittlich 39,2 cM, die 3,2 % des Wildeltergenoms repräsentiert. Zurzeit sind das Wildgerste-Chromosom 6H komplett, sowie die Chromosomen 4H und 7H größtenteils durch S42ILs abgedeckt. Aus einem Pool von 45 potentiellen Introgressionslinien, die bisher mehrere Chromosomensegmente des Wildelters tragen, werden in Zukunft Linien für die noch fehlenden Genombereiche selektiert.

Mit dem Ziel QTL-Effekte, die vorher in der Eltern-Population S42 detektiert wurden (von Korff et al. 2005, 2006, 2008), zu verifizieren sowie zusätzlich neue QTLs und vorteilhafte Wildgerste-Allele zu identifizieren, wurden insgesamt 39 S42ILs in einem einjährigen Feldversuch getestet. Phänotypdaten für sieben agronomische Parameter und zwei Resistenzmerkmale wurden in bis zu drei verschiedenen Umwelten erhoben (Schmalenbach et al. 2008, 2009). Zusätzlich wurden die S42ILs auf Basis von acht verschiedenen Merkmalen hinsichtlich ihrer Malzqualität bewertet (Schmalenbach und Pillen 2009). Alle Geno- und Phänotypdaten der 39 getesteten Linien wurden anschließend im Rahmen einer Linie x Phänotyp-Assoziationsstudie verrechnet. Diese umfasste eine zweifaktorielle Varianzanalyse und einen nachfolgenden Dunnett-Test mit dem rekurrenten Elter ‚Scarlett' als Kontrolle. Ein QTL-Effekt einer Wildelter-Introgression wurde angenommen, falls sich der Merkmalswert der jeweiligen S42IL signifikant ($P<0,05$) von ‚Scarlett' unterschied, entweder über alle getesteten Umwelten

und/oder in einer einzelnen Umwelt. Für alle untersuchten Merkmale wurden insgesamt 148 signifikante Linie x Phänotyp-Assoziationen detektiert. Da mehrere Linien überlappende oder flankierende Introgressionen enthalten, wurden die Assoziationen zu insgesamt 98 QTLs zusammengefasst. Hierbei war die Wildelter-Introgression bei 31,6 % aller lokalisierten QTLs mit einer verbesserten Merkmalsleistung verbunden. Bei 77 QTLs war sowohl der Linien-Haupteffekt als auch der Linie x Umwelt-Interaktionseffekt signifikant, wohingegen bei sechs QTLs nur der Linien-Haupteffekt und bei 15 QTLs nur der Interaktionseffekt signifikant war. Für die Merkmale Mehltau- und Zwergrostresistenz, für alle agronomischen Parameter, außer Kornzahl pro Ähre, sowie für die Malzqualiätsmerkmale α-Amylase-Aktivität, Korngrößenfraktion >2,5 mm und Kolbachindex wurden Wildgerste-Introgressionen identifiziert, die, verglichen mit der Kontrolle, mit einer verbesserten Merkmalsleistung assoziiert waren.

Für 14, in den Populationen S42 und S42IL übereinstimmend untersuchte Merkmale wurden insgesamt 49,5 % aller QTLs der Population S42 und 42,1% der QTLs mit einem vorteilhaften Wildelter-Effekt durch S42ILs verifiziert. Zusätzlich wurden 29 neue QTL-Effekte, die in der Elternpopulation nicht detektiert wurden, in der vorliegenden S42IL-Studie identifiziert. Für die neu untersuchten Merkmale Kornzahl pro Ähre, Korngrößenfraktion >2,5mm und Kolbachindex wurden insgesamt 25 QTLs lokalisiert. Widersprüchliche QTL-Effekte zwischen beiden untersuchten Populationen können möglicherweise auf eine Reduktion von epistatischen Effekten zwischen Wildgerste-Allelen in den S42ILs zurückgeführt werden. Denn während die S42ILs überwiegend nur ein Donorsegment tragen, enthält nahezu jede Linie der S42-Population mehrere Introgressionen gleichzeitig. Da beide Populationen in unterschiedlichen Umwelten getestet wurden, könnten widersprüchliche Effekte auch durch Umwelt-Effekte erklärt werden. Beim Vergleich von QTL-Effekten in überlappenden S42ILs wurden sowohl übereinstimmende, als auch widersprüchliche Ergebnisse festgestellt. Die Detektion von gleichen QTLs in sich überlappenden Linien weist darauf hin, dass möglicherweise ein oder mehrere merkmalsbestimmende Gene in der überlappenden Region lokalisiert sind. Im Gegensatz hierzu können widersprüchliche QTL-Effekte eventuell auf Gene, die in den nicht-überlappenden Wildelter-Regionen lokalisiert sind, zurückgeführt werden. Eine genauere genetische Charakterisierung der S42ILs, basierend auf der Analyse neuer

DNA-Marker, z.B. Illumina SNPs (Rostoks et al. 2006), wird außerdem zeigen, ob in manchen Linien eine oder mehrere bisher unentdeckte nicht-Ziel-Introgressionen vorhanden sind. Als eine weitere Ursache für inkonsistente QTL-Effekte in sich überlappenden S42ILs können epistatische Interaktionen zwischen Donor-Allelen in einer Introgression oder verschiedenen unabhängigen Segmenten vermutet werden.

In der vorliegenden Studie wurden mehrere QTL-Cluster auf verschiedenen Chromosomen lokalisiert. Die Haupt-QTL-Cluster für agronomische Merkmale und Malzqualitätsparameter wurden auf den Chromosomen 2H bzw. 1H identifiziert. Die hier detektierten QTLs waren überwiegend stabil über alle Umwelten und wurden durch QTLs der Elternpopulation S42 bestätigt. Mit dem Ziel die genetische Beschaffenheit von QTL-Clustern aufzuklären, sollen in Zukunft S42ILs mit multiplen QTL-Effekten dazu genutzt werden, eine Feinkartierung der QTL-Regionen sowie eine anschließende kartenbasierte Klonierung der zugrunde liegenden Gene durchzuführen. Eine Analyse sogenannter Sub-ILs, die kleinere Wildelter-Segmente tragen, und eine Anreicherung der Introgressionen mit zusätzlichen molekularen Markern wird eine weitere Unterteilung der QTL-Regionen ermöglichen. Die anschließende kartenbasierte QTL-Klonierung könnte nach einem Kandidatengen-Ansatz erfolgen. So wurden in der hier beschriebenen Studie für mehrere Merkmale Kandidatengene identifiziert. Eine Feinkartierung sowie Transformationsexperimente sollen in zukünftigen Studien Aufschluss darüber geben, ob diese Gene tatsächlich den jeweiligen QTLs zugrunde liegen.

Die vorliegende Studie hat den Nutzen von Introgressionslinien für die QTL-Detektion für komplexe Merkmale in Sommergerste aufgezeigt. Da jede Introgressionslinie nur einen geringen Anteil des Wildgerste-Genoms beinhaltet und alle Linien vollständig homozygot sind, stellen die S42ILs eine stabile genetische Ressource dar, die in Zukunft auf vielfältige Art und Weise genutzt werden soll. Eine präzisere Charakterisierung der Linien auf den Ebenen von Genotyp, Phänotyp, Transkriptom, Proteom und Metabolom ist beabsichtigt. Die Erforschung der genetischen Grundlage, sowie der physiologischen Mechanismen abiotischer Stresstoleranz wird hierbei im Mittelpunkt stehen. Schließlich stellen solche S42ILs, die vorteilhafte Wildgerste-Allele tragen, eine wertvolle Ressource für die Züchtung von Gerstensorten mit verbesserter Merkmalsleistung dar.

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7 Abbreviations

Abbreviation	Explanation
AB-QTL analysis	Advanced backcross quantitative trait locus analysis
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BAC	Bacterial artificial chromosome
BC_i	i-th backcross
BIL	Backcross inbred line
CAPS	Cleaved amplified polymorphic sequence
cDNA	Complementary DNA
cM	Centi Morgan
DArT	Diversity arrays technology
DH	Doubled haploid
DNA	Desoxyribonucleic acid
EBC	European Brewery Convention
EMS	Ethyl methanesulfonate
EST	Expressed sequence tag
F_i	i-th filial generation
GLM	General linear model
Hsp	*Hordeum vulgare* ssp. *spontaneum*
Hv	*Hordeum vulgare* ssp.*vulgare*
IL	Introgression line
InDel	Insertion/deletion
kb	Kilo-base
LSMEANS	Least squares means
MAS	Marker-assisted selection
Mb	Mega-base
MEBAK	Mitteleuropäische Brautechnische Analysenkommission
NCBI	National Center for Biotechnology Information
NIL	Near isogenic line
PCR	Polymerase chain reaction
Pre-IL	Candidate introgression line
QTL	Quantitative trait locus
RCSL	Recombinant chromosome substitution line
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
SCRI	Scottish Crop Research Institute
S_i	i-th selfing generation
SNP	Single nucleotid polymorphism
SSR	Simple sequence repeat
T-DNA	Transfer DNA
TILLING	Targeting Induced Local Lesions in Genomes
YAC	Yeast artificial chromosome

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E R K L Ä R U N G

Hiermit versichere ich an Eides Statt, dass ich die eingereichte Dissertation „Selection and Phenotypic Evaluation of a Wild Barley Introgression Library“ selbstständig angefertigt und diese nicht bereits für eine Promotion oder andere Zwecke an einer anderen Universität eingereicht habe. Weiterhin versichere ich, dass ich die zur Erstellung der Dissertationsschrift verwendeten Arbeiten und Hilfsmittel genau und vollständig angegeben habe.

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