Juvenile drought stress tolerance and QTL detection in

wild barley introgression lines

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1 GENERAL INTRODUCTION

Barley (*Hordeum vulgare* ssp. *vulgare* L., hereafter abbreviated with *Hv*) is one of the most important cereal crops worldwide. As one of the first domesticated species in the "Fertile Crescent" it has been playing an important role for at least 10,000 years; from the beginning of the transition of hunting and gathering to agrarian societies until the present day. While in ancient times barley was most likely mainly used as human food, nowadays its main use is as animal feed and as malt grain.

Barley is grown in a wide range of environments from temperate to semi-arid climates and regarded as the most widely adapted cereal species. It is grown further north, at higher altitudes, and in dryer environments than other cereal grain species (Ullrich 2011).

Apart from its significance in agricultural production, barley is an important model organism in plant science. Its diploid nature, the broad genetic diversity, and the good availability of mutant collections make it a suitable species for applied and basic research questions (Heneen 2011).

In 2012 the international barley sequencing consortium published a first draft sequence of the barley genome, the so called "barley gene space". The physical map comprises 4.98 of the 5.1 gigabases (Gb) of the barley genome and serves as a valuable resource for research and crop improvement (Mayer et al. 2012) towards higher productivity under biotic and abiotic stress factors.

Drought is one of the most severe stress factors worldwide limiting crop yields in many parts of the world. In order to select drought tolerant genotypes, access to exotic germplasm and efficient phenotyping protocols are needed. Already Vavilov (1940) mentioned the positive characteristics of wild relatives of crop species as donors of exotic germplasm to enhance crop varieties. Zamir (2001) argued that systematically developed introgression line libraries could enhance the improvement of crops through wild donor alleles. Introgressions lines (ILs) contain small portions of a wild species donor genome in the genetic background of an elite cultivar. ILs have been developed for many different crop species. They proved to be beneficial for variety development as Hajjar and Hodgkin (2007) summarized in their review. Besides the exploration of genetic diversity phenotyping is a second bottleneck in crop improvement. While high-

throughput genotyping became standard for many crop species, high-throughput phenotyping techniques are still less widely established. Non-invasive, high-throughput plant phenotyping could strongly improve plant breeding and plant physiology research by combining large amounts of genetic data with appropriate phenotypic information (Furbank 2009).

1.1 BARLEY – PRODUCTION, USES, ECONOMIC IMPORTANCE

With a worldwide production of 145 million tons from a 50 million ha growing area in 2013 barley is one of the most important crop species and ranks fourth among the worldwide production of cereals. The European Union produced 60 million tons of barley in 2013. That equals 41.3% of the world production. In Germany barley is the second most produced cereal and with 10.3 million tons Germany is the second biggest producer of barley worldwide (7.1%). Even though barley production has decreased since the 1990s by almost 20% (FAO 2014) barley still is an important crop. Barley gained its importance due to its multipurpose use as animal feed, human food and substrate for malting, which is used for brewing and distilling (Druka et al. 2011). While in ancient times barley was probably mainly used as human food, nowadays its main use is as animal feed and as malt grain. Approximately 55-60% of barley is used as animal feed. Barley is a common feed crop in temperate environments. It is especially popular in regions where the climate is too cold, too dry, or where the growing season is too short for maize production (Ullrich 2011). Traditionally barley has been used in diets of ruminant and non-ruminant livestock and poultry (Blake et al. 2011). Barley is used as grain crop as well as forage. The fibrous hull of barley presents a disadvantage compared to wheat because it is not well digestible by poultry and swine. However, hulless barley varieties that loose the hull during threshing are superior to hulled barley and can serve as a high quality alternative to wheat and maize (Ullrich 2011). As forage barley for grazing and silage production hooded varieties are of advantage. In hooded varieties the *Kap* gene on chromosome 4H deforms the lemma awn into an extra flower (Muller et al. 1995). This extra flower is much softer than the awn and thus, prevents jaw infections (Blake et al. 2011).

The second largest share of barley, between 30 and 40%, is used as substrate for malting. The major part of malt is used for brewing. Smaller amounts are used for distilling and in the food industry. While in principle malt can be produced from every cereal, the vast majority of malt is produced from barley (Schwarz and Li 2011). Barley has been used in beer brewing for several thousand years and thus, barley has been under selection for favorable malt and brew character-

istics for a very long time. Nowadays, knowledge and technology of malting and brewing are highly developed and a major goal in barley breeding is the continuous improvement of quality traits, like good enzyme activity and low β-glucan content.

Today, food barley plays a very minor role on a global scale. Only around 2% is used directly for human consumption (Baik and Ullrich 2008). However, in some cultures barley retained its traditional role as an important food source. Especially in regions with extreme climates barley continues to be a major principal food source, e.g. in the Himalaya, western and eastern Asia and northern and eastern Africa (Ullrich 2011).

In recent years barley experienced a small renaissance as a "health food" in the developed world. The Food and Drug Administration (FDA) of the United States of America issued an endorsement that allows health claims for barley products (Ullrich 2011). The health benefits of barley are based on its soluble fiber content, the β-glucans. Compared to malting barley food barley should have high β-glucan content in order to provide health benefits. Soluble fiber can lower blood cholesterol levels and thus, reduce the risk of coronary heart diseases (Behall et al. 2004a, b). Barley may also lower the glycemic index (Cavallero et al. 2002). Barley can be used in different forms, for example pearled in soups and stews, flaked or ground in breakfast cereals or as flour in bakery flour blends (Baik et al. 2011).

1.2 BARLEY TAXONOMY AND DOMESTICATION

Barley belongs to the tribe Triticeae, which is part of the grass family Poaceae and comprises some of the most important crop species in the world. Wheat (*Triticum* sp.), rye (*Secale cereale*), triticale, and barley (*Hordeum vulgare*) and several forage grass species are amongst them. Within the Triticeae barley belongs to the genus *Hordeum*. According to the gene pool concept which is based on the success rate of crosses between species *Hordeum* has been divided into three gene pools. The primary gene pool consists of cultivated barley (*H. vulgare* ssp. *vulgare*) and its wild progenitor (*H. vulgare* ssp. *spontaneum*, hereafter abbreviated with *Hsp*). No crossing barriers exist between the two subspecies. *H. bulbosum* is the single species belonging to the secondary gene pool. Crosses with barley are possible but show a reduction in crossability and hybrid fertility. All other *Hordeum* species belong to the tertiary gene pool and crosses to cultivated barley are extremely difficult to achieve (von Bothmer et al. 2003). The tribe comprises 31 species. As in all Triticeae species the basic chromosome number of the genus *Hordeum* is x=7 and diploids (2n=2x=14) and polyploids (2n=4x=28 and 2n=6x=42) exist. *Hordeum* species show a high

variation in life forms and populate a wide range of ecological niches. The majority of the species are short or long-lived perennials, but annual species have developed independently in different parts of the world. Most of the *Hordeum* species have the capacity for self- as well as cross-pollination. Some species are more or less obligate inbreeders with cleistogamous flowers (for instance *H. murinum*, *H. intercedes*). Also cultivated barley (*H. vulgare* ssp. *vulgare*) is almost exclusively inbreeding, while the wild ancestor (*H. vulgare* ssp. *spontaneum*) has partly open flowers and a higher rate of cross-pollination. Furthermore, *Hordeum* species exist that are complete outbreeders and some species even developed self-incompatibility mechanisms (e.g. *H. bulbosum*) (von Bothmer and Komatsuda 2011). *Hordeum* occurs widespread in temperate areas, except in Australia. The genus exists in a wide range of environments from sea level up to elevations of 4000-5000 m in the Andean (*H. muticum*, *H. comosum*) and Himalayan mountains (*H. brevisubulatum* ssp. *nevskianum* and ssp. *turkestanicum*) (von Bothmer et al. 2003). There are species adapted to dry or wet pastures habitats, dry steppes, sandy beaches and even saline environments, e.g. the salt tolerant *H. bogdanii* and *H. depressum* and the halophyte *H. marinum*.

A characteristic trait common to all *Hordeum* species is the triplet, consisting of three one-flowered spikelets at each rachis node. The spikelets are sessile in cultivated and wild barley (*H. vulgare* ssp. *spontaneum*) and stalked in all other *Hordeum* species. The lateral spikelets can be fertile as in six-rowed or sterile as in two-rowed barley (von Bothmer and Komatsuda 2011).

Wild barley (*Hsp*) is the progenitor of cultivated barley (*Hv*) and still abundantly present in nature. The natural distribution of wild barley reaches from the eastern Mediterranean area to Afghanistan and has its center of distribution in the Middle East (von Bothmer et al. 2003). Archeological research has found evidence that barley was amongst the early domesticated plants in the “Fertile Crescent”, where the transition from hunter and gatherer to sedentary farmer first took place. Signs of initial domestication in barley date back until 8000 BC (Badr et al. 2000). Characteristic traits of domesticated plants are for example, larger grains or fruits, lack of natural seed dispersal, the loss of seed dormancy and changes in vernalization requirement and photoperiod sensitivity (Doebley et al. 2006).

Wild and cultivated barley belong to the same species. No crossing barriers have been developed between them and spontaneous and artificial crosses are easily obtained (Asfaw and von Bothmer 1990). The most important traits that distinguish domesticated from wild barely are the non-brittle rachis, higher seed weight, and the existence of six-rowed and naked seed varieties (Salamini et al. 2002). The non-brittle rachis facilitates the harvest and prevents seed shattering in the field. The non-brittle rachis phenotype is controlled by the two closely linked genes

btr1 and *btr2* that map to the short arm of chromosome 3H. The recessive allele in either of the genes causes the non-brittle phenotype (Komatsuda and Mano 2002). The most important gene causing a six-rowed spike type is the recessive *vrs1*. *Vrs1* is located on the long arm of chromosome 2H. The dominant allele is present in wild barley and in two-rowed cultivars, whereas the recessive allele is present in all six-rowed cultivars. *Vrs5* or *int*-c, located on chromosome 4HS, is present in many two-rowed cultivars and can cause an intermediate spike type. There are at least three more genes - *vrs2*, *vrs3*, and *vrs4* - that can cause the six-rowed spike type. They are, however, only present in induced mutant lines (Lundqvist et al. 1997).

Husks of hulless or naked barley are easily separable from the grain upon threshing. Hulless barley is preferred as human food, because pearling to remove the hull is not necessary and the kernel is easier to digest. The recessive allele of the *nud* gene on chromosome 7H causes the free threshing phenotype (Taketa et al. 2008). Schmalenbach et al. (2011) mapped the *thresh-1* locus in the wild barley introgression line library S42IL on chromosome 1H. The recessive wild barley allele confers a phenotype which is difficult to thresh. This suggests that the locus might be involved in the domestication of barley.

1.3 BARLEY GENOME MAPPING

Barley plays an important role as genetic model system for the Triticeae (Muñoz-Amatriaín et al. 2014b). Barley has traditionally been regarded as a model system in plant genetics, due to a variety of characteristics (Mayer et al. 2012). Those include the diploid ploidy level, the inbreeding habit, large chromosomal synteny to other Triticeae species and the whole grass family, as well as access to rich collections of mutant lines and other genetic stocks and a large number of well documented germplasm collections (Sato et al. 2003). There is a long history of barley genetics research and mapping, starting with a linkage map with 32 naked eye polymorphisms developed in the first half of the 20th century (Smith 1951) and culminating recently in the publication of a draft genome sequence. In the following a brief overview of the development of linkage maps with genetic markers will be given, subsequently the “barley gene space” will be illustrated.

The first complete molecular maps in barley were primarily generated with restriction fragment length polymorphism (RFLP) markers in the early 1990s (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993). The maps contained between 157 and 295 loci, spanned between 1,096 and 1,453 cM, and were generated with progeny of a single cross. Since RFLP markers were reliable but the technology was rather expensive and slow new technologies were devel-

oped like amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers. RAPD markers contributed only little to barley genome mapping. The AFLP proved to be more reliable than the RAPD technology and thus, several whole genome maps were constructed with AFLP markers (e.g.: Waugh et al. (1997); Qi et al. (1998); Yin et al. (1999)). Compared to RFLP markers AFLPs have the advantage to be relatively fast and inexpensive. The drawback is the dominant nature of the markers, which is not easily transferable among diverse crosses.

Single sequence repeat (SSR) markers are codominant, easy to use, and transferable between different crosses, and therefore became popular for map construction and marker assisted selection (MAS) (Graner et al. 2011). SSR maps were for example published by Ramsay et al. (2000) and Li et al. (2003). In order to facilitate the comparison of studies carried out with different marker systems consensus maps were developed. Karakousis et al. (2003) generated a map with 700 markers spanning 933 cM assembled from SSR, AFLP, and RFLP markers. Varshney et al. (2007) merged marker data from six different mapping populations and created a map consisting of 775 SSR markers spanning 1,068 cM.

Even though SSR markers are very useful new technologies that allow much higher throughput, like the diversity array technology (DArT) and single nucleotide polymorphism (SNP) techniques were developed. The first DArT map in barley was published by Wenzl et al. (2004). The authors mapped 385 markers on a length of 1,137 cM in a Steptoe x Morex double haploid (DH) population. Later consensus maps were produced containing several thousand markers, either DArT markers only (e.g. Alsop et al. (2011)) or maps that include different marker types, for example SSR, RFLP and Sequence Tagged Sites (STS) markers (e.g. Wenzl et al. (2006)).

SNPs require sequence information and with sequencing techniques becoming ever faster and cheaper SNPs have been on the rise for some years (Graner et al. 2011). Rostoks et al. (2005) developed an SNP-based map integrating RFLP, AFLP and SSR markers. The map has a total of 1,237 markers spanning 1,211 cM. Array technologies like the Illumina GoldenGate and the Infinium iSelect SNP assay are widely applied in barley. The technology allows the analysis of several thousand SNPs in parallel and make dense genotyping possible for large numbers of individuals (Gupta et al. 2008). The GoldenGate assay consists of 4596 SNPs arranged in three separate assays (Graner et al. 2011). Two of them, barley oligonucleotide pool assays 1 and 2 (BOPA 1 and 2) were used to construct a consensus genetic map from four doubled-haploid (DH) mapping populations (Close et al. 2009). Muñoz-Amatriaín et al. (2011) developed an improved consensus map including 11 mapping populations. The iSelect assay comprises 7,842 SNPs which are a

combination of 2,832 BOPA SNPs and 5,010 new SNPs discovered from Next Generation Sequencing data. These markers were first mapped in a Morex x Barke F6 recombinant inbred line (RIL) population of 360 individuals (Comadran et al. 2012). Later a consensus map was established by Muñoz-Amatriaín et al. (2014a) merging the two maps published by Muñoz-Amatriaín et al. (2011) and Comadran et al. (2012). The SNP assays are widely used in the barley research community. This has the advantage of simple comparability of quantitative trait locus (QTL) positions between populations.

Genotyping by sequencing (GBS) is a method of de novo SNP detection without prior sequence information. Elshire et al. (2011) proposed the highly multiplexed, reproducible, and simple approach for generation of genome wide SNP markers. GBS uses restriction enzymes to create reduced representation libraries of the target genome. Individual genotypes are barcoded and sequenced in parallel on a next-generation sequencing platform. Poland et al. (2012) developed a protocol for barley using two different restriction enzymes. The SNPs can either be mapped by classical linkage mapping or they can be positioned by alignment to the barley genome sequence via the BLAST procedure (Mascher et al. 2013b).

In 2012 the International Barley Sequencing Consortium published a draft sequence of the barley genome, the so-called barley genome gene space (Mayer et al. 2012). The physical map represents 4.98 of the 5.1 Gb of the barley genome. More than 3.9 Gb are anchored to a high-resolution genetic map. The authors assigned 79,379 transcript clusters by projecting complementary DNA (cDNA) and deep RNA sequence data onto the framework. Of those 26,159 sequences are described as “high-confidence” genes due to homology to other plant genomes.

1.4 QUANTITATIVE TRAIT LOCI ANALYSIS

Two types of phenotypic variation can be distinguished, qualitative and quantitative variation. Qualitative traits are characterized by discrete variation with two or few clearly distinguishable types. Examples from crop breeding are dwarf types in cereals or low erucic acid content in rapeseed. The majority of the traits of interest in plant breeding, however, underlie quantitative or continuous variation. Examples for quantitative traits are yield, thousand kernel weight, nutrient use efficiency, and drought stress tolerance. While one or few genes control qualitative traits, quantitative traits are controlled by large numbers of genes. Quantitative trait loci (QTL) can be defined as loci on chromosomes that are involved in the inheritance of a trait. A QTL may comprise one or several genes. QTL analysis is applied to dissect the genetic basis of a

quantitative trait into QTL. The basis for QTL analyses is linkage disequilibrium (LD) between genetic marker and QTL allele (Becker 2011). For QTL analysis different types of marker systems have been used in barley (compare chapter 1.3). Principally two types of QTL analysis can be distinguished. Genome-wide association studies use LD present in natural or breeding populations. Classical QTL studies use specially constructed biparental populations, for instance recombinant inbred lines, DH-populations or advanced backcross populations (Thomas et al. 2011). Statistical methods are applied to detect correlations between a phenotype and a genetic marker. Statistically significant marker-trait associations are referred to as QTL effects. In some biparental populations, like introgression lines (ILs) each individual is clearly identifiable by one unique introgression (chromosomal segment). In this case an alternative to a marker-based analysis is individual based analysis. In introgression lines each individual harbors one chromosomal segment of a wild donor parent in the background of an elite cultivar. In the analysis each individual is compared to the cultivar parent in order to detect differences in trait performance. If such a difference is detected it can clearly be attributed to the introgressed donor alleles.

1.5 WILD BARLEY INTROGRESSION LINES

The domestication of crop plants has led to a loss of genetic diversity in the crop species populations compared to populations of crop wild relatives (CWR). With the onset of modern breeding this erosion of genetic diversity became even more dramatic. Diverse landraces were replaced by higher yielding but genetically less diverse cultivars (Zamir 2001). Plant breeding, however, needs genetic diversity in order to select for better performing genotypes. The current loss of genetic variation in the elite gene pool tends to limit the breeding success of improved cultivars (Zhao et al. 2010). The introgression of CWR alleles, either from direct progenitors of crops or other closely related species, into the elite breeding gene pool is one strategy to overcome this dilemma (Feuillet et al. 2008). The use of CWR to improve crop performance dates back to the first half of the 20th century (Hajjar and Hodgkin 2007) when it was used in sugarcane improvement. During the 1940s and 1950s it was recognized as a useful source in other major crop breeding programs (Plucknett et al. 1987). Nowadays the use of CWR in crop improvement is well established as Hajjar and Hodgkin (2007) demonstrate in their review. They describe 13 crop species for which cultivars with introgressions from CWR were released between the mid-1980s and 2006. Most varieties were released in tomato (55), followed by potato and rice (12 each), wheat (9), and sunflower (7). However, improving cultivars with the help of

CWR is time-consuming. This might explain the relatively small number of released cultivars over a time span of 20 years. Exotic germplasm resources often carry alleles that confer agriculturally undesirable traits, for example low yield or seed shattering. If they are closely linked to the trait of interest (linkage drag) they hamper the success of introgressing superior alleles. Tanksley and Nelson (1996) proposed the method of "advanced backcross quantitative trait loci analysis" (AB-QTL). The method combines QTL detection and the introduction of favorable exotic alleles from a wild donor parent into an elite cultivar. Several rounds of back crossing to the elite parent reduce linkage drag. Classical QTL studies are carried out with bi-parental populations derived from two elite genotypes. AB-populations enable detection of new alleles not yet present in the breeding gene pool. Zamir (2001) suggested the development of exotic libraries as part of an infrastructure to facilitate introgression breeding. Such exotic libraries consist of a set of introgression lines. Each of these introgression lines contains a marker-defined chromosomal segment of a CWR crossed onto the background of an elite variety. This is achieved by several rounds of backcrossing to the recurrent parent and MAS. A complete library represents the entirety of an exotic genome. This method allows the evaluation of small parts of a CWR genome in an agriculturally adapted genetic background for suitability in plant breeding. Similar to AB populations, introgression lines can facilitate the development of new varieties through reduction of linkage drag. Since they only contain a small part of the exotic donor genome with the gene of interest transfer into breeding gene pools is facilitated.

In barley, many studies have been conducted which investigate the positive effects of wild barley in crosses with cultivated barley on plant performance and their potential use in plant breeding e.g. Baum et al. (2003); Li et al. (2006); Lakew et al. (2011). Pillen et al. (2003) published the first AB-QTL study in barley. Von Korff et al. (2004) developed a BC_2DH population from a cross between the German spring barley cultivar Scarlett (*Hv*) and the Israeli wild barley accession ISR42-8 (*Hsp*). The lines of this S42 population were used in several AB-QTL studies to identify QTL for yield, pathogen resistance and malting quality (von Korff et al. 2005, 2006; von Korff et al. 2008; von Korff et al. 2010; Saal et al. 2011).

By a further round of backcrossing with the recurrent parent Scarlett and subsequent selfing and MAS Schmalenbach et al. (2008) developed 59 ILs (S42ILs) from the S42 population. Each of the S42ILs contains a single or a small number of *Hsp* introgressions. The introgression line library was used to carry out QTL studies to verify QTL from AB-QTL studies and to identify new QTL for pathogen resistance, yield and quality parameters and nutrient use efficiency (Schmalenbach et al. 2008; Schmalenbach et al. 2009; Schmalenbach and Pillen 2009; Hoffmann

et al. 2012; Schnaithmann and Pillen 2013). Naz et al. (2012) studied root architecture of S42ILs and detected QTL for root dry weight and root volume. Later, the S42IL population was extended to 73 lines and the lines were genotyped with a 1,536-SNP Illumina BOPA1 set (Schmalenbach et al. 2011). Six hundred thirty-six informative SNPs and their known map order (Close et al. 2009) allowed the precise localization of the *Hsp* introgressions. The S42IL set represents 87.3% of the wild barley donor genome. Moreover, Schmalenbach et al. (2011) developed segregating high-resolution mapping populations (S42IL-HRs) for 70 S42ILs. Those lines are readily available to facilitate fine mapping and, ultimately, cloning of QTL.

1.6 DROUGHT

Drought, defined as a lack of water availability which limits crops reaching their full yield potential, is the most important abiotic stress factor in crop production worldwide. It is a permanent constraint in many developing countries but causes great yield losses in developed countries too. Drought occurs often in conjunction with other abiotic stress factors like high temperatures (Cattivelli et al. 2011) and radiation. Due to climate change a rise of extreme weather events and a higher frequency of drought events is expected (Tester and Langridge 2010). Also in Europe a change of rainfall patterns is observed as is indicated by the frequency of drought events occurring in spring and early summer. More droughts occur especially in the Mediterranean region as well as in western and eastern Europe (Olesen et al. 2011; Christensen et al. 2013). A main objective of plant breeding, therefore, is the maintenance of plant growth and high yields under drought conditions (Cattivelli et al. 2008).

Plants have developed different mechanisms to deal with drought and these are classically divided into drought escape, avoidance (postponement), and tolerance strategies (Turner 1986). Those three strategies cannot be strictly separated from each other and plants combine elements of different mechanisms (Ludlow 1989; Chaves et al. 2003). Drought escape is achieved by early maturity and the successful reproduction before the onset of severe water deficit (Kumar 2005). This is an important survival strategy in arid regions (Chaves et al. 2003). Under Mediterranean growing conditions drought at the end of the barley life cycle during grain filling is common (Stanca et al. 2003) and, if flowering occurs too late, can lead to yield reductions (Passioura 1996). One breeding strategy for improved yield under drought, therefore, is the selection for early flowering time in order to match the soil moisture supply with the phenological development of the plants (Turner 1986).

The escape mechanism can also be regarded as a drought avoidance strategy. Other avoidance strategies postpone or avoid drought stress by maintaining water in the cells through early closing of stomata and maximizing water acquisition through a large root system that penetrates deep into the soil (Stanca et al. 2003; Bray 2007). Osmotic adjustment is an adaptive mechanism in which osmotic pressure is adjusted by a net increase in solute concentration in the cells in order to maintain cell turgor. The higher solute concentration lowers the water potential of the cells and, thus, facilitates water uptake (Bray 2001; Taiz and Zeiger 2007; Cattivelli et al. 2011). Commonly accumulated solutes include the amino acid proline, sugar alcohols sorbitol and mannitol, and the quaternary amine glycine betaine. Those solutes are called compatible solutes because they do not interfere with enzyme functions in the cell. Apart from these compounds, inorganic ions, especially K^+, play a role. However, since high concentrations of ions can severely inhibit the function of cytosolic enzymes, the accumulation of ions during osmotic adjustment appears to be restricted to the vacuoles.

Finally some plants are able to tolerate dehydration or even desiccation. They can withstand extremely low water potentials or survive complete cellular dehydration by protecting the cells from injury through biochemical and morphological strategies (Bray 2007).

The signaling of osmotic stress in plants is mediated by abscisic acid (ABA) dependent and ABA independent pathways. The plant hormone ABA typically accumulates during osmotic stress. One immediate consequence of this is the closing of stomata to prevent water loss. In response to the rise of ABA concentration the products of ABA-responsive genes accumulate. ABA, therefore, plays an important role in the drought-signaling pathway. However, as studies with ABA-insensitive mutants have shown ABA-independent drought response pathways exist as well. In ABA-regulated genes the promoters contain the so-called ABA responsive element (ABRE), a six –nucleotide sequence that probably binds to transcription factors (TFs) regulated by ABA. ABA regulated TFs include bZIP, MYC, and MYP genes. Some of the genes regulated by osmotic stress also contain an alternative nine-nucleotide regulatory sequence element. This so-called dehydration response element (DRE) is recognized by ABA-independent transcription factors (Shinozaki and Yamaguchi-Shinozaki 2007; Taiz and Zeiger 2007), for example *HvDREB1* (Xu et al. 2009). C-repeat binding factors (CBFs) are also responsive to dehydration. In a second ABA-independent signaling pathway osmotic stress responsive genes are apparently directly controlled by the mitogen-activated protein (MAP) kinase signaling pathway (Shinozaki and Yamaguchi-Shinozaki 2007; Taiz and Zeiger 2007).

During osmotic stress several genes are induced which code for enzymes associated with metabolic pathways that biosynthesize compatible solutes for osmotic adjustment, e.g. betaine aldehyde dehydrogenase (Li et al. 2007). Other important genes regulated by osmotic stress encode for instance the water channel proteins aquaporins, which are associated with membrane transport. Moreover, heat shock proteins are induced which may protect or renature proteins inactivated by desiccation (Taiz and Zeiger 2007). A large group of proteins that accumulate in vegetative tissue during drought stress are LEA (late embryogenesis abundant) proteins. These proteins are typically hydrophilic and strongly bind water. They are suspected to be involved in cellular membrane protection (Bray 2001). Examples for genes encoding LEA proteins are the dehydrin gene family (Choi et al. 1999) and the gene *HVA1* (Bahieldin et al. 2005).

1.7 HIGH-THROUGHPUT PHENOTYPING

While genotyping techniques developed rapidly in the last decades and dense genomic information on important crop species is available at low cost, phenotyping technology has stayed behind, causing a bottleneck for crop improvement. Phenomics, the characterization of the phenome, faces various challenges. Compared to genotyping which is highly mechanized and its technology principally uniform across species, phenotyping is still in its early development. Automated high-throughput phenotyping platforms are available for controlled environments and at field level. However, it is still a long way to achieve a level of automated phenotyping in plant research comparable to genotyping technology (Cobb et al. 2013). Apart from the technological and computational issues to address in plant phenotyping, the complex and dynamic nature of the phenome is challenging. The phenotype can be measured at different organizational levels like cell, organ, whole plant, and the population level. Phenotypes also differ between developmental stages, in different environments and under biotic and abiotic stresses. A complete characterization of the phenotype, unlike the genotype, therefore seems impossible. Prioritizing what to measure will therefore always be an important decision in phenotyping (Houle et al. 2010). In order to tackle the challenges close collaboration between scientists from different disciplines like, biology, computer science, and engineering is needed. Several national and international networks were founded to coordinate phenotyping research and development, e.g. DPPN, EPPN, and IPPN (German, European, and International Plant Phenotyping Network, respectively).

Automated plant imaging allows rapid data collection on large numbers of plants. Compared to conventional methods to determine for instance biomass and measure growth, imaging tech-

nologies possess a range of advantages. Imaging is less time-consuming and labor intensive, moreover the technology is non-destructive. Biomass can be estimated precisely without destructive harvest of the plant which is often not possible with more traditional methods. Therefore, it is possible to carry out repeated measurements on the same plant and thus, follow one individual throughout its development (Munns et al. 2010; Golzarian et al. 2011). Changes in leaf health status, e.g. percentage of necrotic or senescent leaf area, can be estimated more precisely by computer assisted analysis of images.

Controlled environment phenotyping facilities enable fully automated high-throughput imaging of hundreds to thousands of plants per day. In fully automated greenhouses plants can be delivered via conveyor belts to watering, weighing and imaging stations. High-throughput phenotyping facilities of this type are currently in use in various research institutes (e.g. The Plant Accelerator®, Adelaide, Australia; CropDesign, Gent, Belgium; IPK, Gatersleben, Germany; PhenoArch, Montpellier, France; SCREEN house, Jülich, Germany).

High resolution color pictures (RGB pictures), taken from the top and two side views are used to determine the projected shoot area of the plant. The projected shoot area serves as a measure for biomass. Hence, from RGB images taken at several time points, growth curves as well as growth rates can be calculated. The pictures can also be used to determine plant/ leaf color which allows conclusions about plant health to be made. Several other types of plant images can be taken, e.g. infrared, near infrared, and fluorescent images. Scanning with infrared light gives information on plant or leaf temperature, while near infrared imaging sheds light on the plant water content and fluorescent pictures enable conclusions on plant health status.

Automated high-throughput phenotyping facilities are ideal to combine controlled irrigation and phenotyping protocols (Berger et al. 2010). A first application was given by Rajendran et al. (2009) who used a manual imaging system (LemnaTecScanalyzer3D, Wuerselen, Germany) to screen *Triticum monococcum* accessions for salinity tolerance. They developed high-throughput quantification assays to distinguish sodium exclusion, sodium tissue tolerance and osmotic tolerance as the strategies plants use to establish salinity tolerance. Imaging technologies have been successfully used in several studies in *Arabidopsis thaliana*, e.g. Granier et al. (2006) and Leister et al. (1999). The first study investigated nine accessions under different levels of water deficit in the phenotyping facility “PHENOPSIS”. The authors point out the facilitation of the experiment through automated watering of the plants. The reaction to water deficits was documented by images, which allowed following leaf area growth precisely.

1.8 OBJECTIVES

The objectives of this thesis were to evaluate the phenotypic drought stress response of a set of juvenile wild barley introgression lines of the S42IL library. Novel phenotyping methods were tested on the library in a high-throughput phenotyping facility. GBS was applied in order to genotype the S42ILs with higher resolution than was previously achieved and to compare GBS to array based technologies. In detail the research questions were:

1) Do wild barley alleles improve plant performance under moderate and severe drought stress? (Chapters 2.1 and 2.2)

2) Do wild barley alleles improve trait performance per se or can an interaction between genotype and drought treatment be observed? (Chapters 2.1, 2.2 and 3.1)

3) Is imaged based plant phenotyping an appropriate method to characterize juvenile barley plants? And are image based methods accurate enough to estimate plant biomass in the S42IL library? (Chapters 2.1 and 3.1)

4) Are GBS and the positioning of SNPs through alignment to the barley genome sequence reliable methods to obtain denser genotypic characterization of the S42IL library? And is the S42IL map constructed with GBS SNPs comparable to the BOPA1 map? (Chapters 2.2 and 3.4)

2 ORIGINAL PAPERS

This thesis comprises two original papers in which a subset of the S42IL library is evaluated for drought stress response at juvenile growth stage. Chapter 2.1 (Honsdorf et al. 2014a) describes the evaluation of growth parameters in 47 S42ILs under moderate drought stress and well-watered control conditions in a high-throughput phenotyping facility. In chapter 2.2 (Honsdorf et al. 2014b) experiments are described in which 52 S42ILs were grown under severe drought stress and well-watered control conditions. Moreover the chapter includes the results of the genotypic characterization of 55 S42ILs with the method genotyping by sequencing.

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High-Throughput Phenotyping to Detect Drought Tolerance QTL in Wild Barley Introgression Lines

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Abstract

Drought is one of the most severe stresses, endangering crop yields worldwide. In order to select drought tolerant genotypes, access to exotic germplasm and efficient phenotyping protocols are needed. In this study the high-throughput phenotyping platform "The Plant Accelerator", Adelaide, Australia, was used to screen a set of 47 juvenile (six week old) wild barley introgression lines (S42ILs) for drought stress responses. The kinetics of growth development was evaluated under early drought stress and well watered treatments. High correlation ($r = 0.98$) between image based biomass estimates and actual biomass was demonstrated, and the suitability of the system to accurately and non-destructively estimate biomass was validated. Subsequently, quantitative trait loci (QTL) were located, which contributed to the genetic control of growth under drought stress. In total, 44 QTL for eleven out of 14 investigated traits were mapped, which for example controlled growth rate and water use efficiency. The correspondence of those QTL with QTL previously identified in field trials is shown. For instance, six out of eight QTL controlling plant height were also found in previous field and glasshouse studies with the same introgression lines. This indicates that phenotyping juvenile plants may assist in predicting adult plant performance. In addition, favorable wild barley alleles for growth and biomass parameters were detected, for instance, a QTL that increased biomass by approximately 36%. In particular, introgression line S42IL-121 revealed improved growth under drought stress compared to the control Scarlett. The introgression line showed a similar behavior in previous field experiments, indicating that S42IL-121 may be an attractive donor for breeding of drought tolerant barley cultivars.

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Introduction

Barley (*Hordeum vulgare* ssp. *vulgare*, hereafter abbreviated with *Hv*) is ranked fourth among the worldwide production of cereals. Due to its multipurpose use as animal feed, human food and substrate for malting it is one of the most important cereals worldwide [1]. Barley is known to be relatively tolerant to abiotic stresses among the major cereal crops and, thus, is often grown in more marginal sites [2]. However, the process of genetic erosion has been under way in barley since its domestication some 10,000 years ago and, in particular, since the advent of intensive modern elite breeding during the last century [3]. As a result of this process, diverse landraces have been replaced by modern elite cultivars with a much narrower gene pool. Therefore there is limited genetic diversity remaining in the elite barley gene pool for abiotic and biotic stress tolerance. The current loss of genetic variation in the elite gene pool tends to limit the breeding success of improved cultivars [4]. To overcome this problem several authors, e.g. Zamir [5], proposed to use wild relatives of crop species as donors of exotic germplasm to enhance elite varieties. Tanksley and Nelson [6] proposed the method of "advanced backcross quantitative trait loci analysis" (AB-QTL) to introduce exotic genes into modern crop varieties. The method combines QTL detection and the introduction of favorable exotic alleles from a wild donor parent. Lines produced by advanced backcrosses ideally contain only one single introgression from the wild parent and are then referred to as introgression lines (ILs). This is achieved by several rounds of backcrossing to the recurrent parent and marker assisted selection (MAS). A set of ILs ideally represents the whole genome of a wild donor plant in the genetic background of a single elite variety [5].

Pillen et al. [7] published the first AB-QTL study in barley. Von Korff et al. [8] developed a BC_2DH population from a cross between the German spring barley cultivar Scarlett (*Hv*) and the Israeli wild barley accession ISR42-8 (*Hordeum vulgare* ssp. *spontaneum*, hereafter abbreviated with *Hsp*). The lines of this S42 population were used in several AB-QTL studies to identify QTL for yield, pathogen resistance and malting quality traits [9–13].

Schmalenbach et al. [14] used the S42 population to develop 59 ILs (S42ILs) by a further round of backcrossing with the recurrent parent Scarlett and subsequent selfing and MAS. Each of the S42ILs contains a single or a small number of *Hsp* introgressions.

Several QTL studies were conducted to verify QTL from AB-QTL studies and to identify new QTL for pathogen resistance, yield and quality parameters [14–16]. Naz et al. [17] studied root architecture of S42ILs and detected QTL for root dry weight and root volume. Later on, the S42IL population was extended to 73 lines and the lines were genotyped with a 1,536-SNP Illumina BOPA1 set [18]. Six hundred thirty-six informative SNPs and their known map order [19] allowed the precise localization of the *Hsp* introgressions. The S42IL set represents 87.3% of the wild barley donor genome. Moreover, Schmalenbach et al. [18] developed segregating high-resolution mapping populations (S42IL-HRs) for 70 S42ILs. Those lines are readily available to facilitate fine mapping and, ultimately, cloning of QTL.

Drought is one of the main factors limiting yield worldwide [20]. Due to climate change extreme weather events are predicted to occur more frequently and an altered pattern of drought occurrence is expected [21]. Therefore maintaining plant growth and yield under drought remains a major objective for plant breeding [22]. Many studies have been conducted on the impacts of terminal drought stress in cereals, while impacts of drought stress at early developmental stages are less well investigated [23]. However, several authors comment that yield may be enhanced by improved early vigor and a rapid development of maximum leaf area [24,25]. López-Castañeda and Richards [26] reported that on average barley has a higher yield in water limited environments compared to wheat, triticale, and oat. As part of a possible explanation, they pinpointed the faster and more vigorous growth of barley during vegetative development. Variation in this trait is, therefore, likely to be in direct relation to drought stress tolerance and yield.

Conventional methods to determine biomass and measure growth are time-consuming and labor intensive. Often they involve destructive harvest of plants and therefore make repeated measurements on the same plant impossible. New developments in plant imaging technologies allow the estimation of biomass and growth parameters as a non-destructive and rapid alternative to more traditional methods [27,28]. New phenotyping facilities enable automated imaging of plants. Several types of plant images can be taken, e.g. with infrared, near infrared, fluorescent and visible light. Scanning with infrared light gives information on plant or leaf temperature, while near infrared imaging sheds light on the plant water content and fluorescent pictures enable conclusions on plant health status. High resolution color pictures (RGB pictures), taken from the top and two side views are used to determine the projected shoot area of the plant. The projected shoot area serves as a measure for biomass. Hence, from RGB images taken at several time points, growth curves as well as growth rates can be calculated.

In fully automated greenhouses plants can be delivered via conveyor belts to watering, weighing and imaging stations. In these high-throughput phenotyping facilities several hundred individual plants can be imaged per day in a fully automated manner. High-throughput phenotyping facilities of this type are currently in use in various research institutes (e.g. The Plant Accelerator, Adelaide, Australia; CropDesign, Gent, Belgium; IPK Gatersleben, Germany, PhenoArch, Montpellier, France).

Such phenotyping facilities are ideal to combine controlled irrigation and phenotyping protocols [29]. A first application was given by Rajendran et al. [30] who used a manual imaging system (LemnaTecScanalyzer3D, Wuerselen, Germany) to screen *Triticum monococcum* accessions for salinity tolerance. They developed high-throughput quantification assays to distinguish sodium exclusion, sodium tissue tolerance and osmotic tolerance as the strategies plants use to establish salinity tolerance.

In this report, "The Plant Accelerator" was used to screen growth of wild barley ILs under well watered and drought treatments during vegetative growth. The aims were (1) to identify wild barley derived QTL within the set of S42ILs that control drought stress responses and (2) to test the use of non-destructive high-throughput imaging to measure vegetative stage drought response in barley.

We could show that high-throughput imaging provides accurate estimates for biomass development over time. Moreover several drought related QTLs were identified and genotypes detected that may be beneficial in future breeding programs.

Materials and Methods

Plant Material

Forty-seven wild barley ILs of the S42IL library and the recipient parent Scarlett were selected for the experiment. The S42ILs are derived from a cross between the German malting barley variety Scarlett and the Israeli wild barley accession ISR42-8. The 47 ILs possess few *Hsp* chromosome segments and were selected based on SSR and SNP genotyping to represent a large portion, 87.3%, of the ISR42–8 genome [18]. Repeated backcrossing and MAS are described in Schmalenbach et al. [14].

Glass House Cultivation

Two drought stress experiments, with duration of six weeks each, were conducted between end of March and mid of July 2011 in The Plant Accelerator greenhouse facilities in Adelaide, Australia (34°58′16.18″S; 138°38′23.88″E). Forty-eight barley genotypes were grown under a well watered and stress treatments with three replicates per genotype and treatment. Each experiment was designed in three randomized blocks. Control and stress treatments of each genotype were placed next to each other (Fig. 1, Table S1).

Single plants were grown in 2.5 L plastic containers with 2.1 kg of soil (50% UC Davis soil mix, 35% Coco-peat, 15% clay-loam). Three seeds per pot were directly sown into the soil and after germination thinned out, leaving one plant per pot. Plants were pre-grown for two weeks in a regular greenhouse and watering was performed manually to allow optimal germination and seedling establishment. Subsequently, the pots were transferred to the "smart house" where each pot was placed onto a cart on a conveyor belt and the two treatments were applied. Every second day, pots were weighed and watered automatically to 22% gravimetric water content for the well watered treatment and 15% for the stress treatment (Fig. 2). Based on the experience of the first

Figure 1. View of experiment 1 with five-week old barley S42IL plants growing in The Plant Accelerator.
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Figure 2. Barley plants at the weighing and watering unit after leaving the imaging station.
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experiment we adjusted the drought stress in the second experiment to 12% gravimetric water content to slightly increase drought effects. The experiments were carried out under natural lighting with the temperature in the greenhouse kept at a range between 15°C (night) and 22°C (day).

Phenotyping

With the onset of the stress treatment imaging of the plants started. Plant images were captured using a LemnaTec 3D Scanalyzer (LemnaTec, GmbH, Wuerselen, Germany). Every day, three RGB pictures (2056×2454 pixels) were taken of each barley plant, one top view image and two side view images with a 90° horizontal rotation. After background-foreground separation was applied to separate the plant tissue area from the background, pixel numbers per plant were counted and the pixel sum of the three pictures per plant was taken to define the projected shoot area. The shoot area measured over time was used to draw growth curves. For each growth curve, curve fitting with a 6^{th} order polynomial was conducted to adjust for possible missing data points and absolute growth rate [dA/dt] and relative growth rate [(dA/dt)/A] were calculated. For each of the three curves the integral was determined and used as a trait in the statistical analysis. Moreover, six further traits were extracted from the images; caliper length, height, color (as hue angle in the HSI color scheme) and the two parameters shoot area top view and convex hull area to calculate compactness of each plant. At the end of the experiment, barley plants were harvested and above ground biomass, tiller number (TIL), and plant height (HEI) were determined. Fresh biomass was weighed and, subsequently, oven dried to constant weight to determine dry biomass. Water use efficiency (WUE) was calculated by dividing dry biomass at the end of the experiment by the total amount of water added during the four weeks in the "smart house" [mg/g water]. Specific plant weight (SPW) was calculated from the dry weight and the maximum projected shoot area at the end of the experiment. In addition, simple stress indices (SSI) were calculated as follows: SSI = Ts/Tc, where Ts and Tc are the average trait performances of an IL under stress and control conditions, respectively. An overview of trait definitions is given in Table 1.

Genotyping

The S42ILs were genotyped with the 1,536-SNP barley BOPA1 set [19] of the Illumina GoldenGate assay [18]. Six hundred and thirty-six out of the tested 1,536 SNPs were polymorphic and used to characterize the extent of exotic *Hsp* introgressions in each S42IL (see Fig. S1).

Statistical Analysis

Statistical analyses were performed with SAS Enterprise Guide 4.2. [31]. Descriptive statistical parameters (Table S2) were calculated with procedure MEANS. Heritabilities across treatments were calculated as $h^2 = V_G/[V_G + V_{GT}/t + V_{GE}/e + V_{GET}/et + V_R/etr]$, and within treatments as: $h^2 = V_G/[V_G + V_{GE}/e + V_R/er]$. The terms V_G, V_{GT}, V_{GE}, V_{GET} and V_R represent the genotypic, genotype×treatment, genotype×environment, genotype×environment×treatment, and error variance components, respectively, calculated with procedure VARCOMP [31]. The terms t, e, and r indicate the number of treatments, experiments and replicates, respectively. Pearson correlation coefficients between traits were calculated with means across treatments, blocks and experiments and within drought stressed and control treatments, respectively, using the procedure CORR.

Analysis of variance was carried out with the procedure MIXED using model I to test for genotype main effects across treatments and experiments.

Model I:

$$Y_{ijkl} = \mu + L_i + T_j + E_k + L \times T_{ij} + L \times E_{ik} + B\left(E \times T_{kj}\right)_l + \varepsilon_{ijkl}$$

and model II for genotype effects across experiments but within a single treatment.

Model II:

$$Y_{ikl} = \mu + L_i + E_k + L \times E_{ik} + B(E_k)_l + \varepsilon_{ikl}$$

Where μ is the general mean, L_i is the fixed effect of the ith line, T_j is the fixed effect of the jth treatment, E_k is the fixed effect of the kth experiment, $L \times T_{ij}$ is the fixed interaction between the ith line and the jth treatment, $L \times E_{ik}$ is the fixed interaction between the ith line and the kth experiment, $B(E \times T_{kj})_l$ is the random effect of the lth block nested in the interaction between kth experiment and jth treatment, $B(E_k)_l$ is the random effect of the lth block nested in the kth experiment and ε_{ijkl} and ε_{ikl} are the error of Y_{ijkl} and Y_{ikl}, respectively.

Following the mixed model analysis a Dunnett test was conducted where least square means (LSMEANS) of each IL were compared to the control Scarlett. In case an IL revealed a significant ($P<0.05$) deviation in trait performance from Scarlett, as main effect and/or as line×treatment interaction, a line×trait

Table 1. List of evaluated traits.

Trait	Abbreviation	Unit	Method of measurement
Imaging parameters			
Shoot area integral[a]	SAI	kPix[b]	Calculated from pixel sum of three images per plant per day; A
Absolute growth rate integral	AGRI	kPix/d	Calculated from pixel sum of three images per plant per day; dA/dt
Relative growth rate integral	RGRI	d^{-1}	Calculated from pixel sum of three images per plant per day; (dA/dt)/A
Height integral	HEII	kPix	Max. distance from bottom to top of plant
Caliper length integral	CALI	kPix	Max. distance between two points on the object boundary, top view image
Hull area integral	HULI	kPix	Smallest geometrical object without concave parts that covers whole plant, top view image
Shoot area top view integral	SATVI	kPix	Pixel number
Plant hue integral	HUEI	-	Average hue value calculated from all pixels per plant and day
Harvest parameters			
Tiller number	TIL	-	Number of tillers per pot
Height	HEI	cm	Plant height measured from bottom to leaf tip
Biomass dry	BMD	g	Weight of oven dried biomass per pot
Indices			
Water use efficiency	WUE	mg/g water	Harvested biomass per plant/total amount of irrigation water
Specific plant weight	SPW	mg/kPix	Harvested biomass per plant/pixel number per plant at end of experiment
Compactness integral	COMI	-	SATV/HUL per plant per day
Simple stress index	SSI	-	Trait performance under stress/trait performance under control treatment

[a]Integral: calculated for length of entire experiment, respectively.
[b]kilo Pixel.
doi:10.1371/journal.pone.0097047.t001

association was assumed and the presence of a QTL was accepted. If several ILs with overlapping introgressions showed a similar effect, it was assumed that the ILs contained the same QTL. We consider this QTL as the most likely location of the effect and, thus, define a minimum number of QTL needed to explain all identified trait effects. The relative performance (RP) of an IL was calculated as RP (IL) = [LSMEANS (IL) – LSMEANS (Scarlett)] ×100/LSMEANS (Scarlett), where LSMEANS were calculated with model I across treatments, experiments and blocks or with model II across experiments and blocks, separately for each treatment. The detection of significant line by trait associations was conducted for every trait revealing a heritability with $h^2>0\%$ across treatments or within the two watering treatments, respectively.

Results

Trait Performance of S42ILs

For most traits, means were higher under well watered treatment than under drought stress (Fig. 3 and Fig. 4, Table S2), e.g. 2.2 g of biomass dry (BMD) vs. 0.9 g. There were, however, four exceptions. Compactness integral (COMI) was higher under drought treatment than under well watered treatment. The same was true for plant hue integral (HUEI), SPW, and WUE but differences were marginal.

Coefficients of variation (CV) differed strongly between traits (1.5 to 72.1%). Highest CV was calculated for BMD across treatments (72.1%). The lowest CVs were determined for HUEI, varying from 1.5 to 1.6% for the different treatments and the SSI. CV was generally higher under drought than under well watered treatment. The four exceptions were WUE, SPW and height integral (HEII) where CV under well watered treatment was higher and HUEI where CV was the same under both treatments.

Heritability was generally higher under well watered treatment. HEI, HEII and WUE were exceptions and showed higher heritabilities under drought treatment. Highest heritabilities were found for HEI, HEII and caliper integral (CALI) (between 46.2 and 76.8%). Low heritabilities were determined for WUE and HUEI under drought treatment and across treatments, as well as for BMD under drought treatment (15%). Most of the SSI showed heritabilities equal to 0. SSI (HEII), SSI (HUEI), SSI (SPW), SSI (WUE) revealed heritabilities between 4.7 and 14.5%.

Trait Correlations

Highest correlations were found among measured traits and among stress indices (Table S3). However, correlations between measured traits and stress indices were low. The measured traits showed the highest correlations between shoot area integral (SAI) and absolute growth rate integral (AGRI) ($r = 0.99$), BMD and SAI (Fig. 4), BMD and AGRI, shoot area top view integral (SATVI) and SAI and SATVI and AGRI ($r = 0.98$). Most correlations were positive and statistically significant. HEI showed negative correlation with relative growth rate integral (RGRI) and TIL, but values were not statistically significant. COMI showed negative correlations with all traits but HEII. Among stress indices highest correlations were found for SSI (SAI), SSI (AGRI) and SSI (SAI), as well as between SSI (WUE) and SSI (SPW) (with $r>0.93$). Interestingly, WUE, SPW and BMD showed only low correlations ($r<0.43$). Looking at the simple stress index, however, correlations between SSI (WUE), SSI (SPW) and SSI (BMD) were very high ($r = 0.85$ to 0.96). Autocorrelations between drought and well watered treatments of a single trait were high ($r>0.61$) for most traits. HEI with $r = 0.85$ had the highest correlation between treatments. RGRI showed a low but still significant correlation between the treatments with $r = 0.33$. Autocorrelations for SPW and WUE were not significant.

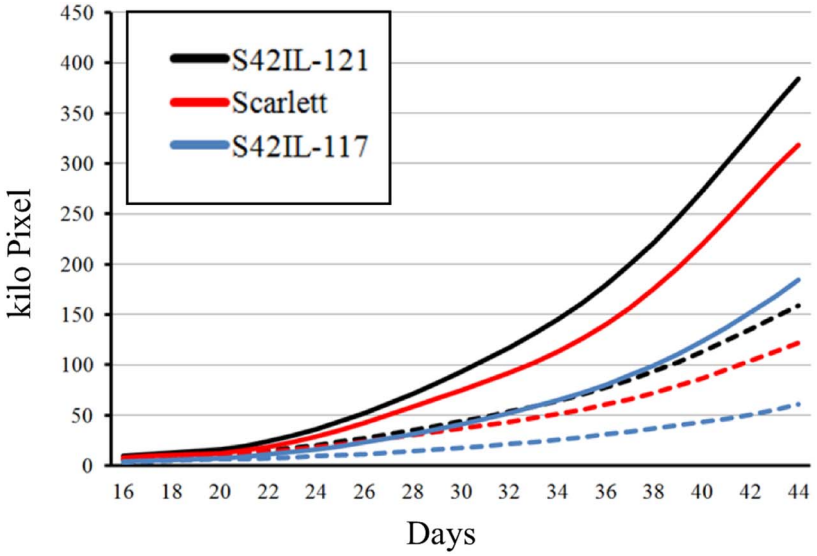

Figure 3. Development of shoot area of S42IL-121, Scarlett and S42IL-117 under well watered (solid line) and drought (dashed line) treatment, respectively.
doi:10.1371/journal.pone.0097047.g003

Mixed Model Analysis of Variance

The mixed model analysis, including fixed line, treatment, and experiment effects (i.e. model I) revealed significant ($P<0.05$) line effects for all investigated traits (Table S4). Treatment had a clear impact on trait performance. For all traits, except WUE and SPW the effect was significant. Line by treatment interaction effects were not significant for any of the measured traits. The experiments had a significant effect on trait performance of all traits except leaf color measured as plant hue integral (HUEI). And line by experiment interaction was significant for all traits but RGRI, COMI, and HEII. In the mixed model analyses for single treatments including fixed line, and experiment effects (i.e. model II), line had a significant effect on trait performance for all traits but HUEI, RGRI, and WUE under well watered and HUEI and SPW under drought conditions (Tables S5 and S6). Also simple stress indices were analyzed with model II (Table S7). The line effect was not significant for any of the simple stress indices.

QTL Detection

QTL were only determined for traits with heritability greater than 0. The Dunnett tests revealed, in total, 63 line effects for eleven out of 14 traits. These effects were detected either across treatments (39), within the drought treatment (15) or within the well watered treatment (9). Several of the measured effects were consistent between the different treatments. Thus, these line effects were summarized to a minimum of 44 QTL (Table 2 and Fig. 5). No QTL were identified for RGRI, SPW, and HUEI. Between two and nine QTL were identified for the traits AGRI, BMD, CALI, COMI, HEI, HEII, hull area integral (HULI), SAI,

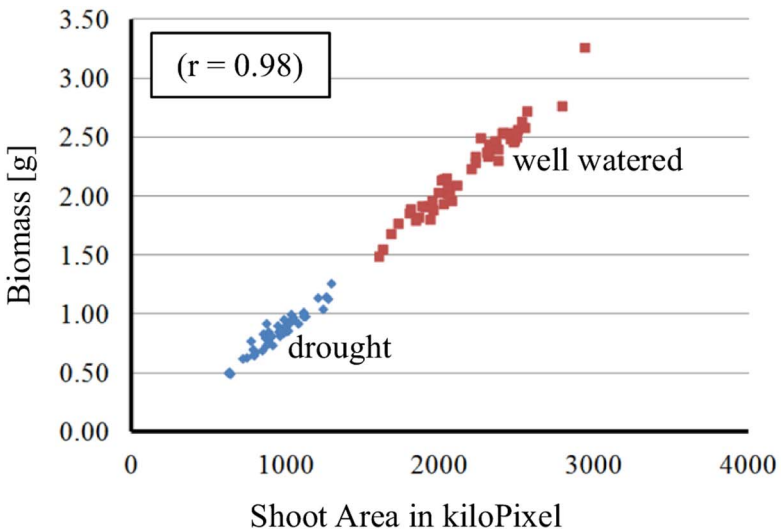

Figure 4. Correlation between biomass and shoot area integral under drought (blue dots) and well watered (red dots) treatment, respectively.
doi:10.1371/journal.pone.0097047.g004

Table 2. List of 44QTL detected for 11 traits in the S42IL-population.

Trait[a]	QTL Name	Position of main introgression[b]	Line	Treatment[c]	LSMEANS Scarlett[d]	LSMEANS IL[e]	Dev. f. Sca	Dev. f. Sca %[g]	Candidate genes[h]	Studies with corresponding QTL[i]
AGRI	QAgri.S42IL-3H	3H; 204.48–255.13	S42IL-115	a	170.84	119.10	−51.74	−30.28		
	QAgri.S42IL-4H	4H; 027.52–064.77	S42IL-117	a, d	170.84	114.69	−56.15	−32.87		
	QAgri.S42IL-6H	6H; 073.90–133.47	S42IL-129	a	170.84	120.89	−49.95	−29.24		
BMD	QBmd.S42IL-3H	3H; 204.48–255.13	S42IL-115	a	1.66	1.10	−0.56	−33.64		
	QBmd.S42IL-4H	4H; 027.52–064.77	S42IL-117	a	1.66	0.99	−0.67	−40.30		
	QBmd.S42IL-4Hb	4H; 074.11–119.06	S42IL-121	a	1.66	2.26	0.60	35.97		
	QBmd.S42IL-6H	6H; 073.90–133.47	S42IL-129	a	1.66	1.10	−0.57	−33.94		IV
CALI	QCali.S42IL-1H	1H; 040.51–089.01	S42IL-103	a, w	19.39	15.83	−3.56	−18.35		
	QCali.S42IL-2H	2H; 102.66–104.81	S42IL-110	a, d	19.39	15.47	−3.92	−20.23		
	QCali.S42IL-3H	3H; 067.01–098.41	S42IL-111	a	19.39	16.52	−2.87	−14.80		
	QCali.S42IL-4H	4H; 027.52–064.77	S42IL-117	a, d	19.39	15.98	−3.42	−17.62		
	QCali.S42IL-4Hb	4H; 074.11–119.06	S42IL-121	a	19.39	22.34	2.94	15.18		
	QCali.S42IL-6H	6H; 073.90–133.47	S42IL-129	a, d, w	19.39	15.02	−4.37	−22.55		
COMI	QComi.S42IL-4H	4H; 027.52–064.77	S42IL-117	a, w	4.10	5.32	1.22	29.77		
	QComi.S42IL-6H	6H; 071.39–132.23	S42IL-128	w	3.82	5.23	1.42	37.13		
HEI	QHei.S42IL-1H	1H; 040.51–089.01	S42IL-103	a, w	49.00	42.75	−6.25	−12.76		
	QHei.S42IL-1Hb	1H; 130.68–173.49	S42IL-143	a	49.00	43.50	−5.50	−11.22	*HvFT-3*[3]	I
	QHei.S42IL-2H	2H; 063.96–110.84	S42IL-109	a	49.00	44.33	−4.67	−9.52	*sdw3*[2], *HvFT4*[3]	I, II, III, IV
	QHei.S42IL-3H	3H; 067.01–098.41	S42IL-111	a	49.00	44.50	−4.50	−9.18		
	QHei.S42IL-3Hb	3H; 154.99–253.73	S42IL-140	a, d, w	49.00	58.17	9.17	18.71	*denso*[1]	I, IV, V
	QHei.S42IL-4H	4H; 074.11–119.06	S42IL-121	a, d, w	49.00	57.75	8.75	17.86		II, IV
	QHei.S42IL-6H	6H; 073.90–133.47	S42IL-129	a	49.00	44.42	−4.58	−9.35		V
	QHei.S42IL-7H	7H; 134.43–193.89	S42IL-137	a, d	49.00	54.67	5.67	11.56		I, II, IV, V
HEII	QHeii.S42IL-1H	1H; 040.51–089.01	S42IL-103	a	11.67	9.85	−1.83	−15.64		
	QHeii.S42IL-1Hb	1H; 130.68–173.49	S42IL-143	a	11.67	10.06	−1.62	−13.85	*HvFT-3*[3]	I
	QHeii.S42IL-4H	4H; 061.15–119.06	S42IL-162	a	11.67	9.99	−1.68	−14.39		
	QHeii.S42IL-4Hb	4H; 171.25–183.54	S42IL-124	a	11.67	10.04	−1.64	−14.02		
	QHeii.S42IL-7H	7H; 176.37–229.66	S42IL-138	a	11.67	10.05	−1.63	−13.93		
HULI	QHuli.S42IL-1H	1H; 040.51–089.01	S42IL-103	a, w	6679	4443	−2236	−33.47		
	QHuli.S42IL-2H	2H; 102.66–104.81	S42IL-110	a, d	6679	4517	−2161	−32.36		
	QHuli.S42IL-3H	3H; 067.01–098.41	S42IL-111	a	6679	4617	−2062	−30.88		
	QHuli.S42IL-3Hb	3H; 204.48–255.13	S42IL-115	a	6679	4616	−2063	−30.89		
	QHuli.S42IL-4H	4H; 027.52–064.77	S42IL-117	a	6679	4327	−2352	−35.21		
	QHuli.S42IL-6H	6H; 073.90–133.47	S42IL-129	a	6679	4179	−2500	−37.42		
SAI	QSai.S42IL-2H	2H; 102.66–104.81	S42IL-110	d	995	625	−369	−37.14		I, (as biomass)

Table 2. Cont.

Trait[a]	QTL Name	Position of main introgression[b]	Line	Treatment[c]	LSMEANS Scarlett[d]	LSMEANS IL[e]	Dev. f. Sca	Dev. f. Sca %[g]	Candidate genes[h]	Studies with corresponding QTL[i]
	QSai.S42IL-4H	4H; 027.52–064.77	S42IL-117	a, d	1654	1116	−538	−32.53		
	QSai.S42IL-6H	6H; 073.90–133.47	S42IL-129	a, d	1654	1151	−504	−30.44		
SATVI	QSatvi.S42IL-2H	2H; 102.66–104.81	S42IL-110	d	398	214	−184	−46.18		
	QSatvi.S42IL-4H	4H; 027.52–064.77	S42IL-117	a	677	437	−240	−35.44		
	QSatvi.S42IL-6H	6H; 073.90–133.47	S42IL-129	a	677	450	−228	−33.64		
TIL	QTil.S42IL-3H	3H; 204.48–255.13	S42IL-115	a	8.17	5.92	−2.25	−27.55		
	QTil.S42IL-4H	4H; 171.25–183.54	S42IL-124	a, d, w	8.17	11.67	3.50	42.86	*VRN-H2*[3]	III, V
WUE	QWue.S42IL-4H	4H; 027.52–064.77	S42IL-117	d	1.97	1.25	−0.72	−36.53		
	QWue.S42IL-6H	6H; 073.90–133.47	S42IL-129	d	1.97	1.17	−0.80	−40.59		

[a]Trait abbreviations are given in Table 1.
[b]By chromosome and cM position.
[c]Treatment under which effect occured, a: main effect, d: under drought treatment, w: under well watered treatment.
[d]LSMEANS Scarlett for the indicated trait and treatment.
[e]LSMEANS for indicated IL for indicated trait and treatment, if under more than one treatment then a.
[f]Deviation from Scarlett = LSMEANS[IL] - LSMEANS [Scarlett].
[g]Deviation from Scarlett in % = (LSMEANS[IL] – LSMEANS [Scarlett])/LSMEANS[Scarlett] *100.
[h]References: [1] Laurie et al. (1995), [2] Gottwald et al. (2004), [3] Wang et al. (2010)
[i]I: Von Korff et al. (2006), II: Schmalenbach et al. (2009), III: Wang et al. (2010), IV: March et al. (in prep.), V: Honsdorf et al. (in prep.).
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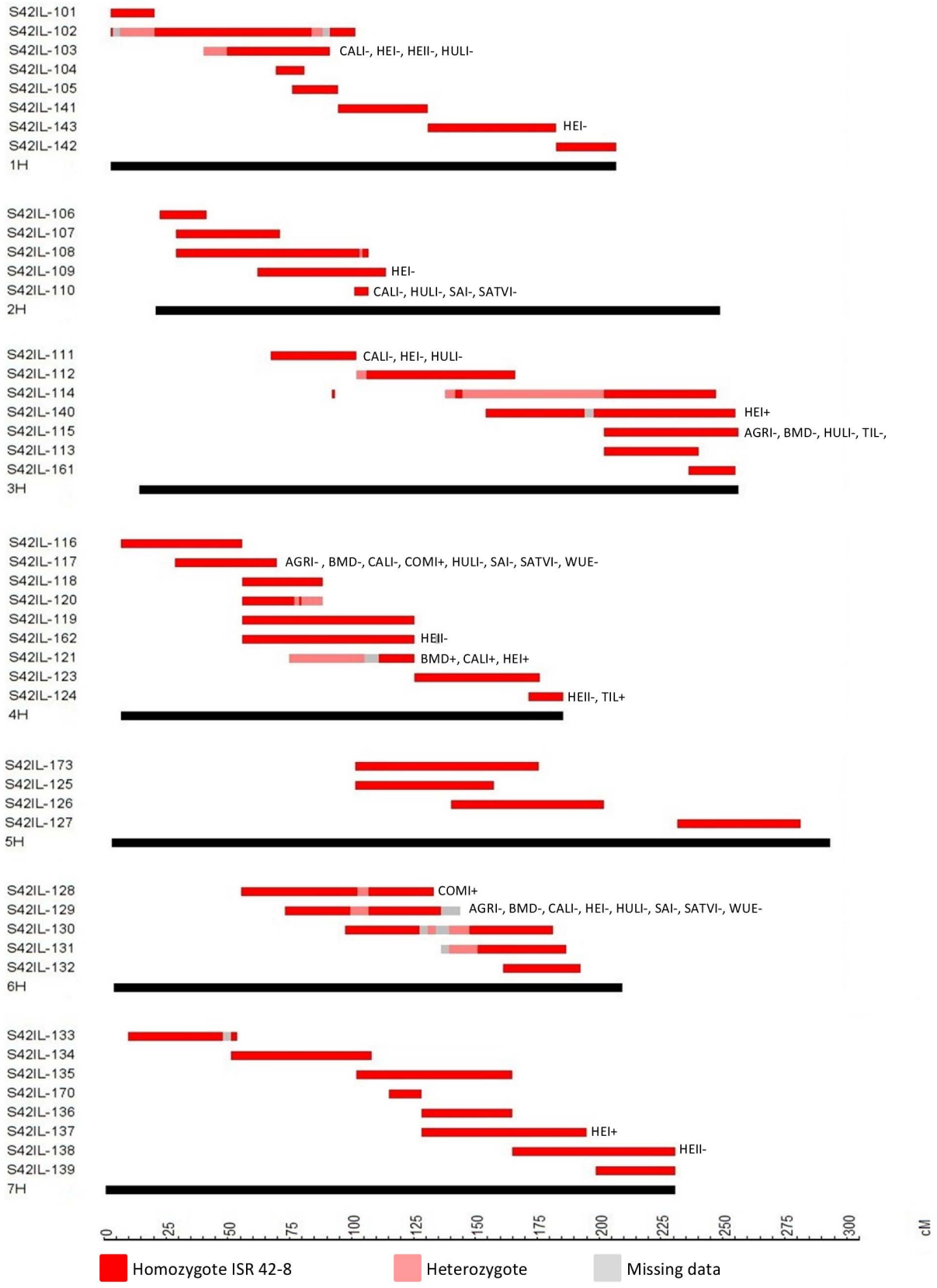

Figure 5. QTL map with indication of S42IL introgressions (Schmalenbach et al. 2011). SNP positions (in cM) are based on Close et al. (2009). QTL are placed right to the S42IL, indicated by trait abbreviations (see Table 1). The sign indicates an increasing (+) or a decreasing (−) *Hsp* effect.
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SATVI, TIL, and WUE. In the following, the QTL are presented for each trait separately.

Absolute Growth Rate Integral (AGRI)

Three QTL were identified for AGRI. The QTL are located on chromosomes 3H, 4H and 6H and the *Hsp* allele in all three QTL reduced the trait performance. Across treatments the *Hsp* alleles reduced the integral of the absolute growth rate by approximately 30%. Under drought conditions the *Hsp* allele of QTL QAgri.S42IL-4H reduced the trait performance by almost 40%.

Biomass Dry (BMD)

For biomass four QTL were identified across treatments. The *Hsp* alleles at QTL QBmd.S42IL-3H and QBmd.S42IL-6H on chromosomes 3H and 6H reduced biomass by approximately 33%. The two QTL QBmd.S42IL-4H and QBmd.S42IL-4Hb on chromosome 4H showed contrary effects. While at the first QTL the *Hsp* allele reduced biomass by 40.3%, at the second QTL it increased it by 36.0%.

Caliper Length Integral (CALI)

Six QTL were detected for caliper length on chromosome 1H, 2H, 3H, 4H and 6H. All QTL were detected across treatments, three and two of them also showed effects for drought and well watered treatments, respectively. In five cases the *Hsp* allele had decreasing effects between 14.8 and 22.6% across treatments. In one case the *Hsp* allele at QTL QCali.S42IL-4Hb increased caliper length by 15.2% compared to Scarlett.

Compactness Integral (COMI)

For COMI two QTL were detected on chromosomes 4H and 6H. The effect of the *Hsp* allele at QComi.S42IL-4H was observed across treatments and under well watered treatment. It increased compactness across treatments by 29.8%. In case of QComi.S42IL 6H the effect was solely observed under well watered treatment and lead to an increase of 27.1% by the *Hsp* allele.

Height (HEI)

The highest number of QTL was detected for plant height. Eight QTL were detected across treatments. Of those, four also showed effects under either one or both of well watered and drought treatment. The QTL are located on all chromosomes except 5H. In five cases the *Hsp* alleles reduced plant height (9.2 to 12.8%). In three cases the *Hsp* allele increased plant height by 11.6 to 18.7%.

Height Integral (HEII)

Five QTL across treatments were detected for HEII on 1H, 4H and 7H. Two of those, QHeii.S42IL-1H and QHeii.S42IL-1Hb, coincided with QTL for manual measurement of HEI. At all detected QTL the *Hsp* alleles reduced HEII by between 13.9 to 15.6%.

Hull Area Integral (HULI)

For HULI six QTL were detected on chromosomes 1H, 2H, 3H, 4H, and 6H. In all cases the *Hsp* alleles reduced the hull area by between 30.9 to 37.4% across treatments. In addition, QHuli.S42IL-1H and QHuli.S42IL-2H showed effects under well watered and drought treatments, respectively.

Shoot Area Integral (SAI)

Three QTL were found for the integral of the projected shoot area. The QTL are located on chromosomes 2H, 4H, and 6H. All three QTL were detected under drought treatment, while, in addition, QSai.S42IL-4H and QSai.S42IL-6H showed effects across treatments. The presence of the *Hsp* allele reduced the projected shoot area between 30.4 and 37.1% across treatments.

Shoot Area Top View Integral (SATVI)

Three QTL were detected for shoot area top view that corresponded to the same QTL detected for SAI on chromosomes 2H, 4H, and 6H. However, only QSatvi.S42IL-2H was detected under drought treatment, while the two other QTL were detected across treatments. In all cases the *Hsp* alleles reduced SATVI between 33.6 and 46.2% compared to Scarlett.

Tiller Number (TIL)

For tiller number two QTL were identified on chromosomes 3H and 4H. The *Hsp* allele at QTil.S42IL-3H reduced tiller number by 27.6% across treatments. QTil.S42IL-4H was detected across treatments and separately within the two treatments. Across both treatments the *Hsp* allele increased the tiller number by 42.9%.

Water Use Efficiency (WUE)

Two QTL were detected for WUE. The *Hsp* allele of QTL QWue.S42IL-4H and QWue.S42IL-6H reduced WUE by 36.5 and 40.6% under drought treatment, respectively.

Relative Growth Rate Integral (RGRI) and Plant Hue Integral (HUEI), Specific Plant Weight (SPW), and Simple Stress Index (SSI)

For RGRI, HUEI, and SPW and the SSIs no QTL were detected in this study.

Discussion

The aim of the study was to validate the use of non-destructive high-throughput phenotyping to measure vegetative drought response in barley and to identify QTL derived from wild barley that control physiological traits related to drought stress. To the authors knowledge this is the first QTL report on drought stress that used a high-throughput phenotyping facility.

Plant growth and the biomass parameters tiller number, plant height, and shoot dry weight of 48 barley genotypes were investigated under drought and well watered treatments. Two week old barley plants were transferred into a high-throughput phenotyping greenhouse, where stress and control treatments were applied automatically. During the following four weeks of cultivation, plants were imaged daily in an automated manner. Images were processed and used as a measure for plant height, caliper length, biomass and, consequently, plant growth, and plant color. After a total of six weeks green plants were harvested. Tiller number, plant height, and shoot dry weight were determined for each plant. Moreover, plant compactness, water use efficiency and specific plant weight, as well as stress indices were calculated.

The mixed model ANOVA revealed a clear effect of the treatment on trait expression. Drought stressed plants had a lower growth rate and subsequently produced less biomass (see Fig. 4). However, there was no significant line×treatment interaction, indicating that the S42ILs reacted similar under drought stress and well watered conditions for the investigated traits. This finding is also supported by high autocorrelations for investigation of traits under drought and control treatments, e.g. 0.77 for biomass (see Table S3).

QTL Detection

In this study 44 QTL were identified in 15 ILs for eleven traits. In eight cases the *Hsp* alleles increased the performance of the trait, while in 36 cases there was a decreasing effect. This is to be expected since wild barley is known to carry many unfavorable alleles as well [5]. In six ILs only one QTL was determined, predominantly for HEI and HEII. Multiple QTL effects were found in nine ILs. For S42IL-115,-117, -121, and -129 QTL for BMD were detected in combination with one or more of the traits AGRI, HEI, SAI, and TIL which is in agreement with the high correlations found between those traits. SAI is a measure for biomass, and AGRI is the first derivative of SAI. Therefore, it was expected that ILs show effects for all three traits, simultaneously. However, this was not always the case. Thus, increasing the number of experiments and replications might be useful to increase the power of QTL detection. In the following, the traits or trait complexes are discussed separately.

Absolute Growth Rate Integral, Shoot Area Integral and Biomass Dry (AGRI, SAI, BMD)

The three traits were highly correlated with each other ($r = 0.98$ and $r = 0.99$). Since their relation may be functional, it appears likely that a single pleiotropic QTL may control the three traits AGRI, SAI and BMD, simultaneously.

For line S42IL-129 a biomass reduction of 33.9% was observed. March et al. (in prep.) found a similar decrease in biomass in that line measured under terminal drought stress. This suggests that biomass production may be partly controlled by similar genes during early and late drought stress occurrence.

Three QTL were detected for SAI. Two of those, namely QSai.S42IL-4H and QSai.S42IL-6H, in line S42IL-117 und S42IL-129, respectively, were in accordance with BMD QTL. The *Hsp* allele, in both lines caused a decrease in the projected shoot area. Due to the high correlation of the traits it can be assumed that QTL for biomass correspond to QTL for shoot area. The *Hsp* allele of the QTL QSai.S42IL-2H on chromosome 2H caused a decrease of 37% in projected shoot area, compared to Scarlett. Von Korff et al. [10] described a QTL related to biomass reduction (QMas.S42-2H.a) in the same region in an AB-QTL field trial. Since this AB-QTL population is the parent population of the S42ILs used in this study, it is likely that the same QTL was detected in both the greenhouse and field trials.

AGRI is directly related to SAI. This might be seen as a reason for the detection of similar QTL for AGRI and SAI and, consequently, BMD. Many QTL studies on growth focus on relative growth rate instead of absolute growth rate. In the present study RGRI showed only a weak correlation to AGRI ($r = 0.35$) and other biomass parameters. Poorter et al. [33] pointed out that in their study QTL for RGR rather co-located with QTL for seed mass than with QTL for biomass. This fits well to the weak correlations found between biomass parameters and RGRI. However, two of the QTL detected for AGRI coincided with locations where previous studies mapped QTL for RGR. Yin et al. [34] reported a minor effect for relative growth rate associated with the *denso* locus on chromosome 3H in a spring barley recombinant inbred line (RIL) population of the cross Prisma×A-pex, which may co-localize with QAgri.S42IL-3H. Poorter et al. [33] conducted QTL studies in a F_2 population derived from a cross between two *Hsp* accessions. They mapped QTL for relative growth rate on chromosomes 1H, 2H, 5H and a minor QTL on 6H. The latter one might be in accordance with QAgri.S42IL-6H.

Tiller Number (TIL)

Two QTL were detected for the trait tiller number on chromosomes 3H and 4H. Wang et al. [35] identified the *VRN-H2* gene on chromosome 4H in introgression line S42IL-124. Whereas S42IL-124 carries a dominant winter-type allele, Scarlett carries the recessive and deleted spring type allele at *Vrn-H2*. S42IL-124 showed an increased tiller number compared to Scarlett. Since *Vrn-H2* is known to have a pleiotropic effect on tiller number [36], we assume that this gene explains the underlying effect of the QTL. Studies on other populations revealed QTL for tiller number on chromosomes 4H as well. In a cross between two wild barley accessions Elberse et al. [37] found a QTL for tiller number on that chromosome. Baum et al. [38] identified a QTL on chromosome 4H where the *Hsp* allele increased the number of tillers and a QTL on chromosome 3H where the *Hsp* allele had a decreasing impact in an Arta×*Hsp* 41-1 RIL population. Those effects might correspond to the QTL detected in this study. Both QTL occurred irrespective of the treatment. Especially QTil.S42IL-4H appears to be a very stable QTL. It was detected across and within treatments and was detected in several studies under varying conditions, in field studies as well as under greenhouse conditions. Moreover, von Korff et al. [10] detected QTL for number of ears, which is directly related to tiller number, in the same genomic regions. On 4H the *Hsp* allele increased the number of ears, while on 3H it has a decreasing effect. This supports the observation of a stable QTL.

Height (HEI) and Height Integral (HEII)

Plant height was determined in two ways. First, height (HEII) was modeled from the images taken during four weeks and the integral of the height growth curve was calculated. Second, height (HEI) was measured manually when plants were harvested after six weeks at the end of the experiment. The correlation between HEI and HEII was relatively low with $r = 0.72$, compared to the correlation between SAI and BMD with $r = 0.98$. While SAI shows a constant increase over time, HEII shows an overall increase, but fluctuation between days may be strong. When a new leaf is unfolded the plant grows higher, however, when the leaf becomes too heavy and bends down, the height of the plant appears to be shorter. At the end of the experiment the length of the stretched plant was measured, which is longer than the upright standing plant. Nevertheless two coinciding QTL were found between HEI and HEII on chromosome 1H.

Six out of eight QTL were already identified in previous field studies with the S42 population and/or the S42ILs, exhibiting similar effects of the same direction in all three studies. QTL QHei.S42IL-3Hb was already detected in von Korff et al. [10] and March et al. (in prep.). In this region also the *denso* dwarfing gene was mapped [39], which may be identical with the semi-dwarf gene *sdw1* [40]. The second largest effect, after QHei.S42IL 3Hb, was associated with QHei.S42IL-4H in S42IL-121. This QTL corresponds to QHei.S42IL-4H.a in Schmalenbach et al. [15]. In both studies the *Hsp* allele increased plant height by 18%. March et al. (in prep.) mapped a QTL for height for S42IL-121 as well. A third QTL (QHei.S42IL-7H) with an increasing effect of the *Hsp* allele was detected on chromosome 7H. Here an effect that was already found in the studies of von Korff et al. [10], Schmalenbach et al. [15] and March et al. (in prep.) could be verified. Moreover two QTL where the *Hsp* allele had a decreasing effect on plant height [10] were verified. In S42IL-143 HEI was reduced by 11% (QHei.S42IL-1Hb) and HEII (QHeii.S42IL-1Hb) by 14%. Von Korff et al. [10] detected a QTL in the same region on chromosome 1H. The flowering time gene *HvFT3* is mapped in the same region and known to have a pleiotropic effect

on plant height [35]. QHei.S42IL-2H in S42IL-109 had reduced height by 9.5%. March et al. (in prep.) and Schmalenbach et al. [15] found the same effect in their studies. Von Korff et al. [10] found a similar effect in the region where the *Hsp* introgression of S42IL-109 was mapped. Moreover two candidate genes are mapped to the chromosomal region. These are the dwarfing gene *sdw3* [41] and the flowering gene *HvFT4*, which is known to have an effect on plant height [35].

All of the HEI QTL in the present experiments were detected across treatments. Six out of eight QTL were also found in previous field and glasshouse studies. The QTL therefore seem to be very stable across locations as well as across treatments. Moreover they seem to be independent of the developmental stage. The present experiments, thus, allowed the verification of effects after six weeks that were previously screened in field experiments after flowering, indicating that phenotyping juvenile plants may be predictive for adult plant performance, at least in regard to growth parameters. The high heritability of 76.8% supports this finding.

For HEII two QTL coincided with previous studies. Besides QHeii.S42IL-1Hb mentioned above, this was QHeii.S42IL-4Hb where the *Hsp* allele reduced height by 14%. This QTL was also detected by von Korff et al. [10] and Wang et al. [35]. Heritability for digitally determined height was lower (61.4%) than for the manually measured one. Determining height by multiple measurements apparently was not an advantage here. However, this may change at a later stage of plant development. After shooting, the plant height is less subjected to bending of leaves and therefore can be measured more precisely by the imaging technique.

Water Use Efficiency (WUE)

Water use efficiency indicates how much biomass a plant can produce per unit water supplied. Thus, increased WUE has the potential to improve yield under drought stress conditions. Measuring WUE in regular greenhouse experiments is time-consuming. Therefore the high-throughput phenomics facility greatly assisted in scoring of water use efficiency through automated watering of pots to specific weights.

In this study the two S42ILs -117 and -129, with wild barley introgressions on chromosomes 4H and 6H, respectively, showed significant differences in WUE compared to Scarlett. Both ILs showed reduced water use efficiency compared to Scarlett. These ILs also produced less biomass. S42IL-117 and S42IL-129, thus, clearly carry unfavorable alleles for this trait. Chen et al. [42] pointed out that WUE itself is difficult to measure under field conditions and that a suitable tool to measure WUE efficiency is missing. Carbon isotope discrimination is a commonly used technique to measure WUE. Teulat et al. [43] used this method and identified QTL for WUE on chromosome 6H in a set of 167 RILs from a cross between Tadmor and Er/Apm and likewise Diab et al. [44] identified a QTL for the same trait on chromosome 4H. The QTL detected in this study may correspond to the ones found in the studies mentioned before and suggest the results from both techniques are correlated.

Compactness Integral, Shoot Area Top View Integral, Hull Area Integral (COMI, SATVI and HULI)

The compactness of a plant describes how much of the hull area is covered by leaves. It was calculated as the ratio of SATVI to HULI. The more compact a plant is, the more ground cover it has with regard to the hull area. Two QTL were detected for this trait.

SATVI and HULI showed a high correlation of $r = 0.9$, however, correlations between COMI and HULI and between COMI and SATVI were only moderate and negative. This indicates that in general, bigger plants take more space and have a lower compactness compared to smaller plants. In the present experiments this was observed by comparing drought stressed and well watered plants. Drought stressed plants showed on average a higher compactness than well watered plants. Jansen et al. [45] report the same effect on a study in *Arabidopsis thaliana*. Compactness shows negative correlation with all other traits evaluated in this experiment, with the exception of HEII ($r = 0.16$). An example for this is S42IL-117. This introgression line has a higher compactness, but reduced biomass, and other growth parameters compared to Scarlett.

SATVI is one of the three parameters that control SAI and, thus, is highly correlated with this trait as well as with BMD and AGRI. As one may expect, for SATVI the same QTL were detected as for SAI. For HULI a total of six QTL were detected. Three of those may be due to high correlations in accordance with SATVI, AGRI and BMD.

Caliper Length Integral (CALI)

Caliper length describes the maximum diameter of the plant. For this trait six QTL were detected. Those were in accordance with QTL for HULI. This can be explained by the close connection of both traits. Hull area is taken as the basis to calculate caliper length and both traits are highly correlated ($r = 0.94$). CALI also shows positive and high correlations with AGRI, SAI, SATVI, HEI, and BMD. Plants with a large diameter cover a larger area, tend to be bigger, have a higher growth rate and a higher biomass than plants with a smaller diameter. Therefore, a lot of information on plant structure can be deduced from the plant diameter.

Stress Indices

Simple stress indices were calculated for each trait as the ratio of the mean plant performance under drought stress versus well watered treatments. In this study no QTL for a SSI was detected. Additionally the authors used two more complex stress indices (modified after Fischer and Maurer [46]), but were not able to detect QTL with those either. A stress index states how well a genotype performs under stress conditions relative to its performance under control conditions. Therefore, to see differences between genotypes for a stress index, a line by treatment interaction is necessary. If all genotypes show a similar growth reaction under stress and control conditions, the initially existing differences between the genotypes may be drastically reduced. In the present experiments line by treatment interactions were not significant and autocorrelations were high between the treatments. This may be the reason why no significant effect for the stress indices was found. This notion is supported by Wang et al. [47]. In their study on "mathematically-derived traits in QTL mapping" the authors pointed out that an increased complexity of the genetic architecture of derived traits (e.g. stress indices) may reduce the power of QTL detection.

High-throughput Phenotyping using The Plant Accelerator

Determination of biomass by manual harvest is tedious and time-consuming. In addition, destructive harvest makes repeated measurements on the same plant impossible. Visual light imaging technologies applied in this study can solve these problems by utilizing the strong correlation between the projected shoot area and the actual biomass [27]. Imaging technologies have been successfully used in several studies in *Arabidopsis thaliana*, e.g. Granier et al. [48] and Leister et al. [49]. The first study

investigated nine accessions under different levels of water deficit in the phenotyping facility "PHENOPSIS". Reaction to water deficits was, amongst other traits, characterized by leaf area growth determined through images. The authors pointed out the importance of the automated watering in their experiment, which enables equal conditions for all plants. A characteristic that was also found very important in the present experiments. Leister et al. [49] described a first approach of using an image based technology for high-throughput growth analysis. They calculated plant area from top view images and found high correlations to plant fresh weight.

In this study the sum of three two-dimensional pictures was used as a measure of plant biomass. In these experiments correlation between SAI and BMD and between AGRI and BMD were very high ($r = 0.98$). The results with six-week old barley plants proved that the sum of three pictures accounts sufficiently for overlapping leaves during early development. Rajendran et al. [30] found the same for *T. monococcum*. However, as Munns et al. [27] pointed out, accuracy may decline when plants become larger and produce multiple shoots. The results of the present study approved that The Plant Accelerator is suited to enable detailed growth analysis of barley plants. The prediction of biomass by the image-based leaf sum gave accurate results when comparing to actual biomass. Growth curves can give detailed information on differences in development of genotypes. For instance, the maximum of the absolute growth rate gives insight into the change from vegetative to generative phase of plant development. The present experiments ran only for six weeks. Therefore not all plants have reached this point. In future experiments this factor should be accounted for by adjusting the duration of the experiment. Automated imaging and the appropriate analysis pipeline make the detection of different developmental stages of plants feasible in high-throughput. With this technique it is possible to detect differences in stress responses between genotypes not only at different time points, but also to account for differences in development at those time points.

Rajendran et al. [30] used non-destructive imaging to screen for different response mechanisms of *T. monococcum* to salt stress. In contrast to conventional salt stress experiments, where tolerance is measured as total biomass production of stressed plants compared to unstressed plants, the growth curves provided through daily imaging gave detailed insight into the tolerance mechanisms of the plants. While osmotically tolerant plants showed a constant growth rate, the growth rate of sodium excluders first dropped than increased after a couple of days. Moreover plant color was analyzed. No stress symptoms occurred on leaves in the present experiments. Due to little variation between the genotypes no QTL was detected for leave color. However, in the experiments by Rajendran et al. [30], color analysis was successfully used to screen leaf damages due to high salt concentrations.

Fluorescence imaging gives information on the health statues of a plant. It allows for detection of leaf senescence and necrosis. However, such symptoms were not observed in the present experiments and, thus, this parameter was not applied. Nonetheless, the technique is readily available. In addition, near infrared (NIR) and infrared (IR) imaging may be useful for future plant growth evaluations and QTL studies. NIR enables the observation of the water status of a plant, while IR is used to determine shoot temperature.

Conclusion

In this study the use of a non-destructive high-throughput phenotyping platform was implemented to map QTL controlling vegetative drought stress responses in barley. Several QTL where the exotic *Hsp* allele had a positive effect on trait performance were detected. In particular, introgression line S42IL-121 showed improved growth under drought stress compared to the recurrent parent Scarlett. The line showed the same behavior in previous field experiments. Thus, this introgression line might be interesting for further breeding.

Moreover, several QTL were detected where the *Hsp* allele had a decreasing effect on trait performance. Especially two QTL for water use efficiency might be interesting for further investigation. In future, interesting effects of S42IL-121 and other S42ILs will be fine mapped with already available high-resolution progeny [18] to further narrow down the QTL region and, ultimately, clone the underlying genes, which caused the observed QTL effects.

Supporting Information

Figure S1 Map of 47 S42ILs, the map contains 636 BOPA1 SNPs.
(PPTX)

Table S1 Experimental layout with genotypes referred to positions (1–100) per replication (1–3).
(XLSX)

Table S2 Descriptive statistics for S42Ils.
(XLSX)

Table S3 Correlations between traits and stress indices.
(XLSX)

Table S4 ANOVA (Model I) results of studied traits.
(XLSX)

Table S5 ANOVA (Model II drought) results of studied traits.
(XLSX)

Table S6 ANOVA (Model II well watered) results of studied traits.
(XLSX)

Table S7 ANOVA (Model II SSI) results of studied traits.
(XLSX)

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Author Contributions

Conceived and designed the experiments: TJM BB MT KP. Performed the experiments: NH TJM BB. Analyzed the data: NH BB. Contributed reagents/materials/analysis tools: BB KP. Wrote the paper: NH KP. Provided comments and corrected the manuscript: TJM BB MT KP.

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Evaluation of juvenile drought stress tolerance and genotyping by sequencing with wild barley introgression lines

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Abstract Drought is a major stress which can seriously limit yield in many crops including barley. Wild barley introgression lines (ILs) like the S42IL library may enhance drought stress tolerance of barley cultivars through the introduction of exotic alleles. The S42IL library was already characterized with 636 Illumina SNPs. New approaches like genotyping by sequencing (GBS) are available for barley to enhance the characterization of ILs. We generated an improved genetic map of the S42IL library, consisting of 4,201 SNPs by adding GBS data. The new map with a total length of 989.2 cM confirmed the extent of wild barley introgressions. Adding GBS data increased the resolution of the S42IL map tenfold from 0.4 to 4.2 markers/cM. This may assist to select possible candidate genes that improve drought tolerance. In four greenhouse experiments, juvenile drought stress response of 52 barley S42ILs was tested to identify quantitative trait loci (QTL). Thirteen S42ILs showed effects for plant biomass and leaf senescence. Subsequently, two verification experiments were conducted with these S42ILs. Nine out of eleven QTL were verified, and 22 additional QTL were detected. For 21 QTL, the *Hsp* allele increased trait performance, indicating the value of wild barley introgressions. For example, S42IL-107 and S42IL-123 produced more biomass under drought. Two different water-saving strategies were observed. S42IL-143 and S42IL-129 both revealed increased relative water content under drought. While S42IL-143 reduced biomass under drought, S42IL-129 maintained a high biomass production. We recommend using S42IL-107, S42IL-123 and S42IL-129 in barley breeding programs to enhance drought tolerance.

Keywords Barley · *Hordeum vulgare* spp. *spontaneum* · Introgression lines · Drought tolerance · Genotyping by sequencing (GBS)

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Introduction

Drought is the most important factor causing yield loss worldwide (Pennisi 2008). With increasing climate

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variability, more extreme weather events are expected and a higher frequency of drought events (Tester and Langridge 2010). Drought that occurs shortly after sowing can lead to inhomogeneous plant development and plant death and, thus, to severe yield losses. While many studies on drought tolerance focus on late developmental stages, drought stress tolerance at juvenile stage has been investigated less intensively (Tyagi et al. 2011a). Drought tolerance at the juvenile stage might have a positive influence on yield. It has been suggested that improved early vigor and rapid development of maximum leaf area may enhance yield performance (Hafid et al. 1998; Lu and Neumann 1998). López-Castañeda and Richards (1994) pointed out that in water limited environments, barley (*Hordeum vulgare* ssp. *vulgare* hereafter abbreviated with *Hv*) yield is on average higher than the yield of wheat, oat and triticale. The authors pointed out that a fast and vigorous barley growth during early vegetative development might play an important role toward final yield. Therefore, variation in this trait might be of importance in breeding for drought tolerance. Several studies were carried out on the investigation of early vigor and biomass production in wild and cultivated barley (e.g., Borràs-Gelonch et al. 2010; Tyagi et al. 2011b; Lakew et al. 2013).

Compared to other cereals, barley has good tolerance to drought, cold and salt stress (Ullrich 2011) and as a result is often grown in marginal environments (Jana and Wilen 2005). However, further progress in breeding toward increased tolerance to abiotic stress is limited by a lack of genetic diversity in the elite barley gene pool. During the process of domestication and especially with the onset of modern breeding, genetic diversity of the barley gene pool has declined. Diverse landraces have been replaced by modern elite varieties with a much narrower gene pool (Zamir 2001). In order to overcome this limitation, Zamir (2001) proposed the use of wild relatives of crop plants to diversify the genetic composition of elite breeding gene pools. In barley, many studies have been conducted that investigate the positive effects of wild barley (*Hordeum vulgare* ssp. *spontaneum*, hereafter abbreviated with *Hsp*) in crosses with *Hv* on plant performance and their potential use in plant breeding (e.g., Baum et al. 2003; Li et al. 2006; Lakew et al. 2011). The method of 'advanced backcross quantitative trait loci analysis' (AB-QTL) was introduced by Tanksley and Nelson (1996). This method integrates the introduction of favorable exotic alleles from wild donor plants and QTL detection. AB-QTL populations are produced by several rounds of backcrossing with the recipient and marker-assisted selection (MAS). Ideally, each line contains only a single introgression from the wild parent and is then referred to as introgression line (IL). The donor segments should ideally cover the entire donor genome (Zamir 2001). The first AB-QTL study in barley was published by Pillen et al. (2003). Von Korff et al. (2004) crossed the German spring barley variety Scarlett with the wild barley accession ISR42-8 from Israel to produce a BC_2DH population (S42). The S42 population was tested in several AB-QTL studies to detect QTL for pathogen resistance, yield and malt quality (von Korff et al. 2005, 2006, 2008, 2010; Saal et al. 2011). Schmalenbach et al. (2008) developed 59 ILs (S42ILs) by applying another round of backcrossing to the S42 population and subsequent selfing and MAS. Each IL contains one or a small number of *Hsp* introgressions. The S42IL library was tested in several QTL studies for verification of QTL previously detected in AB-QTL studies as well as for detection of new QTL for yield, resistance and quality traits (Schmalenbach et al. 2008, 2009; Schmalenbach and Pillen 2009; Hoffmann et al. 2012; Schnaithmann and Pillen 2013). The S42IL library was extended to 73 lines and genotyped with 1,536 SNPs of the Illumina BOPA1 set (Schmalenbach et al. 2011). Of those markers, 636 were polymorphic in the population. Their known map order (Close et al. 2009; Muñoz-Amatriaín et al. 2011) enable precise localization of the *Hsp* introgressions. The complete set of S42ILs represents 87.3 % of the wild barley donor genome. For further enabling of fine-mapping, Schmalenbach et al. (2011) developed segregating high-resolution mapping populations (S42IL-HRs) for 70 S42ILs. Those lines are readily available for fine-mapping and cloning of QTL.

Elshire et al. (2011) recently proposed the highly multiplexed method of genotyping by sequencing (GBS) to generate large numbers of genomewide SNP markers. GBS uses restriction enzymes to create reduced representation libraries of the target genome. Individual genotypes are barcoded and sequenced in parallel on a next-generation sequencing platform. Poland et al. (2012) developed a protocol for barley using two different restriction enzymes. This methodology opens the way to further genetically characterize the S42IL library.

The goals of this study were (1) to identify S42ILs that reveal higher stress tolerance than the recipient cultivar Scarlett, (2) to locate QTL controlling the investigated traits and (3) to further characterize the S42IL library with GBS generated SNPs.

Material and methods

Plant material

The plant material included 55 wild barley ILs of the S42IL library and the elite barley cultivar Scarlett. All 55 S42ILs were genotyped, while for the phenotyping only 52 ILs were used. Due to limited greenhouse space and genotyping capacities, it was not possible to include all 73 S42ILs in the study. We, thus, selected a subset representing a representative proportion of the *Hsp* genome. The S42IL library was generated by an initial cross of the malting barley cultivar Scarlett and the wild barley accession ISR42-8 followed by several rounds of backcrossing, selfing (up to $BC_3S_{4:8}$) and MAS as indicated by Schmalenbach et al. (2008).

Experimental setup

The genotypes were screened for their response to drought stress at the juvenile stage. To assess drought stress response, we conducted dry down experiments in a greenhouse at the 'Kühnfeld' experimental station of the Martin-Luther University in Halle, Germany. In 2011, four experiments were carried out following an identical design, two during spring and two during autumn, each lasting 34 days. The S42ILs were grown with ten plants per pot in 1.5 kg of soil ('Einheitserde Typ ED 73'). Each experiment was carried out with two treatments and in three replications per treatment. Treatments differed in plant available water supply. On the day of sowing, all pots were irrigated with 400 mL of water. One week after germination, automatic watering of control plants by capillary mat irrigation started, whereas drought-stressed plants did not receive any additional water. In spring 2012, two verification experiments were conducted with those 13 genotypes, which in the 2011 experiments had shown a significant deviation in trait performance from Scarlett (Table 3). The selected S42ILs were tested under the same experimental conditions except there were six replications instead of three, and the control plants were hand-watered with 200 mL water every second day, starting one week after planting. Greenhouse conditions were semi-controlled. Temperatures were controlled by heating and ventilation. Target temperatures in the greenhouse were between 14 °C at night and 20 °C during the day. Artificial lighting was supplied between 8 a.m. and 8 p.m. when natural radiation dropped below 20 klx.

Table 1 Investigated traits and trait definitions

Trait	Abbr.	Unit	Method of measurement
Physiological parameters			
Photosystem II efficiency	PAM	Unit free	Six primary leaves per pot measured[a]
Leaf greenness	SPAD	Unit free	Average of three measurements on six primary leaves per pot[b]
Water content	Wcon	g	BMF [g] − BMD [g]
Relative water content	RWC	%	(Wcon/BMF) × 100
Harvest parameters			
Tiller number	TIL	Unit free	Number of tillers per pot
Height	HEI	cm	Plant height measured from bottom to leaf tip
Biomass fresh	BMF	g	Weight of fresh biomass per pot
Biomass dry	BMD	g	Weight of oven dried biomass per pot (after 2 days at 80 °C)
Index			
Simple stress index	SSI	Unit free	Ts/Tc[c]

[a] PAM-2500, WALZ, Effeltrich, Germany

[b] SPAD-502 meter (Minolta Camera Co., Osaka, Japan)

[c] Ts: trait performance of an S42IL under drought, Tc: trait performance of an S42IL under control treatment

Phenotyping

An overview of traits investigated and trait definitions is given in Table 1. To detect drought stress-induced leaf senescence, leaf greenness and photosystem II efficiency was measured on primary leaves using a SPAD-502 meter (Minolta) and a Mini-PAM (Walz), respectively, on the last two days before completion of

the experiment. At the completion of the experiment, all plants were harvested and fresh biomass (BMF), tiller number (TIL) and plant height (HEI) were determined per pot. Subsequently, the plant material was oven dried for two days at 80 °C. Dry biomass (BMD), water content (Wcon) and relative water content (RWC) were calculated for complete above ground plant material per pot. Stress indices were calculated for all genotypes investigated in the verification experiments. Simple stress indices (SSI) were calculated for each trait as: SSI = Ts/Tc, where Ts and Tc are trait performances of an S42IL under stress and control condition, respectively (modified after Fischer and Maurer (1978).

Genotyping

The S42ILs and Scarlett were already genotyped with 636 informative Illumina GoldenGate BOPA1 SNPs (Schmalenbach et al. 2011). For this experiment, the population was additionally characterized with GBS as previously described by Poland et al. (2012). Genomic DNA (200 ng) of individual S42ILs was double digested with *Pst*I–*Msp*I. All individuals were then ligated with unique barcoded adapters and pooled into a single sequencing library and sequenced on a single lane of an Illumina HiSeq 2000 at the Australian Genome Research Facility Ltd (Australia). Bi-allelic SNP markers were called using the Tassel UNEAK pipeline (Lu et al. 2013). Viroblast (Deng et al. 2007) was used to align the GBS and BOPA1 SNP marker sequences to the barley whole genome shotgun (WGS) contigs (Mayer et al. 2012). The BLAST procedure was executed with default settings. The position of the best hit for each SNP marker was used to assemble a first draft of the S42IL genetic map. If a marker seemed to be in the wrong position (e.g., unexpected double crossovers), the second best BLAST hit was evaluated.

Statistical analysis

SAS Enterprise Guide 4.2. (Institute SAS 2008) was used to perform statistical analyses. Descriptive statistics were calculated with the SAS procedure MEANS. We calculated heritability across treatments as $h^2 = V_G/[V_G + V_{GT}/t + V_{GE}/e + V_{GET}/et + V_R/etr]$ and within treatments as $h^2 = V_G/[V_G + V_{GE}/e + V_R/er]$ (Becker 2011). V_G, V_{GT}, V_{GE}, V_{GET} and V_R represent the genotypic, genotype × treatment, genotype × environment, genotype × environment × treatment and error variance components, respectively. Variance components were calculated with the SAS procedure VARCOMP. The procedure CORR was used to calculate Pearson's correlation coefficients between traits using means across treatments, experiments and blocks and, in addition, across experiments and blocks within treatments.

A mixed-model analysis of variance was applied with the SAS procedure MIXED using model I for main effects across treatments:

$$Y_{ijkl} = \mu + L_i + T_j + E_k + L \times T_{ij} + L \times E_{ik} + B(E \times T_{kj})_l + \varepsilon_{ijkl} \quad (1)$$

and model II for effects within each treatment:

$$Y_{ikl} = \mu + L_i + E_k + L \times E_{ik} + B(E_k)_l + \varepsilon_{ikl} \quad (2)$$

In this equation, μ is the general mean, L_i is the fixed effect of the ith line, T_j is the fixed effect of the jth treatment, E_k is the fixed effect of the kth experiment, $L \times T_{ij}$ is the fixed interaction between the ith line and the jth treatment, $L \times E_{ik}$ is the fixed interaction between the ith line and the kth experiment, $B(E \times T_{kj})_l$ is the random effect of the lth block nested in the interaction between kth experiment and jth treatment, $B(E_k)_l$ is the random effect of the lth block nested in the kth experiment and ε_{ijkl} and ε_{ikl} are the random errors of Y_{ijkl} and Y_{ikl}, respectively.

Subsequently, an a posteriori Dunnett test was carried out with procedure MIXED. Least square means (LSMEANS) of each S42IL were compared to the control Scarlett. When an S42IL showed a significant ($P < 0.05$) difference in trait performance to Scarlett, a line × trait association was assumed and the presence of a QTL was accepted. If several S42ILs with overlapping introgressions showed a similar effect, it was assumed that those genotypes contained the same QTL. IL effects were summarized to a minimum number of QTL assuming that only one gene per introgression causes the effect and IL effects in shared introgressions are caused by the same gene. In addition, the most likely location of the QTL was assumed within the target introgression because this is generally the largest introgression segment per IL. However, the precise localization of the QTL has to be validated through fine-mapping in follow-up studies using the available high-resolution S42IL libraries

(Schmalenbach et al. 2011). When two ILs, containing their main introgression at the same location, did not show a similar phenotypic effect, this was regarded a first indication that the QTL may be located in a secondary introgression instead. The relative performance (RP) of an S42IL was calculated as RP[IL] = [LSMEANS (IL) − LSMEANS (Scarlett)] × 100/LSMEANS (Scarlett), where LSMEANS were calculated across treatments, blocks and experiments from model I and, separately for each treatment, across blocks and experiments from model II.

Results

Genotyping by sequencing

A set of 55 S42ILs was genotyped using GBS. The analysis gave sufficient results for all genotypes except S42IL-114. A total of 41,554 putative SNPs were generated. SNPs were filtered based on if they were polymorphic between the two parents Scarlett and ISR42-8, had less than 10 % missing data and were not heterozygous in either parent. Of the resulting GBS markers, 3,744 could be aligned to the barley WGS contigs using BLAST. Additionally, 457 BOPA1 SNPs were included through alignment by the BLAST procedure. The resulting genetic map consisted of 4,201 SNP markers with a total length of 989.2 cM (3.9 Gb) (Fig. 1; Table S1). The target introgressions of the 54 S42ILs represent 84.9 % of the *Hsp* genome. Chromosomes 1H, 4H, 6H and 7H contain only very small regions where the *Hsp* genome is not represented. The biggest gap is on chromosome 5H, where 63.8 % of the *Hsp* chromosome is not represented. The marker density ranged from 0.8 (4H) to 1.3 (5H) SNPs per Mb and from 3.6 (4H) to 5.0 (7H) SNPs per cM. Altogether, 41 out of the 54 S42ILs showed additional introgressions apart from the target introgression. In nine S42ILs, previously uncharacterized nontarget introgressions were identified. Conversely, three S42ILs that previously showed more than one introgression are now solely characterized by the target introgression. Including nontarget introgressions, 86.6 % of the *Hsp* genome is represented by the 54 S42ILs. The average size of the target introgression is 137.3 Mb, ranging from 9.5 to 468.6 Mb. Including all target and nontarget introgressions, each line contains on average 3.4 % of *Hsp* SNP loci, ranging from 0.4 to 8.3 %. The S42ILs carry on average 0.6 % heterozygous loci with a range from 0.0 to 3.0 %. Table 2 provides details for each IL.

Drought stress results 2011 (four experiments with 52 S42ILs)

Descriptive statistics

We investigated six traits under drought and well-watered treatments. Trait performances across all genotypes are given in Table S2. The table shows the trait means, standard deviations, minima, maxima, coefficients of variation (CV) and heritabilities across treatments and within drought and well-watered treatments. In general, mean values were higher under the well-watered treatment. For instance, average BMD production under drought treatment was 1.7 g and under well-watered treatment 4.8 g. Leaf greenness (SPAD) was the only exception. At the end of the experiment, the SPAD value of the drought-stressed plants was higher than that of the well-watered plants. Coefficients of variation ranked from 9.5 % for PAM under well-watered treatment to 83.6 % for BMF across treatments. Variation was generally higher under well-watered treatment. Only the CVs of PAM and SPAD were higher under drought than under well-watered treatment. Heritabilities varied widely between traits and treatments. No heritability ($h^2 = 0$) was found for SPAD and PAM across treatments. For both traits, heritability was also rather low under the two treatments (between 3.4 and 14.8 %). The highest heritability was found for HEI (67.5 % across treatments).

Trait correlations

Trait correlations between drought and well-watered treatments varied from moderate to low for all traits. Therefore, correlations are described separately for drought and well-watered treatment (Table S3). Under drought treatment, the highest correlation was observed between SPAD and PAM ($r = 0.70$) and between BMD and BMF ($r = 0.63$). All other correlations were below $r = 0.50$ and most of them not statistically significant. Under well-watered treatment, BMD and BMF showed very high correlations of $r = 0.97$. SPAD and PAM did not show a significant correlation under well-watered treatment. Relatively

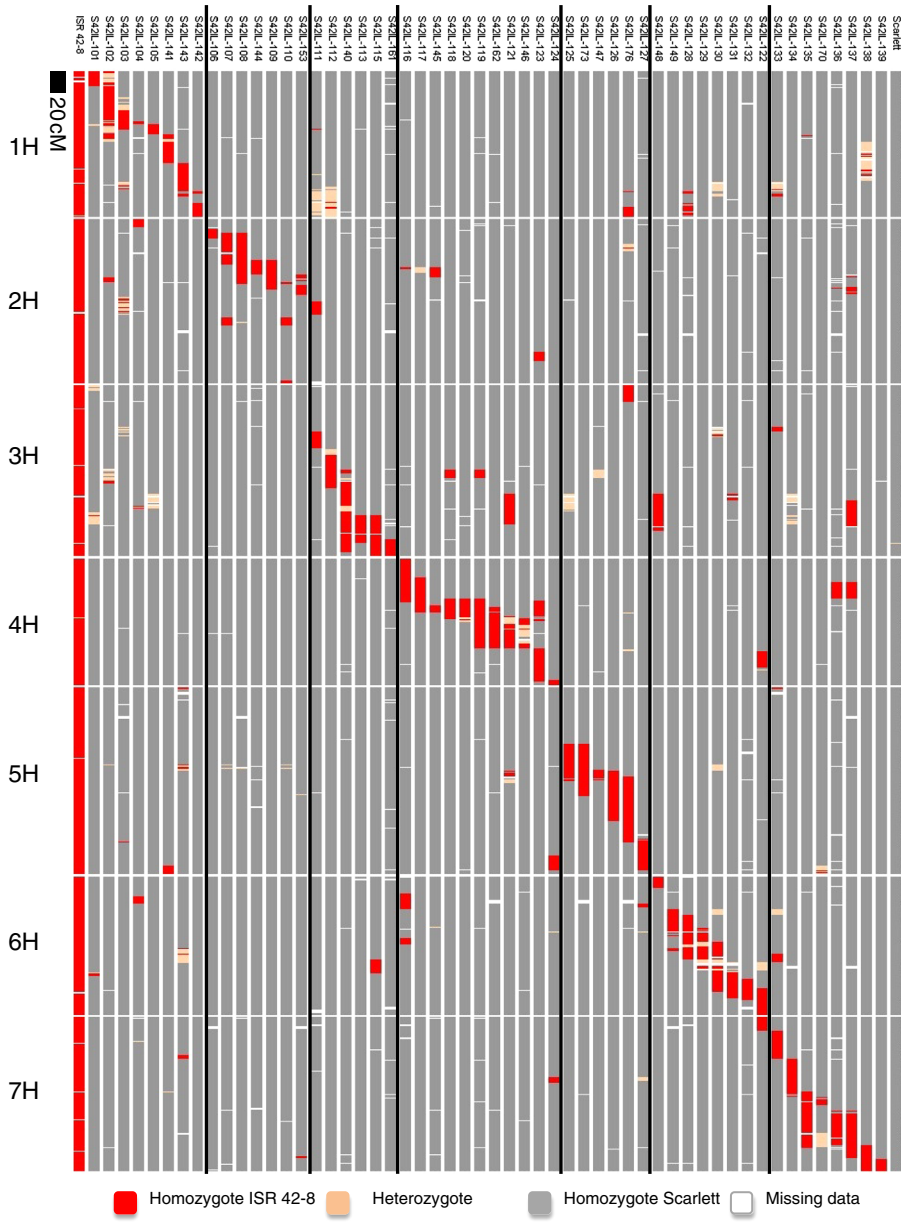

Fig. 1 Map of 54 S42ILs and the parents Scarlett and ISR42-8. The map contains 4,201 SNPs, consisting of 3,744 GBS and 457 BOPA1 SNPs

high correlation coefficients were observed for TIL with BMD and BMF ($r = 0.67$ and $r = 0.69$, respectively).

Mixed-model analysis of variance

The mixed-model analysis of variance included line, treatment and experiment as main effects, as well as the interaction between line and treatment and line and experiment. The analyses revealed significant ($P < 0.05$) line effects and line by treatment interaction effects for all measured traits (Table S4). The treatment had an effect on all traits but SPAD. The experiment had an effect on trait performance for HEI, SPAD, and PAM but not for TIL, BMF, and BMD. Line by experiment interaction effect was only significant for SPAD and PAM.

Table 2 Genetic characterization of position and extent of *Hsp* introgressions of 55 S42ILs based on genotyping by sequencing (GBS) and BOPA1 SNPs

Chr.	S42IL	Pos. of first SNP (cM)[a]	Pos. of last SNP (cM)	Min. size of target intr. (cM)	Pos. of first SNP (Mb)	Pos. of last SNP (Mb)	Min. size of target intr. (Mb)	No. add. intr.	*Hsp* %[b]	Het %	Missing %
1H	S42IL-101	0.0	13.1	13.1	0.0	9.8	9.8	4	1.0	2.6	0.1
	S42IL-102	0.0	61.5	61.5	0.0	361.0	361.0	3	5.8	2.9	0.5
	S42IL-103	24.9	53.3	28.4	13.9	303.3	289.4	4	4.0	1.7	0.5
	S42IL-104	46.5	48.9	2.4	60.3	263.2	202.8	4	2.8	0.2	1.0
	S42IL-105	48.1	57.3	9.2	154.8	329.2	174.5	1	2.1	1.2	0.4
	S42IL-141	57.3	82.5	25.2	322.7	404.6	81.9	2	2.6	0.2	0.2
	S42IL-143	84.1	108.5	24.3	404.6	439.8	35.2	5	3.2	1.3	1.3
	S42IL-142	119.7	132.7	13.0	452.9	464.1	11.1	1	1.3	0.0	0.1
2H	S42IL-106	8.9	18.1	9.3	11.1	20.6	9.5	0	0.9	0.0	0.8
	S42IL-107	12.7	40.8	28.0	17.4	48.4	31.1	2	2.6	0.1	0.7
	S42IL-108	12.7	59.0	46.2	17.4	451.2	433.8	0	5.7	0.2	0.5
	S42IL-144	37.8	50.7	12.9	39.9	74.9	35.0	0	1.6	0.0	1.2
	S42IL-109	37.8	63.5	25.7	39.9	476.5	436.6	0	5.0	0.0	0.0
	S42IL-110	89.9	94.9	5.0	543.0	563.5	20.4	3	1.9	0.1	0.2
	S42IL-153	60.7	68.6	7.9	462.5	495.4	32.9	3	2.7	0.0	0.2
3H	S42IL-111	42.6	56.9	14.3	39.9	401.4	361.6	4	5.1	1.7	0.8
	S42IL-112	57.2	90.7	33.4	404.5	489.5	85.0	1	2.3	2.8	0.2
	S42IL-114	76.0	144.6	68.7	468.1	549.9	81.8	0	0.4	0.4	89.1
	S42IL-140	87.4	148.4	61.0	481.2	556.8	75.6	1	6.2	0.6	0.5
	S42IL-113	117.9	143.1	25.2	520.0	548.9	28.9	0	2.9	0.0	0.4
	S42IL-115	117.9	155.0	37.1	520.0	564.6	44.6	1	4.8	0.0	0.4
	S42IL-161	138.7	155.0	16.3	542.2	564.0	21.8	0	1.4	0.0	3.0
4H	S42IL-116	1.1	40.0	39.0	0.0	36.4	36.4	3	3.2	0.0	0.9
	S42IL-117	18.5	49.9	31.4	11.7	62.8	51.0	1	1.6	0.3	0.1
	S42IL-145	43.3	49.9	6.5	42.3	62.8	20.5	2	1.4	0.1	0.7
	S42IL-118	38.0	54.6	16.6	34.7	405.6	370.9	1	2.8	0.0	0.3
	S42IL-120	38.0	57.3	19.3	34.7	423.5	388.7	0	2.2	0.5	0.8
	S42IL-119	38.0	81.4	43.4	34.7	500.7	465.9	1	5.5	0.0	1.4
	S42IL-162	45.7	81.4	35.7	45.8	500.7	454.8	0	4.8	0.0	0.6
	S42IL-121	53.4	81.4	28.0	367.3	500.7	133.4	2	5.2	1.5	0.4
	S42IL-146	54.6	81.4	26.8	405.6	500.7	95.0	0	1.6	1.1	0.4
	S42IL-123	81.4	112.5	31.2	501.5	539.5	37.9	2	5.7	0.0	0.2
	S42IL-124	110.2	115.2	5.0	531.1	543.0	11.9	2	2.0	0.0	0.2
5H	S42IL-125	51.5	81.3	29.9	387.8	445.8	58.0	1	2.4	1.5	0.3
	S42IL-173	51.5	95.5	44.0	387.8	464.8	77.0	0	3.6	0.0	0.3
	S42IL-147	75.2	81.3	6.1	435.7	445.8	10.0	1	0.9	0.4	0.1
	S42IL-126	75.9	120.7	44.8	438.0	492.6	54.6	0	3.8	0.0	0.1
	S42IL-176	81.3	139.9	58.6	445.8	517.4	71.6	5	8.3	0.5	0.3
	S42IL-127	137.8	165.9	28.1	515.0	549.3	34.3	3	3.4	0.2	0.7
6H	S42IL-148	1.0	10.6	9.7	0.2	9.9	9.7	1	4.3	0.0	0.5
	S42IL-149	30.0	52.2	22.2	20.2	93.0	72.8	1	2.3	0.0	0.4
	S42IL-128	35.6	75.5	39.9	21.9	490.5	468.6	2	6.4	0.4	1.4

Table 2 continued

Chr.	S42IL	Pos. of first SNP (cM)[a]	Pos. of last SNP (cM)	Min. size of target intr. (cM)	Pos. of first SNP (Mb)	Pos. of last SNP (Mb)	Min. size of target intr. (Mb)	No. add. intr.	*Hsp* %[b]	Het %	Missing %
	S42IL-129	46.5	86.3	39.8	39.5	500.8	461.3	0	5.0	0.9	0.2
	S42IL-130	59.9	105.0	45.0	371.9	522.6	150.7	5	4.1	3.0	0.2
	S42IL-131	86.8	110.1	23.4	503.8	524.5	20.6	1	1.9	0.2	0.7
	S42IL-132	94.8	113.2	18.4	511.6	527.6	16.0	1	1.1	0.0	1.6
	S42IL-122	104.8	126.5	21.7	522.6	538.7	16.1	3	4.4	0.4	0.7
7H	S42IL-133	12.7	37.6	24.9	11.9	46.1	34.1	6	3.7	1.0	0.5
	S42IL-134	37.6	70.2	32.6	46.1	231.1	185.0	2	4.2	1.3	0.3
	S42IL-135	67.4	118.5	51.1	132.2	570.2	438.0	1	7.6	0.0	0.7
	S42IL-170	75.2	78.7	3.5	456.7	510.5	53.8	2	1.4	1.9	0.4
	S42IL-136	88.9	116.1	27.2	533.2	566.1	32.9	3	2.9	0.1	3.7
	S42IL-137	88.9	127.5	38.6	533.2	584.8	51.6	5	7.6	0.1	0.6
	S42IL-138	116.1	141.0	24.9	565.8	601.4	35.6	1	3.9	1.8	0.2
	S42IL-139	129.4	141.0	11.6	588.3	601.4	13.1	0	1.5	0.0	0.1

[a] cM data and Mb data of SNPs are based on Mayer et al. (2012)

[b] Percentage of *Hsp*, heterozygous, and missing SNPs per genotype

QTL detection

Following the mixed-model analysis of variance, a Dunnett test was conducted for all traits with heritability >0, to compare the performance of each S42IL with the *Hv* control genotype Scarlett. The test revealed 22 effects across all traits and treatments (Table 3). QTL were detected for BMD, HEI, TIL and PAM. No effects were detected for BMF and SPAD. Some effects were detected across treatments as well as under drought and well-watered treatments. Those effects were summarized to one QTL. If S42ILs with overlapping introgressions showed a similar effect for a trait, it was assumed that this effect was due to the same underlying QTL. In total, eleven QTL, present in 13 S42ILs, were detected for four traits. In the following, the detected QTL will be described in detail.

Biomass dry (BMD)

One QTL for BMD (QBmd.S42IL-7H) was detected on chromosome 7H in this study. Compared to the recipient Scarlett, S42IL-136 showed an increased biomass production across both treatments (16.0 %) and under well-watered treatment (20.0 %).

Height (HEI)

Most QTL in this study were detected for plant height. The six QTL were distributed over chromosomes 2H, 3H, 6H, and 7H. S42IL-107 and S42IL-153 with a main introgression on chromosome 2H and S42IL-129, S42IL-132, and S42IL-122 with introgressions on chromosome 6H reduced plant height between 4.4 and 6.3 % compared to Scarlett. In S42IL-140 and S42IL-137 with main introgressions on 3H and 7H, respectively, the *Hsp* allele increased plant height by 4.7 and 6.6 % across treatments, respectively.

Tiller number (TIL)

Three QTL were detected for tiller number on chromosomes 1H, 4H, and 7H. The two lines S42IL-123 and S42IL-124 with main introgressions on chromosome 4H showed a common QTL. For all three QTL, the *Hsp* allele increased tiller numbers. Under drought stress, S42IL-124 produced 12.8 % more tillers than Scarlett. Across treatments, the three QTL increased tiller numbers by between 23.6 and 25.8 %.

Table 3 List of QTL effects ($P < 0.05$) across four drought experiments in 2011 with 52 S42ILs

Trait	Line	Position of main intr. (Chr. and Pos. in Mb)[a]	QTL name	Treat.[b]	LSMEANS[c]	Dev. f. Sca[d]	Dev. f. Sca %[e]
BMD	S42IL-136	7H; 533-566	QBmd.S42IL-7H	a, w	3.7	0.5	16.0
HEI	S42IL-107	2H; 017-048	QHei.S42IL-2H	d	35.8	−2.4	−6.3
	S42IL-153	2H; 462-495	QHei.S42IL-2Hb	a	42.6	−2.3	−5.1
	S42IL-140	3H; 481-557	QHei.S42IL-3H	a, d, w	47.8	3.0	6.6
	S42IL-149	6H; 020-093	QHei.S42IL-6H	a	42.9	−2.0	−4.4
	S42IL-129	6H; 039-501	QHei.S42IL-6H	a, d	42.8	−2.1	−4.7
	S42IL-132	6H; 512-528	QHei.S42IL-6Hb	a	42.7	−2.2	−4.8
	S42IL-122	6H; 523-539	QHei.S42IL-6Hb	a	42.6	−2.3	−5.1
	S42IL-137	7H; 533-585	QHei.S42IL-7H	a, w	47.0	2.1	4.7
PAM	S42IL-128	6H; 022-491	QPam.S42IL-6H	w	0.6	−0.1	−12.1
TIL	S42IL-143	1H; 405-440	QTil.S42IL-1H	a, d	23.6	3.3	16.2
	S42IL-123	4H; 502-540	QTil.S42IL-4H	a, w	23.8	3.5	17.5
	S42IL-124	4H; 531-543	QTil.S42IL-4H	d	12.8	2.0	18.6
	S42IL-136	7H; 533-566	QTil.S42IL-7H	a, w	25.8	5.5	27.3

[a] Chromosome positions are based on Mayer et al. (2012)

[b] Treatment: a: across treatments, d: under drought treatment, w: under well-watered treatment

[c] Mean trait performance for indicated IL, trait and treatment. If more than one treatment is given, then means across treatments are indicated

[d] Deviation from Scarlett = LSMEANS [IL] − LSMEANS [Scarlett]

[e] Deviation from Scarlett in % = 100 × (LSMEANS [IL] − LSMEANS [Scarlett])/LSMEANS [Scarlett]

Chlorophyll fluorescence (PAM)

One QTL was detected for chlorophyll fluorescence on chromosome 6H. The *Hsp* allele in S42IL-128 reduced chlorophyll fluorescence under well-watered treatment by 12.1 %.

Drought stress results 2012 (two QTL verification experiments with 13 S42ILs)

Descriptive statistics

In 2012, two verification experiments were carried out. Thirteen S42ILs, which had shown effects in 2011, and the recipient Scarlett were grown under drought and well-watered treatments (Table S5). Coefficient of variation varied widely. The lowest variation with 1.8 % was found for relative water content under well-watered treatment. SSI-PAM showed the highest variation with 181.3 %. PAM and SPAD showed high variations under well-watered and drought treatments with values between 51.9 and 92.3 %. CVs for the other traits under well-watered and drought treatments were rather moderate with the highest value of 29.8 %. Variation for traits measured across treatments was high for most traits (58.7–102.8 %) although moderate for HEI and RWC (20.7 and 23.2 %, respectively). Variation for most SSIs was low to moderate with values ranking from 6.8 for SSI-HEI to 48.1% for SSI-Wcon. SSI-SPAD and SSI-PAM showed very high CVs with 82.6 and 181.3 %, respectively.

Heritability was low for many traits that showed high CVs. It was equal to 0 for instance for PAM under drought and well-watered treatments. High heritabilities were observed, for instance, for TIL under well-watered treatment (90.0 %) and drought (86.4 %) and BMD under drought treatment (88.6 %).

Trait correlations

Pearson's correlation coefficients are shown in Table S6. Autocorrelations of traits between drought and well-watered treatments were highest for TIL ($r = 0.76$) and HEI ($r = 0.74$). All other autocorrelation coefficients were not statistically significant.

Between traits, highest correlation was observed for BMF and Wcon ($r = 1.00$). SPAD and PAM showed a high correlation of $r = 0.81$, and TIL showed high correlations with BMF ($r = 0.74$) and Wcon ($r = 0.77$). A strong negative correlation of $r = -0.72$ was observed between RWC and BMD. Among stress indices, strongest correlations were observed between SSI-Wcon and SSI-BMF ($r = 0.95$), SSI-BMD and SSI-BMF ($r = 0.80$), and SSI-Wcon and SSI-RWC ($r = 0.74$). Highest correlations between stress indices and measured traits were observed for RWC and SSI-RWC ($r = 0.98$), and SSI-Wcon and RWC ($r = 0.70$). Several stress indices showed negative correlations with measured traits. Strongest negative correlations were observed for SSI-BMF with BMF ($r = -0.72$) and Wcon ($r = -0.70$) and BMD with SSI-RWC ($r = -0.70$).

Mixed-model analysis of variance

A mixed-model analysis of variance (Tables S7–S10) was carried out for all traits and stress indices. The ANOVA revealed significant line and treatment effects for all traits. Line by treatment interaction was not significant for PAM. The factor experiment did not have an influence on trait performance of BMD. Line by experiment interactions did not have a significant effect on the majority of the traits, but influenced SPAD, TIL and HEI. The SSIs were analyzed with mixed model II. The line effect had a significant effect on all SSIs except SSI-SPAD and SSI-PAM. Experiment showed an effect on all SSIs except SSI-HEI. Line by experiment interaction was not significant for any of the stress indices.

QTL detection

For each trait with heritability >0, a Dunnett test was conducted subsequently to the ANOVA, where each S42IL was compared to Scarlett. In total, 54 effects (Table 4) were detected. Twenty effects were detected across treatments, while 12 were detected under drought and 15 under well-watered treatment. Seven effects were identified for stress indices. The 54 effects were summarized to a minimum of 31 QTL (Fig. 2). QTL were detected for all investigated traits and for five stress indices. No QTL was detected for SSI-BMD, SSI-PAM and SSI-SPAD. In the following, QTL are described separately in more detail.

Biomass dry (BMD)

Four QTL were found for BMD. The QTL were located on chromosomes 1H, 2H, 4H, and 6H. While the first three QTL were only detected as line by treatment interaction effect under drought treatment, the latter QTL was detected across treatments as well as under well-watered treatment. In S42IL-143 and S42IL-129 with introgressions on 1H and 6H, respectively, the *Hsp* allele reduced biomass by 7.5 and 7.3 % compared to Scarlett. The *Hsp* allele increased biomass by 10.6 and 8.5 %, respectively, at the QTL on 2H and 4H in S42IL-107 and S42IL-123.

Biomass fresh (BMF)

For BMF, one QTL was detected on chromosome 7H. The *Hsp* introgression common in S42IL-136 and S42IL-137 showed a biomass increase of 9.2 and 8.2 %, respectively, under well-watered treatment.

Height (HEI)

Seven QTL were found for the trait height on all chromosomes but 4H and 5H. *Hsp* alleles had rather low effects on plant height. In five cases, height was reduced between 4.0 % and 5.5 %. In two cases, the *Hsp* allele increased height by 3.8 and 5.5 %, respectively.

Chlorophyll fluorescence (PAM)

Two QTL in S42IL-123 and S42IL-128, QPam.-S42IL-4H and QPam.S42IL-6H, with main introgressions in chromosomes 4H and 6H, respectively, were detected for the trait chlorophyll fluorescence. Across treatments, the *Hsp* allele caused higher chlorophyll fluorescence at the end of the experiment in S42IL-123 and S42IL-128. The trait performance was increased by 42.0 and 31.4 %, respectively.

Relative water content (RWC)

Two QTL were detected for relative water content across treatments and under drought treatment. One QTL was located on 1H, the second QTL on 6H in both lines S42IL-128 and S42IL-129. The *Hsp* allele increased RWC between 5.8 and 6.9 %.

Table 4 List of QTL effects ($P < 0.05$) across two verification drought experiments in 2012 with 13 S42ILs

Trait	Line	Position of main intr. (Mb)[a]	QTL Name	Treat.[b]	LS-MEANS IL[c]	LS-MEANS Sca[d]	Dev. f. Sca[e]	Dev.f.Sca %[f]	New/ ver.[g]	Studies with corresponding QTL	Candidate genes
BMD	S42IL-143	1H; 405-440	QBmd.S42IL-1H	d	1.53	1.66	−0.13	−7.54	n		
	S42IL-107	2H; 017-048	QBmd.S42IL-2H	d	1.83	1.66	0.18	10.55	n		
	S42IL-123	4H; 502-540	QBmd.S42IL-4H	d	1.80	1.66	0.14	8.54	n		
	S42IL-129	6H; 039-501	QBmd.S42IL-6H	a, w	4.80	5.18	−0.38	−7.33	n	V, VI	
BMF	S42IL-136	7H; 533-566	QBmf.S42IL-7H	w	84.90	77.77	7.13	9.17	n		
	S42IL-137	7H; 533-585		w	84.16	77.77	6.39	8.22	n		
HEI	S42IL-143	1H; 405-440	QHei.S42IL-1H	a	45.54	47.83	−2.29	−4.79	n	I, VI, VII	*HvFT3*[h]
	S42IL-107	2H; 017-048	QHei.S42IL-2H	d	36.58	38.42	−1.83	−4.77	v	I, II, III, V	*Ppd-H1*[h]
	S42IL-153	2H; 462-495	QHei.S42IL-2Hb	a	45.92	47.83	−1.92	−4.01	v	I	
	S42IL-140	3H; 481-557	QHei.S42IL-3H	a, w	50.46	47.83	2.63	5.49	v	I, V, VI	*denso*[i]
	S42IL-149	6H; 020-093	QHei.S42IL-6H	a	45.54	47.83	−2.29	−4.79	v		
	S42IL-122	6H; 523-539	QHei.S42IL-6Hb	a, w	45.21	47.83	−2.63	−5.49	v	I	
	S42IL-137	7H; 533-585	QHei.S42IL-7H	a	49.67	47.83	1.83	3.83	v	I, II, V, VI, VII	
PAM	S42IL-123	4H; 502-540	QPam.S42IL-4H	a	0.29	0.20	0.08	42.01	n		
	S42IL-128	6H; 022-491	QPam.S42IL-6H	a	0.27	0.20	0.06	31.35	n		
RWC	S42IL-143	1H; 405-440	QRwc.S42IL-1H	a, d	0.75	0.71	0.04	5.76	–		
	S42IL-128	6H; 022-491	QRwc.S42IL-6H	a, d	0.75	0.71	0.04	6.27	–		
	S42IL-129	6H; 039-501		a, d	0.76	0.71	0.05	6.89	–		
SPAD	S42IL-143	1H; 405-440	QSpad.S42IL-1H	d	16.30	11.19	5.11	45.64	n	IV	
	S42IL-123	4H; 502-540	QSpad.S42IL-4H	d	18.29	11.19	7.10	63.44	n	IV	
TIL	S42IL-143	1H; 405-440	QTil.S42IL-1H	a, d, w	31.83	27.88	3.96	14.20	v		
	S42IL-107	2H; 017-048	QTil.S42IL-2H	a, w	22.29	27.88	−5.58	−20.03	n		
	S42IL-153	2H; 462-495	QTil.S42IL-2Hb	a, d, w	33.38	27.88	5.50	19.73	n		
	S42IL-123	4H; 502-540	QTil.S42IL-4H	a, w	31.58	27.88	3.71	13.30	v	VII	
	S42IL-124	4H; 531-543		a, d, w	33.79	27.88	5.92	21.23	v	VI	*VRN-H2*[h]
	S42IL-122	6H; 523-539	QTil.S42IL-6H	a, w	31.63	27.88	3.75	13.45	n		
	S42IL-136	7H; 533-566	QTil.S42IL-7H	a, w	31.54	27.88	3.67	13.15	v	VII	
	S42IL-137	7H; 533-585		a, w	31.79	27.88	3.92	14.05	n		
Wcon	S42IL-136	7H; 533-566	QWcon.S42IL-7H	w	75.97	69.08	6.89	9.98	–		
	S42IL-137	7H; 533-585		w	74.98	69.08	5.90	8.54	–		
SSI-BMF	S42IL-129	6H; 039-501	QSsibmf.S42IL-6H	–	0.06	0.05	0.01	22.71	–		

Table 4 continued

Trait	Line	Position of main intr. (Mb)[a]	QTL Name	Treat.[b]	LS-MEANS IL[c]	LS-MEANS Sca[d]	Dev. f. Sca[e]	Dev.f.Sca %[f]	New/ ver.[g]	Studies with corresponding QTL	Candidate genes
SSI-HEI	S42IL-107	2H; 017-048	QSsihei.S42IL-2H	–	0.62	0.67	−0.05	−7.13	–		
SSI-RWC	S42IL-143	1H; 405-440	QSsirwc.S42IL-1H	–	0.69	0.60	0.08	13.87	–		
	S42IL-128	6H; 022-491	QSsirwc.S42IL-6H	–	0.68	0.60	0.08	13.24	–		
	S42IL-129	6H; 039-501		–	0.70	0.60	0.10	16.74	–		
SSI-TIL	S42IL-122	6H; 523-539	QSsitil.S42IL-6H	–	0.21	0.26	−0.05	−18.21	–		
SSI-Wcon	S42IL-129	6H; 039-501	QSsiWcon.S42IL-6H	–	0.05	0.03	0.01	37.60	–		

I: Von Korff et al. (2006), II: Schmalenbach et al. (2009), III: Wang et al. (2010), IV: Schnaithmann and Pillen (2013), V: March et al. (in prep.), VI: Honsdorf et al. (2014), VII Hoffmann et al. (2012)

[a] Chromosome positions are based on Mayer et al. (2012)

[b] a: across treatments, d: under drought treatment, w: under well-watered treatment

[c] Mean trait performance for indicated IL, trait and treatment, if under more than one treatment, then a

[d] Mean trait performance of Scarlett for indicated trait and treatment, if under more than one treatment, then a

[e] Deviation from Scarlett = LSMEANS [IL] − LSMEANS [Scarlett]

[f] Deviation from Scarlett in % = (LSMEANS [IL] − LSMEANS [Scarlett])/LSMEANS [Scarlett] × 100

[g] New trait in 2012 experiments, n: new QTL in 2012 experiments, v: verified QTL from 2011 experiment

[h] Wang et al. (2010)

[i] Laurie et al. (1995)

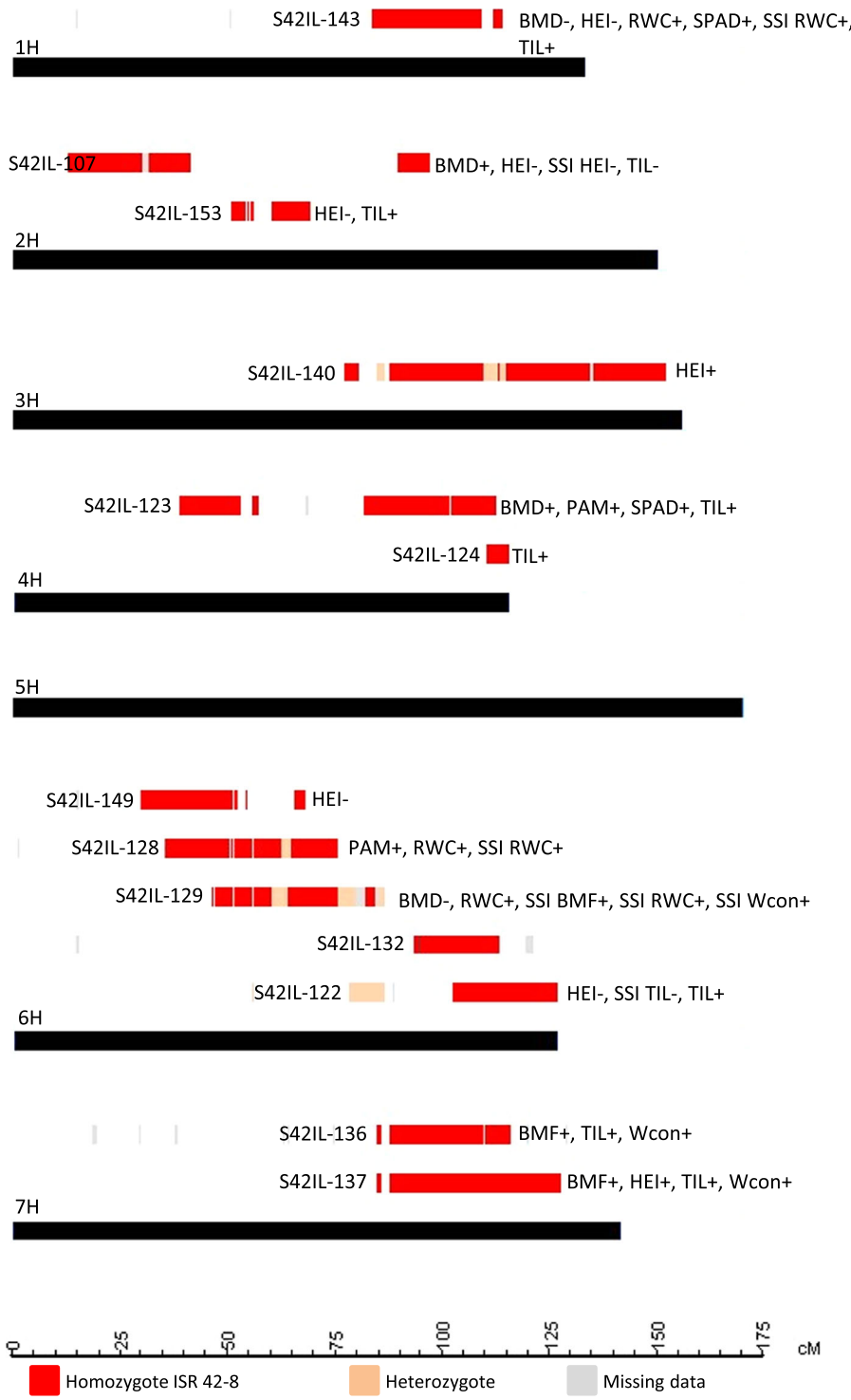

Fig. 2 Genetic map with selected S42IL introgressions. QTL are placed right to the S42ILs, indicated by trait abbreviations (see Table 1). The sign indicates an increasing (+) or decreasing (−) *Hsp* effect

Leaf greenness (SPAD)

For leaf greenness, two QTL were detected by measuring the trait at the end of the experiment. S42IL-143 and S42IL-123 with introgressions on 1H and 4H, respectively, revealed higher greenness under drought treatment. Trait values were increased by 45.6 and 63.4 %, respectively.

Tiller number (TIL)

Six QTL, QTil.S42IL-1H, QTil.S42IL-2H, QTil.S42IL-2Hb, QTil.S42IL-4H, QTil.S42IL-6H, QTil.S42IL-7H, were detected for tiller number on all chromosomes but 3H and 5H. Eight lines showed effects for this trait across treatments as well as under drought and/or well-watered treatment. In one case, the *Hsp* allele decreased tiller number by 20.0 %. In all other cases, the wild barley allele increased tiller number between 13.2 and 21.2 %.

Water content (Wcon)

For Wcon, one QTL on chromosome 7H was detected in S42IL-136 and S42IL-137. Under well-watered treatment, the water content was increased by 10.0 and 8.5 %, respectively.

Stress indices

QTL were detected for five SSIs. Those are SSI-BMF, SSI-HEI, SSI-RWC, SSI-TIL and SSI-Wcon. One QTL was detected for SSI-HEI and SSI-TIL, where the *Hsp* allele caused a reduced stress tolerance compared to Scarlett by 7.1 and 18.2 %, respectively. S42IL-129 with a QTL on 6H revealed a 22.7 % higher stress tolerance for the trait BMF. Two QTL were found for SSI-RWC on chromosome 1H in line S42IL-143 and on 6H in line S42IL-128 and S42IL-129 which increased stress tolerance by 13.2–16.7 %. S42IL-129 showed a QTL effect on chromosome 6H for SSI-Wcon with an increase of 37.6 %.

Discussion

Genotyping

GBS is a highly multiplexed, reproducible, and simple approach for generation of genomewide SNP markers, recently proposed by Elshire et al. (2011). Restriction enzymes are employed to construct reduced representation libraries of the target genome. The DNA samples are barcoded and sequenced in parallel on a next-generation sequencing platform. In this study, 55 S42ILs were genotyped with the method. The aims were to increase the marker density of the genetic map that is currently used for characterization of the S42IL library and to test how GBS compares to a previous map comprised of Illumina Golden Gate BOPA1 SNPs.

A total of 54 out of 55 S42ILs were genotyped successfully through GBS. For S42IL-114, the number of sequence reads obtained was extremely low. The DNA concentration for this genotype met the quality requirements, and we thus conclude that an error must have occurred during library preparation.

Combining the GBS markers with the existing BOPA1 markers resulted in a map which consists of 4,201 SNPs with a total length of 989.2 cM (3.9 Gb) that has high coherence to the published barley physical map (Mayer et al. 2012). The 4,201 SNPs are composed of 3,744 GBS SNPs and 457 BOPA1 SNPs. After aligning to the barley reference genome, only those BOPA markers were included that remained in the same order as published by Muñoz-Amatriaín et al. (2011). New SNP orders appeared especially in situations where several markers had been mapped previously into the same bin. Due to the high mapping resolution of the barley physical map, markers could be mapped to unique positions; thus, the order of markers in bins as well as for adjacent markers changed in 138 cases. Twenty-seven SNPs were mapped to different chromosomes. Since some of these positions exhibited high BLAST *e* values, we decided to exclude those markers with positions that were not in accordance with the previously published map by Muñoz-Amatriaín et al. (2011).

In order to estimate the number of SNPs located within genes, the marker sequences were aligned to the barley nucleotide sequence database 'HC_genes_CDS_Seq' on the IPK BLAST server (http://webblast.ipk-gatersleben.de/barley/) (Deng et al. 2007). The database is composed of RNA-seq and barley full-length cDNA-derived gene predictions. Setting the threshold of the expectation value at 0.0001, 29 % of the SNP markers are estimated to be located in coding gene sequences.

Based on the new genetic map, the set of 54 S42ILs represents 84.9 % of the donor genome if only the

target introgressions are accounted for and 86.6 % if also nontarget introgressions are included. These results confirm results obtained with the BOPA1 set by Schmalenbach et al. (2011). In that study, 73 S42ILs were genotyped and the whole library showed overall representation of the *Hsp* genome of 87.3 % considering only the target introgressions and 89.5 % if secondary introgressions were included. The slightly lower numbers might be explained by fewer S42ILs genotyped with GBS. The inclusion of the GBS SNPs improved the resolution of the map from 0.4 to 4.2 marker/cM. Gaps in the introgression library, especially on 5H (63.3 % BOPA chromosome map, 63.8 % combined GBS and BOPA chromosome map), were also verified by the GBS data. Overall, the results indicate accurate estimates of marker positions can be achieved by aligning marker sequences to the barley genome sequence. A finding also described by Mascher et al. (2013).

Drought stress experiments 2011 versus 2012

In this study, 52 wild barley ILs were screened for biomass development and leaf senescence under drought and well-watered conditions. The aim of the study was to identify genotypes that show a more vigorous growth and delayed leaf senescence compared to the cultivar parent Scarlett. Subsequently, experiments with a subset of 13 S42ILs were performed to verify the results. Two more traits and stress indices were added to the analysis of the verification experiments to obtain more details on the plants' drought responses.

The experimental setup changed from the first set of experiments in 2011 to the verification experiments in 2012. During the first experiments with 52 S42ILs, the plants were located close to each other. This most likely caused shading between plants, which influenced the senescence of lower level leaves. Shading was more intense in well-watered than in drought-stressed plants due to increased growth. This caused earlier initiation of leaf senescence in the well-watered compared to drought-stressed plants. As one of the goals of the experiments was to study drought stress-induced leaf senescence, the design of the verification experiments was modified. In this case, fewer genotypes allowed more space between plants. Consequently, shading was reduced markedly, resulting in higher SPAD and PAM values at the end of the experiment under well-watered treatment. We, therefore, assume that the stress treatment was successful and that the observed leaf senescence was drought stress induced. Due to fewer genotypes in the verification experiments, more replications could be included per genotype. Moreover, automated mat irrigation was replaced by more precise hand watering. As a result of those modifications, heritabilities for almost all traits increased compared to the 2011 experiments. Differences between experiments in 2011 and 2012 also arise from the seasonal differences in which the experiments were conducted. While in 2011 experiments were conducted during spring and autumn, the experiments in 2012 were only conducted during spring. Thus, differences in temperature and day length may be part of the explanation why not all QTL could be verified.

Phenotyping

The drought stress treatment applied to the S42ILs at an early stage of development reduced trait performance of all investigated parameters, with the exception of SPAD values in 2011. The reduction of growth parameters, RWC, and chlorophyll parameters under water deficit is in accordance with previous studies, e.g., Guo et al. (2008) and Teulat et al. (1997). RWC was only measured in the experiments in 2012. The trait showed negative correlation with BMD. A similar observation was reported by Teulat et al. (1997). This could be an indication for two different drought reaction patterns in the S42IL library in terms of stomatal conductance and osmotic adjustment. Some genotypes may decrease their stomatal conductance to prevent water loss. They maintain higher RWC, but due to closed stomata have less CO_2 influx and, thus, reduced biomass production. In contrast, other genotypes might react to the stress by osmotic adjustment. This allows maintenance of high cell turgor and biomass production. For instance, S42IL-143, following the first strategy, showed low BMD and high RWC under drought treatment compared to Scarlett, indicating that more water is preserved at the cost of biomass production. In contrast, S42IL-129, following the second strategy, showed high RWC under drought treatment; however, BMD was not reduced in this genotype. Interestingly, under the well-watered treatment, S42IL-129 produced less biomass, but did not show a difference in RWC compared to Scarlett. In

previous experiments with moderate drought stress, S42IL-129 had lower WUE than Scarlett. Therefore, it seems that under optimal and moderate stress conditions, the *Hsp* introgression in this genotype has a negative effect on biomass production. However, under severe stress, the genotype was able to maintain the biomass production and save water for ongoing physiological processes.

An alternative explanation for the negative correlation between BMD and RWC could be the different growth rates of the genotypes. Plants that grow faster will have used more water at the end of the experiment than slow growing plants and, thus, have a lower RWC. The RWC and, consequently, the stress level were not controlled in our experiments. This can be seen as a shortcoming of the experimental setup, because the effective water stress level may have varied among the genotypes on the day of trait measurements. However, this finding may shed light on the different drought stress avoidance strategies of plants and may allow differentiating between 'water savers' and 'water spenders' under water limited conditions.

We investigated drought stress-induced leaf senescence by SPAD and chlorophyll fluorescence measurements. One hypothesis is that plants that stay green for a longer time under drought stress produce more biomass (Rivero et al. 2007). In the 2012 experiments, SPAD and PAM showed high correlation ($r = 0.81$), but overall there was no significant correlation between chlorophyll content and biomass production. There was, however, one genotype (S42IL-123) that showed delayed leaf senescence and higher BMD under drought. It would be interesting to further investigate this line. For example, it could be used along with lines that showed an effect for RWC to investigate their reaction in recovery experiments.

QTL detection

The verification experiments revealed a minimum number of 31 QTL. This number is based on the assumption that only one gene per introgression causes the QTL effect and effects in shared introgressions are caused by the same gene. The Dunnett test reveals differences in trait performance between an S42IL and the control Scarlett. If an S42IL possesses more than one introgression, it is not possible to ultimately answer the question where the QTL is located. We assumed the most likely QTL position to be located in the main introgression since the main introgression is usually the largest one. However, further experiments and analyses with the high-resolution S42IL library are required to finally determine QTL positions (Schmalenbach et al. 2011). In 21 out of the 31 QTL, the ISR42-8 allele enhanced trait performance, while in ten cases, it diminished trait performance. Under drought treatment, this relation was even more in favor of the *Hsp* allele. Out of eleven detected QTL, the *Hsp* allele had a positive effect for nine. This is in contrast to previous experiments performed with the S42IL library. In a study by Honsdorf et al. (2014), the S42ILs were screened for drought stress response under moderate water stress in a high-throughput phenotyping facility. In these experiments, the majority of the detected QTL showed a negative effect on trait performance in the presence of the *Hsp* allele. Therefore, it appears that under moderate stress the *Hv* alleles were preferable, and under severe stress, the *Hsp* alleles were beneficial. This is in accordance with the assumption that wild barley from Israel is more adapted to drought conditions than a malting barley variety from Germany. It is also in accordance with the observation that genotypes with a high yielding potential perform better in most environments, but that under severe stress drought tolerant genotypes with lower yield potential may realize higher yields (Blum 2005).

Nine out of eleven QTL discovered in 2011 were verified in the 2012 experiments. In addition, 22 new QTL were detected. Eleven of the 31 QTL were also described in previous studies on the S42 and the S42IL libraries. In the following, the verified QTL as well as new QTL detected in the verification experiments will be discussed separately for traits or trait complexes. Table 4 provides an additional overview of accordance with QTL in previous studies.

Biomass (BMD and BMF)

Four QTL were detected for BMD and one for BMF. QTL detected for BMD and BMF were different from each other. This is in accordance with the low correlation between the two traits and can also be explained by the negative correlation between BMD and RWC. Plants with a high RWC tend to have low biomass and vice versa. The *Hsp* allele at QBmd.S42IL-6H caused a

reduction in BMD. March et al. (in prep.) observed a reduction of biomass in this genotype under terminal drought stress after anthesis and Honsdorf et al. (2014) as an across treatment effect in a study on moderate early drought stress. QBmd.S42IL-6H, thus, seems to be a stable QTL, across environments and developmental stages. The QTL QBmf.S42IL-7H for BMF was detected in S42IL-136 and S42IL-137, which posses overlapping introgressions. Under well-watered treatment, the *Hsp* introgressions increased BMF by 9.2, respectively, 8.2 %. This is in accordance with the QTL detected for water content. The water content was 10.0, respectively, 8.5 % higher in the two genotypes. Both lines also showed increased tiller number. This may indicate that a fast development and a high growth rate could be responsible for the higher biomass of the two genotypes.

Height (HEI)

Seven QTL were detected for plant height on all chromosomes but 4H and 5H. Most QTL were detected across treatments. This is in accordance with the high autocorrelation for this trait. One QTL, however, was detected solely under drought stress. This is QHei.S42IL-2H in S42IL-107. This line possesses the *Ppd-H1* gene (Wang et al. 2010), which causes early flowering. The genotype thus has less time for vegetative development and reaches its final height earlier than other genotypes. The height reduction in S42IL-107 was already observed by von Korff et al. (2006), Schmalenbach et al. (2009), Wang et al. (2010) in field experiments and drought experiments in the greenhouse by March et al. (in prep.).

In two of the detected QTL, the *Hsp* allele reduced plant height, and in five cases, it increased plant height. All detected QTL had rather small effects on plant height, exhibiting deviation from Scarlett between -5.5 and 5.5 %. However, six out of the seven QTL were detected in one or more previous studies on the S42 and S42IL populations (Table 4). Previous studies included field, greenhouse and hydroponic experiments at different growth stages and under different treatments. The verification of six out of seven effects shows that these QTL are very stable. This, together with the high heritability of the trait, suggests that phenotyping of growth parameters at the juvenile stage can be predictive for adult plant performance.

Tiller number (TIL)

Six QTL were detected for tiller number. All QTL showed an effect across treatments and under well-watered treatment. Additionally, the *Hsp* alleles at three QTL also caused higher number of tillers under the severe drought stress applied. At QTL QTil.S42IL-2H in S42IL-107, the *Hsp* allele caused a reduction of tiller number by 20 %. This line with the early flowering gene *Ppd-H1* (Wang et al. 2010) also revealed reduced plant height and might be explained by a shorter duration of the vegetative growth phase, which is favorable under dry land conditions. In all other QTL, the *Hsp* allele increased tiller number between 13.2 and 21.2 %. QTil.S42IL-4H was already detected in previous studies by Hoffmann et al. (2012) in S42IL-123 and Honsdorf et al. (2014) in S42IL-124. Wang et al. (2010) mapped the vernalization gene *VRN-H2* in line S42IL-124, which has a pleiotropic effect on tiller number (Karsai et al. 2006) and thus may be the underlying cause of the increase in tiller number in this experiment. QTil.S42IL-7H confirms results by Hoffmann et al. (2012). They reported the same QTL in two-week-old plants in a hydroponic system, indicating that those effects can already be detected at a very early developmental stage. Four additional QTL on chromosomes 1H, 2H, and 6H were detected that had not been reported in the S42IL library before. Gyenis et al. (2007) studied an advanced backcross population of the cross between the cultivar Harrington and the wild barley OUH602 for morphological traits and detected tiller number QTL on 1H, 2H, and 6H as well. However, while in Gyenis et al. (2007) the *Hsp* alleles reduced tiller number in all cases, in the S42IL library most *Hsp* alleles had an increasing effect on tiller number. It might be interesting to generate genotypes that possess two or more QTL for tiller number under drought and to test if these QTL act additively.

Chlorophyll (SPAD and PAM)

The two chlorophyll parameters SPAD and PAM showed a very high correlation coefficient of $r = 0.81$ across treatments. The traits did not show significant correlation with any other trait. Two QTL were detected for PAM on chromosomes 4H and 6H and for SPAD on 1H and 4H, respectively. The two QTL for photosystem II efficiency were detected across

treatments. In both cases, the *Hsp* allele caused an increase of PSII efficiency at the end of the experiment by 42.0, respectively, 31.4 % compared to Scarlett. For chlorophyll content measured with the SPAD-meter, the QTL were detected under drought treatment. The *Hsp* allele had an increasing effect on trait performance as well. The plants had 45.6, respectively, 63.4 % higher leaf greenness compared to Scarlett at the end of the experiment and, thus, delayed leaf senescence. The QTL on 4H in S42IL-123 was detected for both traits. Therefore, a common genetic control possibly underlies both traits. PAM QTL were only detected across treatments and SPAD QTL only under drought treatment. It should be mentioned that for the QTL in line S42IL-123, the effects were also detected under drought for PAM and across treatments for SPAD. However, heritability for those traits was 0. Thus, those effects were excluded from QTL detection. The high correlation between the traits possibly indicates a functional relationship; higher chlorophyll content might cause higher PSII efficiency. The high correlation between chlorophyll content and chlorophyll fluorescence also suggests that the simpler and cheaper measurement with the SPAD-meter provides already a good estimate for PSII efficiency in a QTL study. Kumagai et al. (2009) describe high correlation between SPAD measurements and Fv/Fm values in rice. Ma et al. (1995) in a study on soybean suggested SPAD as a rapid alternative for assessing photosynthesis in field trials. The SPAD QTL in S42IL-123 was detected in previous experiments with the S42IL library by Schnaithmann and Pillen (2013) on nitrogen use efficiency. However, while under drought stress the SPAD value increased, under N deficiency it was decreased. Guo et al. (2008) investigated chlorophyll parameters in a barley recombinant inbred line population and detected QTL for PS II efficiency and chlorophyll content on chromosomes 2H, 4H, and 5H. The first one might correspond to the QTL detected in our study. We, thus, conclude that S42IL-123 may be a promising genotype to further investigate the effect of delayed leaf senescence on plant performance.

Water content (RWC and Wcon)

One QTL was detected for water content. This locus, QWcon.S42IL-7H, is located on 7H and was detected only under well-watered treatment. S42IL-136 and S42IL-137 possess overlapping introgressions and show a similar increase in water content. Both lines also show a higher BMF. However, neither of the two lines had increased BMD or RWC. Under well-watered conditions, these plants were able to store more water, under drought conditions no deviation in trait performance compared to Scarlett was observed.

Two QTL were detected for RWC. At the two loci on chromosomes 1H and 6H, the *Hsp* allele increased RWC across treatments and under drought treatment. QRwc.S42IL-1H was detected in S42IL-143. This line had reduced BMD under drought treatment. It stores more water at the cost of reduced biomass production, thus, seems to be less water use efficient. An alternative reason may be a slow growth rate, which leads to a slower use of water and, thus, a higher RWC. In the case of QTL QRwc.S42IL-6H, detected in S42IL-128 and S42IL-129, RWC was also increased by the *Hsp* allele across treatments and under drought treatment. In contrast to S42IL-143, S42IL-128 did not show a decrease in biomass production and S42IL-129 had no biomass reduction under drought stress. In a previous experiment, S42IL-129 showed reduced water use efficiency under moderate drought stress (Honsdorf et al. 2014). These results indicate that stress severity interacts with trait performance. A genotype that performs less good under optimal or moderate stress conditions might perform well under severe stress (Blum 2005). This line therefore would be interesting for further investigation, e.g., gene expression analysis. It might also be interesting to further compare S42IL-143 with S42IL-128 and S42IL-129 and investigate the difference in their response to drought, e.g., osmotic adjustment and stomatal conductance. QTL for RWC were detected for instance by Teulat et al. (1997). They detected two QTL for RWC on 1H and 6H in a drought stress study of the recombinant inbred line population Er x Apm under growth chamber conditions in juvenile barley plants. The QTL might correspond to the ones we detected in this study. In a study of the same population under Mediterranean field conditions, the QTL on 6H was confirmed in adult plants (Teulat et al. 2003). This is an example that genes/QTL may simultaneously control both juvenile and adult drought stress responses. In this region, a cluster of dehydrine genes is mapped. They are known to play a role in drought stress tolerance (Campbell and Close 1997). These genes may be involved in the effects reported for S42ILs.

Stress indices

Six QTL for the stress indices SSI-BMF, SSI-HEI, SSI-RWC, SSI-TIL and SSI-Wcon were identified. No QTL were detected for SSI-BMD, SSI-PAM and SSI-SPAD. A stress index expresses how well a genotype performs under stress compared to the control treatment. In four of the six QTL, the *Hsp* allele improved the stress index. For instance, S42IL-129 with a main introgression on 6H showed 22.7 % better stress index for BMF than Scarlett. This genotype also showed higher SSI-Wcon. This is consistent with the high correlations ($r = 0.95$) between both traits. Two QTL were detected for SSI-RWC in genotypes S42IL-143, S42IL-128 and S42IL-129. All three genotypes showed higher RWC under drought compared to Scarlett and, hence, improved performance compared to Scarlett. One QTL was detected for SSI-HEI and SSI-TIL, respectively. In both cases, the *Hsp* allele caused higher stress susceptibility. For SSI-HEI, the effect was observed in S42IL-107. Under drought treatment, this genotype grew slower than Scarlett. S42IL-122 revealed the QTL for SSI-TIL. The genotype developed more tillers under well-watered treatment. S42IL-107 reveals higher stress susceptibility than Scarlett and also decreases performance under stress in absolute numbers. S42IL-122, however, shows higher relative decrease of tiller number and, thus, higher susceptibility, but absolute numbers show that the genotype performs as well as Scarlett under stress. We, thus, conclude that stress indices can provide valuable information about the relative extent of trait reduction under stress. However, when selection is based on an appropriate stress index, the absolute performance of the genotypes should also be considered.

Conclusions

In this study, 55 S42ILs were genotyped with GBS. A revised map consisting of 3,744 GBS SNPs and 457 BOPA1 SNPs was generated through alignment of marker sequences to the barley genome sequence. The extent and position of introgressions shows that mapping SNPs against the barley genome sequence produces reliable results, even though the genome sequence is not yet complete. The results show that GBS produces robust marker data and that the method could be used as a cost-effective alternative to chip-based SNP genotyping.

In four drought stress experiments, 13 S42ILs were identified which showed effects for biomass and senescence parameters. In two subsequent verification experiments, 31 QTL were detected for the investigated traits and stress indices. The *Hsp* allele had a positive effect on trait performance for 21 QTL. That shows that wild barley introgressions can be a valuable resource for enhancing plant performance under drought stress. For instance, S42IL-107 and S42IL-123 produced more biomass under drought. In a future experiment, the most promising S42ILs should be tested under drought conditions in field trials. When the positive *Hsp* effects are confirmed under field conditions, the respective S42ILs could be included in a barley breeding program to improve drought tolerance during early plant development.

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3 General Discussion

The two original papers present results on drought stress experiments conducted with the wild barley introgression line library S42IL. Both experiments were carried out with plants at the juvenile growth stage. However, treatments varied significantly between the two sets of experiments, having ample of different effects on the plants. In the following the experiments as well as general ideas about plant experiments in greenhouses will be discussed (3.1). Further possible candidate genes involved in drought response are discussed (3.2). Chapter 3.3 deals with the genetic characterization of the S42IL library with GBS. In chapter 3.4 QTL regions are described including all published S42IL QTL studies. The last chapter (3.5) contains future prospects.

3.1 Drought stress experiments

Phenotyping experiments of plants can be carried out in a variety of ways. Plants can be cultivated under controlled or semi-controlled conditions in growth chambers or greenhouses, where either all or a selection of environmental parameters are regulated. Another option is to cultivate plants in experimental gardens or under agricultural or ecological conditions. For the latter alternatives environmental conditions are less controlled. The advantage is that results of such experiments are better transferable to the plants in their natural environment. In order to allow generalizations of the results replicability as well as reproducibility are required. Replication means that the same researcher achieves the same results when repeating an experiment. When different laboratories are able to find the same results in independent experiments it is referred to as reproducibility. Achievement of high replicability and reproducibility are often challenging. Both parameters are expected to be met when environmental factors are similar between experiments (Poorter et al. 2012). Best conditions for that offer experiments in growth chambers. In greenhouse experiments environmental effects can be extenuated by cooling, heating, shading or additional lighting. However, environmental factors always play a role in greenhouses and cannot be controlled completely. The drought stress experiments carried out in the two original papers showed the difficulty to reproduce an experiment under the same conditions in greenhouses. The experiments that were carried out in the 'Plant Accelerator' showed experiment effects for almost all of the 14 investigated traits as well as for eleven out of 14 simple stress indices. Across treatments as well as under drought treatment leaf color was the only trait

that did not show a significant interaction with the factor experiment. However, for leaf color the genotype effect on trait performance was not statistically significant under either of the treatments. This indicates that little variation for the trait is present in the population. Under well watered treatment plant height was the only trait for which the factor experiment did not have a significant influence on trait performance. In contrast line by experiment interaction under well watered treatment was not significant for any trait but plant height. Under drought, however, interaction between line and experiment was significant for all but four of the digitally determined traits, namely, the integrals of compactness of the plants, plant height, plant color, and relative growth rate.

Differences between the experiments might be explained by differences in radiation intensity and day length variations between the experiments. The greenhouse facilities were not equipped with assimilation lighting. Thus, it was not possible to attenuate the seasonal differences in light intensity and day length. Moreover the stronger experiment effect under drought might be explained by differences in water supply. From the first to the second experiment water supply for drought treated plants was reduced from 15 und 12% water capacity. Control plants received the same amount of water in both experiments.

Poorter et al. (2012) demonstrate in their review on methods in plant biology experiments that even if experimental conditions are strictly controlled as in growth chambers reproducibility is still a challenge. Massonnet et al. (2010) conducted a study in which ten different laboratories carried out an experiment with the same protocol. The authors investigated leaf growth and molecular phenotypes of three different Arabidopsis genotypes. The results show large variation between but also within the laboratories. The authors argue that the different results in the laboratories might be explained by slight differences in the experimental set up. Even though all laboratories used the same protocol, differences may result from equipment of the facilities. The authors pinpoint differences in evaporative demand, distance between lamps and plants, and light quality. Differences within the same facility can for instance be caused by uneven light distribution or non-uniform air movement in the greenhouse (reviewed in Poorter et al. (2012). The results by Massonnet et al. (2010) show that even small differences between experimental setups can have large effects. They put emphasis on the importance of documenting the exact environmental conditions and argue that phenotypic data should be interpreted with great care when comparing results across independent experiments.

Drought stress experiments can be carried out in a variety of ways. The type of drought stress applied should depend on the question to be answered. Drought responses can vary widely between different treatments and timing of treatment.

Poorter et al. (2012) list five different ways to apply drought stress in plant experiments, each with its own advantages and disadvantages. In hydroponic systems osmotic stress can be applied through application of osmolytes like polyethylene glycol (Lagerwer et al. 1961). The advantage of the method is that a constant and known water potential can be applied to the roots. However, the method is rather suitable for short experiments of one to two days (Poorter et al. 2012) since the treatment has negative side effects like a lack of O_2 supply (Mexal et al. 1975; Munns et al. 2010).

Drought stress treatments in solid substrates can be applied in a variety of ways. Dry down experiments conducted by withholding irrigation from a defined point in time is one strategy that is suitable for field and pot experiments. In field experiments this can be achieved with rain-out shelters. Poorter et al. (2012) argue that dry down experiments in pots are less suitable to investigate drought response. Large plants in small pots might be exposed to drought too quick to adapt. Small plants in large pots at the contrary might not experience drought, since sufficient water is stored in the substrate.

In the drought stress experiments conducted at Kühnfeld experimental station we used the dry down method in pot experiments. We found that pre-experiments with different starting water levels were necessary to determine a suitable experimental setup. However, after establishing a dry down system with appropriate initial water content the method was suitable to allow the plants to develop and to detect genotype differences under the stress treatment.

Another method is the regular re-watering of plants with a fixed amount of water. Both methods have the drawback that plants of different sizes are subjected to different stress levels when exposed to the same amount of water. Bigger plants therefore, experience stress earlier than smaller plants, simply by growing faster. This has to be considered when interpreting the results (Poorter et al. 2012). However, the method is suitable for example when it is of interest how much biomass a plant can produce with a given amount of water.

An alternative is to water pots to a certain weight in regular time intervals. This is the method that was used in the drought experiments in the 'Plant Accelerator'. Plants were watered to weight every second day; to 15% water capacity for drought (12% in the second experiment) and 22% for control treatment. In this case the soil water content is kept at a similar level for all genotypes. This has the consequence that water spenders will be supplied with more water as

water savers. When irrigation quantities are recorded water use efficiency can be calculated. When weighing and watering is done in an automated manner as in the 'Plant Accelerator' the method is suitable also for large experiments.

Fernandez and Reynolds (2000) proposed an interesting method to overcome weighing of pots and still assuring constant soil water content. They placed water conductive Styrofoam blocks into tubs with different levels of water. Pots were placed on top of the Styrofoam blocks. The bottom of the pots was replaced with a fine mash, allowing water flow and inhibiting penetration of roots. With variable water levels different levels of stress severity can be attained. Good capillary contact is essential (Poorter et al. 2012) which can be achieved by soaking the blocks thoroughly in water (Fernandez and Reynolds 2000).

With all the different methods to impose drought stress it should be kept in mind that many plants react to drought by alteration of the root system. With deeper roots they are able to reach deeper water layers. While this is an important mechanism in natural and agricultural environments it cannot be simulated in regular pot experiments (Poorter et al. 2012). This might also be one reason that often different QTL for drought tolerance are detected in pot and field experiments. For appropriate investigation of root systems more elaborate experimental setups are necessary. Rhizo-lysimeters provide good conditions for free root development and enable the investigation of root architecture (Eberbach et al. 2013). Facilities like GROWSCREEN-rhizo at Jülich plant phenotyping center enable automated high-throughput phenotyping of roots (Nagel et al. 2012).

The two original papers describe experiments which investigate drought stress responses in juvenile barley plants. For paper one, plants were grown in the 'Plant Accelerator' in Adelaide, Australia. The 'Plant accelerator' is a high-throughput phenotyping facility equipped with the Lemnatec 3D Scanalyzer. Plants are located on carts on conveyor belts which allow automated delivery of plants to weighing, watering, and imaging stations. The experiments published in the second paper were carried out in a regular greenhouse at "Kühnfeld" experimental station in Halle, Germany. Both experiments use the S42IL library. In both experiments space was limited. Therefore not the whole library of 73 genotypes was investigated. A set of 47 and 52 genotypes was grown in Adelaide and Halle, respectively. Forty-six genotypes were present in both studies. However, only five concurrent QTL were detected. The explanation might be the different experimental setups. In Adelaide plants were grown with one plant per pot for a total of six weeks. All plants were grown for two weeks under control conditions before the drought treatment started. The treatment was carried out by drying down the pots to 15% soil water capacity (12% in the

second experiment). After reaching this level pots were re-watered every second day to the target water capacity. The stress level applied was moderate. Plants showed a clear growth reduction compared to the control but constant growth was recorded and no signs of leaf damage (e.g. senescence) detected. In Halle ten plants per pot were grown. Experiments had a duration of 34 days. At the beginning of the experiment all pots were irrigated with 400 mL of water to ensure equal germination conditions for all individuals. After one week control plants were watered regularly while drought treated plants did not receive any additional water. Plants, thus, were subjected to severe drought stress. Biomass and tillering were clearly reduced compared to the control plants and drought stress induced leaf senescence was visible to the naked eye.

The set of traits evaluated differed in the two studies. Nine out of fourteen of the traits evaluated in Adelaide were image based, two calculated traits had an image component, while only three traits were determined manually at the end of the experiment. In the second study all traits were determined manually. Leaf greenness and photosystem II efficiency were determined at the end of the experiment before harvest in order to quantify leaf senescence. At completion of the experiment biomass, tiller number, and plant height were measured. Three traits, namely dry biomass (BMD), height (HEI), and tiller number (TIL) were recorded in both studies. For the three traits 14 and 17 QTL were detected in the studies in Adelaide and Halle, respectively. Only five QTL were concurrent between the two studies; three QTL for HEI and one QTL each for BMD and TIL. None of the QTL was drought stress specific. All effects were discovered across treatments and some were additionally visible under drought and/ or well watered treatment. That no concurrent drought specific QTL was detected in both studies shows how different the treatments affected the investigated genotypes. Some QTL are effective over all treatments. Those QTL are interesting and especially useful because they can improve a cultivar under uncertain climatic conditions, i.e. be productive under optimum and stress conditions. Blum (2005) points out that the axiom that high yielding varieties will perform well in most environments is not wrong, but that the important point in the definition is “most environments”. At a certain stress level, cross over effects arise and genotypes with lower yield under non-stress conditions outperform high yielding varieties. The results of our two studies on the S42ILs can be interpreted in this way. Some genotypes perform better under control and moderate stress conditions. Under severe drought, however, other genotypes are favorable. This shows that the S42IL library contains enough diversity to improve the parental genotype Scarlett under varying drought conditions for vegetative biomass parameters. As an example the two genotypes S42IL-107 and -123 produced 10.6 and 8.5% more biomass compared to Scarlett under severe drought. Under mod-

erate drought and control conditions at the contrary, no difference to Scarlett was detected. Other genotypes as for instance S42IL-121 show improved biomass production of 36% across moderate drought and control conditions, but no improvement under severe stress was detected.

Correct and homogenous phenotyping is another challenging part in obtaining reproducible results. While some traits can easily be measured in the same way by different investigators, e.g. weight of complete above ground biomass. Other traits, where a certain amount of (subjective) estimation is required are subjected to higher fluctuations between different people, e.g. percentage of senescent leaf area. Moreover, phenotyping of large populations is very time-consuming. Due to limited time (or human) resources often repeated measurements are not possible. Repeated measurement of the same trait throughout an experiment, however, might allow obtaining better estimates of phenotypic values. With automated high-throughput plant imaging constraints of manual phenotyping could be overcome. In our experiments with the LemnaTecScanalyzer3D plant biomass was determined image-based every day over a time span of four weeks. The correlation to manually determined weight of biomass at the end of the experiment was very high (r=0.98). Heritability for the image based biomass estimate, however, was clearly higher than for manually determined biomass. Under drought treatment heritability was doubled from 15 to 30% and under well watered treatment it was increased by 10% from 43 to 53%. This indicates that repeated measurements can be beneficial for a selection process. Image based automated phenotyping offers a solution for time effective neutral characterization of plants. Computer based image analysis, for example for percentage of necrotic or senescent leaf area can be automated as well and are thus independent of varying judgments between different researchers. More neutral phenotyping is possible. This, for instance, might improve comparability of measurements in breeding programs carried out in different locations and by different investigators.

3.2 DROUGHT CANDIDATE GENES IN THE S42IL-LIBRARY

In order to understand the genetic basis of plant traits quantitative and molecular genetic approaches are applied. This also applies to the dissection of plants´ response to drought and other abiotic stresses (Tondelli et al. 2006). Plenty of QTL studies have been conducted in many types of crops, including barley, aiming to shed light onto the genetic architecture of drought tolerance, e.g. Baum et al. (2003), Diab et al. (2004), Mohammadi et al. (2008), and Varshney et al. (2012). The results of QTL studies, as also presented in this thesis, allow the connection of phenotypic

variation with specific alleles of QTL. The analysis of stress response of plants with molecular genetic methods led to the identification of genes involved in stress perception, signal transduction, and regulation of target genes by transcription factors (Tondelli et al. 2006). Most of the basic research was carried out in *Arabidopsis thaliana*. By exploiting genome synteny genetic information is transferred from model species to crop species. In this way stress responsive genes including drought responsive genes have been identified in barley (Cattivelli et al. 2011).

The candidate gene (CG) approach facilitates the linkage of findings from quantitative and molecular genetic approaches. In plant genetics CGs are defined as all genes putatively involved in trait variation. The integration of CG into QTL maps can help to connect "anonymous" markers with functional genes and, hence, functional genes with phenotypes (Pflieger et al. 2001).

In barley candidate genes for drought stress response have been described in several studies, for example in Choi et al. (1999), Malatrasi et al. (2002), Tondelli et al. (2006), Cseri et al. (2011), and Karami et al. (2013).

In this thesis several QTL were detected under drought stress for biomass and physiological parameters like water use efficiency and drought stress induced leaf senescence. In order to connect these phenotypes not only with chromosomal regions of undefined purpose but to genes of interest, CG genes were positioned on the S42IL map. Thirty-four CG genes known to be involved in drought response or leaf senescence were selected from publications by Choi et al. (1999), Tondelli et al. (2006), Cseri et al. (2011), Al Abdallat et al. (2014), and Christiansen and Gregersen (2014). The genes include 17 TFs and 17 target genes. The selection of TFs were comprised of ten NAC TFs, three MYB TFs, and two TFs belonging to the CBF/DREB family. Target genes included eleven dehydrin genes and the genes *HVA1*, *HvARH1*, *HvNHX1*, *HvNUD*, *HvP1*, and *SRG6*. A full list is given in Table 1. The CGs were aligned to the barley reference genome using the BLAST procedure. Subsequently CG positions were located on the S42IL map (Honsdorf et al. 2014b). At this stage no sequence comparisons between the S42ILs and Scarlett were conducted. Therefore it is not possible to say that the alleles differ between an IL and the cultivar parent. However, the position of the CG and the phenotypic effect might be a first indication for differences between the genotypes and are a good base for further investigations.

Thirty-one out of the 34 CGs were located inside of wild barley introgressions considering all 55 S42ILs represented in the map. One *MYB*, and two *NAC* TFs were not located inside a target introgression. One (*dhn9*) of the 31 remaining genes was located in an S42IL that was not phenotyped in either of the studies. Thirteen of the thirty remaining CGs co-located with QTL detected under drought, well watered, or across treatments. Eight genes co-located with QTL detected

specifically under drought stress. These include three dehydrin genes, *HVA1*, *HvP1*, and the TFs *HvNAC008*, *HvNAC013*, and *HvMYB2*. Twenty-one QTL detected under drought treatment were associated with CGs across both studies. The QTL were detected in seven different S42ILs. In the following these QTL and the potentially underlying genes will be discussed by genotype.

S42IL-143

The genotype S42IL-143 has a main wild barley introgression on chromosome 1H. Under severe drought stress as applied in the experiments conducted at "Kühnfeld" experimental station the IL revealed five QTL effects. BMD was reduced in this genotype by 7.5% compared to Scarlett. Relative water content (RWC) and TIL were increased by 14.5 and 13.8%, respectively. The IL also showed delayed leaf senescence, indicated by a SPAD value which was 45.6% higher at the end of the experiment. The stress index for RWC (SSI-RWC) showed that for this trait S42IL-143 is more stress resistant than Scarlett. S42IL-143 harbors the *HVA1* and the TF *HvMYB2* in its main introgression. *HVA1* is a member of LEA group 3 proteins. The gene has been shown to confer drought tolerance in barley (Qian et al. 2007). It was shown that different transcript levels were related to differences in dehydration tolerance. Transgenic approaches in wheat (Bahieldin et al. 2005) and rice (Xu et al. 1996) showed that the expression of barley *HVA1* may confer drought tolerance in other species as well. It has been shown in several studies that MYB TFs, like *HvMYB2*, are involved in signaling pathways of drought response. For example Urao et al. (1993) showed that *Atmyb2* in *Arabidopsis* is induced under dehydration as well as under salinity stress and ABA treatment. In *Arabidopsis* ABA mediates the induction of the dehydration responsive gene *rd22*. Abe et al. (2003) showed that *Arabidopsis* lines overexpressing *AtMYB2* were more sensitive to ABA and therefore also the expression of *rd22* was increased.

While S42IL-143 shows a slightly reduced BMD production under drought all other QTL show an improvement in trait performance compared to Scarlett. Allelic differences in the two CGs between S42IL-143 and the cultivar parent might explain the differences in trait performance.

S42IL-107

Three QTL were detected in S42IL-107 under drought treatment. The main introgression of S42IL-107 is located on chromosome 2H. The *Hsp* allele increased BMD under drought by 10.6%. HEI was reduced under drought stress by 4.8%. The genotype also reduced HEI stronger under drought than Scarlett as indicated by the stress index. Two NAC TFs are located in the introgression on chromosome 2H; *HvNAC008* and *HvNAC013*. NAC TFs are involved in ABA-dependent and

-independent signaling pathways for drought response (Shinozaki and Yamaguchi-Shinozaki 2007). Moreover, they also have important functions in responses to other stresses and in plant development (Nakashima et al. 2014). For example NACs are involved in the senescence process in plants (Breeze et al. 2011). Christiansen and Gregersen (2014) studied the regulation of NAC genes during senescence of flag leaves and found an up-regulation of *HvNAC008* and *HvNAC013*. In our experiments we did not detect a co-location of NAC TFs and senescence QTL. This might be explained by the fact the we were studying drought stress induced senescence while Christiansen and Gregersen (2014) were investigating natural senescence.

S42IL-128 and -129

S42IL-128 and -129 contain an overlapping introgression on chromosome 6H. Therefore, QTL detected in both studies are assumed to depend on the same underlying genetic effect and considered as the same QTL. S42IL-129 was the only genotype that revealed QTL effects under drought overlapping between the studies at the experimental station "Kühnfeld" in Halle, Germany and in the 'Plant Accelerator' in Adelaide, Australia. In the experiments in Halle both S42IL-128 and -129 had increased RWC by 14.7 and 17.4%, respectively. Both lines also showed an improvement in the stress index for RWC compared to Scarlett. Additionally S42IL-129 revealed better values for the stress indices for fresh biomass (SSI-BMF) and absolute water content (SSI-Wcon). In this IL BMF and Wcon under drought treatment were reduced less strongly than in Scarlett. Both ILs, thus, show improved performance under drought compared to Scarlett. In the experiments carried out in the 'Plant Accelerator' S42IL-129 showed a reduction of trait performance under drought compared to the parent genotype. The integral of caliper length (CALI) which corresponds to the plant diameter was reduced by 27.3%. Integral of the shoot area (SAI) is used as a measure for biomass and was reduced by 37.2% and water use efficiency (WUE) decreased by 40.6%. The contradictory performance of the genotype in both studies might be explained by different levels of drought severity. Under moderate drought, as applied in the 'Plant Accelerator' the *Hsp* introgressions conferred a disadvantage. Under severe drought, however, the wild barley introgressions led to a clearly better trait performance. The lines contain the dehydrin gene *dhn8*. Choi et al. (1999) studied the expression of dehydrin genes in barley under drought, ABA, and cold acclimation treatment. They found that *dhn8* in contrast to most other dehydrins was detectable at control conditions. Under dehydration it was down-regulated while the other ten dehydrin genes studied were up-regulated. Grossi et al. (1995) reported a rapid response of the gene to dehydration which happened prior to an increase in ABA accumulation. They detected an up-regulation of the gene expression with drought stress. However, this was

described as a transiently up-regulation (Choi et al. 1999). Both studies suggest that *dhn8* is expressed in an ABA-independent pathway. The gene is only induced weakly by ABA and typical ABRE-related elements are missing. The activity of *dhn8* under control and drought conditions might explain that a QTL was detected under both moderate and severe drought stress in S42IL-129.

S42IL-117

Under moderate drought stress four QTL were detected in S42IL-117. The genotype has its main *Hsp* introgression on chromosome 4H where *dhn6* is located. Under drought treatment S42IL-117 showed clear reduction of biomass and WUE compared to Scarlett. Integral of the absolute growth rate (AGRI) was reduced by 39.3%, CALI by 24.4%, and SAI and WUE by 36.5%. Suprunova et al. (2004) studied the expression of dehydrin genes in drought sensitive and tolerant wild barley genotypes under drought treatment. They detected differences in *dhn6* expression levels between resistant and sensitive genotypes. Early (3h) after the onset of drought *dhn6* was expressed at higher level in resistant as compared to sensitive genotypes. The authors assume also that the expression starts earlier. However, that was not investigated in their study. Twelve and 24 hours after the stress was applied the sensitive genotypes showed higher expression levels. The authors suggest that the resistance might be caused by one or more mechanisms including earlier perception of the water stress, more efficient signaling pathways and transcriptional activators, and higher expression of the *dhn* genes. Our results suggest that the *Hsp* introgression present in S42IL-117 carries an unfavorable allele for *dhn6*, leaving Scarlett more drought tolerant in comparison.

S42IL-140

S42IL-140 is characterized by the main introgression on chromosome 3H. Under drought treatment height was increased by 21.7%. The position of *dhn10* was mapped to chromosome 3H (Choi et al. 1999) and the gene is located inside the introgression of S42IL-140. In expression analyses Choi et al. (1999) showed that *dhn10* is induced by dehydration.

S42IL-137

HEI was increased by 13.7% in S42IL-137 under drought treatment. The main introgression is located on chromosome 7H in the region where the gene *HVP1* is mapped. Fukuda et al. (2004) cloned *HVP1* which encodes a vacuolar H^+-inorganic pyrophospatase. They studied gene expression under osmotic stress through mannitol application and reported up-regulation of the gene under the treatment. The gene might be involved in drought response in S42IL-137.

Table 1: List of 34 candidate genes involved in drought response and leaf senescence

Gene name	GenBank	Chr.	Position cM[a]	Position Mb[a]	Expect value[b]	Inside introgression? S42IL-
HvNAC007	AK249749.1	1H	48.67	247	5E-25	102, 103, 104, 105
DRF1	AY223807.1	1H	57.29	331	0	102, 141
HvARH1	Z48360.1	1H	76.84	399	0	141
HvMYB2	X70880.1	1H	86.54	412	0	143
HVA1	X78205.1	1H	90.30	417	0	143
HvNAC008	FR821737.1	2H	29.39	36	0	107, 108
HvNAC013	AK376297.1	2H	39.04	40	1E-74	107, 108, 144, 109
HvNHX1	AB089197.1	2H	41.86	52	0	108, 144, 109
HvMYB4	X99973.1	2H	72.45	504	0	-
DREB1	DQ012941.1	3H	45.63	46	0	111
dhn11	AF043086.1	3H	56.20	400	0	111
dhn10	AF043095.1	3H	105.03	505	0	114, 140
dhn6	AF043091.1	4H	43.34	42	0	117, 145, 118, 120, 119
HvNAC005	AK251058.1	4H	52.97	361	0	118, 120, 119, 162
HvNAC001	AK250475.1	5H	41.74	41	0	-
HvNAC027	AK368213.1	5H	44.38	278	0	-
HvNAC046	AK252960.1	5H	62.50	413	1E-162	125, 173
HvABI5	AY156992.1	5H	65.97	421	0	125, 173
HvNAC010	FR821754.1	5H	84.51	454	0	173, 126, 176
HvMYB1	X70879.1	5H	98.72	474	0	126, 176
dhn1	AF043087.1	5H	118.06	488	0	126, 176
dhn2	AF043088.1	5H	118.06	488	0	126, 176
dhn9	AF043094.1	5H	136.11	511	0	176
HvNAC003	AK249102.1	5H	149.10	530	0	127
HvSNAC1	JF796130.1	5H	149.24	530	0	127
dhn8	AF043093.1	6H	59.26	364	0	128, 129
dhn3	AF043089.1	6H	94.90	513	0	130, 131, 132
dhn4	AF043090.1	6H	94.90	513	0	130, 131, 132
dhn5	AF043096.1	6H	94.90	513	0	130, 131, 132
dhn7	AF043092.1	6H	95.61	514	0	130, 131, 132
HvNAC016	AK366470.1	7H	70.21	234	2E-37	135
SRG6	AJ300144.1	7H	73.58	425	0	135
HvNUD	AP009567.1	7H	78.36	510	0	135, 170
HvP1	AB032839.1	7H	108.73	562	0	135, 136, 137

[a] cM data and Mb data of SNPs are based on Mayer et al. 2012

[b] The expect (E) value describes the number of hits that can be expected by chance when searching a database of a particular size,
here candidate gene sequences were matched against the barley draft genome sequence (Mayer et al. 2012)

3.3 GENOTYPING BY SEQUENCING

GBS is a highly multiplexed, reproducible, and simple approach for generation of genome wide SNP markers. The technique was recently proposed by Elshire et al. (2011) and protocols for barley were developed by Poland et al. (2012). In GBS restriction enzymes are employed to construct reduced representation libraries of the target genome. Subsequently, DNA samples are barcoded and sequenced in parallel on next-generation sequencing platforms. The S42IL library was already characterized with 636 Illumina BOPA1 SNPs. In order to increase the marker density 55 S42ILs and the parental genotypes Scarlett and ISR42-8 were genotyped with GBS in this thesis (see chapter 2.2). Besides achieving a denser genetic characterization of the S42ILs the aim was to test how well the GBS generated map compares to the map comprised of BOPA1 SNPs.

The analysis produced sufficient sequence reads for all genotypes except S42IL-114. The DNA concentration for this genotype met the quality requirements. We thus conclude that an error must have occurred during library preparation. For this genotype only 2,000 sequence tags were obtained. A sequence tag is defined as unique sequence read in a genotype. For all other genotypes between 256,462 and 513,891 sequences tags were counted. The number of sequence tags per genotype followed the normal distribution. SNP calling was carried out using the TASSEL UNEAK pipeline (Lu et al. 2013) by identifying tag pairs. In a subsequent quality assurance step tags that had less than three reads in a genotype were marked as missing values in that specific genotype. The same was applied to heterozygous genotypes that showed an allele ratio different from 1:1. This resulted in the generation of 41,554 SNPs. Subsequently, the SNPs were filtered based on the following criteria: SNPs are polymorphic between the two parents Scarlett and ISR42-8, have less than 10% missing data, are not heterozygous in either parent, and do not have missing values for either of the parents. The filtering reduced the number of SNPs drastically. The random sequencing applied to the GBS library and the low coverage caused a high number of missing values. Therefore, filtering for less than 10% missing data had the largest effect reducing the number of SNPs from 41,554 to 7,113. Further filtering based on the other three criteria resulted in 3,744 SNPs that were used for constructing the S42IL genetic map. In the parent ISR42-8 3,087 markers were heterozygous after filtering for 10% missing values which were subsequently removed. It might make sense to include these markers in a future version of the map. For wild barley a certain amount of heterozygosity is expected. The reason we excluded these markers lies in the nature of the map we use. Typically, as in the map published by Schmalenbach

et al. (2009) three marker classes are distinguished; *Hv*, *Hsp*, and heterozygous, where heterozygous is defined as *Hv*/*Hsp*. Therefore, heterozygous markers in a parent genotype could be confounded with heterozygosity in the offspring and marker genotypes might not be attributed with 100% reliability to either of the parents. However, the large number of "lost" SNPs might be reason enough to change the map from the way it was used up to now.

The remaining 3,744 GBS SNPs were aligned to the barley WGS contigs (Mayer et al. 2012) using ViroBLAST (Deng et al. 2007). Additionally, 457 BOPA1 SNPs were included into the map using the same procedure. The combination of the two marker sets resulted in a map comprising 4,201 SNPs with a total length of 989.2 cM (3.9 Gb). The map has high coherence to the published barley physical map (Mayer et al. 2012).

Based on the new genetic map, the set of 54 S42ILs represents 84.9% of the donor genome if only the target introgressions are considered. When non-target introgressions are accounted for as well 86.6% is represented. On chromosomes 1H, 4H, 6H, and 7H only very small parts of the *Hsp* genome are not represented. The biggest gap is found on chromosome 5H, where 63.8% of the *Hsp* chromosome is not represented. These findings verify results obtained with the BOPA1 set reported by Schmalenbach et al. (2011). In their study 73 S42ILs were genotyped. The whole library represented 87.3% of the *Hsp* genome considering only the target introgressions and 89.5% if secondary introgressions were included as well. The slightly higher numbers might be explained by higher number of S42ILs genotyped in the study by Schmalenbach et al. (2011). Gaps in the introgression library, where the *Hsp* genome is missing, for example on 5H (63.3% BOPA chromosome map, 63.8% combined GBS and BOPA chromosome map) were also confirmed by the GBS data. The marker density ranged from 3.6 (4H) to 5.0 (7H) SNPs per cM or 0.8 (4H) to 1.3 (5H) SNPs per Mega base (Mb). Compared to the map containing only BOPA1 SNPs the inclusion of GBS SNPs improved the resolution of the map from 0.4 to 4.2 marker/cM.

In order to estimate the number of SNPs located within genes, the marker sequences were aligned to the barley nucleotide sequence database "HC_genes_CDS_Seq" on the IPK BLAST server (http://webblast.ipk-gatersleben.de/barley/) (Deng et al. 2007). The database contains RNA-seq and barley full length cDNA derived gene predictions. With the threshold of the expectation value set to 0.0001, 29% of the SNP markers were estimated to be located in coding gene sequences.

Overall the results indicate that accurate estimates of marker positions can be achieved through alignment of marker sequences to the barley genome sequence. This finding is shared by Mascher et al. (2013b). GBS proved to be a valuable and cost-effective method for generation of SNPs in the S42IL library. In order to overcome the large number of missing values and obtain a

higher number of reliable SNPs a second round of sequencing with the same protocol could be applied to the genotypes. Doubling the number of sequence reads would most likely improve the number of reads per sequence.

3.4 QTL REGIONS IN THE S42IL-LIBRARY

The S42IL-library has been characterized thoroughly for a large number of traits under diverse growing conditions and at different developmental stages. Initially, 59 S42ILs were developed by Schmalenbach et al. (2008). A subset of 39 S42ILs was tested in field trials for resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei* L.) and leaf rust (*Puccinia hordei* L.) (Schmalenbach et al. 2008). The 39 genotypes can be regarded as the core set of the S42ILs. It was studied in most of the later conducted QTL studies. The studies by Schmalenbach and Pillen (2009) and Schmalenbach et al. (2009) investigated the performance of the 39 genotypes for eight malting quality traits and seven agronomic traits, respectively. Wang et al. (2010) reinvestigated the core set for the same agronomic traits and described associations of photoperiod and vernalization genes to QTL for flowering and agronomic traits. Hoffmann et al. (2012) tested 42 S42ILs, including the core set. They investigated ten shoot and root related traits of juvenile plants in a hydroponic system applying two different nitrogen supply treatments. Schnaithmann and Pillen (2013) used a smaller set of 28 S42ILs in a QTL study on nitrogen stress tolerance under greenhouse conditions in adult plants. In that study 15 traits including morphological traits, grain parameters, and nitrogen and carbon content were considered. In the two studies by Honsdorf et al. (2014a) and Honsdorf et al. (2014b) 47, respectively 52 S42ILs were screened for drought stress response at juvenile development under greenhouse conditions. In the first study 14 traits were evaluated which included manually determined and image based growth parameters. The second study included eight growth and leaf senescence related traits. In both studies stress indices were calculated for all traits. Two further studies by Schmalenbach et al. (2011) and Naz et al. (2012) focused on one, respectively two, S42ILs. In the first study the *thresh-1* locus, initially detected in S42IL-143 was fine-mapped, employing the S42IL-HR mapping populations. The second study compared S42IL-126 and -176 to narrow the chromosomal region for two root traits and tiller number.

This summary shows that over the last years a large amount of information was gathered on the S42IL library. In order to facilitate comparison of different studies and co-localization of QTL for different traits QTL regions were determined for all traits detected in the studies mentioned

above. This was done by summarizing traits evaluated in different studies to trait groups. Subsequently, overlapping QTL were integrated to QTL regions. A complete list can be found in Table 1 of the appendix. It comprises all original QTL with information on QTL location, treatment, trait effect, and reference of the original study as well as locations of QTL regions. In total 49 traits were evaluated in the S42IL library. Eleven traits were evaluated in more than one study. The QTL were summarized to 228 QTL regions. The number of QTL regions per trait varied between one and twelve. Most QTL regions were detected for plant height. Figure 1 shows QTL regions for eight selected traits. Only traits that were studied in at least two research papers were included in the figure. The traits are biomass, days to heading, grains per ear, height, leaf greenness, thousand grain weight, tiller number, and yield. Altogether 71 QTL regions were detected for the eight traits. Six QTL regions were detected for yield, seven for thousand grain weight, eight for biomass and leaf greenness each, ten for days to heading, grains per ear, and tiller number, respectively, and twelve for plant height. On chromosomes 3H, 4H, 6H, and 7H QTL regions for all eight traits were localized. On chromosome 5H only QTL for five traits were detected. This might be explained by the large gap of wild barley alleles present on this chromosome. A share of 15.1% of the ISR42-8 (*Hsp*) alleles is not present in the S42IL library. The largest part of these 'missing' introgressions is located on chromosome 5H (Honsdorf et al. 2014b). This might explain that relatively few QTL are detected on this chromosome. Across all studies 419 genetic effects were detected. In 231 cases the *Hsp* allele had a trait reducing effect; in 188 cases trait performance was enhanced. Trait reducing or enhancing *Hsp* effects cannot be strictly assigned to positive and negative effects. Examples are earlier and later flowering dates. Both may have advantages, depending on the context. Early flowering can be an advantage to escape late season drought; while later flowering can be helpful to escape late frost periods in spring. In summary, the S42ILs harbor many useful alleles that may improve cultivar performance. For practical application in breeding programs the S42ILs can be directly crossed with elite breeding lines. In order to fine-map and eventually clone QTL further experiments are necessary. The investigation of S42ILs might be seen as a pre-screening for QTL effects. The rather small number of S42IL genotypes is good to handle in various phenotyping procedures. The size of the *Hsp* introgressions, however, is quite large. QTL regions provide a quick and simple overview of chromosomal regions related to phenotypes. The regions are large and therefore can just provide a rough first overview. As pointed out in the original papers (Chapters 2.1 and 2.2) a minimum number of one QTL is expected when an S42IL reveals a trait effect compared to Scarlett. When two S42ILs with overlapping introgressions show the same phenotypic effect it is assumed that this is due to the

same underlying QTL. The consolidation of QTL to QTL regions showed that in some regions several S42ILs overlap for the same trait. Overlaps, however, occur in different locations of the chromosomes. It might be assumed that more than one QTL is present in a genotype with two

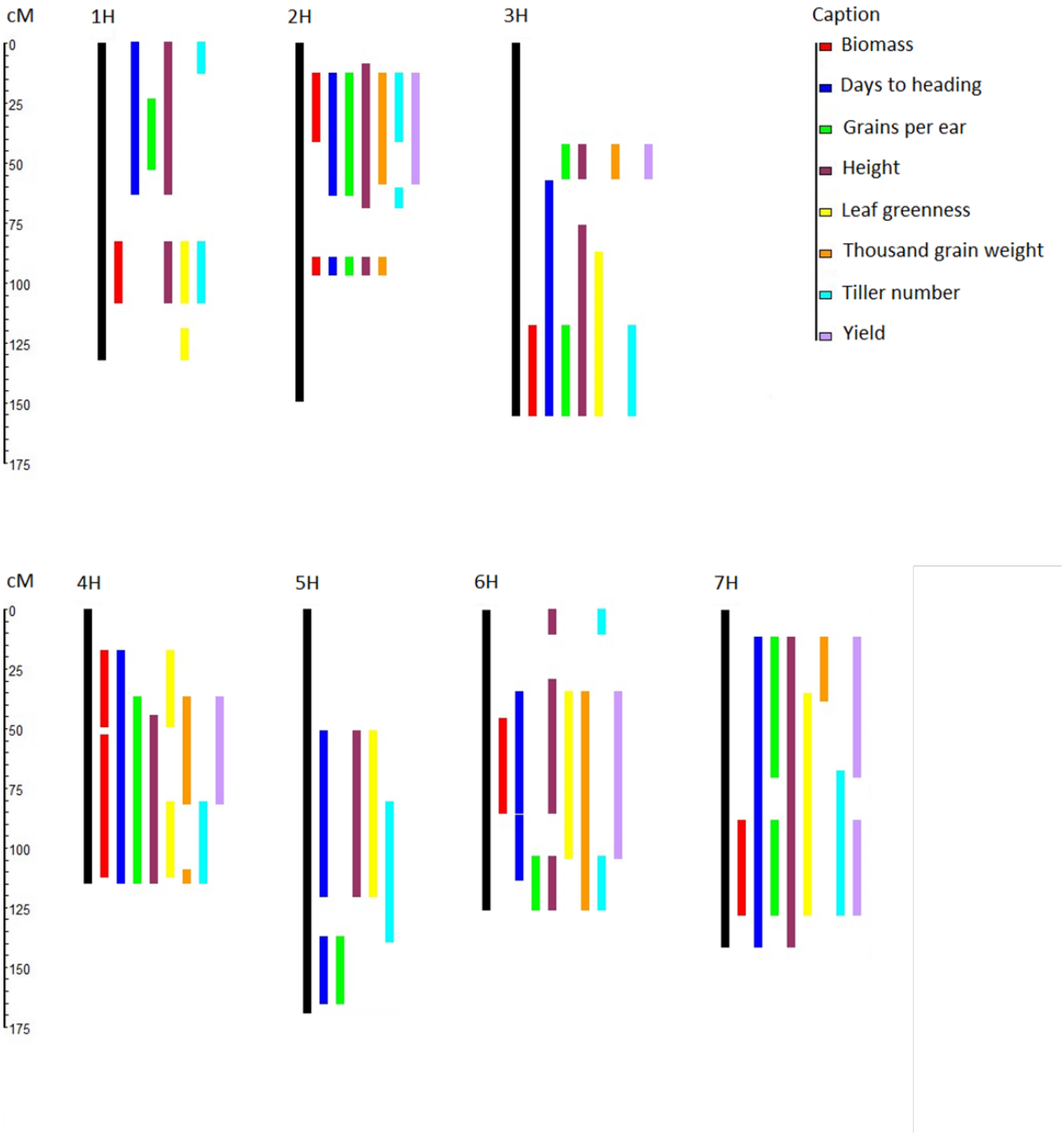

Figure 1: Overview of QTL regions for eight traits in the S42IL-library, black bars indicate the length of chromosomes, colored bars show QTL regions in which genetic effects for the eight listed traits were detected. All listed traits were investigated in at least two studies.

overlaps. On the other hand the assumption that a QTL is located in overlapping regions might be doubted. It might as well be that two or more independent QTL are active. Elucidation can only be achieved through further studies with the S42IL-HRs. Those harbor small introgressions and therefore enable fine-mapping of QTL effects.

3.5 Future Prospects

As demonstrated in chapter 2.1 and 2.2 several S42ILs were identified that show better performance under moderate and severe drought conditions compared to the cultivar Scarlett. The presented results were obtained in greenhouse experiments. In future S42ILs of interest should be grown in field sites to test if positive *Hsp* effects are also active under natural drought conditions. Genotypes with confirmed enhanced performance under field conditions might be integrated into breeding programs to improve tolerance to water limited conditions in juvenile plants.

Further phenotyping experiments could be carried out where the recovery ability of S42ILs after the exposure to drought is tested. Especially S42IL-123 that showed delayed leaf senescence under drought is of interest here. Moreover further characterization of the S42IL library with high-throughput imaging could be interesting to follow the plant development until maturity. Root development could be investigated with imaging methods, for example with the GROWSCREEN Rhizo at Jülich Plant Phenotyping Center.

In order to better describe the molecular base of differences between S42ILs and Scarlett fine-mapping of QTL and ultimate cloning of underlying candidate genes could be carried out employing the S42IL-HR population. Moreover, exome capture could be applied as described by Mascher et al. (2013a) to analyze differences in coding regions between drought tolerant genotypes and Scarlett. Moreover drought response could be studied at additional 'Omics' levels like transcriptome, proteome, and metabolome. Apart from characterizing existing introgression lines in more detail on phenotypic and molecular level, new introgression libraries could be developed. Koumproglou et al. (2002) proposed the 'Stepped Aligned Inbred Recombinant Strains' (STAIRS) approach. This type of library allows rapid fine-mapping of QTL.

4 SUMMARY

Drought is one of the most severe stresses, limiting crop yields worldwide. A main objective of plant breeding, therefore, is the development of varieties which maintain plant growth and high yields under water limited conditions. In order to achieve this goal access to exotic plant material and efficient phenotyping protocols are needed. Wild barley introgression lines (ILs) may facilitate the introduction of exotic alleles into elite breeding gene pools. ILs harbor a small chromosomal segment of a crop wild relative (CWR) donor genome in the genetic background of an adapted variety. Thus, small pieces of CWR genome can be evaluated for beneficial influence on trait performance without the negative influence of characteristics typically found in wild plants, e.g. seed shattering or brittleness.

In this thesis a set of wild barley introgression lines of the S42IL library was tested for its response to drought stress at the juvenile development stage. Subsequently, quantitative trait loci (QTL) were localized for growth and physiological parameters. Two different studies were conducted. The first study was carried out at the high-throughput phenotyping platform 'Plant Accelerator' in Adelaide, Australia. In this study 47 S42ILs were grown under moderate drought and well watered control conditions. Plants were imaged daily in an automated manner. The images were used to calculate plant biomass and related growth parameters. Image based biomass estimates were compared to biomass weight in order to evaluate the accuracy of the system. In the second study plant biomass and leaf senescence parameters were studied in 52 S42ILs under severe drought stress and well watered control conditions. The experiments were carried out under regular greenhouse conditions at 'Kühnfeld' experimental station of Martin-Luther University in Halle, Germany. In addition to plant phenotyping 55 S42ILs were genetically characterized with SNP markers generated by genotyping by sequencing (GBS). The aim was to achieve a denser coverage than previously attained with the Illumina BOPA1 chip and to compare results obtained by GBS and BOPA1.

The improved genetic map of the S42IL library consists of 4,201 SNPs composed of 3,744 GBS and 457 BOPA1 SNPs. The new map with a total length of 989.2 cM confirmed the extent of wild barley introgressions. Adding GBS data increased the resolution of the S42IL map 10-fold from 0.4 to 4.2 markers/cM.

Both studies revealed beneficial wild barley alleles that might improve drought tolerance in barley cultivars. The studies showed that under severe and moderate drought conditions tolerance is conferred by different QTL.

High correlation (r = 0.98) between image based biomass estimates and actual biomass were shown in the experiments at the 'Plant Accelerator'. This demonstrates the suitability of the system to accurately and non-destructively estimate biomass. QTL were located, which contributed to the genetic control of growth under drought stress. In total, 44 QTL for eleven out of 14 investigated traits were mapped. The correspondence of QTL with QTL identified in previous studies on the S42IL library was shown. For instance, six out of eight QTL controlling plant height were also found in previous field and glasshouse studies. Favorable wild barley alleles for growth and biomass parameters were detected, for instance, a QTL that increased biomass by approximately 36%. In particular, introgression line S42IL-121 revealed improved growth under drought stress compared to the control Scarlett. The introgression line showed a similar behavior in previous field experiments, indicating that S42IL-121 may be an attractive donor for breeding of drought tolerant barley cultivars.

In the experiments conducted at Martin-Luther University in Halle 52 S42ILs were tested in four independent experiments. Thirteen S42ILs showed effects for plant biomass and leaf senescence. Subsequently, two verification experiments were conducted with these 13 S42ILs. Nine out of eleven QTL were verified and 22 additional QTL were detected. For 21 QTL the wild barley allele increased trait performance. For traits like biomass this indicates the value of wild barley introgressions. For example, S42IL-107 and -123 produced more biomass under drought. Two different water-saving strategies were observed. S42IL-143 and -129 both revealed increased relative water content under drought. While S42IL-143 reduced biomass under drought, S42IL-129 maintained a high biomass production.

In future S42ILs of interest should be grown under drought conditions in field experiments to test if positive wild barley effects are active under natural drought. Genotypes with confirmed enhanced performance under field conditions might be integrated into breeding programs to improve tolerance to water limited conditions in juvenile plants. The two studies suggest that S42IL-121 might be of interest under moderate drought stress. S42IL-107, -123, and -129 could be beneficial in barley breeding programs to enhance tolerance to severe drought stress.

5 ZUSAMMENFASSUNG

Trockenheit ist einer der wichtigsten Stressfaktoren in der Pflanzenproduktion und gefährdet Ernteerträge in vielen Teilen der Erde. Ein wichtiges Ziel der Pflanzenzüchtung ist es daher Sorten zu entwickeln, die Wachstum auch unter wasserlimitierten Bedingungen aufrechterhalten und hohe Erträge produzieren. Um dieses Ziel zu erreichen muss Zugang zu exotischem, genetisch diversem Pflanzenmaterial sowie zu effizienten Phänotypisierungsprotokollen gegeben sein. Wildgerstenintrogressionslinien (ILs) können die Einführung von exotischen Allelen in Elitegenpools von Züchtungsprogrammen vereinfachen. ILs besitzen ein kleines Chromosomenstück von einer verwandten Wildpflanze im genetischen Hintergrund einer angepassten Kulturpflanze. Das Segment kann so auf positive Eigenschaften getestet werden ohne dass agronomisch negative Eigenschaften der Wildpflanzen wie z.B. Spindelbrüchigkeit ins Gewicht fallen.

In dieser Dissertation wurden Trockenstressantworten von Jungpflanzen der Wildgerstenintrogressionslinienbibliothek S42IL getestet. Anschließend wurden QTL (quantitative trait loci) für physiologische und Wachstumsparameter bestimmt. Die erste Studie wurde an der Hochdurchsatzphänotypisierungsplattform „Plant Accelerator“ in Adelaide in Australien durchgeführt. In dieser Studie wurden 47 S24ILs unter moderatem Trockenstress und gut gewässerten Kontrollbedingungen getestet. In einem automatisieren Verfahren wurden jeden Tag Bilder von allen Pflanzen aufgenommen. Auf Grundlage der Bilder wurden Biomasse und Wachstumsparameter berechnet. Bildbasierte Schätzwerte für Biomasse und gewogene Biomasse wurden korreliert um die Genauigkeit des Systems zu bestimmen. In der zweiten Studie wurden Wachstums- und Blattseneszenzparameter in 52 S42ILs unter starkem Trockenstress und gut gewässerten Kontrollbedingungen untersucht. Diese Untersuchungen wurden in einem regulären Gewächshaus auf dem Versuchsgut „Kühnfeld“ der Martin-Luther-Universität in Halle durchgeführt. Zusätzlich zur Phänotypisierung der Pflanzen wurden 55 der S42ILs mit durch „genotyping by sequencing“ (GBS) generierten SNP Markern genetisch charakterisiert. Ziel war es, eine höhere Abdeckung mit genetischen Markern im Vergleich zur bereits existierenden Karte mit Illumina BOPA1 SNPs zu erreichen und einen Vergleich zwischen genetischen Karten auf GBS und BOPA1 Basis durchzuführen.

Die verbesserte genetische Karte der S42ILs besteht aus 4.201 SNPs, welche sich aus 3.744 GBS und 457 BOPA1 SNPs zusammensetzen. Die neue Karte mit einer Länge von 989,3 cM bestätigt die Ausdehnung der Wildgerstenintrogressionen. Das Hinzufügen der GBS SNPs erhöhte die Auflösung der Karte um das Zehnfache, von 0,4 auf 4,2 Marker/cM.

Beide Studien zeigten positive Effekte von Wildgerstenallelen welche die Trockenstresstoleranz in Gerstensorten verbessern könnten. Die Studien zeigten auch, dass Toleranz unter moderatem und starkem Stress von unterschiedlichen QTL vermittelt wird.

Die Ergebnisse der Studien im „Plant Accelerator“ zeigten, dass bildbasierte Biomasseschätzungen und tatsächliche Biomasse hoch korreliert waren ($r = 0.98$). Die hohe Korrelation zeigt, dass das System geeignet ist, um Biomasseproduktion akkurat und nicht-invasiv zu bestimmen. QTL, die zur genetischen Kontrolle von Wachstum unter Trockenstress beitragen, wurden bestimmt. Insgesamt wurden 44 QTL für elf von 14 untersuchten Merkmalen gefunden. Es konnte gezeigt werden, dass einige QTL mit solchen aus früheren Studien mit der S42IL Bibliothek übereinstimmen. Beispielsweise wurden sechs von acht QTL für Pflanzenhöhe bereits in früheren Studien beschrieben. Wildgerstenallele mit positivem Einfluss auf Wachstums- und Biomasseparameter wurden detektiert. Darunter war z.B. ein QTL der die Biomasseproduktion um etwa 36% erhöht. Die Linie S42IL-121 zeigte verbessertes Wachstum unter Kontroll- und Stressbedingungen im Vergleich zu Scarlett. In vorhergegangenen Feldexperimenten zeigte S42IL-121 einen ähnlichen Phänotyp. Das deutet darauf hin, dass dieser Genotyp interessant für ein Züchtungsprogramm für trockentolerante Gerste seien könnte.

In den Experimenten an der Martin-Luther-Universität wurden 52 S42ILs in vier unabhängigen Experimenten getestet. Dreizehn S42ILs zeigten veränderte Merkmalsausprägung für Biomasse und Seneszenzparameter im Vergleich zu Scarlett. Im Anschluss wurden zwei Verifizierungsexperimente mit den 13 Genotypen durchgeführt. Neun von elf QTL konnten verifiziert werden. Außerdem wurden 22 zusätzliche QTL entdeckt. Bei 21 der QTL steigerte das Wildgerstenallel die Merkmalsausprägung. Für Merkmale wie z.B. Biomasse zeigt dies den Wert von Wildgerstenintrogressionen. Zum Beispiel produzierten S42IL-107 und -123 im Vergleich zu Scarlett mehr Biomasse unter Trockenstress. Zwei unterschiedliche Wassernutzungsstrategien wurden deutlich. S42IL-143 und -129 hatten beide einen erhöhten relativen Wassergehalt unter Trockenstress. Während S42IL-143 unter Trockenstress weniger Biomasse produzierte, hielt S42IL-129 eine hohe Biomasseproduktion bei.

In Zukunft sollten S42ILs, die in Gewächshausversuchen interessante Eigenschaften unter Trockenstress gezeigt haben, unter Feldbedingen getestet werden, um zu sehen ob die positiven Wildgersteneffekte auch unter natürlichen Trockenstressbedingungen aktiv sind. Lässt sich der positive Effekt unter Feldbedingungen bestätigen könnten die betreffenden S42ILs in Züchtungsprogramme integriert werden, um die Toleranz von Jungpflanzen gegen Wassermangel zu stärken. Aus den Studien geht hervor, dass S42IL-121 bei moderater Trockenheit interessant

sein könnte. Unter starkem Stress könnten S42IL-107, -123 und –129 positive Effekte in ein Gerstenzüchtungsprogramm einbringen um Trockenstresstoleranz zu stärken.

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9 ABBREVIATIONS

Abbreviation	Explanation
ABA	Abscisic acid
ABRE	ABA responsive element
AFLP	Amplified fragment length polymorphism
AGRI	Absolute growth rate integral
ANOVA	Analysis of variance
BC_2	Backcross two
BLAST	Basic local alignment search tool
BMD	Biomass dry
BMF	Biomass fresh
BOPA	Barley oligonucleotide pool assay
CALI	Caliper length integral
CBF	C-repeat binding factors
CCS	Chromosome substitution strain
cDNA	Complementary DNA
CG	Candidate gene
cM	Centi Morgan
COMI	Compactness integral
CV	Coefficient of variation
CWR	Crop wild relative
DArT	Diversity arrays technology
DH	Doubled haploid
DNA	Desoxyribonucleic acid
DPPN	Deutsches Pflanzenphänotypisierungsnetzwerk
DRE	Dehydration responsive element
EPPN	European plant phenotyping network
FDA	Food and drug administration
Gb	Gigabase
GBS	Genotyping by squencing
GEA	Grains per ear
HEA	Days to heading
HEI	Height
HEII	Height integral
Het	Heterozygous
HR	High resolution
Hsp	*Hordeum vulgare* ssp. *spontaneum*
HUEI	Plant hue integral

Abbreviation	Explanation
HULI	Hull area integral
Hv	*Hordeum vulgare* ssp.*vulgare*
IL	Introgression line
IPPN	International plant phenotyping network
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant
LSMEANS	Least squares means
MAP	Mitogen-activated protein
MAS	Marker assisted selection
PAM	Photosystem II efficiency
PS II	Photosystem II
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RGB	Red green blue
RGRI	Relative growth rate integral
RIL	Recombinant inbred line
RNA	Ribonucleic acid
RP	Relative performance
RWC	Relative water content
SAI	Shoot area integral
SATVI	Shoot area top view integral
SNP	Single nucleotid polymorphism
SPAD	Leaf greenness measure
SPW	Specific plant weight
SSI	Simple stress index
SSR	Simple sequence repeat
STAIRS	Stepped aligned inbred recombinant strains
STS	Sequence tagged sites
TF	Transcription factor
TGW	Thousand grain weight
TIL	Tiller number
Wcon	Water content
WGS	Whole genome shotgun
WUE	Water use efficiency

Appendix Table 1: Compilation of all QTL mapped in the S42IL library and published until October 2014 ; QTL regions indicate connected introgressions of ILs showing an effect for the same trait

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Biomass	BMD	QBmd.S42IL-1H	S42IL-143	d	-7.5	Honsdorf et al. 2014b	1H; 84.1-108.5	1H; 84.1-108.5
	BMD	QBmd.S42IL-2H	S42IL-107	d	10.6	Honsdorf et al. 2014b	2H; 12.7-40.8	2H; 12.7-40.8
	SAI	QSai.S42IL-2H	S42IL-110	d	-37.1	Honsdorf et al. 2014a	2H; 89.9-94.9	2H; 89.9-94.9
	BMD	QBmd.S42IL-3H	S42IL-115	a	-33.6	Honsdorf et al. 2014a	3H; 117.9-155	3H; 117.9-155
	SDW	QSdw.S42IL-3H	S42IL-115	N25	58.2	Hoffmann et al. 2012	3H; 117.9-155	
	BMD	QBmd.S42IL-4H	S42IL-117	a	-40.3	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	SAI	QSai.S42IL-4H	S42IL-117	a, d	-32.5	Honsdorf et al. 2014a	4H; 18.5-49.9	
	BMD	QBmd.S42IL-4Hb	S42IL-121	a	36.0	Honsdorf et al. 2014a	4H; 53.4-81.4	4H; 53.4-112.5
	SDW	QSdw.S42IL-4H	S42IL-121	N25	53.0	Hoffmann et al. 2012	4H; 53.4-81.4	
	BMD	QBmd.S42IL-4H	S42IL-123	d	8.5	Honsdorf et al. 2014b	4H; 81.4-112.5	
	BMD	QBmd.S42IL-6H	S42IL-129	a	-33.9	Honsdorf et al. 2014a	6H ; 46.5-86.3	6H ; 46.5-86.3
	SAI	QSai.S42IL-6H	S42IL-129	a, d	-30.4	Honsdorf et al. 2014a	6H ; 46.5-86.3	
	BMD	QBmd.S42IL-6H	S42IL-129	a, w	-7.3	Honsdorf et al. 2014b	6H ; 46.5-86.3	
	BMF	QBmf.S42IL-7H	S42IL-136	w	9.2	Honsdorf et al. 2014b	7H ; 88.9-116.1	7H ; 88.9-127.5
	BMF	QBmf.S42IL-7H	S42IL-137	w	8.2	Honsdorf et al. 2014b	7H ; 88.9-127.5	
	SDW	QSdw.S42IL-7H	S42IL-136	N25	53.6	Hoffmann et al. 2012	7H ; 88.9-116.1	
	SDW	QSdw.S42IL-7H	S42IL-137	N25	55.0	Hoffmann et al. 2012	7H ; 88.9-127.5	
C content straw	C_CONT_STR	QC_cont_str.S42IL-2H	S42IL-108	N1	-4.2	Schnaithmann and Pillen 2013	2H; 12.7-59	2H; 12.7-59
	C_CONT_STR	QC_cont_str.S42IL-2H.b	S42IL-153	N0	-4.7	Schnaithmann and Pillen 2013	2H; 60.7-68.6	2H; 60.7-68.6
	C_CONT_STR	QC_cont_str.S42IL-7H	S42IL-137	N1	6.2	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	7H ; 88.9-127.5
C/N straw	CN_STR	QCn_str.S42IL-2H	S42IL-153	All	-15.5	Schnaithmann and Pillen 2013	2H; 60.7-68.6	2H; 60.7-68.6
Caliper integral	CALI	QCali.S42IL-1H	S42IL-103	a, w	-18.4	Honsdorf et al. 2014a	1H; 24.9-53.3	1H; 24.9-53.3
	CALI	QCali.S42IL-2H	S42IL-110	a, d	-20.2	Honsdorf et al. 2014a	2H; 89.9-94.9	2H; 89.9-94.9
	CALI	QCali.S42IL-3H	S42IL-111	a	-14.8	Honsdorf et al. 2014a	3H; 42.6-56.9	3H; 42.6-56.9
	CALI	QCali.S42IL-4H	S42IL-117	a, d	-17.6	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	CALI	QCali.S42IL-4Hb	S42IL-121	a	15.2	Honsdorf et al. 2014a	4H; 53.4-81.4	4H; 53.4-81.4
	CALI	QCali.S42IL-6H	S42IL-129	a, d, w	-22.6	Honsdorf et al. 2014a	6H ; 46.5-86.3	6H ; 46.5-86.3
Compactness integral	COMI	QComi.S42IL-4H	S42IL-117	a, w	29.8	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	COMI	QComi.S42IL-6H	S42IL-128	w	37.1	Honsdorf et al. 2014a	6H ; 35.6-75.5	6H ; 35.6-75.5
Days to heading	HEA	QHea.S42IL-1H.a	S42IL-102	L + I	2.9	Schmalenbach et al. 2009	1H; 0-61.5	1H; 0-61.5
	HEA	QHea.S42IL-1H.a	S42IL-103	L + I	3.1	Schmalenbach et al. 2009	1H; 24.9-53.3	
	HEA	QHea.S42IL-1H.b	S42IL-105	L + I	-6.4	Schmalenbach et al. 2009	1H; 48.1-57.3	
	HEA	QHea.S42IL-2H.a	S42IL-107	L + I	-15.7	Schmalenbach et al. 2009	2H; 12.7-40.8	2H; 12.7-63.5
	HEA	QHea.S42IL-2H.a	S42IL-108	L + I	-15.5	Schmalenbach et al. 2009	2H; 12.7-59	
	HEA	QHea.S42IL-2H	S42IL-108	All + N0 + N1	-27.8	Schnaithmann and Pillen 2013	2H; 12.7-59	
	HEA	QHea.S42IL-2H	S42IL-107	L+I	-12.7	Wang et al. 2010	2H; 12.7-40.8	
	HEA	QHea.S42IL-2H	S42IL-108	L+I	-11.2	Wang et al. 2010	2H; 12.7-59	
	HEA	QHea.S42IL-2H.b	S42IL-109	I	-2.1	Schmalenbach et al. 2009	2H; 37.8-63.5	
	HEA	QHea.S42IL-2H.b	S42IL-110	L + I	-5.9	Schmalenbach et al. 2009	2H; 89.9-94.9	2H; 89.9-94.9

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Days to heading	HEA	QHea.S42IL-3H	S42IL-112	N1	-3.5	Schnaithmann and Pillen 2013	3H; 57.2-90.7	3H; 57.2-155
(continued)	HEA	QHea.S42IL-3H.a	S42IL-114	L + I	-5.2	Schmalenbach et al. 2009	3H; 76-144.6	
	HEA	QHea.S42IL-3H	S42IL-140	All + N1	-5.1	Schnaithmann and Pillen 2013	3H; 87.4-148.4	
	HEA	QHea.S42IL-3H.a	S42IL-115	I	-2.1	Schmalenbach et al. 2009	3H; 117.9-155	
	HEA	QHea.S42IL-3H	S42IL-115	All + N1	-3.6	Schnaithmann and Pillen 2013	3H; 117.9-155	
	HEA	QHea.S42IL-4H	S42IL-117	All + N1	-3.7	Schnaithmann and Pillen 2013	4H; 18.5-49.9	4H; 18.5-115.2
	HEA	QHea.S42IL-4H.a	S42IL-120	L + I	2.6	Schmalenbach et al. 2009	4H; 38-57.3	
	HEA	QHea.S42IL-4H	S42IL-119	N1	-5.4	Schnaithmann and Pillen 2013	4H; 38-81.4	
	HEA	QHea.S42IL-4H.b	S42IL-121	L + I	-9.9	Schmalenbach et al. 2009	4H; 53.4-81.4	
	HEA	QHea.S42IL-4H.b	S42IL-123	All + N0 + N1	-5.5	Schnaithmann and Pillen 2013	4H; 81.4-112.5	
	HEA	QHea.S42IL-4H.c	S42IL-124	L + I	2.9	Schmalenbach et al. 2009	4H; 110.2-115.2	
	HEA	QHea.S42IL-4H	S42IL-124	L+I	3.4	Wang et al. 2010	4H; 110.2-115.2	
	HEA	QHea.S42IL-5H.a	S42IL-125	L + I	-6.2	Schmalenbach et al. 2009	5H; 51.5-81.3	5H; 51.5-120.7
	HEA	QHea.S42IL-5H	S42IL-126	All + N1	-4.9	Schnaithmann and Pillen 2013	5H; 75.9-120.7	
	HEA	QHea.S42IL-5H.b	S42IL-127	All + N1	-4.1	Schnaithmann and Pillen 2013	5H; 137.8-165.9	5H; 137.8-165.9
	HEA	QHea.S42IL-6H	S42IL-128	N1	-3.8	Schnaithmann and Pillen 2013	6H ; 35.6-75.5	6H; 35.6-86.3
	HEA	QHea.S42IL-6H.a	S42IL-129	I	-2.1	Schmalenbach et al. 2009	6H ; 46.5-86.3	
	HEA	QHea.S42IL-6H.b	S42IL-131	N1	-4.2	Schnaithmann and Pillen 2013	6H ; 86.8-110.1	6H; 86.8-113.2
	HEA	QHea.S42IL-6H.b	S42IL-132	I	2.4	Schmalenbach et al. 2009	6H ; 94.8-113.2	
	HEA	QHea.S42IL-7H.a	S42IL-133	L + I	4.2	Schmalenbach et al. 2009	7H ; 12.7-37.6	7H; 12.7-141
	HEA	QHea.S42IL-7H	S42IL-133	L	2.9	Wang et al. 2010	7H ; 12.7-37.6	
	HEA	QHea.S42IL-7H.b	S42IL-134	L + I	-4.7	Schmalenbach et al. 2009	7H ; 37.6-70.2	
	HEA	QHea.S42IL-7H	S42IL-134	All + N1	-4.7	Schnaithmann and Pillen 2013	7H ; 37.6-70.2	
	HEA	QHea.S42IL-7Hb	S42IL-134	L+I	-6.0	Wang et al. 2010	7H ; 37.6-70.2	
	HEA	QHea.S42IL-7H.b	S42IL-135	I	-2.1	Schmalenbach et al. 2009	7H ; 67.4-118.5	
	HEA	QHea.S42IL-7H	S42IL-135	All + N0 + N1	-7.1	Schnaithmann and Pillen 2013	7H ; 67.4-118.5	
	HEA	QHea.S42IL-7H.c	S42IL-137	L + I	-9.2	Schmalenbach et al. 2009	7H ; 88.9-127.5	
	HEA	QHea.S42IL-7H	S42IL-137	All + N0 + N1	-4.8	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	
	HEA	QHea.S42IL-7H	S42IL-138	N1	-4.9	Schnaithmann and Pillen 2013	7H ; 116.1-141	
Fine-grind extract of	FGE	QFge.S42IL-1H.a	S42IL-102	L+I	-3.2	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
malt	FGE	QFge.S42IL-1H.a	S42IL-103	L+I	-2.9	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	FGE	QFge.S42IL-1H.a	S42IL-105	L	-1.6	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	FGE	QFge.S42IL-4H.a	S42IL-121	L+I	-2.6	Schmalenbach and Pillen 2009	4H; 53.4-81.4	4H; 53.4-81.4
	FGE	QFge.S42IL-6H.a	S42IL-128	L+I	-2.3	Schmalenbach and Pillen 2009	6H ; 35.6-75.5	6H ; 35.6-86.3
	FGE	QFge.S42IL-6H.a	S42IL-129	L+I	-2.2	Schmalenbach and Pillen 2009	6H ; 46.5-86.3	
	FGE	QFge.S42IL-7H.a	S42IL-137	L+I	-2.3	Schmalenbach and Pillen 2009	7H ; 88.9-127.5	7H ; 88.9-127.5
Friability of malt	FRI	QFri.S42IL-1H.a	S42IL-102	L+I	-20.0	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
	FRI	QFri.S42IL-1H.a	S42IL-103	L+I	-15.7	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	FRI	QFri.S42IL-1H.a	S42IL-105	L+I	-16.3	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	FRI	QFri.S42IL-4H.a	S42IL-118	I	-18.0	Schmalenbach and Pillen 2009	4H; 38-54.6	4H; 38-81.4
	FRI	QFri.S42IL-4H.a	S42IL-119	I	-19.0	Schmalenbach and Pillen 2009	4H; 38-81.4	
	FRI	QFri.S42IL-4H.a	S42IL-121	L	-11.6	Schmalenbach and Pillen 2009	4H; 53.4-81.4	

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Grain area	G_AREA	QArea.S42IL-1H	S42IL-102	All + N0	6.7	Schnaithmann and Pillen 2013	1H; 0-61.5	1H; 0-61.5
	G_AREA	QArea.S42IL-1H.b	S42IL-143	All + N0 + N1	15.8	Schnaithmann and Pillen 2013	1H; 84.1-108.5	1H; 84.1-108.5
	G_AREA	QArea.S42IL-2H	S42IL-108	N0	9.4	Schnaithmann and Pillen 2013	2H; 12.7-59	2H; 12.7-59
	G_AREA	QArea.S42IL-3H	S42IL-111	N0	7.9	Schnaithmann and Pillen 2013	3H; 42.6-56.9	3H; 42.6-56.9
	G_AREA	QArea.S42IL-4H	S42IL-119	All + N0 + N1	7.5	Schnaithmann and Pillen 2013	4H; 38-81.4	4H; 38-81.4
	G_AREA	QArea.S42IL-6H	S42IL-130	N0	9.4	Schnaithmann and Pillen 2013	6H ; 59.9-105	6H ; 59.9-105
Grain length	G_LENGTH	QG_length.S42IL-1H	S42IL-102	All + N0 + N1	7.3	Schnaithmann and Pillen 2013	1H; 0-61.5	1H; 0-61.5
	G_LENGTH	QG_length.S42IL-1H.b	S42IL-143	All + N0 + N1	25.2	Schnaithmann and Pillen 2013	1H; 84.1-108.5	1H; 84.1-108.5
	G_LENGTH	QG_length.S42IL-7H	S42IL-137	All + N1	5.8	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	7H ; 88.9-127.5
Grain protein content	GPC	QGpc.S42IL-1H.a	S42IL-102	L+I	5.9	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
	GPC	QGpc.S42IL-1H.a	S42IL-105	L+I	6.6	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	GPC	QGpc.S42IL-4H.a	S42IL-121	L+I	7.7	Schmalenbach and Pillen 2009	4H; 53.4-81.4	4H; 53.4-81.4
	GPC	QGpc.S42IL-6H.a	S42IL-128	L	4.7	Schmalenbach and Pillen 2009	6H ; 35.6-75.5	6H ; 35.6-86.3
	GPC	QGpc.S42IL-6H.a	S42IL-129	L+I	8.9	Schmalenbach and Pillen 2009	6H ; 46.5-86.3	
	GPC	QGpc.S42IL-7H.a	S42IL-137	L+I	7.4	Schmalenbach and Pillen 2009	7H ; 88.9-127.5	7H ; 88.9-127.5
Grain sieving fraction >	GSF	QGsf.S42IL-1H.a	S42IL-102	L+I	-12.7	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
2.5 mm	GSF	QGsf.S42IL-1H.a	S42IL-103	L+I	-6.4	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	GSF	QGsf.S42IL-2H.a	S42IL-107	L+I	8.4	Schmalenbach and Pillen 2009	2H; 12.7-40.8	2H; 12.7-63.5
	GSF	QGsf.S42IL-2H.b	S42IL-108	I	-6.1	Schmalenbach and Pillen 2009	2H; 12.7-59	
	GSF	QGsf.S42IL-2H.b	S42IL-109	L+I	-12.0	Schmalenbach and Pillen 2009	2H; 37.8-63.5	
	GSF	QGsf.S42IL-2H.c	S42IL-110	L	6.7	Schmalenbach and Pillen 2009	2H; 89.9-94.9	2H; 89.9-94.9
	GSF	QGsf.S42IL-3H.a	S42IL-114	I	-10.2	Schmalenbach and Pillen 2009	3H; 76-144.6	3H; 76-155
	GSF	QGsf.S42IL-3H.a	S42IL-115	I	-10.0	Schmalenbach and Pillen 2009	3H; 117.9-155	
	GSF	QGsf.S42IL-4H.a	S42IL-119	L	6.2	Schmalenbach and Pillen 2009	4H; 38-81.4	4H; 38-115.2
	GSF	QGsf.S42IL-4H.b	S42IL-121	I	-11.9	Schmalenbach and Pillen 2009	4H; 53.4-81.4	
	GSF	QGsf.S42IL-4H.b	S42IL-123	L+I	-8.0	Schmalenbach and Pillen 2009	4H; 81.4-112.5	
	GSF	QGsf.S42IL-4H.c	S42IL-124	L+I	-7.2	Schmalenbach and Pillen 2009	4H; 110.2-115.2	
	GSF	QGsf.S42IL-4H.b	S42IL-122	L+I	-14.1	Schmalenbach and Pillen 2009	6H ; 104.8-126.5	6H ; 104.8-126.5
	GSF	QGsf.S42IL-7H.a	S42IL-134	L+I	-7.5	Schmalenbach and Pillen 2009	7H ; 37.6-70.2	7H ; 37.6-141
	GSF	QGsf.S42IL-7H.a	S42IL-135	L	-6.6	Schmalenbach and Pillen 2009	7H ; 67.4-118.5	
	GSF	QGsf.S42IL-7H.b	S42IL-137	L+I	-10.3	Schmalenbach and Pillen 2009	7H ; 88.9-127.5	
	GSF	QGsf.S42IL-7H.b	S42IL-138	L+I	-7.7	Schmalenbach and Pillen 2009	7H ; 116.1-141	
Grain width	G_WIDTH	QG_width.S42IL-2H	S42IL-108	N0	5.4	Schnaithmann and Pillen 2013	2H; 12.7-59	2H; 12.7-59
	G_WIDTH	QG_width.S42IL-3H	S42IL-111	N0	5.4	Schnaithmann and Pillen 2013	3H; 42.6-56.9	3H; 42.6-56.9
	G_WIDTH	QG_width.S42IL-4H	S42IL-119	N0	6.8	Schnaithmann and Pillen 2013	4H; 38-81.4	4H; 38-81.4
	G_WIDTH	QG_width.S42IL-6H	S42IL-150	All + N0 + N1	-6.7	Schnaithmann and Pillen 2013	6H; 73.9-82.43	6H; 35.6-110.1
	G_WIDTH	QG_width.S42IL-6H	S42IL-128	N1	-4.9	Schnaithmann and Pillen 2013	6H ; 35.6-75.5	
	G_WIDTH	QG_width.S42IL-6H.b	S42IL-130	N0	7.7	Schnaithmann and Pillen 2013	6H ; 59.9-105	
	G_WIDTH	QG_width.S42IL-6H.b	S42IL-131	N0	6.5	Schnaithmann and Pillen 2013	6H ; 86.8-110.1	
	G_WIDTH	QG_width.S42IL-7H	S42IL-134	N1	-5.0	Schnaithmann and Pillen 2013	7H ; 37.6-70.2	7H ; 37.6-70.2

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Grains per ear	GEA	QGea.S42IL-1H.a	S42IL-103	I	-16.3	Schmalenbach et al. 2009	1H; 24.9-53.3	1H; 24.9-53.3
	GEA	QGea.S42IL-2H.a	S42IL-107	L + I	-26.8	Schmalenbach et al. 2009	2H; 12.7-40.8	2H; 12.7-63.5
	GEA	QGea.S42IL-2H.a	S42IL-108	L + I	-14.2	Schmalenbach et al. 2009	2H; 12.7-59	
	GEA	QGea.S42IL-2H	S42IL-108	N1	-32.1	Schnaithmann and Pillen 2013	2H; 12.7-59	
	GEA	QGea.S42IL-2H	S42IL-107	L+I	-26.8	Wang et al. 2010	2H; 12.7-40.8	
	GEA	QGea.S42IL-2H	S42IL-108	L+I	-14.2	Wang et al. 2010	2H; 12.7-59	
	GEA	QGea.S42IL-2H.b	S42IL-109	L + I	-19.5	Schmalenbach et al. 2009	2H; 37.8-63.5	
	GEA	QGea.S42IL-2H	S42IL-109	L+I	-19.5	Wang et al. 2010	2H; 37.8-63.5	
	GEA	QGea.S42IL-2H.b	S42IL-110	L + I	-20.5	Schmalenbach et al. 2009	2H; 89.9-94.9	2H; 89.9-94.9
	GEA	QGea.S42IL-3H.a	S42IL-111	L + I	-16.6	Schmalenbach et al. 2009	3H; 42.6-56.9	3H; 42.6-56.9
	GEA	QGea.S42IL-3H	S42IL-111	L+I	-16.6	Wang et al. 2010	3H; 42.6-56.9	
	GEA	QGea.S42IL-3H	S42IL-115	N1	13.7	Schnaithmann and Pillen 2013	3H; 117.9-155	3H; 117.9-155
	GEA	QGea.S42IL-4H	S42IL-119	N0	-22.8	Schnaithmann and Pillen 2013	4H; 38-81.4	4H; 38-115.2
	GEA	QGea.S42IL-4H.a	S42IL-121	L + I	-10.8	Schmalenbach et al. 2009	4H; 53.4-81.4	
	GEA	QGea.S42IL-4H.a	S42IL-123	L + I	-10.3	Schmalenbach et al. 2009	4H; 81.4-112.5	
	GEA	QGea.S42IL-4H.b	S42IL-124	I	-17.6	Schmalenbach et al. 2009	4H; 110.2-115.2	
	GEA	QGea.S42IL-4H	S42IL-124	I	-17.6	Wang et al. 2010	4H; 110.2-115.2	
	GEA	QGea.S42IL-5H	S42IL-127	N0	-56.8	Schnaithmann and Pillen 2013	5H; 137.8-165.9	5H; 137.8-165.9
	GEA	QGea.S42IL-4H.a	S42IL-122	I	-14.6	Schmalenbach et al. 2009	6H ; 104.8-126.5	6H ; 104.8-126.5
	GEA	QGea.S42IL-7H.a	S42IL-133	I	-17.6	Schmalenbach et al. 2009	7H ; 12.7-37.6	7H ; 12.7-70.2
	GEA	QGea.S42IL-7H	S42IL-133	N1	-13.3	Schnaithmann and Pillen 2013	7H ; 12.7-37.6	
	GEA	QGea.S42IL-7H	S42IL-133	I	-17.6	Wang et al. 2010	7H ; 12.7-37.6	
	GEA	QGea.S42IL-7H.a	S42IL-134	L + I	-9.8	Schmalenbach et al. 2009	7H ; 37.6-70.2	
	GEA	QGea.S42IL-7Hb	S42IL-134	L+I	-9.8	Wang et al. 2010	7H ; 37.6-70.2	
	GEA	QGea.S42IL-7H.b	S42IL-136	I	-14.6	Schmalenbach et al. 2009	7H ; 88.9-116.1	7H ; 88.9-127.5
	GEA	QGea.S42IL-7H.b	S42IL-137	I	-20.6	Schmalenbach et al. 2009	7H ; 88.9-127.5	
Growth rate	AGRI	QAgri.S42IL-3H	S42IL-115	a	-30.3	Honsdorf et al. 2014a	3H; 117.9-155	3H; 117.9-155
	AGRI	QAgri.S42IL-4H	S42IL-117	a, d	-32.9	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	AGRI	QAgri.S42IL-6H	S42IL-129	a	-29.2	Honsdorf et al. 2014a	6H ; 46.5-86.3	6H ; 46.5-86.3
Hartong 45 °C, extract at 45 °C	VZ45	QVZ45.S42IL-1H.a	S42IL-102	L+I	-9.8	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
	VZ45	QVZ45.S42IL-1H.a	S42IL-103	L+I	-10.5	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	VZ45	QVZ45.S42IL-1H.a	S42IL-105	L+I	-7.6	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	VZ45	QVZ45.S42IL-4H.a	S42IL-121	L+I	-6.9	Schmalenbach and Pillen 2009	4H; 53.4-81.4	4H; 53.4-81.4
	VZ45	QVZ45.S42IL-5H.a	S42IL-125	L+I	-10.1	Schmalenbach and Pillen 2009	5H; 51.5-81.3	5H; 51.5-120.7
	VZ45	QVZ45.S42IL-5H.a	S42IL-126	L+I	-8.4	Schmalenbach and Pillen 2009	5H; 75.9-120.7	
	VZ45	QVZ45.S42IL-6H.a	S42IL-128	L	-6.8	Schmalenbach and Pillen 2009	6H ; 35.6-75.5	6H ; 35.6-75.5

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Height	HEI	QHei.S42IL-1H.a	S42IL-102	ALL	7.0	Hoffmann et al. 2012	1H; 0-61.5	1H; 0-61.5
	HEI	QHei.S42IL-1H	S42IL-103	a, w	-12.8	Honsdorf et al. 2014a	1H; 24.9-53.3	
	HEII	QHeii.S42IL-1H	S42IL-103	a	-15.6	Honsdorf et al. 2014a	1H; 24.9-53.3	
	HEI	QHei.S42IL-1H.a	S42IL-105	L + l	11.3	Schmalenbach et al. 2009	1H; 48.1-57.3	
	HEI	QHei.S42IL-1Hb	S42IL-143	a	-11.2	Honsdorf et al. 2014a	1H; 84.1-108.5	1H; 84.1-108.5
	HEII	QHeii.S42IL-1Hb	S42IL-143	a	-13.9	Honsdorf et al. 2014a	1H; 84.1-108.5	
	HEI	QHei.S42IL-1H	S42IL-143	a	-4.8	Honsdorf et al. 2014b	1H; 84.1-108.5	
	HEI	QHei.S42IL-1H.b	S42IL-143	ALL+N100+N25	-18.1	Hoffmann et al. 2012	1H; 84.1-108.5	
	HEI	QHei.S42IL-2H.a	S42IL-106	N25	12.9	Hoffmann et al. 2012	2H; 8.9-18.1	2H; 8.9-68.6
	HEI	QHei.S42IL-2H.a	S42IL-107	L + l	-14.6	Schmalenbach et al. 2009	2H; 12.7-40.8	
	HEI	QHei.S42IL-2H	S42IL-107	d	-4.8	Honsdorf et al. 2014b	2H; 12.7-40.8	
	HEI	QHei.S42IL-2H	S42IL-107	L+l	-15.1	Wang et al. 2010	2H; 12.7-40.8	
	HEI	QHei.S42IL-2H	S42IL-108	L	-5.3	Wang et al. 2010	2H; 12.7-59	
	HEI	QHei.S42IL-2H.a	S42IL-109	L + l	-9.6	Schmalenbach et al. 2009	2H; 37.8-63.5	
	HEI	QHei.S42IL-2H	S42IL-109	a	-9.5	Honsdorf et al. 2014a	2H; 37.8-63.5	
	HEI	QHei.S42IL-2H.b	S42IL-109	ALL+N25	6.9	Hoffmann et al. 2012	2H; 37.8-63.5	
	HEI	QHei.S42IL-2H	S42IL-109	L+l	-9.5	Wang et al. 2010	2H; 37.8-63.5	
	HEI	QHei.S42IL-2Hb	S42IL-153	a	-4.0	Honsdorf et al. 2014b	2H; 60.7-68.6	
	HEI	QHei.S42IL-2H.b	S42IL-110	N25	12.1	Hoffmann et al. 2012	2H; 89.9-94.9	2H; 89.9-94.9
	HEI	QHei.S42IL-3H	S42IL-111	a	-9.2	Honsdorf et al. 2014a	3H; 42.6-56.9	3H; 42.6-56.9
	HEI	QHei.S42IL-3H.a	S42IL-114	L + l	17.5	Schmalenbach et al. 2009	3H; 76-144.6	3H; 76-155
	HEI	QHei.S42IL-3Hb	S42IL-140	a, d, w	18.7	Honsdorf et al. 2014a	3H; 87.4-148.4	
	HEI	QHei.S42IL-3H	S42IL-140	a, w	5.5	Honsdorf et al. 2014b	3H; 87.4-148.4	
	HEI	QHei.S42IL-3H	S42IL-115	ALL+N25	10.9	Hoffmann et al. 2012	3H; 117.9-155	
	HEII	QHeii.S42IL-4H	S42IL-162	a	-14.4	Honsdorf et al. 2014a	4H; 45.7-81.4	4H; 45.7-115.2
	HEI	QHei.S42IL-4H.a	S42IL-121	L + l	18.1	Schmalenbach et al. 2009	4H; 53.4-81.4	
	HEI	QHei.S42IL-4H	S42IL-121	a, d, w	17.9	Honsdorf et al. 2014a	4H; 53.4-81.4	
	HEI	QHei.S42IL-4H	S42IL-121	ALL+N25	7.3	Hoffmann et al. 2012	4H; 53.4-81.4	
	HEI	QHei.S42IL-4H	S42IL-123	ALL+N25	7.8	Hoffmann et al. 2012	4H; 81.4-112.5	
	HEII	QHeii.S42IL-4Hb	S42IL-124	a	-14.0	Honsdorf et al. 2014a	4H; 110.2-115.2	
	HEI	QHei.S42IL-4H	S42IL-124	L	-4.4	Wang et al. 2010	4H; 110.2-115.2	
	HEI	QHei.S42IL-5H.a	S42IL-125	L + l	15.1	Schmalenbach et al. 2009	5H; 51.5-81.3	5H; 51.5-120.7
	HEI	QHei.S42IL-5H	S42IL-126	N25	13.3	Hoffmann et al. 2012	5H; 75.9-120.7	
	HEI	QHei.S42IL-6H	S42IL-148	ALL	7.5	Hoffmann et al. 2012	6H ; 1-10.6	6H ; 1-10.6
	HEI	QHei.S42IL-6H	S42IL-149	a	-4.8	Honsdorf et al. 2014b	6H ; 30-52.2	6H ; 30-86.3
	HEI	QHei.S42IL-6H	S42IL-129	a	-9.4	Honsdorf et al. 2014a	6H ; 46.5-86.3	
	HEI	QHei.S42IL-6Hb	S42IL-122	a, w	-5.5	Honsdorf et al. 2014b	6H ; 104.8-126.5	6H ; 104.8-126.5
	HEI	QHei.S42IL-7H.a	S42IL-133	ALL+N100	-9.1	Hoffmann et al. 2012	7H ; 12.7-37.6	7H ; 12.7-141
	HEI	QHei.S42IL-7H.a	S42IL-134	L + l	11.4	Schmalenbach et al. 2009	7H ; 37.6-70.2	
	HEI	QHei.S42IL-7H.a	S42IL-134	N100	-12.8	Hoffmann et al. 2012	7H ; 37.6-70.2	
	HEI	QHei.S42IL-7H	S42IL-134	L+l	9.2	Wang et al. 2010	7H ; 37.6-70.2	

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Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Height (continued)	HEI	QHei.S42IL-7H.b	S42IL-135	ALL+N25	9.6	Hoffmann et al. 2012	7H ; 67.4-118.5	
	HEI	QHei.S42IL-7H.b	S42IL-137	L + I	19.7	Schmalenbach et al. 2009	7H ; 88.9-127.5	
	HEI	QHei.S42IL-7H	S42IL-137	a, d	11.6	Honsdorf et al. 2014a	7H ; 88.9-127.5	
	HEI	QHei.S42IL-7H	S42IL-137	a	3.8	Honsdorf et al. 2014b	7H ; 88.9-127.5	
	HEI	QHei.S42IL-7H.b	S42IL-136	ALL+N25	8.4	Hoffmann et al. 2012	7H ; 88.9-116.1	
	HEI	QHei.S42IL-7H.b	S42IL-137	ALL+N25	12.8	Hoffmann et al. 2012	7H ; 88.9-127.5	
	HEII	QHeii.S42IL-7H	S42IL-138	a	-13.9	Honsdorf et al. 2014a	7H ; 116.1-141	
	HEI	QHei.S42IL-7H.c	S42IL-138	ALL+N25	8.9	Hoffmann et al. 2012	7H ; 116.1-141	
	HEI	QHei.S42IL-7H.c	S42IL-139	N25	12.9	Hoffmann et al. 2012	7H ; 129.4-141	
Hull area integral	HULI	QHuli.S42IL-1H	S42IL-103	a, w	-33.5	Honsdorf et al. 2014a	1H; 24.9-53.3	1H; 24.9-53.3
	HULI	QHuli.S42IL-2H	S42IL-110	a, d	-32.4	Honsdorf et al. 2014a	2H; 89.9-94.9	2H; 89.9-94.9
	HULI	QHuli.S42IL-3H	S42IL-111	a	-30.9	Honsdorf et al. 2014a	3H; 42.6-56.9	3H; 42.6-56.9
	HULI	QHuli.S42IL-3Hb	S42IL-115	a	-30.9	Honsdorf et al. 2014a	3H; 117.9-155	3H; 117.9-155
	HULI	QHuli.S42IL-4H	S42IL-117	a	-35.2	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	HULI	QHuli.S42IL-6H	S42IL-129	a	-37.4	Honsdorf et al. 2014a	6H ; 46.5-86.3	6H ; 46.5-86.3
Kolbach index	KOL	QKol.S42IL-1H.a	S42IL-102	L+I	-11.5	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
	KOL	QKol.S42IL-1H.a	S42IL-103	L+I	-7.0	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	KOL	QKol.S42IL-1H.a	S42IL-105	L+I	-8.2	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	KOL	QKol.S42IL-3H.a	S42IL-115	I	-7.0	Schmalenbach and Pillen 2009	3H; 117.9-155	3H; 117.9-155
	KOL	QKol.S42IL-4H.a	S42IL-116	L	5.1	Schmalenbach and Pillen 2009	4H; 1.1-40	4H; 1.1-40
	KOL	QKol.S42IL-4H.b	S42IL-121	L+I	-10.1	Schmalenbach and Pillen 2009	4H; 53.4-81.4	4H; 53.4-81.4
	KOL	QKol.S42IL-5H.a	S42IL-125	L+I	-7.3	Schmalenbach and Pillen 2009	5H; 51.5-81.3	5H; 51.5-120.7
	KOL	QKol.S42IL-5H.a	S42IL-126	L+I	-7.1	Schmalenbach and Pillen 2009	5H; 75.9-120.7	
	KOL	QKol.S42IL-6H.a	S42IL-129	L+I	-6.4	Schmalenbach and Pillen 2009	6H ; 46.5-86.3	6H ; 46.5-105
	KOL	QKol.S42IL-6H.a	S42IL-130	I	-8.8	Schmalenbach and Pillen 2009	6H ; 59.9-105	
	KOL	QKol.S42IL-7H.a	S42IL-133	I	-10.6	Schmalenbach and Pillen 2009	7H ; 12.7-37.6	7H ; 12.7-37.6
Leaf greenness	SPAD	QSpad.S42IL-1H	S42IL-143	d	45.6	Honsdorf et al. 2014b	1H; 84.1-108.5	1H; 84.1-108.5
	CC_HEA	QCc_hea.S42IL-1H	S42IL-142	N0	-8.0	Schnaithmann and Pillen 2013	1H; 119.7-132.7	1H; 119.7-132.7
	CC_HEA	QCc_hea.S42IL-3H	S42IL-140	N0	-14.0	Schnaithmann and Pillen 2013	3H; 87.4-148.4	3H; 87.4-155
	CC_HEA	QCc_hea.S42IL-3H	S42IL-115	N0	-7.2	Schnaithmann and Pillen 2013	3H; 117.9-155	
	CC_HEA	QCc_hea.S42IL-3H	S42IL-115	N1	4.6	Schnaithmann and Pillen 2013	3H; 117.9-155	
	CC_HEA	QCc_hea.S42IL-4H	S42IL-117	N0	-6.3	Schnaithmann and Pillen 2013	4H; 18.5-49.9	4H; 18.5-49.9
	CC_HEA	QCc_hea.S42IL-4H.b	S42IL-123	N0	-7.8	Schnaithmann and Pillen 2013	4H; 81.4-112.5	4H; 81.4-112.5
	PAM	QPam.S42IL-4H	S42IL-123	a	42.0	Honsdorf et al. 2014b	4H; 81.4-112.5	
	SPAD	QSpad.S42IL-4H	S42IL-123	d	63.4	Honsdorf et al. 2014b	4H; 81.4-112.5	
	CC_HEA	QCc_hea.S42IL-5H	S42IL-125	N0	-7.4	Schnaithmann and Pillen 2013	5H; 51.5-81.3	5H; 51.5-120.7
	CC_HEA	QCc_hea.S42IL-5H	S42IL-126	N1	5.5	Schnaithmann and Pillen 2013	5H; 75.9-120.7	
	PAM	QPam.S42IL-6H	S42IL-128	a	31.4	Honsdorf et al. 2014b	6H ; 35.6-75.5	6H ; 35.6-105
	CC_HEA	QCc_hea.S42IL-6H	S42IL-130	N1	5.2	Schnaithmann and Pillen 2013	6H ; 59.9-105	
	CC_HEA	QCc_hea.S42IL-7H	S42IL-134	N0	-7.9	Schnaithmann and Pillen 2013	7H ; 37.6-70.2	7H ; 37.6-127.5
	CC_HEA	QCc_hea.S42IL-7H	S42IL-135	N0	-8.2	Schnaithmann and Pillen 2013	7H ; 67.4-118.5	
	CC_HEA	QCc_hea.S42IL-7H	S42IL-135	N1	6.8	Schnaithmann and Pillen 2013	7H ; 67.4-118.5	
	CC_HEA	QCc_hea.S42IL-7H	S42IL-137	All + N0	-4.3	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Leaf length	LL	QLl.S42IL-1H	S42IL-143	ALL+N100+N25	-14.3	Hoffmann et al. 2012	1H; 84.1-108.5	1H; 84.1-108.5
	LL	QLl.S42IL-2H	S42IL-107	N100	-9.3	Hoffmann et al. 2012	2H; 12.7-40.8	2H; 12.7-59
	LL	QLl.S42IL-2H	S42IL-108	N100	-9.7	Hoffmann et al. 2012	2H; 12.7-59	
	LL	QLl.S42IL-3H.a	S42IL-113	N100	-9.1	Hoffmann et al. 2012	3H; 117.9-143.1	3H; 117.9-155
	LL	QLl.S42IL-3H.b	S42IL-115	N25	15.0	Hoffmann et al. 2012	3H; 117.9-155	
	LL	QLl.S42IL-4H	S42IL-121	N25	11.8	Hoffmann et al. 2012	4H; 53.4-81.4	4H; 53.4-112.5
	LL	QLl.S42IL-4H	S42IL-123	ALL+N25	9.7	Hoffmann et al. 2012	4H; 81.4-112.5	
	LL	QLl.S42IL-5H	S42IL-126	N25	13.8	Hoffmann et al. 2012	5H; 75.9-120.7	5H; 75.9-120.7
	LL	QLl.S42IL-6H	S42IL-131	N100	-9.5	Hoffmann et al. 2012	6H ; 86.8-110.1	6H ; 86.8-110.1
	LL	QLl.S42IL-7H.a	S42IL-133	N100	-10.4	Hoffmann et al. 2012	7H ; 12.7-37.6	7H ; 12.7-141
	LL	QLl.S42IL-7H.a	S42IL-134	ALL+N100	-8.6	Hoffmann et al. 2012	7H ; 37.6-70.2	
	LL	QLl.S42IL-7H.b	S42IL-135	ALL+N25	8.0	Hoffmann et al. 2012	7H ; 67.4-118.5	
	LL	QLl.S42IL-7H.b	S42IL-136	N25	18.9	Hoffmann et al. 2012	7H ; 88.9-116.1	
	LL	QLl.S42IL-7H.b	S42IL-137	ALL+N25	10.4	Hoffmann et al. 2012	7H ; 88.9-127.5	
	LL	QLl.S42IL-7H.c	S42IL-138	ALL+N25	8.1	Hoffmann et al. 2012	7H ; 116.1-141	
Leaf rust	LR	QLr.S42IL-2H.a	S42IL-107	L+I	38.8	Schmalenbach et al. 2008	2H; 12.7-40.8	2H; 12.7-40.8
	LR	QLr.S42IL-2H.b	S42IL-110	L+I	30.6	Schmalenbach et al. 2008	2H; 89.9-94.9	2H; 89.9-94.9
	LR	QLr.S42IL-4H.a	S42IL-123	L+I	-34.7	Schmalenbach et al. 2008	4H; 81.4-112.5	4H; 81.4-112.5
	LR	QLr.S42IL-4H.a	S42IL-122	L+I	-34.7	Schmalenbach et al. 2008	6H ; 104.8-126.5	6H ; 104.8-126.5
	LR	QLr.S42IL-7H.a	S42IL-138	I	-41.5	Schmalenbach et al. 2008	7H ; 116.1-141	7H ; 116.1-141
Leaf to root ratio	LRR	QLrr.S42IL-2H	S42IL-107	ALL+N100	-23.7	Hoffmann et al. 2012	2H; 12.7-40.8	2H; 12.7-40.8
	LRR	QLrr.S42IL-5H	S42IL-126	ALL+N100	24.1	Hoffmann et al. 2012	5H; 75.9-120.7	5H; 75.9-120.7
	LRR	QLrr.S42IL-6H	S42IL-128	ALL+N100	-20.9	Hoffmann et al. 2012	6H ; 35.6-75.5	6H ; 35.6-75.5
	LRR	QLrr.S42IL-7H	S42IL-135	N100	-21.0	Hoffmann et al. 2012	7H ; 67.4-118.5	7H ; 67.4-118.5
Lodging at harvest	LAH	QLah.S42IL-1H.a	S42IL-105	I	100.0	Schmalenbach et al. 2009	1H; 48.1-57.3	1H; 48.1-57.3
	LAH	QLah.S42IL-2H.a	S42IL-109	L + I	-40.0	Schmalenbach et al. 2009	2H; 37.8-63.5	2H; 37.8-63.5
	LAH	QLah.S42IL-2H	S42IL-109	L	-30.1	Wang et al. 2010	2H; 37.8-63.5	
	LAH	QLah.S42IL-2H.a	S42IL-110	L	-34.3	Schmalenbach et al. 2009	2H; 89.9-94.9	2H; 89.9-94.9
	LAH	QLah.S42IL-4H.a	S42IL-121	L + I	74.3	Schmalenbach et al. 2009	4H; 53.4-81.4	4H; 53.4-112.5
	LAH	QLah.S42IL-4H.b	S42IL-123	L + I	40.0	Schmalenbach et al. 2009	4H; 81.4-112.5	
	LAH	QLah.S42IL-7H.a	S42IL-137	L + I	88.6	Schmalenbach et al. 2009	7H ; 88.9-127.5	7H ; 88.9-141
	LAH	QLah.S42IL-7H.b	S42IL-138	L + I	-37.1	Schmalenbach et al. 2009	7H ; 116.1-141	
	LAH	QLah.S42IL-1H	S42IL-138	L	-30.1	Wang et al. 2010	7H ; 116.1-141	
N content grain	N_CONT_G	QN_cont_g.S42IL-1H	S42IL-141	All + N1	13.8	Schnaithmann and Pillen 2013	1H; 57.3-82.5	1H; 57.3-82.5
	N_CONT_G	QN_cont_g.S42IL-2H	S42IL-153	N1	-12.6	Schnaithmann and Pillen 2013	2H; 60.7-68.6	2H; 60.7-68.6
	N_CONT_G	QN_cont_g.S42IL-6H	S42IL-128	All + N1	13.4	Schnaithmann and Pillen 2013	6H ; 35.6-75.5	6H ; 35.6-75.5
	N_CONT_G	QN_cont_g.S42IL-7H	S42IL-137	N1	-11.7	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	7H ; 88.9-127.5
N content straw	N_CONT_STR	QN_cont_str.S42IL-6H	S42IL-128	N1	17.8	Schnaithmann and Pillen 2013	6H ; 35.6-75.5	6H ; 35.6-75.5
	N_CONT_STR	QN_cont_str.S42IL-7H	S42IL-137	N1	-15.3	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	7H ; 88.9-127.5
Number of ears/m²	EAR	QEar.S42IL-2H.a	S42IL-107	I	41.1	Schmalenbach et al. 2009	2H; 12.7-40.8	2H; 12.7-63.5
	EAR	QEar.S42IL-2H.a	S42IL-108	L + I	29.9	Schmalenbach et al. 2009	2H; 12.7-59	
	EAR	QEar.S42IL-2H	S42IL-107	I	41.1	Wang et al. 2010	2H; 12.7-40.8	

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Number of ears/m² (continued)	EAR	QEar.S42IL-2H	S42IL-108	L+I	27.0	Wang et al. 2010	2H; 12.7-59	
	EAR	QEar.S42IL-2H.a	S42IL-109	L + I	29.3	Schmalenbach et al. 2009	2H; 37.8-63.5	
	EAR	QEar.S42IL-2H	S42IL-109	L+I	26.8	Wang et al. 2010	2H; 37.8-63.5	
	EAR	QEar.S42IL-6H.a	S42IL-132	I	40.4	Schmalenbach et al. 2009	6H ; 94.8-113.2	6H ; 94.8-113.2
Number of leaves	LN	QLn.S42IL-2H	S42IL-109	N25	28.3	Hoffmann et al. 2012	2H; 37.8-63.5	2H; 37.8-63.5
	LN	QLn.S42IL-4H	S42IL-123	ALL	21.3	Hoffmann et al. 2012	4H; 81.4-112.5	4H; 81.4-115.2
	LN	QLn.S42IL-4H	S42IL-124	ALL+N100	19.1	Hoffmann et al. 2012	4H; 110.2-115.2	
	LN	QLn.S42IL-6H	S42IL-148	N100	21.0	Hoffmann et al. 2012	6H ; 1-10.6	6H ; 1-10.6
	LN	QLn.S42IL-7H.a	S42IL-133	ALL	19.6	Hoffmann et al. 2012	7H ; 12.7-37.6	7H ; 12.7-37.6
	LN	QLn.S42IL-7H.b	S42IL-135	N100	18.8	Hoffmann et al. 2012	7H ; 67.4-118.5	7H ; 67.4-118.5
	LN	QLn.S42IL-7H.c	S42IL-139	N25	27.5	Hoffmann et al. 2012	7H ; 129.4-141	7H ; 129.4-141
Powdery mildew	PM	QPm.S42IL-1H.a	S42IL-101	L+I	-52.5	Schmalenbach et al. 2008	1H; 0-13.1	1H; 0-61.5
	PM	QPm.S42IL-1H.a	S42IL-102	L+I	-66.1	Schmalenbach et al. 2008	1H; 0-61.5	
	PM	QPm.S42IL-1H.b	S42IL-104	L+I	-45.8	Schmalenbach et al. 2008	1H; 46.5-48.9	
	PM	QPm.S42IL-2H.a	S42IL-110	L+I	25.4	Schmalenbach et al. 2008	2H; 89.9-94.9	2H; 89.9-94.9
	PM	QPm.S42IL-4H.a	S42IL-118	I	28.6	Schmalenbach et al. 2008	4H; 38-54.6	4H; 38-54.6
	PM	QPm.S42IL-4H.b	S42IL-123	L+I	42.4	Schmalenbach et al. 2008	4H; 81.4-112.5	4H; 81.4-115.2
	PM	QPm.S42IL-4H.c	S42IL-124	L+I	-25.4	Schmalenbach et al. 2008	4H; 110.2-115.2	
	PM	QPm.S42IL-7H.a	S42IL-135	L	-22.0	Schmalenbach et al. 2008	7H ; 67.4-118.5	7H ; 67.4-141
	PM	QPm.S42IL-7H.a	S42IL-137	L+I	-32.2	Schmalenbach et al. 2008	7H ; 88.9-127.5	
	PM	QPm.S42IL-7H.a	S42IL-138	L+I	-45.8	Schmalenbach et al. 2008	7H ; 116.1-141	
Root biomass	RDW	QRdw.S42IL-1H.a	S42IL-102	ALL+N100	33.0	Hoffmann et al. 2012	1H; 0-61.5	1H; 0-61.5
	RDW	QRdw.S42IL-1H.b	S42IL-143	N100	-28.4	Hoffmann et al. 2012	1H; 84.1-108.5	1H; 84.1-108.5
	RDW	QRdw.S42IL-2H	S42IL-109	ALL+N100+N25	41.9	Hoffmann et al. 2012	2H; 37.8-63.5	2H; 37.8-63.5
	RDW	QRDW.S42IL-5H	S42IL-176	-	+	Naz et al. 2012	5H; 81.3-139.9	5H; 81.3-139.9
Root length	RL	QRl.S42IL-1H.a	S42IL-141	ALL+N100	13.0	Hoffmann et al. 2012	1H; 57.3-82.5	1H; 57.3-82.5
	RL	QRl.S42IL-1H.b	S42IL-143	ALL+N100	-15.5	Hoffmann et al. 2012	1H; 84.1-108.5	1H; 84.1-108.5
	RL	QRl.S42IL-1H.c	S42IL-142	N25	18.2	Hoffmann et al. 2012	1H; 119.7-132.7	1H; 119.7-132.7
	RL	QRl.S42IL-2H	S42IL-106	ALL+N100	14.6	Hoffmann et al. 2012	2H; 8.9-18.1	2H; 8.9-40.8
	RL	QRl.S42IL-2H	S42IL-107	ALL+N100+N25	19.0	Hoffmann et al. 2012	2H; 12.7-40.8	
	RL	QRl.S42IL-3H	S42IL-113	N25	18.7	Hoffmann et al. 2012	3H; 117.9-143.1	3H; 117.9-143.1
	RL	QRl.S42IL-4H.a	S42IL-121	N25	21.4	Hoffmann et al. 2012	4H; 53.4-81.4	4H; 53.4-81.4
	RL	QRl.S42IL-4H.b	S42IL-124	ALL+N100+N25	17.0	Hoffmann et al. 2012	4H; 110.2-115.2	4H; 110.2-115.2
	RL	QRl.S42IL-5H	S42IL-126	N100	-20.8	Hoffmann et al. 2012	5H; 75.9-120.7	5H; 75.9-120.7
	RL	QRl.S42IL-6H.a	S42IL-148	ALL+N25	15.2	Hoffmann et al. 2012	6H ; 1-10.6	6H ; 1-10.6
	RL	QRl.S42IL-6H.b	S42IL-128	ALL+N100+N25	20.9	Hoffmann et al. 2012	6H ; 35.6-75.5	6H ; 35.6-105
	RL	QRl.S42IL-6H.b	S42IL-129	ALL+N100+N25	16.4	Hoffmann et al. 2012	6H ; 46.5-86.3	
	RL	QRl.S42IL-6H.b	S42IL-130	ALL+N100+N25	19.7	Hoffmann et al. 2012	6H ; 59.9-105	
	RL	QRl.S42IL-7H.a	S42IL-133	ALL+N100+N25	-17.7	Hoffmann et al. 2012	7H ; 12.7-37.6	7H ; 12.7-118.5
	RL	QRl.S42IL-7H.a	S42IL-134	ALL	-15.1	Hoffmann et al. 2012	7H ; 37.6-70.2	
	RL	QRl.S42IL-7H.b	S42IL-135	ALL+N100+N25	25.7	Hoffmann et al. 2012	7H ; 67.4-118.5	
Root volume	RV	QRV.S42IL-5H	S42IL-176	-	+	Naz et al. 2012	5H; 81.3-139.9	5H; 81.3-139.9

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Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Shoot area top view	SATVI	QSatvi.S42IL-2H	S42IL-110	d	-46.2	Honsdorf et al. 2014a	2H; 89.9-94.9	2H; 89.9-94.9
integral	SATVI	QSatvi.S42IL-4H	S42IL-117	a	-35.4	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	SATVI	QSatvi.S42IL-6H	S42IL-129	a	-33.6	Honsdorf et al. 2014a	6H ; 46.5-86.3	6H ; 46.5-86.3
Shoot to root ratio	SRR	QSrr.S42IL-1H	S42IL-143	ALL+N100+N25	35.1	Hoffmann et al. 2012	1H; 84.1-108.5	1H; 84.1-108.5
	SRR	QSrr.S42IL-2H	S42IL-107	ALL+N100	16.3	Hoffmann et al. 2012	2H; 12.7-40.8	2H; 12.7-40.8
	SRR	QSrr.S42IL-4H	S42IL-123	N100	18.4	Hoffmann et al. 2012	4H; 81.4-112.5	4H; 81.4-112.5
	SRR	QSrr.S42IL-5H	S42IL-126	ALL+N100	13.5	Hoffmann et al. 2012	5H; 75.9-120.7	5H; 75.9-120.7
	SRR	QSrr.S42IL-7H	S42IL-133	ALL+N100+N25	30.2	Hoffmann et al. 2012	7H ; 12.7-37.6	7H ; 12.7-37.6
Simple stress index	SSIBMF	QSsibmf.S42IL-6H	S42IL-129	-	22.7	Honsdorf et al. 2014b	6H ; 46.5-86.3	6H ; 46.5-86.3
Simple stress index	SSIHEI	QSsihei.S42IL-2H	S42IL-107	-	-7.1	Honsdorf et al. 2014b	2H; 12.7-40.8	2H; 12.7-40.8
Simple stress index	SSIRWC	QSsirwc.S42IL-1H	S42IL-143	-	13.9	Honsdorf et al. 2014b	1H; 84.1-108.5	1H; 84.1-108.5
relative water content	SSIRWC	QSsirwc.S42IL-6H	S42IL-128	-	13.2	Honsdorf et al. 2014b	6H ; 35.6-75.5	6H ; 35.6-86.3
	SSIRWC	QSsirwc.S42IL-6H	S42IL-129	-	16.7	Honsdorf et al. 2014b	6H ; 46.5-86.3	
Simple stress index	SSITIL	QSsitil.S42IL-6H	S42IL-122	-	-18.2	Honsdorf et al. 2014b	6H ; 104.8-126.5	6H ; 104.8-126.5
Simple stress index	SSIWcon	QSsiWcon.S42IL-6H	S42IL-129	-	37.6	Honsdorf et al. 2014b	6H ; 46.5-86.3	6H ; 46.5-86.3
Straw per plant	STR	QStr.S42IL-1H	S42IL-102	All + N1	16.5	Schnaithmann and Pillen 2013	1H; 0-61.5	1H; 0-61.5
	STR	QStr.S42IL-2H	S42IL-108	N1	-11.5	Schnaithmann and Pillen 2013	2H; 12.7-59	2H; 12.7-59
	STR	QStr.S42IL-3H	S42IL-140	N1	11.6	Schnaithmann and Pillen 2013	3H; 87.4-148.4	3H; 87.4-148.4
	STR	QStr.S42IL-4H	S42IL-119	All + N1	14.0	Schnaithmann and Pillen 2013	4H; 38-81.4	4H; 38-81.4
	STR	QStr.S42IL-5H	S42IL-127	All + N0	-14.0	Schnaithmann and Pillen 2013	5H; 137.8-165.9	5H; 137.8-165.9
	STR	QStr.S42IL-7H	S42IL-137	All + N1	20.1	Schnaithmann and Pillen 2013	7H ; 88.9-127.5	7H ; 88.9-127.5
Thousand grain weight	TGW	QTgw.S42IL-2H.a	S42IL-107	L + I	7.9	Schmalenbach et al. 2009	2H; 12.7-40.8	2H; 12.7-59
	TGW	QTgw.S42IL-2H.a	S42IL-108	I	13.1	Schmalenbach et al. 2009	2H; 12.7-59	
	TGW	QTgw.S42IL-2H	S42IL-107	L+I	4.6	Wang et al. 2010	2H; 12.7-40.8	
	TGW	QTgw.S42IL-2H	S42IL-108	I	13.1	Wang et al. 2010	2H; 12.7-59	
	TGW	QTgw.S42IL-2H.b	S42IL-110	L	7.0	Schmalenbach et al. 2009	2H; 89.9-94.9	2H; 89.9-94.9
	TGW	QTgw.S42IL-3H	S42IL-111	N0	15.9	Schnaithmann and Pillen 2013	3H; 42.6-56.9	3H; 42.6-56.9
	TGW	QTgw.S42IL-4H.a	S42IL-119	L	8.7	Schmalenbach et al. 2009	4H; 38-81.4	4H; 38-81.4
	TGW	QTgw.S42IL-4H	S42IL-119	All + N0	13.0	Schnaithmann and Pillen 2013	4H; 38-81.4	
	TGW	QTgw.S42IL-4H.a	S42IL-121	I	12.3	Schmalenbach et al. 2009	4H; 53.4-81.4	
	TGW	QTgw.S42IL-4H.c	S42IL-124	L + I	-8.8	Schmalenbach et al. 2009	4H; 110.2-115.2	4H; 110.2-115.2
	TGW	QTgw.S42IL-4H	S42IL-124	L+I	-6.6	Wang et al. 2010	4H; 110.2-115.2	
	TGW	QTgw.S42IL-6H	S42IL-150	All + N1	-12.8	Schnaithmann and Pillen 2013	6H; 73.9-82.43	6H; 35.6-126.5
	TGW	QTgw.S42IL-6H	S42IL-128	L	-4.6	Wang et al. 2010	6H ; 35.6-75.5	
	TGW	QTgw.S42IL-6H.a	S42IL-130	L + I	7.7	Schmalenbach et al. 2009	6H ; 59.9-105	
	TGW	QTgw.S42IL-6H.b	S42IL-130	N0	19.3	Schnaithmann and Pillen 2013	6H ; 59.9-105	
	TGW	QTgw.S42IL-6H	S42IL-130	L+I	5.9	Wang et al. 2010	6H ; 59.9-105	
	TGW	QTgw.S42IL-4H.b	S42IL-122	L + I	-10.8	Schmalenbach et al. 2009	6H ; 104.8-126.5	
	TGW	QTgw.S42IL-7H	S42IL-133	All + N1	11.8	Schnaithmann and Pillen 2013	7H ; 12.7-37.6	7H ; 12.7-37.6
Thresability	Thr	QThr.S42IL-1H	S42IL-143	-	+	Schmalenbach et al. 2011	1H; 84.1-108.5	1H; 84.1-108.5

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Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Tiller number	TN	QTn.S42IL-1H	S42IL-101	N100	22.9	Hoffmann et al. 2012	1H; 0-13.1	1H; 0-13.1
	TIL	QTil.S42IL-1H	S42IL-143	a, d, w	14.2	Honsdorf et al. 2014b	1H; 84.1-108.5	1H; 84.1-108.5
	TIL	QTil.S42IL-2H	S42IL-107	a, w	-20.0	Honsdorf et al. 2014b	2H; 12.7-40.8	2H; 12.7-40.8
	TIL	QTil.S42IL-2Hb	S42IL-153	a, d, w	19.7	Honsdorf et al. 2014b	2H; 60.7-68.6	2H; 60.7-68.6
	TIL	QTil.S42IL-3H	S42IL-115	a	-27.6	Honsdorf et al. 2014a	3H; 117.9-155	3H; 117.9-155
	TIL	QTil.S42IL-4H	S42IL-123	a, w	13.3	Honsdorf et al. 2014b	4H; 81.4-112.5	4H; 81.4-115.2
	TN	QTn.S42IL-4H	S42IL-123	ALL+N100	26.4	Hoffmann et al. 2012	4H; 81.4-112.5	
	TIL	QTil.S42IL-4H	S42IL-124	a, d, w	42.9	Honsdorf et al. 2014a	4H; 110.2-115.2	
	TIL	QTil.S42IL-4H	S42IL-124	a, d, w	21.2	Honsdorf et al. 2014b	4H; 110.2-115.2	
	ΔTIL	QΔTIL.S42IL-5H	S42IL-176	-	+	Naz et al. 2012	5H; 81.3-139.9	5H; 81.3-139.9
	TN	QTn.S42IL-6H	S42IL-148	N100	30.7	Hoffmann et al. 2012	6H ; 1-10.6	6H ; 1-10.6
	TIL	QTil.S42IL-6H	S42IL-122	a, w	13.5	Honsdorf et al. 2014b	6H ; 104.8-126.5	6H ; 104.8-126.5
	TN	QTn.S42IL-7H	S42IL-135	N100	25.5	Hoffmann et al. 2012	7H ; 67.4-118.5	7H ; 67.4-127.5
	TIL	QTil.S42IL-7H	S42IL-136	a, w	13.2	Honsdorf et al. 2014b	7H ; 88.9-116.1	
	TIL	QTil.S42IL-7H	S42IL-137	a, w	14.1	Honsdorf et al. 2014b	7H ; 88.9-127.5	
	TN	QTn.S42IL-7H	S42IL-136	N100	22.7	Hoffmann et al. 2012	7H ; 88.9-116.1	
Viscosity of wort	VIS	QVis.S42IL-1H.a	S42IL-102	L+I	5.0	Schmalenbach and Pillen 2009	1H; 0-61.5	1H; 0-61.5
	VIS	QVis.S42IL-1H.a	S42IL-103	L+I	5.6	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	VIS	QVis.S42IL-1H.a	S42IL-105	I	4.5	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	VIS	QVis.S42IL-6H.a	S42IL-130	I	5.6	Schmalenbach and Pillen 2009	6H ; 59.9-105	6H ; 59.9-105
Water content	RWC	QRwc.S42IL-1H	S42IL-143	a, d	5.8	Honsdorf et al. 2014b	1H; 84.1-108.5	1H; 84.1-108.5
	RWC	QRwc.S42IL-6H	S42IL-128	a, d	6.3	Honsdorf et al. 2014b	6H ; 35.6-75.5	6H ; 35.6-86.3
	RWC	QRwc.S42IL-6H	S42IL-129	a, d	6.9	Honsdorf et al. 2014b	6H ; 46.5-86.3	
	Wcon	QWcon.S42IL-7H	S42IL-136	w	10.0	Honsdorf et al. 2014b	7H ; 88.9-116.1	7H ; 88.9-127.5
	Wcon	QWcon.S42IL-7H	S42IL-137	w	8.5	Honsdorf et al. 2014b	7H ; 88.9-127.5	
Water use efficiency	WUE	QWue.S42IL-4H	S42IL-117	d	-36.5	Honsdorf et al. 2014a	4H; 18.5-49.9	4H; 18.5-49.9
	WUE	QWue.S42IL-6H	S42IL-129	d	-40.6	Honsdorf et al. 2014a	6H ; 46.5-86.3	6H ; 46.5-86.3
Yield	YLD	QYld.S42IL-2H	S42IL-107	L+I	-8.6	Wang et al. 2010	2H; 12.7-40.8	2H; 12.7-59
	YLD	QYld.S42IL-2H	S42IL-108	L+I	-8.5	Wang et al. 2010	2H; 12.7-59	
	YLD	QYld.S42IL-3H	S42IL-111	L	-6.1	Wang et al. 2010	3H; 42.6-56.9	3H; 42.6-56.9
	YLD	QYld.S42IL-4H.a	S42IL-118	I	11.8	Schmalenbach et al. 2009	4H; 38-54.6	4H; 38-81.4
	YLD	QYld.S42IL-4H.b	S42IL-121	L +I	-17.7	Schmalenbach et al. 2009	4H; 53.4-81.4	
	YLD	QYld.S42IL-6H	S42IL-128	L	-5.8	Wang et al. 2010	6H ; 35.6-75.5	6H ; 35.6-105
	YLD	QYld.S42IL-6H	S42IL-130	L+I	-6.4	Wang et al. 2010	6H ; 59.9-105	
	YLD	QYld.S42IL-7H.a	S42IL-133	L	-11.1	Schmalenbach et al. 2009	7H ; 12.7-37.6	7H ; 12.7-70.2
	YLD	QYld.S42IL-7H	S42IL-133	L+I	-9.1	Wang et al. 2010	7H ; 12.7-37.6	
	YLD	QYld.S42IL-7H.a	S42IL-134	I	-18.0	Schmalenbach et al. 2009	7H ; 37.6-70.2	
	YLD	QYld.S42IL-7Hb	S42IL-134	L+I	-8.6	Wang et al. 2010	7H ; 37.6-70.2	
	YLD	QYld.S42IL-7H.b	S42IL-137	L +I	-17.9	Schmalenbach et al. 2009	7H ; 88.9-127.5	7H ; 88.9-127.5

[1, 2, 3] for legend see last page of table

Appendix Table 1 continued

Trait	Trait abbr.[1]	QTL name[2]	S42ILname	Treatment[3]	Dev f. Sca %	Reference	Pos. QTL	Pos. QTL region
Yield per plant	YDP	QYdp.S42IL-2H	S42IL-108	N1	-16.2	Schnaithmann and Pillen 2013	2H; 12.7-59	2H; 12.7-59
	YDP	QYdp.S42IL-4H	S42IL-119	N1	20.1	Schnaithmann and Pillen 2013	4H; 38-81.4	4H; 38-81.4
	YDP	QYdp.S42IL-5H	S42IL-126	N1	20.0	Schnaithmann and Pillen 2013	5H; 75.9-120.7	5H; 75.9-120.7
	YDP	QYdp.S42IL-5H.b	S42IL-127	N0	-58.7	Schnaithmann and Pillen 2013	5H; 137.8-165.9	5H; 137.8-165.9
	YDP	QYdp.S42IL-7H	S42IL-135	N1	15.9	Schnaithmann and Pillen 2013	7H ; 67.4-118.5	7H ; 67.4-118.5
α-amylase activity of	AA	QAa.S42IL-1H.a	S42IL-101	L	-18.1	Schmalenbach and Pillen 2009	1H; 0-13.1	1H; 0-61.5
	AA	QAa.S42IL-1H.a	S42IL-102	L+I	-27.1	Schmalenbach and Pillen 2009	1H; 0-61.5	
	AA	QAa.S42IL-1H.b	S42IL-103	L+I	-18.7	Schmalenbach and Pillen 2009	1H; 24.9-53.3	
	AA	QAa.S42IL-1H.b	S42IL-105	L+I	-15.1	Schmalenbach and Pillen 2009	1H; 48.1-57.3	
	AA	QAa.S42IL-4H.a	S42IL-116	L+I	20.0	Schmalenbach and Pillen 2009	4H; 1.1-40	4H; 1.1-40
	AA	QAa.S42IL-4H.b	S42IL-124	L+I	16.2	Schmalenbach and Pillen 2009	4H; 110.2-115.2	4H; 110.2-115.2
	AA	QAa.S42IL-5H.a	S42IL-125	L+I	-32.5	Schmalenbach and Pillen 2009	5H; 51.5-81.3	5H; 51.5-120.7
	AA	QAa.S42IL-5H.a	S42IL-126	L	-20.6	Schmalenbach and Pillen 2009	5H; 75.9-120.7	
	AA	QAa.S42IL-6H.a	S42IL-130	L	-14.9	Schmalenbach and Pillen 2009	6H ; 59.9-105	6H ; 59.9-105
	AA	QAa.S42IL-7H.a	S42IL-133	I	-28.6	Schmalenbach and Pillen 2009	7H ; 12.7-37.6	7H ; 12.7-37.6

[1] Trait abbreviation as in original publication

[2] QTL name as in original publication

[3]Treatment abbreviations (for more details see respective publications):

a: all (across treatments), d: drought, w: well watered

All: across treaments, N0: low N, 10 mg N/plant, N1: high N, 30 mg N/ plant

N25: 0.5 mM N solution (25%), N100: 2 mM N solution (100%)

L: line main effect, I: line by environment interaction effect

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DECLARATION UNDER OATH

Eidesstattliche Erklärung/ *Declaration under Oath*

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

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

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