Increasing productivity of barley cultivars using a wild barley introgression library in fields under nitrogen stress and diverse preceding crop combinations

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1. Introduction

A major challenge of global agriculture is the economic, ecological and sustainable production of crops irrespective of the existing conditions (Christen 2000). Modern cereal production is essential for feeding the world's population. However, the projected growth of the population until 2050 demands to double food productivity. Agriculture intensification is or at least should be limited to secure environmental sustainability, public health, long-term food security and terminate ecosystem expansion (Tilman *et al.* 2011). In this enormous challenge advancement, data knowledge and efficiency will be the major guidelines. One key solution is the crop itself. The formation of plant traits is a result of the interaction of genetic and environmental factors. In order to gain insights into this complex synergy, cultivated crops are tested under various field conditions. Phenotypic, sensor-based and analytical techniques can be used to test important aspects of adapted agricultural land use. These aspects include yield enhancement and stability, nutrient uptake, and resistance to biotic and abiotic environmental stresses. The cultivation conditions cannot only be very different between certain locations, but also highly problematic for optimal plant development. Extreme agricultural conditions can often only be counteracted by limited exogenous strategies, such as irrigation, fertilization, adding of rhizosphere colonizing microorganisms and crop protection, which must meet legal and economic conditions (Yang *et al.* 2009). Therefore, in addition to good agricultural practice, plant breeding contributes significantly to the securing of available agricultural goods and intermediates. During the last decades, the genetic potential of cultivated species has been investigated by using new crossing and selection methods. For example, the use of related wild species as crossing partners for established crops is a promising method. In particular, the introduction of introgression lines in 1992 made it possible to identify genetic regions that encode genes controlling a quantitative phenotypic trait, which enables the breeding of improved genotypes (Eshed *et al.* 1992). In this regard, wild species, related to crop species, may serve as a source of genetic variation, which has been lost during domestication (Tanksley and McCouch 1997).

1.1 Origin, Botany and Developmental Physiology of Barley

According to the taxonomic classification of Species 2000 & ITIS Catalog of Life (Roskov *et al.* 2000) more than 40 species belong to the genus *Hordeum*, which is assigned to the family of *Poaceae* (sweet grasses). Together with many other important cereal genera it forms the subfamily of *Triticeae* (Jacobsen and von Bothmer 1995). Diverse developmental and cultivation processes produced countless populations across all continents. Four sections can be distinguished by a rough classification according to morphological features: *Hordeum*, *Anisolepis*, *Stenostachys* and *Critesion*

(Bothmer *et al.* 1991). However, a more accurate classification of the *Hordeum* species would distinguish in three gene pools (Von Bothmer *et al.* 2003). The primary gene pool includes cultivated barley (*H. vulgare L.*) and the wild form (*H. vulgare ssp. spontaneum*). Crossings of these species are unproblematic and leads to fertile offspring. Only the species *H. bulbosum* is associated with the secondary gene pool, which can be crossed with species of the first group under extensive molecular methods due to genetic barriers. All remaining *Hordeum* species are summarized in the tertiary gene pool. Crossings of representatives from this gene pool with the first pool are extremely laborious to almost impossible, due to their distant genetic relation.

Starting from one karyotype with seven chromosomes barley varieties with diploid (2n = 14), tetraploid (4n = 28) and hexaploid (6n = 42) sets of chromosomes developed. In Germany, *H. murinum, H. marinum, H. jubatum, H. bulbosum* and *H. vulgar*e are the most widespread barley species. From these only the cultured six-rowed barley (*H. vulgare L. ssp. vulgare*) and two-rowed barley (*H. vulgare L. ssp. distichon*) are suitable for cultivation.

Barley is one of the oldest cultivated crops in the world with a high adaptive capacity compared to other major cereals (Rawson *et al.* 1988; Delogu *et al.* 1998; Garthwaite *et al.* 2005). The domestication of barley dates back about 10.000 years to the Neolithic Revolution. From this time onwards, cereals were domesticated from wild grasses in several distinct places of the world. Morrell and Clegg (2007) described two geographically independent cultivation origins of wild barley. One domestication zone of modern barley varieties, used in Europe and America, was located in the Fertile Crescent in the Middle East. In this region, i.e. in today's Israel, Syria, south-eastern Turkey to northern Iraq and Iran, archaeological finds of plant and seed residues were discovered, which point to the historic use of barley. The second centre of barley domestication was located in southern Central Asia (today's Kyrgyzstan, Afghanistan and West Pakistan) and resulted in a wide variety of barley cultivars in Central Asia and the Far East.

The development of one of the world's oldest crops, barley (*Hordeum vulgare*, abbreviated with *Hv)*, can be attributed to the targeted use of *Hordeum vulgare ssp. spontaneum* (abbreviated with *Hsp)*. Analyses of 6.000-year-old barley seeds from the Yoram Cave in Israel showed that the DNA of these grains hardly differs from today's cultivated barley of this area, but displays several differences compared to the wild forms (Mascher *et al.* 2016). Thus, cultivation of barley developed early. The use of barley in Europe started around 5.000 BC, facilitated by the trade-related introduction from the Middle East (Jones *et al.* 2011).

During the domestication process some genes mutated spontaneously. An important characteristic for the commercial use of wild barley as a crop was the selection for wild barley plants whose grains

were fixed at the ears and remain at the rachis until harvest. The fixation is a result of a mutation in the complementary genes Non-brittle rachis 1 *(btr1)* and Non-brittle rachis 2 (*btr2)* (Pourkheirandish *et al.* 2015). These genes are responsible for rachis fragility and spike axis stability. Only when both genes are dominantly expressed, a furrow is formed at the base of the spikelet to release ripe seeds from the infructescence. Therefore, one of these two genes have to be recessive in all cultivars (*btr1*-type: *btr1Btr2*, *btr2*-type: *Btr1btr2*) preventing furrow formation. The exact locus responsible for spindle fragility differs between varieties from East and West Asia due to their distinct domestication origins.

Another mutation during the domestication processes in the Fertile Crescent resulted in an altered number of rows of grains on the ear. Whether barley is two- or more-rowed is determined by the gene six-rowed spike 1 *(VRS1)* (Pourkheirandish and Komatsuda 2008). Both wild barley (two-rowed) and cultivated two-rowed barley bear the dominant form of the gene. Mutations in this gene led to fertile lateral spikelets. Thus, in six-rowed barley the recessive allele of *vrs1* is inherited. Presumably, many multiple-rowed genotypes originate from different ancestors as this spontaneous mutation can occur relatively frequently.

These natural mutations (non-brittle rachis and six-rowed spike) are the result of functional impairments in transcription factor genes (Pourkheirandish and Komatsuda 2007).While *brt1/brt2* were located on the short arm of chromosome 3H, the *vrs1* was identified on chromosome 2HL (Fedak *et al.* 1972; Lundqvist 1997; Komatsuda and Mano 2002). The *btr1* gene had been fine-mapped to a 0.84 cM region by using AFLP markers (Azhaguvel *et al.* 2006).

Characteristics of many sweet grasses are annual growth, reproduction mainly by self-fertilization and reduced flower organs. Depending on the environmental adaptation, a distinction is made between spring and winter barley. Winter barley is able to germinate at ground temperatures of a minimum of 0 °C with positive effects on accelerated germination from 3 - 5 °C upwards (Fettell *et al.* 2010). Winter barley requires a vernalization to initiate the reproductive phase. This progress is mainly associated with the epistatic genes *VrnH1, VrnH2* and *VrnH3.* These genes control the requirements of vernalization and photoperiodic sensitivity, so that the plant can tolerant cold temperatures (Distelfeld *et al.* 2009). In contrast to winter barley spring barley is sensitive to frost, thus the temperature of the soil should be consistently above 1 °C for germination (Anderson *et al.* 1995). The warmer the soil at sowing time the faster the seedlings develop. However, the optimum of 20 °C is never reached in temperate latitudes. For the development of the radicle from the primordium, sufficient moisture and ventilation are needed. In the first days, the radicle grows downwards to supply the seedling with water and nutrients. Shortly thereafter, horizontal lateral roots grow from the main root, which takes over the task of anchoring and transporting water and nutrients

to the stems (Naz *et al.* 2014). Further branching results in lateral roots of ascending order creating an active supply network for aboveground biomass. However, cultivated barley has a rather weak total homorhizy root system compared to its wild relatives (Grando and Ceccarelli 1995; Naz *et al.* 2014).

At the same time as the primary root system establishes, the white acrospire grows and finally breaks through the surface, approximately after 6 - 8 days (MacLeod and Palmer 1966). Within the next 3 - 5 days, the first leaf develops by stretching the coleoptile. The process of leaf formation is temperature-dependent and the thermal exposure time controls the number of leaves each plant develops. Winter barley forms about 13 - 15 leaves on the main stem, while spring barley generally develops only 8 - 10 leaves. At the 3-leaf stage, the young plant forms tillers (Anderson *et al.* 1995). The growth of side tillers is an important yield component. The formation of the spikes and spikelets starts already at the end of tillering within the double ring stage (Kirby and Appleyard 1987). During the subsequent stem elongation some stems are reduced. Each plant develops an average of three fully formed stems, each with a single ear. Additionally, the grain number per ear is determined in the stage of stem elongation. After the formation of several ‘three single-flowered’ spikelets in a distichous manner at each rachis internode the ear is fully developed and the booting begins. Already before the entire spike liberates from the leaf sheath of the flag leaf the plant begins to flower (Anderson *et al.* 1995). The self-pollination begins in the middle of the spike and spreads to the top and bottom within a few days (Briggs 1978). In this stage of fertilization and grain formation, the plant is extremely susceptible to biotic stress (heat and drought), which can lead to severe grain reduction (Bennett *et al.* 1973). The grain formation begins with length growth, which is followed by broad growth resulting in the maximum surface of the grain. After the stages of flowering, milk maturity and dough maturity the plant forms a hard grain at full maturity. The ripening is followed by the over-ripening of the grains and the death of the plant (Lancashire *et al.* 1991).

The limiting factor for the cultivation of spring barley in the northern hemispheres is the short vegetation period of 110 - 140 days. An early sowing is the basis for an optimal production of grain formation with favourable protein values. Therefore, the timeframe from end of February to end of March is beneficial (Farack *et al.* 2011). To achieve maximum yield, dry and slightly warmed soil for sowing, low mineral nitrogen release and uniform precipitation distribution over the vegetation period is needed. Waterlogged, humus-rich soils lead to uncontrolled nitrogen availability. In order to ensure well-aerated soil, sufficient stubble work as well as the incorporation of crop residues into the soil is important. Sunflowers and cereals, outstand with low amounts of nitrogen in plant residues and thus, are suitable preceding crops for spring barley (Farack *et al.* 2011).

1.2 Economic importance

The world population has grown by 1 - 2% every year from 1950 to 2015. According to population projections (intermediate scenario), the annual growth may decrease by 2100, but the absolute population is predicted to reach 11 billion people (DESA 2019). Due to this steady increase in the world's population and the raising consumption of goods, for example from emerging countries, the future development of efficient crop plants is of high importance. In addition, due to settlement construction, commercial space and traffic routes a decreasing acreage per capita worldwide is recorded. Thus, there is a need in focusing on economic and biological viable crops, which guarantee a secure food supply even under difficult climatic conditions. The profitable farming of any crop is determined by specific biotic and abiotic environmental conditions of each agricultural landscape.

With a total amount of 47.8 million ha cultivation area, barley ranks fourth in the world's cultivated market fruits (Statista 2018, USDA Foreign Agricultural Service). Between 1961 and 1979, the barley harvest multiplied by 2.5 times from 71.1 million tons to 175.7 million tons. In the past 20 years, a 20% decline in cultivated area was observed across the world. The largest barley acreages in Europe are located in Russia, France and Spain, and the highest average yields were recorded in Belgium, Netherlands and Ireland (FAOSTAT, 2018). With a total acreage of 1.6 million ha and an average yield of 59.1 dt ha^{-1} Germany ranks 3^{th} and 5^{th} in Europe, respectively (FAOSTAT, 2018). Of the 1.6 million ha 21% were used for spring barley cultivation. 1.9% of German barley cultivation area, 30.000 ha, are used for organic farming, which is until today of negligible importance.

The harvested grains of winter and spring barley are widely used for animal feed, food, brewing, distilling and malt coffee production. High quality standards are demanded for any of the aforementioned products, but particularly for barley malt used in the brewing process. A healthy, well-formed grain with high germination, low protein content of 9 - 11.5%, varietal purity, a fine glume, 60 - 80% starch content and absence of toxins are important criteria for the classification of malting barley (Bundessortenamt 2018). In the malting process 22 kg of barley grains results in about 17 kg of malt, from which in turn almost 100 litres of beer can be brewed.

1.3 Cultivation of barley

In 1842 the German chemist J. von Liebig described the importance of a healthy soil and restocking extracted substances with chemical fertilizers marking the beginning of the industrial production of nutrients (von Liebig 1842). New fertilizers such as potash and ammonia were developed replacing human and livestock faeces. Nitrogen quickly became the most important synthetic mineral fertilizer. Due to its leading role F. Haber, C. Bosch, and E. Johnson tried with huge efforts to find a commercial

way for the synthesis of ammonia made in the early 20th century (Erisman *et al.* 2008). Initially, the production and consumption of fertilizer steadily increased, but with the Fertilizer Act in 1977 by the German Federal Ministry of Food and Agriculture application limits corresponding to the cultivation area were introduced. These fertilizer ordinances (‘Düngeverordnung’) have been repeatedly specified and, above all, include lower permitted average N balance over 3 years. As a result, an efficient nitrogen application strategy has become increasingly important. Excessive fertilization increases the farmer's costs disproportionately on the one hand and, on the other hand, carries the risk of leaching and as a result groundwater pollution.

A "good practice" fertilizer management in malting barley production would be to give a single dose of restrained nitrogen fertilization to avoid high protein levels at harvest. Depending on the presence of mineral nitrogen in the soil, its delivery to the plant and other possible inflows and losses, an early fertilization (prior to the 3-leaf stage) is recommended (Farack *et al.* 2011). The concentration value of nitrogen for malting barley should total around 140 kg N ha^{-1}. Suitable fertilizers for barley crops are synthetic agents with rapid plant availability of nitrogen, such as urea, calcareous ammonium nitrate or sulphur-containing nitrogen fertilizer. To avoid or reduce an unwanted nitrogen surplus, it is also important to achieve a high rate of uptake by the plants. The so-called nitrogen utilization efficiency (NUE) is an assessment that calculates the ability of a genotype to respond to N-supplies (Dobermann 2005). Balancing between applicability and accuracy, various definition and indices are known in scientific literature for determining NUE (Dobermann 2005).

In addition to other factors such as tillage, seed drill technology and fertilization, the opportunity to combine crops into a special rotation for agronomic and economic advantage is well known by agronomists and farmers. The use of crop rotations, i.e. an expedient succession and change of crop species to obtain both soil fertility and optimal yields has been known since the 8th century, in addition to the use of fertilizer and implements (Britannica 1998). In the Middle Ages it was common to work with a three-field crop rotation, which required a mandatory fallow (Britannica 1998). During the Agrarian Revolution various systems up to an eight-year crop rotation were introduced, which impressively proves the high value attached to preceding crops. Nowadays, preceding crops are often used on arable land since they have a more advantageous effect on the almost yearly changing market crops. The present crop rotation system consists of individual crop rotation fields with one-year main crop, and sometimes associated catch crops, composed to crop rotation elements. This system results in balanced crop yields and risk distribution in the long term. Depending on their self-compatibility, the same crops can be arranged as crop rotation modules directly behind each other (Bundesinformationszentrum Landwirtschaft 2019). The focus is laid on economic and ecological

benefits, given by a chronologically and optimally adapted crop rotation. Nevertheless, preceding crop impacts can be crucial for growth, development, yield productivity, and susceptibility to biotic and abiotic stresses. All cereals contribute to humus draining with their erosive capacity. In contrast, some foliage crops, such as legumes and agricultural grass mixtures promote humus formation through their soil-conserving crop and root residues (Kolbe and Zimmer 2015). However, other foliage crops like beets and potatoes instead leave a low amount of plant residues in soil after harvest. The first element of a crop rotation is a foliage crop and the rotation ends with the last cereal crop. As a result, crop rotation elements can be of different lengths (Bundesinformationszentrum Landwirtschaft 2019). For an optimal crop rotation, economic, plant and phytosanitary aspects have to be considered (Kolbe and Zimmer 2015).

Spring barley is a self-compatible crop (Nelson *et al.* 1988). However, a permanent spring barley rotation would lead to yield losses. Spring barley has lower demands on preceding crops than for example wheat, although the amount of grain nitrogen is a limiting factor. Especially, for malting barley suitable crops cultivated the year before are winter wheat, winter rye or potato, due to their low nitrogen supply. In contrast, legumes as well as oilseed rape, which supply high nitrogen amounts and consequently high protein content affecting the malting process, should be avoided as preceding crops (Farack *et al.* 2011).

In recent years, the agricultural sector has recognized that not a single strategy such as area growth, pesticide or fertilizer use, crop rotation, tillage or breeding high-performance varieties ensures economically and environmentally optimal production, but their balanced interplay with regard to the environmental impact.

1.4 Barley genetics and breeding

Barley as a model plant

Barley is a monocotyledonous species and a widespread object of research in plant breeding (Meinke *et al.* 1998). It is also well suited for molecular biology investigations with its self-pollination property and simple diploid genetics. Further benefits are a short growing season, diverse cultivation regions and easy reproducibility. In past decades, knowledge about basic genetics, biotechnological tools for common breeding methods as well as structural and functional analyses were gained. Furthermore, the complete genome of barley was sequenced (Mascher *et al.* 2017). In public barley databases such as the National Center for Biotechnology Information (NCBI), the European Barley Database (EBDB) or GrainGenes data and gene information of more than 155.000 barley accessions are

managed and published (Sreenivasulu *et al.* 2008). These archived information are an important tool for targeted genetic studies.

Genome research in barley

Due to the large average genome size of cereals the isolation of individual resistance genes (R genes) was difficult in the beginning. However, due to its use as a model plant, numerous research tools were developed to investigate the genome of cultivated barley. In 1987, David Burke described a procedure that allowed the creation of clone-based DNA libraries. The yeast artificial cloning method (YAC) uses artificial yeast-based chromosomes in which genomic inserts of the species of interest are sub-cloned. The design of a YAC allows extremely large segments of genetic material (160-1000 kb) to be inserted (Anand *et al.* 1989; Kleine *et al.* 1993). With the help of this technique the gene that conveys resistance against mildew, *Rar1*, was isolated (Shirasu *et al.* 1999). As an alternative to the rather expensive and labour-intensive YAC method, the bacterial artificial cloning (BAC) method was developed in 1997 for resistance gene isolation (Mejia and Monaco 1997). BAC produced DNA libraries have insert sizes of around 150 kb. Using this method, the mildew resistance genes *Mla1* and *Mla6* (Zhou *et al.* 2001; Halterman *et al.* 2001), the gene encoding for yellow mosaic virus resistance *rym4* (Pellio *et al.* 2005) and the resistance gene against black rust *rpg1* were identified (Kilian *et al.* 1997).

Based on a random ‘loss-of-function’ point mutation another powdery mildew resistance (MlR) was discovered as early as 1942. The responsible gene *Mlo* was described in detail by Jørgensen (1992) but the procedure was only fully understood in 2004 in a research collaboration (Piffanelli *et al.* 2004) Finally, in 2017 the first complete genome of a spring barley variety ('Morex') was decoded (Mascher *et al.* 2017).

In addition to the cultivated barley genome, the wild barley genome is also of considerable interest to scientists. It might be possible that lost genes can be found in an exotic background. In 1993, the gene encoding for qualitative resistance to mildew (*Mla*) from the subspecies *H. spontaneum* was identified (Jahoor and Fischbeck 1993). In addition to this gene, further mildew resistance genes at loci *Mlj*, *Mlt* and *Mlf* were identified in the following years (Schönfeld *et al.* 1996). Until today, more than 20 resistance genes against mildew are known in barley. All of them, except for the recessive gene *mlo*, are race-specific. Furthermore, in the wild species, *H. bulbosum*, resistance genes against several important barley diseases were detected. For example, Ruge *et al.* (2003); (2006) identified two genes on chromosome 6H and 2H, which lead to resistance against yellow mosaic virus ($Rym14^{Hb}$, $Rym16^{Hb}$). In addition, the genes *Ryd4hb* and *Rph22* conveying resistance against *Rhynchosporium*

commune on chromosome 4H (Pickering *et al.* 2006), against yellow dwarf virus on chromosome 3H (Scholz *et al.* 2009) and against brown rust on chromosome 2H (Johnston *et al.* 2013) were sequenced from the secondary gene pool. Aside from the qualitative trait research, many attempts were made to record quantitative resistance and polygenic inheritance of traits. New methods such as introgression breeding, cis-gene plants and CRISPR/CAS editing will help to understand the missing links (Schouten *et al.* 2006; Wright *et al.* 2016; Hernandez *et al.* 2020).

QTL mapping in barley

A quantitative trait locus (QTL) is an identified genomic section whose influence on the quantitative variation of a trait could be demonstrated by phenotypic studies. In the context of QTL analyses, the heredity of complex traits, controlled by several genes simultaneously can be detected. Locating of QTL is the first step in the identification of candidate genes for quantitative traits. In the QTL-restricted region, only those molecular markers are assessed that showed an association regarding the polygenic trait. The proximity to the target gene is determined by the marker density in this section, and/or by studies of the functional relationship of previously identified genes in this region. Despite the early idea of Thoday (1961) to characterize the regional assignment of polygenes by linkage analyses with DNA markers, the first QTL in tomato (Bernatzky and Tanksley 1986), maize (Helentjaris *et al.* 1986) and wheat (Gill *et al.* 1991) could be identified after the development of restriction fragment length polymorphism (RFLP) markers. The method is based on restriction fragments of different sizes, which are tagged with radioactive DNA probes. By comparing the length of segments between different individuals, polymorphisms resulting from insertion, deletion, inversion, translocation or point mutations can be recognized. The advantages of this type of markers are their codominant inheritance, their good reproducibility and their ability to detect hetero- and homozygote characteristics. The RFLPs are also characterized by a good comparability between genetic maps (for example wheat and barley). At the beginning of the 90th, a map with sufficient marker saturation for barley was compiled (Kleinhofs *et al.* 1993; Melchinger *et al.* 1994). Since the creation of RFLP maps, a time-consuming and labour-intensive method, new methods were sought to replace these markers. With the invention of the polymerase chain reaction (PCR) an American research team led by Mullis *et al.* (1986) achieved a breakthrough in molecular marker technology leading to fast methods with PCR-based markers such as microsatellites, amplified fragment-length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), sequence-tagged site (STS) markers and single nucleotide polymorphism (SNP) (Semagn *et al.* 2006). All markers are based on polymorphism and differ mainly in the size of the DNA fragments, which are used to detect

differences between the individual genotypes (Bernardo 2008). The monetary effort of techniques also differs (Bernardo 2008).

The basis of QTL mapping is formed by genetic maps, which are mostly created through the combination of markers. Two approaches for the analysis of QTL are available, which differ in sample size and the systematic use of markers. While linkage studies of biparental population requires fewer markers that systematically screen in small sample size (10-20 cM) to uncover QTL, association studies need many diverse accessions as parents to identify QTL through dense markers (Verdeprado *et al.* 2018). Thus, association studies achieve a higher statistical power. Association studies detect specific gene effects even with little impact on the trait, because markers have to be close to the QTL for significant detection, but the significance of the relationship to the phenotype is marginal and the false discovery rate is higher than in linkage studies (Kaler and Purcell 2019). In contrast, very rare alleles can be discovered by linkage studies (Verdeprado *et al.* 2018).

For the detection of quantitative gene loci in barley, many studies with different raw materials and experimental questions have been conducted. The first publications on QTL in barley were published by Heun (1992) and Hackett *et al.* (1992) on quantitative mildew resistance and plant height, respectively. Hayes *et al.* (2003) summarized the number of reports on QTL and divided it into five classifications. For abiotic and biotic stress factors, agronomic traits, quality criteria and other characteristics, 757 QTL were found. The utilized 131 phenotypes and 44 different populations showed a high variation between the studies (Hayes *et al.* 2003). Especially in Europe and North America, QTL mapping in barley has been strongly promoted, while regions such as Australia, Russia and North Africa, which are heavily involved in barley cultivation, account for only a small proportion of research efforts. Due to their different initial crosses, QTL studies are hardly comparable and transferable (Hayes *et al.* 2003). However, despite the large number of parental genotypes, ranging from elite species to wild species from all continents, some of the crossing parents are represented more often. From 1992 to 2018 elite varieties such as 'Morex' (America), 'Steptoe' (America), 'Harrington' (Canada), 'Scarlett' (Germany), 'L94' (Ethiopia), 'Franklin' (Australia) and 'Vada' (Netherlands) were frequently used as crossbred parents for QTL studies (Qi *et al.* 1998a; Qi *et al.* 1998b; Qi *et al.* 2000; Igartua *et al.* 2000; Marquez-Cedillo *et al.* 2000; Marcel *et al.* 2007; Ullrich *et al.* 2008; Schmalenbach *et al.* 2009; Wang *et al.* 2010a; Naz *et al.* 2014). The wild species accessions ‘ISR42-8’ (Israel) and ‘Er/Apm’ (Tunisia) were also involved multiple times in initial crosses for a wide variety of mapping analyses (Teulat *et al.* 1998; Von Korff *et al.* 2004; Saal *et al.* 2011; Honsdorf *et al.* 2017). In addition to the widely used double haploid technique (DH), recombinant inbred populations (RIL) and near-isogenic lines (NILs) have also been applied. These techniques differ in the way of establishing the filial generations after the initial crossing. Double

haploid lines do not split their genetic characteristics in the next generations and are completely homozygous as they were created by the doubling of the parental chromosomes. In the RIL, however, the F1 generation is selfed several rounds to achieve a high degree of homozygosity of the parental genes. The different F1 plants have diverse compositions of the hereditary system. NILs arise from frequent backcrossing with the recurrent parent and are almost identical to it with the exception of one chromosome segment. NILs are mainly used for fine mapping and as control plants in the evaluation of genetically modified plants, while DH lines rather serve for rough mapping. Despite a wide variety of parent material, environments, markers, techniques for producing test populations and statistical programs, QTL discovery for certain traits were confirmed using reference markers across multiple studies. Thus, genetic trait positions can be targeted for future barley breeding projects through marker-assisted selection (MAS) (Collard *et al.* 2005).

Introgression breeding

Due to high selection pressure, especially self-pollinators are marked by restricted genetic diversity (Russell *et al.* 1997; Ellis *et al.* 2000). The commercially cultivated crops are derived from only a limited number of old land races. While domestication of crops increased their productivity, it also narrowed their genetic diversity (Zamir 2001). Such a reduction in genetic structures can range from limited allele potential to inbreeding depression. Thus, even in modern plant breeding with high-quality techniques shortage of genetic variation is a major issue. To overcome these genetic constraints the use of wild relatives is an appropriate research approach. Wild species are evolutionarily adapted to adverse environmental conditions over millions of years and have a rich gene reservoir. Researchers agree that wild species introgressions can increase the degree of genetic variation way higher than pure mutations (Stebbins 1959). Thus, cross breeding with distant relatives recreates a gene pool, which was lost during domestication. Extensive genetic studies should thereby identify valuable agricultural qualities, mainly resistance characteristics. This method has its origin in the spontaneous crossing of cultivated tomatoes and wild tomatoes in South America. As early as 1953, the potential of spontaneous introgression of alien genes was described (Anderson 1953). He first introduced the concept of introgression back in 1938 and with the introduction of the scatter diagram also provided a method for describing it (Anderson and Hubricht 1938). Since then, genome-wide analyses of introgression have been conducted in many crops including rapeseed, sunflower, rice and wheat (Heiser Jr 1951; Second 1982; Kison and Neumann 1993; Jørgensen *et al.* 1994). The significant difference to hybridization is that specific genes of one species are permanently integrated into the genetic background of another species (Gottlieb 1972; Heiser 1973).

The focused development of introgression lines takes place through the selection of a high-performing cultural parent and a donor, which is usually a related wild species accession. An initial crossing of these parents creates a filial generation with foreign genetic information in the genomic background of the cultivated crop. The amount of exotic genome is diminished by repeated marker-assisted backcrossing resulting in lines with either a single wild species introgression or small multiple introgressions. Subsequently, the so-called pre-introgression lines are repeatedly selfed to achieve a high homozygosity of the alien alleles, and finally selected by screening for specific research questions.

The expected results of this breeding concept are in particular an increase in genetic diversity, a transfer of environmental adaptation, the origin of completely new properties, and the emergence of taxonomically new ecotypes (Rieseberg and Wendel 1993).

Although the idea of introgression breeding is promising some difficulties regarding its evaluation exist. Many features are very complex due to the functional interaction of multiple genes. They need to be transmitted from the wild parent through several generations of time-consuming backcrossing and selfing (Jacobsen and Hutten 2006). Since this process of backcrossing is associated with gene linkage, targeted transfers of individual trait characteristics can be limited and often there is a negative impact of introgressions on other relevant traits. Due to this crossing process, it is not possible to directly improve already existing varieties, but it always leads to new genotypes. In addition, dominant major genes could be superimposing genes with minor impact on traits, therefore their targeted detection is extremely challenging (Jacobsen and Hutten 2006).

S42IL Population

This thesis is based on the S42 introgression line (IL) population developed by Von Korff *et al.* (2004) and Schmalenbach *et al.* (2008). The ILs were generated from an initial cross of the German spring barley cultivar ‘Scarlett’ and the Israeli wild barley accession ‘ISR42-8’ (*Hordeum vulgare ssp. spontaneum*). The wild barley accession shows an increased genetic diversity compared to commercial varieties, and thus, also serves as a potential donor of favourable genes and, consequently, of positive trait effects. A set of candidate introgression lines (pre-ILs) were selected from the BC_2DH population. The amount of exotic genome was diminished by repeated marker assisted backcrossing resulting in lines with either a single small introgression or multiple introgressions (Von Korff *et al.* 2004). After selecting a set of lines representing the completely exotic genome in the background of the elite parent the agronomic performance of such an IL population could be evaluated by phenotypic assessment and QTL analyses (Von Korff *et al.* 2006).

Several QTL studies were conducted with S42ILs to evaluate genetic regions causing variation in agronomic traits, followed by fine mapping of promising QTL. The usefulness of the exotic S42IL population and its precursor population S42 was proven in numerous studies in greenhouse and field trials. In particular, a broad spectrum of important agronomic traits were associated with QTL like mildew and leaf rust resistance (Schmalenbach *et al.* 2008), malting quality (Schmalenbach and Pillen 2009), growth phenology (Wang *et al.* 2010b), threshability (Schmalenbach *et al.* 2011) as well as yield and yield-related parameters (Von Korff *et al.* 2006; Schmalenbach *et al.* 2009; Schmalenbach and Pillen 2009; Saal *et al.* 2011; Schmalenbach *et al.* 2011; Schnaithmann and Pillen 2013; Honsdorf *et al.* 2014a; Honsdorf *et al.* 2014b; Honsdorf *et al.* 2017). Furthermore, Honsdorf *et al.* (2014a) and Honsdorf *et al.* (2014b) reported drought stress tolerance in traits when exotic alleles were introgressed from wild barley. Likewise, greenhouse trials with root and shoot related parameters (Hoffmann *et al.* 2012; Naz *et al.* 2012) as well as nutrient accumulation (Reuscher *et al.* 2016; Soleimani *et al.* 2017) revealed several QTL. A major obstacle in QTL detection is the phenotypical expression of agronomic traits under various conditions. Most quantitative traits are controlled by up to hundreds of QTL (Mackay *et al.* 2009). Some have stronger influence than others on the trait, but the majority have only small effects and their expression is strongly depending on the environment. To uncover the genetic basis of phenotypical variation, which may only occur under certain developmental or environmental conditions, it might be useful to study a segregating population under different management combinations in the field, like diverse preceding crops and varying nitrogen fertilization regimes, as already known from cropping system evaluation for large scale changes (Huggins and Pan 1993; Atuahene-Amankwa *et al.* 2004).

So far, however, little is known about the preceding crop effect on QTL detection and the influence of exotic barley alleles on nitrogen utilization under contrasting N fertilization levels in the field.

1.5 Objectives

The effect of different preceding crops and the interaction with nitrogen fertilization has been described several times for various environments and input levels in many arable crops. However, the link and effect on the genetic level has not been studied. For this purpose, within the frame of this thesis the population S42IL was investigated in multi-environment field trials for different agronomic traits aiming for impact assessments of fertilizer and pre-crop management on QTL detection.

The following main objective were examined:

Quantification of phenotypical variation in the barley S42IL population and identification of QTL in field

with underpart

I. S42IL evaluation by different nitrogen fertilizer managements (N_0 and N_1)
II. S42IL evaluation by using two different preceding crops (winter wheat and winter oilseed rape)
III. Effect of complex genotype×management interactions
IV. Suitable exotic alleles for targeted regulation of single or multiple trait characteristics to increase genetic diversity and productivity of the elite barley gene pool

Partial results of the presented work in chapter **2, 3.2** and **4.2** have been published at Zahn *et al.* (2020).

2. Materials and Methods

Plant material and genotyping

For the present study, the S42IL library was used. Resulting from initial crossing of wild barley alleles of the Israeli accession 'ISR42-8' (*Hordeum vulgare* ssp. *spontaneum*) into the gene pool of the elite barley variety 'Scarlett' (*Hordeum vulgare* ssp. *vulgare*) a pre-IL library emerged (Von Korff *et al.* 2004). To diminish the amount of exotic genome the pre-ILs were several times backcrossed with the recurrent parent 'Scarlett' followed by repeated rounds of selfing, as described in Schmalenbach *et al.* (2008). During IL development marker-assisted selection was conducted, ensuring the holistic representation of the wild barley genome in a set of introgression lines resulting in a population of 73 S42ILs. Genotyping of the final ILs was performed by using the 50k Illumina Infinium iSelect SNP Array (Bayer *et al.* 2017). The chip incorporates 44.040 working SNP markers, including 6.251 from the previous 9k iSelect platform (Comadran *et al.* 2012). Each line contains a single wild barley introgression, which overlaps with the neighbouring S42IL, occasionally accompanied by few additional small wild barley segment(s). Due to repetition and space limitation, a representative set of 49 out of 73 wild barley introgression lines (S42ILs) were selected for the field experiments (Table 1). *Hsp* genome coverage of main introgression in the genetic background of 'Scarlett' were calculated by using SNP marker information of 50k Illumina Infinium iSelect SNP Array. Ratio of each line's targeted wild segment (overlapping excluded) per chromosome were summarized and stated in percentage.

Table 1 Overview S42IL genotypes

Genotype	Position of Target Introgression (in bp)[a]		Position of Target Introgression (in cM)[b]		Tested in Merbitz	Tested in Morgenrot	S42IL-HR (g)[c]
S42IL_101	1H	145.558 – 8.512.325	1H	0.2 – 12.5	✓	✓	715
S42IL_102	1H	6.817.863 – 435.913.226	1H	0.2 – 62.3	✓	✓	673
S42IL_103	1H	21.469.219 – 395.873.777	1H	32.2 – 53.7	✓	✓	968
S42IL_104	1H	30.067.246 – 350.841.749	1H	46.3 – 48.8	✓		703
S42IL_105	1H	294.733.886 – 415.571.566	1H	48.1 – 57.3	✓	✓	293
S42IL_106	2H	11.388.475 – 21.160.027	2H	8.9 – 17.6	✓	✓	865
S42IL_107	2H	15.870.298 – 45.504.095	2H	12.5 – 41.2	✓	✓	607
S42IL_108	2H	11.388.475 – 448.196.379	2H	12.5 – 59.1	✓	✓	290
S42IL_109	2H	35.947.807 – 546.773.586	2H	33.9 – 62.7	✓	✓	984
S42IL_110	2H	371.627.646 – 492.798.654	2H	89.5 – 97.8	✓	✓	47
S42IL_111	3H	37.347.545 – 462.188.024	3H	43.1 – 55.2	✓	✓	1062
S42IL_112	3H	470.835.287 – 555.710.032	3H	59.0 – 90.9	✓	✓	1312
S42IL_113	3H	584.504.621 – 611.086.196	3H	120.7 – 142.2	✓		396
S42IL_114	3H	539.584.685 – 614.035.558	3H	75.9 – 144.9	✓		1392
S42IL_115	3H	584.504.621 – 626.117.822	3H	120.7 – 155.0	✓	✓	791

Genotype	Position of Target Introgression (in bp)[a]		Position of Target Introgression (in cM)[b]		Tested in Merbitz	Tested in Morgenrot	S42IL-HR (g)[c]
S42IL_116	4H	3.446.418 – 27.585.310	4H	1.1 – 40.0	✓	✓	724
S42IL_117	4H	10.269.158 – 51.562.622	4H	17.8 – 49.9	✓	✓	529
S42IL_118	4H	23.533.385 – 499.157.928	4H	35.9 – 54.6	✓	✓	957
S42IL_119	4H	23.533.385 – 582.142.994	4H	35.9 – 81.2	✓	✓	978
S42IL_120	4H	23.533.385 – 511.278.021	4H	35.9 – 57.5	✓		1352
S42IL_121	4H	449.709.848 – 582.142.994	4H	51.9 – 81.2	✓	✓	952
S42IL_122	6H	554.471.635 – 573.188.979	6H	103.8 – 126.6	✓	✓	1007
S42IL_123	4H	581.386.307 – 613.240.968	4H	85.6 – 111.3	✓	✓	1174
S42IL_124	4H	610.289.980 – 624.031.203	4H	110.2 – 115.2	✓	✓	1339
S42IL_125	5H	432.238.110 – 494.930.941	5H	51.5 – 81.3	✓	✓	1095
S42IL_126	5H	483.227.444 – 531.535.501	5H	76.2 – 120.3	✓	✓	400
S42IL_127	5H	555.146.452 – 588.682.025	5H	138.5 – 162.5	✓	✓	1349
S42IL_128	6H	24.312.046 – 520.657.754	6H	38.0 – 74.6	✓	✓	1394
S42IL_129	6H	39.540.308 – 522.957.998	6H	47.5 – 79.6	✓	✓	303
S42IL_130	6H	405.001.747 – 556.416.320	6H	59.9 – 105.2	✓	✓	1250
S42IL_131	6H	537.117.533 – 559.123.981	6H	87.9 – 108.3	✓		1244
S42IL_132	6H	543.532.745 – 560.864.079	6H	94.9 – 108.3	✓	✓	185
S42IL_133	7H	13.654.310 – 40.714.264	7H	12.7 – 37.6	✓	✓	302
S42IL_134	7H	40.201.971 – 209.754.781	7H	37.6 – 68.4	✓	✓	1468
S42IL_135	7H	109.879.629 – 605.849.299	7H	67.8 – 118.5	✓	✓	29
S42IL_136	7H	555.814.735 – 602.198.212	7H	84.6 – 110.8	✓	✓	1137
S42IL_137	7H	559.557.978 – 618.510.494	7H	86.0 – 127.5	✓	✓	948
S42IL_138	7H	602.433.103 – 634.078.525	7H	110.8 – 141.1	✓	✓	841
S42IL_139	7H	620.873.823 – 634.078.525	7H	129.5 – 141.1	✓	✓	474
S42IL_140	3H	542.309.062 – 622.267.947	3H	86.2 – 148.2	✓	✓	2096
S42IL_141	1H	415.060.486 – 465.545.124	1H	58.4 – 80.2	✓	✓	504
S42IL_142	1H	509.013.513 – 522.028.097	1H	122.1 – 132.7	✓	✓	348
S42IL_143	1H	467.558.386 – 502.524.381	1H	82.6 – 112.3	✓	✓	1180
S42IL_144	2H	35.147.418 – 67.176.356	2H	33.9 – 50.1	✓		421
S42IL_148	6H	2.190.636 – 9.170.563	6H	0.3 – 11.3	✓	✓	1519
S42IL_149	6H	21.306.993 – 106.489.196	6H	30.0 – 51.0	✓		1659
S42IL_153	2H	75.534.489 – 560.473.324	2H	60.7 – 68.6	✓	✓	1183
S42IL_161	3H	608.443.317 – 624.054.943	3H	139.6 – 154.9	✓		i.p.
S42IL_176	5H	492.638.739 – 559.392.120	5H	81.3 – 140.1	✓	✓	-
‘Scarlett’					✓	✓	-

[a] based on RefSeq 2.0 (Monat *et al.* 2019), [b] based on Honsdorf *et al.* (2017), [c]Grams (g) of seed available for each BC4S2 population. ‘i.p.’ indicates that the HR population is currently in preparation through field multiplication.

In total, based on the RefSeq 2.0 position of SNPs the S42IL library of 49 lines covers 84.3% of the ‘ISR42-8’ genome. Except for 5H, the wild barley introgressions cover most of the genetic background of ‘Scarlett’ on all chromosomes (Figure 1).

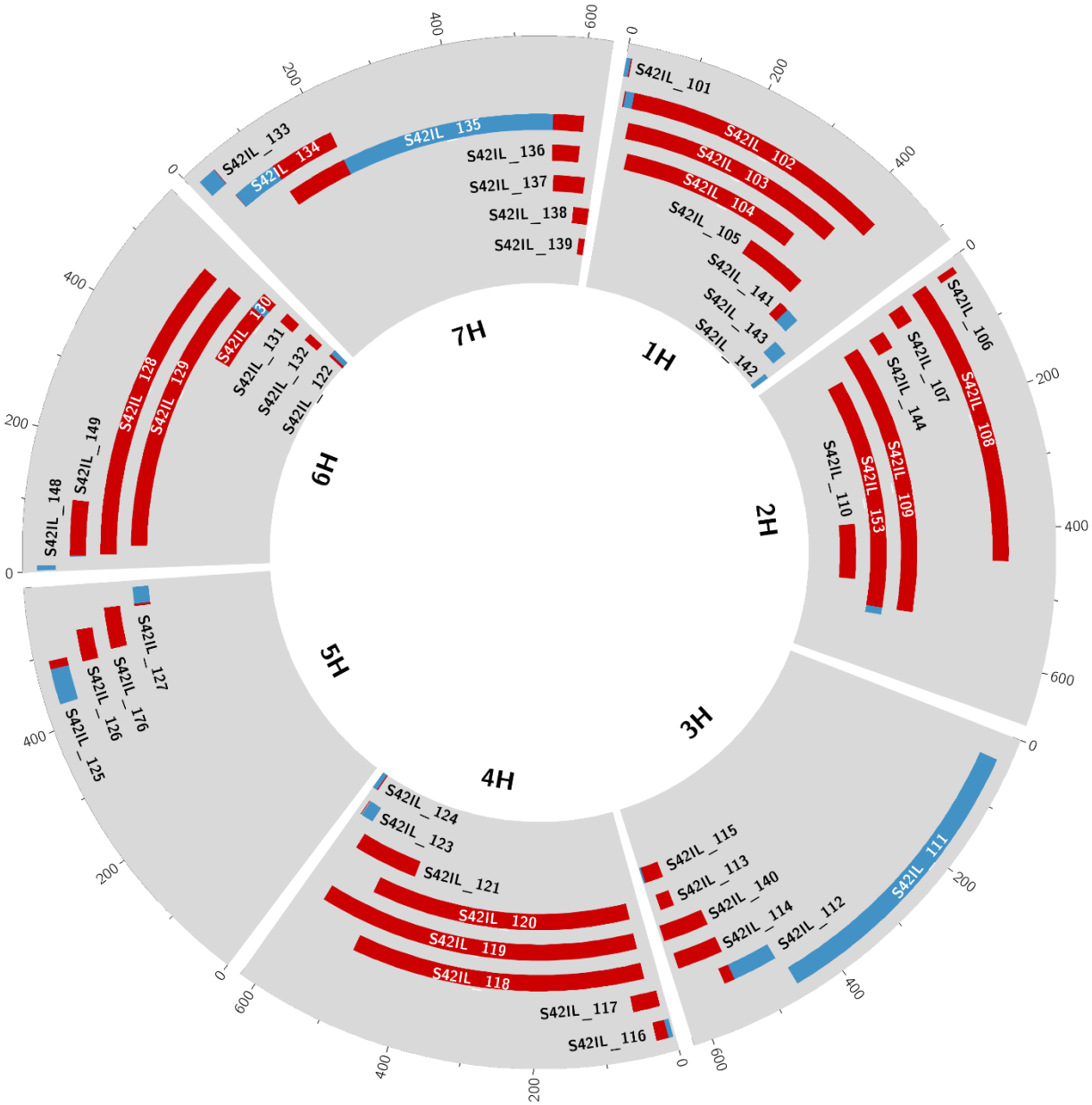


Figure 1 Physical map positions of target introgressions of the representative set of 49 wild barley introgression lines

Circular genome visualization and data visualization with Circos (Krzywinski et al. 2009);
Outer ring: Physical position of SNPs in Mbp based on RefSeq 2.0 (Monat et al. 2019);
Centrals rings (with grey background): extension of target introgressions (red colour: overlapping part of ILs, blue colour: singular part of ILs only represented in one IL);
Inner ring: barley chromosomes

Experimental setup

The field experiments were carried out for two years (2015 and 2016) in Morgenrot, Germany (51°47'19.2"N 11°12'14.5"E) and four years (2015 to 2018) in Merbitz, Germany (51°36'38.6"N 11°53'27.8"E). The soil textures in Morgenrot and Merbitz were loam (pH: 7.0) and sandy loam (pH: 6.9), respectively. Both locations represent dry areas with a yearly average temperature of 8.8 °C and a precipitation of 550 mm per year in Morgenrot, and 9.5 °C and 450 mm in Merbitz, respectively.

The plants were grown in 5.25 m² (3.5 m × 1.5 m) plots arranged in a randomized split-plot design in three replications. The trials were sown in spring with a seed density of 300 grains m^{-2}. In Morgenrot, 41 S42ILs and the reference parent 'Scarlett' were grown whereas in Merbitz 49 S42ILs and 'Scarlett' were cultivated (Table 1). Prior to sowing, available soil mineral nitrogen (Nmin) was measured following the description of VDLUFA (Association of German Agriculture Analytic and Research Institutes). All genotypes were cultivated under low (N_0 = 60 - 80 kg N ha^{-1}) and high (N_1 = 100 - 120 kg N ha^{-1}) target nitrogen fertilization. For the N_1 treatment, calcium ammonium nitrate was applied at BBCH 31 (Lancashire *et al.* 1991) after subtracting soil Nmin from target nitrogen fertilization (Table 2). Additionally, two different preceding crops, winter wheat (WW) and winter oilseed rape (WR), were included in the field trial in Merbitz. Both preceding crops were grown as quality food crop and harvested before seedbed preparation was conducted. Following site-specific recommendations crop management treatments (growth regulators, herbicides, insecticides, and fungicides) were applied.

Table 2 Nitrogen treatments in A) Merbitz and B) Morgenrot

A) Merbitz

Preceding crop	***Fertilization level***		**N-Fertilization in kg ha^{-1}**							
			2015		*2016*		*2017*		*2018*	
		Target N	Nmin	N-apply	Nmin	N-apply	Nmin	N-apply	Nmin	N-apply
Winter wheat	N_0	60 - 80	63	20	41	0	52	0	60	0
	N_1	100 - 120	63	60	41	60	52	60	60	60
Winter oilseed rape	N_0	60 - 80	76	0	46	0	60	0	106	0
	N_1	100 - 120	76	40	46	60	60	60	106	60

B) Morgenrot

Preceding crop	***Fertilization level***		**N- Fertilization in kg ha^{-1}**			
			2015		*2016*	
		Target N	Nmin	N-apply	Nmin	N-apply
Winter wheat	N_0	60 - 80	82	0	-	0
	N_1	100 - 120	82	40	-	30

Phenotypic evaluation

During the field trials, 12 and 15 yield-related and developmental traits were evaluated in Morgenrot and Merbitz, respectively (Table 3).

Table 3 Quantitative traits, assessed in Morgenrot and Merbitz

Parameter	Abbreviation	Unit	Assessment	Measurement
Shooting	SHO	d	Visual	Evaluation per plot when 50% of main tillers showed stem elongation, number of days after sowing
Heading	HEA	d	Visual	Evaluation per plot when 50% of main tillers showed 2 cm of visible awns, number of days after sowing
Maturity	MAT	d	Visual	Assessment per plot when 50% of main tillers showed hard dough, number of days after sowing
Height	HEI	cm	Ruler	Average height of 10 ears without awns
Ears per m²	EAR	-	Counting	Based on the average number of ears counted from a 0.5 m row per plot
Grain yield [a]	YLD	dt ha^{-1}	Harvester [a]	Grain weight calculated from harvest of the whole plot
Thousand grain weight [b]	TGW	g	Marvin [b]	Average weight of 1,000 grains extrapolated from a sample of 10 ears
Grain length [b]	GRL	mm	Marvin [b]	Average length of grain from 10 ears
Grain width [b]	GRW	mm	Marvin [b]	Average width of grain from 10 ears
Grain area [b]	GRA	mm²	Marvin [b]	Average grain surface based on grain length and grain width
Grains per ear [b]	GEA	-	Marvin [b]	Average number of grains per ear based on 10 ears
Grain protein content	GPC	% of dry matter	Foss Infratec	Measured average value of 300g grain per plot
Chlorophyll content [c]	SPAD	Index	SPAD-502 Plus	Measured average of 10 flag leaves at BBCH 61 (Lancashire et al. 1991)
Senescence [c]	SEN	Scale (1-10)	Visual	Assessment of average flag leaf yellowing per plot during ripening (BBCH principal growth stage 8) (Pask et al. 2012)
Lodging [c]	LOD	Scale (1-9)	Visual	Assessment of average plant lodging per plot

[a] *Harvest data in 2015 is absent in Merbitz due to extensive lodging*

[b] Assessed by *MARVIN seed analyzer (GTA Sensorik GmbH, Neubrandenburg, Germany)*

[c] *Only assessed in Merbitz*

Statistical analysis

Significant genotype and treatment effects were determined by analysis of variance (ANOVA) for each trait by fitting a linear mixed model in SAS software version 9.2 (procedure MIXED; SAS Institute, 2008). Due to different test factors and introgression lines used, the statistical analyses were split in two multi-environment trials: the nitrogen trial [A] in years 2015 and 2016 with two nitrogen treatments at both test sites, Morgenrot and Merbitz, and the preceding crop trial [B] in years 2015-2018 with two preceding crops in Merbitz. The following two models were applied:

$$[A] \qquad Y_{ijkl} = \mu + G_i + N_j + E_k + B_l + G_i \times N_j + B_l \times N_j + \varepsilon_{ijkl}$$

where μ is the general mean of trait Y, G_i is the fixed effect for each of the i = 42 genotypes (41 S42ILs and 'Scarlett'), N_j is the fixed effect for each of the j = 2 N levels, E_k is the fixed effect for each of the k = 4 environments, B_l is the random block effect, $G_i \times N_j$ is the fixed interaction effect between *i*th genotype and *j*th treatment, $B_l \times N_j$ is the random interaction effect between *l*th block and *j*th N level and ε_{ijkl} is the random error effect of Y. Least squares means (LSMeans) were calculated for factors G and G×N.

$$[B] \qquad Y_{ijklm} = \mu + G_i + N_j + P_k + E_l + B_m + G_i \times N_j + G_i \times P_k + \varepsilon_{ijklm}$$

where μ is the general mean of trait Y, G_i is the fixed effect for each of the i = 50 genotypes (49 S42ILs and 'Scarlett'), N_j is the fixed effect for each of the j = 2 N levels, P_k is the fixed effect for each of the k = 2 preceding crops, E_l is the random effect for each of the l = 4 environments, B_m is the random block effect, $G_i \times N_j$ is the fixed interaction effect between *i*th genotype and *j*th nitrogen treatment, $G_i \times P_k$ is the fixed interaction effect between *i*th genotype and *k*th preceding crop and ε_{ijklm} is the random error effect of Y. Based on the mixed model, least squares means (LSMeans) for the factors G, G×N and G×P were calculated.

Pearson's correlation coefficients were calculated for all traits based on estimated LSMeans across replications and years for each nitrogen treatment and preceding crop. In addition, the auto-correlation of a trait between N_0 and N_1 as well as WW and WR was calculated.

Based on models A and B broad-sense heritabilities for each trait was estimated with 'PROC VARCOMP', assuming all factors random, across all environments according to Holland *et al.* (2003):

$$[C] \quad h^2 = 100 \times \frac{\sigma^2 G}{\sigma^2 G + \left(\frac{\sigma^2 GE}{e}\right) + \left(\frac{\sigma^2 GN}{n}\right) + \left(\frac{\sigma^2 GEN}{en}\right) + \left(\frac{\sigma^2 \varepsilon}{enpr}\right)}$$

$$[D] \; h^2 = 100 \times \frac{\sigma^2 G}{\sigma^2 G + \left(\frac{\sigma^2 GE}{e}\right) + \left(\frac{\sigma^2 GN}{n}\right) + \left(\frac{\sigma^2 GP}{p}\right) + \left(\frac{\sigma^2 GEN}{en}\right) + \left(\frac{\sigma^2 GEP}{ep}\right) + \left(\frac{\sigma^2 GENP}{enp}\right) + \left(\frac{\sigma^2 \varepsilon}{enpr}\right)}$$

where σ^2G is the variance component of the genotype (G), σ^2GE is the variance component of genotype×environment, σ^2GN is the variance component of genotype×nitrogen level, σ^2GP is the variance component of genotype×preceding crop level, σ^2GEN is the variance component of genotype×environment×nitrogen level, σ^2GEP is the variance component of genotype×environment×preceding crop, σ^2GENP is the variance component of genotype×environment×nitrogen×preceding crop and $\sigma^2\varepsilon$ is the experimental error variance component with e, n, p and r being the number of environments, nitrogen levels, preceding crops and replications, respectively.

QTL detection

A Dunnett test was performed to examine the significance of genotypic differences between individual S42ILs and the recurrent parent 'Scarlett' (Dunnett 1955). Since each S42IL differed from the recurrent parent 'Scarlett' in the target introgression, the presence of a QTL was assumed in the introgressed segment, if an S42IL significantly deviated from the control 'Scarlett'.

For the nitrogen trial [A] the presence of a QTL was accepted if a S42IL revealed a significant LSMeans difference from 'Scarlett' with $p<0.05$. For the preceding crop trial [B] a more critical p-value below 0.001 in a treatment-dependent effect after false discovery rate (FDR) correction was used to accept QTL detection. If lines, carrying overlapping or flanking introgressions, showed similar significant effects (i.e. a joint increase or decrease of trait value with respect to 'Scarlett'), a single QTL was assumed. The relative performance (RP) of a S42IL, describing its deviation from 'Scarlett' in %, was calculated by the following equation:

$$RP(IL) = 100 \times \frac{LSMeans(S42IL) - LSMeans('Scarlett')}{LSMeans('Scarlett')}$$

3. Results

3.1 Genotyping

All results of the 41 and 49 S42IL lines, respectively, with their introgressions were illustrated with the latest genotyping data of the Illumina Infinium iSelect SNP Array (Appendix eTable 1). The position of the target introgression were precisely assigned to base pair positions based on the current RefSeq 2.0 version of the barley genome (Monat et al. 2019 and Figure 1). An accurate information that these 49 tested lines cover 84.3% of the 'ISR42-8' genome were calculated as described before. While the chip revealed small introgressions in S42IL_101, _106, _107, _113, _115, _116, _117, _122, _123, _124, _126, _127, _131, _132, _133, _136, _137, _138, _139, _141, _142, _143, _144, and _148, large introgressions were carried by S42IL_102, _103, _104, _108, _109, _111, _118, _119, _120, _128, _129, _135 and _153. Serious gaps were present at the end of chromosomes 2H and 5H, and at the beginning of chromosome 3H, where solely S42IL_111, with none overlapping region, was localized. Additionally, overlapping of entire lines were found. Five previously unknown small overlaps were detected for S42IL_105 and _141, S42IL_109 and _110, S42IL_119 and _123, S42IL_125 and _176, as well as S42IL_133 and _134.

3.2 Field evaluation of wild barley introgression lines in a nitrogen trial

The study was split into two multi-environment trials. The nitrogen trial [A] was carried out with 41 S42ILs studied under two nitrogen fertilization regimes across two-years and two locations. The preceding crop trial [B] was carried out with 49 S42ILs studied under two nitrogen fertilization regimes and two preceding crops across four-years, but only at one location, Merbitz. To ensure better traceability first descriptive statistic followed by QTL detection are presented for each trial separately. Subsequently, the outcome of both multi-environment trials are compared.

Descriptive Statistics

In Table 4 the descriptive statistics of the measured traits are listed comparing the S42IL population and 'Scarlett' in a nitrogen trial. Having the genetic background of the parent 'Scarlett' the majority of the assessed 41 S42ILs manifested similar phenotypes. However, in a few genotypes strong deviations in trait expression could be found both decreasing and increasing the trait compared to 'Scarlett'. Especially for EAR and YLD a large dispersion led to high coefficients of variation. Higher nitrogen input (N_1) only had a significant influence on HEI, EAR, YLD and GPC resulting in higher means than under N_0.

Table 4 Descriptive statistics of the nitrogen trial

Trait	Genotype	N_0					N_1				
		N	Mean	Min	Max	CV	N	Mean	Min	Max	CV
SHO	S42IL	473	52.77	39	64	11.5	488	52.90	39	64	11.6
	'Scarlett'	12	54.83	49	62	10.5	12	54.33	49	61	8.8
HEA	S42IL	473	69.61	53	82	8.6	488	69.63	53	82	8.7
	'Scarlett'	12	70.42	63	77	7.2	12	70.75	60	79	8.4
MAT	S42IL	473	103.38	86	117	7.6	488	103.61	88	117	7.6
	'Scarlett'	12	103.83	91	112	7.1	12	104.25	91	114	8.0
HEI*	S42IL	473	65.02	40	96	14.5	488	65.72	42	100	14.5
	'Scarlett'	12	63.83	55	77	10.4	12	65.25	53	76	10.9
EAR*	S42IL	473	779.16	400	1360	19.6	488	831.97	368	1712	21.8
	'Scarlett'	12	844.00	576	1136	18.5	12	830.67	416	1296	30.8
YLD*	S42IL	350	59.94	29.6	77.0	16.2	365	62.92	38.2	81.6	16.6
	'Scarlett'	9	60.95	40.4	71.8	20.2	9	63.58	49.9	78.6	15.4
TGW	S42IL	473	47.90	34.8	56.7	7.4	488	47.43	36.5	56.1	7.4
	'Scarlett'	12	48.14	42.5	53.0	5.8	12	47.31	42.0	54.3	8.5
GRL	S42IL	473	8.15	5.6	10.8	9.0	488	8.23	6.3	10.5	8.0
	'Scarlett'	12	7.87	6.0	8.7	12.1	12	8.18	7.0	8.7	7.8
GRW*	S42IL	473	3.83	3.3	4.2	4.3	488	3.81	3.2	4.2	4.6
	'Scarlett'	12	3.89	3.6	4.1	4.0	12	3.81	3.5	4.0	4.1
GRA	S42IL	473	21.36	14.9	27.8	10.6	488	21.44	16.0	26.8	9.8
	'Scarlett'	12	20.99	16.3	23.8	12.5	12	21.35	17.6	23.5	10.1
GEA	S42IL	473	22.75	10.6	28.2	9.4	488	22.96	7.5	29.0	11.1
	'Scarlett'	12	23.47	20.6	25.7	7.1	12	23.41	19.5	25.9	8.4
GPC*	S42IL	228	10.38	8.8	12.9	7.9	242	11.40	9.8	13.3	5.4
	'Scarlett'	6	10.19	9.1	11.2	8.8	6	11.09	10.6	11.8	3.7
SPAD	S42IL	246	46.52	32.9	56.9	10.7	246	46.60	31.7	57.0	11.2
	'Scarlett'	6	47.37	37.8	53.0	11.4	6	46.38	38.3	56.0	12.7

N = Number of observations, Mean = Mean value, Min = Minimum, Max = Maximum, CV = Coefficient of variation (%), * = significant ($p<0.05$) nitrogen level difference based on ANOVA. Trait abbreviations are defined in Table 3.

The analysis of variance revealed significant effects ($p<0.05$) in nitrogen treatment on HEI, EAR, GRW, YLD and GPC (Table 5). Only GRW was significantly increased under N_0 whereas the nitrogen limitation (N_0) provoked a significant reduction in HEI, EAR, YLD and GPC compared to N_1. Except for GPC, significant genotype main effects were observed for all assessed traits ($p<0.01$) (Table 5). In HEI and GRW significant genotype×nitrogen treatment effects occurred (Table 5).

Table 5 ANOVA list of significant trait effects of genotype, nitrogen and genotype*nitrogen

Trait	Effect	NumDF	DenDF	FValue	ProbF
SHO*	Genotype	41	756	112.61	0.000
SHO	Nitrogen treatment	1	11	0.09	0.770
SHO	Genotype*Nitrogen treatment	41	756	1.06	0.364
HEA*	Genotype	41	756	144.59	0.000
HEA	Nitrogen treatment	1	11	0.24	0.635
HEA	Genotype*Nitrogen treatment	41	756	1.05	0.384
MAT*	Genotype	41	756	18.04	0.000
MAT	Nitrogen treatment	1	11	1.09	0.318
MAT	Genotype*Nitrogen treatment	41	756	0.96	0.550
HEI*	Genotype	41	756	49.86	0.000
HEI	Nitrogen treatment	1	11	4.41	0.060
HEI*	Genotype*Nitrogen treatment	41	756	1.42	0.046
EAR*	Genotype	41	756	3.44	0.000
EAR*	Nitrogen treatment	1	11	8.73	0.013
EAR	Genotype*Nitrogen treatment	41	756	1.02	0.444
YLD*	Genotype	41	551	12.35	0.000
YLD*	Nitrogen treatment	1	8	16.05	0.004
YLD	Genotype*Nitrogen treatment	41	551	0.86	0.721
TGW*	Genotype	41	756	10.91	0.000
TGW	Nitrogen treatment	1	11	1.27	0.283
TGW	Genotype*Nitrogen treatment	41	756	1.34	0.076
GRL*	Genotype	41	756	12.02	0.000
GRL	Nitrogen treatment	1	11	0.86	0.375
GRL	Genotype*Nitrogen treatment	41	756	1.02	0.433
GRW*	Genotype	41	756	9.04	0.000
GRW*	Nitrogen treatment	1	11	13.62	0.004
GRW*	Genotype*Nitrogen treatment	41	756	1.41	0.047
GRA*	Genotype	41	756	7.73	0.000
GRA	Nitrogen treatment	1	11	0.02	0.903
GRA	Genotype*Nitrogen treatment	41	756	1.20	0.187
GEA*	Genotype	41	756	16.01	0.000
GEA	Nitrogen treatment	1	11	0.54	0.479
GEA	Genotype*Nitrogen treatment	41	756	1.01	0.463
GPC	Genotype	41	347	1.19	0.200
GPC*	Nitrogen treatment	1	5	28.17	0.003
GPC	Genotype*Nitrogen treatment	41	347	0.61	0.970
SPAD*	Genotype	41	512	4.76	0.000
SPAD	Nitrogen treatment	1	5	3.34	0.127
SPAD	Genotype*Nitrogen treatment	41	512	1.22	0.136

NumDF= Numerator degrees of freedom, DenDF= denominator degrees of freedom, ProbF= significance probability value associated with the F Value. *= significant ($p<0.05$) difference based on ANOVA. Trait abbreviations are defined in Table 3.

Calculated broad-sense heritabilities were high for all traits except for EAR and GPC ($h^2 = 0.67$ and $h^2 = 0.22$, respectively, Table 6).

Table 6 Heritabilities

Trait	σ^2_G	σ^2_E	σ^2_N	σ^2_{GN}	σ^2_{GE}	σ^2_{GEN}	σ^2_ε	**h^2[%]**
SHO	10.38	31.62	0.00	0.00	1.17	0.00	2.29	**96.40**
HEA	6.31	38.65	0.00	0.00	0.28	0.04	1.00	**98.19**
MAT	0.97	80.40	0.00	0.00	0.17	0.12	1.58	**88.66**
HEI	39.84	35.76	0.07	0.43	1.82	0.68	20.55	**96.11**
EAR	2320.88	2631.97	1260.28	0.00	0.00	1640.39	22813.46	**66.76**
YLD	6.44	108.94	5.94	0.00	5.63	0.00	16.71	**75.37**
TGW	1.56	7.29	0.02	0.10	0.48	0.00	4.90	**80.66**
GRL	0.04	0.43	0.00	0.00	0.00	0.00	0.13	**86.42**
GRW	0.00	0.03	0.00	0.00	0.00	0.00	0.01	**78.03**
GRA	0.17	5.07	0.00	0.01	0.04	0.00	0.79	**78.20**
GEA	1.23	1.62	0.00	0.00	0.86	0.00	2.23	**79.97**
GPC	0.01	0.00	0.39	0.00	0.02	0.00	0.39	**22.05**
SPAD	1.60	31.13	0.06	0.15	0.00	0.28	8.04	**78.21**

σ^2_G, σ^2_E, σ^2_N, σ^2_{GN}, σ^2_{GE}, σ^2_{GEN} and σ^2_ε correspond to the genotype, environment, nitrogen treatment, genotype×nitrogen treatment, genotype×environment, genotype×environment×nitrogen treatment, and error variance component, respectively. Trait abbreviations are defined in Table 3.

Pearson correlation coefficients between twelve quantitative traits, separately calculated for N_0 and N_1, are displayed in Table 7. A total of 55 significant correlations were found. Except for GPC, Pearson correlations between N_0 and N_1 were significant for all traits ($p<0.05$). For plant developmental traits (SHO, HEA, MAT) similar results could be found under both nitrogen levels. These three traits were highly positive correlated ($r > 0.81$). The same holds true for TGW, GRL and GRA. Correlations between grain components were generally slightly stronger under N_1 than under N_0. In this study, a higher correlation of GRA with GRL ($r_{N0} = 0.81$ and $r_{N1} = 0.82$) than with GRW ($r = 0.23$ for both) were observed. TGW revealed high correlations with GRW ($r_{N0} = 0.53$ and $r_{N1} = 0.67$) and GRA ($r = 0.72$ for both), whereas GRL ($r_{N0} = 0.30$ and $r_{N1} = 0.29$) was only moderately correlated. The trait HEI was significantly negatively correlated with SHO, HEA, MAT, EAR and YLD under N_0 as well as in N_1. The correlation matrices for YLD showed homogenous findings for SHO, HEA, MAT, HEI and GPC under both nitrogen levels. Significant moderate positive

correlations between YLD and plant developmental traits were detected for both nitrogen levels (r= 0.32 – 0.48), while negative correlations between YLD and HEI (r_{N0} = -0.50 and r_{N1} =-0.59) as well as GPC (r_{N0} = -0.35 and r_{N1} = -0.29) occurred. Interestingly, YLD was significantly positively correlated with EAR (r_{N0} = 0.47) only under N_0. Furthermore, results for correlations of YLD and SPAD also strongly differed comparing N_0 with N_1 (r_{N0} = -0.29 and r_{N1} = 0.15). Under N_0 YLD correlated negatively with SPAD whereas weak positive correlation can be seen under N_1. Additionally, low positive correlations were detected for SPAD with TGW (r_{N0} = 0.31 and r_{N1} = 0.25) and GRA (r_{N0} = 0.34 and r_{N1} = 0.11).

Table 7 Pearson correlation coefficients between 13 traits under two nitrogen levels (N_0 and N_1)

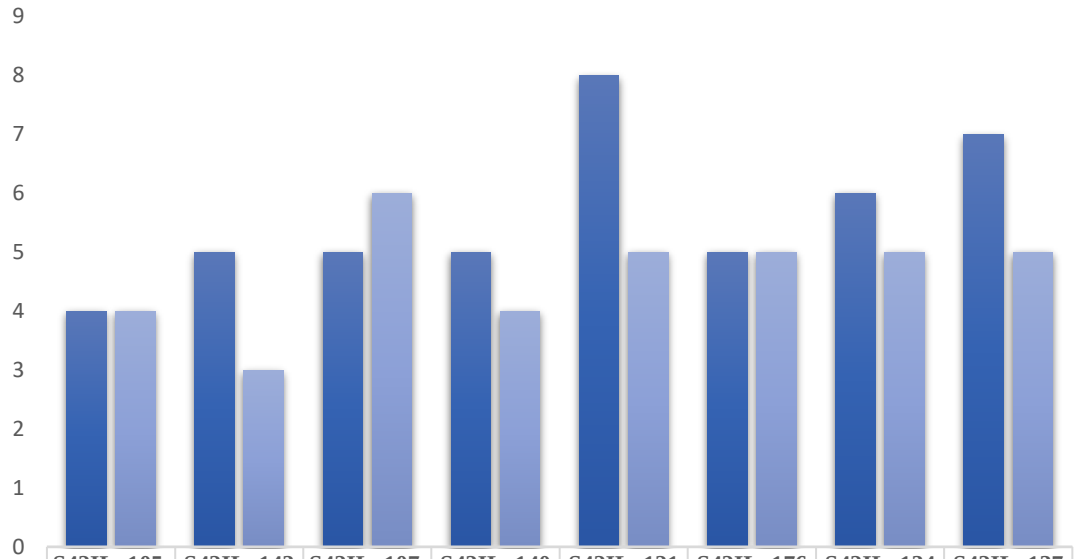
N_0 / N_1	SHO	HEA	MAT	HEI	EAR	YLD	TGW	GRL	GRW	GRA	GEA	GPC	SPAD
SHO	0.98 *	0.85 *	0.89 *	-0.79 *	0.11	0.37 *	-0.29	0.10	0.03	-0.02	0.22	-0.25	-0.15
HEA	0.81 *	0.99 *	0.82 *	-0.45 *	-0.06	0.32 *	-0.32 *	0.12	-0.07	-0.04	0.36 *	-0.12	-0.40 *
MAT	0.86 *	0.87 *	0.89 *	-0.61 *	0.10	0.37 *	-0.15	0.15	0.06	0.09	0.28	-0.10	-0.28
HEI	-0.74 *	-0.32 *	-0.44 *	0.96 *	-0.39 *	-0.50 *	0.28	0.15	-0.16	0.19	0.02	0.28	-0.03
EAR	0.16	-0.14	0.01	-0.47 *	0.56 *	0.47 *	-0.21	-0.35 *	0.12	-0.34 *	-0.12	-0.18	-0.11
YLD	0.45 *	0.34 *	0.48 *	-0.59 *	0.19	0.83 *	0.04	-0.29	0.20	-0.23	0.21	-0.35 *	-0.29
TGW	-0.28	-0.29	-0.19	0.25	-0.34 *	-0.11	0.78 *	0.30	0.53 *	0.72 *	-0.27	-0.08	0.31 *
GRL	0.01	0.08	0.13	0.25	-0.12	-0.26	0.29	0.83 *	-0.30	0.81 *	-0.07	0.14	0.25
GRW	-0.11	-0.28	-0.20	-0.12	-0.16	-0.03	0.67 *	-0.30	0.73 *	0.23	-0.11	-0.10	0.12
GRA	-0.13	-0.11	-0.05	0.27	-0.26	-0.28	0.72 *	0.82 *	0.23	0.69 *	-0.19	0.06	0.34 *
GEA	0.27	0.46 *	0.45 *	0.03	-0.19	0.05	-0.34 *	-0.09	0.02	-0.16	0.89 *	0.04	-0.29
GPC	-0.09	-0.03	0.01	0.22	-0.10	-0.29	0.10	0.00	0.04	0.08	-0.05	0.23	-0.09
SPAD	-0.16	-0.38 *	-0.26	-0.15	0.01	0.15	0.25	0.01	0.23	0.11	-0.38 *	-0.21	0.47 *

Correlations within N_0 are represented in the upper triangle, while N1 is represented in the lower triangle. The values in the diagonal represent the auto-correlation of a trait between N_0 and N_1. * $p<0.05$. Trait abbreviations are defined in Table 3.

QTL detection in nitrogen trial

To detect S42ILs, which are significantly different from ‘Scarlett’, a mixed model ANOVA followed by a post-hoc Dunnett test was conducted. Considering all traits, 32 lines showed significant deviations from ‘Scarlett’ revealing 77 putative QTL (Appendix Table 1). In Table 8 eight outstanding S42ILs are listed, showing significant effects for several traits simultaneously. Most QTL findings overlapped between S42IL_121 and _137, primarily for plant development and growth (SHO, HEA, MAT, HEI) as well as grain yield parameters (YLD, GRA, GRL, TGW). Hereafter, each trait will be described in detail.

Table 8 Outstanding S42ILs with QTL for several traits under N_0 or N_1



	S42IL_105 1H	S42IL_143 1H	S42IL_107 2H	S42IL_140 3H	S42IL_121 4H	S42IL_176 5H	S42IL_134 7H	S42IL_137 7H
QTL under N0	4	5	5	5	8	5	6	7
QTL under N1	4	3	6	4	5	5	5	5

Plant development (SHO, HEA, MAT)

For SHO, HEA and MAT thirteen, twelve and seven QTL were identified, respectively (Appendix Table 1). On each chromosome plant development QTL were mapped. High variations in the number of days to achieve these stages were detected compared to ‘Scarlett’. The spectrum ranged from -10 days in SHO by line S42IL_140, having an introgression on 3H, to +3 days by line S42IL_176 and _143, having an introgression on 5H and 1H, respectively. A part of the introgression lines could only be detected for the growth stages SHO (S42IL_106, _115) or HEA (S42IL_102, _103, _109) while seven S42ILs showed continous significant deviations from ‘Scarlett’ at all three developmental stages (S42IL_105, _107, _140, _121, _148, _134, _137).

Ears per m² (EAR)

No QTL was found for EAR.

Plant height (HEI)

In twelve S42ILs, eleven QTL across all chromosomes, were detected controlling plant height (Appendix Table 1). The *Hsp* alleles decreased plant height in lines S42IL_107, S42IL_109, S42IL_122, S42IL_124 and S42IL_138 with the strongest effect in line 109 (up to -14.5%). Contrary, seven lines (S42IL_105, _121, _125, _134, _137, _140 and _148) showed an increase in plant height. The highest increase was observed on chromosome 3H (S42IL_140) with 29.5% higher plants.

Grain yield (YLD)

Six QTL were identified for grain yield across all chromosomes except for 2H (Appendix Table 1). All significant S42ILs showed a decrease in grain yield between 10.1% and 13.1% for both nitrogen levels related to a grain harvest loss of 4.5-8.0 dt ha^{-1}. Overall, more significant QTL were found under N_1 than under N_0. Remarkably, the highest yield loss of 13% appeared under N_1 level identified on chromosome 6H (S42IL_148).

Grain yield components: Thousand grain weight (TGW), Grain area (GRA), Grain length (GRL), Grain width (GRW) and Grains per ear (GEA)

Four QTL for TGW were detected on chromosomes 4H, 5H and 6H (Appendix Table 1). Three lines (S42IL_124, _128, _176) showed a decrease by 6.1-7.5% in TGW compared to 'Scarlett', while line S42IL_119 and S42IL_121 with target introgressions on 4H showed an increase in TGW by 6.0-7.3% and 9.4%, respectively. Eighteen QTL were found for grain shape including four for GRA, six for GRL and eight for GRW (Appendix Table 1). For GRA, the *Hsp* alleles at all QTL provoked an increase with the strongest effect in line S42IL_121, where trait performance extended by 10.5% under N_0 treatment. All QTL for GRL deviated positively from 'Scarlett', while all QTL regions for GRW showed a decrease. The strongest effect for GRL was found in S42IL_102, _121 and _143 increasing the values up to 15.2% across both treatments. In the remaining lines the *Hsp* alleles resulted in increased values under N_0 level compared to 'Scarlett' varying between 5.1-8.9%. For the traits GRA, GRL and GRW, the most pronounced effects were detected under N_0 levels.

All *Hsp* alleles at QTL regions for GEA deviated negatively from 'Scarlett'. S42IL_107, S42IL_109 and S42IL_110 showed the strongest decrease under N_1 with up to 17.1% less grains per ear.

Grain protein content (GPC)

No QTL was found for GPC.

Chlorophyll content (SPAD)

Two QTL, located on chromosomes 1H and 2H were associated with SPAD (Appendix Table 1). Line S42IL_142, with a target introgression on 1H, revealed the highest decrease under N_0 with a chlorophyll reduction of -11.3% compared to 'Scarlett'. On the contrary, *Hsp* alleles on 2H led to higher SPAD values. The highest increase of 16.3% was found under N_1, present in S42IL_110.

3.3 Field evaluation of wild barley introgression lines in a preceding crop trial

Descriptive Statistics

In Table 9 the statistics of measured traits are listed comparing the S42IL population and 'Scarlett' in a preceding crop trial with winter wheat (WW) and winter rape seed (WR) as variants. All traits showed a considerable amount of variation between the 49 S42ILs. A decreased degree of variation from SHO to MAT could be observed. The parameter LOD showed an extremely high coefficient of variation (CV). Also, high CV values were obtained for EAR and SEN. The comparison between WW and WR revealed that in ten traits mean values were highest after WR. The majority of the assessed 49 S42ILs manifested similar phenotypes like their parent 'Scarlett'. However, in a few genotypes strong deviations in trait expression could be found, both decreasing and increasing the trait compared to 'Scarlett'. With a few exceptions, growth parameters like SHO, HEA, MAT, SEN and LOD showed no differences in Mean, Min and Max growing after WW or WR. Noticeable higher mean values were measured for HEI, YLD, TGW and SPAD after WR. Equally, the mean number of EAR increased after WR, but, in S42ILs, were highest after WW. Surprisingly, Mean, Min and Max of GPC were higher after WW than WR. Remarkably, the highest yield in single plots across all treatments was found in the S42 population rather than in the reference cultivar 'Scarlett' (Appendix eTable 2).

Table 9 Descriptive statistics of the preceding crop trial

		WW					WR				
Trait	Genotype	N	Mean	Min	Max	CV	N	Mean	Min	Max	CV
SHO	S42IL	1175	48.9	29	63	17.0	1176	48.6	28	63	17.7
	'Scarlett'	24	50.4	36	61	16.1	24	49.3	37	61	16.6
HEA*	S42IL	1175	65.0	45	82	13.5	1176	64.4	45	82	14.4
	'Scarlett'	24	65.8	52	79	13.7	24	65.8	52	79	14.4
MAT*	S42IL	1175	97.7	78	117	10.9	1176	100.1	87	119	7.9
	'Scarlett'	24	98.5	85	114	10.9	24	100.5	91	114	8.2
HEI*	S42IL	1175	60.9	31	100	22.4	1176	66.9	49	101	13.6
	'Scarlett'	24	60.6	40	77	19.0	24	65.3	50	80	12.6
EAR*	S42IL	1175	710.2	224	1712	34.1	1176	792.4	320	1600	27.6
	'Scarlett'	24	746.7	352	1296	37.3	24	804.7	496	1584	35.8
YLD*	S42IL	867	56.0	13.9	82.5	26.2	872	75.3	41.8	102.3	10.5
	'Scarlett'	18	56.2	28.0	70.2	25.3	17	78.4	67.7	85.0	6.5
TGW*	S42IL	1170	44.6	28.7	55.9	9.0	1174	47.2	32.3	60.2	8.9
	'Scarlett'	24	44.3	36.4	49.1	8.6	24	47.1	37.4	54.4	9.7
GRA	S42IL	1174	20.1	13.7	25.6	12.0	1175	20.5	15.2	25.7	11.3
	'Scarlett'	24	19.7	15.8	23.0	12.7	24	19.8	15.7	23.2	10.7
GRL	S42IL	1173	7.8	5.6	10.0	10.8	1175	7.7	6.2	10.5	11.5
	'Scarlett'	24	7.6	6.1	8.7	11.8	24	7.4	6.1	9.1	11.4
GRW*	S42IL	1173	3.7	3.2	4.2	5.1	1175	3.8	3.2	4.2	4.4
	'Scarlett'	24	3.7	3.4	4.1	5.3	24	3.8	3.5	4.1	4.1
GEA*	S42IL	1173	22.2	12.2	29.80	11.2	1175	23.4	11.1	31.1	11.7
	'Scarlett'	24	22.7	19.5	25.9	8.5	24	24.1	16.8	28.0	11.3
GPC	S42IL	582	15.1	10.5	18.8	12.0	578	14.8	9.6	18.2	10.0
	'Scarlett'	12	14.5	11.4	17.2	12.7	11	14.4	11.7	15.9	11.1
SPAD*	S42IL	1175	47.4	31.7	58.9	9.6	1176	50.1	34.0	61.0	10.9
	'Scarlett'	24	47.1	39.1	55.0	7.8	24	50.6	39.8	57.5	10.1
SEN*	S42IL	1175	8.2	1.0	10.0	22.3	1175	7.1	1.0	10.0	33.4
	'Scarlett'	24	7.8	5.0	9.0	16.1	24	6.3	3.0	10.0	38.0
LOD*	S42IL	1175	3.0	1.0	9.0	72.1	1176	3.9	1.0	9.0	72.4
	'Scarlett'	24	2.8	1.0	7.0	68.8	24	3.3	1.0	7.0	74.4

N = Number of observations, Mean = Mean value, Min = Minimum, Max = Maximum, CV = Coefficient of variation (%), * = significant ($p<0.001$) preceding crop difference based on ANOVA. Trait abbreviations are defined in Table 3.

Eleven agronomic traits were significantly ($p<0.05$) influenced by the different preceding crops (Table 10). WR as a previous crop caused higher average values in growth and grain parameters, compared to results of spring barley cultivated after WW. Significant genotype main effects as well as year main effects were observed for all assessed traits ($p<0.001$). Additionally, statistically significant interactions between genotypes and preceding crops were found for four traits (SHO, YLD, GPC, SEN, Table 10).

Table 10 ANOVA list of significant trait effects of genotype, nitrogen and genotype*nitrogen

Trait	Effect	NumDF	DenDF	FValue	ProbF
SHO*	Genotype	49	2180	118.70	0.000
SHO	Preceding crop	1	2180	2.14	0.144
SHO*	Genotype*Preceding crop	49	2180	1.38	0.043
HEA*	Genotype	49	2180	136.54	0.000
HEA*	Preceding crop	1	2180	10.01	0.002
HEA	Genotype*Preceding crop	49	2180	0.62	0.982
MAT*	Genotype	49	2180	20.60	0.000
MAT*	Preceding crop	1	2180	4.27	0.039
MAT	Genotype*Preceding crop	49	2180	0.90	0.662
HEI*	Genotype	49	2180	43.08	0.000
HEI*	Preceding crop	1	2180	7.91	0.000
HEI	Genotype*Preceding crop	49	2180	1.05	0.388
EAR*	Genotype	49	2180	3.12	0.000
EAR*	Preceding crop	1	2180	7.91	0.005
EAR	Genotype*Preceding crop	49	2180	1.13	0.245
YLD*	Genotype	49	1591	8.19	0.000
YLD*	Preceding crop	1	1591	18.87	0.000
YLD*	Genotype*Preceding crop	49	1591	3.61	0.000
TGW*	Genotype	49	2173	14.27	0.000
TGW*	Preceding crop	1	2173	13.17	0.000
TGW	Genotype*Preceding crop	49	2173	0.90	0.664
GRL*	Genotype	49	2177	14.01	0.000
GRL	Preceding crop	1	2177	0.31	0.577
GRL	Genotype*Preceding crop	49	2177	1.07	0.351
GRW*	Genotype	49	2177	11.68	0.000
GRW*	Preceding crop	1	2177	13.14	0.000
GRW	Genotype*Preceding crop	49	2177	1.24	0.120
GRA*	Genotype	49	2177	9.99	0.000
GRA	Preceding crop	1	2177	3.78	0.052
GRA	Genotype*Preceding crop	49	2177	1.08	0.324
GEA*	Genotype	49	2177	17.27	0.000
GEA*	Preceding crop	1	2177	24.63	0.000
GEA	Genotype*Preceding crop	49	2177	1.19	0.170
GPC*	Genotype	49	1012	6.31	0.000

Trait	Effect	NumDF	DenDF	FValue	ProbF
GPC	Preceding crop	1	1012	0.34	0.563
GPC*	Genotype*Preceding crop	49	1012	2.95	0.000
SPAD*	Genotype	49	2180	8.34	0.000
SPAD*	Preceding crop	1	2180	59.03	0.000
SPAD	Genotype*Preceding crop	49	2180	0.90	0.674
SEN*	Genotype	49	2179	23.52	0.000
SEN*	Preceding crop	1	2179	9.38	0.002
SEN*	Genotype*Preceding crop	49	2179	1.39	0.039
LOD*	Genotype	49	2180	15.50	0.000
LOD*	Preceding crop	1	2180	14.26	0.000
LOD	Genotype*Preceding crop	49	2180	0.99	0.500

NumDF= Numerator degrees of freedom, DenDF= denominator degrees of freedom, ProbF= significance probability value associated with the F Value. *= significant ($p<0.05$) difference based on ANOVA. Trait abbreviations are defined in Table 3.

Calculated broad-sense heritabilities were moderate for EAR, YLD and GPC ($0.39 \leq h^2 \leq 0.55$). All remaining traits revealed high heritabilities ranging from 0.73 to 0.97 (Table 11).

Table 11 Heritabilities

Trait	σ^2_G	σ^2_E	σ^2_P	σ^2_{GP}	σ^2_{GE}	σ^2_{GEP}	σ^2_ε	**h^2[%]**
SHO	6.6	82.3	0.0	0.0	0.3	0.3	2.2	**97.2**
HEA	6.4	97.3	0.1	0.0	0.7	0.2	1.5	**96.5**
MAT	2.2	94.3	0.0	0.0	0.6	0.0	2.4	**90.2**
HEI	18.0	100.4	13.2	0.0	3.3	0.4	20.6	**91.1**
EAR	621.4	26369.0	2066.8	378.2	546.0	0.0	27778.2	**39.3**
YLD	2.7	0.0	122.5	2.8	2.5	5.3	33.4	**41.1**
TGW	1.8	4.9	2.3	0.0	0.6	0.0	9.3	**83.6**
GRL	0.0	0.6	0.0	0.0	0.0	0.0	0.2	**83.8**
GRW	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**76.2**
GRA	0.2	5.6	0.0	0.0	0.1	0.0	1.1	**76.4**
GEA	1.3	1.9	0.6	0.0	0.5	0.0	3.3	**84.1**
GPC	0.1	2.7	0.0	0.0	0.0	0.2	1.5	**54.8**
SPAD	1.0	18.1	3.8	0.0	0.0	0.6	7.2	**77.2**
SEN	0.8	0.9	0.2	0.0	0.2	0.1	1.7	**89.1**
LOD	0.4	3.6	0.3	0.0	0.4	0.1	1.3	**72.7**

σ^2_G, σ^2_E, σ^2_P, σ^2_{GP}, σ^2_{GE}, σ^2_{GEP} and σ^2_ε correspond to the genotype, environment, preceding crop treatment, genotype× preceding crop treatment, genotype×environment, genotype×environment× preceding crop treatment, and error variance component, respectively. Trait abbreviations are defined in Table 3.

Pearson correlations among all traits are listed separately for WR and WW in Table 12, together with auto-correlations of a trait between both preceding crops. Auto-correlations for YLD, EAR and GPC were comparatively low (<0.52). Depending on the preceding crop, a multitude of different correlations with other traits could be found for these traits. Moderate positive correlations between YLD and the traits EAR, TGW, GRW as well as SPAD were found after WW (r_{WW} = 0.31 to 0.45). Negative correlations were detected for YLD and growth stages (r_{WW} = -0.43 to -0.24) as well as GPC (r_{WW} = -0.41). Equally, after WR a moderate positive correlation of YLD with EAR (r_{WR} = 0.51) and a negative correlation between YLD and GPC (r_{WR}= -0.32) were observed. However, major differences were found for TGW showing no correlation with YLD after WR. Moreover, after WR the correlation of YLD and GEA with 0.31 was opposite to the outcome after WW (r_{WW} = -0.23). Equal discrepancies in the correlation results appeared for TGW and EAR (r_{WW}= -0.27; r_{WR}= 0.10), EAR and GPC (r_{WW}= -0.37; r_{WR}= -0.02) as well as EAR and SPAD (r_{WW}= -0.06; r_{WR}= 0.27).

Table 12 Pearson correlation coefficients between 15 traits after two preceding crops (WW and WR)

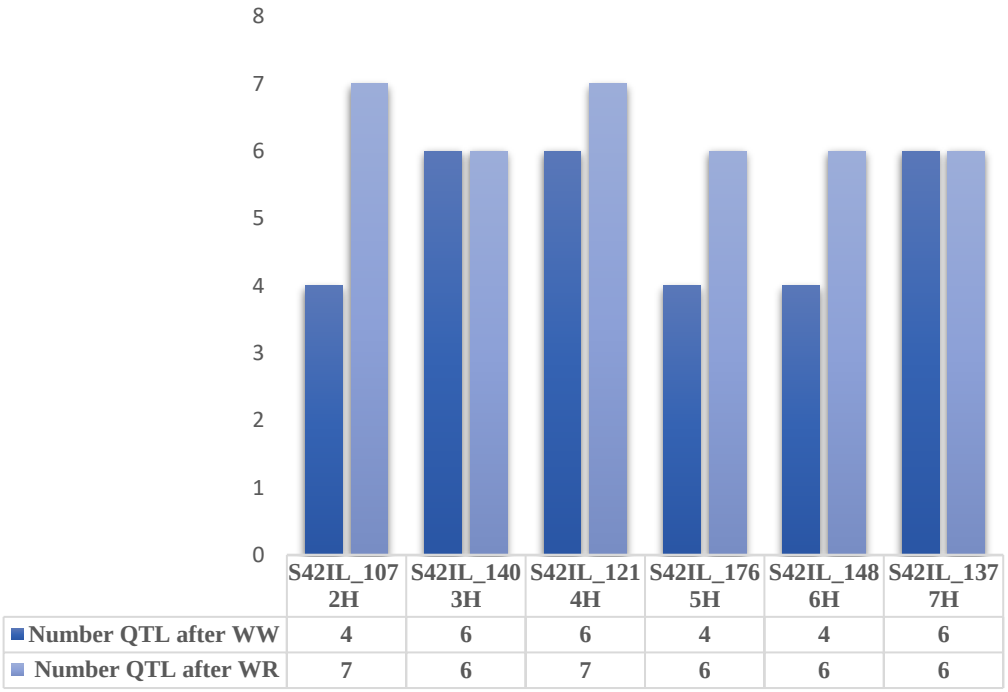
WW / WR	SHO	HEA	MAT	HEI	EAR	YLD	TGW	GRA	GRL	GRW	GEA	GPC	SPAD	SEN	LOD
SHO	0.98 *	0.84 *	0.79 *	-0.76 *	-0.03	-0.24	-0.55 *	-0.30 *	-0.01	-0.36 *	0.31 *	-0.03	-0.18	-0.74 *	-0.87 *
HEA	0.82 *	0.99 *	0.80 *	-0.41 *	-0.16	-0.43 *	-0.56 *	-0.26	0.02	-0.44 *	0.55 *	0.04	-0.42 *	-0.76 *	-0.65 *
MAT	0.80 *	0.87 *	0.93 *	-0.37 *	-0.22	-0.39 *	-0.41 *	-0.10	0.17	-0.40 *	0.41 *	0.06	-0.44 *	-0.79 *	-0.64 *
HEI	-0.74 *	-0.34 *	-0.33 *	0.95 *	-0.30 *	-0.10	0.49 *	0.40 *	0.21	0.15	-0.01	0.10	-0.22	0.40 *	0.70 *
EAR	0.17	-0.06	-0.09	-0.48 *	0.47 *	0.31 *	-0.27	-0.34 *	-0.25	-0.11	-0.28	-0.37 *	-0.06	0.13	0.10
YLD	0.07	0.00	-0.06	-0.28	0.51 *	0.52 *	0.40 *	0.11	-0.08	0.45 *	-0.23	-0.41 *	0.32 *	0.30 *	0.12
TGW	-0.46 *	-0.43 *	-0.34 *	0.29	0.10	0.10	0.89 *	0.75 *	0.32 *	0.70 *	-0.41 *	0.02	0.19	0.34 *	0.42 *
GRA	-0.22	-0.11	0.04	0.38 *	-0.06	-0.19	0.63 *	0.83 *	0.84 *	0.20	-0.41 *	0.06	0.07	0.18	0.37 *
GRL	0.07	0.16	0.29	0.23	-0.09	-0.27	0.11	0.80 *	0.86 *	-0.30 *	-0.30 *	-0.01	-0.02	-0.03	0.21
GRW	-0.19	-0.28	-0.29	-0.13	0.18	0.29	0.58 *	-0.10	-0.60 *	0.81 *	-0.13	0.12	0.21	0.28	0.13
GEA	0.29 *	0.48 *	0.40 *	-0.05	-0.22	0.31 *	-0.44 *	-0.38 *	-0.20	-0.05	0.87 *	0.10	-0.37 *	-0.34 *	-0.35 *
GPC	-0.17	-0.11	-0.13	0.23	-0.02	-0.32 *	-0.19	0.04	0.05	-0.12	-0.08	0.35 *	0.18	0.00	-0.05
SPAD	-0.19	-0.48 *	-0.55 *	-0.28	0.27	0.20	0.05	-0.25	-0.28	0.23	-0.19	0.09	0.80 *	0.25	0.01
SEN	-0.85 *	-0.82 *	-0.90 *	0.48 *	-0.02	0.06	0.33 *	0.08	-0.17	0.21	-0.30 *	0.20	0.42 *	0.92 *	0.60 *
LOD	-0.76 *	-0.65 *	-0.68 *	0.47 *	-0.04	-0.01	0.35 *	0.30 *	0.14	0.01	-0.33 *	-0.05	0.16	0.68 *	0.88 *

Correlations within winter wheat (WW) are represented in the upper triangle, while winter oilseed rape (WR) is represented in the lower triangle. The values in the diagonal represent the auto-correlation of a trait between winter wheat (WW) and winter oilseed rape (WR). * $p<0.05$. Trait abbreviations are defined in Table 3.

QTL Detection

A total of 69 presumed QTL were detected for 14 out of 15 traits across both preceding crops (Appendix Table 2). In Table 13 outstanding S42 lines are listed, showing significant effects for several traits simultaneously. Most QTL were found in S42IL_121 and _137, primarily for plant development and growth (SHO, HEA, MAT, HEI, SEN) as well as grain yield parameters (TGW, GRA, GRL). Hereafter, each trait will be described in detail.

Table 13 Outstanding lines with QTL for several traits after WW or WR



	S42IL_1072H	S42IL_1403H	S42IL_1214H	S42IL_1765H	S42IL_1486H	S42IL_1377H
Number QTL after WW	4	6	6	4	4	6
Number QTL after WR	7	6	7	6	6	6

Plant development (SHO, HEA, MAT, SEN)

For SHO and HEA, the QTL analysis revealed similar finding after WR and WW. Ten QTL were shared between both traits and preceding crops (Appendix Table 2). The highest effect for SHO was observed in S42IL_140, possessing an introgressed *Hsp* segment on chromosome 3H. This line reduced the period until shooting by 7 - 8 days compared to 'Scarlett', while S42IL_107 (target introgression on 2H) showed the highest effect on HEA reducing time to heading by 8 - 9 days. For SHO and HEA significant preceding crop-dependent effects were also identified for S42IL_109, _115, _124 and _138, however with relatively low effects. A comparatively shorter growth period in SHO was observed after WW than after WR, as revealed by lower relative performances of *Hsp* QTL alleles. Interestingly, it was opposite for HEA. A clearly reduced number of only three detected QTL

was observed for MAT. Two significant lines (S42IL_107 and S42IL_140) were obtained after both preceding crops, while S42IL_148 only showed significant effects after WR.

Significant SEN increases were identified across all chromosomes (Appendix Table 2). Nine significant lines, summarized to seven QTL, were identified after WR of which only S42IL_140 and S42IL_176 could also be found after WW. Only in S42IL_176 lower senescence scores of -33.6% (WR) and -36.4% (WW) were found, while in all other six QTL regions an increase in SEN occurred. The highest increases were revealed in S42 lines 107, 114 and 140 (46.6 - 50.7%) after WR. S42IL_114 and S42IL_140 carry an overlapping introgression on chromosome 3H (Figure 1).

Plant height and lodging (HEI, LOD)

The trait HEI showed very similar findings after both preceding crops but with two more significant S42 lines (109 and 124) after WW (Appendix Table 2). In total, nine potential QTL were spread over all chromosomes. A wide range of QTL effects of up to +14 cm in plant height compared to 'Scarlett' were obtained almost always showing a higher relative performance after WW. However, the two WW specific QTL on 2H and 4H showed the only HEI decreasing effects (-7 cm). Five QTL for LOD were found. Except for the QTL associated with S42IL_107, the four remaining QTL overlapped with HEI QTL.

Ear number (EAR)

No QTL was found for EAR.

Grain yield (YLD)

Three QTL, present in S42IL_103, _111 and _176, were found for YLD (Appendix Table 2). All QTL appeared only after WR. No significant QTL were detected after WW. All three chromosome regions (1H, 3H, 5H) decreased YLD by 14.6 - 23.1% as compared to 'Scarlett'.

Grain yield components: Thousand grain weight (TGW), Grain area (GRA), Grain length (GRL), Grain width (GRW) and Grains per ear (GEA)

Only a single QTL on chromosome 4H was observed for TGW, which led to an increase in TGW by 9.8% compared to 'Scarlett' after WW (Appendix Table 2). For GRA two QTL were found on 1H and 4H after both preceding crops and one on 7H only after WW. All Hsp QTL alleles showed trait-increasing effects of 7 - 10% (Appendix Table 2). For GRL four out of seven QTL were obtained

after both preceding crops, while one was exclusively found after WW on chromosome 2H and two only after WR on chromosome 4H and 5H (Appendix Table 2). All QTL effects increased trait values by up to 13% as compared to 'Scarlett'. For GRW, however, both QTL, detected in S42IL_102 and _176, were connected with decreasing trait effects. These *Hsp* introgressions corresponded to two QTL increasing GRL. For GEA three QTL were observed. The S42 lines 107 and 109 carrying overlapping introgression on 2H (Figure 1), that showed strong GEA reduction effects of up to -18% after both preceding crops.

Grain protein content (GPC)

Four significant lines (S42IL_129, _130, _133 and _134) were found for GPC (Appendix Table 2). Wild barley introgressions at two detected QTL led to an increase of GPC by 11 - 14% as compared to 'Scarlett'. S42 lines 129 and 130 have an overlapping introgression on 6H, while lines 133 and 134 overlap on 7H (Figure 1). Remarkably, significant lines could only be found after WW.

Chlorophyll content (SPAD)

One significant QTL effect was localized on chromosome 2H for chlorophyll content, solely after WR (Appendix Table 2). Here, the measured SPAD index was increased by 9.6%.

3.4 QTL detection in complex field management interaction

In this chapter the two independent multi-environment trials, presented before, will be compared. Due to the lack of the experimental factor preceding crop at the test site Morgenrot a joint statistical model cannot be established across both multi-environment trials. Instead, QTL results for both treatment factors (nitrogen level and preceding crop) will be presented and used to define sensitive and solid QTL. Whereas the former QTL were only described under one specific management, the latter were now enlighted across both managements, nitrogen fertilization and preceding crops (Figure 3).

Overall, 94 significant QTL were associated with 14 out of the 15 assessed traits. These QTL were found for 33 out of the 49 S42 barley introgression lines studied. Among all traits the highest number of QTL were identified for SHO and HEA (Table 14). No QTL was found for EAR. Comparing both multi-environment trials, 77 and 69 QTL were found for the nitrogen trial and the preceding crop trial, respectively, with 33 super-solid QTL shared between both trials and detected in each treatment. Among them 28 were found in plant growth traits (nine in SHO, ten in HEA, two in MAT) and five in grain components (three in GRL, one in GEA, one GRW). Two super-solid QTL at chromosome

2H and 3H were equally found across SHO, HEA and MAT (Appendix 2 and 3).
Out of 77 QTL of the nitrogen trial, 49 solid QTL were present under both N levels whereas 19 and eight sensitive QTL were exclusively present under the N_0 and N_1 levels, respectively. Likewise, out of 69 QTL of the preceding crop trial, 46 solid QTL were present after both preceding crops, whereas 17 and eight sensitive QTL were exclusively present after winter rape seed and winter wheat, respectively. The ratio of environmental solid and sensitive QTL varied between traits (Table 14).

Table 14 Number of QTL per trait and classification as solid (common) or sensitive (level-specific) QTL

Trait	N_0	N_1	Total	N-Solid	Sensitive	WW	WR	Total	P-Solid	Sensitive
SHO	11	11	13	10	3	11	12	12	11	1
HEA	12	10	12	10	2	11	10	11	10	1
MAT	7	6	7	6	1	2	3	3	2	1
HEI	11	10	11	10	1	9	7	9	7	2
YLD	3	5	6	2	4	0	3	3	0	3
TGW	3	4	4	3	1	1	0	1	0	1
GRL	6	3	6	3	3	5	6	7	6	1
GRW	7	2	8	1	7	1	2	2	1	1
GRA	4	1	4	1	3	3	2	3	2	1
GEA	3	4	4	3	1	2	3	3	2	1
GPC	0	0	0	0	0	2	0	2	0	2
SPAD	1	1	2	0	2	0	1	1	0	1
SEN	-	-	-	-	-	2	7	7	2	5
LOD	-	-	-	-	-	3	5	5	3	2

N= nitrogen, WW= winter wheat, WR= winter oilseed rape, P= preceding crop.
Trait abbreviations are defined in Table 3.

Not only introgression lines with significant deviations from the recurrent parent ‘Scarlett’ were of interest, but also lines that showed a tendency to outperform ‘Scarlett’ in seasons under extreme drought stress or heavy rainfall events. For the nitrogen trials five lines (S42IL_109, _122, _123, _135 and _136) showed a non-significant average tendency across both N-level to increase yield by of 2.6 - 4.0 dt ha^{-1} (4.2 - 6.5%) compared to ‘Scarlett’ (Appendix eTable 3). Under the N_1-treatment, the yield of S42IL_122 even increased by 5.9 dt ha^{-1} (9.2%) compared to ‘Scarlett’. Likewise, three lines (S42IL_109, _122 and _136) showed a non-significant average tendency to increase yield in the preceding crop trial. Especially, after the preceding crop WW the yield of S42IL_122 and S42IL_136 increased by 1.6 dt ha^{-1} (2.4%) compared to ‘Scarlett’ (Appendix eTable 3).
Additionally, line S42IL_123 showed lower grain yield loss than average and high crop stability over all years at both test sites despite suboptimal weather conditions. The average monthly precipitation and monthly temperature of the four experimental years at Morgenrot and Merbitz are

given in Appendix Table 3. Drought stress periods and heavy rainfall events occurred throughout the growing season across all years 2015 to 2018 at the test sites in Morgenrot and Merbitz (Figure 2). In 2015 and 2018, the months May and June (shooting and booting phase) were characterized by few precipitations at both test sites. After this time of poor water availability heavy rainfall events followed in July and August in 2015 during grain filling and ripening phase, while 2018 severe drought continuously affected plants until harvest. The years 2016 and 2017 experienced more balanced precipitations during the growing season with one heavy rainfall event in July 2017 (around BBCH 80).

Although harsh growing conditions were prevalent in Morgenrot during the season, acceptable average grain yields of 59.6 dt ha^{-1} and 70.7 dt ha^{-1} were harvested for S42IL_123 in 2015 and 2016, respectively, resulting in a yield loss of only 15.7% compared to the average yield loss of 29.1% for all lines between 2015 and 2016 (Appendix eTable 3 and 4). In Merbitz, average grain yields of 67.5, 69.9 and 64.4 dt ha^{-1} were harvested for S42IL_123 in 2016, 2017 and 2018, respectively (Appendix eTable 3). Similar to the results for Morgenrot in 2015 and 2016, the loss in grain yield in the drought stress year 2018, was only 6.7% compared to the average loss of 18.3% across all other plots, calculated by the mean of years 2016 and 2017 against year 2018 (Appendix eTable 3 and 4)

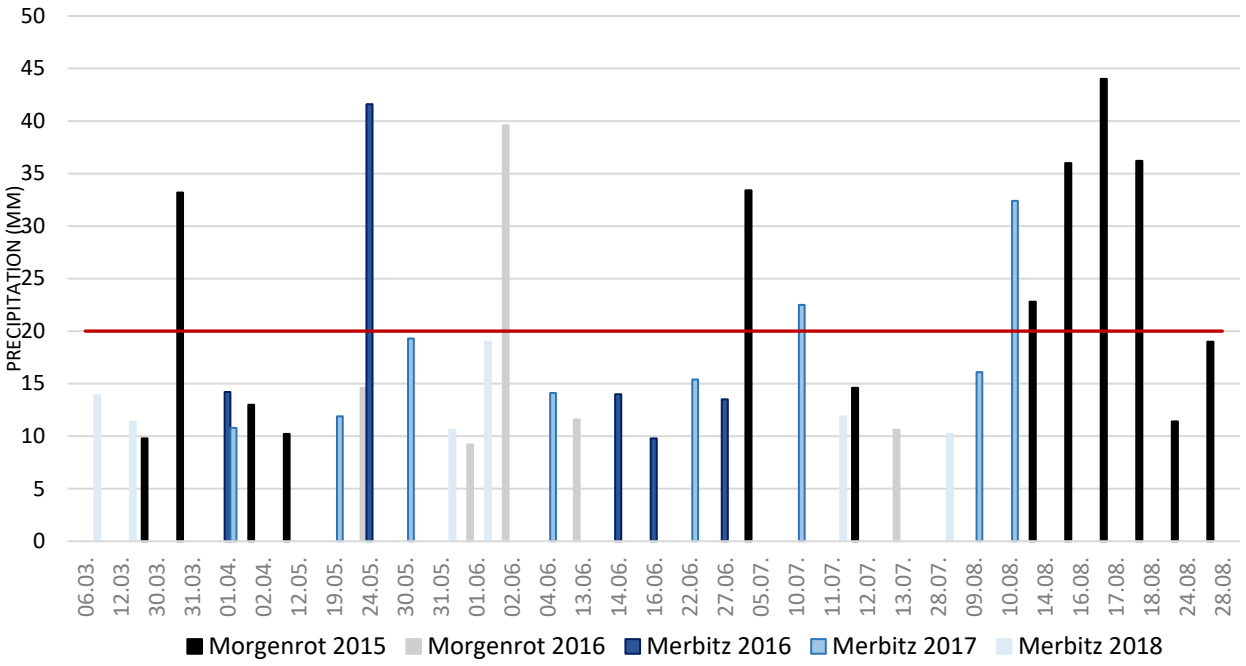


Figure 2 Heavy rainfall events during growing seasons 2015 to 2018 in Merbitz and Morgenrot
Red line = benchmark of 20 mm precipitation for heavy rainfall events;
Only precipitation events above 9 mm are represented.

After considering and analysing each multi-environment trial separately the treatments of this study (N_0, N_1, WW and WR) were evaluated in groups to compare their QTL detection power (Figure 3). This causes four possible cultivation management scenarios:

A) preceding crop winter wheat and two nitrogen levels in barley (WW under N_0 & N_1),
B) preceding crop winter oilseed rape and two nitrogen levels in barley (WR under N_0 & N_1),
C) two preceding crops and nitrogen stress in barley (WR & WW under N_0), and
D) two preceding crops and high nitrogen availability in barley (WR & WW under N_1).

Most QTL (62) were detected in scenario C (WR & WW under N_0), closely followed by the scenario B (WR under N_0 & N_1) with 60 QTL. The lowest number of discovered QTL appeared in scenario D with 45 QTL.

Thirty-five environmentally stable QTL were identified across all four scenarios (Appendix 4). This included QTL controlling the traits SHO, HEA, MAT, HEI, GRA, GRL, GEA and SEN. As the effect of these introgressed *Hsp* QTL alleles emerged under all four managements scenarios, i consider those QTL as major QTL acting independent from the tested field managements. Regarding the interaction effects genotype×preceding crop and genotype×nitrogen, ten sensitive QTL were

detected (Appendix 4). These QTL acted on the traits HEA (1), HEI (1), YLD (1), GRA (1) GPC (1), SEN (4) and LOD (1) representing minor QTL.

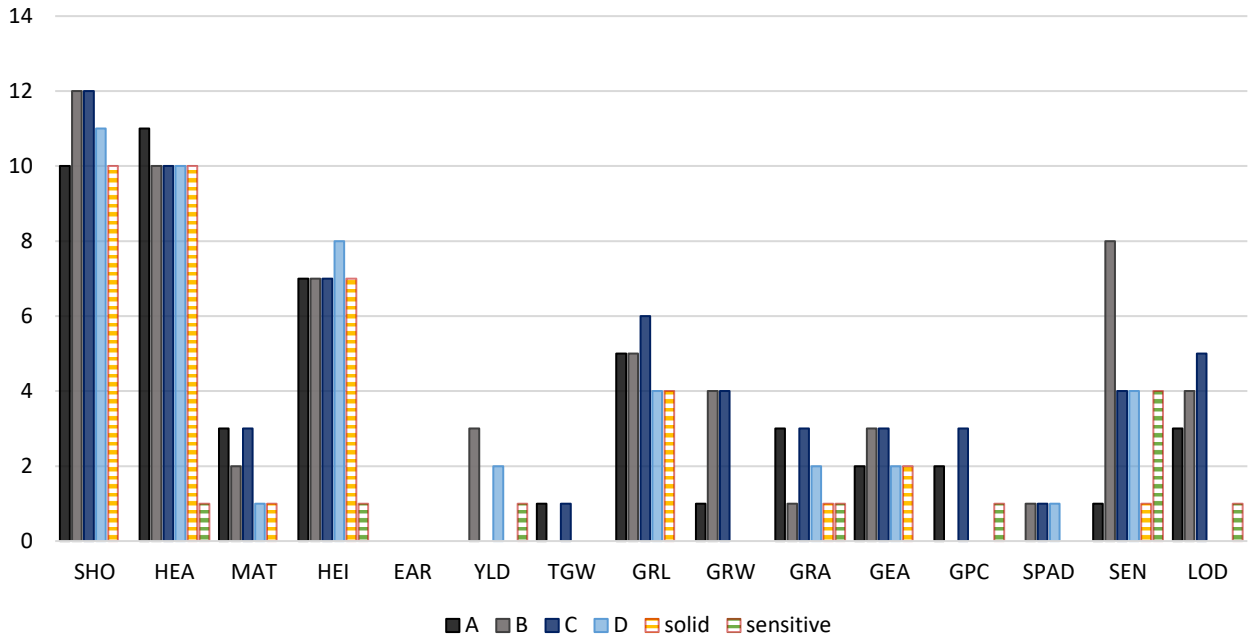


Figure 3 Number of QTL detected in four cultivation management scenarios (A to D)
A) preceding crop winter wheat and two nitrogen levels in barley (WW under N_0 & N_1),
B) preceding crop winter oilseed rape and two nitrogen levels in barley (WR under N_0 & N_1),
C) two preceding crops and nitrogen stress in barley (WR & WW under N_0), and
D) two preceding crops and high nitrogen availability in barley (WR & WW under N_1).
Solid QTL: detectable across both management factors, nitrogen fertilization and preceding crops
Sensitive QTL: only detectable under one management factor.

4. Discussion and future prospects

Both, selection and cultivation of barley varieties have to be adapted to local climate conditions and regional resources, which stresses the importance of versatile breeding schemes such as crossbreeding with wild donors, transferring phenotype-genotype relations and evaluating various crop management treatments. Therefore, the present study evaluated the impact of different preceding crops (winter wheat (WW) and winter oilseed rape (WR)) and two contrasting N treatments (N_0 and N_1) on QTL detection using the wild barley introgression population S42IL. This study was conducted by two multi-year trials, the first as a two-year nitrogen trial located at two field sites in Saxony-Anhalt, Morgenrot and Merbitz, and the second as a four-year preceding crop trial located only in Merbitz. Both trials were carried out with 41 and, respectively, 49 wild barley introgression lines of the S42IL population. As a result, a comprehensive database of multiple evaluated traits for the S42IL population under various field conditions was established and various new QTL were described for 14 developmental and agronomical studied traits located on all seven barley chromosomes. With the help of these new results, first insights into QTL detection depending on different preceding crops and nitrogen fertilization were gained.

4.1 Genotyping

The genotyping of the S42ILs with the Infinium 50k iSelect SNP array allowed to precisely characterize the extension of the *Hsp* introgressions in each of the 49 studied lines. The advantages for this study are on the one hand highest accuracy in defining introgressions and on the other hand, a clear understanding which S42ILs are quite redundant and can be replaced for further field studies. Supported by the physical positioning (in Mbp) of SNPs to delimit introgressions, it became apparent that using recombination rates (in cM) to define positions of SNPs on barley chromosomes, as used in previous S42IL QTL studies, is of limited information. By comparing the current physical map with the cM map used in Honsdorf *et al.* (2017) i could find that a number of *Hsp* introgressions were previously undetected or not seen to overlap with introgressions in other S42ILs. In accordance to Honsdorf *et al.* (2017) sub-introgression were determined to some extent.

4.2 Nitrogen- and preceding crop-based evaluation of S42ILs

The aim of this study was the detection and mapping of QTL in wild barley introgression lines (S42ILs), which arose from backcrossing the German elite spring barley cultivar ‘Scarlett’ with the Israeli wild barley accession ‘ISR42-8’. The importance of the effects of nitrogen variation, N_0 and N_1, and preceding crops, winter wheat (WW) and winter oilseed rape (WR), on QTL detection were

evaluated in field trials. Since 2008, 20 studies on S42IL performance under field, greenhouse and hydroponic conditions were conducted (Schmalenbach *et al.* 2008; Schmalenbach *et al.* 2009; Schmalenbach and Pillen 2009; Wang *et al.* 2010a; El Soda *et al.* 2010; Saal *et al.* 2011; Schmalenbach *et al.* 2011; Hoffmann *et al.* 2012; March *et al.* 2012; Schnaithmann and Pillen 2013; Honsdorf *et al.* 2014a; Honsdorf *et al.* 2014b; Naz *et al.* 2014; Reuscher *et al.* 2016; Reinert *et al.* 2016; Honsdorf *et al.* 2017; Soleimani *et al.* 2017; Brauch *et al.* 2018; Muzammil *et al.* 2018; Zahn *et al.* 2020).

Furthermore, related introgression studies examined various *Hsp* crossbreeds and their performance in the field. For instance, QTL for yield structures, growth habits and harvest index of the accession 'Hsp 41-1' and 'Hsp 41-5' under drought stress were investigated by Lakew *et al.* (2013). Baum *et al.* (2003) extended these investigation efforts in field by searching QTL for vigor, cold damage, chlorophyll content, tiller number and grain protein content. The team of Li *et al.* (2005) used the crossing between 'Brenda' and 'HS584' to research the effects of wild introgressions on traits such as GEA, TGW, EAR, HEA and HEI . Furthermore, Nice *et al.* (2019) checked malting qualities, YLD, HEA, HEI and LOD of introgression lines derived from 'Harrington (GRIN-CA, CN 39242)' × 'OUH602 (PI 682043)'. Additionally, increased disease resistance was tested (Åhman and Bengtsson 2019). However, conducting experiments with nitrogen treatments in the field require a high workload and only one of the aforementioned studies was conducted as a field-based nitrogen stress trial. In that trial the advanced backcross doubled haploid (BC_2DH) population S42, the precursor population of S42IL, was characterized (Saal *et al.* 2011). Thus, little is known about QTL detection of S42IL in a nitrogen-stress related field experiment. Even more impressive, no investigations have been conducted on different preceding crops with regard to QTL analysis. However, the results of this thesis clearly demonstrate the relevance of these factors for breeding.

Descriptive statistic

The application of higher N fertilization conditions (N_1) led to a significant ($p<0.05$) increase in the traits HEI, EAR, GRW, YLD and GPC. These results are in accordance with several nitrogen related studies (Baethgen *et al.* 1995; Hussain *et al.* 2006; El-Habbal *et al.* 2010). The impact of preceding crops on the traits of the subsequent crop are extensive, as can be seen in the large number of significant trait differences between WR and WW. In both multi-environment trials the traits EAR, YLD and GPC are the most challenging for interpretation. They showed a wide variation of data, consequently resulting in a high CV. Low heritabilities of these traits indicate a strong impact of diverse environmental conditions that can hardly be considered in their entirety. Furthermore, the

auto-correlation of these traits was low, indicating that the degree of genotype by treatment interaction is high for EAR, YLD and GPC. Additionally, statistically significant interactions between genotypes×preceding crops were found for SHO, YLD, GPC and SEN as well as significant genotype×nitrogen treatment effects for HEI and GRW revealing that the phenotypic expression of the tested genotypes were instable for these traits under multi-environments.

QTL Detection

In total, 77 QTL related to nitrogen treatments were found. Effects were detected under both N treatments but some were clearly stronger either under N_0 or under N_1. Eight QTL less, namely 69 QTL, were found regarding the preceding crop treatments. This may be due to the fact, that a stricter $p_{(FDR)}$ value was assumed for the preceding crop trial. Since the bigger raw data set of the four-year preceding crop trial led to an enormous rate of QTL detections using the standard $p_{(FDR)}<0.05$ threshold, a lower error threshold of $p_{(FDR)}<0.001$ was selected. This impressively shows the huge impact of adversarial preceding crops in QTL detection. Although a higher amount of available nitrogen in soil after WR than after WW were detected, as reported previously (Sieling and Christen 1997; Ryan *et al.* 2006) the observed ANOVA and QTL differences between WR and WW cannot only be explained by different N supply as no similar results between the different N treatments were detected. Preceding crops not only affect the nitrogen availability for the following arable crop but also influence extensive ecological procedures (biotic and abiotic) over the years. The effects of different preceding crops are highly complex as they cause biotic (nutrients, parasites, microorganism) and abiotic (soil structure, salinity, soil moisture) short to medium term changes in the soil environment (Sieling and Christen 1997; Sieling *et al.* 2005; Kirkegaard *et al.* 2008; Sieling and Christen 2015).

Both multi-environmental trial showed a list of outstanding S42ILs, which showed significant effects for several traits simultaneously. In particular, S42 lines 121, 137, 143, 140 and 148 stand out. These five lines carry a secondary introgression on 3H and thus, it can be assumed that alternative traits effects arrive from this segment. Interestingly, at this chromosomal location the candidate gene GA20OX2, accountable for gibberellin-regulated developmental processes and NaCl-controlled primary root and root hair growth, is located (Rieu *et al.* 2008; Lv *et al.* 2018).

Comparing detection of solid and environmentally sensitive QTL, both multi-environment trials revealed interesting quantitative proportions. While 27 out of 77 QTL were environmentally sensitive in the nitrogen trial, with 19 QTL specific for N_0 and eight specific QTL for N_1, 25 out of 69 QTL were environmentally sensitive in the preceding crop trial, with 17 QTL specific for WR and eight

QTL specific for WW. Thus, 35.1% and 36.2% of QTL were nitrogen- or preceding crop-dependent. These results indicate that the preceding crop also is a major factor for phenotypic performance of the subsequent spring barley genotype. In general, oilseed rape improves soil structure and nitrogen supply while cereal crops such as winter wheat often lower the availability of nutrients and, due to reduced root density, soil pores (Sieling et al. 2005; Kolbe and Zimmer 2015). Thus, the diverse trait performance of spring barley following the studied two preceding crops might be triggered by their substantially diverging environmental impacts. This hypothesis is supported by previous studies (Schönhammer and Fischbeck 1987; Chan and Heenan 1996; Chalk 1997; Kirkegaard et al. 2008; Sieling and Christen 2015).

So far, little is known about QTL-trait-nitrogen relationships and almost nothing about QTL-trait-preceding crop relationships in field. To confirm the represented data regarding the preceding crop effects on spring barley studies with other pre-crops like barley itself or rye should be considered for verifying results presented here and detect further solid and sensitive QTL. Additionally, preceding crop trials combined with expanded stress condition like drought or salt stress need to be conducted investigating multi-environmental responses of introgression lines more accurate.

Growth phenology

Hsp introgressions affecting developmental stages of plants, as previously reported for heading (Von Korff *et al.* 2006; Schmalenbach *et al.* 2008; Schnaithmann and Pillen 2013) seem to be environmentally solid. We found similar results in our studies for plant development (SHO, HEA, MAT) comparing QTL detection after N_0 and N_1 with 26 out of 31 QTL being detected under both nitrogen levels as well as WW and WR with 23 out of 26 QTL being detected under both preceding crops. These findings also reflect the high heritability of these traits.

All three growth stages (SHO, HEA and MAT) were accelerated similarly in S42IL_105, _107, _140, _121, _137, _134 and _148 as compared to the parental control ‘Scarlett’ under both nitrogen levels. After analysing preceding crop data, lines 107, 140 and 148 were also found stable across SHO, HEA and MAT. In general, a higher variance in the early growth stage SHO than in the later stages HEA and MAT were found. During juvenile plant development, high plant growth rates were detected. In cereals, this so called exponential phase is predominated by SHO (Pope 1932). Therefore, the effects of wild alleles are more pronounced in SHO through stronger plant growth. Thus, it can be assumed that the assessment and evaluation of multifarious QTL detection of wild alleles controlling growth stages can be conducted in early stages. The strongest effect in this study was detected for S42IL_140 and _148, which showed a reduction in the number of days until SHO by up to 9.7 days (17.7%)

under the N_0 treatment and 8.3 days (16.5%) after WW, respectively. S42IL_140 has a target introgression on chromosome 3H (542.3 - 622.3 Mbp) where the semi-dwarf gene *sdw1/HvGA20ox2* is located. Interestingly, although line 148 has its target introgression on chromosome 6H, a secondary introgression on chromosome 3H exists where the gene *sdw1/HvGA20ox2* can be found. Additionally, four other lines, also characterized by earlier SHO, carry a target (S42IL_115) or a secondary introgression (S42ILs 121, 148, 137) in this region on chromosome 3H, covering the same locus. The observed effect was also found by (Maurer *et al.* 2016). The authors also reported that *Hsp* alleles at this locus cause increased plant height phenotypes and explained early development in growth stages.

The introgression line with the highest impact on HEA and MAT in both multi-environment trials was S42IL_107 with an acceleration of heading time by 3 - 9 days. This line carries an introgression at the distal end of the short arm of chromosome 2H containing the flowering gene *Ppd-H1,* known to accelerate plant development at all three growth stages (Turner *et al.* 2005; Maurer *et al.* 2015; Maurer *et al.* 2016; Wiegmann *et al.* 2019). Even though not showing the fastest development in early plant growth, this line also significantly reduced SHO by 4 - 7 days.

Moreover, four QTL on choromosomes 1H, 4H, 5H and 7H were found in the nitrogen trial that significantly delayed SHO and HEA. Only three of them were detected in the preceding crop trial, namely 1H, 4H and 5H. These late developing lines can be beneficial under optimal climate conditions, where early plant development would lead to undesirable premature ripening and lower yield. Otherwise, rapid early crop canopy establishment could suppress weed growth and pest pressure (Lemerle *et al.* 2006). In addition, early plant development could give benefits with regard to heat-related shorter growing seasons. As a result of plant adaption a slight shift towards selecting early barley genotypes can be recognized in different breeding programs (Josephides 1992; Deniz 2007; Gracia *et al.* 2012; Ogrodowicz *et al.* 2017).

Although plant growth is a multilateral combination of genetic and environmental factors (Gregory 1926; Cushman and Bohnert 2000) some introgression lines shared significant effects exclusively at a single growth stage. Potentially, these introgression are carrying genes that control only a short developmental time interval. For example, the nitrogen data revealed QTL in S42IL_106, _115 and _135 only for SHO and S42IL_102 and _103 only in HEA. QTL exclusivly present at one growth stage in the preceding crop trial were S42IL_143 and _138 in SHO as well as S42IL_110 in HEA. This phenomen was also reported by Dhanagond *et al.* (2019) for barley and by Li *et al.* (2006b) for rice. Dhanagond *et al.* (2019) reported QTL for tiller number, growth rate and biomass, which only appeared in drought stress phase or recovery phase. Therefore, detected QTL were grouped related to their time of appearance. In rice, plant growth rate is controlled by different genes activated at

diverse growth stages (Li *et al.* 2006b). Both reports partitioned plant development in consecutive time-related QTL analyses to obtain more precise genetic information for each growth stage.

In summary, numerous new growth-related QTL that contribute to the understanding of yield-related development dynamics under various locations and conditions were discovered in this studies.

Regarding nitrogen fertilization, the field experiments revealed similar effects under both multi-environment trials. Although it is described that N-stressed plants flower earlier (Birch and Long 1990), the genetic influence of the *Hsp* introgressions seemed to overlay the impact of low N input on the S42IL population. Examining preceding crop data most QTL for growth stages were equivalent to the nitrogen findings. This finding indicates a promising strategy to select for a wide range of environmental conditions and locations.

Effects on plant developmental stages, as well as HEI and LOD were often linked and controlled by the same QTL. For HEI, most QTL were in common under N_0 and N_1 as well as after WW and WR. Assesing plant height, extremely large plants with up to 29.5% increased HEI were measured in S42IL_121, _137, _140 and _148. This might be caused by the presence of the *Hsp* allele at the *sdw1/HvGA20ox*$_2$ locus. Based on greater HEI these plants tended to lodge, leading to significantly reduced grain yield. Remarkably, for HEI only four QTL in the nitrogen trial and two QTL in the preceding crop trial, which were congruent, with wild barley introgressions were found causing a decrease in plant height. Both, environmental solid and sensitive QTL were located. While inferior in number compared to solid QTL, sensitive QTL showed the most extreme effects for HEI. Thus again, in HEI major as well as minor QTL effects were reliably identified under different environments as suggested by Tanksley (1993). The detection of stable QTL has the great advantage of environmental independently selection and utilization, whereas the selection of sensitive QTL may result in more pronounced effects for a trait.

Like mentioned before a high correlation between lodging and height as well as developmental traits were detected in this study, which was also reported in Wych *et al.* (1985). Four out of six detected QTL for higher LOD also showed an increased plant height and all six QTL for LOD displayed significant earlier development and higher SEN. Except for S42IL_107, all lines significant for LOD harbour introgressions carrying the *sdw1* locus bearing the gene *HvGA20ox*$_2$. Mutations in this gene are known to cause semi-dwarf phenotypes and earlier flowering (Jia *et al.* 2015a). Greater plant height and earlier plant development of S42ILs are caused by wild barley alleles resulting in increased lodging and senescence. Remarkably, five out of seven QTL for SEN were only detected after WR suggesting that some of the introgressed genes might be highly sensitive to the preceding crop used. EAR is an important yield formation trait, which is challenging to be evaluated in field trials. Due to

high workload, help of assistants are required for this trait, which unfortunately seemed to cause low repeatability and heritability. Consequently, QTL detection rates are poor.

Grain yield

Most studies of spring barley populations derived from crosses with wild barley were focused on the identification of quantitative trait loci that are related to yield potential. This is due to the importance of its stable genetic expression for breeders. As Kromdijk *et al.* (2013) pointed out, the detection of QTL in quantitatively inherited traits is often highly vulnerable to environmental impacts, crop management and the production system. Unfortunately, one of the most complex traits is grain yield. In this study, some promising yield observations in S42ILs outperformed the recurrent parent control 'Scarlett'. However, these individual values showed no continuity across the experiment and consequently led to no yield increasing QTL. In this study, six yield-reducing QTL were detected for the nitrogen multi-environment trial. The decreasing *Hsp* effect was, for example, present in S42ILs, which carry an introgression at the *sdw1/HvGA20ox*$_2$ locus, possibly affecting YLD because of stronger lodging (Kuczyńska *et al.* 2013). Again, it is important to note that final grain yield not only is regulated by genetic control but is also highly affected by environmental impacts and agronomic treatments. Additionally, a multitude of major and minor QTL for plant development, located on different chromosome regions, may interact while realizing final grain yield (Larson *et al.* 1996; Slafer 2003). In this regard, a key event is heading and flowering time. These growth stages influence reproductive success and crop yield improvement (Jung and Muller 2009). According to various sources (Baum *et al.* 2003; Li *et al.* 2006a; Cuesta-Marcos *et al.* 2009; Wang *et al.* 2010b), effects both for HEA and YLD were simultaneously detected in several S42ILs (S42IL_121, _134, _140, _143, _148 and _176) in the nitrogen study. However, QTL showing either late or early flowering reduced YLD.

Additionally, three different QTL for YLD were found only after WR, of which just one was uniform to the six aforementioned QTL, indicating the importance of genotype×nitrogen and genotype×preceding crop interactions. Nice *et al.* (2019) detected a decreasing yield QTL in the crossbred of 'Harrington (GRIN-CA, CN 39242)' × 'OUH602 (PI 682043)' at chromosome region 3H, there wild segments in S42IL_111 also showed significantly minor yield.

The average high yield of 'Scarlett' and small coefficient of variation for 'Scarlett' found after WR compared to WW might have boosted QTL detection. In this study, drought stress in 2018 resulted in very few ears per m² highly influencing the harvested grain yield. This effect was more pronounced after WW suggesting that WR as preceding crop was able to compensate for the

unfavorable weather condition in regard to biotic and abiotic factors. This connection was determined by a relatively poor auto-correlation of EAR (r= 0.47) for both preceding crops as well as different correlations of EAR and YLD after WW and WR (r_{WW}= 0.31 and r_{WR}=0.51). The lack of data consistency regarding YLD of previous S42IL studies is caused by its genetic complexity and its dependency on the applied cultivation strategy. Most lines with a significant decrease in YLD were found in the nitrogen-based experiments. Nonetheless, stronger QTL effects for this trait were observed in the S42IL population in the preceding crop trial. These results indicate that a large number of contrasting environments and contrasting management practices are needed to detect significant and meaningful effects for grain yield.

Another important factor that influences plant development and therefore yield is root growth. Naz *et al.* (2014) described an extensive root system for S42IL_176 compared to 'Scarlett' in a greenhouse experiment. In this study, the line was simultaneously found significant in both multi-environment trials indicating to contain a solid and pleiotropic QTL causing effects for SHO, HEA and GRW across nitrogen levels and preceding crops. In accordance with Passioura (1983) and Ayad *et al.* (2010), no beneficial effect of the large root system on grain yield could be observed for S42IL_176 in the present field experiment. On the contrary, this specific line revealed a significantly lower YLD compared to 'Scarlett', which was reduced by -6.4 dt ha^{-1} in nitrogen data and -13.7 dt ha^{-1} in preceding crop data.

Grain parameters and Yield components

Due to the genetic complexity underlying the trait grain yield, acquired results are often not statistically significant. Grain yield is to a lesser extent controlled by genetic main effect than grain components (Kumar *et al.* 2013; Ahmadi *et al.* 2016), which we also observed in the present study. Therefore, few QTL were detected, which, additionally, were environmental sensitive. Grain yield is mainly determined by ears per square meter, grains per ear and thousand grain weight. Therefore, these agronomic determinants were investigated in more detail for a better understanding of the grain yield of S42ILs. Studying traits like TGW, GEA, GRL, GRW and GRA revealed various QTL, both, environmental stable and sensitive. However, these grain parameters are strongly connected with each other (Groh *et al.* 2001; Ayoub *et al.* 2002; Breseghello and Sorrells 2007). In cereals, TGW is characterized by grain size and shape. Again, grain size and shape are controlled largely by GRA. Finally, GRL and GRW determine GRA.

No QTL was found for TGW in S42IL_143 although this line showed the highest increase in GRL compared to 'Scarlett'. A similar performance was detected for S42IL_102 that showed a slightly stronger reduction in GRW and a comparably weaker increase in GRL than S42IL_143, also resulting

in no QTL for TGW. In accordance with Ramya *et al.* (2010) an explanation for these observations might be the compensatory effect for GRL and GRW, which was detected in these lines. Considering the link of GRL, GRW and TGW, the results of this study confirmed similar findings of Backes *et al.* (1995). Correlation for GRL and GRW was negative, while GRW had a higher impact on TGW than GRL.

In the nitrogen trial some important facts were be observed. Positive TGW performance was found in S42IL_119 as well S42IL_121, which contain overlapping introgressions on chromosome 4H between 449.7 Mbp and 582.1 Mbp. In addition, both lines displayed another unique favourable *Hsp* characteristic. Compared to 'Scarlett' an increase in GRL and GRA was detected but no significant decrease in GRW was measured resulting in higher TGW. This could be explained by introgressed wild alleles specifically affecting GRL. Consequently, no compensatory impact of GRL and GRW was detected for S42IL_119 and S42IL_121 but rather an improvement in TGW. In addition, line S42IL_121 carries the exotic allele at the *sdw1/HvGA20ox*$_2$ locus, which is known to positively affect TGW (Maurer *et al.* 2016).

Furthermore, an additional outstanding attribute was found in S42IL_121 for GEA. Although two S42ILs (119 and 121) formed significantly higher TGW only S42IL_121 did not show lower GEA. Unfortunately, similar effects were not found in the preceding crop trial and thus this extraordinary result cannot be explained yet. Additionally, the *Hsp* introgression of S42IL_121 overlapped with other S42IL introgressions, which did not show these effects and in accordance with Kjær and Jensen (1996) a negative correlation between TGW and GEA was found in this study.

In barley, the trait GPC, like YLD, is under polygenic control with relatively low heritability. However, low protein contents are necessary in spring barley to assure high malting qualities. Many QTL on all chromosomes have been mapped with either decreasing or increasing effect on grain protein content (Oziel *et al.* 1996; Bezant *et al.* 1997; Emebiri *et al.* 2005). The trait grain protein content is highly influenced by nitrogen fertilization management and by the rate of nitrogen remobilization, affected by preceding crop biotic effects. Thus, the environment-dependent efficiency of nitrogen processing of the whole plant is of interest. In this study, a strong nitrogen dependence ($p<0.01$), was detected, but no QTL could be identified for GPC, which is most likely due to a low heritability of the trait and the presence of genotype-independent effects. In case of the nitrogen trial, it can be assumed that the environmental impact on this trait was stronger than the genetic background ($h^2 = 0.22$). However, in case of the preceding crop trial, four S42ILs with two protein-increasing QTL on chromosomes 6H and 7H were detected after WW with a maximum increase of 2.0% in S42IL_129 and _133. Likewise, a field study by Schmalenbach *et al.* (2009) revealed six QTL,

which raised GPC, whereby S42IL_129 also showed the highest increase. Comparing different GPC studies of S42IL, a new QTL on chromosome 7H could be revealed in two overlapping lines (S42IL_133 and _134) (von Korff *et al.* 2008; Schmalenbach and Pillen 2009). GPC is reported to be very sensitive regarding environmental changes (Bertholdsson 1999). The highest impact on grain parameters is exerted at the maturation phase. Hence, the supply of water and nutrients is of utmost importance for the formation of grains and their filling with carbon and nitrogen. A reason for these environmental sensitive observations might be the differences in water supply and nutrient uptake efficiency of the individual lines grown after each preceding crop system. Modern barley cultivars are known to form a poorer developed root system than their wild relatives (Naz *et al.* 2014; Arifuzzaman *et al.* 2016; Sayed *et al.* 2017). Since winter wheat as a preceding crop provides poorer soil properties than winter oilseed rape, differences in root growth caused by the S42IL wild alleles might result in a more pronounced effect. In this study, the preceding crop had no significant main effect but a highly significant ($p<0.001$) genotype×preceding crop interaction was observed.
Although YLD and GPC were negatively correlated (r_{WW}= -0.41 and r_{WR}= -0.32), the detected QTL between both traits differ. This offers the opportunity for breeders to introgress desired wild barley alleles to break the negative correlation of YLD and GPC by increasing GPC without YLD loss.

In summary, assessing the impact of nitrogen fertilization on yield and yield components is often difficult because of compensatory effects between grain parameters. Nevertheless, different N fertilizer strategies, especially nitrogen stress, provides specific insights in *Hsp* expression. Additionally, in respect to the preceding crop effects on yield, protein content, thousand grain weight and number of ears on the subsequent crop diverse studies showed a significant relation to the environmental conditions caused by the previous market crop (Zimmermann *et al.* 1984; Papastylianou 1987; Váňová *et al.* 2011; Sieling and Christen 2015; O'Donovan *et al.* 2017). These starting conditions are defined by N replacement, soil structure, soil root penetration, water supply, nutrient availability and disease protection (McEwen *et al.* 1990; Angers *et al.* 1993; Kirkegaard *et al.* 2008). The cited studies showed that biotic and abiotic factors have strong impacts on the regulation of agronomic traits, even on traits with high heritability. We conclude that the preceding crop effect, which often is neglected, should be considered more carefully with regard to phenotypic evaluations in the breeding frame for grain yield and yield components. Many useful QTL mentioned in this study, nitrogen-related as well as preceding crop-related, showed favourable effects for far-reaching breeding approaches. In conclusion, assessing grain components is a promising approach for selecting QTL to improve yield.

Chlorophyll content

Photosynthesis, operated by chlorophyll molecules, is perhaps the most important biological process for the energy supply of plants. As the flag leaf receives the highest amount of light, the carbohydrate production at this location is a main source for grain filling (Yap and Harvey 1972; Tungland *et al.* 1987). Differences in chlorophyll content level leads to varying photosynthetic activities and thereby to differences in carbohydrate production. Diagnostic methods like the handheld SPAD-502 Plus chlorophyll meter provide information about the leaf greenness and consequently, the N content and N supply for grain filling. This device provides a non-destructive technique and is commonly used in sustainable agriculture and as an effective tool for selection of high N genotypes in breeding. High correlation between SPAD and N content in leaves as well as YLD were verified in various studies (Spaner *et al.* 2005; Izsáki and Németh 2007). Although significantly differing between WW and WR, unexpectedly, chlorophyll values of flag leaves were not significantly influenced by the two different nitrogen levels tested ($p = 0.13$). One reason for this result could be that the differences in nitrogen treatment were too small to show an impact on this trait. Significant introgression effects for chlorophyll content in the nitrogen trial were detected on chromosome 1H and 2H. Line S42IL_110 showed significantly higher chlorophyll values as well as significantly faster plant development. In contrast, line S42IL_142 revealed significantly lower chlorophyll values. For the preceding crop trial one QTL associated with chlorophyll content was identified. An *Hsp* allele in S42IL_107 caused a significant increase in chlorophyll content and was also associated with earlier plant growth and SEN as well as higher risk in LOD. As mentioned before, it is noteworthy that an introgression line can carry additional non-target *Hsp* alleles, which can impede the assignment of the QTL to the main target introgression. In case of S42IL_107 and _110 an overlapping second introgression is present on chromosome 2H (603.7 - 611.1 Mbp). Since both lines showed similar significantly higher chlorophyll values, it can be hypothesized that the causative QTL might be located on chromosome 2H.

No genotype×preceding crop or genotype×nitrogen treatment interaction were found for this trait and yet these minor genes were solely detected in N_0 or N_1 or after WR. Showing only small positive effects, environmental sensitivity is assumed for this trait. Nevertheless, the comparison of these results with those of greenhouse trials of Schnaithmann and Pillen (2013) and Honsdorf *et al.* (2014b) revealed three new QTL for SPAD.

4.3 Management interaction effects

Probably the most unique, most important and most far-reaching finding of this study is that the influence of previous crops in connection with other test factors on QTL detection is high. Phenotyping for QTL at field level is extremely complex, because of the interplay of molecular pathways, multiple environment-dependent processes and intra- and interplant competition. Breeding programs are aware that genotypes perform differently in different environments. All quantitative traits are sensitive to contrasting environments and show varying degrees of genotype×environment interactions. This constitutes a major obstacle to the ongoing breeding efforts. By selecting favourable plants, particularly self-pollinated plants with a high differential genotypic sensitivity, two strategies are of interest. On one hand a suitable location can be chosen that offers optimal plant growth and yield conditions or on the other hand plants can be grown under stress inducing conditions. In this regard, a high number of tested environments will allow a stronger genotype adaptation analysis to identify superior and stable genotypes as well as extraordinary outliers for particular environments.

In this trial four cultivation systems were used demonstrating the importance of stress induced nitrogen fertilization with preceding crop variation for QTL detection. In accordance with studies in barley (Dhanagond *et al.* 2019) and rice (Li *et al.* 2003), QTL tend to show varied degrees of responses to different environments. In our studies the most QTL were found by analysing barley phenotype data within low nitrogen levels using both preceding crops, directly followed by the system using two nitrogen levels and solely the preceding crop winter oilseed rape. In these two systems also most environmental sensitive QTL were found. Apparently, the type of cultivation system had a strong influence on identifying special minor wild alleles. The heterogeneity of the response to specific environments and cultural practices complicated the simple use of a major QTL or gene in a wide range of environments for some traits like YLD, GPC and SPAD. Observed low QTL effects or non-identification of QTL probably arise from the lack of a multidimensional environmental variable in the statistical model considering flexible dimensions of QTL×environment characterisation.

Despite recognizing environmental sensitive QTL, the localization of solid wild allele effects, performing well across a wide range of environments, is of particular interest. We identified environmental stable QTL for traits like plant development, height, grain shape, number of grains per ear and senescence. Advantageously, these QTL are active across different environments. Nevertheless, each of these major QTL has a different degree of stability and therefore detectability.

Although solid QTL effects modify traits significantly in the same direction, their expression can be stronger in one environment but weaker in another.

Finally, the next section will focus on novel and unique QTL as well as remarkable non-significant *Hsp* effects.

Comparing published S42ILs studies with our multi-environment trial, one innovation was the use of a nitrogen-preceding crop trial in the field. Furthermore, QTL detection in the three traits SHO, MAT and SEN was an absolute novelty. For these traits, 28 novel QTL could be found. This not only increased the phenotype to genotype transfer but also foster the understanding of growth stage-dependent QTL. The observed correlations of some traits, such as height and lodging or plant growth and senescence, can now be genetically explained and proven by the detection of similar QTL for these characteristics.

Furthermore, 33 unique QTL in already frequently examined plant traits were identified in this study compared to all S42IL publications. They were attributed to 10 traits like SEN (5 novel QTL), HEA (4 novel QTL), HEI (1 novel QTL), YLD (3 novel QTL), TGW (2 novel QTL), GRL (5 novel QTL), GRW (6 novel QTL), GRA (3 novel QTL), GPC (1 novel QTL) and SPAD (3 novel QTL). Some of these QTL must be environmental sensitive and produce very marginal effects, since they appeared only in one multi-environment trial of this experiment. That may be true for the *Hsp* introgressions in S42IL_109, _110, _124, _138, _140, _142, _143 and _148, whose significant effects for YDL, GRW, GRL, GRA and SPAD were unique in the nitrogen treatment. Additionally, S42IL_103, _107, _120, _123 and _135 showed unique effects in the preceding crop trial. The potential use of such weak QTL is probably very low, because of their limited range of application. Nevertheless, breeders' selection of major QTL effects usually is associated with an unintended simultaneously selection of minor effects in this gene region linked to polygenic traits. Therefore, that mere knowledge about such minor environmental effects may help during the selection process.

Due to climate change, the frequency of extreme weather events increased markedly over the last decades and is predicted to increase further and with more extreme events during the coming decades (Meehl *et al.* 2000; Hov *et al.* 2013). Matching these weather forecasts, the growing seasons of the experimental trials were confronted with either heavy rainfall events or drought periods. Since line S42IL_123 displayed a more stable and to some extent even higher grain yield under difficult climate conditions than 'Scarlett', this line might be a valuable choice for breeding programs concerning drought management. Similar favourable behaviour of S42IL_123 were reported in dry down experiments by Honsdorf *et al.* (2014b). Moreover, S42IL_123 showed higher biomass, photosystem-II efficiency, chlorophyll content and tiller number. With regard to increasing extreme weather events, like heat and drought periods or intense rainfall, it will be necessary to rely on genotypes that

are more tolerant. For this purpose, the respective introgression on 4H represents an interesting target.

4.4 Favourable *Hsp* regions

Among many useful QTL detected in this study some QTL alleles appear more favourable to increase productivity and genetic diversity among a wide variety of environmental factors. A list of those *Hsp* QTL alleles is given in Table 15.

Table 15 Selected favourable QTL alleles derived from *Hordeum spontaneum*

Region	Advantages	Lines	Candidate gene
2H | 15.9 – 45.5 Mbp	Fast plant development, high chlorophyll content, short straw	S42IL_107 S42IL_108 S42IL_109	Ppd-H1
2H | 371.6 – 492.8 Mbp	Fast plant development, high chlorophyll content, stable yield under stress condition	S42IL_110	
4H | 449.7 – 582.1 Mbp	Fast plant development, improved grain components	S42IL_119 S42IL_120 S42IL_121 S42IL_123	
6H | 554.5 – 573.2 Mbp	Stable yield under stress condition, high number of ear per m^{-2}, short straw	S42IL_122	
7H | 559.6 – 618.5 Mbp	Fast plant development, improved grain components, stable yield under stress condition	S42IL_137 S42IL_138	

4.5 Future prospects

Switching from greenhouse to field trials and monitor the S42IL under natural cultivation was a fully success. Previous results not only were confirmed but also new tested traits and QTL were provided. However, this effort should be extended in many ways.

On the one hand, further investigation in phenotyping root traits, disease resistance, deficiency symptoms and time-related testing series could bring a more holistic view on QTL researches in spring barley. At this point high-throughput phenotyping has a great potential, managing immense time consuming traits and revealing phenotype-genotype interaction in a non-invasive way. A large variety of computerized phenotyping platforms were developed nowadays like the Field Scanalyzer (Lemnatec), FieldScan (Phenospex) PhenoRob (Jülich), Robotanist (Carnegie Mellon University), Phenoscope (INRA), AgRover (Fraunhofer Institute) etc. as well as commercial use of ERT, MRI, X-ray CT, Drones for plant phenotyping (Virlet *et al.* 2017; Mueller-Sim *et al.* 2017; Sytar *et al.*

2018; Wang *et al.* 2019; Dhanagond *et al.* 2019). These systems would be an excellent opportunity to investigate the S42ILs even more extensive and rapid, because phenotyping will replacing genotyping as the limitation factor (Cobb *et al.* 2013). On the other hand, continued attempts to use multi environmental trials should be combined with before mentioned practices for best prediction of future plant performance.

At level of narrowing down QTL regions, linkage analysis and genome-wide analysis can provide the most power by combining these two techniques, due to their described limitation in chapter 1 (Mammadov *et al.* 2015; Li *et al.* 2016). High resolution mapping, positional cloning, insertional mutagenesis and candidate genes are the main approaches for QTL cloning of strong and useful alleles (Salvi and Tuberosa 2007). Map-based cloning delivers an understanding of the functional basis of traits as well as identified genes and the genetic mechanics of quantitative traits (Salvi and Tuberosa 2007). A high-resolution mapping population (BC_4S_2 HR) already were generated by Schmalenbach *et al.* (2011) and seed have to be further multiplied. Using this HR will be a resource to analyze strong *Hsp* QTL with high precision.

One opportunity to transfer targeted favorable alleles or genes into modern high-performance barley cultivars is marker-assisted backcross breeding (MAB). MAB is a traditionally and routinely used method in introgression breeding programs (Frisch and Melchinger 2005). Accepted as a conventional method all over the world, MAB could be applied in S42IL-HR populations.

Another promising way to insert specific selected segments into modern barley varieties is the fast method of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) genes. Despite its efficiency and simplicity compared with other genome editing techniques, the technical issues and the classification as a genetically modifying technique by Court of Justice of the European Union are currently prevent broad access to the European market.

Not only the exact localization and transfer of genes are of importance, but the accurate nature of involvement of investigated genes or their gene product in the plant characteristics. This can be clarified, for example, with the help of expression studies (transcriptomic).

Expression studies are part of the 'omics'. The interplay of biological sciences that ends with -omics aims to better understand complex regulatory mechanisms in the plant and thus to generate a holistic knowledge. (Vailati-Riboni *et al.* 2017). Precisely, this study was able to show how differently genes were expressed in multi-environments and therefore, a holistic approach on the 'omics' level can be recommended for the S42IL.

5. Summary

In the past decades, researchers elaborately tried to transfer potential loci and genes from crop wild relatives into the elite cultivar background in response to environmental changes. Modern agriculture has to reconcile the demand for increasing efficiency and care of plants to efficiently use resources like water, nutrients, soil and energy. However, the current gene pool of arable crops shows only minimal genetic diversity. One way to overcome this reduced adaptability of elite cultivars to diverse climate conditions and cultivation sites is to gain advantages from including related wild alleles in breeding studies. Using introgression libraries in field trials enables the collection of a wide range of phenotypic information in the context of hands-on requirements while considering multiple cultivation factors. With this multi-environment trial, two field studies using barley introgression lines of the S42IL population under different cultivation regimes were conducted. With the help of the presented results first insights into the complex topic of combining different preceding crops and nitrogen fertilization for QTL analysis were gained.

The field trials in Merbitz and Morgenrot revealed that the fertilizer regime exerted less influence than the preceding crop on both the individual phenotypic assessment and the QTL detection. The data suggests that the specific environmental conditions, studied by testing different preceding crops or nitrogen levels, may lead to stronger trait expression of the introgressed wild genes. More precisely, increased nitrogen stress conditions combined with particular preceding crops may benefit QTL detection in field trials for grain parameters. In contrast, traits such as plant development grain yield and chlorophyll content showed highest phenotypic deviation when cultivated under advantageous conditions (i.e. preceding crop WR with both nitrogen level). This clearly demonstrates how strong environment×QTL interaction intervene with the detection power, especially for environmentally sensitive QTL.

In both studies a total of 94 QTL were described for the 49 lines investigated, with various environmentally solid or sensitive as well as unique QTL. Across all barley chromosome 33 super-solid QTL were detected for plant development, grain yield components and senescence. These QTL alleles are eminently useful in barley breeding programs for non-specific cultivation habitat. In this study 10 management-dependent sensitive QTL where found for yield, height, lodging, grain area, grain protein content and senescence. This finding demonstrate the high potential of using multiple preceding crops combined with nitrogen fertilization regime.

In order to achieving a holistic approach of QTL detection in field trials, four novel traits were assessed. So far S42IL studies did not considered the traits shooting (13 novel QTL), maturity (9 novel QTL), lodging (5 novel QTL) and senescence (7 novel QTL). Furthermore, novel QTL were

identified for already frequently examined traits like heading (4 novel QTL), plant height (1 novel QTL), grain yield (3 novel QTL), thousand grain weight (2 novel QTL), grain length (5 novel QTL), grain width (6 novel QTL), grain area (3 novel QTL), grain protein content (1 novel QTL) and chlorophyll content (3 novel QTL).

Additionally, both multi-environment trials revealed favourable wild barley introgressions on chromosomes 2H, 3H, 4H, 5H and 7H combining a number of favourable characteristics like fast plant development, high chlorophyll content, stable yield under stress conditions and improved grain components.

Evaluating introgression lines in future experimental setups like growing S42ILs after additional preceding crops or under diverse nutrient stress managements may be advisable to extend investigating wild barley allele adaption to environmental conditions.

6. Zusammenfassung

Forscher suchen bereits seit 30 Jahren potentielle Loci von Wildgerstenlinien, um einzelne Gene oder Genabschnitte in Elitesorten zu übertragen und somit auf anstehende Umweltveränderungen zu reagieren. Die moderne Landwirtschaft muss Produktionssteigerung mit einem ressourcenschonenden Einsatz von Wasser, Nährstoffen, Boden und Energie in Einklang bringen. Der gegenwärtige Genpool der Ackerkulturen weist jedoch nur eine eingeschränkte genetische Vielfalt auf. Eine Möglichkeit, die verminderte Anpassungsfähigkeit von Elitesorten an unterschiedliche Klimabedingungen und Anbauorte zu überwinden, besteht darin, durch Introgressionsstudien vorteilhafte Wildallele zu gewinnen. Die Verwendung von Introgressionsbibliotheken in Feldversuchen ermöglicht die Sammlung einer breiten Palette phänotypischer Informationen im Kontext unterschiedlichster Anbaufaktoren und -anforderungen. In dieser Studie wurde ein vierjähriger Praxisversuch durchgeführt, bei dem Gerstenintrogressionslinien der S42IL-Population verwendet und in verschiedenen Anbauregimen kombiniert wurden. Erste Einblicke in die Komplexität der QTL-Analyse im Feld verbunden mit verschiedenen Vorfruchtarten sowie Stickstoffdüngungen konnten mit Hilfe der vorliegenden Ergebnisse gewonnen werden. Hierbei zeigten die Feldversuche in Merbitz und Morgenrot, dass das gewählte Düngungsregime weniger Einfluss auf die individuelle phänotypische Ausprägung und auf den QTL-Nachweis hatte als die Vorfruchtwirkung. Die Ergebnisse deuten darauf hin, dass spezifische Umweltbedingungen durch die verschiedenen Vorfrüchte oder den Stickstoffgehalt geschaffen wurden, welche zu einer stärkeren und teilweise vorteilhaften Merkmalsausprägung der eingekreuzten Wildallele führten. In den durchgeführten Versuchen wurde die Fähigkeit der QTL-Identifikation für Kornparameter begünstigt, wenn die Ausgangslage ein erhöhter Stickstoffstress in Kombination mit beiden Vorfrüchten war. Im Gegensatz dazu zeigten Merkmale wie Pflanzenentwicklung, Kornertrag und Chlorophyllgehalt die höchsten phänotypischen Abweichungen, wenn sie unter vorteilhaften Bedingungen angebaut wurden (Vorfrucht WR mit beiden Stickstoffstufen). Dies zeigt deutlich, wie stark die Umwelt×QTL-Interaktion in Studien eingreifen kann, insbesondere bei umweltsensitiven QTL.

In beiden Teilstudien wurden insgesamt 94 QTL für die 49 untersuchten Linien beschrieben. Über das gesamte Gerstengenom wurden 33 robuste QTL für Pflanzenentwicklung, Kornertragskomponenten und Seneszenz nachgewiesen. Diese könnten hervorragend in Gerstenzuchtprogrammen für unspezifische Kultivierungsräume genutzt werden. In dieser Studie wurden außerdem 10 anbauregimeabhängige QTL für Ertrag, Pflanzenhöhe, Lager, Kornfläche, Kornproteingehalt und Seneszenz gefunden. Die Ergebnisse zeigen ebenfalls das hohe Potenzial der

Verwendung mehrerer Vorfrüchte in Kombination mit verschiedenen Stickstoffstufen für QTL Analysen auf.

Um einen ganzheitlichen Ansatz der QTL-Erkennung auf dem Feld zu erreichen, wurden vier neue Merkmale bewertet. Bisher wurden in S42IL-Studien die Pflanzenmerkmale Schossen (13 neue QTL), Reife (9 neue QTL), Lager (5 neue QTL) und Seneszenz (7 neue QTL) nicht berücksichtigten. Darüber hinaus wurden neue QTL für bereits häufig untersuchte Merkmale wie den folgenden identifiziert: Blüte (4 neue QTL), Pflanzenhöhe (1 neue QTL), Kornertrag (3 neue QTL), Tausendkorngewicht (2 neue QTL), Kornlänge (5 neue QTL), Kornbreite (6 neue QTL), Kornfläche (3 neue QTL), Kornproteingehalt (1 neue QTL) und Chlorophyllgehalt (3 neue QTL).

Darüber hinaus zeigten beide Studien günstige Effekte der Wildgerstenintrogressionen auf den Chromosomen 2H, 3H, 4H, 5H sowie 7H, die eine Vielzahl vorteilhafter Eigenschaften wie schnelle Pflanzenentwicklung, hoher Chlorophyllgehalt, stabiler Ertrag unter Stressbedingungen und verbesserte Kornparameter kombinierten.

Zukünftige Versuchsanordnungen, wie der Anbau von zusätzlichen Vorfrüchten oder von neuen Nährstoffstressvarianten, könnten für weitere Evaluierungsstudien von Introgressionslinien von großem Nutzen sein.

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8. Abbreviations

ANOVA	Analyse of variance
BC	Before Christ
BC_2DH	2. backcross doubled haploid population
°C	Degree Celsius
CRISPR	Clustered regularly interspaced short palindromic repeats
CAS	CRISPR-associated
DNA	Deoxyribonucleic acid
(1-7)H	Barley chromosomes 1-7
IL	Introgression line
Kb	Kilo base
Kg	Kilogramm
Mbp	Mega base pair
N	Nitrogen
NUE	Nitrogen use efficiency
QTL	Quantitative trait locus (loci)
WR	Winter oilseed rape
WW	Winter wheat

9. List of Figures

10. List of Tables

11. Appendix

Appendix eTable 1 – 4 attached on CD (at hardcover)

Appendix eTable 1 SNP marker information of 50k Illumina Infinium iSelect SNP Array for 49 S42ILs
Appendix eTable 2 Raw data of trait experiments with barley S42ILs
Appendix eTable 3 Multi-environment YLD data
Appendix eTable 4 Overview of average YLD in each environment

Appendix Table 1 Nitrogen trial: Results of Dunnett test of significant genotype effects for 11 traits, calculated for each treatment (N_0 or N_1).

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GEA	QGea.S42.2H	2H	S42IL_107	N_1	19.5	-4.00	-17.02	0.000	*PpdH1* *[a]
GEA	QGea.S42.2H	2H	S42IL_107	N_0	19.7	-3.79	-16.21	0.000	*PpdH1* *[a]
GEA	QGea.S42.2H	2H	S42IL_109	N_1	19.5	-4.01	-17.06	0.000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H	2H	S42IL_109	N_0	19.9	-3.51	-14.99	0.000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H	2H	S42IL_110	N_1	19.5	-4.02	-17.13	0.000	*[HvCEN* *[c]*]
GEA	QGea.S42.2H	2H	S42IL_110	N_0	20.5	-2.98	-12.81	0.000	*[HvCEN* *[c]*]
GEA	QGea.S42.3H	3H	S42IL_111	N_0	19.6	-3.88	-16.70	0.000	*HvGI* *[h]; *HvFT2* *[h]
GEA	QGea.S42.3H	3H	S42IL_111	N_1	20.7	-2.79	-11.88	0.000	*HvGI* *[h]; *HvFT2* *[h]
GEA	QGea.S42.4H	4H	S42IL_123	N_1	20.8	-2.70	-11.47	0.001	*HvGI* *[h]; *HvFT2* *[h]
GEA	QGea.S42.4H	4H	S42IL_123	N_0	21.3	-2.18	-9.42	0.043	
GEA	QGea.S42.7H	7H	S42IL_134	N_1	21.4	-2.06	-8.86	0.037	*HvCO1* *[g]
GRA	QGra.S42.2H	2H	S42IL_109	N_0	22.4	1.42	6.74	0.001	*HvFT4* *[b]; *HvCEN* *[c]
GRA	QGra.S42.4H	4H	S42IL_119	N_1	22.5	1.11	5.18	0.030	
GRA	QGra.S42.4H	4H	S42IL_119	N_0	22.3	1.29	6.15	0.004	
GRA	QGra.S42.4H	4H	S42IL_121	N_0	23.2	2.17	10.36	0.000	*[sdw1/HvGA20ox2* *[d]*]
GRA	QGra.S42.7H	7H	S42IL_137	N_0	22.1	1.11	5.24	0.030	*[sdw1/HvGA20ox2* *[d]*]
GRA	QGra.S42.1H	1H	S42IL_143	N_0	22.6	1.70	8.10	0.000	

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GRL	QGrl.S42.1H.a	1H	S42IL_102	N_0	8.7	0.78	8.90	0.000	
GRL	QGrl.S42.1H.a	1H	S42IL_102	N_1	8.80	0.63	7.36	0.000	
GRL	QGrl.S42.1H.a	1H	S42IL_103	N_0	8.3	0.44	5.06	0.027	
GRL	QGrl.S42.2H	2H	S42IL_109	N_0	8.6	0.68	8.85	0.000	*HvFT4* *[b]; *HvCEN* *[c]
GRL	QGrl.S42.4H	4H	S42IL_119	N_0	8.4	0.48	6.32	0.006	
GRL	QGrl.S42.4H	4H	S42IL_121	N_0	8.7	0.81	10.10	0.000	*[sdw1/HvGA20ox2* *[d]]
GRL	QGrl.S42.7H	7H	S42IL_137	N_1	8.66	0.48	5.61	0.005	*[sdw1/HvGA20ox2* *[d]]
GRL	QGrl.S42.7H	7H	S42IL_137	N_0	8.5	0.66	8.33	0.000	*[sdw1/HvGA20ox2* *[d]]
GRL	QGrl.S42.1H.b	1H	S42IL_143	N_1	8.85	0.68	7.96	0.000	
GRL	QGrl.S42.1H.b	1H	S42IL_143	N_0	9.1	1.22	15.24	0.000	
GRL	QGrl.S42.5H	5H	S42IL_176	N_0	8.4	0.50	6.30	0.005	*Vrn-H1* *[e]; *[HvELF3* *[f]]
GRW	QGrw.S42.1H.a	1H	S42IL_102	N_0	3.73	-0.16	-4.06	0.000	
GRW	QGrw.S42.1H.b	1H	S42IL_143	N_0	3.77	-0.12	-3.08	0.007	
GRW	QGrw.S42.2H	2H	S42IL_107	N_1	3.92	0.11	2.85	0.016	*PpdH1* *[a]
GRW	QGrw.S42.4H	4H	S42IL_124	N_0	3.76	-0.12	-3.15	0.009	*Vrn-H2* *[e]
GRW	QGrw.S42.5H	5H	S42IL_176	N_1	3.68	-0.13	-3.73	0.001	*Vrn-H1* *[e]; *[HvELF3* *[f]]
GRW	QGrw.S42.5H	5H	S42IL_176	N_0	3.76	-0.13	-3.38	0.002	*Vrn-H1* *[e]; *[HvELF3* *[f]]
GRW	QGrw.S42.6H	6H	S42IL_148	N_0	3.75	-0.13	-3.43	0.001	*[sdw1/HvGA20ox2* *[d]]
GRW	QGrw.S42.7H.a	7H	S42IL_134	N_0	3.78	-0.10	-2.69	0.035	*HvCO1* *[g]
GRW	QGrw.S42.7H.b	7H	S42IL_135	N_0	3.78	-0.11	-2.87	0.012	
GRW	QGrw.S42.7H.b	7H	S42IL_137	N_0	3.76	-0.12	-3.19	0.003	*[sdw1/HvGA20ox2* *[d]]
HEA	QHea.S42.1H.a	1H	S42IL_102	N_0	72.3	1.88	2.67	0.00	
HEA	QHea.S42.1H.a	1H	S42IL_102	N_1	72.5	1.67	2.35	0.00	
HEA	QHea.S42.1H.a	1H	S42IL_103	N_0	72.1	1.73	2.46	0.00	
HEA	QHea.S42.1H.b	1H	S42IL_105	N_0	66.9	-3.52	-5.00	0.00	
HEA	QHea.S42.1H.b	1H	S42IL_105	N_1	67.0	-3.75	-5.30	0.00	
HEA	QHea.S42.2H	2H	S42IL_107	N_0	61.7	-8.68	-12.33	0.00	*PpdH1* *[a]
HEA	QHea.S42.2H	2H	S42IL_107	N_1	61.8	-9.00	-12.71	0.00	*PpdH1* *[a]
HEA	QHea.S42.2H	2H	S42IL_108	N_1	62.9	-7.92	-11.18	0.00	*PpdH1* *[a] ; HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H	2H	S42IL_108	N_0	62.3	-8.08	-11.48	0.00	*PpdH1* *[a] ; HvFT4* *[b]; *HvCEN* *[c]

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEA	QHea.S42.2H	2H	S42IL_109	N_1	69.1	-1.67	-2.35	0.00	*HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H	2H	S42IL_110	N_1	67.9	-2.92	-4.12	0.00	*[HvCEN* *[c]*]
HEA	QHea.S42.2H	2H	S42IL_110	N_0	67.2	-3.17	-4.50	0.00	*[HvCEN* *[c]*]
HEA	QHea.S42.4H.a	4H	S42IL_121	N_1	67.0	-3.75	-5.30	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.4H.a	4H	S42IL_121	N_0	65.7	-4.69	-6.67	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.4H.b	4H	S42IL_124	N_0	71.9	1.54	2.19	0.03	*Vrn-H2* *[e]
HEA	QHea.S42.5H.a	5H	S42IL_125	N_0	67.7	-2.75	-3.91	0.00	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.5H.a	5H	S42IL_125	N_1	68.0	-2.83	-4.00	0.00	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.7H.a	7H	S42IL_134	N_1	67.2	-3.58	-5.06	0.00	*HvCO1* *[g]
HEA	QHea.S42.7H.a	7H	S42IL_134	N_0	66.3	-4.07	-5.78	0.00	*HvCO1* *[g]
HEA	QHea.S42.7H.b	7H	S42IL_137	N_1	66.8	-4.00	-5.65	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.7H.b	7H	S42IL_137	N_0	66.2	-4.25	-6.04	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.3H	3H	S42IL_140	N_1	66.2	-4.58	-6.47	0.00	*sdw1/ HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_140	N_0	65.8	-4.65	-6.60	0.00	*sdw1/ HvGA20ox2* *[d]
HEA	QHea.S42.1H.c	1H	S42IL_143	N_0	72.2	1.84	2.62	0.00	
HEA	QHea.S42.6H	6H	S42IL_148	N_0	65.7	-4.73	-6.72	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.6H	6H	S42IL_148	N_1	66.0	-4.75	-6.71	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.5H.b	5H	S42IL_176	N_0	73.7	3.33	4.74	0.00	*Vrn-H1* *[e]; *[HvELF3* *[f]*]
HEA	QHea.S42.5H.b	5H	S42IL_176	N_1	74.1	3.33	4.71	0.00	*Vrn-H1* *[e]; *[HvELF3* *[f]*]
HEI	QHei.S42.1H	1H	S42IL_105	N_1	74.8	9.50	14.55	0.00	
HEI	QHei.S42.1H	1H	S42IL_105	N_0	78.3	14.47	22.68	0.00	
HEI	QHei.S42.2H	2H	S42IL_107	N_1	57.9	-7.42	-11.36	0.00	*PpdH1* *[a]
HEI	QHei.S42.2H	2H	S42IL_107	N_0	57.0	-6.82	-10.68	0.01	*PpdH1* *[a]
HEI	QHei.S42.2H	2H	S42IL_109	N_1	55.8	-9.50	-14.55	0.00	*HvFT4* *[b]; *HvCEN* *[c]
HEI	QHei.S42.3H	3H	S42IL_140	N_1	82.7	17.42	26.67	0.00	*sdw1/ HvGA20ox2* *[d]
HEI	QHei.S42.3H	3H	S42IL_140	N_0	82.6	18.81	29.48	0.00	*sdw1/ HvGA20ox2* *[d]
HEI	QHei.S42.4H.a	4H	S42IL_121	N_1	77.8	12.50	19.14	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEI	QHei.S42.4H.a	4H	S42IL_121	N_0	80.6	16.82	26.36	0.00	*[sdw1/HvGA20ox2* *[d]*]
HEI	QHei.S42.4H.b	4H	S42IL_124	N_0	54.9	-8.94	-14.02	0.00	*Vrn-H2* *[e]; *Bmy1* *[i]
HEI	QHei.S42.4H.b	4H	S42IL_124	N_1	57.5	-7.78	-11.91	0.00	*Vrn-H2* *[e]; *Bmy1* *[i]

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEI	QHei.S42.5H	5H	S42IL_125	N_1	71.7	6.42	9.83	0.01	*Dhn1/Dhn2* *[j]
HEI	QHei.S42.5H	5H	S42IL_125	N_0	73.5	9.67	15.15	0.00	*Dhn1/Dhn2* *[j]
HEI	QHei.S42.6H.a	6H	S42IL_148	N_1	78.4	13.08	20.04	0.00	*[sdw1/HvGA20ox2* *[d]]
HEI	QHei.S42.6H.a	6H	S42IL_148	N_0	80.2	16.44	25.77	0.00	*[sdw1/HvGA20ox2* *[d]]
HEI	QHei.S42.6H.b	6H	S42IL_122	N_0	56.9	-6.92	-10.84	0.00	
HEI	QHei.S42.7H.a	7H	S42IL_134	N_1	75.7	10.42	15.95	0.00	*HvCO1* *[g]
HEI	QHei.S42.7H.a	7H	S42IL_134	N_0	75.0	11.23	17.60	0.00	*HvCO1* *[g]
HEI	QHei.S42.7H.b	7H	S42IL_137	N_1	79.2	13.92	21.31	0.00	*[sdw1/HvGA20ox2* *[d]]
HEI	QHei.S42.7H.b	7H	S42IL_137	N_0	81.6	17.75	27.82	0.00	*[sdw1/HvGA20ox2* *[d]]
HEI	QHei.S42.7H.c	7H	S42IL_138	N_0	57.5	-6.33	-9.93	0.02	*HvFT3* *[b]
HEI	QHei.S42.7H.c	7H	S42IL_138	N_1	59.3	-6.00	-9.19	0.03	*HvFT3* *[b]
MAT	QMat.S42.1H	1H	S42IL_105	N_1	102.17	-2.08	-2.00	0.01	
MAT	QMat.S42.1H	1H	S42IL_105	N_0	101.73	-2.10	-2.03	0.01	
MAT	QMat.S42.2H	2H	S42IL_107	N_1	100.83	-3.42	-3.28	0.00	*PpdH1* *[a]
MAT	QMat.S42.2H	2H	S42IL_107	N_0	100.24	-3.60	-3.46	0.00	*PpdH1* *[a]
MAT	QMat.S42.4H	4H	S42IL_121	N_0	101.94	-1.89	-1.82	0.04	*[sdw1/HvGA20ox2* *[d]]
MAT	QMat.S42.4H	4H	S42IL_121	N_1	101.75	-2.50	-2.40	0.00	*[sdw1/HvGA20ox2* *[d]]
MAT	QMat.S42.7H.a	7H	S42IL_134	N_0	101.61	-2.22	-2.14	0.00	*HvCO1* *[g]
MAT	QMat.S42.7H.b	7H	S42IL_137	N_1	102.17	-2.08	-2.00	0.01	*[sdw1/HvGA20ox2* *[d]]
MAT	QMat.S42.7H.b	7H	S42IL_137	N_0	101.08	-2.75	-2.65	0.00	*[sdw1/HvGA20ox2* *[d]]
MAT	QMat.S42.3H	3H	S42IL_140	N_1	101.92	-2.33	-2.24	0.00	*sdw1/ HvGA20ox2* *[d]
MAT	QMat.S42.3H	3H	S42IL_140	N_0	100.92	-2.92	-2.81	0.00	*sdw1/ HvGA20ox2* *[d]
MAT	QMat.S42.6H	6H	S42IL_148	N_1	101.75	-2.50	-2.40	0.00	*[sdw1/HvGA20ox2* *[d]]
MAT	QMat.S42.6H	6H	S42IL_148	N_0	100.98	-2.85	-2.75	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.1H.a	1H	S42IL_105	N_1	47.55	-6.75	-12.43	0.00	
SHO	QSho.S42.1H.a	1H	S42IL_105	N_0	46.66	-8.14	-14.86	0.00	
SHO	QSho.S42.2H.a	2H	S42IL_106	N_0	51.95	-2.85	-5.19	0.00	
SHO	QSho.S42.2H.a	2H	S42IL_107	N_1	48.5	-5.83	-10.74	0.00	*PpdH1* *[a]
SHO	QSho.S42.2H.a	2H	S42IL_107	N_0	48.1	-6.72	-12.26	0.00	*PpdH1* *[a]
SHO	QSho.S42.2H.a	2H	S42IL_108	N_1	50.2	-4.08	-7.52	0.00	*PpdH1* *[a] ; HvFT4* *[b]; *HvCEN* *[c]

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
SHO	QSho.S42.2H.a	2H	S42IL_108	N_0	49.6	-5.25	-9.58	0.00	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
SHO	QSho.S42.2H.b	2H	S42IL_110	N_0	52.3	-2.50	-4.56	0.01	*[HvCEN* *[c]]
SHO	QSho.S42.3H	3H	S42IL_115	N_0	52.3	-2.50	-4.56	0.01	
SHO	QSho.S42.4H.a	4H	S42IL_121	N_1	47.9	-6.42	-11.82	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.4H.a	4H	S42IL_121	N_0	46.2	-8.64	-15.76	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.4H.b	4H	S42IL_124	N_1	56.6	2.27	4.18	0.04	*Vrn-H2* *[e]; *Bmy1* *[i]
SHO	QSho.S42.5H.a	5H	S42IL_125	N_0	49.6	-5.17	-9.43	0.00	*Dhn1/Dhn2* *[j]
SHO	QSho.S42.5H.a	5H	S42IL_125	N_1	49.1	-5.17	-9.52	0.00	*Dhn1/Dhn2* *[j]
SHO	QSho.S42.7H.a	7H	S42IL_134	N_1	48.7	-5.58	-10.28	0.00	*HvCO1* *[g]
SHO	QSho.S42.7H.a	7H	S42IL_134	N_0	48.4	-6.43	-11.74	0.00	*HvCO1* *[g]
SHO	QSho.S42.7H.b	7H	S42IL_135	N_1	52.0	-2.25	-4.14	0.04	
SHO	QSho.S42.7H.b	7H	S42IL_135	N_0	52.0	-2.83	-5.17	0.00	
SHO	QSho.S42.7H.b	7H	S42IL_137	N_1	46.0	-8.25	-15.19	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.7H.b	7H	S42IL_137	N_0	45.6	-9.25	-16.88	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.3H	3H	S42IL_140	N_1	44.7	-9.58	-17.65	0.00	*sdw1/ HvGA20ox2* *[d]
SHO	QSho.S42.3H	3H	S42IL_140	N_0	45.1	-9.73	-17.76	0.00	*sdw1/ HvGA20ox2* *[d]
SHO	QSho.S42.1H.b	1H	S42IL_143	N_0	58.2	3.44	6.28	0.00	
SHO	QSho.S42.1H.b	1H	S42IL_143	N_1	57.4	3.08	5.68	0.00	
SHO	QSho.S42.6H	6H	S42IL_148	N_0	45.6	-9.19	-16.77	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.6H	6H	S42IL_148	N_1	45.0	-9.25	-17.03	0.00	*[sdw1/HvGA20ox2* *[d]]
SHO	QSho.S42.5H.b	5H	S42IL_176	N_1	57.5	3.17	5.83	0.00	*Vrn-H1* *[e]; *[HvELF3* *[f]]
SHO	QSho.S42.5H.b	5H	S42IL_176	N_0	57.1	2.28	4.16	0.04	*Vrn-H1* *[e]; *[HvELF3* *[f]]
SPAD	QSpad.S42.2H	2H	S42IL_110	N_1	53.5	7.53	16.34	0.000	*[HvCEN* *[c]]
SPAD	QSpad.S42.1H	1H	S42IL_142	N_0	41.2	-5.27	-11.34	0.043	
TGW	QTgw.S42.4H.a	4H	S42IL_119	N_0	51.0	2.89	6.01	0.038	
TGW	QTgw.S42.4H.a	4H	S42IL_119	N_1	50.8	3.45	7.29	0.004	
TGW	QTgw.S42.4H.a	4H	S42IL_121	N_0	52.6	4.54	9.44	0.000	*[sdw1/HvGA20ox2* *[d]]
TGW	QTgw.S42.4H.b	4H	S42IL_124	N_0	44.6	-3.59	-7.46	0.006	*Vrn-H2* *[e]; *Bmy1* *[i]
TGW	QTgw.S42.4H.b	4H	S42IL_124	N_1	44.0	-3.31	-6.99	0.011	*Vrn-H2* *[e]; *Bmy1* *[i]
TGW	QTgw.S42.5H	5H	S42IL_176	N_1	44.1	-3.17	-6.69	0.013	*Vrn-H1* *[e]; *[HvELF3* *[f]]

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
TGW	QTgw.S42.5H	5H	S42IL_176	N_0	45.2	-2.91	-6.06	0.046	*Vrn-H1* *[e]; *[HvELF3* *[f]]
TGW	QTgw.S42.6H	6H	S42IL_128	N_1	44.4	-2.93	-6.18	0.033	
YLD	QYld.S42.1H	1H	S42IL_143	N_1	56.9	-6.58	-10.36	0.022	
YLD	QYld.S42.3H	3H	S42IL_140	N_0	54.6	-6.39	-10.47	0.044	*sdw1/ HvGA20ox2* *[d]
YLD	QYld.S42.4H	4H	S42IL_121	N_0	53.9	-7.07	-11.60	0.014	*[sdw1/HvGA20ox2* *[d]]
YLD	QYld.S42.4H	4H	S42IL_121	N_1	56.4	-7.14	-11.25	0.008	*[sdw1/HvGA20ox2* *[d]]
YLD	QYld.S42.5H	5H	S42IL_176	N_1	57.1	-6.43	-10.12	0.028	*Vrn-H1* *[e]; *[HvELF3* *[f]]
YLD	QYld.S42.6H	6H	S42IL_148	N_1	55.2	-8.34	-13.13	0.001	*[sdw1/HvGA20ox2* *[d]]
YLD	QYld.S42.7H	7H	S42IL_134	N_0	54.5	-6.47	-10.60	0.039	*HvCO1* *[g]
YLD	QYld.S42.7H	7H	S42IL_134	N_1	56.8	-6.73	-10.60	0.017	*HvCO1* *[g]

[a] Abbreviation of traits, see Table 3, [b] Chromosomal location, [c] Least squares means, calculated for the indicated nitrogen level (N_0 and N_1), [d] Difference = LSMEANS [IL] - LSMEANS [Scarlett], [e] Relative performance, [f] fdr corrected Dunnett test probability of difference between S42IL and control 'Scarlett', *[a] Turner *et al.* (2005), *[b] Faure *et al.* (2007), *[c] Comadran *et al.* (2012), *[d] Jia *et al.* (2015b), *[e] Yan *et al.* (2004), *[f] Faure *et al.* (2012); Zakhrabekova *et al.* (2012), *[g] Griffiths *et al.* (2003), *[h] Wang *et al.* (2010a), *[i] Clark *et al.* (2003). *[j] Choi *et al.* (2000), [ ] Candidate genes on sub-introgression

Appendix Table 2 Preceding crop trial: Results of Dunnett test of significant genotype effects for 14 traits, calculated for each preceding crop (winter wheat or winter rape seed).

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans[c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GEA	QGea.S42.2H.a	2H	S42IL_107	WR	19.85	-4.28	-17.73	0.0000	*PpdH1* *[a]
GEA	QGea.S42.2H.a	2H	S42IL_109	WR	20.23	-3.90	-16.18	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H.a	2H	S42IL_107	WW	18.80	-3.86	-17.03	0.0000	*PpdH1* *[a]
GEA	QGea.S42.2H.a	2H	S42IL_109	WW	18.90	-3.75	-16.55	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H.b	2H	S42IL_110	WR	20.66	-3.47	-14.40	0.0000	*[HvCEN* *[c]]
GEA	QGea.S42.2H.b	2H	S42IL_110	WW	19.30	-3.35	-14.81	0.0000	*[HvCEN* *[c]]
GEA	QGea.S42.3H	3H	S42IL_111	WR	21.03	-3.10	-12.86	0.0000	
GPC	QGpc.S42.6H	6H	S42IL_130	WW	16.06	1.58	10.94	0.0001	

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans[c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GPC	QGpc.S42.6H	6H	S42IL_129	WW	16.51	2.03	14.05	0.0000	*HvNAM-1* *[l]
GPC	QGpc.S42.7H	7H	S42IL_134	WW	16.06	1.58	10.94	0.0001	*HvCO1* *[g]
GPC	QGpc.S42.7H	7H	S42IL_133	WW	16.50	2.03	13.99	0.0000	*VRN-H3* *[h]
GRA	QGra.S42.1H	1H	S42IL_143	WR	21.21	1.38	6.98	0.0009	
GRA	QGra.S42.1H	1H	S42IL_143	WW	21.14	1.44	7.31	0.0004	
GRA	QGra.S42.4H	4H	S42IL_121	WR	21.62	1.79	9.04	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRA	QGra.S42.4H	4H	S42IL_121	WW	21.70	2.00	10.15	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRA	QGra.S42.7H	7H	S42IL_137	WW	21.25	1.55	7.87	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRL	QGrl.S42.1H.a	1H	S42IL_102	WR	8.18	0.80	10.85	0.0000	
GRL	QGrl.S42.1H.a	1H	S42IL_102	WW	8.32	0.77	10.15	0.0000	
GRL	QGrl.S42.1H.a	1H	S42IL_103	WR	7.90	0.53	7.18	0.0007	
GRL	QGrl.S42.1H.b	1H	S42IL_143	WR	8.40	1.02	13.90	0.0000	
GRL	QGrl.S42.1H.b	1H	S42IL_143	WW	8.51	0.96	12.66	0.0000	
GRL	QGrl.S42.2H	2H	S42IL_109	WW	8.17	0.62	8.17	0.0000	*HvFT4 *[b]; HvCEN *[c]*
GRL	QGrl.S42.4H.a	4H	S42IL_121	WW	8.21	0.66	8.77	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRL	QGrl.S42.4H.a	4H	S42IL_121	WR	7.97	0.60	8.08	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRL	QGrl.S42.4H.b	4H	S42IL_123	WR	7.92	0.54	7.34	0.0004	
GRL	QGrl.S42.5H	5H	S42IL_176	WR	8.21	0.84	11.36	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
GRL	QGrl.S42.7H	7H	S42IL_137	WW	8.22	0.68	8.94	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRL	QGrl.S42.7H	7H	S42IL_137	WR	8.02	0.64	8.70	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
GRL	QGrl.S42.7H	7H	S42IL_135	WR	7.91	0.53	7.23	0.0006	
GRW	QGrw.S42.1H	1H	S42IL_102	WR	3.69	-0.13	-3.49	0.0003	
GRW	QGrw.S42.5H	5H	S42IL_176	WW	3.57	-0.16	-4.30	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
GRW	QGrw.S42.5H	5H	S42IL_176	WR	3.67	-0.15	-4.03	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
HEA	QHea.S42.1H	1H	S42IL_105	WR	61.63	-4.17	-6.33	0.0000	
HEA	QHea.S42.1H	1H	S42IL_105	WW	62.29	-3.46	-5.26	0.0000	
HEA	QHea.S42.2H.a	2H	S42IL_107	WR	56.50	-9.29	-14.12	0.0000	*PpdH1 *[a]*
HEA	QHea.S42.2H.a	2H	S42IL_107	WW	57.13	-8.63	-13.12	0.0000	*PpdH1 *[a]*
HEA	QHea.S42.2H.a	2H	S42IL_108	WR	57.67	-8.12	-12.35	0.0000	*PpdH1 *[a] ; HvFT4 *[b]; HvCEN *[c]*

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans[c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEA	QHea.S42.2H.a	2H	S42IL_108	WW	58.63	-7.13	-10.84	0.0000	*PpdH1* *[a] ; *HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.a	2H	S42IL_109	WR	63.50	-2.29	-3.48	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.b	2H	S42IL_110	WR	61.63	-4.17	-6.33	0.0000	*[HvCEN* *[c]*]
HEA	QHea.S42.2H.b	2H	S42IL_110	WW	62.21	-3.54	-5.39	0.0000	*[HvCEN* *[c]*]
HEA	QHea.S42.3H	3H	S42IL_113	WR	62.54	-3.25	-4.94	0.0000	
HEA	QHea.S42.3H	3H	S42IL_113	WW	63.54	-2.21	-3.36	0.0000	
HEA	QHea.S42.3H	3H	S42IL_114	WR	60.72	-5.07	-7.70	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_114	WW	61.66	-4.09	-6.23	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_140	WR	60.88	-4.92	-7.47	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_140	WW	61.42	-4.33	-6.59	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.4H.a	4H	S42IL_120	WR	62.71	-3.08	-4.69	0.0000	
HEA	QHea.S42.4H.a	4H	S42IL_120	WW	63.33	-2.42	-3.68	0.0000	
HEA	QHea.S42.4H.a	4H	S42IL_121	WR	60.92	-4.87	-7.41	0.0000	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.4H.a	4H	S42IL_121	WW	61.83	-3.92	-5.96	0.0000	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.4H.b	4H	S42IL_124	WW	67.83	2.08	3.17	0.0001	*Vrn-H2* *[e]; *Bmy1* *[i]
HEA	QHea.S42.5H.a	5H	S42IL_125	WR	62.83	-2.96	-4.50	0.0000	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.5H.a	5H	S42IL_125	WW	63.63	-2.13	-3.23	0.0001	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.5H.b	5H	S42IL_176	WR	69.33	3.54	5.38	0.0000	*Vrn-H1* *[e]; *[HvELF3* *[f]*]
HEA	QHea.S42.5H.b	5H	S42IL_176	WW	69.95	4.20	6.39	0.0000	*Vrn-H1* *[e]; *[HvELF3* *[f]*]
HEA	QHea.S42.6H	6H	S42IL_148	WR	60.63	-5.17	-7.85	0.0000	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.6H	6H	S42IL_148	WW	61.08	-4.67	-7.10	0.0000	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.7H.a	7H	S42IL_134	WR	61.67	-4.12	-6.27	0.0000	*HvCO1* *[g]
HEA	QHea.S42.7H.a	7H	S42IL_134	WW	62.29	-3.46	-5.26	0.0000	*HvCO1* *[g]
HEA	QHea.S42.7H.b	7H	S42IL_137	WR	61.46	-4.33	-6.59	0.0000	*[sdw1/HvGA20ox2* *[d]*]
HEA	QHea.S42.7H.b	7H	S42IL_137	WW	61.58	-4.17	-6.34	0.0000	*[sdw1/HvGA20ox2* *[d]*]
HEI	QHei.S42.1H	1H	S42IL_105	WR	74.13	8.83	13.53	0.0000	
HEI	QHei.S42.1H	1H	S42IL_105	WW	70.42	9.83	16.23	0.0000	
HEI	QHei.S42.2H	2H	S42IL_109	WW	53.79	-6.79	-11.21	0.0003	*HvFT4* *[b]; *HvCEN* *[c]
HEI	QHei.S42.3H	3H	S42IL_114	WW	70.41	9.83	16.23	0.0000	*sdw1/HvGA20ox2* *[d]
HEI	QHei.S42.3H	3H	S42IL_114	WR	75.76	10.47	16.04	0.0000	*sdw1/HvGA20ox2* *[d]

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans[c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEI	QHei.S42.3H	3H	S42IL_140	WW	71.50	10.92	18.02	0.0000	*sdw1/HvGA20ox2* *[d]
HEI	QHei.S42.3H	3H	S42IL_140	WR	76.63	11.33	17.36	0.0000	*sdw1/HvGA20ox2* *[d]
HEI	QHei.S42.4H.a	4H	S42IL_121	WW	70.12	9.54	15.75	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.4H.a	4H	S42IL_121	WR	76.29	11.00	16.85	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.4H.b	4H	S42IL_124	WW	53.50	-7.08	-11.69	0.0001	*Vrn-H2 *[e]; Bmy1 *[i]*
HEI	QHei.S42.5H	5H	S42IL_125	WR	71.88	6.58	10.08	0.0006	*Dhn1/Dhn2 *[j]*
HEI	QHei.S42.5H	5H	S42IL_125	WW	68.42	7.83	12.93	0.0000	*Dhn1/Dhn2 *[j]*
HEI	QHei.S42.6H	6H	S42IL_148	WW	70.62	10.04	16.57	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.6H	6H	S42IL_148	WR	75.46	10.17	15.57	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.7H.a	7H	S42IL_134	WR	73.92	8.62	13.21	0.0000	*HvCO1 *[g]*
HEI	QHei.S42.7H.a	7H	S42IL_134	WW	69.79	9.21	15.20	0.0000	*HvCO1 *[g]*
HEI	QHei.S42.7H.b	7H	S42IL_137	WW	71.46	10.88	17.95	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.7H.b	7H	S42IL_137	WR	79.17	13.87	21.25	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.2H	2H	S42IL_107	WR	5.50	2.17	65.00	0.0000	*PpdH1 *[a]*
LOD	QLod.S42.3H	3H	S42IL_140	WW	4.75	1.92	67.65	0.0002	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.3H	3H	S42IL_114	WR	5.35	2.01	60.42	0.0005	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.3H	3H	S42IL_140	WR	5.42	2.08	62.50	0.0000	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.4H	4H	S42IL_121	WR	5.17	1.83	55.00	0.0007	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.6H	6H	S42IL_148	WR	5.67	2.33	70.00	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.6H	6H	S42IL_148	WW	5.17	2.33	82.35	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.7H	7H	S42IL_137	WW	5.08	2.25	79.41	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.7H	7H	S42IL_137	WR	5.67	2.33	70.00	0.0000	*[sdw1/HvGA20ox2 *[d]]*
MAT	QMat.S42.2H	2H	S42IL_107	WW	94.00	-4.46	-4.53	0.0000	*PpdH1 *[a]*
MAT	QMat.S42.2H	2H	S42IL_107	WR	96.17	-4.33	-4.31	0.0000	*PpdH1 *[a]*
MAT	QMat.S42.3H	3H	S42IL_140	WW	95.88	-2.58	-2.62	0.0001	*sdw1/HvGA20ox2* *[d]
MAT	QMat.S42.3H	3H	S42IL_140	WR	98.12	-2.37	-2.36	0.0008	*sdw1/HvGA20ox2* *[d]
MAT	QMat.S42.6H	6H	S42IL_148	WR	97.79	-2.71	-2.69	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.2H	2H	S42IL_107	WR	9.54	3.21	50.66	0.0000	*PpdH1 *[a]*
SEN	QSen.S42.3H	3H	S42IL_113	WR	8.38	2.04	32.24	0.0002	
SEN	QSen.S42.3H	3H	S42IL_140	WW	9.83	2.04	26.20	0.0002	*sdw1/HvGA20ox2 *[d]*

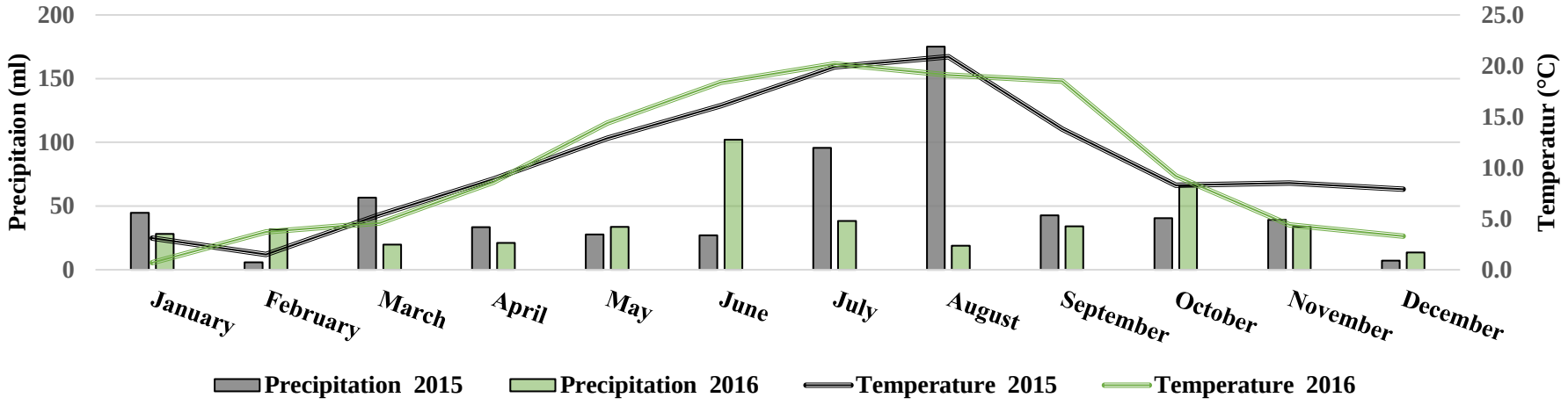
Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans[c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
SEN	QSen.S42.3H	3H	S42IL_114	WR	9.29	2.95	46.61	0.0000	*sdw1/HvGA20ox2* *[d]
SEN	QSen.S42.3H	3H	S42IL_140	WR	9.38	3.04	48.03	0.0000	*sdw1/HvGA20ox2* *[d]
SEN	QSen.S42.4H	4H	S42IL_121	WR	8.58	2.25	35.53	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.5H	5H	S42IL_176	WW	4.96	-2.83	-36.36	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SEN	QSen.S42.5H	5H	S42IL_176	WR	4.21	-2.13	-33.55	0.0001	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SEN	QSen.S42.6H	6H	S42IL_148	WR	8.88	2.54	40.13	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.7H.a	7H	S42IL_134	WR	8.33	2.00	31.58	0.0003	*HvCO1 *[g]*
SEN	QSen.S42.7H.b	7H	S42IL_137	WR	8.63	2.29	36.18	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SHO	QSho.S42.1H.a	1H	S42IL_105	WW	44.83	-5.54	-11.00	0.0000	
SHO	QSho.S42.1H.a	1H	S42IL_105	WR	44.38	-4.92	-9.97	0.0000	
SHO	QSho.S42.1H.b	1H	S42IL_143	WW	52.75	2.37	4.71	0.0001	
SHO	QSho.S42.1H.b	1H	S42IL_143	WR	53.42	4.12	8.37	0.0000	
SHO	QSho.S42.2H	2H	S42IL_107	WW	45.00	-5.38	-10.67	0.0000	*PpdH1 *[a]*
SHO	QSho.S42.2H	2H	S42IL_107	WR	45.25	-4.04	-8.20	0.0000	*PpdH1 *[a]*
SHO	QSho.S42.2H	2H	S42IL_108	WW	47.04	-3.33	-6.62	0.0000	*PpdH1 *[a]; HvFT4 *[b]; HvCEN *[c]*
SHO	QSho.S42.2H	2H	S42IL_108	WR	46.63	-2.67	-5.41	0.0000	*PpdH1 *[a]; HvFT4 *[b]; HvCEN *[c]*
SHO	QSho.S42.3H	3H	S42IL_140	WW	42.13	-8.25	-16.38	0.0000	*sdw1/HvGA20ox2 *[d]*
SHO	QSho.S42.3H	3H	S42IL_140	WR	42.17	-7.13	-14.45	0.0000	*sdw1/HvGA20ox2 *[d]*
SHO	QSho.S42.3H	3H	S42IL_114	WW	44.57	-5.80	-11.51	0.0000	*sdw1/HvGA20ox2 *[d]*
SHO	QSho.S42.3H	3H	S42IL_114	WR	44.47	-4.82	-9.78	0.0000	*sdw1/HvGA20ox2 *[d]*
SHO	QSho.S42.3H	3H	S42IL_113	WW	46.79	-3.58	-7.11	0.0000	
SHO	QSho.S42.3H	3H	S42IL_113	WR	46.29	-3.00	-6.09	0.0000	
SHO	QSho.S42.3H	3H	S42IL_115	WW	47.92	-2.46	-4.88	0.0000	
SHO	QSho.S42.4H.a	4H	S42IL_121	WW	44.50	-5.88	-11.66	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SHO	QSho.S42.4H.a	4H	S42IL_121	WR	44.42	-4.88	-9.89	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SHO	QSho.S42.4H.a	4H	S42IL_120	WW	47.50	-2.88	-5.71	0.0000	
SHO	QSho.S42.4H.a	4H	S42IL_120	WR	46.67	-2.63	-5.33	0.0000	
SHO	QSho.S42.4H.b	4H	S42IL_124	WW	52.63	2.25	4.47	0.0003	*Vrn-H2 *[e]; Bmy1 *[i]*
SHO	QSho.S42.4H.b	4H	S42IL_124	WR	52.38	3.08	6.26	0.0000	*Vrn-H2 *[e]; Bmy1 *[i]*
SHO	QSho.S42.5H.a	5H	S42IL_125	WW	46.50	-3.88	-7.69	0.0000	

Trait [a]	QTL in S42IL	Chr [b]	Line	Effect	LSMeans[c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
SHO	QSho.S42.5H.a	5H	S42IL_125	WR	46.63	-2.67	-5.41	0.0000	
SHO	QSho.S42.5H.b	5H	S42IL_176	WW	52.99	2.61	5.19	0.0000	*Vrn-H1* *[e]; [*HvELF3* *[f]]
SHO	QSho.S42.5H.b	5H	S42IL_176	WR	52.33	3.04	6.17	0.0000	*Vrn-H1* *[e]; [*HvELF3* *[f]]
SHO	QSho.S42.6H	6H	S42IL_148	WW	42.04	-8.33	-16.54	0.0000	[*sdw1/HvGA20ox*$_2$ *[d]]
SHO	QSho.S42.6H	6H	S42IL_148	WR	42.96	-6.33	-12.85	0.0000	[*sdw1/HvGA20ox*$_2$ *[d]]
SHO	QSho.S42.7H.a	7H	S42IL_134	WW	45.67	-4.71	-9.35	0.0000	*HvCO1* *[g]
SHO	QSho.S42.7H.a	7H	S42IL_134	WR	45.17	-4.13	-8.37	0.0000	*HvCO1* *[g]
SHO	QSho.S42.7H.b	7H	S42IL_137	WW	42.92	-7.46	-14.81	0.0000	[*sdw1/HvGA20ox*$_2$ *[d]]
SHO	QSho.S42.7H.b	7H	S42IL_137	WR	42.46	-6.83	-13.86	0.0000	[*sdw1/HvGA20ox*$_2$ *[d]]
SHO	QSho.S42.7H.c	7H	S42IL_138	WR	52.13	2.83	5.75	0.0000	*HvFT3* *[b]
SPAD	QSpad.S42.2H	2H	S42IL_107	WR	55.40	4.83	9.55	0.0000	*PpdH1* *[a]
TGW	QTgw.S42.4H	4H	S42IL_121	WW	48.61	4.32	9.75	0.0000	[*sdw1/HvGA20ox*$_2$ *[d]]
YLD	QYld.S42.1H	1H	S42IL_103	WR	67.37	-11.46	-14.54	0.0000	
YLD	QYld.S42.3H	3H	S42IL_111	WR	60.66	-18.17	-23.05	0.0000	*HvGI* *[h]; *HvFT2* *[h]
YLD	QYld.S42.5H	5H	S42IL_176	WR	65.17	-13.66	-17.32	0.0000	*Vrn-H1* *[e]; [*HvELF3* *[f]]

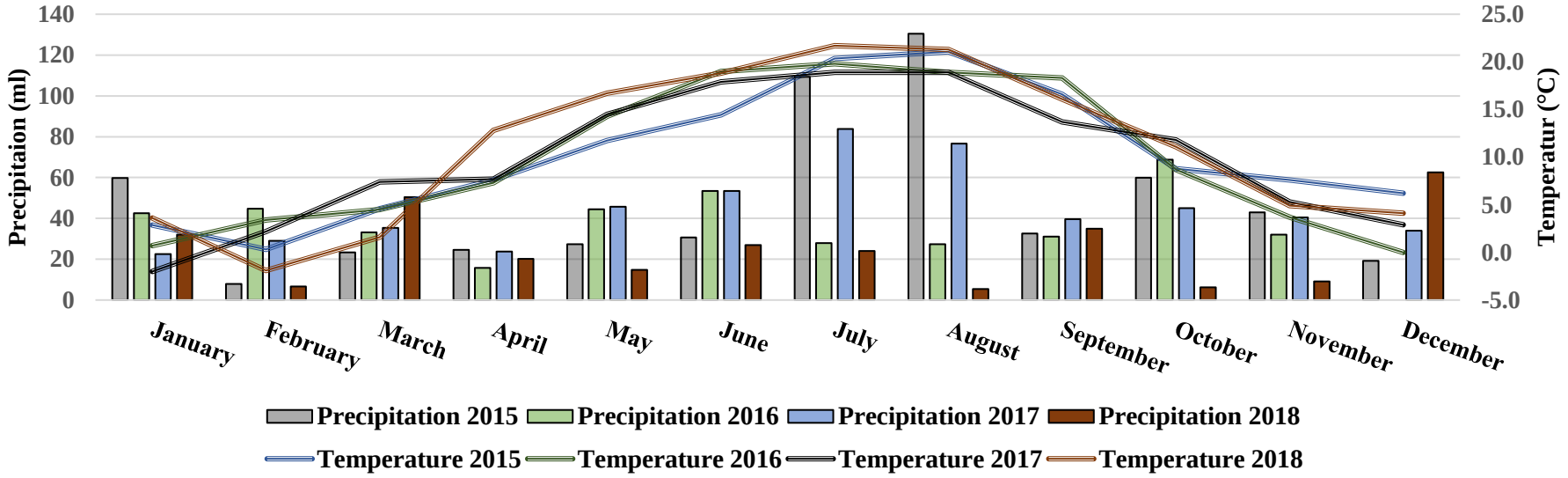
[a] Abbreviation of traits, see Table 3, [b] Chromosomal location, [c] Least squares means, calculated for the indicated preceding crop winter wheat (WW) and winter oilseed rape (WR), [d] Difference = LSMEANS [IL] - LSMEANS [Scarlett], [e] Relative performance, [f] fdr corrected Dunnett test probability of difference between S42IL and control 'Scarlett', *[a] Turner *et al.* 2005, *[b] Faure *et al.* (2007), *[c] Comadran *et al.* (2012), *[d] Jia *et al.* (2015b), *[e] Yan *et al.* (2004), *[f] Faure *et al.* (2012); Zakhrabekova *et al.* (2012), *[g] Griffiths *et al.* (2003), *[h] Wang *et al.* (2010a), *[i] Clark *et al.* (2003). *[j] Choi *et al.* (2000), *[l] Distelfeld *et al.* (2008), [ ] Candidate genes on sub-introgression

Appendix Table 3 Average monthly climate data in A) Morgenrot and B) Merbitz

A) Morgenrot



B) Merbitz



Appendix Table 4 Multi-environmental trial: Results of Dunnett test of significant genotype effects for 14 traits, calculated for each Scenario [A) preceding crop winter wheat and two nitrogen levels in barley (WW under N_0 & N_1), B) preceding crop winter oilseed rape and two nitrogen levels in barley (WR under N_0 & N_1), C) two preceding crops and nitrogen stress in barley (WR & WW under N_0), and D) two preceding crops and high nitrogen availability in barley (WR & WW under N_1)].

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GEA	QGea.S42.2H.a	2H	S42IL_107	A	18.80	-3.86	-17.03	0.0000	*PpdH1* *[a]
GEA	QGea.S42.2H.a	2H	S42IL_107	B	19.85	-4.28	-17.73	0.0000	*PpdH1* *[a]
GEA	QGea.S42.2H.a	2H	S42IL_107	C	19.17	-4.39	-18.63	0.0000	*PpdH1* *[a]
GEA	QGea.S42.2H.a	2H	S42IL_107	D	19.48	-3.75	-16.14	0.0000	*PpdH1* *[a]
GEA	QGea.S42.2H.a	2H	S42IL_109	A	18.90	-3.75	-16.55	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H.a	2H	S42IL_109	B	20.23	-3.90	-16.18	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H.a	2H	S42IL_109	C	19.68	-3.88	-16.47	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H.a	2H	S42IL_109	D	19.46	-3.77	-16.25	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GEA	QGea.S42.2H.b	2H	S42IL_110	A	19.30	-3.35	-14.81	0.0000	*[HvCEN* *[c]*]
GEA	QGea.S42.2H.b	2H	S42IL_110	B	20.66	-3.47	-14.40	0.0000	*[HvCEN* *[c]*]
GEA	QGea.S42.2H.b	2H	S42IL_110	C	20.25	-3.30	-14.01	0.0000	*[HvCEN* *[c]*]
GEA	QGea.S42.2H.b	2H	S42IL_110	D	19.70	-3.53	-15.19	0.0000	*[HvCEN* *[c]*]
GEA	QGea.S42.3H	3H	S42IL_111	B	21.03	-3.10	-12.86	0.0000	*HvGI* *[h]; *HvFT2* *[h]
GEA	QGea.S42.3H	3H	S42IL_111	C	20.89	-2.66	-11.30	0.0005	*HvGI* *[h]; *HvFT2* *[h]
GPC	QGpc.S42.1H	1H	S42IL_102	C	15.52	1.47	10.44	0.0009	
GPC	QGpc.S42.1H	1H	S42IL_105	C	15.53	1.48	10.56	0.0008	
GPC	QGpc.S42.6H	6H	S42IL_129	A	16.51	2.03	14.05	0.0000	*HvNAM-1* *[l]
GPC	QGpc.S42.6H	6H	S42IL_129	C	15.74	1.69	12.04	0.0000	*HvNAM-1* *[l]
GPC	QGpc.S42.6H	6H	S42IL_130	A	16.06	1.58	10.94	0.0004	
GPC	QGpc.S42.7H	7H	S42IL_133	A	16.50	2.02	13.99	0.0000	*VRN-H3* *[h]
GPC	QGpc.S42.7H	7H	S42IL_133	C	15.65	1.60	11.39	0.0001	*VRN-H3* *[h]
GPC	QGpc.S42.7H	7H	S42IL_134	A	16.06	1.58	10.94	0.0004	*HvCO1* *[g]
GRA	QGra.S42.2H	2H	S42IL_109	C	21.43	1.64	8.30	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
GRA	QGra.S42.4H	4H	S42IL_121	A	21.70	2.00	10.15	0.0000	*[sdw1/HvGA20ox2* *[d]*]

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GRA	QGra.S42.4H	4H	S42IL_121	B	21.62	1.79	9.04	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRA	QGra.S42.4H	4H	S42IL_121	C	21.85	2.07	10.47	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRA	QGra.S42.4H	4H	S42IL_121	D	21.47	1.72	8.71	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRA	QGra.S42.7H	7H	S42IL_137	A	21.25	1.55	7.87	0.0001	*[sdw1/HvGA20ox2 *[d]]*
GRA	QGra.S42.7H	7H	S42IL_137	C	21.22	1.44	7.27	0.0006	*[sdw1/HvGA20ox2 *[d]]*
GRA	QGra.S42.1H	1H	S42IL_143	A	21.13	1.43	7.27	0.0007	
GRA	QGra.S42.1H	1H	S42IL_143	D	21.23	1.49	7.53	0.0006	
GRL	QGrl.S42.1H.a	1H	S42IL_102	A	8.32	0.77	10.15	0.0000	
GRL	QGrl.S42.1H.a	1H	S42IL_102	B	8.18	0.80	10.85	0.0000	
GRL	QGrl.S42.1H.a	1H	S42IL_102	C	8.21	0.84	11.42	0.0000	
GRL	QGrl.S42.1H.a	1H	S42IL_102	D	8.28	0.73	9.60	0.0000	
GRL	QGrl.S42.1H.a	1H	S42IL_103	C	7.94	0.57	7.74	0.0002	
GRL	QGrl.S42.2H	2H	S42IL_109	A	8.17	0.62	8.17	0.0000	*HvFT4 *[b]; HvCEN *[c]*
GRL	QGrl.S42.2H	2H	S42IL_109	C	8.12	0.75	10.18	0.0000	*HvFT4 *[b]; HvCEN *[c]*
GRL	QGrl.S42.4H	4H	S42IL_121	A	8.21	0.66	8.77	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.4H	4H	S42IL_121	B	7.97	0.60	8.08	0.0002	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.4H	4H	S42IL_121	C	8.10	0.73	9.89	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.4H	4H	S42IL_123	C	7.92	0.55	7.41	0.0003	
GRL	QGrl.S42.7H	7H	S42IL_135	C	7.95	0.57	7.80	0.0001	
GRL	QGrl.S42.7H	7H	S42IL_137	A	8.23	0.67	8.94	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.7H	7H	S42IL_137	B	8.02	0.64	8.70	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.7H	7H	S42IL_137	C	8.12	0.75	10.18	0.0000	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.7H	7H	S42IL_137	D	8.12	0.57	7.50	0.0003	*[sdw1/HvGA20ox2 *[d]]*
GRL	QGrl.S42.1H.b	1H	S42IL_143	A	8.50	0.95	12.58	0.0000	
GRL	QGrl.S42.1H.b	1H	S42IL_143	B	8.40	1.02	13.90	0.0000	
GRL	QGrl.S42.1H.b	1H	S42IL_143	C	8.41	1.04	14.13	0.0000	
GRL	QGrl.S42.1H.b	1H	S42IL_143	D	8.49	0.93	12.35	0.0000	
GRL	QGrl.S42.5H	5H	S42IL_176	B	8.21	0.84	11.36	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
GRL	QGrl.S42.5H	5H	S42IL_176	C	8.00	0.63	8.57	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
GRL	QGrl.S42.5H	5H	S42IL_176	D	8.18	0.63	8.33	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
GRW	QGrw.S42.1H.a	1H	S42IL_102	B	3.69	-0.13	-3.49	0.0001	
GRW	QGrw.S42.1H.a	1H	S42IL_102	C	3.68	-0.14	-3.60	0.0000	
GRW	QGrw.S42.4H	4H	S42IL_124	B	3.70	-0.12	-3.16	0.0008	*Vrn-H2* *[e]; *Bmy1* *[i]
GRW	QGrw.S42.4H	4H	S42IL_124	C	3.69	-0.12	-3.28	0.0002	*Vrn-H2* *[e]; *Bmy1* *[i e]
GRW	QGrw.S42.1H.b	1H	S42IL_143	B	3.70	-0.12	-3.16	0.0008	
GRW	QGrw.S42.1H.b	1H	S42IL_143	C	3.69	-0.13	-3.38	0.0001	
GRW	QGrw.S42.5H	5H	S42IL_176	A	3.57	-0.16	-4.29	0.0000	*Vrn-H1* *[e]; *[HvELF3* *[f]]
GRW	QGrw.S42.5H	5H	S42IL_176	B	3.67	-0.15	-4.03	0.0000	*Vrn-H1* *[e]; *[HvELF3* *[f]]
GRW	QGrw.S42.5H	5H	S42IL_176	C	3.63	-0.19	-4.85	0.0000	*Vrn-H1* *[e]; *[HvELF3* *[f]]
HEA	QHea.S42.1H	1H	S42IL_105	A	62.29	-3.46	-5.26	0.0000	
HEA	QHea.S42.1H	1H	S42IL_105	B	61.63	-4.17	-6.33	0.0000	
HEA	QHea.S42.1H	1H	S42IL_105	C	61.88	-3.79	-5.77	0.0000	
HEA	QHea.S42.1H	1H	S42IL_105	D	62.04	-3.83	-5.82	0.0000	
HEA	QHea.S42.2H.a	2H	S42IL_107	A	57.13	-8.62	-13.12	0.0000	*PpdH1* *[a]
HEA	QHea.S42.2H.a	2H	S42IL_107	B	56.50	-9.29	-14.12	0.0000	*PpdH1* *[a]
HEA	QHea.S42.2H.a	2H	S42IL_107	C	56.88	-8.79	-13.39	0.0000	*PpdH1* *[a]
HEA	QHea.S42.2H.a	2H	S42IL_107	D	56.75	-9.12	-13.85	0.0000	*PpdH1* *[a]
HEA	QHea.S42.2H.a	2H	S42IL_108	A	58.63	-7.12	-10.84	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.a	2H	S42IL_108	B	57.67	-8.12	-12.35	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.a	2H	S42IL_108	C	57.75	-7.92	-12.06	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.a	2H	S42IL_108	D	58.54	-7.33	-11.13	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.a	2H	S42IL_109	B	63.50	-2.29	-3.48	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.a	2H	S42IL_109	C	63.63	-2.04	-3.11	0.0000	*HvFT4* *[b]; *HvCEN* *[c]
HEA	QHea.S42.2H.b	2H	S42IL_110	A	62.21	-3.54	-5.39	0.0000	*[HvCEN* *[c]]
HEA	QHea.S42.2H.b	2H	S42IL_110	B	61.63	-4.17	-6.33	0.0000	*[HvCEN* *[c]]
HEA	QHea.S42.2H.b	2H	S42IL_110	C	61.83	-3.83	-5.84	0.0000	*[HvCEN* *[c]]
HEA	QHea.S42.2H.b	2H	S42IL_110	D	62.00	-3.87	-5.88	0.0000	*[HvCEN* *[c]]
HEA	QHea.S42.3H	3H	S42IL_113	A	63.54	-2.21	-3.36	0.0001	
HEA	QHea.S42.3H	3H	S42IL_113	B	62.54	-3.25	-4.94	0.0000	
HEA	QHea.S42.3H	3H	S42IL_113	C	62.88	-2.79	-4.25	0.0000	

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEA	QHea.S42.3H	3H	S42IL_113	D	63.21	-2.67	-4.05	0.0000	
HEA	QHea.S42.3H	3H	S42IL_114	A	61.64	-4.11	-6.25	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_114	B	60.74	-5.05	-7.68	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_114	C	61.02	-4.65	-7.08	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_114	D	61.36	-4.51	-6.85	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.4H	4H	S42IL_120	B	62.71	-3.08	-4.69	0.0000	
HEA	QHea.S42.4H	4H	S42IL_120	C	63.04	-2.62	-4.00	0.0000	
HEA	QHea.S42.4H	4H	S42IL_120	D	63.00	-2.87	-4.36	0.0000	
HEA	QHea.S42.4H.a	4H	S42IL_120	A	63.33	-2.42	-3.68	0.0000	
HEA	QHea.S42.4H	4H	S42IL_121	B	60.92	-4.87	-7.41	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.4H	4H	S42IL_121	C	61.29	-4.37	-6.66	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.4H	4H	S42IL_121	D	61.46	-4.42	-6.70	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.4H.a	4H	S42IL_121	A	61.83	-3.92	-5.96	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.4H.b	4H	S42IL_124	A	67.83	2.08	3.17	0.0003	*Vrn-H2* *[e]; *Bmy1* *[i]
HEA	QHea.S42.5H.a	5H	S42IL_125	A	63.63	-2.12	-3.23	0.0002	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.5H.a	5H	S42IL_125	B	62.83	-2.96	-4.50	0.0000	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.5H.a	5H	S42IL_125	C	63.33	-2.33	-3.55	0.0000	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.5H.a	5H	S42IL_125	D	63.13	-2.75	-4.17	0.0000	*Dhn1/Dhn2* *[j]
HEA	QHea.S42.7H.a	7H	S42IL_134	A	62.29	-3.46	-5.26	0.0000	*HvCO1* *[g]
HEA	QHea.S42.7H.a	7H	S42IL_134	B	61.67	-4.12	-6.27	0.0000	*HvCO1* *[g]
HEA	QHea.S42.7H.a	7H	S42IL_134	C	61.83	-3.83	-5.84	0.0000	*HvCO1* *[g]
HEA	QHea.S42.7H.a	7H	S42IL_134	D	62.13	-3.75	-5.69	0.0000	*HvCO1* *[g]
HEA	QHea.S42.7H.b	7H	S42IL_137	A	61.58	-4.17	-6.34	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.7H.b	7H	S42IL_137	B	61.46	-4.33	-6.59	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.7H.b	7H	S42IL_137	C	61.29	-4.37	-6.66	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.7H.b	7H	S42IL_137	D	61.75	-4.12	-6.26	0.0000	*[sdw1/HvGA20ox2* *[d]]*
HEA	QHea.S42.3H	3H	S42IL_140	A	61.42	-4.33	-6.59	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_140	B	60.88	-4.92	-7.47	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_140	C	61.38	-4.29	-6.54	0.0000	*sdw1/HvGA20ox2* *[d]
HEA	QHea.S42.3H	3H	S42IL_140	D	60.92	-4.96	-7.53	0.0000	*sdw1/HvGA20ox2* *[d]

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEA	QHea.S42.6H	6H	S42IL_148	A	61.08	-4.67	-7.10	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEA	QHea.S42.6H	6H	S42IL_148	B	60.63	-5.17	-7.85	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEA	QHea.S42.6H	6H	S42IL_148	C	60.92	-4.75	-7.23	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEA	QHea.S42.6H	6H	S42IL_148	D	60.79	-5.08	-7.72	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEA	QHea.S42.5H.b	5H	S42IL_176	A	69.94	4.19	6.37	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
HEA	QHea.S42.5H.b	5H	S42IL_176	B	69.33	3.54	5.38	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
HEA	QHea.S42.5H.b	5H	S42IL_176	C	69.91	4.24	6.46	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
HEA	QHea.S42.5H.b	5H	S42IL_176	D	69.38	3.50	5.31	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
HEI	QHei.S42.1H	1H	S42IL_105	A	70.42	9.83	16.23	0.0000	
HEI	QHei.S42.1H	1H	S42IL_105	B	74.13	8.83	13.53	0.0000	
HEI	QHei.S42.1H	1H	S42IL_105	C	72.33	9.96	15.97	0.0000	
HEI	QHei.S42.1H	1H	S42IL_105	D	72.21	8.71	13.71	0.0000	
HEI	QHei.S42.2H	2H	S42IL_107	D	56.92	-6.58	-10.37	0.0008	*PpdH1 *[a]*
HEI	QHei.S42.3H	3H	S42IL_114	A	70.42	9.84	16.24	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.3H	3H	S42IL_114	B	75.75	10.46	16.02	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.3H	3H	S42IL_114	C	75.02	12.65	20.28	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.3H	3H	S42IL_114	D	71.15	7.65	12.05	0.0001	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.4H	4H	S42IL_121	A	70.13	9.54	15.75	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.4H	4H	S42IL_121	B	76.29	11.00	16.85	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.4H	4H	S42IL_121	C	74.46	12.08	19.37	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.4H	4H	S42IL_121	D	71.96	8.46	13.32	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.5H	5H	S42IL_125	A	68.42	7.83	12.93	0.0002	*Dhn1/Dhn2 *[j]*
HEI	QHei.S42.5H	5H	S42IL_125	B	71.88	6.58	10.08	0.0000	*Dhn1/Dhn2 *[j]*
HEI	QHei.S42.5H	5H	S42IL_125	C	70.29	7.92	12.69	0.0000	*Dhn1/Dhn2 *[j]*
HEI	QHei.S42.5H	5H	S42IL_125	D	70.00	6.50	10.24	0.0010	*Dhn1/Dhn2 *[j]*
HEI	QHei.S42.7H.a	7H	S42IL_134	A	69.79	9.21	15.20	0.0000	*HvCO1 *[g]*
HEI	QHei.S42.7H.a	7H	S42IL_134	B	73.92	8.63	13.21	0.0000	*HvCO1 *[g]*
HEI	QHei.S42.7H.a	7H	S42IL_134	C	71.46	9.08	14.56	0.0000	*HvCO1 *[g]*
HEI	QHei.S42.7H.a	7H	S42IL_134	D	72.25	8.75	13.78	0.0000	*HvCO1 *[g]*
HEI	QHei.S42.7H.b	7H	S42IL_137	A	71.46	10.87	17.95	0.0000	*[sdw1/HvGA20ox2 *[d]]*

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
HEI	QHei.S42.7H.b	7H	S42IL_137	B	79.17	13.88	21.25	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.7H.b	7H	S42IL_137	C	62.54	14.00	0.27	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.7H.b	7H	S42IL_137	D	74.25	10.75	16.93	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.3H	3H	S42IL_140	A	71.50	10.92	18.02	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.3H	3H	S42IL_140	B	76.63	11.33	17.36	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.3H	3H	S42IL_140	C	74.42	12.04	19.31	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.3H	3H	S42IL_140	D	73.71	10.21	16.08	0.0000	*sdw1/HvGA20ox2 *[d]*
HEI	QHei.S42.6H	6H	S42IL_148	A	70.63	10.04	16.57	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.6H	6H	S42IL_148	B	75.46	10.17	15.57	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.6H	6H	S42IL_148	C	73.54	11.17	17.90	0.0000	*[sdw1/HvGA20ox2 *[d]]*
HEI	QHei.S42.6H	6H	S42IL_148	D	72.54	9.04	14.24	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.2H	2H	S42IL_107	B	5.50	2.17	65.00	0.0001	*PpdH1 *[a]*
LOD	QLod.S42.2H	2H	S42IL_107	C	4.50	2.25	100.00	0.0000	*PpdH1 *[a]*
LOD	QLod.S42.3H	3H	S42IL_114	C	4.57	2.32	103.13	0.0000	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.4H	4H	S42IL_121	C	4.67	2.42	107.41	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.7H	7H	S42IL_137	A	5.08	2.25	79.41	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.7H	7H	S42IL_137	B	5.67	2.33	70.00	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.7H	7H	S42IL_137	C	5.17	2.92	129.63	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.3H	3H	S42IL_140	A	4.75	1.92	67.65	0.0001	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.3H	3H	S42IL_140	B	5.42	2.08	62.50	0.0002	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.3H	3H	S42IL_140	C	4.83	2.58	114.81	0.0000	*sdw1/HvGA20ox2 *[d]*
LOD	QLod.S42.6H	6H	S42IL_148	A	5.17	2.33	82.35	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.6H	6H	S42IL_148	B	5.67	2.33	70.00	0.0000	*[sdw1/HvGA20ox2 *[d]]*
LOD	QLod.S42.6H	6H	S42IL_148	C	5.17	2.92	129.63	0.0000	*[sdw1/HvGA20ox2 *[d]]*
MAT	QMat.S42.2H	2H	S42IL_107	A	94.00	-4.46	-4.53	0.0000	*PpdH1 *[a]*
MAT	QMat.S42.2H	2H	S42IL_107	B	96.17	-4.33	-4.31	0.0000	*PpdH1 *[a]*
MAT	QMat.S42.2H	2H	S42IL_107	C	94.92	-4.58	-4.61	0.0000	*PpdH1 *[a]*
MAT	QMat.S42.2H	2H	S42IL_107	D	95.25	-4.21	-4.23	0.0000	*PpdH1 *[a]*
MAT	QMat.S42.3H	3H	S42IL_140	A	95.87	-2.58	-2.62	0.0000	*sdw1/HvGA20ox2 *[d]*
MAT	QMat.S42.3H	3H	S42IL_140	C	96.79	-2.71	-2.72	0.0000	*sdw1/HvGA20ox2 *[d]*

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
MAT	QMat.S42.6H	6H	S42IL_148	A	96.12	-2.33	-2.37	0.0004	*[sdw1/HvGA20ox2 *[d]]*
MAT	QMat.S42.6H	6H	S42IL_148	B	97.79	-2.71	-2.69	0.0002	*[sdw1/HvGA20ox2 *[d]]*
MAT	QMat.S42.6H	6H	S42IL_148	C	96.79	-2.71	-2.72	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.1H	1H	S42IL_105	B	8.20	1.87	29.49	0.0001	
SEN	QSen.S42.2H	2H	S42IL_107	B	9.54	3.21	50.66	0.0000	*PpdH1 *[a]*
SEN	QSen.S42.2H	2H	S42IL_107	C	9.63	2.33	32.00	0.0000	*PpdH1 *[a]*
SEN	QSen.S42.2H	2H	S42IL_107	D	9.54	2.71	39.63	0.0000	*PpdH1 *[a]*
SEN	QSen.S42.3H	3H	S42IL_113	B	8.38	2.04	32.24	0.0000	
SEN	QSen.S42.3H	3H	S42IL_114	B	9.29	2.96	46.70	0.0000	*sdw1/HvGA20ox2 *[d]*
SEN	QSen.S42.3H	3H	S42IL_114	C	9.65	2.36	32.34	0.0000	*sdw1/HvGA20ox2 *[d]*
SEN	QSen.S42.4H	4H	S42IL_121	B	8.58	2.25	35.53	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.7H.a	7H	S42IL_134	B	8.33	2.00	31.58	0.0000	*HvCO1 *[g]*
SEN	QSen.S42.7H.b	7H	S42IL_137	B	8.63	2.29	36.18	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.3H	3H	S42IL_140	B	9.38	3.04	48.03	0.0000	*sdw1/HvGA20ox2 *[d]*
SEN	QSen.S42.3H	3H	S42IL_140	C	9.63	2.33	32.00	0.0000	*sdw1/HvGA20ox2 *[d]*
SEN	QSen.S42.3H	3H	S42IL_140	D	9.58	2.75	40.24	0.0000	*sdw1/HvGA20ox2 *[d]*
SEN	QSen.S42.6H	6H	S42IL_148	B	8.88	2.54	40.13	0.0000	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.6H	6H	S42IL_148	C	9.33	2.04	28.00	0.0001	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.6H	6H	S42IL_148	D	8.96	2.12	31.10	0.0006	*[sdw1/HvGA20ox2 *[d]]*
SEN	QSen.S42.5H	5H	S42IL_176	A	4.96	-2.83	-36.36	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SEN	QSen.S42.5H	5H	S42IL_176	B	4.21	-2.13	-33.55	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SEN	QSen.S42.5H	5H	S42IL_176	C	4.79	-2.50	-34.29	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SEN	QSen.S42.5H	5H	S42IL_176	D	4.38	-2.46	-35.98	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SHO	QSho.S42.1H.a	1H	S42IL_105	A	44.83	-5.54	-11.00	0.0000	
SHO	QSho.S42.1H.a	1H	S42IL_105	B	44.38	-4.92	-9.97	0.0000	
SHO	QSho.S42.1H.a	1H	S42IL_105	C	44.08	-5.96	-11.91	0.0000	
SHO	QSho.S42.1H.a	1H	S42IL_105	D	45.13	-4.50	-9.07	0.0000	
SHO	QSho.S42.2H	2H	S42IL_107	A	45.00	-5.38	-10.67	0.0000	*PpdH1 *[a]*
SHO	QSho.S42.2H	2H	S42IL_107	B	45.25	-4.04	-8.20	0.0000	*PpdH1 *[a]*
SHO	QSho.S42.2H	2H	S42IL_107	C	45.21	-4.83	-9.66	0.0000	*PpdH1 *[a]*

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
SHO	QSho.S42.2H	2H	S42IL_107	D	45.04	-4.58	-9.24	0.0000	*PpdH1* *[a]
SHO	QSho.S42.2H	2H	S42IL_108	A	47.04	-3.33	-6.62	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
SHO	QSho.S42.2H	2H	S42IL_108	B	46.63	-2.67	-5.41	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
SHO	QSho.S42.2H	2H	S42IL_108	C	46.88	-3.17	-6.33	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
SHO	QSho.S42.2H	2H	S42IL_108	D	46.79	-2.83	-5.71	0.0000	*PpdH1* *[a]; *HvFT4* *[b]; *HvCEN* *[c]
SHO	QSho.S42.3H	3H	S42IL_113	A	46.79	-3.58	-7.11	0.0000	
SHO	QSho.S42.3H	3H	S42IL_113	B	46.29	-3.00	-6.09	0.0000	
SHO	QSho.S42.3H	3H	S42IL_113	C	46.29	-3.75	-7.49	0.0000	
SHO	QSho.S42.3H	3H	S42IL_113	D	46.79	-2.83	-5.71	0.0000	
SHO	QSho.S42.3H	3H	S42IL_114	A	44.56	-5.81	-11.54	0.0000	*sdw1/HvGA20ox$_2$* *[d]
SHO	QSho.S42.3H	3H	S42IL_114	B	44.48	-4.81	-9.76	0.0000	*sdw1/HvGA20ox$_2$* *[d]
SHO	QSho.S42.3H	3H	S42IL_114	C	44.24	-5.80	-11.59	0.0000	*sdw1/HvGA20ox$_2$* *[d]
SHO	QSho.S42.3H	3H	S42IL_114	D	44.80	-4.82	-9.72	0.0000	*sdw1/HvGA20ox$_2$* *[d]
SHO	QSho.S42.3H	3H	S42IL_115	A	47.92	-2.46	-4.88	0.0003	
SHO	QSho.S42.4H	4H	S42IL_120	A	47.50	-2.88	-5.71	0.0000	
SHO	QSho.S42.4H.a	4H	S42IL_120	B	46.67	-2.62	-5.33	0.0000	
SHO	QSho.S42.4H.a	4H	S42IL_120	C	47.38	-2.67	-5.33	0.0000	
SHO	QSho.S42.4H.a	4H	S42IL_120	D	46.79	-2.83	-5.71	0.0000	
SHO	QSho.S42.4H	4H	S42IL_121	A	44.50	-5.88	-11.66	0.0000	*[sdw1/HvGA20ox$_2$* *[d]]*
SHO	QSho.S42.4H.a	4H	S42IL_121	B	44.42	-4.87	-9.89	0.0000	*[sdw1/HvGA20ox$_2$* *[d]]*
SHO	QSho.S42.4H.a	4H	S42IL_121	C	43.92	-6.12	-12.24	0.0000	*[sdw1/HvGA20ox$_2$* *[d]]*
SHO	QSho.S42.4H.a	4H	S42IL_121	D	45.00	-4.63	-9.32	0.0000	*[sdw1/HvGA20ox$_2$* *[d]]*
SHO	QSho.S42.4H.b	4H	S42IL_124	B	52.38	3.08	6.26	0.0000	*Vrn-H2* *[e]; *Bmy1* *[i]
SHO	QSho.S42.4H.b	4H	S42IL_124	C	52.54	2.50	5.00	0.0000	*Vrn-H2* *[e]; *Bmy1* *[i]
SHO	QSho.S42.4H.b	4H	S42IL_124	D	52.46	2.83	5.71	0.0000	*Vrn-H2* *[e]; *Bmy1* *[i]
SHO	QSho.S42.5H.a	5H	S42IL_125	A	46.50	-3.88	-7.69	0.0000	*Dhn1/Dhn2* *[j]
SHO	QSho.S42.5H.a	5H	S42IL_125	B	46.63	-2.67	-5.41	0.0000	*Dhn1/Dhn2* *[j]
SHO	QSho.S42.5H.a	5H	S42IL_125	C	46.67	-3.37	-6.74	0.0000	*Dhn1/Dhn2* *[j]
SHO	QSho.S42.5H.a	5H	S42IL_125	D	46.46	-3.17	-6.38	0.0000	*Dhn1/Dhn2* *[j]
SHO	QSho.S42.7H.a	7H	S42IL_134	A	45.67	-4.71	-9.35	0.0000	*HvCO1* *[g]

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
SHO	QSho.S42.7H.a	7H	S42IL_134	B	45.17	-4.12	-8.37	0.0000	*HvCO1* *[g]
SHO	QSho.S42.7H.a	7H	S42IL_134	C	45.13	-4.92	-9.83	0.0000	*HvCO1* *[g]
SHO	QSho.S42.7H.a	7H	S42IL_134	D	45.71	-3.92	-7.89	0.0000	*HvCO1* *[g]
SHO	QSho.S42.7H.b	7H	S42IL_137	A	42.92	-7.46	-14.81	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.7H.b	7H	S42IL_137	B	42.46	-6.83	-13.86	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.7H.b	7H	S42IL_137	C	42.25	-7.79	-15.57	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.7H.b	7H	S42IL_137	D	43.13	-6.50	-13.10	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.7H.c	7H	S42IL_138	B	52.13	2.83	5.75	0.0000	
SHO	QSho.S42.7H.c	7H	S42IL_138	C	52.63	2.58	5.16	0.0000	
SHO	QSho.S42.3H	3H	S42IL_140	A	42.13	-8.25	-16.38	0.0000	*sdw1/HvGA20ox$_2$ *[d]*
SHO	QSho.S42.3H	3H	S42IL_140	B	42.17	-7.12	-14.45	0.0000	*sdw1/HvGA20ox$_2$ *[d]*
SHO	QSho.S42.3H	3H	S42IL_140	C	42.25	-7.79	-15.57	0.0000	*sdw1/HvGA20ox$_2$ *[d]*
SHO	QSho.S42.3H	3H	S42IL_140	D	42.04	-7.58	-15.28	0.0000	*sdw1/HvGA20ox$_2$ *[d]*
SHO	QSho.S42.1H.b	1H	S42IL_143	A	52.75	2.37	4.71	0.0006	
SHO	QSho.S42.1H.b	1H	S42IL_143	B	53.42	4.13	8.37	0.0000	
SHO	QSho.S42.1H.b	1H	S42IL_143	C	53.21	3.17	6.33	0.0000	
SHO	QSho.S42.1H.b	1H	S42IL_143	D	52.96	3.33	6.72	0.0000	
SHO	QSho.S42.6H	6H	S42IL_148	A	42.04	-8.33	-16.54	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.6H	6H	S42IL_148	B	42.96	-6.33	-12.85	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.6H	6H	S42IL_148	C	42.67	-7.37	-14.74	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.6H	6H	S42IL_148	D	42.33	-7.29	-14.69	0.0000	*[sdw1/HvGA20ox$_2$ *[d]]*
SHO	QSho.S42.5H.b	5H	S42IL_176	A	52.99	2.61	5.19	0.0001	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SHO	QSho.S42.5H.b	5H	S42IL_176	B	52.33	3.04	6.17	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SHO	QSho.S42.5H.b	5H	S42IL_176	C	52.41	2.37	4.74	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SHO	QSho.S42.5H.b	5H	S42IL_176	D	52.92	3.29	6.63	0.0000	*Vrn-H1 *[e]; [HvELF3 *[f]]*
SPAD	QSpad.S42.2H	2H	S42IL_107	B	55.40	4.83	9.55	0.0000	*PpdH1 *[a]*
SPAD	QSpad.S42.2H	2H	S42IL_107	C	52.89	4.34	8.94	0.0002	*PpdH1 *[a]*
SPAD	QSpad.S42.2H	2H	S42IL_107	D	53.03	3.88	7.89	0.0007	*PpdH1 *[a]*
TGW	QTgw.S42.4H	4H	S42IL_121	A	48.61	4.32	9.75	0.0003	*[sdw1/HvGA20ox$_2$ *[d]]*
TGW	QTgw.S42.4H	4H	S42IL_121	C	50.51	4.08	8.78	0.0002	*[sdw1/HvGA20ox$_2$ *[d]]*

Trait [a]	QTL in S42IL	Chr [b]	Line	Scenario	LSMeans [c]	Diff [d]	RP of S42IL [e]	$P_{(fdr)}$ value [f]	Candidate genes
YLD	QYld.S42.1H	1H	S42IL_103	B	67.37	-11.48	-14.56	0.0000	
YLD	QYld.S42.3H	3H	S42IL_111	B	60.66	-18.19	-23.07	0.0000	*HvGI* [*h]; *HvFT2* [*h]
YLD	QYld.S42.3H	3H	S42IL_111	D	57.66	-10.49	-15.39	0.0000	*HvGI* [*h]; *HvFT2* [*h]
YLD	QYld.S42.5H	5H	S42IL_176	B	65.17	-13.68	-17.35	0.0000	*Vrn-H1* [*e]; *[HvELF3* [*f]]
YLD	QYld.S42.5H	5H	S42IL_176	D	55.19	-12.97	-19.03	0.0000	*Vrn-H1* [*e]; *[HvELF3* [*f]]

[a] Abbreviation of traits, see Table 3, [b] Chromosomal location, [c] Least squares means, calculated for the indicated scenario, [d] Difference = LSMEANS [IL] - LSMEANS [Scarlett], [e] Relative performance, [f] fdr corrected Dunnett test probability of difference between S42IL and control 'Scarlett', *[a] Turner *et al*. 2005, *[b] Faure *et al.* (2007), *[c] Comadran *et al.* (2012), *[d] Jia *et al.* (2015b), *[e] Yan *et al.* (2004), *[f] Faure *et al.* (2012); Zakhrabekova *et al.* (2012), *[g] Griffiths *et al.* (2003), *[h] Wang *et al.* (2010a), *[i] Clark *et al.* (2003). *[j] Choi *et al.* (2000), *[l] Distelfeld *et al.* (2008), [ ] Candidate genes on sub-introgression

12. Curriculum vitae

Name:	**Sarah Zahn**
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Place of birth:	**Dresden**

WORK EXPERIENCE

05/2014 – 11/2014	**Research assistant at Bayer Crop Science** *Gatersleben*

EDUCATION

07/2020 – 01/2021	**PhD student at the Chair of Plant Breeding** *Institute of Agricultural and Nutritional Science, Martin-Luther-University Halle*
04/2015 – 09/2019	**PhD student at the Chair of Agronomy and Organic Farming** *Institute of Agricultural and Nutritional Science, Martin-Luther-University Halle*
10/2012 – 03/2015	**Master of Science degree in agriculture science** *Agricultural and Nutritional Science, MLU Halle-Wittenberg*
10/2009 – 09/2012	**Bachelor of Science degree in agriculture science** *Agricultural and Nutritional Science, MLU Halle-Wittenberg*

Halle (Saale), 24 March 2021

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14. 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.

Halle (Saale), 24 March 2021 _____

Datum/*Date* Unterschrift/*Signature*