Genetic regulation of juvenile plant growth under contrasting levels of phosphorus, potassium and carbon dioxide nutrition in the wild barley introgression library S42IL

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1 General introduction

Cultivated barley (*Hordeum vulgare* ssp. *vulgare*) is one of the most important crops in the world after rice, maize and, wheat. Mainly, this crop is used for malt and beer production, but also for direct human consumption and animal feed (Gupta et al. 2010; Druka et al. 2011). Furthermore, barley is also known as a model plant for physiological and genetic studies due to a short life cycle, a wide genetic variation, its diploid nature and the availability of a complete genome sequence (Bengtsson 1992; Forster et al. 2000; IBGS 2012). Domestication of barley started approximately 10,000 years ago in the Fertile Crescent (Badr et al. 2000), ultimately leading to a reduction of genetic diversity available in our present barley gene pool compared to its progenitor, wild barley *(Hordeum vulgare ssp. spontaneum,* Tanksley and McCouch 1997). Wild ancestors of cultivated plants are useful sources for improving important traits such as disease resistance. For instance, resistance to powdery mildew (Jorgensen 1992) and leaf rust (Ivandic et al. 1998) could be increased due to the introgression of exotic barley germplasm.

Growth and yield performance of plants depend on various growth conditions such as nutrient availabiliy, water supply and temperature. The frequently discussed climate change must also be considered, whether it is caused by natural or human influences. At present, it is assumed that it is caused mainly by human-induced global warming. Climate change also means rising carbon dioxide concentrations of the atmosphere. This not only leads to higer temperatures and reducing rainfall, but also has a direct impact on plant growth. This effect is referred to as the "CO_2 fertilization effect" and is plant type dependent. In C3 plants like barley, which account for about 90% of land plants of the earth, a doubling of CO_2 concentration increases yield by 20% (Busch et al. 2013).

For an optimal plant nutrition and yield, both phosphorus and potassium as important macronutrients are essential. Phosphorus is required in some key function of the plant and promotes root growth and tillering. Potassium affects plant growth and reproduction. Phosphorus and potassium resources are limited in agriculture. Deficiency leads to reduction of plant growth and yield. The economic benefits of chemical fertilizer on crop production are well documented, but it is assumed that the costs of fertilizer in crop cultivation will increase in the future.

The expected food and energy needs of a growing global population require the application of advanced technologies to optimize the breeding progress in essential plant breeding programs. Therefore, introducing of new and adapted varieties with acceptable and stable yields even under stress condition is an important challenge for future plant breeding and crop production.

An expected use of germplasm and genes which are able to exhibit an improved phosphorus or potassium use efficiency and favourable reactions against increased CO_2 concentration should help.

1.1 Barley – taxonomy, origin and domestication

Together with einkorn and emmer the self-pollinated barley is known as the first cultivated cereal which was used and domesticated by humans. In the past, barley was most important as food for human nutrition. Surpassed by rice, corn and wheat in many countries barley lost its prominence. Only 2% are still used for human consumption. High soluble dietary fiber of the barley grain can have positive effects on humans as well as a reduced risk of serious human diseases such as type II diabetes, cardiovascular disease and colorectal cancers (Mayer et al. 2012). Today barley plays a main role in feeding (55%-60%) and malt production (30%-40%). Barley is the fourth abundant cereal after maize, wheat and rice based on tonnage and acreage (Mayer et al. 2012). In 2014/2015 the world barley production was 141.2 million tons which could be increased to 3.2% in 2015/2016 to 145.8 million tons (http://www.statista.com/statistics/271973/world-barley-production-since-2008).

Barley (*Hordeum vulgare* L.) belongs to the genus *Hordeum* in the tribe *Triticeae*, the economically most important group of the grass family, the Poaceae, with most of them are hulled (with a tough, inedible outer hull around the seed). But there are also hulless, naked barley. The genus *Hordeum* with about 32 species comprises both annual (*H. vulgare* and *H. marinum*) and perennial (*H. bulbosum*) species. In these species, chromosome number is based on x =7. *Hordeum* species form a polyploid series with diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42) ploidy level. Cultivated barley, (*Hordeum vulgare* ssp. *vulgare* L., hereafter abbreviated with *Hv*) and the wild type of cultivated barley (*Hordeum vulgare* ssp. *spontaneum* C. Koch, hereafter abbreviated with *Hsp*) are diploid.

The genus *Hordeum* can be classified into three gene pools based on several criteria including the possibility of interspecific hybridizations and molecular and cytogenetic analyses (Zhang et al. 2001). The primary gene pool contains the cultivated barley (H*v*), landraces and the wild barley (H*sp*). The species of this pool are diploid, annual and predominantly autogamous. Crosses within this pool are easily possible and give fertile offspring. Only *H. bulbosum* belongs to the secondary gene pool. Crosses with cultivated barley are limited and carried out for the

production of doubled haploids. The remaining *Hordeum* species belong to the tertiary gene pool. They are far distant species and difficult to be crossed.

Due to frost tolerance, vernalization requirements and photoperiod sensitivity, three different genotypic groups can be distinguished for barley, namely winter, facultative and spring barley (Szücs et al. 2007; Tondelli et al. 2014). Winter barley which is known as vernalization-sensitive needs a defined requirement of low temperatures for the transition from the vegetative to the reproductive growth phase (Tondelli et al. 2014). Both winter and facultative types differ in tolerance to vernalization to each other and to spring barley. In addition, these varieties reveal different response to day length. Winter barley varies in sensitivity to day length while facultative barley may be sensitive. In the case of spring varieties, sensitivity or insensitivity to short day length is not relevant (von Zitzewitz et al. 2011).

Barley cultivation is widely distributed and also temperate regions are suitable for cultivation. Von Bothmer (1992) reported about barley diversity centers in central and western Asia, western North America, southern South America and in the Mediterranean region. The high heterogeneity of barley landraces was one of the reasons for high adaptability to different environments until the late 19th century (Box 2008). Barley is also more tolerant to drought, soil salinity and alkalinity compared to other cereals. The wide ecological adaptability is a main factor for cultivation and production of barley for several thousand years.

Human attempts to domesticate cultivated barley from a wild type (*H. vulgare* ssp. *spontaneum*) started about 10,000 years ago in the Fertile Crescent (Badr et al. 2000). Intensive breeding of cultivars, supported by modern sciences and based on repeated crosses between exotic and elite genotypes in combination with subsequent selections, has narrowed the gene base in many species including barley.

Domestication led to the reduction of genetic diversity of modern varieties compared to their wild relatives. This may cause an increase of epidemic disease susceptibility. Despite this narrowing of the genetic diversity during domestication, two major gene changes have taken place in barley.These are non-brittle rachis and six row spike (Sakuma et al. 2011). First, brittle spikes (Btr, shattering) at maturity and grain fall are reasons for introducing non-brittle rachis (*brt*) in cultivated barley (non-brittle is controlled by two tightly linked complementary genes *brt1*, *brt2* on the short arm of chromosome 3H). The second key event of barley domestication changed the number of seed rows on the spike from the dominant wild-type two-rowed barley (*Vrs1*) to the recessive six-rowed barley (*vrs1*). Six row spike barley increases the yield potential relative to two row spike (Sakuma et al. 2011).

Domesticated barley is hulless (free-threshing) or naked. Hulless is controlled by the *nud* gene which is located on the long arm of chromosome 7H (Taketa et al. 2008), Before eating no extra

dehulling process. is necessary. Kinner et al. (2011) demonstrated that naked barley could be a suitable source for food production due to high amounts of β-glucans.
Other useful domestication efforts include the improvement of traits such as disease and insect resistance, seed recovery, increase of yield performance and reduction of seed dormancy. During the past 100 years grain yield increased doubled.
After cross breeding of wild and cultivated species not all traits are positively affected, e.g. yield, caused by hybrid sterility and unfertile offspring due to disturbed chromosome recombination and linkage drag (Grandillo and Tanksley 2005). An effective plant breeding is successful in obtaining favorable varieties or genes which provide as renewable resources for energy and food with respect to future challenges such as global climate change and population growth.

1.2 Barley genome and molecular marker application

Genome sequencing projects started with two model plants: 1. *Arabidopsis thaliana* (The *Arabidopsis* Genome sequencing, 2000) and 2. rice (*Oryza sativa*, International rice Genome Sequencing project, 2005). Using the complete genome sequence, it is possible to identify genes and to determine their functions.

Barley is an important model plant for genetics and genomics of the *Triticeae* and, therefore, for physiological and genetic studies (Bengtsson 1992; Forster et al. 2000). Reasons are: cultivated barley is diploid with 14 chromosomes (2n = 2x = 14, 1H to 7H), a self-pollinator, annual with a short life cycle, has a wide natural genetic variation and a complete genetic map available. The haploid genome size of barley is about 5.3 Gbp (giga base pairs) with more than 80% repetitive DNA. It is one of the largest sequenced genome and about 14 times bigger than the rice genome (Sakuma et al. 2011). The availability of molecular markers, comprehensive EST (expressed sequence tag) collections, BAC (bacterial artificial chromosome) libraries, mutant collections, DNA arrays as well as the production of double haploids and transformation protocols support the study of the barley genome (Sreenivasulu et al. 2008). They reported 437,713 ESTs (expressend sequence tags) from various cDNA libraries of different plant developmental stages and tissues could be created with respect to abiotic and biotic stresses in barley in a period of five years. Zhang et al. (2004) referred to 110,981 ESTs from 22 cDNA libraries of barley and showed 25,224 putative unique sequences for barley. Detection of genes are implicated in specific plant metabolic pathways which was the first uses

of EST collections (Ohlrogge and Benningt 2000). With regards to low cost of this technology, it is possible to generate thousands of DNA sequences to identify a target gene, information about gene expression and metabolism.

To understand the genotype-phenotype relationship, knowledge of allelic variation in gene expression is essential. Three main classes of allelic variation within a genome of the same species are 1. segmental insertions/deletions (InDels), 2. single nucleotide polymorphisms (SNPs) and 3. microsatellites or simple sequence repeats (SSRs) due to differences in the number of tandem repeats at a particular locus (Mammadov et al. 2012). Molecular markers were created to identify these diversities in individuals of a certain progeny at DNA level and are divided into three groups based on detection method and throughput (Mammadov et al. 2012): **a)** low-throughput, hybridization-based markers such as RFLP (restriction fragment length polymorphism) as first generation markers, most commonly used in the late nineties for mapping of quality traits and resistance against several diseases; **b)** medium-throughput, PCR (polymerase chain reaction)-based markers of the second generation containing random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) or microsatellites. Advantages of SSR markers are their multi-allelic nature, co-dominant inheritance, relative abundance, extensive genome coverage and easy detection by PCR (Powell et al. 1996). They are widely used to construct genetic maps in plant breeding. The development of these markers by conventional method is expensive and complicated. Therefore, alternatives for the development of markers (low cost, short time) were important in plant breeding; **c)** high-throughput (HTP) sequence-based markers such as SNPs (single nucleotide polymorphisms, Mammadov et al. 2012). SNP markers are based on a change of a single base within the genome. They are common, highly abundant, bi-allelic in nature, more stable relative to SSRs and, therefore, suitable for genetic association studies (Giordano et al. 1999; Kota et al. 2001; Kota et al. 2003; Mammadov et al. 2012). Kota et al. (2003) reported a new strategy of computer algorithm SNiPping by EST database which is able for rapid SNP identification in barley. Later Thiel et al. (2004) showed a new computer program, SNP2CAPS, for SNP analysis. The conversion of SNP sites into cleaved amplified polymorphic sequence (CAPS) markers has been facilitated by this program.

Low-throughput genotyping based on gel procedures enabled high-throughput technologies that are capable of simultaneously studying thousands of markers based on single nucleotide polymorphisms. These technologies include the Illumina Golden Gate Bead Arrays with 1,524 genome wide SNPs in barley (Rostoks et al. 2006) and the Diversity Arrays Technology (DArT),

which is able to identify and type DNA variation without the need for any sequence information, as described by Wenzl et al. (2004) for barley.

The development of the 22 K Barley 1 GeneChip from EST sequences without knowledge of the fully sequenced genome was reported by Close et al. (2004). It is used for the detection of single-feature polymorphisms (SFPs) that contain both single nucleotide polymorphisms (SNPs) and polymorphisms which are generated during mRNA processing (Luo et al. 2007; Rostoks et al. 2005). Zhu and Salmeron (2007) reported a high correlation between SNP and SFP markers. They described that some favorable characteristics of SNP markers such as abundance, frequency and ease of diagnosis also apply for SFP markers. Luo et al. (2007) pointed out that SFPs markers are useful genetic markers which potentially suggest a physical link to the structural genes themselves. Ophir et al. (2010) explained the useful application of SFP technology for performing discovery and fine mapping of traits. These markers can also detect genetic variation (frequently a SNP or small indel) through their sequence. Rostoks et al. (2005) detected more than 10,000 SFP in barley.

A further high-throughput technology is known as next generation sequencing (NGS) which makes it possible to generate the DNA sequence of a total plant genome in a short time and high sequence capacity in a never before possible speed (Varshney et al. 2009). This technology is able to analyse genomic resources and develop molecular markers for the identification of kinship relationships of plants and for the selection of desired traits in plant breeding. Molecular markers are widely used in far-distant crosses and alien gene introgression, analysis of expression, QTL mapping, genetic association mapping and population genetics (Varshney et al. 2009). This includes parental genotyping of mapping populations or wild relatives which can expedite marker development (e.g. SSR (simple sequence repeats), SNP). These markers are important tools to create genetic maps, for QTL detection and also for monitoring alien genome introgression in the case of wild crosses. Genetic association or population biology is one of the main application of NGS (Varshney et al. 2009).

In addition to other markers (e. g. morphological, biochemical) molecular markers are also an unique tool in plant breeding. In general, they can be used indirectly for trait selection if this marker is linked with the trait of interest. This marker-assisted selection (MAS) can be used even at juvenile growth stages and could save time and costs, resulting in a fast selection process with high accuracy. This has been already successfully applied to both qualitative (monogenic) and quantitative (polygenic, QTL) disease resistance. Well known examples of MAS in barley breeding are the use of the recessive resistance genes *rym*4 (resistance to yellow

mosaic virus complex) and the *mlo* resistance against barley powdery mildew (Miedaner and Korzun 2012). In the last 10 years great efforts have been made to access the complete sequences of the barley genome. The International barley genome sequencing consortium (IBGSC) was able to develop a physical map (4.98 Gb of the entire barley genome), integrating more than 3.9 Gb into a high resolution genetic map (Mayer et al. 2012). Now, barley is completely sequenced as largest sequence genome with 5.2 billion base pairs (Mascher et al. 2017). This so-called reference genome will generally influence the basic research in cereals (Keller and Krattinger 2017). In 2016 an international team published the genome sequencing of 6000 years old barley grain. This barley genome is the oldest ever reconstruct plant genome. The research team found that the cultivated, 6000 years old varieties genetically clearly different from this wild ancestor. However, they are quite similar with today's domesticated varieties (Mascher 2016). This study opens up new insights into the origin of today's crop and the domestication process. In the future new and improved barley cultivars can be bred with better disease resistance and adaptation to changed environmental and climatic conditions.

1.3 Introgression lines in barley

The domestication of today's crops started about 10,000 years ago in the Fertile Crescent (Badr et al. 2000). This ultimately led to a reduction in genetic diversity, as is the case with our current barley gene pool compared to its wild ancestors (Tankley and McCouch 1997). However, wild ancestors are still useful sources for improving important plant traits such as resistance to diseases, tolerance to biotic and abiotic stress, quality and yield. The introgression of exotic germplasm for trait improving during the breeding of new varieties was reviewed by Hajjar and Hodgkin (2007). A successful use in barley is the introgression of the *mlo* (mildew resistance locus o on the long arm of the barley chromosome 4H) gene from an Ethiopian landrace into European spring barley (Jorgensen 1992).

One strategy to transfer exotic genes into modern cultivars is the AB-QTL (advanced backcross-quantitative trait locus) approach (Tanksley and Nelson 1996), which was successfully applied to main crops such as rice (Xiao et al. 1998), maize (Ho et al. 2002), barley (Pillen et al. 2003, Pillen et al. 2004) and wheat (Huang et al. 2003). A further approach of utilization is given by the development of introgression lines (ILs). Zamir (2001) defined that each IL includes a small chromosome segment of an exotic donor parent in the background of the elite recipient parent. Ideally, a complete set of introgression lines contains the entire genome of the donor. Based on the genetic diversity present in such ILs, useful genes

improving agronomic traits may be found. Keurentjes et al. (2007) reported that ILs can act as an ideal source to detect small QTL effects. Initially ILs were developed and tested in tomato (Eshed et al. 1992). Subsequently, ILs were also obtained in other crops including rice (Guo et al. 2013) and wheat (Liu et al. 2006; Pestsova et al. 2001). First ILs in barley were introduced by Schmalenbach et al. (2008).

The S42IL population originated from the crossing of the German spring barley (*Hordeum vulgare* ssp. *vulgare* (Hv) elite cultivar 'Scarlett' and the Israeli wild barley (*Hordeum vulgare* ssp. *spontaneum* (Hsp) accession 'ISR42-8' (Figure 1). Advanced backcrossing with 'Scarlett' and subsequent marker-assisted selection culminated in a set of 73 S42ILs in BC_3S_4 generation (Schmalenbach et al. 2011). Each introgression line is characterized by a specific *Hsp* segment of the wild donor parent (Figure 2), which was incorporated into the background of the cultivated recipient 'Scarlett'.

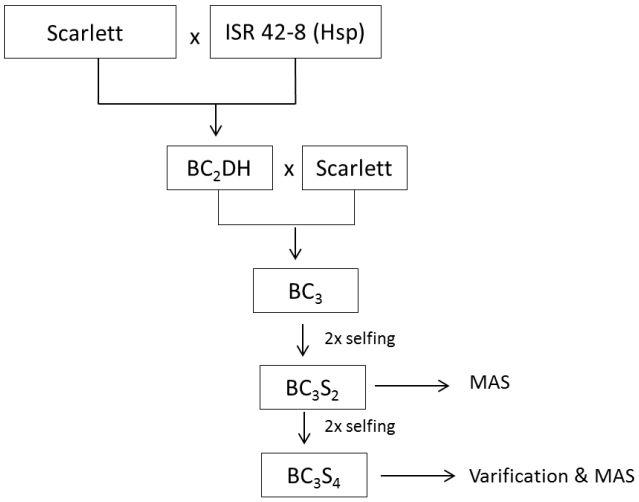

Figure 1: Crossing scheme for the development of the S42IL population (Schmalenbach et al. 2008)

Schmalenbach et al. (2008), Schmalenbach et al. (2009), Schmalenbach and Pillen (2009) and Wang et al. (2010) analyzed and detected wild barley QTLs of the introgression library S42IL controlling yield-related traits, malting quality, flowering time and resistance against pathogens. Later on, the barley ILs were also used to detect QTLs for drought tolerance (Honsdorf et al. 2014a; Honsdorf et al. 2014b) and nitrogen stress tolerance, studied in glasshouses (Schnaithmann and Pillen 2013) and in hydroculture (Hoffmann et al. 2012).

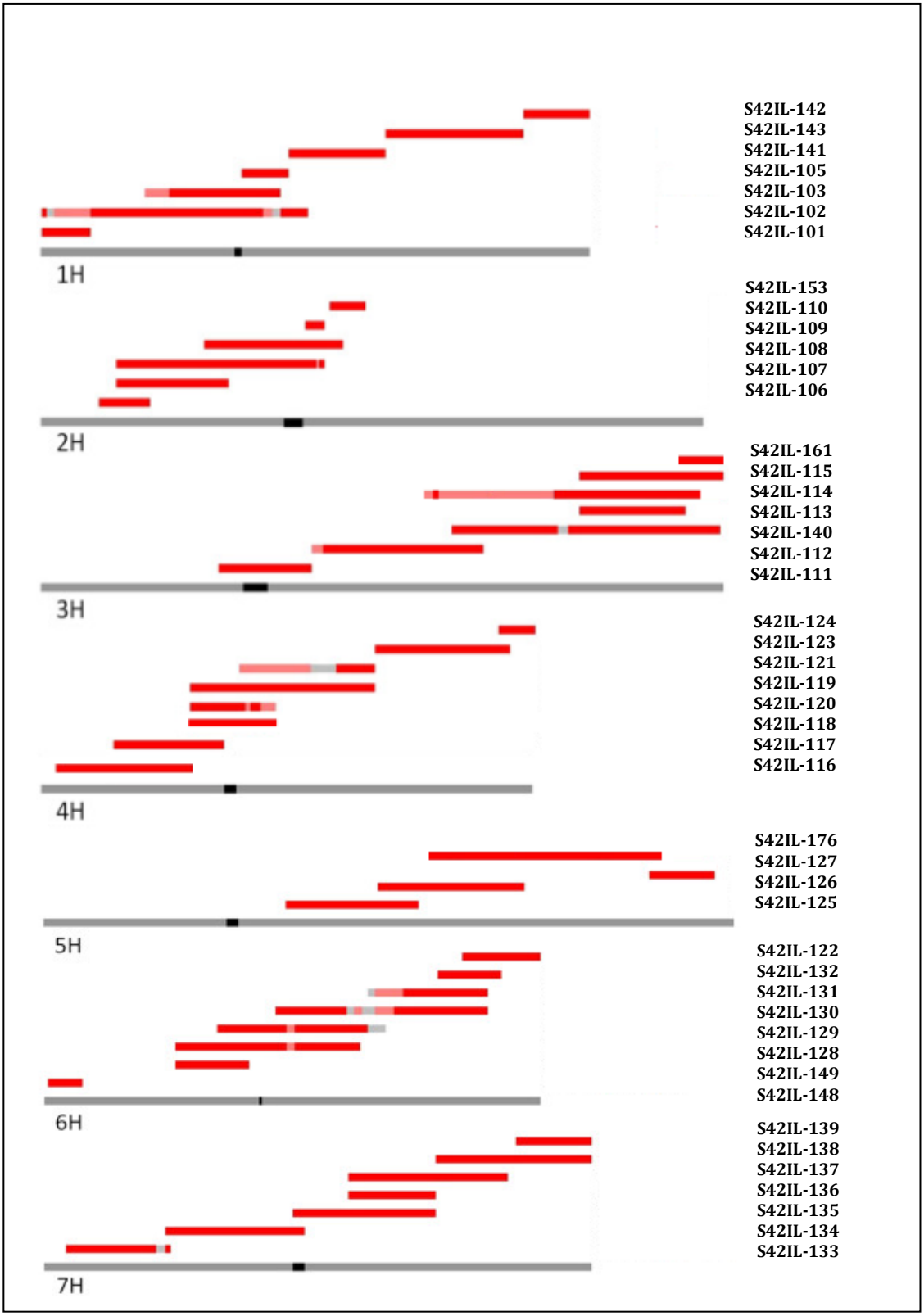

Figure 2: Position of the wild barley introgressions on barley chromosomes 1H to 7H (grey bars; Black box within the chromosome represents the centromere region. In each chromosome homozygous *Hsp* loci, heterozygous *Hsp* loci and loci without SNP data are presented in red, pink and light grey, respectively).

1.4 Global climate change and plant cultivation

In recent years, climate change and its possible effects such as rising temperatures and carbon dioxide (CO_2) concentration in the atmosphere are increasingly being discussed. This change can be caused by a ever-growing world population and an associated increased demand for food and energy. Human activites such as burning of fossil fuels and altered land surface uses promote higher carbon dioxide and other gas concentrations. The atmospheric CO_2 concentration fluctuated over the past 400,000 years between 200 and 300 ppm. At the end of the last ice age about 10,000 years ago it was about 200 ppm. The CO_2 concentration increased significantly since the pre-industrial level of 280 ppm to 379 ppm today (Craufurd and Wheeler 2009). That means a CO_2 concentration increase by 30% from the pre-industrial to the current CO_2 level. It is assumed that this concentration will double by the end of this century (Moore et al. 1999). A climate change from which negative results are expected for the living conditions in most parts of the world is presumably connected with this development. But not only temperature and CO_2 changes are to be expected. In addition, increased CO_2 leads to changes in precipitation. Based on the IPCC (Intergovernmental Panel on Climate Change) reports, a maximum global mean temperature increase of 6.4°C and a reduction of the annual precipitation at the end of this century are predicted (Reddy et al. 2010). These changes will have direct and indirect positive or negative effects on physiological, morphological and developmental processes in plant development and growth. Most studies are conducted to investigate the physiological plant response under rising CO_2 concentration.

Carbon dioxide (CO_2) as basic substrate for photosynthesis is essential for plant growth. Carbohydrates are synthesized using sunlight and are indispensable for the formation of plant structures and as an energy beneficiary for metabolic processes (Taub 2010). The response of plants to increasing CO_2 depends both on the plant age (Ingvardsen and Veierskov 1994; Stitt 1991) and the duration of exposure. Prominent is particularly the so-called "CO_2 fertilization effect" because the increased concentration also leads to an improved absorption of the gas, in particular in C3 plants such as barley, to an increased growth and photosynthesis rate (Bowes 1993). Consequently, more biomass is formed and higher yield performance is the result, along with an improved water use efficiency and a reduction of transpiration (Drake and Gonzalez-Meler 1997). But even opposite effects of increasing CO_2 concentration have been reported. The down regulation of photosynthesis (van Oosten et al. 1994) decreases the nitrogen concentration in a plant (Cotrufo et al. 1998) and the acceleration of senescence as in barley and wheat (Fangmeier et al. 2000; Zhu et al. 2009). This in turn causes a change in gene expression as a long-term impact of higher CO_2 concentration, which is attributed to an

increase of non-structural carbohydrates (sucrose and starch) and an imbalance between sink and source in plants such as wheat (Nie et al. 1995) and tomato (van Oosten et al. 1994). Positive effects include an increase of photosynthesis, leaf area, tiller number, biomass and yield (Clausen et al. 2011; Fangmeier et al. 2000; Rao et al. 1995). Thus, Fangmeier et al. (2000) found a 38% increase in biomass and yield at high CO_2 concentration in barley, caused by a higher tiller production and tiller survival. An increase in grain yield was also reported by Jitla et al. (1997) in rice. In addition to this CO_2 impact on biomass, flowering time, leaf senescence and water use efficiency are affected. Sicher and Bunce (1998) reported that the reduction of photosynthetic rate accelerates senescence in barley and wheat under high CO_2. On the one hand, the effect of rising CO_2 on water availability corresponded with a lower transpiration, since the opening of the stomata necessary for CO_2 absorption can be reduced. On the other hand, leaf surface temperature is increased by the lower evaporation cooling effect. Wullschleger et al. (2002) observed that rising CO_2 concentrations resulted in an increase of root volume by an improved carbon allocation to root growth. This enables plants to absorb water from deeper soil layers. All together improves water use efficiency and leads to improved drought stress tolerance. Plants cultivated under salt stress conditions had an improved salt tolerance under elevated CO_2 (Schwarz and Gale 1984).

In summary, it can be stated that rising CO_2 not only leads to higher temperatures, but usually directly favors photosynthesis, plant growth and yield. This is called 'CO_2 fertilization effect'. Most crops are either C3 plants such as wheat, rice, barley, potatoes and soya bean or C4 plants such as corn, sorghum and sugarcane. In C3 plants which account for 90% of the earth's landplants, a doubling of atmospheric CO_2 concentration acts in a yield increase of about 20%. In contrast C4 plants with a special mechanism to store carbon dioxide only realize a yield increase of a few percent. Compared with C4 plants, C3 plants are the bigger calorie provider in the world. Ninety % of the required global amount of calories are provided by twelve plant species with a C3 photosynthetic pathway (Reddy et al. 2010).

1.5 Hydroponic plant cultivation

There are different ways to cultivate plants. Originally, plants grow in soil. The soil itself is not necessary for plant cultivation, but the mineral nutrients contained therein are essential for plant growth. If these nutrients are available to the plants together with water, no more soil is necessary as root substrate. With hydroponics plants can grow in an aqueous nutrient solution instead of soil. In the 19th century, plants were cultivated soilless in nutrient solutions. These

hydroponics are used to study plant nutrition (Epstein and Bloom 2005), but also to commercialize plant production in the greenhouse. According to Taiz and Zeiger (2008) different techniques are distinguished: **1**. the hydroponic growth system, the classical hydroculture, where the roots of the experimental plants are constantly in the nutrient solution enriched with oxygen; **2**. the nutrient-film growth system, where the oxygen-enriched nutrient solution rinses the roots in the cultivation container over a slope; **3**. the Aeroponic growth system, where the roots are constantly sprayed with the nutrient solution; and **4**. the ebb and flow system. where the nutrient solution in the root space periodically rises and falls again. With hydroponics plant growth can be simulated with a defined nutrient solution (including pH value and oxygen content) comparable to the soil (Epstein and Bloom 2005).

Hydroponics as one possibility of agriculture does not use soil but nutrient-rich water solutions for plant nutrition. The basic prerequisites for a hydroculture medium are the supply of plants with the necessary nutrients, an adequate supply of oxygen to the roots and the adjustment of a plant-specific optimal pH value (Epstein and Bloom 2005). Both macronutrients (nitrogen, potassium, calcium, phosphorus, sulfur and magnesium) and micronutrients (chlorine, iron, boron, manganese, zinc, copper and molybdenum) are essential for plant metabolism. Nickel, silicon, cobalt and vanadium are also used depending on the culture plant type to be investigated (Epstein and Bloom 2005). An optimal concentration is to be set here since young plants are very sensitive to excessive nutrient concentrations (Taiz and Zeiger 2008). With large amounts of nutrient media, which circulate constantly in the hydroculture system, this is usually no problem. Furthermore, plants can be grown under controlled environmental conditions. An advantage of hydroculture systems compared to soil as a medium is that natural occurring environmental effects can be reduced (Beatty et al. 2010). This also makes it possible to better control the root space, which facilitates the phenotyping of root and also shoot features. Hydroponics requires no fertile soil area, less water and pesticides compared to traditional agriculture, but also the examination of plant response to environmental stresses and of plant-microbe interactions.

Plant cultivation in water culture was applied in mint plants by John Woodward in 1699 (Singh and Davidson 2016). He noted that less-pure water sources were better suited for cultivating the plants than distilled water. This led to the determination of the essential nutrients required by plants. In 1842 the nine elements which are necessary for plant growing, were found.

The soil-free hydroponic system is ideally suited for plant growth under defined nutrient solution composition in a short (only juvenile) growth time. In addition, root growth is not affected by soil and nutrients are more easily accessible. The investigation of deficiencies and toxicities of macro- and micronutrients is also possible with low costs. The special advantage of

hydroponics is the possibility of plant phenotyping (shoots and roots), mainly by root investigations in a short time. In particular, the root remains almost undamaged, which is hard to achieve in a solid culture medium (Epstein and Bloom 2005).

1.6 Plant cultivation under nutrient stress

Each plant places high demands on nutrient supply. For optimal growth and development several macro- and micronutrients are necessary in different quantities, which occur in soil, water and air. Carbon (C), hydrogen (H) and oxygen (O) are primarily derived from water (H_2O) and air (CO_2). These elements are the basic prerequisites for carbohydrate synthesis (photosynthesis), plant structure and the energy supply of plants. The classification of the nutrient elements in macro- and micronutrients is based on the quantity required by the plant (Warne 2014). Plants need high amounts of the six essential macroelements which comprises two groups: the three primary core nutrients (nitrogen (N), phosphorus (P) and potassium (K), involved in enzyme functions and biochemical processes which controlling growth and yield in plants) and the secondary nutrients calcium (Ca), magnesium (Mg) and sulphur (S). The 10 essential microelements are consumed by the plants in considerably smaller quantities, such as iron (Fe), chlorine (Cl), manganese (Mn), zinc (Zn), sodium (Na), copper (Cu), boron (B), molybdenum (Mo), cobalt (Co) and nickel (Ni). A lack of these trace elements can lead to considerable losses in yield and quality.

Natural soil should contain all the essential macro- and micronutrients needed for growth. However, the amount of these nutrients is constantly consumed by plants (absorbed as cations or anions, mainly by the root), which can subsequently lead to a reduction in plant growth and yield and soil fertility. This is also the case when only one nutrient is insufficiently present, while all others are sufficiently available or supplemented. If a nutrient deficiency is observed, it should be remedied as soon as possible. The later this happens, the longer the plants are exposed to physiological stress, which reduces their productivity.

Even today it can be observed that plants suffer from nutrient lacks in some soils. The optimal use of mineral fertilizers can prevent this, but requires appropriate knowledge for a correct fertilizer quantity and composition and fertilization time. The economic benefits of fertilizers on crop yield are well documented. However, it is not enough to continously monitor and improve soils. In fact we must fully understand the effect of each nutrient on plant development and growth. But also the interaction between different nutrients and the plant reaction to the nutrient imbalance in the soil must be considered.

The availability of necessary nutrients to achieve an optimum yield and quality is important, too, when growing barley. The successful production of barley is also dependent on the availability of the three macronutrients nitrogen, phosphorus and potassium, and to a lesser extent on manganese, copper and zinc. In this study we focus on the response of plants of the wild barley introgression line (S42IL) library to different levels of phosphorus and potassium supply. These two macronutrients are needed to a considerable amount for plant growth and development and are first described separately.

1.6.1 Phosphorus

Natural phosphorus (P) reserves are less and less available. Both organic and inorganic phosphorus are found abundantly in the soil, but mostly in insoluble forms. Plant growth is thus limited by the low availability of free phosphate (Pi). Phosphorus is a major macronutrient in plants and plays a crucial role in many life processes (Bovill et al. 2013). Phosphate promotes both shoot and root growth and is involved in key physiological processes such as energy storage, photosynthesis activity and cell division. Phosphorus is a component of nucleotides and, thus, required for storage and expression of genetic information. Therefore, phophates are indispensable for young plants. The highest phosphorus concentration is found in the developing plant parts, such as roots, shoots and the vascular tissue.

A limiting factor for plant growth is the limited absorption of phosphorus by the root. Inorganic phosphates in ionic form are very easily absorbed. Organic phosphate on the other side does not. The phosphate form in solution is dependent on the pH value. Plants can absorb two forms of P ions, HPO_4^{2-} or $H_2PO_4^-$. Therefore, the phosphoric acid (H_3PO_4) dissociates in an acidic environment to $H_2PO_4^-$ and in a neutral environment to HPO_4^{2-}. The pH value of the soil influences the subsequent transport of the soluble phosphorus ions into the plants, whereby about 0.1% of the total P-concentration can be utilized by plants (Sharma et al. 2013).

Phosphate deficiency may result in reduced growth and yield. Plant responses to phosphorus starvation are caused by changes in morphological (influence on shoot and root growth), physiological (increase of phosphorus uptake and mobilization of internal phosphorus) and metabolic (change of primary and secondary metabolism) processes (Hammond et al. 2004). Visible symptoms of phosphorus deficiency in plants are growth inhibition, weak tillering (dark to blue-green leaves, purple shoots (anthocyanin) and leave sheaths). Anthocyanin protects nucleic acids from UV damage and chloroplasts from photoinhibitory damage (Amtmann et al. 2005). Zhang et al. (2014) explained responses of plants to phosphorus

deficiency such as increased phosphorus uptake and internal phosphorus recycling by mechanisms such as intensification of acid phosphatase secretion, increase of transcription factors and phosphorus transporters and changes in root morphology. This includes the reduction of primary root length and an increase of root hair and lateral root elongation. Under low phosphorus treatment Hammond and White (2011) also confirmed the importance of re-location of carbon from shoots to roots resulting in an increase of root size relative to shoot size. In addition, the induction of sugar signaling pathways and changes in expression of genes and proteins, which are associated with plant responses to phosphorus starvation, are also important (Hammond and White 2011).

The economic benefits of fertilization on crop production are well documented and application of phosphate fertilizers supports plant growth and increase yield. The application of phosphate fertilizers is, however, cost intensive and may lead to environmental harm (negative effects on soils (fertility and microbial composition) and waters). As noted by Vance et al. (2003), improving phosphate use efficiency (PUE) is an important ecological and economic aspect. Assuring crop yield and quality that grow in low phosphate containing soils would be advantageous (Clark and Duncan 1991). For this, interactions between genotype, environment and cultivation management have to be taken into account to improve PUE (Manschadi et al. 2014). In general, nutrient use efficiency (NUE) is defined by Moll et al. (1982) as the ratio of crop yield by nutrient supply from soil and fertilizer. NUE can be further partitioned into the product of nutrient acquisition efficiency (NAE) and nutrient utilization efficiency (NUtE), which is defined as the total nutrient in the above-ground plant organs at maturity per unit of nutrient supply and the crop seed yield per unit of nutrient taken up, respectively. Manschadi et al. (2014) also emphasized the close association of enhanced phosphorus uptake with root size and root architecture. Several studies in this regard were conducted. QTLs for phosphate uptake (PUP) and PUE were detected in wheat (Su et al. 2009). In rice, Wissuwa et al. (1998) mapped QTLs for dry weight and phosphate uptake and Ni et al. (1998) found QTLs for phosphorus deficiency tolerance. QTL mapping has also been described for other plant species including *Arabidopsis* (Reymond et al. 2006), *Brassica* (Yang et al. 2011) and maize (Li et al. 2010; Zhu et al. 2005).

In adddition, phosphate starvation response (PSR) genes which, induced, can make both internally and externally bound phosphorus available to plants. They encode enzymes to compensate for phosphorus deficiency. PSR enzymes cause the release of organically bound phosphorus from different sources, for example, from soil, plant storage tissue, senescent

organs and intracellular compartments. The genes are classified in two groups with ‘early’ and ‘late’genes.

‘Early’ genes respond quickly and often not specifically to phosphorus starvation. They are also associated with increasing expression with genes which coding general stress-related proteins and different transcription factors (Amtmann et al. 2005; Chen et al. 2007; Devaiah et al. 2007a; Devaiah et al. 2007b; Franco-Zorrilla et al. 2004; Mukatira et al. 2001; Nilsson et al. 2007). Four transcription factors, including PHR1 (phosphate starvation response 1), are induced in *Arabidopsis* in response to P deficiency (Chen et al. 2007; Devaiah et al. 2007a; Devaiah et al. 2007b; Nilsson et al. 2007). The *phr1* mutant in *Arabidopsis* resulted in a reduction of anthocyanin and sugar and starch accumulation, as well as a modified phosphorus allocation between root and shoot.

‘Late’ genes damage (Amtmann et al. 2005) is responsible for changes in morphology, physiology or metabolism of plants with prolonged P deficiency and generally improve the acquisition of P or promote the efficient use of P within the plant (Hammond et al. 2004; Vance et al. 2003). An increased P uptake from the soil (through a more effective root surface) and an improved phosphate use effeciency within the plant are late responses of plants. Therefore, phosphorus starvation leads to root changes, such as increased lateral root formation, prolonged or decreased length of the primary root (Hodge 2004).

1.6.2 Potassium

Analogous to phosphorus, potassium deficiency is in the following considered in more detail. Potassium is the eighth most abundant element of the earth with a proportion of about 2.5% in the lithosphere (Shin 2014). Its absorption by the plant root is dependent on the soil type. Freely available potassium is present in the soil in positively charged inorganic potassium ions (K^+), which are needed to distribute potassium in the corresponding concentrations into the cell compartments after uptake (Gierth and Mäser 2007). Maintenance of an adequate potassium concentration in all plant cells is essential for plant growth and reproduction. The required potassium concentration for optimal enzyme activation in the cytosol is about 100 µM. Constant potassium concentration in vacuoles is variable and depends on the potassium status of the plant. The vacuole acts as a K^+ reservoir to maintain a constant concentration in the cytosol under potassium starvation. Therefore numerous transporters of K^+ and K^+ channels are involved in potassium homeostasis to provide the suitable cytosolic potassium concentration. Most of the potassium is bound to clay minerals and humus so that potassium ions can easily be released into the soil if required. Potassium, which is firmly bound in crystal

lattices of silicates, can't be absorbed by plants. Soils often have a low basic potassium concentration. But also leaching and intensive agriculture contribute to the potassium deficiency of soils. Consequently the lack of adequate absorption of potassium by root from soil is one of the limiting factors for yield and quality in crops. An improved genetic K utilization efficiency (KUE) of crop plants can contribute to the solution of this problem.

Potassium is one of the big macronutrients, which is also involved in numerous life processes of plant growth and reproduction (Schachtman and Liu 1999). The amount of K in plant tissues can vary considerably. Characteristic in comparison to others is its high concentration in the plant, which can reach up to 8% of plant dry weight (Grabov 2007). But a potassium concentration of less than 10 g / kg dry weight is responsible for the corresponding deficiency symptoms in most species (Gierth and Mäser 2007). Potassium plays vital roles in growth, stress adaptation and central metabolic processes such as photosynthesis, protein biosynthesis, osmoregulation, turgor controlled movements and maintenance of plasma membrane potential. Wang et al. (2013) pointed out the significant role of potassium in growth and cell metabolism, which improve plant resistance to diseases, animal pests, drought, salinity, cold and frost. Also potassium is implicated in other activities such protein synthesis (Römheld and Kirkby 2010), activation of enzyme (Maathuis 2009), movement of solutes in the phloem (White and Karley 2010), photosynthesis activity (Hermans et al. 2006), biotic and abiotic stress and crop quality (Römheld and Kirkby 2010), osmotic regulation and neutralization of negative electrical charge from organic molecules (Grabov 2007).

Low potassium deficiency does not directly lead to visible symptoms due to the high rate of redistribution between mature and developing tissues. Later, chlorosis and necrosis are visible on the mature leaves (Römheld and Kirkby 2010). Other negative effects of potassium deficiency in plants are the inhibition of shoot growth and development of generative organs such as seeds (Römheld and Kirkby 2010), associated with decreased crop quality and yield performance. One way to avoid potassium deficiency and to maintain yield performance and improve disease resistance in food crops is the use of appropriate fertilizer. The use of fertilizer has increased by 4.4% per year worldwide from 1999 to 2005 and it was anticipated that this amount increased to 12% during the last decade (Aleman et al. 2011). According to Wang and Wu (2015), the global potassium fertilizer consumption will continuously increase from 28.6 Mt potassium oxide (K_2O) in 2012 to 33.2 Mt in 2016. The practically oriented application of fertilizer generally helps to mitigate or to prevent the negative effects of potassium deficiency, but can also contribute to environmental, ecological and economic problems. Rengel and Damon (2008) pointed out that the cultivation of genotypes with

improved uptake and utilization efficiency of the nutrient instead of chemical fertilizer usage has a positive effect on environment. Another possibility is, therefore, the breeding of potassium efficient genotypes which are able to achieve a sufficient yield performance with low potassium concentration in the soil (Rengel and Damon 2008). Shin (2014) postulated four strategies for improving the potassium use efficiency: 1) increased root volume, 2) increased potassium uptake efficiency from soil and translocation into plants, 3) increased potassium mobility in the soil and 4) breeding of new varieties with improved potassium efficiency by marker-assisted selection as a result of the identification and utilization of potassium-associated QTLs. Increase of root hair elongation has been resulted in pea, red clover, alfalfa, barley and ryegrass (Høgh-Jensen and Pedersen 2003). Significant differences in potassium utilization and uptake efficiency between various genotypes could be found in several crops such as wheat (Damon and Rengel 2007), canola (Damon et al. 2007), barley (Shivay et al. 2003) and rice (Yang et al. 2003; Yang et al. 2004).

When potassium supply is low, plants must try to ensure a stable potassium concentration in their cells and tissues via potassium uptake and transport. Plants react to potassium stress by altering gene expression. Changes of gene expression of potassium transporter and channels are induced by potassium deficiency (Ashley et al. 2006). A large number of transporter genes are generally found in higher plants (13 in *Arabidopsis* and 17 in rice (Shin 2014). In *Arabidopsis* and rice, candidate genes such as jasmonic acid-related enzymes, cell wall proteins, Ca^{2+} signal proteins, protein kinase and ion transporter families could be localized (Ruan et al. 2015). In two Tibetan wild barley genotypes transcription factors under low potassium were detected (Zeng et al. 2014).
Other influencing variables in K deficient plants are reactive oxygen species (ROS), plant hormones, enzyme activation and amino acid level. ROS such as H_2O_2 are involved in many physiological processes. An increase of root hair length in barley plants (Høgh-Jensen and Pedersen 2003) could be described with ROS. The concentration of plant hormones such as auxin and ethylene is altered in response to potassium stress. Potassium is also involved in enzyme activation in plants. A high number of these enzymes are implicated in sugar and nitrogen metabolism, where potassium acts as a 'K sensor'.
Another effect of potassium deficiency was observed on amino acid level. In *Arabidopsis* changes in glutamine and glutamate (increase and decrease, respectively) under low potassium could be observed (Amtmann et al. 2005).

Reduced fertilizer use and degraded soil fertility led to studies on the adaptability of crop plants to low potassium (Høgh-Jensen and Pedersen 2003). An improved K utilization

efficiency (KUE) of crop plants can help to overcome K deficiency problems. Different plant species and even different varieties (genotypes) of the same species often show differences in this utilization efficiency. This can be used for the selection or the genetic modification of the KUE. The KUE is particularly dependent on the K acquisition capability of the plant roots. It is assumed that the KUE can be positively influenced by the optimization of the plant root architecture and the root K uptake activity, but also by the K transport and the translocation into the plant tissues and organs. The selection within the natural variation or found QTLs can be valuable sources for the breeding improvement of our cultivated plants (Wang and Wu 2015).

1.7 Hyperspectral imaging (HSI)

Hyperspectal imaging (HSI) is used in various research areas, such as geology (Resmini et al. 1997), environment (Clark et al. 1997), medicine (Ravn et al. 2008), agriculture (Lawrence et al. 2003), food quality and safety (Qiao et al. 2007).

This promising advanced method, assessing intrinsic biochemical compounds of plants at a higher sensitivity and at an increased spatial resolution, is hyperspectral imaging (Arvidsson et al. 2011, Furbank and Tester 2011, Backhaus and Seiffert 2013; Dale et al. 2013). The aim of the imaging analysis of plants is to analyze the development and growth as well as the physiological and qualitative characteristics of plants. Meanwhile, imaging automation is quite cost-effective and universally applicable. The technical progress of available complex imaging systems led to high-throughput plant phenotyping. Plant phenotyping is based on automated imaging methods to allow genetic and molecular analyses of different plant genotypes, QTL analyses, but also statements on gene expression with respect to changes in environmental conditions (Fahlgren et al. 2015). Especially phenotyping experiments in the greenhouse and growth chamber benefit from controllable environmental conditions and plant growth. Bucksch et al. (2014) demonstrated that defined environmental conditions are suitable for root phenotyping. For the effective use of modern imaging methods, phenotypic data must be linked with the corresponding molecular data.

In order to determine the nutritional status of plants, macro- and micronutrients need to be quantified in a destructive approach to draw conclusions on nutrient deficiencies or inadequate nutrient supply of plants and the need to fertilize. Generally, chemical analysis in the laboratory requires wet digestion of plant samples to determine the nutrient status in plant tissues. However, chemical analysis is time consuming and costly for large sample sizes. In

plant breeding non-destructive, rapid and cheap methods for the detection of the nutrition status of plants are of increasing interest. This includes near-infrared spectroscopy (NIRS), a standard method already established during the past decades (Cabrera-Bosquet et al. 2012). This method is used for quantitative and quality assessments of agricultural products like seeds, flour, fibers, etc. with respect, for example, to determine contents of moisture, protein, fat, starch and fibers. The advantages of NIRS include short analysis time with an easy-to-handle measurement and without the use of chemicals.

Multispectral imaging systems used differed from hyperspectral imaging systems in terms of the number of spectral bands and the spectral resolution (Elmasry et al. 2012). While the number of spectral bands in multispectral imaging systems is very small (typically less than 10 bands), hundreds of contiguous and regularly spaced bands are the rule in hyperspectral images. Hyperspectral images are generated by reflected, transmitted or emitted light in ultraviolet (200-400 nm), visible and very near-infrared (400-1000 nm) as well as near-infrared (900-1700 nm) and shortwave-infrared range (1000-2500 nm) as a function of the type of detector (Elmasry et al. 2012). HSI relies on a mobile data input using a hyperspectral camera and their evaluation with a special software. The hyperspectral imaging technique simultaneously provides spatial information and spectral information for each pixel in the image. This three-dimensional information (3D cube) can be analyzed to determine the physical and chemical properties of the experimental samples (Elmasry et al. 2012; Lorente et al. 2012). The biochemical composition of each recorded camera pixel can be determined after modelling the reflectance spectrum from a number of calibration samples, which were chemically analyzed beforehand. The software then provides a prediction of the trait of interest to be determined based on a hyperspectral fingerprint. In our study, this refers to the nutrient concentration which is present in the tissue.

Applying HSI Zhang et al. (2013) were able to detect the distribution of macronutrient concentrations in leaves of oilseed rape. With the HSI technique it was also possible to estimate the accumulation of leaf nitrogen in wheat (Yao et al. 2010), the chlorophyll distribution in cucumber leaves (Zou et al. 2011), to predict sweetness and amino acid content in soybean (Monteiro et al. 2007) and to explore abiotic stress like salt tolerance (Sytar et al. 2017).

1.8 Objectives

The present study focused on wild barley QTLs controlling phosphorus (P), potassium (K) and carbon dioxide effects (CO_2) in the barley population S42IL. For this, root and shoot growth as well as nutrient concentration under two levels of P and K supply (control and low) and shoot growth under two levels of CO_2 supply (control: 380 ppm, high: 760 ppm) of juvenile S42IL plants were analysed. Our goal was to locate exotic barley alleles, present within the introgression lines, which improve trait performance either within a single treatment (P10, P100, K0, K100, N CO_2 and H CO_2) or across both treatments in comparison to the control genotype 'Scarlett'. This work may assist to detect desirable ILs and QTLs, exhibiting improved growth and nutrient concentration under P or K deficiency as well as growth changes under increased CO_2, which may be utilized in future barley breeding programs:

- Identification of S42ILs showing a significant increase or decrease in plant growth compared to the recipient parent 'Scarlett' under different nutrient treatments (P or K in hydroculture experiments) and increased CO_2 concentration (in growth chamber tests)
- Detection of QTLs controlling phosphorus or potassium stress tolerance and plant response to rising CO_2 concentration in the atmosphere.

2 Material and methods

All experiments of the present study were conducted in the greenhouse (hydroponics) and in a growth chamber (carbon dioxide) of the experimental stations of the Martin-Luther-University Halle-Wittenberg in 'Heide Süd' and 'Kühnfeld' in 2013 and 2014, respectively. The individual experiments lasted 14 or 15 days each and took place under two different treatment levels of phosphorus, potassium (control and reduced) and atmospheric carbon dioxide (control and elevated).

2.1 Plant material

In all three different investigations juvenile barley (*Hordeum vulgare*) plants of the wild barley introgression library S42IL (Schmalenbach et al. 2008) and the German elite barley cultivar 'Scarlett' as control genotype were grown. From this set, 47 introgression lines (Table 1) were selected in order to represent a maximum of 87% of the wild barley *Hsp* donor genome.

Table 1: List of 47 wild barley introgression lines studied (source: Schmalenbach et al. 2011)

Introgression on barley chromosome	**Number of investigated lines**	**Name of S42 introgression line**
1H	7	101, 102, 103, 105, 141, 142, 143
2H	6	106, 107, 108, 109, 110, 153
3H	7	111, 112, 113, 114, 115, 140, 161
4H	9	116, 117, 118, 119, 120, 121, 122, 123, 124
5H	4	125, 126, 127, 176
6H	7	128, 129, 130, 131, 132, 148, 149
7H	7	133, 134, 135, 136, 137, 138, 139
Total	47	

2.2 Methods

2.2.1 Hydroponic plant cultivation

Hydroponic culture experiments with juvenile barley plants under two treatment levels were carried out in a greenhouse compartment of the experimental station of the Martin-Luther-University Halle-Wittenberg in 'Heide Süd' (Fig. 3). Prior to each 14-day long experiment seeds were protected against seed-borne diseases by application of 2 µl/g Landor CT, Bayer CropScience (Monheim, Germany) and germinated in petri dishes at room temperature. After 3 days, barley seedlings were selected for hydroculture, bearing a short coleoptile and 3 to 5 rootlets, approximately 2 cm in size. The roots of the selected seedlings were passed through

1.5 ml Eppendorf tubes with open bases. One hundred-forty Eppendorf tubes with seedlings were placed in a perforated tray organized in a 10 x 14 grid. Subsequently, the tray was placed on top of a box filled with a nutrient solution as reported in Hoffmann et al. (2012). To avoid border effects due to varying light and space conditions 'Scarlett' seedlings were planted in the 44 outer holes of each tray. These border plants were not used for scoring. A different number of boxes were used. But each box held 2 blocks, i.e. a tray with 48 barley seedlings per block, namely 47 introgression lines and the recurrent parent 'Scarlett' (Fig. 3). Within a block, seedlings were completely randomized. The boxes holding the barley seedlings of the same treatment were connected to a 200 L nutrient solution tank, to secure a continuously circulation of the nutrient solution through a pump (Hoffmann et al. 2012). Plant roots were completely immersed within the nutrient solution of the boxes. In addition, the roots were continuously supplied with oxygen by air bubbles generated by an air pump to support the root system. During the experiments, half of the boxes were connected to the tank with the control or reduced nutrient solution.

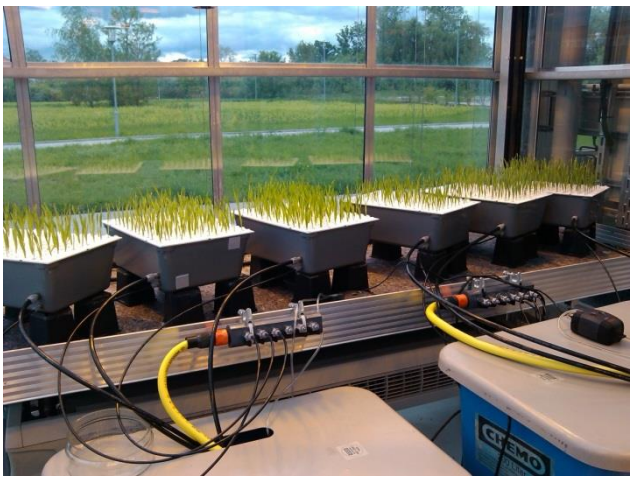

Figure 3: Hydroponic system with two tanks (foreground) providing reduced and control nutrient solutions for three boxes each. A box holds 96 barley test plants.

The composition of the standard nutrient solution was taken from Hoffmann et al. (2012). Chemicals for preparation of the hydroponic media were supplied by Merck (Darmstadt, Germany), with the exception of $CaCl_2$ supplied by Carl Roth (Karlsruhe, Germany). Electric conductivity (EC) and pH value of the solutions in the 200 L tanks were checked daily. If needed, pH was adjusted within a range from 5.5 to 6.0 using hydrochloric acid or potassium hydroxide in phosphorus expriments. In potassium experiments sodium hydroxide was used instead of potassium hydroxide. Cultivation of plants was carried out for 14 days under a 16h photoperiod and a day/night temperature shift of 24°C/16°C, respectively. After harvesting

shoot and root samples were oven-dried at 105°C for two days. Thereafter, the samples were stored for further analysis.

2.2.1.1 Phosphorus

From May to August 2013, hydroponic culture experiments with juvenile barley plants under two treatments of P supply, i.e. control P level (100 µM phosphate = P100) and low P level (10 µM phosphate = P10) were carried out. In total, 4 experiments, each with 2 simultaneous treatments (P10 and P100) per experiment were done. Per treatment, 3 boxes in experiments 1 and 2 and 2 boxes in experiments 3 and 4, respectively, were used for plant cultivation. Across experiments, each genotype was, thus, cultivated in 20 replicates per treatment, i.e. 4 experiments * 2.5 boxes x 2 blocks.

In the nutrient solutions phosphate concentration varied between 100 µM (P100) and 10 µM (P10) to study effects of P deficiency in seedling plants (see Table 2).

Table 2: Composition of standard (P100) and low (P10) phosphorus nutrient solutions

Component	**Concentration P100 (in µM)**	**Concentration P10 (in µM)**
$Ca(NO_3)_2*4H_2O$	2000.00	2000.00
MES[a]	2400.00	2400.00
K_2SO_4	700.00	745.00
$MgSO_4$	500.00	500.00
KCl	100.00	100.00
KH_2PO_4	100.00	10.00
Na-EDTA[b]	50.00	50.00
$Fe(ll)SO_4*7H_2O$	50.00	50.00
H_3BO_3	10.00	10.00
$MnSO_4*H_2O$	0.50	0.50
$CuSO_4*5H_2O$	0.20	0.20
$ZnSO_4*7H_2O$	0.10	0.10
$(NH_4)_6Mo_7O_{24}$	0.01	0.01

[a]Na-ethylenediaminetetraacetic acid
[b]2-(N-morpholino) ethanesulfonic acid (MES) adjusted to pH 5.5-6

2.2.1.2 Potassium

From August to November 2013 a total of five hydroponic experiments with juvenile barley plants under two treatments of K supply were carried out, each with 2 simultaneous treatments (K0 and K100) per experiment. Per treatment, 3 boxes in experiments 1 and 2 and 2 boxes in experiments 3, 4 and, 5, respectively, were used for plant cultivation. Across experiments, each genotype was, thus, cultivated in 24 replicates per treatment, i.e. 5 experiments * 2.4 boxes x 2 blocks.

In the nutrient solutions potassium concentration varied to study effects of potassium deficiency in seedling plants. Potassium chloride (KCl) and potassium sulfate (K_2SO_4) were absent in the reduced solution. Potassium was replaced by sodium in the form of sodium chloride (NaCl, see Table 3) due to its function as an osmoticum in the vacuole to maintain physiologically active potassium concentrations in the cytoplasm (Gierth and Mäser 2007). The concentration of potassium dihydrogen phosphate (KH_2PO_4) remained unchanged with 100 µM in the control (K100) and 0 µM in the reduced (K0) nutrient solution.

Table 3: Composition of standard (K100) and low (K0) potassium nutrient solutions

Component	**Concentration K100 (in µM)**	**Concentration K0 (in µM)**
$Ca(NO_3)_2*4H_2O$	2000.00	2000.00
MES[a]	2400.00	2400.00
K_2SO_4	700.00	0
$MgSO_4$	500.00	500.00
KCl	100.00	0
NaCl	0	100.00
KH_2PO_4	100.00	100.00
Na-EDTA[b]	50.00	50.00
$Fe(II)SO_4*7H_2O$	50.00	50.00
H_3BO_3	10.00	10.00
$MnSO_4*H_2O$	0.50	0.50
$CuSO_4*5H_2O$	0.20	0.20
$ZnSO_4*7H_2O$	0.10	0.10
$(NH_4)_6Mo_7O_{24}$	0.01	0.01

[a]Na-ethylenediaminetetraacetic acid
[b]2-(N-morpholino) ethanesulfonic acid (MES) adjusted to pH 5.5-6

2.2.2 Plant cultivation in a CO_2 growth chamber

The growth chamber tests in the experimental station (‘Kühnfeld’) served to further characterize the introgression lines with regard to their reaction to different carbon dioxide concentrations in the atmosphere. The 47 selected introgression lines as well as the control variety 'Scarlett' were examined in five different 15-day experiments from January to June 2014. Only juvenile barley plants were evaluated under two different CO_2 concentrations, i.e. control CO_2 concentration (380 ppm, N CO_2, three experiments) and high CO_2 concentration (stress, 760 ppm, H CO_2, two experiments). In order to reach a maximum plant number, all seeds were germinated in petri dishes on moist filter paper at room temperature. Per line about 30 seeds were separately germinated. After about 3 days, germinated seeds with well-developed coleoptile and radicles were transferred to the soil (Einheitserde, CL ED73) filled trays. In each experiment 384 plants in four trays (96 plants per tray) were cultivated. This means 8 replications per genotype with two replicates in each tray (2x 48 plants). Within each replication (48-block with all genotypes) a complete randomization was performed. The barley plants grew under defined conditions in a growth chamber (Percival, AR-95LC9X, Figure 4): 16h photoperiod, 65% relative humidity and 24°C / 16°C temperature (day / night) both with control as well as elevated CO_2 concentration. Every 20 minutes, all parameters inside the climate chamber were recorded including CO_2 concentration. The plants were regularly watered by hand. For this purpose, the climate chamber had to be opened resulting in short-term fluctuations in the CO_2 concentration inside the growth chamber.

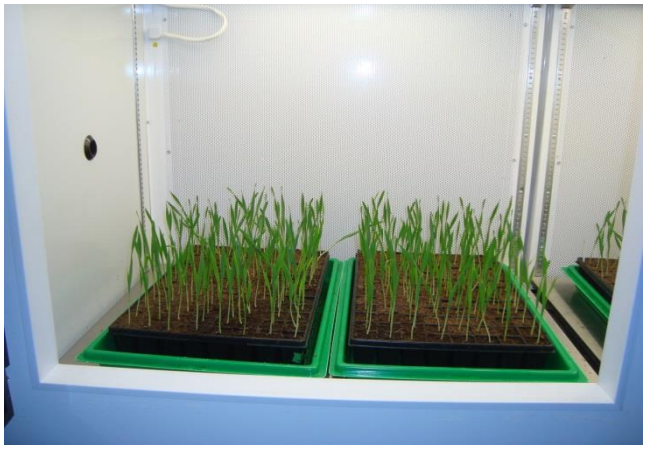

Figure 4: Plant cultivation in the growth chamber. A tray holds 96 barley test plants.

2.2.3 Plant phenotyping

2.2.3.1 Plant phenotyping in the hydroponic experiments

The development of juvenile barley plants was studied under control and reduced nutrient treatments both for phosphorus and potassium. For this purpose, a total of 62 traits were measured. This included eight morphological traits, 24 nutrient concentration traits and stress indices calculated for six morphological and 24 nutrient traits, respectively. Trait abbreviations and the methods of trait determination are listed in Tables 4, 5 and 6.

In phosphorus experiments carbon and nitrogen concentrations in roots and shoots were determined from 10 mg of ground samples using a C/N analyzer (Vario EL, Elementar Analysis System, Hanau, Germany) at the Chair of Plant Nutrition of the Martin-Luther-University following Egle et al. (2015). In potassium experiments both nutrients were determinded by hyperspectral imaging (HSI) at the Departement of Physiology and Cell Biology at the Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben.

All other nutrients were also analyzed at the Departement of Physiology and Cell Biology based on hyperspectral imaging (HSI). For calibration of HSI data at the Fraunhofer Institute for Factory Operation and Automation (IFF) Magdeburg a set of 71 (9,2%) and 195 (20.3%) test plants, randomly chosen in all P and K experiments, was examined by inductively coupled plasma (ICP)-optical emission spectrometry (ICP-OES), respectively. First, oven-dried samples were digested with HNO_3 in polytetrafluoroethylene vials in a pressurized microwave digestion system (UltraCLAVE IV, MLS GmbH).

Table 4: List of 8 evaluated morphological traits in hydroculture experiments

Morphological traits	Abbreviation	Method of measurement	Unit
Tiller number	TN	Number of tillers per plant	
Plant height	HEI	Length of the longest shoot per plant from basis to leaf tip	cm
Shoot dry weight	SDW	Weight of shoots per plant after drying at 105°C for 2 days	mg
Root length	RL	Length of the longest root per plant from crown to root tip	cm
Root dry weight	RDW	Weight of roots per plant after drying at 105°C for 2 days	mg
Shoot root length ratio	SRLR	= HEI / RL	
Shoot root weight ratio	SRWR	= SDW / RDW	
Biomass	BMD	= SDW + RDW	mg

Table 5: List of 24 evaluated nutrient concentration traits in hydroculture experiments

Nutrient trait	Abbre-viation	Method of measurement	Unit
Calcium concentration root	CaCR	Hyperspectral imaging (HSI)	µg/g DW
Carbon concentration root[1]	CCR	Elemental analyzer (EL) Hyperspectral imaging (HSI)	% DW % DW
Copper concentration root	CuCR	Hyperspectral imaging (HSI)	µg/g DW
Iron concentration root	FeCR	Hyperspectral imaging (HSI)	µg/g DW
Potassium concentration root	KCR	Hyperspectral imaging (HSI)	µg/g DW
Magnesium concentration root	MgCR	Hyperspectral imaging (HSI)	µg/g DW
Manganese concentration root	MnCR	Hyperspectral imaging (HSI)	µg/g DW
Sodium concentration root	NaCR	Hyperspectral imaging (HSI)	µg/g DW
Nitrogen concentration root[1]	NCR	Elemental analyzer (EL) Hyperspectral imaging (HSI)	% DW % DW
Phosphate concentration root	PCR	Hyperspectral imaging (HSI)	µg/g DW
Sulfur concentration root	SCR	Hyperspectral imaging (HSI)	µg/g DW
Zinc concentration root	ZnCR	Hyperspectral imaging (HSI)	µg/g DW
Calcium concentration shoot	CaCS	Hyperspectral imaging (HSI)	µg/g DW
Carbon concentration shoot[1]	CCS	Elemental analyzer (EL) Hyperspectral imaging (HSI)	% DW % DW
Copper concentration shoot	CuCS	Hyperspectral imaging (HSI)	µg/g DW
Iron concentration shoot	FeCS	Hyperspectral imaging (HSI)	µg/g DW
Potassium concentration shoot	KCS	Hyperspectral imaging (HSI)	µg/g DW
Magnesium concentration shoot	MgCS	Hyperspectral imaging (HSI)	µg/g DW
Manganese concentration shoot	MnCS	Hyperspectral imaging (HSI)	µg/g DW
Sodium concentration shoot	NaCS	Hyperspectral imaging (HSI)	µg/g DW
Nitrogen concentration shoot[1]	NCS	Elemental analyzer (EL) Hyperspectral imaging (HSI)	% DW % DW
Phosphate concentration shoot	PCS	Hyperspectral imaging (HSI)	µg/g DW
Sulfur concentration shoot	SCS	Hyperspectral imaging (HSI)	µg/g DW
Zinc concentration shoot	ZnCS	Hyperspectral imaging (HSI)	µg/g DW

[1]Determination of nutrients: EL – in phosphorus experiments, HSI – in potassium experiments

Subsequently, wet lab nutrient concentrations were analyzed for each calibration sample by ICP-OES using a iCAP 6500 Dual OES spectrometer (Thermo Fisher Scientific, Erlangen, Germany) and the protocol of Schmid et al. (2014).

To develop calibration curves, the same oven-dried samples, analyzed with ICP-OES, were subjected to HSI using the HySpex SWIR-320m-e hyperspectral camera (Norsk Elektro Optikk AS, Skedsmokorset, Norway). The camera provides hyperspectral reflectance data derived from 256 spectral bands per pixel across the short wavelength infrared range (SWIR) of 970-2500 nm. Based on ICP-OES wet lab data and HSI reflectance data a regression curve was fitted for each nutrient applying the machine learning model *radial basis function* (RBF) network (Backhaus and Seiffert 2013, 2014). Subsequently, root and shoot samples (phosphorus: 384, potassium: 480) were subjected to hyperspectral image acquisition. Based on the fitted regression models nutrient concentrations for each sample were predicted. In Table 5 an overview of the 24 measured traits under reduced P and K treatments is given.

For six morphological and 24 nutrient concentration traits, stress indices were calculated (Table 6).

Table 6: List of 30 calculated stress index traits in hydroculture experiments

Abbreviation	**Trait**	**Method of measurement**
TN_SI	Stress index of tiller number	$= TN_S / TN_{Con}$
HEI_SI	Stress index of plant height	$= HEI_S / HEI_{Con}$
SDW_SI	Stress index of shoot dry weight	$= SDW_S / SDW_{Con}$
RL_SI	Stress index of root length	$= RL_S / RL_{Con}$
RDW_SI	Stress index of root dry weight	$= RDW_S / RDW_{Con}$
BMD_SI	Stress index of biomass	$= BMD_S / BMD_{Con}$
CaR_SI and CaS_SI	Stress index of calcium root / shoot	$= Ca_S / Ca_{Con}$
CR_SI and CS_SI	Stress index of carbon root / shoot	$= C_S / C_{Con}$
CuR_SI and CuS_SI	Stress index of copper root / shoot	$= Cu_S / Cu_{Con}$
FeR_SI and FeS_SI	Stress index of Iron root / shoot	$= Fe_S / Fe_{Con}$
KR_SI and KS_SI	Stress index of potassium root / shoot	$= K_S / K_{Con}$
MgR_SI and MgS_SI	Stress index of magnesium root / shoot	$= Mg_S / Mg_{Con}$
MnR_SI and MnS_SI	Stress index of manganese root / shoot	$= Mn_S / Mn_{Con}$
NaR_SI and NaS_SI	Stress index of sodium root / shoot	$= Na_S / Na_{Con}$
NR_SI and NS_SI	Stress index of nitrogen root / shoot	$= N_S / N_{Con}$
PR_SI and PS_SI	Stress index of phosphate root / shoot	$= P_S / P_{Con}$
SR_SI and SS_SI	Stress index of sulfur root / shoot	$= S_S / S_{Con}$
ZnR_SI and ZnS_SI	Stress index of zinc root / shoot	$= Zn_S / Zn_{Con}$

S, Con: stress and control treatment

2.2.3.2 Plant phenotyping in the CO_2 growth chamber experiments

At the end of each experiment five morphological and their stress index traits were measured after 15 days of growth under control and stress CO_2 level (Table 7). These traits were tiller number, plant height and fresh and dry weight shoot. In addition, reddish pigmentation of seedlings were scored. Therefore, a visual assessment of plant pigmentation was carried out. The presumption was that this pigmentation is caused by a considerable coloring group called anthocyanins. This assumption was confirmed by a later chemical analysis of this pigmentation (Sommer 2016) in two further experiments in 2016. For this reason, this pigmentation of the plants is referred to hereinafter as anthocyanin (AC) with a classification in two levels – 0 and 1 without and with anthocyanin formation, respectively.

Table 7: List of investigated morphological and stress index traits in growth chamber experiments

Trait	**Abbreviation**	**Method of measurment**	**Unit**
Tiller number	TN	Number of tiller per plant	
Plant height	HEI	Length of the longest shoot per plant from basis to leaf tip	mm
Shoot Fresh Weight	SFW	Weight of shoots per plant after 15 days	g
Shoot Dry Weight	SDW	Weight of shoots per plant after drying at 105°C for 2 days	g
Anthocyanin formation	AC	score 1 and 0 with and without anthocyanin formation, respectively	
Stress index of TN	TN_SI	$= TN_{CO2(760)} / TN_{CO2con}$	
Stress index of HEI	HEI_SI	$= HEI_{CO2(760)} / HEI_{CO2con}$	
Stress index of SFW	SFW_SI	$= SFW_{CO2(760)} / SFW_{CO2con}$	
Stress index of SDW	SDW_SI	$= SDW_{CO2(760)} / SDW_{CO2con}$	
Stress index of AC	AC_SI	$= AC_{CO2(760)} / AC_{CO2con}$	

2.2.4 Statistical analysis

The analysis of phenotype data was conducted with SAS Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA). A quality check was carried out on the mean raw data where outliers, representing trait values lower or higher than 2 standard deviations, were excluded from further data analysis. Least squares means (LSmeans) for 'Scarlett' and the introgression lines were calculated with procedure MIXED. Pearson correlation coefficients between traits were determined across treatments and within treatments with procedure CORR. Variance components were calculated with procedure VARCOMP. Broad-sense heritability across

treatments (with equation 1 for morphological traits (MT, different levels of phosphorus, potassium and CO_2) and equation 2 for nutrient concentation traits (NCT, different levels of phosphorus and potassium) and within treatments (with equation 3 for morphological traits (P10, P100, K0, K100, N CO_2 and H CO_2) and equation 4 for nutrient concentration traits (P10, P100, K0, K100) were calculated as follows:

Eq. 1 (MT, across treatments): $h^2 = 100 \times V_G / (V_G + V_{GT}/t + V_{GE}/e + V_{GTE}/te + V_R/rte)$

Eq. 2 (NCT, across treatments): $h^2 = 100 \times V_G / (V_G + V_{GT}/t + V_{GE}/e + V_R/rte)$

Eq. 3 (MT, within treatment): $h^2 = 100 \times V_G / (V_G + V_{GE}/e + V_R/re)$

Eq. 4 (NCT, within treatment): $h^2 = 100 \times V_G / (V_G + V_R/re)$,

where variance components are genotype (V_G), genotype-by-treatment (V_{GT}), genotype-by-experiment (V_{GE}), genotype-by-treatment-by-experiment (V_{GTE}) and error (V_R), with t, e and r are number of treatments, experiments and replications within experiments, respectively. Also, Eq. 3 and Eq.4 were applied to calculate the broad-sense heritability of stress index (SI) traits. The stress index per IL was determined applying the following formula:

$SI = T_S / T_{Con}$

where T_S and T_{Con} are the mean trait performances of an IL under stress (T_S) and control (T_{Con}) treatments.

P and K experiments

To detect significant line-by-trait associations, four mixed models were calculated with procedure MIXED where models 1 and 2 were applied to morphological traits across and within treatments (P10, P100, K0, K100), respectively and models 3 and 4 were applied to nutrient concentration traits across and within treatments (P10, P100, K0, K100), respectively. Models 2 and 4 were also applied to SI traits.

Model 1 (MT, across treatments): $Y = \mu + E_i + T_j + G_k + G_k \times E_i + G_k \times T_j + Box_l(T_j) + Block_m(Box_l) + e_{ijklm}$

Model 2 (MT, within treatment): $Y = \mu + E_i + G_k + G_k \times E_i + Box_l + Block_m(Box_l) + e_{ijklm}$

Model 3 (NCT, across treatments): $Y = \mu + E_i + T_j + G_k + G_k \times T_j + e_{ijklm}$

Model 4 (NCT, within treatment): $Y = \mu + G_k + e_{ijklm}$

where μ is the general mean, E_i is the fixed effect of the *i*th experiment, T_j is the fixed effect of the *j*th treatment, G_k is the fixed effect of the *k*th genotype, $G_k \times E_i$ is the fixed interaction effect

between the *k*th genotype and the *i*th experiment, $G_k \times T_j$ is the fixed interaction effect between the *k*th genotype and the *j*th treatment, $Box_l (T_j)$ is the random effect of the *l*th box, nested in the *j*th treatment, $Block_m(Box_l)$ is the random effect of the *m*th block, nested in the *l*th box and e is the random error term, calculated from *i*=4 experiments with *j*=2 treatments per experiment, *l*=2 or 3 boxes or 4 trays per treatment, *m*=2 blocks per box / tray and *i*=48 genotypes per block (i.e. 47 S42ILs and 'Scarlett').

CO_2 experiments

To detect significant line-by-trait associations, two mixed models were calculated with procedure MIXED where models 5 and 6 were applied to morphological traits across and within treatments (N CO_2 and H CO_2).

Model 5 (across CO_2 treatments): $Y= \mu + G_i + T_j + G_i \times T_j + Exp_k(T_j) + Plate_m + Block_l + Plate_m \times Block + e_{ijklm}$

Model 6 (within CO_2 treatment): $Y= \mu + G_i + Exp_k + Plate_m + Block_l + Plate_m \times Block_l + e_{iklm}$

where μ is the general mean, G_i is the fixed effect of the *i*th genotype, T_j is the fixed effect of the *i*th treatment, $G_i \times T_j$ is the fixed interaction effect of the *i*th genotype with *j*th treatment, $Exp_k(T_j)$ is the random effect of *k*th experiment nested in *j*th treatment, $Plate_m$ is the random effect of the *m*th plate, $Block_l$ is the random effect of the *l*th Block and $Plate_m$ x $Block_l$ is the random interaction effect of *m*th Plate with *l*th block.

Subsequently, a post-hoc Dunnett test was carried out to compare LSmeans between the introgression lines and the control genotype 'Scarlett'. Next, the obtained raw P values of the Dunnett test were adjusted for multiple testing by FDR implemented in procedure MULTTEST. In case the LSmeans of an introgression line was significantly different from 'Scarlett' across treatments or within a single treatment with P(FDR)<0.05, a line-by-trait association was accepted. The relative performance (RP) of each introgression line compared to 'Scarlett' was calculated as follows:

RP(S42IL) = (LSmeans[S42IL] - LSmeans[Scarlett]) × 100 / LSmeans[Scarlett].

Finally, a significant line-by-trait association effect is assumed to be caused by a QTL, located within the introgressed chromosomal segment of the introgression line. If two overlapping ILs show a similar line-by-trait association effect (same sign of effect), the causative QTL is assumed to be located within the overlapping segment of the two S42ILs.

3 Results

3.1 Phosphorus investigation

3.1.1 Morphological traits - trait performance and correlations

Trait performances of the 47 introgression lines and 'Scarlett' are listed in Table 8. The data are summarized in Table 8 containing number of observations (N), means, standard deviation (SD), coefficient of variation (CV) and heritability (h^2) for all 62 traits, across treatments and separately for low and adequate P supplies, P10 and P100, respectively. As expected, the means of all morphological traits studied were higher under P100 than under P10 with the highest increase observed for shoot dry weight (+64%). For example, mean biomass accumulation under P10 and P100 is shown in Fig. 5. P deficiency often results in reduced dry biomass. Under low P treatment, the average dry biomass dropped from 175.90 to 119.30 mg (Table 8). The highest and lowest CV was attributed to tiller number with 42% and 0% under P100 and P10 treatments, respectively. Shoot root weight ratio under P10 showed the highest heritability with 85.8% whereas tiller number under P10 revealed no heritability.

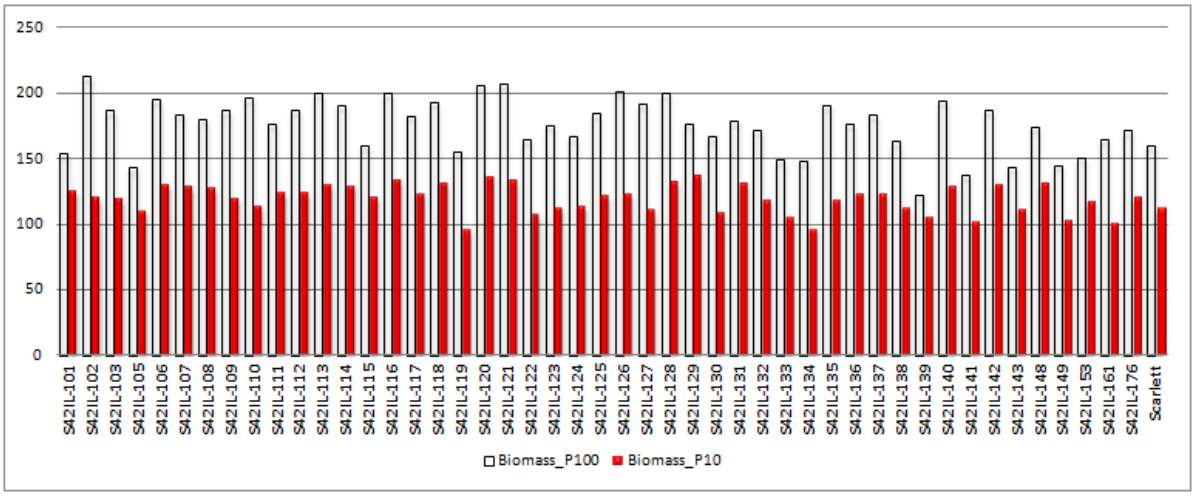

Figure 5: Mean biomass accumulation (in mg) of S42ILs and Scarlett cultivated under P10 and P100 treatments.

Pearson correlations between eight morphological traits across treatments are given in Table 9. Across treatments, eight significant correlations were observed. The highest positive correlation was found between biomass and shoot dry weight (r=0.97), followed by biomass and root dry weight (r=0.91). In addition, plant height revealed positive correlations with

Table 8: Morphological trait performance and heritability of 47 ILs and Scarlett, across and within P treatments

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
TN	Across	1874	1.2	0.5	38.7	0.0
TN	P10	957	1.0	0.0	0.0	0.0
TN	P100	917	1.4	0.6	42.0	41.9
HEI	Across	1819	26.0	5.7	22.1	76.9
HEI	P10	908	22.0	3.4	15.3	73.2
HEI	P100	911	30.0	4.7	15.8	70.0
SDW	Across	1832	98.1	38.3	39.1	45.6
SDW	P10	916	74.3	21.2	28.6	55.0
SDW	P100	916	121.8	36.8	30.2	53.8
RL	Across	1844	28.8	8.0	27.7	73.3
RL	P10	920	27.8	7.4	26.7	77.5
RL	P100	924	29.8	8.4	28.2	55.0
RDW	Across	1839	50.1	18.2	36.3	52.7
RDW	P10	922	45.5	15.5	34.1	58.6
RDW	P100	917	54.7	19.5	35.6	61.2
SRLR	Across	1672	0.9	0.2	26.0	67.9
SRLR	P10	832	0.8	0.2	24.5	71.9
SRLR	P100	840	1.0	0.2	22.4	56.2
SRWR	Across	1737	2.0	0.5	23.3	74.0
SRWR	P10	872	1.6	0.3	18.0	85.8
SRWR	P100	865	2.3	0.4	16.5	63.7
BMD	Across	1798	147.7	52.9	35.8	45.2
BMD	P10	897	119.3	34.3	28.8	51.7
BMD	P100	901	175.9	53.1	30.2	57.6

[a] Trait abbreviations are given in Tables 4, 5 and 6.
[b] Across: across both P treatments, P100 and P10: within treatment
[c] Number of observations
[d] Mean value
[e] Standard deviation
[f] Coefficient of variation (in %)
[g] Heritability (in %)

shoot dry weight, root dry weight and biomass (0.65, 0.70 and 0.68, respectively). Finally, shoot dry weight and root dry weight showed a positive correlation with r=0.80. Negative significant correlations across treatments were found between shoot root length ratio and root length (r=-0.74) and between shoot root weight ratio and root dry weight (r=-0.62).

Pearson correlations between morphological traits were also calculated within P treatments (Table 10). Under P10 the strongest positive and negative correlations were found between shoot dry weight and biomass (r=0.92) and between shoot root length ratio and root length (r=-0.84). Under P100 the strongest positive and negative correlations were found again between shoot dry weight and biomass (r=0.98) and between shoot root weight ratio and root dry weight (r=-0.64).

Table 9: Pearson correlation coefficients between morphological traits across P treatments

	TN	HEI	SDW	RL	RDW	SRLR	SRWR	BMD
TN								
HEI	0.24							
SDW	0.49	**0.65**						
RL	0.17	0.52	0.26					
RDW	0.39	**0.70**	**0.80**	0.45				
SRLR	0.00	0.16	0.23	**-0.74**	0.04			
SRWR	0.03	-0.37	-0.07	-0.44	**-0.62**	0.24		
BMD	0.49	**0.68**	**0.97**	0.31	**0.91**	0.19	-0.29	

(Bold values indicate significant correlations with P<0.001)

Trait auto correlations between P10 and P100 were positive and highly significant (P<0.001) for each morphological trait, except tiller number (diagonal in Table 10). The highest auto correlation coefficient with 0.83 was observed for plant height, followed by root length with 0.82.

Table 10: Pearson correlation coefficients between morphological traits under P10 (bottom left triangle) and P100 (upper right triangle)

	TN	HEI	SDW	RL	RDW	SRLR	SRWR	BMD
TN		0.18	0.50	0.22	0.41	-0.02	0.02	0.48
HEI	**0.83**		**0.70**	0.46	**0.66**	0.38	-0.32	**0.69**
SDW	0.50	**0.72**		0.33	**0.85**	0.29	-0.20	**0.98**
RL	0.51	0.23	**0.82**		0.41	**-0.60**	-0.35	0.32
RDW	**0.62**	**0.67**	0.52	**0.74**		0.17	**-0.64**	**0.93**
SRLR	-0.02	0.05	**-0.84**	-0.20	**0.67**		0.09	0.29
SRWR	-0.35	0.06	**-0.54**	**-0.63**	0.44	**0.73**		-0.36
BMD	**0.59**	**0.92**	0.37	**0.88**	-0.06	-0.26	**0.71**	

(Bold values indicate significant correlations with P<0.001,
Diagonal: auto correlations between P10 and P100)

3.1.2 Nutrient concentration traits - trait performance and correlations

In Table 11 trait performances of the 47 introgression lines and 'Scarlett' are given across treatments and separately within P10 and P100 treatments for 24 nutrient concentration traits and 30 stress index traits. As for morphological traits mean nutrient values increased in most cases from P10 to P100 treatment, except for carbon concentration of root and shoot concentration of calcium, iron and phosphorus. The highest CV was found for zinc concentration root stress index (ZnR_SI) across treatments (202.8%). The highest heritabilities were detected for copper concentration shoot (49.8%), sodium concentration shoot (47.7%) and zinc concentration root (46.9%) across treatments, within P10 and within P100, respectively.

Table 11: Nutrient concentration trait performance and heritability of 47 ILs and Scarlett, across and within P treatments

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
CaCR	Across	371	2003.6	216.2	10.8	0.0
CaCR	P10	185	1955.7	209.0	10.7	0.0
CaCR	P100	186	2051.2	213.3	10.4	2.9
CCR	Across	352	39.4	2.4	6.1	27.5
CCR	P10	179	39.9	1.0	2.6	1.5
CCR	P100	173	38.9	3.2	8.2	0.0
CuCR	Across	370	88.0	52.6	59.8	14.1
CuCR	P10	185	82.1	53.9	65.7	7.4
CuCR	P100	185	93.9	50.8	54.1	0.0
FeCR	Across	369	2809.7	770.2	27.4	3.7
FeCR	P10	185	2567.6	800.6	31.2	0.0
FeCR	P100	184	3053.0	655.4	21.5	0.0
KCR	Across	365	40502.4	8854.5	21.9	0.0
KCR	P10	183	35799.1	7222.2	20.2	13.5
KCR	P100	182	45231.5	7769.7	17.2	18.3
MgCR	Across	373	4866.0	1994.7	41.0	39.0
MgCR	P10	188	4499.0	2105.8	46.8	0.0
MgCR	P100	185	5239.0	1805.4	34.5	8.5
MnCR	Across	366	29.0	7.7	26.8	36.6
MnCR	P10	183	24.4	5.7	23.5	4.3
MnCR	P100	183	33.5	6.8	20.3	18.0
NaCR	Across	368	1427.7	319.0	22.3	27.2
NaCR	P10	182	1262.6	226.3	17.9	4.5
NaCR	P100	186	1589.2	314.3	19.8	27.3
NCR	Across	356	4.3	1.1	24.4	0.0
NCR	P10	186	3.5	0.4	10.4	25.0

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
NCR	P100	170	5.2	0.8	15.8	0.0
PCR	Across	370	3252.9	865.9	26.6	13.5
PCR	P10	185	2700.3	617.9	22.9	0.0
PCR	P100	185	3805.5	712.5	18.7	25.2
SCR	Across	370	3373.8	355.9	10.5	34.8
SCR	P10	181	3107.0	163.0	5.2	0.0
SCR	P100	189	3629.4	298.4	8.2	25.1
ZnCR	Across	369	318.9	199.0	62.4	17.2
ZnCR	P10	183	287.0	190.1	66.2	0.0
ZnCR	P100	186	350.3	203.0	58.0	46.9
CaCS	Across	366	30452.6	7860.8	25.8	0.0
CaCS	P10	185	32093.6	8162.3	25.4	30.0
CaCS	P100	181	28775.4	7183.3	25.0	17.1
CCS	Across	362	40.3	1.1	2.7	35.4
CCS	P10	181	40.2	0.8	2.1	12.5
CCS	P100	181	40.4	1.3	3.2	23.0
CuCS	Across	368	14.3	6.7	46.9	49.8
CuCS	P10	185	8.9	4.2	47.6	21.1
CuCS	P100	183	19.8	3.6	18.1	31.0
FeCS	Across	362	217.4	55.1	25.4	0.0
FeCS	P10	181	242.6	54.7	22.5	37.5
FeCS	P100	181	192.2	42.8	22.3	0.0
KCS	Across	358	138612.2	36108.5	26.1	0.0
KCS	P10	176	119814.1	28567.9	23.8	31.6
KCS	P100	182	156790.6	33266.7	21.2	0.0
MgCS	Across	362	2244.7	471.4	21.0	12.6
MgCS	P10	180	2049.4	445.1	21.7	14.1
MgCS	P100	182	2437.9	414.4	17.0	9.6
MnCS	Across	363	72.4	8.0	11.1	0.0
MnCS	P10	182	70.8	8.2	11.6	24.9
MnCS	P100	181	74.1	7.4	10.1	2.0
NaCS	Across	362	485.3	153.6	31.7	0.0
NaCS	P10	179	390.6	110.9	28.4	47.7
NaCS	P100	183	577.9	131.6	22.8	0.0
NCS	Across	364	5.1	1.2	23.9	35.1
NCS	P10	184	4.0	0.6	14.4	4.2
NCS	P100	180	6.1	0.5	8.6	23.7
PCS	Across	363	3084.1	433.6	14.1	20.0
PCS	P10	183	3188.1	477.4	15.0	34.9
PCS	P100	180	2978.3	355.2	11.9	21.6
SCS	Across	367	3510.8	230.3	6.6	32.9
SCS	P10	182	3418.9	254.3	7.4	0.0
SCS	P100	185	3601.2	159.1	4.4	0.0

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
ZnCS	Across	365	916.6	157.3	17.2	6.1
ZnCS	P10	185	806.0	118.0	14.6	25.3
ZnCS	P100	180	1030.2	101.9	9.9	25.0
TN_SI	Across	914	0.8	0.3	31.5	36.9
HEI_SI	Across	869	0.7	0.1	17.7	27.5
SDW_SI	Across	884	0.6	0.2	31.4	33.5
RL_SI	Across	888	1.0	0.3	33.2	15.7
RDW_SI	Across	886	0.9	0.4	40.9	33.1
BMD_SI	Across	853	0.7	0.2	32.4	36.4
CaR_SI	Across	180	1.0	0.1	14.0	7.4
CR_SI	Across	162	1.0	0.1	7.8	0.0
CuR_SI	Across	178	1.5	2.1	135.7	0.0
FeR_SI	Across	178	0.9	0.2	27.8	0.0
KR_SI	Across	174	0.8	0.2	19.3	14.0
MgR_SI	Across	181	1.0	0.7	71.9	0.0
MnR_SI	Across	174	0.8	0.2	25.7	0.0
NaR_SI	Across	176	0.8	0.2	21.7	6.2
NR_SI	Across	165	0.7	0.1	18.5	0.0
PR_SI	Across	178	0.7	0.2	23.2	0.0
SR_SI	Across	178	0.9	0.1	8.7	5.4
ZnR_SI	Across	177	1.7	3.5	202.8	21.7
CaS_SI	Across	174	1.2	0.5	39.1	38.2
CS_SI	Across	171	1.0	0.0	2.7	0.0
CuS_SI	Across	178	0.5	0.3	59.8	0.0
FeS_SI	Across	170	1.3	0.5	37.5	11.1
KS_SI	Across	167	0.8	0.3	32.6	6.6
MgS_SI	Across	171	0.9	0.2	26.5	22.0
MnS_SI	Across	171	1.0	0.1	14.8	23.9
NaS_SI	Across	171	0.7	0.3	36.0	7.7
NS_SI	Across	172	0.7	0.1	12.0	0.0
PS_SI	Across	173	1.1	0.2	19.4	22.6
SS_SI	Across	177	1.0	0.1	7.5	0.0
ZnS_SI	Across	174	0.8	0.1	16.0	23.2

[a] Trait abbreviations are given in Tables 4, 5 and 6.
[b] Across: across both P treatments, P100 and P10: within treatment
[c] Number of observations
[d] Mean value
[e] Standard deviation
[f] Coefficient of variation (in %)
[g] Heritability (in %)

Pearson correlations between 24 nutrient concentration and 30 stress index traits across treatments are given in Table 12. The highest positive correlation was found between sulfur concentration root and sodium concentration root (r=0.88). The highest negative correlation was found between Na concentration shoot and P concentration shoot (r=-0.77). P concentration root revealed significant positive correlations (r>0.60) with root concentrations of iron, manganese, sodium, sulfur and zinc. In contrast, P concentration shoot revealed significant negative correlations (r<-0.59) with shoot concentrations of manganese, sodium and zinc.

Pearson correlations between nutrient concentration traits were also calculated within P treatments (Table 13). Under P10 the strongest positive and negative correlations were found between sodium concentration root and iron concentration root (r=0.71) and between magnesium concentration root and calcium concentration root (r=-0.77). Under P100 the strongest positive and negative correlations were found between sodium concentration root and sulfur concentration root (r=0.90) and between sodium concentration root and copper concentration root (r=-0.71).

The trait auto correlations (diagonal in Table 13) were often low and non-significant (P<0.05) with the only exception of carbon concentration shoot where P10 and P100 treatments revealed a positive correlation of r=0.51.

Table 12: Pearson correlation coefficients between 24 nutrient concentration traits across P treatments

Trait	CaCR	CCR	CuCR	FeCR	KCR	MgCR	MnCR	NaCR	NCR	PCR	SCR	ZnCR	CaCS	CCS	CuCS	FeCS	KCS	MgCS	MnCS	NaCS	NCS	PCS	SCS	ZnCS
CaCR																								
CCR	0.08																							
CuCR	0.27	-0.06																						
FeCR	0.08	-0.01	-0.18																					
KCR	0.31	-0.04	-0.15	0.48																				
MgCR	-0.52	-0.06	-0.48	0.52	0.01																			
MnCR	0.13	-0.08	-0.27	**0.59**	**0.54**	0.16																		
NaCR	-0.1	-0.05	-0.53	**0.69**	0.49	**0.65**	**0.61**																	
NCR	0.1	0.22	0.14	0.11	-0.02	-0.03	-0.16	0.02																
PCR	0.33	-0.02	-0.08	**0.77**	0.5	0.33	**0.62**	**0.64**	0.01															
SCR	-0.08	-0.15	-0.41	**0.72**	0.51	**0.67**	0.53	**0.88**	-0.02	**0.65**														
ZnCR	-0.19	-0.02	-0.27	**0.59**	0.22	**0.76**	0.39	**0.72**	0.01	**0.6**	**0.66**													
CaCS	-0.3	0.12	-0.05	-0.01	-0.24	0.26	-0.22	0.15	0.24	-0.15	0.11	0.02												
CCS	-0.04	0.17	-0.06	-0.05	-0.14	-0.09	-0.06	-0.12	-0.27	-0.24	-0.13	-0.15	0.14											
CuCS	0.15	0.05	-0.21	0.11	0	0.07	0.13	0.12	-0.2	0.14	0.22	0.11	-0.21	0.11										
FeCS	-0.32	-0.13	0.02	-0.01	-0.4	0.41	-0.22	0.07	0.2	-0.16	0.07	0.09	0.38	-0.21	-0.44									
KCS	-0.17	0.01	-0.08	-0.01	-0.35	0.12	0.04	0.03	-0.24	-0.01	0.08	0.07	0.02	0.13	**0.65**	-0.05								
MgCS	0.14	-0.17	-0.18	0.3	0.36	0.28	0.32	0.39	0.08	0.36	0.35	0.31	-0.05	-0.34	-0.47	0.26	**-0.56**							
MnCS	-0.3	-0.16	-0.17	0.02	-0.31	0.39	0.02	0.27	-0.08	0.01	0.2	0.24	0.22	-0.24	-0.02	0.58	0.43	0.18						
NaCS	-0.28	-0.02	-0.18	0	-0.35	0.3	0.09	0.2	-0.23	-0.06	0.23	0.08	0.38	0.04	0.32	0.31	**0.68**	-0.21	**0.72**					
NCS	-0.11	-0.1	0.16	0.21	-0.02	0.19	0	0.17	0.11	0.31	0.25	0.22	0.12	-0.42	0.36	-0.06	0.35	-0.12	0.21	0.3				
PCS	0.24	0.13	0.24	-0.19	0.2	-0.44	-0.27	-0.34	0.33	-0.08	-0.34	-0.21	-0.31	-0.11	-0.24	-0.32	-0.42	-0.03	**-0.62**	**-0.77**	-0.12			
SCS	0.28	0.23	0.18	-0.14	0.06	-0.35	-0.16	-0.14	0.14	0.04	-0.18	-0.3	0.14	-0.03	0.21	-0.3	-0.03	-0.4	-0.32	-0.16	0.15	0.27		
ZnCS	0.04	-0.16	-0.26	0.37	0.1	0.42	0.4	0.58	0.02	0.32	**0.57**	0.35	0.21	-0.16	0.12	0.27	0.16	0.35	**0.55**	**0.58**	0.14	**-0.59**	-0.23	

(Bold values indicate significant correlations with P<0.001)

Table 12 (continued): Pearson correlation coefficients between 30 stress index of morphological and nutrient concentration traits across P treatments

Trait	TN_SI	HEI_SI	SDW_SI	RL_SI	RDW_SI	BMD_SI	CaR_SI	CR_SI	CuR_SI	FeR_SI	KR_SI	MgR_SI	MnR_SI	NaR_SI	NR_SI	PR_SI	SR_SI	ZnR_SI	CaS_SI	CS_SI	CuS_SI	FeS_SI	KS_SI	MgS_SI	MnS_SI	NaS_SI	NS_SI	PS_SI	SS_SI	ZnS_SI
TN_SI																														
HEI_SI	-0.24																													
SDW_SI	0.17	0.51																												
RL_SI	0.05	0.29	0.19																											
RDW_SI	0.23	0.51	**0.84**	0.45																										
BMD_SI	0.19	0.53	**0.96**	0.30	**0.93**																									
CaR_SI	0.22	-0.02	0.02	-0.06	-0.12	-0.04																								
CR_SI	-0.05	0.02	-0.01	-0.17	-0.16	-0.03	0.19																							
CuR_SI	-0.14	0.07	0.09	0.19	0.14	0.16	0.08	0.18																						
FeR_SI	-0.03	0.15	0.20	0.21	0.27	0.27	-0.06	-0.04	0.17																					
KR_SI	0.45	-0.03	0.26	-0.10	0.14	0.21	0.45	0.27	-0.27	0.15																				
MgR_SI	0.16	0.11	0.27	0.18	0.33	0.34	-0.46	-0.09	-0.18	0.42	-0.04																			
MnR_SI	0.37	0.12	0.43	0.02	0.29	0.41	0.25	0.13	-0.14	0.22	**0.68**	0.01																		
NaR_SI	0.19	0.17	0.41	0.09	0.36	0.40	0.03	-0.01	-0.18	0.45	0.51	0.44	0.50																	
NR_SI	-0.16	-0.16	-0.07	0.11	-0.16	-0.08	-0.03	0.10	0.03	0.03	0.04	-0.20	0.11	-0.09																
PR_SI	0.08	0.20	0.30	0.20	0.27	0.31	0.27	0.10	-0.06	0.46	0.53	0.15	0.45	**0.54**	0.18															
SR_SI	0.15	0.22	0.41	0.16	0.42	0.43	-0.01	0.02	-0.06	**0.55**	0.48	0.42	0.49	**0.80**	-0.01	**0.58**														
ZnR_SI	0.27	0.11	0.14	-0.19	0.09	0.11	-0.03	0.12	-0.19	0.25	0.30	0.25	0.28	**0.57**	-0.19	0.21	0.35													
CaS_SI	-0.09	0.09	0.05	-0.01	0.01	0.03	-0.19	-0.04	-0.10	0.05	-0.12	0.13	-0.03	-0.16	-0.15	0.01	-0.17	-0.13												
CS_SI	0.09	-0.01	0.07	0.04	0.10	0.07	0.04	-0.05	-0.14	0.05	0.08	0.10	-0.07	0.16	-0.11	-0.18	0.14	0.14	0.16											
CuS_SI	0.00	-0.14	-0.11	-0.06	-0.10	-0.09	0.17	0.03	-0.10	-0.39	0.18	-0.20	0.19	-0.05	0.16	-0.05	-0.12	-0.09	-0.44	-0.03										
FeS_SI	-0.23	0.17	0.14	0.18	0.12	0.11	-0.29	-0.21	-0.10	0.14	-0.27	0.15	-0.18	-0.01	-0.10	-0.11	-0.06	-0.15	**0.55**	0.30	**-0.54**									
KS_SI	0.04	-0.05	-0.07	-0.07	-0.02	-0.03	0.13	0.07	0.04	-0.40	-0.11	-0.07	-0.01	0.04	-0.24	-0.18	-0.08	-0.07	-0.22	0.17	0.36	-0.06								
MgS_SI	0.10	-0.03	0.21	0.10	0.14	0.13	-0.24	-0.17	0.02	0.20	-0.01	0.08	-0.01	-0.08	0.09	-0.05	0.07	-0.02	0.18	-0.06	-0.41	0.17	**-0.63**							
MnS_SI	-0.25	-0.03	0.15	0.16	0.21	0.18	-0.43	-0.04	0.12	-0.05	-0.40	0.15	-0.13	-0.04	-0.08	-0.19	-0.08	-0.14	0.14	0.28	-0.04	0.39	0.45	-0.19						
NaS_SI	-0.13	0.02	0.04	0.28	0.18	0.12	-0.10	-0.01	0.29	-0.02	-0.30	0.01	-0.06	-0.12	-0.15	-0.02	-0.14	-0.21	0.21	0.13	0.10	0.15	0.40	-0.40	**0.66**					
NS_SI	-0.07	0.07	0.07	0.15	0.22	0.15	0.12	-0.07	0.07	0.06	0.14	-0.24	0.28	0.04	0.12	0.25	0.12	-0.10	-0.11	0.03	0.19	-0.14	0.19	-0.08	0.19	0.27				
PS_SI	0.16	0.01	-0.03	-0.09	-0.04	-0.03	0.06	-0.04	-0.13	0.05	0.18	0.28	0.08	0.30	0.11	-0.08	0.20	0.37	-0.33	0.12	0.09	-0.22	-0.03	0.07	-0.33	-0.42	-0.05			
SS_SI	0.08	0.01	-0.08	0.35	0.06	-0.01	0.18	-0.14	0.05	0.12	0.04	0.03	0.13	-0.07	0.07	0.10	-0.01	-0.25	0.17	-0.06	0.13	-0.01	-0.07	-0.10	-0.05	0.27	0.01	0.02		
ZnS_SI	-0.32	0.22	0.35	0.28	0.33	0.33	-0.25	-0.11	0.06	0.08	-0.13	-0.03	0.14	0.12	0.02	0.15	0.18	-0.14	0.20	0.12	-0.05	0.39	0.12	-0.03	0.65	0.50	0.14	-0.42	-0.01	

Table 13: Pearson correlation coefficients between 24 nutrient concentration traits under P10 (bottom left triangle) and P100 (upper triangle)

Trait	CaCR	CCR	CuCR	FeCR	KCR	MgCR	MnCR	NaCR	NCR	PCR	SCR	ZnCR	CaCS	CCS	CuCS	FeCS	KCS	MgCS	MnCS	NaCS	NCS	PCS	SCS	ZnCS
CaCR	0.05	0.25	0.17	0.08	0.03	-0.22	-0.08	0.02	0.04	0.36	-0.06	0.09	-0.32	0.01	0.27	-0.30	0.01	0.11	-0.24	-0.27	-0.16	0.08	0.25	-0.23
CCR	0.01	0.16	0.10	0.01	0.08	-0.08	-0.07	-0.01	0.38	0.07	-0.05	0.11	-0.01	0.12	0.19	-0.25	0.16	-0.26	-0.08	0.06	0.15	0.05	0.13	-0.18
CuCR	0.23	-0.04	0.15	-0.18	-0.46	**-0.58**	-0.48	**-0.71**	0.17	-0.23	**-0.61**	**-0.55**	0.02	-0.05	-0.16	0.09	0.04	-0.25	0.09	0.08	-0.03	-0.03	0.14	-0.27
FeCR	-0.15	0.01	-0.01	0.29	**0.61**	**0.58**	**0.54**	**0.56**	-0.11	**0.74**	**0.70**	**0.58**	-0.11	-0.13	-0.16	-0.10	-0.28	0.51	-0.23	-0.37	0.28	0.18	-0.03	0.08
KCR	**0.65**	0.08	0.10	0.10	0.07	**0.54**	**0.57**	**0.80**	0.01	**0.63**	**0.78**	**0.63**	-0.22	0.00	0.02	-0.26	-0.25	0.32	-0.20	-0.31	-0.02	0.25	-0.03	0.09
MgCR	**-0.77**	-0.14	-0.22	0.49	**-0.59**	0.29	0.41	**0.68**	-0.27	0.50	**0.78**	**0.74**	-0.02	-0.20	0.03	-0.01	-0.10	0.36	-0.13	-0.03	0.14	0.10	-0.13	0.28
MnCR	0.46	0.03	0.02	0.33	**0.68**	-0.30	0.23	**0.75**	-0.09	0.48	**0.65**	**0.61**	-0.23	0.02	-0.03	-0.10	-0.07	0.52	0.04	-0.14	0.07	0.06	-0.28	0.37
NaCR	-0.14	-0.20	-0.01	**0.71**	0.04	0.49	0.32	0.12	-0.09	**0.61**	**0.90**	**0.83**	-0.27	-0.07	0.10	-0.24	-0.11	0.49	-0.10	-0.19	0.09	0.21	-0.17	0.26
NCR	0.12	-0.17	-0.03	0.08	0.01	0.03	0.18	0.20	-0.17	0.04	-0.16	0.04	0.08	0.09	-0.14	-0.23	-0.12	-0.14	-0.16	-0.24	0.06	0.39	0.17	-0.16
PCR	0.38	-0.14	0.09	**0.64**	0.45	-0.01	**0.61**	**0.59**	0.23	0.19	**0.68**	**0.68**	-0.27	-0.24	0.02	-0.37	-0.27	0.45	-0.39	-0.43	0.25	0.29	0.11	-0.13
SCR	-0.05	-0.23	0.12	**0.61**	0.20	0.31	0.41	**0.67**	0.26	**0.63**	0.23	**0.78**	-0.19	-0.13	0.11	-0.20	-0.16	0.44	-0.22	-0.22	0.15	0.18	-0.04	0.24
ZnCR	-0.38	-0.17	0.18	0.52	-0.25	**0.57**	0.02	0.60	0.07	0.30	0.37	0.17	-0.28	-0.13	0.07	-0.38	-0.11	0.43	-0.21	-0.24	0.14	0.28	-0.17	0.12
CaCS	-0.31	-0.21	-0.22	0.25	-0.29	0.42	-0.07	0.35	0.07	0.09	0.17	0.05	-0.04	0.14	-0.42	**0.56**	-0.12	-0.05	-0.06	0.12	0.13	-0.17	0.14	-0.06
CCS	-0.14	0.08	0.06	0.05	-0.17	0.12	-0.16	-0.03	-0.32	-0.09	-0.01	-0.03	0.06	0.51	0.11	-0.06	0.08	-0.22	0.06	0.16	**-0.53**	-0.23	0.08	0.00
CuCS	0.19	-0.23	0.02	-0.06	0.18	-0.18	0.36	0.07	0.16	0.18	0.17	0.01	-0.25	0.21	0.29	-0.34	0.45	-0.40	0.03	0.35	-0.07	-0.29	0.14	0.20
FeCS	-0.39	-0.06	-0.11	0.24	-0.43	0.53	-0.24	0.20	-0.04	0.00	0.10	0.19	0.49	-0.13	**-0.62**	-0.05	0.21	-0.03	0.35	0.33	-0.05	-0.38	-0.21	0.28
KCS	-0.07	-0.12	-0.07	-0.04	-0.19	0.11	0.06	0.21	0.10	0.12	0.16	0.16	-0.04	0.18	**0.55**	-0.23	0.01	**-0.54**	**0.68**	**0.78**	-0.10	-0.49	-0.13	0.35
MgCS	-0.10	0.08	0.02	0.11	0.08	0.17	-0.14	-0.08	0.14	-0.05	0.01	-0.07	0.08	-0.30	**-0.55**	0.36	**-0.66**	0.19	-0.18	-0.39	0.21	0.30	-0.22	0.19
MnCS	-0.41	-0.15	-0.09	0.19	-0.47	**0.54**	-0.19	0.39	0.28	0.14	0.31	0.36	0.36	-0.16	-0.12	**0.54**	0.48	0.06	-0.05	**0.70**	-0.19	-0.37	-0.27	**0.58**
NaCS	-0.12	-0.20	0.02	0.21	-0.29	0.30	0.09	0.42	0.24	0.29	0.40	0.20	0.40	0.06	0.23	0.25	0.63	-0.27	0.76	0.18	-0.11	**-0.55**	-0.24	0.43
NCS	0.21	-0.16	0.10	0.11	-0.05	-0.06	0.06	0.16	0.37	0.33	0.33	0.06	-0.08	-0.13	0.36	-0.19	**0.54**	-0.27	0.31	0.45	0.39	0.06	-0.07	-0.06
PCS	0.26	0.12	0.05	-0.27	0.29	-0.35	-0.12	-0.29	-0.17	-0.27	-0.33	-0.19	-0.35	0.00	0.01	-0.33	-0.14	-0.08	-0.52	**-0.63**	-0.11	0.12	0.17	-0.30
SCS	0.19	0.00	-0.02	-0.08	0.20	-0.25	0.25	0.04	0.16	0.03	-0.05	-0.29	0.21	-0.15	0.31	-0.15	0.11	-0.29	-0.08	0.11	0.08	0.19	0.28	-0.30
ZnCS	0.00	-0.29	0.06	0.37	-0.08	0.28	0.25	**0.58**	0.31	0.53	**0.58**	0.31	0.36	-0.16	0.02	0.39	0.13	0.13	**0.60**	**0.70**	0.25	**-0.62**	-0.01	0.12

(Bold values indicate significant correlations with P<0.001,
Diagonal: auto correlations between P10 and P100)

Pearson correlations between ICP wet lab data and the predicted ICP data were calculated based on 71 joint root and shoot samples (Table 14). For all ten evaluated traits positive significant correlations (P<0.05) were detected within the range of r=0.57 (magnesium) and r=0.92 (iron).

Table 14: Pearson correlation coefficients of nutrient oncentrations between ICP and HSI

Nutrient	Correlation coefficient
Ca	0.77
Cu	0.90
Fe	0.92
K	0.70
Mg	0.57
Mn	0.84
Na	0.64
P	0.74
S	0.84
Zn	0.68

3.1.3 QTL identification

All 62 investigated morphological and nutrient concentration traits were used for QTL identification. First, a MIXED model ANOVA was run for each trait across and within P treatments (Table S1, Supporting Information). Significant (P<0.05) treatment effects between P10 and P100 were observed for all traits except root length and carbon concentration shoot. Also, significant (P<0.05) genotype effects across and within P treatments were observed for all morphological traits (Table S1, Supporting Information). In addition, significant genotype effects for two nutrient concentration traits and five stress index traits were observed for at least one analysis, across or within P10 or P100 treatments. Genotype-by-treatment interactions were only significant for morphological traits except plant height and root length. Experiment effects were significant for all morphological traits, six morphological stress index traits and 17 nutrient concentration traits (Table S1, Supporting Information).

Following the MIXED model ANOVA, a post-hoc Dunnett test was applied with subsequent FDR adjustment of raw P values in order to identify significant (P-FDR<0.05) trait by line associations (Table 15). For 39 S42ILs (excluding S42ILs -101, -103, -111, -115, -117, -132, -138 and -153) the Dunnett test revealed significant deviations from the recurrent parent ‘Scarlett’ for at least one trait studied (Table 15). Based on SNP mapping knowledge about overlapping S42IL introgressions, line-by-trait associations were summarized to 91 QTLs,

present as 74, 44 and 40 QTL effects across treatments, within P10 and within P100, respectively. QTLs were observed for a total of 13 traits (eight morphological traits, two shoot nutrient concentration traits (PCS and NaCS) and three stress index traits (TN_SI, RDW_SI and CaS_SI). The highest number of QTLs were found for S42IL-126 and -133 (7 QTLs each), followed by S42IL-128, -129, -140 and -143 (6 QTLs each) with *Hsp* introgressions on chromosome arms 5HL, 7HS, 6HL, 6HL, 3HL and 1HL, respectively (Schmalenbach et al. 2011). *Hsp* alleles, present within the introgression, increased trait performance in 64 cases. The highest number of favorable *Hsp* allele effects were found for S42IL-126 (6) and S42ILs -128, -129 and -140 (5 each). In the following, QTLs controlling eight morphological traits, two nutrient concentration traits and three stress index traits are described in detail (see Table 15).

QTLs for morphological traits

TN: Only one S42IL showed significant association with tiller number (Table 15). This QTL with an increasing *Hsp* effect was located on chromosome 1H. Line S42IL-142 produced on average 0.31 and 0.61 extra tillers compared to 'Scarlett' across treatments and under P100 treatment, respectively.

HEI: With 20 S42ILs, 14 QTLs on all barley chromosomes were detected for plant height. Five of these QTLs reduced plant height. The strongest *Hsp* effects were found in S42IL-143 (chromosome 1H) revealing plant heights reduced by 3.79, 3.29 and 4.32 cm across treatments, within P10 and P100, respectively. In contrast, *Hsp* alleles increased plant height relative to 'Scarlett' in 15 lines representing all barley chromosomes except 1H. The *Hsp* allele of S42IL-121 (chromosome 4H) increased plant height by 5.11, 3.99 and 6.41 cm across treatments, within P10 and P100, respectively.

SDW: For shoot dry weight twelve QTLs were identified on 19 S42ILs. These QTLs were located on all barley chromosomes. Eleven out of 12 detected QTLs revealed an increase in shoot dry weight due to the presence of the *Hsp* allele. The strongest increase was detected for QSdw.S42IL-4H.b (S42IL-121) with a plus of 28.30, 17.47 and 38.76 mg across treatments, within P10 and P100, respectively. In contrast, the exotic allele at QSdw.S42Il-7H.b (S42IL-139) showed a dry weight reduced by 14.28 mg across treatments compared to 'Scarlett'.

RL: In 14 lines nine QTLs for root length were found on chromosomes 1H to 7H. At seven QTLs, the *Hsp* allele reduced root length. The strongest reduction of root length across treatments

and under P100 was found in S42IL-176 (QRl.S42IL-5H) with 7.91 and 8.86 cm compared to 'Scarlett', respectively and under P10 in S42IL-133 (QRl.S42IL-7Ha) with 9.07 cm. In contrast, at two QTLs on chromosomes 6H and 7H S42ILs were associated with an increased root length (Maximum: +7.65 cm in S42IL-135 under P10 treatment).

RDW: In nine S42ILs eight QTLs controlling root dry weight were found on chromosomes 1H, 4H, 6H and 7H. The strongest increasing *Hsp* effect was detected in S42IL-129 (QRdw.S42IL-6H) with +12.38 and +12.26 mg across treatments and within P10, respectively. Under treatment P100 only S42IL-102 was associated with an increasing root dry weight effect of +18.08 mg compared to 'Scarlett'.

SRLR: In 18 S42ILs nine QTLs controlling shoot root length ratio were located on all barley chromosomes except chromosome 6H. *Hsp* alleles increased SRLR in all lines except one (S42IL-124). The strongest effects were observed in S42IL-102 with an increase of 26.45% and in S42ILs -140 and -121 across treatments and within P10, respectively. The *Hsp* allele in line S42IL-109 increased shoot root length ratio by 30.13% under P100.

SRWR: In 19 S42ILs 14 QTLs controlling shoot root weight ratio were detected on all barley chromosomes. *Hsp* alleles increased SRWR in all lines except three (S42ILs -109, –128 and -129). The strongest *Hsp* effects were found in S42IL-133 (QSrwr.S42IL-7H.a) with an increase of SRWR by 41.26%, 66.08% and 24.41%, compared to 'Scarlett', across treatments, within P10 and P100, respectively.

BMD: For biomass, eight QTLs were identified. *Hsp* alleles increased biomass in all lines except one (S42IL-133). The strongest effect was found in S42IL-121 (QBmd.S42IL-4H.b) increasing biomass by 25.61% across treatments and by 31.51% within P100 treatment. Only S42IL-133 was associated with a biomass reduced by 16.69% across treatments.

QTLs for nutrient concentration traits

Altogether, for two nutrient concentration traits significant deviations of S42ILs from 'Scarlett' were detected. These traits are presented in the following.

NaCS: Only one QTL was detected for sodium concentration shoot. At QNacs.S42IL-1H (S42IL-143) the exotic allele was associated with a reduction of NaCS across treatments by 38.30% compared to 'Scarlett'.

PCS: In 14 S42ILs twelve QTLs controlling P concentration shoot were detected on all barley chromosomes. *Hsp* alleles increased PCS in all lines, however, only under P100 treatment. The strongest *Hsp* effect was found in S42IL-116 (QPcs.S42IL-4H.a) with an increase of PCS by 39.66%.

QTLs for stress index traits

A stress index, defined as the ratio of trait performance under P10 and P100, was calculated for each trait. In total, QTLs for three stress index traits were detected. These traits are presented in the following.

TN_SI: For tiller number stress index one QTL was identified. At QTn_si.S42IL-1H (present in S42IL-142) the exotic allele was associated with a reduction of the tiller number stress index by 30.09% compared to 'Scarlett'.

RDW_SI: For root dry weight stress index one QTL was identified. At QRdw_si.S42IL-7H (present in S42IL-139) the exotic allele was associated with an increase of the root dry weight stress index by 46.61% compared to 'Scarlett'.

CaS_SI: For calcium concentration shoot stress index one QTL was identified. At QCas_si.S42IL-5H (present in S42IL-125) the exotic allele was associated with an increase of the calcium concentration shoot index by 148.18% compared to 'Scarlett'.

Table 15: Results of Dunnett test of significant genotype effects for 62 traits, calculated across (ALL) and within treatments (P10 or P100).

						Effect across treatments			Effect within P10			Effect within P100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, corresponding QTL[g]**
TN	QTn.S42IL-1H	All, P100	S42IL-142	1H	188.50-205.07	1.42	0.31	27.65				1.84	0.61	49.96	
HEI	QHei.S42IL-1H.a	All	S42IL-105	1H	74.40-90.92	23.19	-1.99	-7.91							
HEI	QHei.S42IL-1H.b	All	S42IL-141	1H	94.86-127.71	23.04	-2.14	-8.51							
HEI	QHei.S42IL-1H.c	All, P10, P100	S42IL-143	1H	130.68-173.49	21.40	-3.79	-15.04	17.99	-3.29	-15.47	24.66	-4.32	-14.92	HvFT31, *1)
HEI	QHei.S42IL-2H	All, P100	S42IL-109	2H	63.96-110.84	27.71	2.52	10.02				33.08	4.09	14.12	Flt-2L2, Sdw33, *1), *5)
HEI	QHei.S42IL-3H	All	S42IL-112	3H	104.39-161.43	26.99	1.81	7.17							
HEI		All, P10	S42IL-114	3H	138.00-245.49	28.01	2.82	11.21	23.91	2.63	12.36				Sdw1 (denso) 4, 5
HEI		All, P10	S42IL-140	3H	154.99-253.73	27.85	2.67	10.59	23.51	2.23	10.47				Sdw1 (denso) 4, 5, *5)
HEI	QHei.S42IL-4H.a	All	S42IL-116	4H	5.42-47.80	27.29	2.11	8.37							
HEI	QHei.S42IL-4H.b	All, P10, P100	S42IL-118	4H	61.15-83.58	28.88	3.69	14.66	23.99	2.71	12.74	33.75	4.76	16.42	
HEI		All, P10, P100	S42IL-121	4H	74.11-119.06	30.30	5.11	20.30	25.27	3.99	18.75	35.39	6.41	22.10	*1)
HEI	QHei.S42IL-4H.c	All, P10	S42IL-123	4H	128.85-172.32	28.16	2.97	11.81	24.04	2.76	12.96				*1)
HEI	QHei.S42IL-5H	All, P10	S42IL-126	5H	145.57-200.12	27.25	2.06	8.18	23.25	1.98	9.28				ari-e.GP4, *1)
HEI	QHei.S42IL-6H.a	All, P10	S42IL-128	6H	71.39-132.23	28.04	2.86	11.34	23.77	2.49	11.69				
HEI		All, P10, P100	S42IL-129	6H	73.90-133.47	27.88	2.69	10.67	23.32	2.04	9.59	32.42	3.44	11.85	
HEI		All, P10	S42IL-130	6H	98.66-180.69	27.22	2.04	8.08	23.49	2.21	10.39				
HEI	QHei.S42IL-6H.b	All, P10	S42IL-148	6H	3.28-10.73	27.52	2.33	9.25	24.18	2.90	13.63				*1)
HEI	QHei.S42IL-6H.c	All	S42IL-149	6H	71.39-82.43	23.31	-1.88	-7.46							
HEI	QHei.S42IL-7H.a	All, P10	S42IL-133	7H	17.32-51.93	22.30	-2.89	-11.48	18.13	-3.15	-14.78				brh16, *1)
HEI	QHei.S42IL-7H.b	All, P10, P100	S42IL-135	7H	101.23-152.29	29.28	4.09	16.25	25.14	3.86	18.13	33.00	4.01	13.83	*1)
HEI		All, P10	S42IL-137	7H	134.43-193.89	29.24	4.06	16.11	24.87	3.59	16.87				*1)
SDW	QSdw.S42IL-1H.a	All	S42IL-102	1H	1.10-98.23	100.74	13.40	15.35							

						Effect across treatments			Effect within P10			Effect within P100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, corresponding QTL[g]**
SDW	QSdw.S42IL-1H.b	All	S42IL-142	1H	188.50-205.07	100.52	13.19	15.10							
SDW	QSdw.S42IL-2H	All	S42IL-106	2H	22.35-34.31	105.02	17.69	20.25							
SDW		All, P10	S42IL-107	2H	34.31-66.78	105.47	18.14	20.77	82.35	14.93	22.15				
SDW		All	S42IL-108	2H	34.31-104.81	100.92	13.58	15.55							
SDW	QSdw.S42IL-3H	All, P10	S42IL-113	3H	204.48-239.73	104.87	17.54	20.09	81.72	14.31	21.22				
SDW		All	S42IL-114	3H	138.00-245.49	103.85	16.52	18.91							
SDW		All	S42IL-140	3H	154.99-253.73	105.26	17.93	20.53							
SDW	QSdw.S42IL-4H.a	All	S42IL-116	4H	5.42-47.80	105.06	17.73	20.30							
SDW	QSdw.S42IL-4H.b	All	S42IL-118	4H	61.15-83.58	105.28	17.95	20.55							
SDW		All, P10, P100	S42IL-120	4H	61.15-83.58	109.93	22.60	25.88	82.98	15.57	23.09	136.48	29.25	27.28	
SDW		All, P10, P100	S42IL-121	4H	74.11-119.06	115.64	28.30	32.41	84.88	17.47	25.91	145.98	38.76	36.15	*1)
SDW	QSdw.S42IL-5H	All, P100	S42IL-126	5H	145.57-200.12	106.34	19.01	21.77				138.16	30.94	28.86	
SDW	QSdw.S42IL-6H.a	All	S42IL-128	6H	71.39-132.23	102.75	15.42	17.65							
SDW		All, P10	S42IL-129	6H	73.90-133.47	102.17	14.84	16.99	81.92	14.50	21.51				
SDW	QSdw.S42IL-6H.b	All	S42IL-148	6H	3.28-10.73	100.38	13.05	14.94							
SDW	QSdw.S42IL-6H.c	P10	S42IL-131	6H	140.00-180.69				83.25	15.83	23.49				
SDW	QSdw.S42IL-7H.a	All	S42IL-135	7H	101.23-152.29	101.48	14.15	16.21							
SDW	QSdw.S42IL-7H.b	All	S42IL-139	7H	198.70-229.66	73.06	-14.28	-16.35							
RL	QRl.S42IL-1H.a	All, P10	S42IL-102	1H	1.10-98.23	23.55	-6.15	-20.72	21.26	-6.71	-23.99				*2)
RL		All, P10, P100	S42IL-105	1H	74.40-90.92	22.63	-7.07	-23.79	21.46	-6.51	-23.28	24.08	-7.22	-23.08	
RL	QRl.S42IL-1H.b	All	S42IL-143	1H	130.68-173.49	24.69	-5.01	-16.88							*1)
RL	QRl.S42IL-2H	P10	S42IL-110	2H	102.66-104.81				22.93	-5.04	-18.01				
RL	QRl.S42IL-3H	All, P10	S42IL-140	3H	154.99-253.73	24.39	-5.31	-17.88	21.71	-6.26	-22.38				
RL		All	S42IL-161	3H	239.73-253.73	26.06	-3.64	-12.26							
RL	QRl.S42IL-4H	All, P10	S42IL-119	4H	61.15-119.06	23.95	-5.75	-19.35	23.37	-5.60	-20.02				*2)

						Effect across treatments			Effect within P10			Effect within P100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, corresponding QTL[g]**
RL	QRl.S42IL-5H	All, P10	S42IL-125	5H	104.73-154.37	25.74	-3.96	-13.33	22.87	-5.10	-18.24				*2), *5)
RL		All	S42IL-126	5H	145.57-200.12	24.80	-4.90	-16.50							ari-e.GP2, *1)
RL		All, P10, P100	S42IL-176	5H	154.37-234.98	21.79	-7.91	-26.63	21.33	-6.64	-23.75	22.44	-8.86	-28.31	*2)
RL	QRl.S42IL-6H	All, P10	S42IL-128	6H	71.39-132.23	35.01	5.31	17.89	35.59	7.63	27.26				*1)
RL		All, P10	S42IL-129	6H	73.90-133.47	35.06	5.36	18.03	33.80	5.83	20.85				*1)
RL	QRl.S42IL-7H.a	All, P10, P100	S42IL-133	7H	17.32-51.93	21.96	-7.74	-26.07	18.89	-9.07	-32.44	24.82	-6.48	-20.69	*1)
RL	QRl.S42IL-7H.b	All, P10	S42IL-135	7H	101.23-152.29	34.63	4.93	16.59	35.62	7.65	27.34				*1)
RDW	QRdw.S42IL-1H.a	All, P100	S42IL-102	1H	1.10-98.23	58.16	10.75	22.68				68.51	18.08	35.86	*1), *2), *5)
RDW	QRdw.S42IL-1H.b	All, P10	S42IL-143	1H	130.68-173.49	34.88	-12.52	-26.41	33.14	-11.23	-25.31				*1)
RDW	QRdw.S42IL-4H.a	All	S42IL-116	4H	5.42-47.80	56.53	9.13	19.26							*2)
RDW	QRdw.S42IL-4H.b	All	S42IL-120	4H	61.15-83.58	57.44	10.04	21.18							
RDW	QRdw.S42IL-4H.c	P10	S42IL-119	4H	61.15-119.06				33.19	-11.17	-25.18				
RDW	QRdw.S42IL-6H	All	S42IL-128	6H	71.39-132.23	58.53	11.13	23.48							
RDW		All, P10	S42IL-129	6H	73.90-133.47	59.78	12.38	26.11	56.62	12.26	27.64				*2)
RDW	QRdw.S42IL-7H.a	All, P10	S42IL-133	7H	17.32-51.93	34.58	-12.82	-27.04	29.78	-14.58	-32.87				*2), *5)
RDW	QRdw.S42IL-7H.b	All	S42IL-139	7H	198.70-229.66	37.80	-9.60	-20.25							
SRLR	QSrlr.S42IL-1H	All, P10, P100	S42IL-102	1H	1.10-98.23	1.10	0.23	26.45	1.01	0.22	27.30	1.21	0.26	26.83	
SRLR		All, P10, P100	S42IL-105	1H	74.40-90.92	1.05	0.18	20.22	0.93	0.14	17.95	1.15	0.20	20.69	
SRLR	QSrlr.S42IL-2H	All, P100	S42IL-109	2H	63.96-110.84	1.03	0.15	17.43				1.24	0.29	30.13	
SRLR		All, P10	S42IL-110	2H	102.66-104.81	1.00	0.13	14.90	0.95	0.16	20.41				
SRLR	QSrlr.S42IL-3H	P100	S42IL-112	3H	104.39-161.43							1.13	0.18	18.44	
SRLR		All	S42IL-114	3H	138.00-245.49	0.99	0.11	13.15							
SRLR		All, P10, P100	S42IL-140	3H	154.99-253.73	1.10	0.23	25.93	1.03	0.23	29.48	1.18	0.23	23.90	
SRLR	QSrlr.S42IL-4H.a	All, P100	S42IL-118	4H	61.15-83.58	1.00	0.13	15.05				1.17	0.21	22.32	
SRLR		All, P10, P100	S42IL-121	4H	74.11-119.06	1.08	0.20	23.36	1.03	0.23	29.48	1.12	0.17	17.80	

						Effect across treatments			Effect within P10			Effect within P100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, corresponding QTL[g]**
SRLR		All, P10, P100	S42IL-119	4H	61.15-119.06	1.04	0.16	18.57	0.94	0.15	19.02	1.12	0.16	17.09	
SRLR	QSrlr.S42IL-4H.b	P100	S42IL-123	4H	128.85-172.32							1.12	0.16	17.06	
SRLR	QSrlr.S42IL-4H.c	All, P10	S42IL-124	4H	171.25-183.54	0.77	-0.10	-11.93	0.64	-0.15	-19.04				
SRLR	QSrlr.S42IL-5H	All, P10	S42IL-125	5H	104.73-154.37	0.98	0.11	12.63	0.96	0.17	21.29				
SRLR		All, P10, P100	S42IL-126	5H	145.57-200.12	1.05	0.18	20.52	0.95	0.16	19.86	1.17	0.21	22.34	*1)
SRLR		All, P10, p100	S42IL-176	5H	154.37-234.98	1.10	0.23	26.08	1.01	0.22	27.69	1.17	0.21	22.43	
SRLR	QSrlr.S42IL-7H.a	All, P10	S42IL-133	7H	17.32-51.93	1.02	0.14	16.29	1.00	0.21	26.56				
SRLR	QSrlr.S42IL-7H.b	All, P100	S42IL-136	7H	134.43-152.29	0.99	0.11	13.13				1.11	0.15	15.81	
SRLR		All, P10, P100	S42IL-137	7H	134.43-193.89	1.07	0.20	23.09	1.00	0.21	26.43	1.17	0.21	22.48	
SRWR	QSrwr.S42IL-1H.a	All	S42IL-141	1H	94.86-127.71	2.00	0.14	7.74							
SRWR	QSrwr.S42IL-1H.b	All, P10, P100	S42IL-143	1H	130.68-173.49	2.34	0.48	25.98	1.96	0.41	26.56	2.68	0.52	24.22	*1)
SRWR	QSrwr.S42IL-2H.a	All, P10, P100	S42IL-107	2H	34.31-66.78	2.11	0.25	13.72	1.74	0.19	12.32	2.48	0.32	14.68	*1)
SRWR	QSrwr.S42IL-2H.b	All, P10	S42IL-109	2H	63.96-110.84	1.70	-0.16	-8.53	1.40	-0.15	-9.83				
SRWR	QSrwr.S42IL-3H	All, P10	S42IL-140	3H	154.99-253.73	2.03	0.18	9.65	1.81	0.26	16.73				
SRWR	QSrwr.S42IL-4H.a	All	S42IL-118	4H	61.15-83.58	2.00	0.14	7.69							
SRWR		All, P10	S42IL-119	4H	61.15-119.06	2.07	0.21	11.40	1.82	0.27	17.62				
SRWR		All, P10	S42IL-121	4H	74.11-119.06	2.03	0.17	9.43	1.78	0.23	14.57				
SRWR	QSrwr.S42IL-4H.b	All, P100	S42IL-123	4H	128.85-172.32	2.03	0.18	9.48				2.45	0.29	13.58	*1)
SRWR	QSrwr.S42IL-5H.a	All, P10, P100	S42IL-126	5H	145.57-200.12	2.09	0.24	12.67	1.72	0.17	11.11	2.47	0.31	14.26	*1)
SRWR		All, P100	S42IL-176	5H	154.37-234.98	2.05	0.19	10.34				2.45	0.29	13.28	
SRWR	QSrwr.S42IL-5H.b	P10	S42IL-127	5H	231.75-276.77				1.76	0.21	13.30				
SRWR	QSrwr.S42IL-6H.a	All	S42IL-122	6H	180.69-208.13	2.00	0.15	8.08							
SRWR	QSrwr.S42IL-6H.b	P10	S42IL-128	6H	71.39-132.23				1.38	-0.17	-11.13				
SRWR		All	S42IL-129	6H	73.90-133.47	1.68	-0.17	-9.31							
SRWR	QSrwr.S42IL-6H.c	All, P100	S42IL-148	6H	3.28-10.73	2.07	0.21	11.43				2.46	0.30	13.83	

						Effect across treatments			Effect within P10			Effect within P100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, corresponding QTL[g]**
SRWR	QSrwr.S42IL-7H.a	All, P10, P100	S42IL-133	7H	17.32-51.93	2.62	0.77	41.26	2.57	1.02	66.08	2.69	0.53	24.41	*1)
SRWR		All	S42IL-134	7H	51.93-107.44	1.99	0.14	7.55							
SRWR	QSrwr.S42IL-7H.b	All, P10, P100	S42IL-137	7H	134.43-193.89	2.07	0.22	11.75	1.73	0.18	11.53	2.42	0.26	12.17	
BMD	QBmd.S42IL-1H	ALL, P100	S42IL-102	1H	1.10-98.23	158.84	24.12	17.90				200.44	42.80	27.15	
BMD	QBmd.S42IL-2H	All	S42IL-106	2H	22.35-34.31	158.85	24.12	17.91							
BMD	QBmd.S42IL-3H	All	S42IL-113	3H	204.48-239.73	158.07	23.35	17.33							
BMD		All	S42IL-114	3H	138.00-245.49	156.77	22.05	16.37							
BMD		All	S42IL-140	3H	154.99-253.73	156.98	22.25	16.52							
BMD	QBmd.S42IL-4H.a	All	S42IL-116	4H	5.42-47.80	162.17	27.45	20.37							
BMD	QBmd.S42IL-4H.b	All, P100	S42IL-118	4H	61.15-83.58	157.57	22.84	16.96				187.71	30.06	19.07	
BMD		All, P100	S42IL-120	4H	61.15-83.58	166.25	31.53	23.40				200.03	42.39	26.89	
BMD		All, P100	S42IL-121	4H	74.11-119.06	169.22	34.50	25.61				20.32	49.68	31.51	*3)
BMD	QBmd.S42IL-5H	All	S42IL-126	5H	145.57-200.12	157.04	22.32	16.56							
BMD	QBmd.S42IL-6H	All	S42IL-128	6H	71.39-132.23	161.27	26.54	19.70							
BMD		All	S42IL-129	6H	73.90-133.47	156.88	22.16	16.45							*3), *4)
BMD	QBmd.S42IL-7H	All	S42IL-133	7H	17.32-51.93	112.24	-22.48	-16.69							
NaCS	QNacs.S42IL-1H	All	S42IL-143	1H	130.68-173.49	349.34	-216.87	-38.30							
PCS	QPcs.S42IL-1H	P100	S42IL-143	1H	130.68-173.49							3139.15	720.73	29.80	
PCS	QPcs.S42IL-2H	P100	S42IL-106	2H	22.35-34.31							3093.24	674.83	27.90	
PCS	QPcs.S42IL-3H	P100	S42IL-113	3H	204.48-239.73							3109.84	691.42	28.59	
PCS	QPcs.S42IL-4H.a	P100	S42IL-116	4H	5.42-47.80							3377.57	959.15	39.66	
PCS	QPcs.S42IL-4H.b	P100	S42IL-119	4H	61.15-119.06							3336.08	917.66	37.94	
PCS	QPcs.S42IL-5H.a	P100	S42IL-125	5H	104.73-154.37							3251.72	833.30	34.46	
PCS		P100	S42IL-126	5H	145.57-200.12							3111.08	692.66	28.64	
PCS	QPcs.S42IL-5H.b	P100	S42IL-127	5H	231.75-276.77							3104.15	685.74	28.35	HvPHT1;67

						Effect across treatments			Effect within P10			Effect within P100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, correspon-ding QTL[g]**
PCS	QPcs.S42IL-6H.a	P100	S42IL-130	6H	98.66-180.69							3134.60	716.18	29.61	
PCS	QPcs.S42IL-6H.b	P100	S42IL-149	6H	71.39-82.43							3338.59	920.17	38.05	
PCS	QPcs.S42IL-7H.a	P100	S42IL-133	7H	17.32-51.93							3204.06	785.65	32.49	
PCS	QPcs.S42IL-7H.b	P100	S42IL-136	7H	134.43-152.29							3273.35	854.93	35.35	
PCS		P100	S42IL-137	7H	134.43-193.89							3290.93	872.51	36.08	
PCS	QPcs.S42IL-7H.c	P100	S42IL-139	7H	198.70-229.66							3198.30	779.88	32.25	
TN_SI	QTn_si.S42Il-1H	ALL	S42IL-142	1H	188.50-205.07	0.62	-0.27	-30.09							
RDW_SI	QRdw_si.S42IL-7H	ALL	S42IL-139	7H	198.70-229.66	1.34	0.43	46.61							
CaS_SI	QCacs_si.S42IL-5H	ALL	S42IL-125	5H	104.73-154.37	2.44	1.46	148.18							

[a] Trait abbreviations are explained in Tables 4, 5 and 6.
[b] Significant Hsp effect under investigated treatment: across treatments (All), low P (P10) and control P (P100), respectively
[c] Location of chromosome and introgression target (cM) based on Schmalenbach et al. (2011)
[d] Least squares means of IL across treatments, P10 and P100, respectively
[e] Difference between LSmeans [S42IL] and LSmeans [Scarlett] across treatments, P10 and P100, respectively
[f] Relative performance (in %): LSmeans [S42IL] - LSmeans [Scarlett] ×100 / LSmeans [Scarlett]
[g] References of candidate genes: 1: Wang et al. (2010), 2: Chen et al. (2009), 3: Gottwald et al. (2004), 4: Chloupek et al. (2006), 5: Laurie et al. (1993), 6: Dahleen et al. (2005), 7: Pacak et al. (2016)
[g] References of corresonding QTLs: *1), *2),*3),*4) and *5) indicate corresponding Hsp effects in identical S42ILs, reported by Hoffmann et al. (2012), Naz et al. (2014), Honsdorf et al. (2014a), Honsdorf et al. (2014b) and Arifuzzaman et al. (2014), respectively

3.2 Potassium investigation

3.2.1 Morphological traits - trait performance and correlations

Trait performances of the 47 introgression lines and 'Scarlett' are listed in Table 16 containing number of observations (N), means, standard deviation (SD), coefficient of variation (CV) and heritability (h^2) for all 8 traits, across treatments and separately for low and adequate K supplies, K0 and K100, respectively. As expected, the means of all morphological traits studied were higher under K100 than under K0 with one exception for root length. Only root length revealed a higher mean value under reduced K. For example, mean biomass accumulation under K0 and K100 is shown in Fig. 6. Under low K treatment, the average dry biomass dropped from 157.5 to 135.2 mg (Table 16).

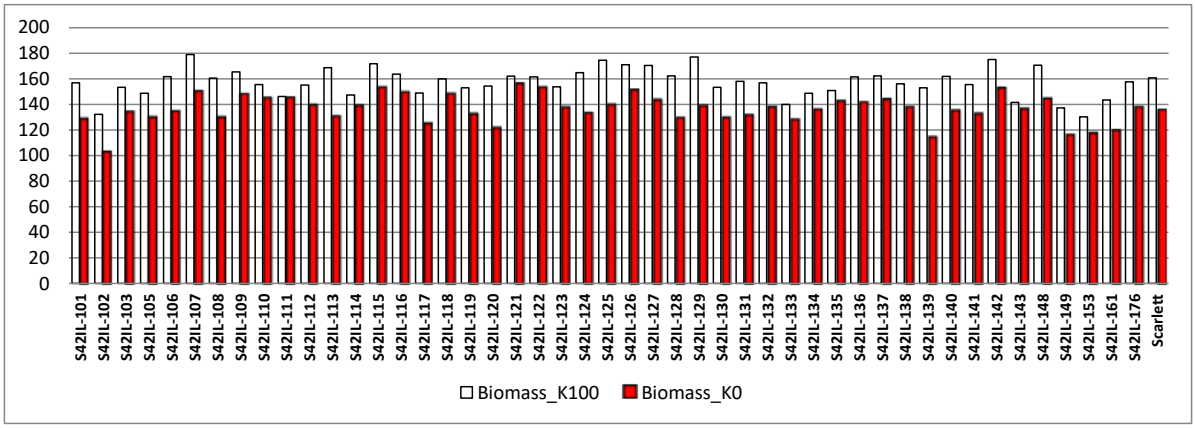

Figure 6: Mean biomass accumulation (in mg) of S42ILs and Scarlett cultivated under K0 and K100 treatments.

The highest and lowest CV was observed under control K for tiller number (40.1%) and HEI (12.8%). The heritabilities for TN, RDW and BMD were low (less than 50%) independent of the treatment level. The highest heritability could be found for RL (74.7%) across both treatments.

Pearson correlations between eight morphological traits across treatments are given in Table 17. Across treatments, seven significant correlations were observed. The highest positive correlation was found between biomass and shoot dry weight (r=0.97), followed by biomass and root dry weight (r=0.91). In addition, plant height revealed positive correlations with shoot dry weight, root dry weight and biomass (0.68, 0.56 and 0.66, respectively). Finally, shoot dry weight and root dry weight showed a positive correlation with r=0.82. A negative significant correlation across treatments was found between shoot root length ratio and root length with r=-0.73.

Table 16: Trait performance and heritability of 47 ILs and Scarlett, across and within K treatments

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^{2} [g]
	All	2198	1.2	0.5	37.4	23.2
	K0	1100	1.1	0.3	25.3	n.e
TN	K100	1098	1.4	0.6	40.1	30.3
	All	2195	32.5	4.7	14.5	65.9
	K0	1102	32.1	5.1	15.9	66.8
HEI	K100	1093	33.0	4.2	12.8	64.4
	All	2175	100.2	30.2	30.2	47.9
	K0	1096	89.7	26.7	29.8	55.1
SDW	K100	1079	110.9	29.8	26.9	32.6
	All	2209	34.6	8.5	24.5	74.7
	K0	1106	37.5	6.1	16.3	66.8
RL	K100	1103	31.7	9.5	29.9	68.8
	All	2189	46.5	14.6	31.5	29.5
	K0	1098	45.7	14.6	32.0	34.2
RDW	K100	1091	47.3	14.6	30.9	22.2
	All	2084	1.0	0.3	26.9	68.3
	K0	1051	0.9	0.1	16.3	67.6
SRLR	K100	1033	1.1	0.3	27.3	63.6
	All	2093	2.2	0.4	18.5	63.2
	K0	1049	2.0	0.4	18.8	53.8
SRWR	K100	1044	2.4	0.3	13.9	61.3
	All	2134	146.3	42.0	28.7	39.0
	K0	1076	135.2	38.7	28.6	47.0
BMD	K100	1058	157.5	42.2	26.8	31.0

[a] Trait abbreviations are given in Tables 4, 5 and 6.
[b] Across: across both K treatments, K100 and K0: within treatment
[c] Number of observations
[d] Mean value
[e] Standard deviation
[f] Coefficient of variation (in %)
[g] Heritability (in %)

Pearson correlations between morphological traits were also calculated within K treatments (Table 18). Under K0 the strongest positive and negative correlations were found between shoot dry weight and biomass (r=0.97) and between shoot root length ratio and root length (r=-0.61). Under K100 the strongest positive and negative correlations were found again between shoot dry weight and biomass (r=0.96) and between shoot root length ratio and root length (r=-0.-78).

Table 17: Pearson correlation coefficients between morphological traits across K treatments

	TN	HEI	SDW	RL	RDW	SRLR	SRWR	BMD
TN								
HEI	0.00							
SDW	0.35	**0.68**						
RL	0.05	0.39	0.34					
RDW	0.16	**0.56**	**0.82**	0.47				
SRLR	-0.12	0.31	0.13	**-0.73**	-0.07			
SRWR	0.36	0.25	0.32	-0.10	-0.22	0.23		
BMD	0.31	**0.66**	**0.97**	0.40	**0.91**	0.06	0.14	

(Bold values indicate significant correlations with P<0.001)

Trait auto correlations between K0 and K100 were positive and highly significant (P<0.001) for each morphological trait, except tiller number (diagonal in Table 18). The highest auto correlation coefficient with 0.84 was observed for root length, followed by plant height with 0.78.

Table 18: Pearson correlation coefficients between morphological traits under K0 (bottom left triangle) and K100 (upper right triangle)

	TN	HEI	SDW	RL	RDW	SRLR	SRWR	BMD
TN	0.49	-0.11	0.38	0.06	0.12	-0.23	0.33	0.30
HEI	0.21	**0.78**	**0.59**	0.47	**0.64**	0.11	-0.03	**0.60**
SDW	0.32	**0.77**	**0.72**	0.34	**0.79**	-0.02	0.19	**0.96**
RL	0.02	0.31	0.31	**0.84**	0.44	**-0.78**	-0.11	0.36
RDW	0.17	0.49	**0.77**	0.44	**0.63**	-0.08	-0.35	**0.90**
SRLR	0.18	**0.54**	0.35	**-0.61**	0.00	**0.74**	0.07	-0.04
SRWR	0.31	0.42	0.37	-0.08	-0.24	0.44	**0.59**	-0.03
BMD	0.29	**0.71**	**0.97**	0.38	**0.89**	0.23	0.18	**0.67**

(Bold values indicate significant correlations with P<0.001,
Diagonal: auto correlation between K0 and K100)

3.2.2 Nutrient concentration traits - trait performance and correlations

In Table 19 trait performances of the 47 introgression lines and 'Scarlett' are given across treatments and separately within K0 and K100 treatments for 24 nutrient concentration traits and 30 stress index traits. As for morphological traits mean nutrient values increased in more than half of the investigated traits (13 from 24) from K0 to K100 treatment. Coefficient of

variation in 20 out of 24 nutrient concentration traits was higher than 10% (at least under one treatment), with the highest and lowest CV for NaCS (72.6%) and CCS (5.6%) under control potassium nutrition, respectively. The highest and lowest CV were found for magnesium concentration root stress index (MgR_SI, 516.3%) and carbon concentration shoot stress index (CS_SI, 7.2%) across treatments. The highest heritabilities estimated were detected for potassium concentration shoot (54.7%), manganese concentration shoot (47.2%) and carbon concentration root (49.2%) across treatments, within K0 and within K100, respectively. For the stress index traits magnesium concentration shoot (MgS_SI) showed the highest heritability (38.2%).

Table 19: Trait performance and heritability of 47 ILs and Scarlett, across and within K treatments

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
CaCR	All	458	10869.3	2087.1	19.2	27.2
	K0	231	10733.5	1840.9	17.2	18.8
	K100	227	11007.6	2306.8	21.0	8.4
CCR	All	462	64.3	6.5	10.1	0.0
	K0	228	65.7	6.5	9.8	26.7
	K100	234	62.9	6.2	9.9	49.2
CuCR	All	455	204.4	36.5	17.9	36.6
	K0	226	211.2	35.8	17.0	5.8
	K100	229	197.8	36.0	18.2	12.9
FeCR	All	460	2709.7	543.9	20.1	0.0
	K0	230	2669.9	546.8	20.5	0.0
	K100	230	2749.5	539.3	19.6	4.1
KCR	All	458	265711.6	41116.7	15.5	37.1
	K0	230	245343.8	41363.3	16.9	0.0
	K100	228	286258.0	28864.6	10.1	13.7
MgCR	All	449	5875.9	3087.8	52.5	24.3
	K0	232	7891.1	2639.2	33.4	6.4
	K100	217	3721.3	1816.6	48.8	36.4
MnCR	All	460	32.9	4.2	12.8	35
	K0	231	31.5	4.4	14.1	0.0
	K100	229	34.2	3.4	10.0	13.0
NaCR	All	464	4693.0	2232.1	47.6	0.0
	K0	231	5586.9	2334.3	41.8	5.2
	K100	233	3806.7	1719.4	45.2	0.0
NCR	All	460	14.3	1.3	9.3	0.0
	K0	227	14.8	1.3	8.8	25.8
	K100	233	13.8	1.1	8.2	0.0

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
PCR	All	458	11196.6	2006.7	17.9	1.3
	K0	230	10973.2	1970.4	18.0	11.5
	K100	228	11422.0	2022.0	17.7	2.0
SCR	All	459	901.9	349.2	38.7	35.5
	K0	231	885.5	337.6	38.1	20.8
	K100	228	918.5	360.6	39.3	20.6
ZnCR	All	459	699.2	211.8	30.3	17.4
	K0	232	635.9	202.1	31.8	30.4
	K100	227	763.9	202.1	26.5	0.0
CaCS	All	438	1307.5	924.2	70.7	0.5
	K0	220	1520.5	1012.0	66.6	47.0
	K100	218	1092.6	770.8	70.6	15.1
CCS	All	455	80.9	5.4	6.7	0.0
	K0	226	83.0	5.6	6.7	24.3
	K100	229	78.8	4.4	5.6	0.0
CuCS	All	426	30.8	18.3	59.3	0.0
	K0	214	30.7	19.4	63.3	19.8
	K100	212	30.9	17.1	55.2	39.6
FeCS	All	462	1873.3	180.5	9.6	0.0
	K0	231	1922.8	201.7	10.5	1.4
	K100	231	1823.8	140.4	7.7	35.3
KCS	All	455	186009.6	32671.3	17.6	54.7
	K0	228	168906.7	32800.6	19.4	45.1
	K100	227	203187.8	21728.8	10.7	0.0
MgCS	All	452	4019.7	1264.5	31.5	7.2
	K0	224	4979.2	927.1	18.6	24.5
	K100	228	3077.0	728.9	23.7	3.0
MnCS	All	456	27.6	4.0	14.3	40.5
	K0	228	25.5	3.8	15.1	47.2
	K100	228	29.8	2.7	9.1	0.0
NaCS	All	440	942.5	651.6	69.1	0.0
	K0	224	1036.2	674.6	65.1	0.0
	K100	216	845.4	613.4	72.6	0.0
NCS	All	462	9.3	0.9	9.7	4.1
	K0	232	9.8	0.9	8.9	0.0
	K100	230	8.8	0.7	7.7	14.8
PCS	All	460	12709.0	1574.8	12.4	49.6
	K0	233	12114.9	1660	13.7	43.8
	K100	227	13318.8	1212.8	9.1	1.2
SCS	All	464	2669.6	206.5	7.7	48.9
	K0	232	2600.6	211.3	8.1	45.6

Trait [a]	Treatment [b]	N [c]	Mean [d]	SD [e]	CV [f]	h^2 [g]
SCS	K100	232	2738.6	176.9	6.5	20.0
ZnCS	All	456	708.3	105.1	14.8	0.0
	K0	229	655.2	102.3	15.6	4.2
	K100	227	761.8	77.1	10.1	0.0
TN_SI	All	1057	0.9	0.3	29.8	2.8
HEI_SI	All	1055	1.0	0.2	15.7	28.8
SDW_SI	All	1041	0.8	0.3	30.1	12.6
RL_SI	All	1063	1.3	0.4	34.3	22.4
RDW_SI	All	1048	1.0	0.4	37.0	18.3
BMD_SI	All	1005	0.9	0.3	29.5	20.5
CaR_SI	All	218	1.0	0.3	33.9	0.0
CR_SI	All	222	1.1	0.1	13.4	37.9
CuR_SI	All	215	1.1	0.2	21.1	0.0
FeR_SI	All	221	1.0	0.2	18.8	0.0
KR_SI	All	218	0.9	0.2	18.1	0.0
MgR_SI	All	209	6.1	31.6	516.3	0.0
MnR_SI	All	220	0.9	0.2	16.7	0.0
NaR_SI	All	225	1.7	0.9	52.3	0.0
NR_SI	All	221	1.1	0.1	10.7	9.1
PR_SI	All	219	1.0	0.2	22.0	1.3
SR_SI	All	220	1.1	0.6	55.0	0.0
ZnR_SI	All	219	0.9	0.3	31.0	0.0
CaS_SI	All	202	2.9	7.3	254.8	36
CS_SI	All	217	1.1	0.1	7.2	19.7
CuS_SI	All	190	1.8	3.1	174.8	18.0
FeS_SI	All	223	1.1	0.1	12.9	24.2
KS_SI	All	216	0.8	0.1	17.2	10.9
MgS_SI	All	212	1.7	0.5	31.2	38.2
MnS_SI	All	216	0.9	0.1	14	28.7
NaS_SI	All	203	2.5	3.7	147.2	35.0
NS_SI	All	223	1.1	0.1	9.9	0.0
PS_SI	All	220	0.9	0.1	14.4	6.7
SS_SI	All	226	1.0	0.1	8.7	0.0
ZnS_SI	All	217	0.9	0.1	15.3	0.0

[a] Trait abbreviations are given in Tables 4, 5 and 6.
[b] Across: across both K treatments, K100 and K0: within treatment
[c] Number of observations
[d] Mean value
[e] Standard deviation
[f] Coefficient of variation (in %)
[g] Heritability (in %)

Pearson correlations between 24 nutrient concentration and 30 stress index traits across treatments are given in Table 20. The highest positive correlation was found between manganese concentration shoot and potassium concentration shoot (r=0.94), followed by manganese concentration root and potassium concentration root with r=0.87. The highest negative correlation was found between sulfur concentration root and calcium concentration root (r=-0.82), followed by sulfur concentration root and phosphorus concentration root with r=-0.81. K concentration root revealed a significant positive and a negative correlation (r>0.60) with phosphorus and sulfur concentration root, respectively. In contrast, K concentration shoot only revealed a significant positive correlations (r=0.61) with phosphorus concentration shoot.

Pearson correlations between nutrient concentration traits were also calculated within K treatments (Table 21). Under K0 the strongest positive and negative correlations were found between manganese concentration root and potassium concentration root (r=0.95) and between sulfur concentration root and calcium concentration root (r=-0.91). Under K100 the strongest positive and negative correlations were also found between manganese concentration root and potassium concentration root (r=0.86) and between sulfur concentration root and calcium concentration root (r=-0.70).

Table 20: Pearson correlation coefficients between 24 nutrient concentration traits across K treatments

Trait	CaCR	CCR	CuCR	FeCR	KCR	MgCR	MnCR	NaCR	NCR	PCR	SCR	ZnCR	CaCS	CCS	CuCS	FeCS	KCS	MgCS	MnCS	NaCS	NCS	PCS	SCS	ZnCS
CaCR																								
CCR	0.29																							
CuCR	-0.05	-0.51																						
FeCR	-0.48	-0.29	0.23																					
KCR	0.32	-0.52	0.44	-0.22																				
MgCR	0.35	0.37	-0.25	-0.22	-0.20																			
MnCR	0.19	-0.52	0.41	-0.33	**0.87**	-0.18																		
NaCR	0.22	0.37	-0.34	-0.38	-0.36	0.30	-0.25																	
NCR	0.37	0.20	-0.38	**-0.68**	0.17	0.18	0.29	0.49																
PCR	**0.58**	-0.16	0.00	-0.53	**0.63**	0.12	**0.62**	0.01	0.47															
SCR	**-0.82**	0.00	-0.11	**0.58**	**-0.64**	-0.20	**-0.61**	-0.09	-0.51	**-0.81**														
ZnCR	-0.44	-0.57	0.56	0.51	0.29	-0.53	0.25	-0.42	-0.32	-0.15	0.18													
CaCS	0.14	0.07	-0.27	0.10	-0.03	-0.05	-0.14	-0.13	0.00	-0.01	-0.07	-0.02												
CCS	0.13	-0.08	-0.07	0.10	0.03	-0.16	0.00	-0.08	-0.13	0.03	-0.09	-0.05	0.51											
CuCS	-0.01	0.13	0.11	0.11	-0.07	-0.07	-0.13	0.01	-0.22	-0.32	0.15	0.03	0.10	0.16										
FeCS	-0.14	0.01	-0.08	-0.07	-0.14	0.12	0.00	0.13	0.21	-0.09	0.09	-0.03	-0.09	-0.18	-0.04									
KCS	0.28	0.05	-0.03	-0.04	0.44	-0.17	0.33	-0.24	0.21	0.41	-0.40	0.10	0.09	-0.10	-0.27	-0.14								
MgCS	0.11	-0.25	-0.05	-0.19	0.14	-0.05	0.27	0.24	0.08	0.24	-0.12	-0.19	-0.10	0.30	0.11	0.01	-0.42							
MnCS	0.17	-0.03	0.00	0.07	0.41	-0.24	0.30	-0.24	0.18	0.41	-0.32	0.19	0.07	-0.10	-0.31	-0.24	**0.94**	-0.38						
NaCS	0.08	0.18	-0.07	-0.04	-0.07	0.28	-0.11	0.13	-0.11	0.13	-0.05	-0.01	0.04	-0.08	0.10	0.01	-0.14	0.00	-0.12					
NCS	0.00	0.32	-0.26	0.04	-0.40	0.27	-0.43	0.09	-0.12	-0.18	0.14	-0.18	0.28	0.28	0.24	-0.06	-0.18	-0.21	-0.23	0.04				
PCS	0.33	0.24	-0.33	-0.09	0.10	-0.04	0.00	-0.11	0.16	0.29	-0.26	-0.25	0.33	0.36	-0.10	-0.17	**0.61**	-0.17	**0.59**	-0.24	0.16			
SCS	-0.15	-0.32	0.27	-0.06	0.07	-0.09	0.19	0.20	0.04	0.07	0.03	0.28	-0.58	-0.40	-0.10	0.10	-0.32	0.36	-0.24	0.11	**-0.54**	**-0.61**		
ZnCS	-0.09	-0.14	0.11	0.02	0.18	0.00	0.29	0.10	0.21	0.17	-0.09	0.15	**-0.58**	-0.48	-0.32	0.32	0.36	-0.02	0.36	-0.06	-0.45	-0.17	0.42	

(Bold values indicate significant correlations with P<0.001)

Table 20 (continued): Pearson correlation coefficients between of morphological and nutrient concentration stress index traits across K treatments

Trait	TN_SI	HEI_SI	SDW_SI	RL_SI	RDW_SI	BMD_SI	CaR_SI	CR_SI	CuR_SI	FeR_SI	KR_SI	MgR_SI	MnR_SI	NaR_SI	NR_SI	PR_SI	SR_SI	ZnR_SI	CaS_SI	CS_SI	CuS_SI	FeS_SI	KS_SI	MgS_SI	MnS_SI	NaS_SI	NS_SI	PS_SI	SS_SI	ZnS_SI
TN_SI																														
HEI_SI	-0.07																													
SDW_SI	0.27	**0.75**																												
RL_SI	0.12	0.38	0.47																											
RDW_SI	0.17	**0.66**	**0.76**	0.48																										
BMD_SI	0.23	**0.71**	**0.94**	0.43	**0.86**																									
CaR_SI	-0.07	-0.06	-0.01	0.06	0.08	0.04																								
CR_SI	-0.04	-0.25	-0.21	-0.33	-0.33	-0.17	0.29																							
CuR_SI	0.05	0.02	0.01	0.08	-0.01	-0.02	-0.27	-0.42																						
FeR_SI	0.00	-0.23	-0.17	0.02	-0.13	-0.15	-0.20	-0.27	**0.59**																					
KR_SI	0.04	0.06	0.02	0.03	0.08	0.00	0.40	-0.11	0.26	0.00																				
MgR_SI	-0.03	0.10	0.03	-0.05	-0.10	-0.01	-0.09	0.07	0.04	0.00	-0.19																			
MnR_SI	0.06	-0.02	-0.10	-0.11	-0.05	-0.12	0.27	-0.11	0.14	-0.08	**0.86**	-0.01																		
NaR_SI	-0.26	0.05	-0.04	-0.12	-0.03	-0.02	-0.09	0.37	-0.28	-0.26	-0.28	-0.06	-0.15																	
NR_SI	0.00	-0.31	-0.20	-0.24	-0.18	-0.14	**0.53**	0.50	-0.50	-0.33	0.15	0.13	0.20	0.21																
PR_SI	0.01	-0.06	-0.06	0.18	0.07	-0.04	**0.63**	0.01	-0.15	-0.29	**0.54**	-0.01	0.52	-0.27	0.46															
SR_SI	-0.12	-0.04	0.03	0.02	0.02	0.06	**-0.64**	-0.28	0.24	0.46	-0.45	0.18	-0.48	-0.01	-0.41	-0.50														
ZnR_SI	0.13	0.06	0.11	-0.01	0.07	0.11	**-0.66**	-0.32	0.36	0.45	-0.18	0.15	-0.08	-0.15	-0.40	-0.42	**0.61**													
CaS_SI	0.25	0.09	0.12	0.00	0.28	0.14	0.17	-0.38	-0.04	0.10	0.30	-0.02	0.27	-0.27	0.13	0.12	-0.05	0.13												
CS_SI	0.00	0.12	0.23	0.24	0.29	0.26	0.18	-0.04	-0.14	0.08	-0.11	-0.02	-0.15	-0.04	-0.03	0.17	0.03	-0.01	0.14											
CuS_SI	0.23	0.07	0.18	0.21	0.16	0.16	0.07	0.28	-0.24	-0.35	-0.14	-0.08	-0.11	0.36	0.12	-0.08	-0.28	-0.29	-0.09	0.15										
FeS_SI	0.06	-0.02	-0.05	0.02	-0.26	-0.09	-0.16	0.20	-0.03	0.01	0.01	0.03	0.06	0.00	0.00	-0.10	-0.01	0.14	-0.17	-0.31	0.01									
KS_SI	-0.10	-0.11	-0.17	0.06	-0.05	-0.15	0.00	-0.01	0.17	0.13	0.21	0.08	0.12	0.14	-0.11	-0.04	0.05	-0.17	-0.04	-0.32	-0.01	0.22								
MgS_SI	-0.08	0.40	0.24	0.21	0.20	0.17	-0.01	-0.14	-0.20	-0.10	-0.22	0.04	-0.21	-0.06	-0.20	-0.01	0.05	0.04	0.07	0.53	0.06	-0.38	-0.52							
MnS_SI	-0.23	-0.06	-0.17	0.10	-0.01	-0.13	0.03	0.07	0.14	0.10	0.15	0.17	0.05	0.18	-0.06	-0.01	0.04	-0.21	-0.10	-0.23	0.00	0.10	**0.95**	-0.44						
NaS_SI	0.12	-0.14	-0.22	-0.06	-0.28	-0.27	0.00	0.14	-0.15	0.07	-0.13	-0.15	-0.19	0.03	-0.07	-0.10	-0.02	-0.26	-0.14	-0.23	-0.07	-0.02	0.05	0.18	0.04					
NS_SI	-0.17	-0.24	-0.08	-0.08	-0.08	-0.01	0.31	0.29	-0.01	0.25	0.18	-0.09	0.19	0.16	0.21	0.21	-0.03	-0.08	-0.13	0.04	-0.06	0.13	0.16	-0.29	0.23	0.03				
PS_SI	-0.24	-0.07	-0.09	0.10	0.17	-0.08	0.10	-0.11	-0.06	0.01	0.11	0.08	0.12	0.17	-0.07	0.16	0.14	-0.14	0.05	0.08	0.12	-0.08	0.56	-0.16	0.59	-0.02	0.29			
SS_SI	0.07	0.19	0.14	0.06	0.09	0.13	-0.21	-0.02	-0.22	-0.30	-0.19	0.26	-0.13	-0.05	0.09	-0.14	0.08	0.15	0.11	0.06	0.04	-0.13	-0.32	0.33	-0.28	-0.12	**-0.60**	-0.30		
ZnS_SI	-0.03	-0.11	-0.25	0.01	-0.13	-0.26	-0.16	-0.04	0.08	-0.07	0.17	0.22	0.14	-0.04	-0.03	0.03	0.09	0.09	-0.04	-0.44	-0.19	0.27	0.44	-0.51	0.43	-0.06	-0.15	0.13	0.13	

Table 21: Pearson correlation coefficients between 24 nutrient concentration traits under K0 (bottom left triangle) and K100 (upper triangle)

	CaCR	CCR	CuCR	FeCR	KCR	MgCR	MnCR	NaCR	NCR	PCR	SCR	ZnCR	CaCS	CCS	CuCS	FeCS	KCS	MgCS	MnCS	NaCS	NCS	PCS	SCS	ZnCS
CaCR	0.23	0.19	0.00	-0.28	0.19	0.13	0.00	0.18	0.25	0.24	**-0.70**	-0.24	0.18	0.27	0.16	-0.16	-0.07	0.11	-0.20	-0.16	0.04	0.26	-0.17	-0.16
CCR	0.44	0.02	**-0.59**	-0.24	**-0.57**	0.46	-0.53	0.48	0.46	-0.21	0.06	**-0.64**	-0.11	-0.02	0.21	0.01	-0.12	-0.28	-0.11	0.36	0.35	0.13	-0.26	-0.27
CuCR	-0.23	-0.38	0.15	0.25	0.46	-0.23	0.33	-0.44	-0.42	-0.05	-0.15	0.59	-0.03	-0.19	-0.20	-0.05	0.00	0.00	0.04	-0.26	-0.24	-0.38	0.30	0.32
FeCR	-0.52	-0.45	0.49	-0.04	-0.32	0.11	-0.37	-0.36	-0.41	-0.53	0.51	0.34	0.08	-0.18	-0.06	-0.22	-0.02	-0.10	0.17	-0.05	0.18	-0.07	-0.05	0.14
KCR	0.35	-0.24	0.30	0.01	0.41	-0.46	**0.86**	-0.27	0.04	**0.60**	**-0.60**	0.40	0.06	0.13	-0.13	-0.10	0.23	0.29	0.10	-0.37	-0.33	-0.03	0.12	0.14
MgCR	**0.64**	0.44	-0.36	**-0.63**	0.07	0.13	-0.34	0.34	0.19	-0.21	0.05	-0.45	-0.17	0.02	0.25	-0.05	-0.05	-0.13	-0.05	0.13	0.23	-0.01	-0.05	-0.05
MnCR	0.31	-0.17	0.28	-0.18	**0.86**	0.15	0.42	-0.07	0.15	**0.56**	-0.49	0.31	0.01	0.12	-0.17	0.03	0.25	0.33	0.14	-0.36	-0.29	-0.02	0.16	0.17
NaCR	0.18	0.36	-0.40	**-0.56**	-0.40	0.35	-0.23	-0.03	**0.54**	-0.04	-0.19	-0.42	-0.12	-0.05	0.22	0.17	-0.15	0.10	-0.15	0.17	0.13	-0.09	0.11	-0.02
NCR	**0.54**	0.37	-0.42	**-0.69**	0.15	0.50	0.30	0.42	-0.03	0.33	-0.41	-0.20	-0.02	0.00	-0.03	0.14	0.14	-0.08	0.14	0.03	0.07	0.12	-0.12	0.00
PCR	**0.76**	0.07	-0.16	-0.43	**0.63**	0.52	**0.64**	0.04	0.51	0.23	**-0.65**	0.08	0.00	0.12	-0.08	0.10	0.23	0.33	0.12	-0.13	-0.13	0.26	0.14	0.07
SCR	**-0.91**	-0.26	0.12	0.47	**-0.60**	**-0.53**	**-0.60**	-0.01	**-0.56**	**-0.85**	0.30	-0.02	-0.12	-0.19	-0.08	0.03	-0.12	-0.22	0.05	0.19	0.10	-0.13	-0.06	0.00
ZnCR	**-0.69**	-0.47	**0.54**	**0.65**	0.03	**-0.66**	0.00	-0.32	-0.46	-0.48	0.53	0.07	0.11	-0.03	-0.13	-0.15	0.21	-0.03	0.31	-0.26	-0.18	-0.14	0.29	0.18
CaCS	0.15	0.07	-0.12	0.10	0.09	0.06	-0.05	-0.20	0.00	0.07	-0.16	-0.16	0.16	0.19	0.00	-0.07	-0.03	0.01	0.07	0.07	0.07	0.02	-0.12	-0.27
CCS	0.04	-0.06	-0.03	0.25	-0.13	0.00	-0.16	-0.13	-0.15	0.04	0.00	-0.04	0.43	0.11	0.13	-0.15	-0.09	0.36	-0.15	-0.21	0.03	0.27	-0.05	-0.26
CuCS	-0.10	0.17	0.08	0.03	-0.14	-0.07	-0.08	0.14	-0.02	-0.25	0.17	-0.01	0.01	0.18	-0.27	-0.08	-0.31	0.11	-0.33	-0.07	0.18	0.14	-0.07	-0.42
FeCS	-0.15	0.14	-0.02	0.07	-0.11	-0.07	-0.07	0.01	-0.01	-0.34	0.11	0.22	-0.04	-0.29	0.04	0.00	-0.23	-0.09	-0.22	-0.03	0.06	-0.11	0.04	0.22
KCS	0.34	0.14	-0.01	-0.01	0.37	-0.09	0.23	-0.09	0.12	0.30	-0.38	-0.17	0.21	-0.12	-0.10	0.05	**0.54**	-0.26	**0.85**	-0.21	-0.24	0.38	-0.08	0.31
MgCS	-0.10	-0.18	-0.13	-0.03	-0.15	0.11	-0.03	0.10	-0.06	0.04	0.14	-0.13	-0.13	0.46	0.16	-0.24	**-0.57**	0.04	-0.26	-0.03	-0.25	-0.13	0.44	-0.01
MnCS	0.31	0.14	0.01	-0.02	0.36	-0.11	0.25	-0.04	0.12	0.31	-0.34	-0.16	0.15	-0.14	-0.10	-0.10	**0.95**	**-0.53**	0.32	-0.10	-0.19	0.30	0.02	0.39
NaCS	0.24	0.12	0.16	0.09	0.35	0.20	0.27	0.03	-0.14	0.20	-0.24	0.02	0.13	-0.11	-0.02	0.16	0.05	-0.08	0.05	0.07	0.00	-0.26	0.05	-0.19
NCS	0.14	0.20	-0.06	0.06	-0.05	0.22	-0.11	0.04	-0.08	0.01	-0.09	-0.08	0.46	0.31	-0.06	-0.04	0.03	-0.27	0.05	0.22	0.12	0.20	**-0.53**	-0.40
PCS	0.21	0.11	-0.14	-0.02	0.11	-0.01	0.03	-0.02	0.00	0.19	-0.18	-0.22	0.45	0.24	-0.11	-0.13	**0.67**	-0.23	**0.68**	-0.11	0.20	0.52	-0.50	-0.29
SCS	-0.20	-0.16	-0.02	-0.16	-0.12	-0.05	0.02	0.23	0.14	-0.08	0.19	0.21	**-0.73**	-0.34	0.05	0.03	-0.44	0.29	-0.40	-0.11	**-0.59**	**-0.58**	0.41	0.47
ZnCS	-0.06	-0.03	0.14	-0.06	0.21	-0.07	0.32	0.02	0.15	0.10	-0.04	0.14	**-0.55**	**-0.64**	-0.18	0.29	0.33	-0.32	0.34	-0.01	-0.31	-0.01	0.26	0.17

(Bold values indicate significant correlations with P<0.001, Diagonal: auto correlatation between K0 and K100)

The trait auto correlations (diagonal in Table 21) were often low and non-significant (P<0.05) with the only exception of potassium concentration shoot where K0 and K100 treatments revealed a positive correlation of r=0.54.

Pearson correlations between ICP wet lab data and the predicted ICP data were calculated based on 195 root and shoot samples (Table 22). For all 12 evaluated traits positive significant correlations (P<0.05) were detected within the range of r=0.21 (manganese) and r=0.84 (copper).

Table 22: Pearson correlation coefficients of nutrient concentrations between ICP and HSI

Nutrient	Correlation coefficient
Ca	0.70
C	0.28
Cu	0.84
Fe	0.75
K	0.76
Mg	0.76
Mn	0.21
Na	0.32
N	0.37
P	0.34
S	0.67
Zn	0.66

3.2.3 QTL identification

All 62 investigated morphological and nutrient concentration traits were used for QTL identification. First, a MIXED model ANOVA was run for each trait across and within K treatments (Table S2, Supporting Information). Significant (P<0.05) treatment effects between K0 and K100 were observed for all traits except seven (plant height, root length, root dry weight, biomass, carbon concentration root, sulfur concentration root and copper concentration root). Also, significant (P<0.05) genotype effects across and within K treatments were observed for all morphological traits (Table S2, Supporting Information). In addition, significant genotype effects for five and one nutrient concentration shoot traits were observed for across (CaCS, KCS, MnCS, PCS, SCS) and within K100 (FeCS) treatments, respectively. No significant genotype effect could be found within K0. Genotype-by-treatment interactions were only significant for the three morphological traits tiller number, shoot root weight ratio and

biomass. Experiment effects were significant for all morphological, all nutrient concentration and all six morphological stress index traits (Table S2, Supporting Information).

Following the MIXED model ANOVA, a post-hoc Dunnett test was applied with subsequent FDR adjustment of raw P values in order to identify significant (P-FDR<0.05) trait by line associations (Table 23). For 36 S42ILs (excluding S42ILs -106, -108, -113, -114, -124, -131, -132, -134, -141, -149 and -161) the Dunnett test revealed significant deviations from the recurrent parent 'Scarlett' for at least one trait studied. Based on SNP mapping knowledge about overlapping S42IL introgressions, line-by-trait associations were summarized to 58 QTLs, present as 56, 27 and 32 QTL effects across treatments, within K0 and within K100, respectively. QTLs were observed for a total of 10 traits (all morphological traits except tiller number, one shoot nutrient concentration trait (CaCS) and two stress index traits (CaS_SI and MgS_SI). The highest number of QTLs were found for seven ILs (S42IL-102, -110, -115, -121, -140, -153 and -176 (4 QTLs each) with *Hsp* introgressions on chromosome arms 1HL, 2HL, 3HL, 4HL, 3HL, 2HL and 5HL, respectively (Schmalenbach et al. 2011), followed by 7 S42ILs (3 QTLs each, Table 23, Figure 8). *Hsp* alleles, present within the introgression, increased trait performance in 67 cases. The highest number of favorable *Hsp* allele effects were found for four ILs (S42IL-110, -115, -121 and -140 (4 QTLs each), followed by three ILs (S42ILs -107, -123 and -137 (3 QTLs each). In the following, QTLs controlling seven morphological traits, one nutrient concentration traits and two stress index traits are described in detail (see Table 23).

QTLs for morphological traits

In total of 52 QTLs were detected for seven out of eight investigated morphological traits. The highest QTL number (16 QTLs) was found for plant height compared to other traits in this study. For TN no QTL was observed. In the following paragraphs QTL are explained for each trait separately.

HEI: Sixteen QTLs on all seven barley chromosomes were found for plant height in 26 S42ILs. *Hsp* alleles had an increasing effect on HEI with one exception. The detected QTL on the long arm of chromosome 1H in S42IL-143 reduced plant height by 2.87 cm (shorter than 'Scarlett') under control K. In contrast, *Hsp* alleles increased plant height relative to 'Scarlett' in 25 lines representing all barley chromosomes except 1H. The *Hsp* allele of S42IL-121 (chromosome

4HL) increased plant height by 5.75, 6.38 and 5.28 cm across treatments, within K0 and K100, respectively.

SDW: For shoot dry weight six QTLs were identified on six S42ILs. These QTLs were located on barley chromosomes 1H, 2H, 3H and 4H. Four out of 6 detected QTLs revealed an increase in shoot dry weight due to the presence of the *Hsp* allele. The strongest increase was detected for QSdw.S42IL-3H (S42IL-115) with a plus of 15.49 mg across treatments. In contrast, the exotic allele at QSdw.S42IL-1H.a (S42IL-102) showed a dry weight reduced by 23.71, 21.76 and 25.38 mg across treatments, within K0 and K100, respectively, compared to 'Scarlett'.

RL: In nine introgression lines seven QTLs for root length were found on all barley chromosomes except 3H. At four QTLs in six lines, the *Hsp* allele reduced root length. The strongest reduction of root length across treatments and within K0 and K100 was found in S42IL-176 (QRl.S42IL-5H) with 6.41, 5.28 and 7.64 cm compared to 'Scarlett', respectively. In contrast, three QTLs on chromosomes 2H, 6H and 7H in the lines S42ILs-107, -128, -135, respectively, were associated with an increased root length (Maximum: +5.78 and 7.44 cm in S42IL-135 across treatments and under K100 treatment, respectively).

RDW: In two S42ILs (S42IL-102 and -153) two QTLs controlling root dry weight reduction were found on chromosomes 1H and 2H. The strongest decreasing *Hsp* effect was detected in S42IL-102 (QRdw.S42IL-1H) with 10.23 and 13.31 mg across treatments and within K0, respectively. Under treatment K100 no QTL was identified for this trait.

SRLR: In 20 S42ILs eleven QTLs controlling shoot root length ratio were located on all barley chromosomes. *Hsp* alleles increased SRLR in all lines. The strongest effect was observed in S42IL-126 with an increase of 32.29, 19.92 and 39.40% across treatments and within K0 and K100, respectively. The *Hsp* allele effects in lines S42IL-105 and -176 were slightly lower in all three treatments.

SRWR: In eight S42ILs eight QTLs controlling shoot root weight ratio were detected on all barley chromosomes except 6H. *Hsp* alleles increased SRWR in all lines except one (S42ILs-109: reduction by 9.60 and 10.22% across treatments and within K100, respectively). The strongest *Hsp* effect was found in S42IL-123 (QSrwr.S42IL-4H.c) with an increase of SRWR by 13.29 and 19.24%, compared to 'Scarlett,' across treatments and within K0, respectively. Slightly lesser *Hsp* alleles effects were found for line S42IL-143 with an increase by 12.71, 14.08 and 12.61% across treatments and within K0 and K100, respectively.

BMD: For biomass, two QTLs were identified in two lines (S42IL-102 (chromosome 1H) and -153 (chromosome 2H). *Hsp* alleles reduced biomass in both lines. The strongest effect was found in S42IL-102 (QBmd.S42IL-1H) by 23.35 and 26.32% across treatments and within K0 treatment. In S42IL-153 a biomass reduction by 19.63% across treatments was observed.

QTLs for nutrient concentration traits

Only for one nutrient concentration trait significant deviations of S42ILs from 'Scarlett' were detected.

CaCS: Two QTLs with increasing effects were detected for calcium concentration shoot in three ILs (S42IL-110, chromosome 2H and S42IL-115 and -140, chromosome 3H). At QCacs.S42IL-3H (S42IL-140) the exotic allele was associated with an increase of CaCS across treatments by 161.18% compared to 'Scarlett'.

QTLs for stress index traits

A stress index, defined as the ratio of trait performance under K0 and K100, was calculated for each trait. In total, QTLs for two stress index traits were detected. These traits are presented in the following.

CaS_SI: For calcium concentration shoot stress index two QTLs were identified in two ILs (S42IL-110 and -153) represent on chromosome 2H. At Qcas_si.S42IL-2H.B (present in S42IL-153) the exotic allele was associated with an increase of the calcium concentration shoot index by 1802.42% across treatments compared to 'Scarlett'.

MgS_SI: Two QTLs were found for magnesium concentration shoot index in the two lines (S42IL-143 and -139) on chromosomes 1H and 7H, respectively. MgS_SI was increased in both lines relative to 'Scarlett'. The highest increasing *Hsp* effect was observed in line S42IL-143 at QMg_si.S42IL-1H by 152.59% across treatments.

Table 23: Results of Dunnett test of significant genotype effects for 62 traits, calculated across (ALL) and within treatments (K0 or K100).

						Effect across treatments			Effect within K0			Effect within K100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, correspon-ding QTL[g]**
HEI	QHei.S42IL-1H	K100	S42IL-143	1H	130.68-173.49							27.95	-2.87	-9.31	HvFT31, *1), *3), *6)
HEI	QHei.S42IL-2H.a	ALL, K100	S42IL-107	2H	34.31-66.78	33.87	3.12	10.13				34.88	4.06	13.17	Ppd-H11
HEI	QHei.S42IL-2H.b	ALL, K0, K100	S42IL-109	2H	63.96-110.84	34.49	3.74	12.15	34.35	3.86	12.65	34.44	3.62	11.75	Flt-2L2, Sdw33, *1), *5), *6)
HEI		ALL, K0, K100	S42IL-110	2H	102.66-104.81	34.03	3.27	10.63	34.14	3.64	11.95	33.77	2.95	9.58	*1)
HEI	QHei.S42IL-3H.a	ALL, K0	S42IL-111	3H	67.01-98.41	33.14	2.39	7.76	33.98	3.49	11.46				
HEI	QHei.S42IL-3H.b	ALL, K100	S42IL-112	3H	104.39-161.43	33.21	2.45	7.98				33.25	2.43	7.89	*6)
HEI	QHei.S42IL-3H.c	ALL, K0, K100	S42IL-115	3H	204.48-255.13	35.02	4.27	13.87	34.64	4.15	13.60	35.22	4.40	14.29	
HEI		ALL, K0, K100	S42IL-140	3H	154.99-253.73	33.97	3.21	10.44	33.70	3.21	10.53	34.18	3.37	10.92	Sdw1 (denso) 4, 5, *3), *4)*5), *6)
HEI	QHei.S42IL-4H.a	ALL	S42IL-116	4H	5.42-47.80	32.68	1.93	6.26							*6)
HEI	QHei.S42IL-4H.b	ALL, K0, K100	S42IL-118	4H	61.15-83.58	34.76	4.00	13.01	34.94	4.45	14.60	34.34	3.52	11.43	*6)
HEI		ALL, K0, K100	S42IL-119	4H	61.15-119.06	34.38	3.62	11.79	34.27	3.78	12.40	34.29	3.47	11.25	
HEI		ALL, K0, K100	S42IL-121	4H	74.11-119.06	36.51	5.75	18.71	36.87	6.38	20.92	36.10	5.28	17.14	*1), *3), *6)
HEI	QHei.S42IL-4H.c	ALL, K0, K100	S42IL-123	4H	128.85-172.32	35.4	4.65	15.11	35.56	5.06	16.61	35.08	4.26	13.83	*1), *6)
HEI	QHei.S42IL-5H.a	K100	S42IL-125	5H	104.73-154.37							33.45	2.63	8.53	
HEI		ALL, K0, K100	S42IL-126	5H	145.57-200.12	33.94	3.19	10.37	33.16	2.67	8.75	34.61	3.79	12.31	ari-e.GP4, *1), *6)
HEI		ALL, K0	S42IL-176	5H	154.37-234.98	33.02	2.26	7.36	33.47	2.98	9.76				
HEI	QHei.S42IL-5H.b	ALL, K100	S42IL-127	5H	231.75-276.77	33.14	2.39	7.75				33.35	2.53	8.21	
HEI	QHei.S42IL-6H.a	ALL, K100	S42IL-128	6H	71.39-132.23	33.1	2.34	7.62				34.20	3.38	10.97	*6)
HEI		ALL, K0, K100	S42IL-129	6H	73.90-133.47	34.22	3.46	11.26	33.67	3.18	10.41	34.52	3.70	12.02	*6)
HEI	QHei.S42IL-6H.b	ALL, K100	S42IL-130	6H	98.66-180.69	33.36	2.60	8.46				33.85	3.04	9.86	*6)
HEI		ALL, K0, K100	S42IL-122	6H	180.69-208.13	33.4	2.64	8.59	33.18	2.69	8.83	33.41	2.59	8.42	
HEI	QHei.S42IL-6H.c	ALL, K0, K100	S42IL-148	6H	3.28-10.73	34.4	3.65	11.86	34.13	3.64	11.95	34.37	3.56	11.54	*1), *6)
HEI	QHei.S42IL-7H.a	ALL, K0, K100	S42IL-135	7H	101.23-152.29	34.62	3.86	12.56	34.46	3.97	13.00	34.81	3.99	12.96	*1), *6)

						Effect across treatments			Effect within K0			Effect within K100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, correspon-ding QTL[g]**
HEI		ALL, K0, K100	S42IL-136	7H	134.43-152.29	33.95	3.19	10.38	33.94	3.45	11.31	33.97	3.15	10.22	*1)
HEI		ALL, K0, K100	S42IL-137	7H	134.43-193.89	36.14	5.38	17.50	36.60	6.11	20.04	35.37	4.55	14.77	*1), *3), *6)
HEI	QHei.S42IL-7H.b	ALL, K100	S42IL-138	7H	176.37-229.66	33.57	2.81	9.14				34.00	3.19	10.34	*1)
SDW	QSdw.S42IL-1H.a	ALL, K0, K100	S42IL-102	1H	1.10-98.23	75.17	-23.71	-23.98	66.23	-21.76	-24.73	83.68	-25.38	-23.27	
SDW	QSdw.S42IL-1H.b	ALL	S42IL-142	1H	188.50-205.07	113.51	14.63	14.80							*6)
SDW	QSdw.S42IL-2H.a	ALL	S42IL-107	2H	34.31-66.78	113.37	14.49	14.65							
SDW	QSdw.S42IL-2H.b	ALL	S42IL-153	2H	108.71-120.83	82.61	-16.27	-16.45							
SDW	QSdw.S42IL-3H	ALL	S42IL-115	3H	204.48-255.13	114.37	15.49	15.67							
SDW	QSdw.S42IL-4H	ALL	S42IL-121	4H	74.11-119.06	112.33	13.45	13.60							*1), *6)
RL	QRl.S42IL-1H	ALL	S42IL-102	1H	1.10-98.23	30.62	-4.36	-12.46							*2), *6)
RL		ALL, K0	S42IL-105	1H	74.40-90.92	29.59	-5.38	-15.39	33.05	-5.26	-13.73				*6)
RL	QRl.S42IL-2H	ALL, K100	S42IL-107	2H	34.31-66.78	39.9	4.92	14.06				36.74	5.77	18.64	
RL	QRl.S42IL-4H	ALL, K0	S42IL-117	4H	27.52-64.77	31.01	-3.97	-11.35	33.34	-4.97	-12.97				
RL	QRl.S42IL-5H	ALL, K100	S42IL-126	5H	145.57-200.12	29.74	-5.24	-14.97				24.50	-6.47	-20.89	ari-e.GP2, *1), *6)
RL		ALL, K0, K100	S42IL-176	5H	154.37-234.98	28.57	-6.41	-18.33	33.02	-5.28	-13.79	23.33	-7.64	-24.67	*2), *6)
RL	QRl.S42IL-6H	ALL, K100	S42IL-128	6H	71.39-132.23	39.2	4.22	12.06				36.50	5.54	17.88	*1), *6)
RL	QRl.S42IL-7H.a	ALL, K100	S42IL-133	7H	17.32-51.93	31.02	-3.96	-11.32				25.27	-5.70	-18.39	*1), *6)
RL	QRl.S42IL-7H.b	ALL, K100	S42IL-135	7H	101.23-152.29	40.74	5.76	16.46				38.41	7.44	24.02	*1), *6)
RDW	QRdw.S42IL-1H	ALL, K0	S42IL-102	1H	1.10-98.23	36.64	-10.23	-21.83	33.23	-13.31	-28.60				
RDW	QRdw.S42IL-2H	ALL	S42IL-153	2H	108.71-120.83	39.02	-7.85	-16.76							
SRLR	QSrlr.S42IL-1H.a	ALL	S42IL-101	1H		1.01	0.11	11.65							
SRLR	QSrlr.S42IL-1H.b	ALL,K0, K100	S42IL-103	1H	40.51-89.01	1.05	0.15	16.14	0.90	0.10	12.52	1.22	0.19	18.21	
SRLR		ALL, K0, K100	S42IL-105	1H	74.40-90.92	1.13	0.23	25.15	0.93	0.13	16.43	1.34	0.31	30.15	*6)
SRLR	QSrlr.S42IL-2H	ALL, K0	S42IL-109	2H	63.96-110.84	1.01	0.10	11.47	0.89	0.09	11.55				*6)
SRLR		ALL, K0, K100	S42IL-110	2H	102.66-104.81	1.07	0.17	18.72	0.88	0.09	11.11	1.31	0.28	27.15	*6)
SRLR	QSrlr.S42IL-3H	ALL, K0	S42IL-112	3H	104.39-161.43	1.02	0.11	12.40	0.89	0.10	12.40				*6)
SRLR		K0	S42IL-115	3H	204.48-255.13				0.89	0.09	11.68				

						Effect across treatments			Effect within K0			Effect within K100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, correspon-ding QTL[g]**
SRLR		ALL, K0	S42IL-140	3H	154.99-253.73	1.04	0.14	15.55	0.93	0.14	17.21				*6)
SRLR	QSrlr.S42IL-4H.a	ALL, K0, K100	S42IL-117	4H	27.52-64.77	1.06	0.15	16.99	0.90	0.10	13.02	1.23	0.20	19.22	
SRLR	QSrlr.S42IL-4H.b	ALL, K0	S42IL-118	4H	61.15-83.58	1.06	0.15	17.03	0.98	0.18	22.64				
SRLR		ALL, K0, K100	S42IL-119	4H	61.15-119.06	1.1	0.19	21.33	0.96	0.17	20.74	1.24	0.21	20.57	*6)
SRLR		ALL	S42IL-120	4H	61.15-83.58	1.02	0.11	12.59							
SRLR		ALL, K0	S42IL-121	4H	74.11-119.06	1.04	0.13	14.82	0.95	0.15	18.94				*6)
SRLR	QSrlr.S42IL-4H.c	K0	S42IL-123	4H	128.85-172.32				0.92	0.13	15.80				*6)
SRLR	QSrlr.S42IL-5H	ALL, K100	S42IL-125	5H	104.73-154.37	1.03	0.12	13.77				1.21	0.18	17.31	*6)
SRLR		ALL, K0, K100	S42IL-126	5H	145.57-200.12	1.2	0.29	32.29	0.94	0.14	17.92	1.44	0.41	39.40	*6)
SRLR		ALL, K0, K100	S42IL-176	5H	154.37-234.98	1.13	0.23	25.34	0.98	0.19	23.47	1.32	0.28	27.47	*6)
SRLR	QSrlr.S42IL-6H	K0	S42IL-148	6H	3.28-10.73				0.89	0.10	12.42				
SRLR	QSrlr.S42IL-7H.a	ALL, K100	S42IL-133	7H	17.32-51.93	1.05	0.14	15.68				1.22	0.18	17.87	*6)
SRLR	QSrlr.S42IL-7H.b	ALL, K0, K100	S42IL-137	7H	134.43-193.89	1.1	0.19	21.16	0.97	0.17	21.27	1.22	0.18	17.75	*6)
SRWR	QSrwr.S42IL-1H	ALL, K0, K100	S42IL-143	1H	130.68-173.49	2.4	0.27	12.71	2.19	0.27	14.08	2.62	0.29	12.61	*1)
SRWR	QSrwr.S42IL-2H	ALL, K100	S42IL-109	2H	63.96-110.84	1.92	-0.20	-9.60				2.09	-0.24	-10.22	*6)
SRWR	QSrwr.S42IL-3H	ALL	S42IL-140	3H	154.99-253.73	2.29	0.17	7.81							*6)
SRWR	QSrwr.S42IL-4H.b	ALL, K100	S42IL-121	4H	74.11-119.06	2.33	0.20	9.60				2.57	0.25	10.58	*6)
SRWR	QSrwr.S42IL-4H.c	ALL, K0	S42IL-123	4H	128.85-172.32	2.41	0.28	13.29	2.29	0.37	19.24				*1), *6)
SRWR	QSrwr.S42IL-5H	ALL, K100	S42IL-176	5H	154.37-234.98	2.38	0.25	11.79				2.64	0.31	13.52	*6)
SRWR	QSrwr.S42IL-7H.a	ALL, K100	S42IL-133	7H	17.32-51.93	2.34	0.21	9.83				2.58	0.26	11.31	*1), *6)
SRWR	QSrwr.S42IL-7H.b	ALL	S42IL-137	7H	134.43-193.89	2.29	0.16	7.54							*6)
BMD	QBmd.S42IL-1H	ALL, K0	S42IL-102	1H	1.10-98.23	111.93	-34.10	-23.35	99.62	-35.58	-26.32				
BMD	QBmd.S42IL-2H	ALL	S42IL-153	2H	108.71-120.83	125.46	-30.65	-19.63							
CaCS	QCacs.S42IL-2H	ALL	S42IL-110	2H	102.66-104.81	1999.17	1179.38	143.86							
CaCS	QCacs.S42IL-3H	ALL	S42IL-115	3H	204.48-255.13	1954.91	1135.13	138.47							
CaCS		ALL	S42IL-140	3H	154.99-253.73	2141.13	1321.34	161.18							
CaS_SI	Qcas_si.S42IL-2H.a	ALL	S42IL-110	2H	102.66-104.81	1.81	0.32	21.62							

						Effect across treatments			Effect within K0			Effect within K100			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**Chr.[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene, correspon-ding QTL[g]**
CaS_SI	Qcas_si.S42IL-2H.B	ALL	S42IL-153	2H	108.71-120.83	28.39	26.89	1802.42							
MgS_SI	QMgs_si.S42IL-1H	ALL	S42IL-143	1H	130.68-173.49	3.18	1.92	152.59							
MgS_SI	QMgs_si.S42IL-7H	ALL	S42IL-139	7H	198.70-229.66	2.52	1.26	100.03							

[a] Trait abbreviations are explained in Tables 4, 5 and 6.
[b] Significant Hsp effect under investigated treatment: across treatments (All), low K (K0) and control K (K100), respectively
[c] Location of chromosome and introgression target (cM) based on Schmalenbach et al. (2011)
[d] Least squares means of IL across treatments, K0 and K100, respectively
[e] Difference between LSmeans [S42IL] and LSmeans [Scarlett] across treatments, K0 and K100, respectively
[f] Relative performance (in %): LSmeans [S42IL] - LSmeans [Scarlett] ×100 / LSmeans [Scarlett]
[g] References of candidate genes: 1: Wang et al. (2010), 2: Chen et al. (2009), 3: Gottwald et al. (2004), 4: Chloupek et al. (2006), 5: Laurie et al. (1993)
[g] References of corresonding QTLs: *1), *2),*3),*4), *5) and *6) indicate corresponding Hsp effects in identical S42ILs, reported by Hoffmann et al. (2012), Naz et al. (2014), Honsdorf et al. (2014a), Honsdorf et al. (2014b), Arifuzzaman et al. (2014) and Soleimani et al. (2017), respectively.

3.3 Carbon dioxide investigation

3.3.1 Morphological and stress index traits - trait performance and correlations

Trait performances of the 47 introgression lines and 'Scarlett' are listed in Table 24 containing number of observations (N), means, standard deviation (SD), coefficient of variation (CV) and heritability (h^2) for five morphological traits and five stress index traits, across CO_2 treatments and separately for increased (760 ppm, H CO_2) and control CO_2 (380 ppm, N CO_2) supplies. Mean values of three out of five morphological traits were higher under high CO_2, namely tiller number, shoot fresh weight (above-ground biomass) and shoot dry weight with the highest increase observed for SFW (+18%). Plant height and anthocyanin formation exhibited lower means under high CO_2. For example, mean above-ground biomass accumulation under H CO_2 and N CO_2 is shown in Fig. 7. Under high CO_2 treatment, the average fresh and dry biomass rose from 1.77 and 0.19 to 2.09 and 0.20 g, respectively (Table 24).

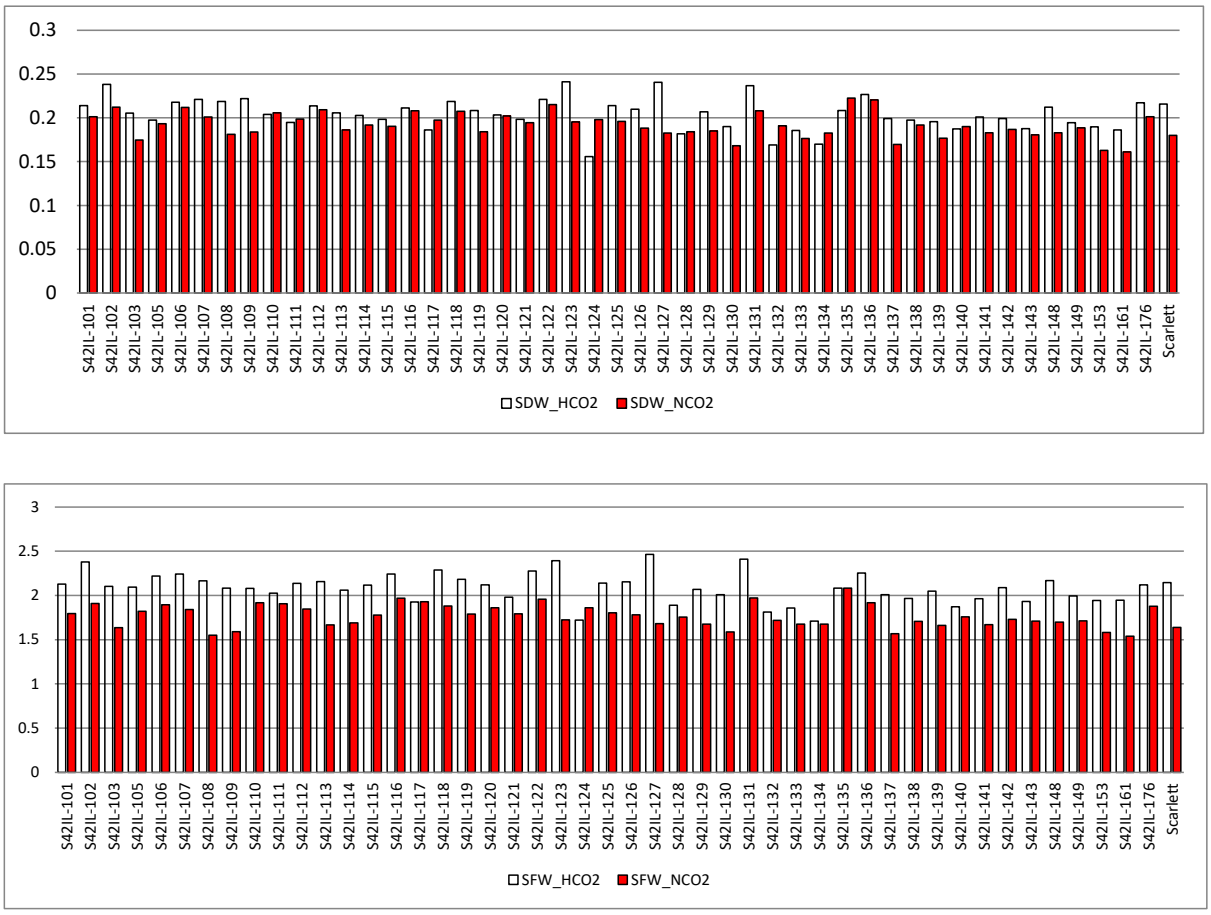

Figure 7: Mean above-ground biomass accumulation (in g) of S42ILs and Scarlett cultivated under H CO_2 and N CO_2treatments (top: shoot dry weight, below: shoot fresh weight).

The highest and lowest CV of the morphological traits was observed under H CO_2 for anthocyanin (63.1%) and HEI (9.2%). Anthocyanin formation showed a strong difference (27.3%) in CV between control and high CO_2 compared to the other traits with a maximum of 4.4% for shoot fresh weight. For all investigated morphological traits heritability under control CO_2 treatment was increased compared to the rised concentration and was higher than 26% across and within treatments. The highest and lowest heritabilities could be found for plant height with 73.8% and 26.6% across both treatments and within H CO_2, respectively. For the calculated stress index traits the highest and lowest CV was found for SDW_SI (84.7%) and HEI_SI (18.3%). The highest and lowest heritabilities could be found for anthocyanin (AC_SI, 49.2%) and plant height (HEI_SI, 12.5%).

Table 24: Trait performance and heritability of morphological and stress index traits

Trait [a]	**Treatment** [b]	**N** [c]	**Mean** [d]	**SD** [e]	**CV** [f]	**h^2** [g]
	Across	1915	2.95	0.54	18.35	64.32
	H CO_2	767	3.09	0.59	19.13	48.04
TN	N CO_2	1148	2.85	0.48	16.90	51.99
	Across	1915	384.63	39.22	10.20	73.79
	H CO_2	767	382.56	35.13	9.18	26.60
HEI	N CO_2	1148	386.02	41.69	10.80	59.88
	Across	1909	1.89	0.51	27.09	54.54
	H CO_2	763	2.09	0.49	23.31	29.84
SFW	N CO_2	1146	1.77	0.49	27.72	65.95
	Across	1909	0.20	0.05	26.81	66.26
	H CO_2	763	0.20	0.05	26.75	29.91
SDW	N CO_2	1146	0.19	0.05	26.50	66.84
	Across	1915	0.82	0.39	47.14	58.86
	H CO_2	767	0.72	0.45	63.06	66.59
AC	N CO_2	1148	0.89	0.32	35.75	72.93
TN_SI	Across	764	1.16	0.41	35.75	29.54
HEI_SI	Across	764	1.03	0.19	18.26	12.53
SFW_SI	Across	760	1.45	0.97	67.16	24.52
SDW_SI	Across	760	1.32	1.11	84.71	16.90
AC_SI	Across	649	0.73	0.44	60.57	49.15

[a] Trait abbreviations are given in Table 7.
[b] Across: across both CO_2 treatments, H CO_2 and N CO_2: 760 ppm and 380 ppm, respectively
[c] Number of observations
[d] Mean value
[e] Standard deviation
[f] Coefficient of variation (in %)
[g] Heritability (in %)

Pearson correlations between the ten morphological and stress index traits across treatments are given in Table 25. All correlations between morphological traits were positive and low with one exception. The highest positive significant correlation was found between shoot fresh weight and shoot dry weight (r=0.93). The lowest correlations were observed between AC and SFW (r=0.07) and SDW (r=0.06). Six of ten correlations between the stress index traits were positively significant with the highest correlation between SFW_SI and HEI_SI of r=0.89.

Table 25: Pearson correlation coefficients between morphological and stress index traits across CO_2 treatments

	TN	HEI	SFW	SDW	AC	TN_SI	HEI_SI	SFW_SI	SDW_SI	AC_SI
TN										
HEI	0.40									
SFW	0.42	0.42								
SDW	0.36	0.29	**0.93**							
AC	0.18	0.29	0.07	0.06						
TN_SI	-0.33	-0.27	-0.07	0.02	-0.21					
HEI_SI	-0.19	-0.25	-0.19	-0.10	-0.11	**0.66**				
SFW_SI	-0.17	-0.29	-0.14	-0.02	-0.07	**0.75**	**0.89**			
SDW_SI	-0.17	-0.24	-0.13	-0.06	-0.11	**0.66**	**0.79**	**0.88**		
AC_SI	0.13	0.21	0.07	0.07	0.77	-0.05	0.13	0.13	0.06	

(Bold values indicate significant correlations with P<0.001)

Pearson correlations between morphological traits were also calculated within CO_2 treatments (Table 26). Only shoot fresh weight showed positive and significant correlations with tiller number (r = 0.54), plant height (r = 0.61) and shoot dry weight (r = 0.91) under control CO_2. In contrast, only SFW and SDW exhibited a strong significant and positive correlation (r=0.95) under high CO_2. In summary, it can be seen that the correlations between these two traits were the highest correlations under all three investigated CO_2 treatments. In general, low correlations with the other traits can be observed for anthocyanin formation and here especially under elevated CO_2 treatment.

Trait auto correlations between N CO_2 and H CO_2 were positive low but not significant (diagonal in Table 26) with correlations between r=0.32 (SFW) and r=0.46 (AC).

Table 26: Pearson correlation coefficients between morphological traits under N CO_2 (bottom left triangle) and H CO_2 (upper right triangle)

	TN	HEI	SFW	SDW	AC
TN	0.40	0.42	0.50	0.47	0.11
HEI	0.52	0.44	0.48	0.43	0.27
SFW	**0.54**	**0.61**	0.32	**0.95**	0.11
SDW	0.47	0.45	**0.91**	0.41	0.14
AC	0.34	0.36	0.23	0.25	0.46

(Bold values indicate significant correlations with P<0.001,
Diagonal: auto correlation between H CO_2 and N CO_2)

3.3.2 QTL identification

All ten investigated morphological and stress index traits were used for QTL identification. First, a MIXED model ANOVA was run for each trait across and within CO_2 treatments (Table S3, Supporting Information). Significant treatment effects between H CO_2 and N CO_2 could not be observed for morpholgoicial traits. But significant (P<0.05) genotype effects across and within CO_2 treatments were found. In addition, significant genotype effects for two of five stress index traits (TN_SI and AC_SI) were observed across CO_2 treatments. Genotype-by-treatment interactions were significant for two of five morphological traits (SFW and AC).

Following the MIXED model ANOVA, a post-hoc Dunnett test was applied with subsequent FDR adjustment of raw P values in order to identify significant (P-FDR<0.05) trait by line associations (Table 27). For twelve out of the 47 S42ILs (S42ILs -102, -107, -108, -116, -117, -121, -122, -124, -131, -135, -136 and -176) the Dunnett test revealed significant deviations from the recurrent parent 'Scarlett' for at least one trait studied. Based on SNP mapping knowledge about overlapping S42IL introgressions, line-by-trait associations were summarized to 12 QTLs, present as 5, 11 and 4 QTL effects across treatments, control and high CO_2 treatment, respectively. QTLs were observed for all five morphological traits but none for stress index traits. The highest number of QTLs were found for three ILs (S42IL-122, -124 and -135 (2 QTLs each) with *Hsp* introgressions on chromosome arms 6HL, 4HL and 7HL, respectively (Schmalenbach et al. 2011), followed by the other nine S42ILs with only one QTL each). *Hsp* alleles, present within the introgression, increased trait performance in nine cases. Most of the favorable *Hsp* allele effects (6 QTLs) were found under control CO_2 treatment. In the following, QTLs controlling the morphological traits are described in detail (see Table 27).

TN: Only S42IL-107 with an introgression on the short arm of chromosome 2H showed a significant association with tiller number (Table 27). The *Hsp* alleles reduced tiller numberby 0.40 relative to the control genotype 'Scarlett' across treatments.

HEI: Two QTLs, one each in one introgression line, were found for plant height. QTLs were located on barley chromosomes 2H (S42IL-108) and 4H (S42IL-121). The detected QTL on 2H decreased plant height relative to 'Scarlett' by 27.1 and 31.2 mm across treatments and control CO_2 concentration, respectively. On the contrary S42IL-121 increased HEI by 29.8 mm or 7.85% under control CO_2. Under high CO_2 concentration no QTL was identified.

SFW: In five introgression lines three QTLs for shoot fresh weight were found on chromosomes 4H (S42IL-116 and -117), 6H (S42IL-122 and -131) and 7H (S42IL-135). All QTLs were identified under control CO_2. The QTL in line S42IL-131 was additionally found across treatments. All *Hsp* alleles involved increased shoot fresh weight between 19.2 and 27.1 % (S42IL-135) under control CO_2. The strongest positive *Hsp* effect was observed in line S42IL-135. Across treatments the effect in line S42IL-131 was lower with 15.9%.

SDW: For shoot dry weight three QTLs were detected in four S42ILs. These QTLs were found on the same chromosomes compared to SFW, namely 4H (S42IL-124), 6H (S42IL-122) and 7H (S42IL-135 and -136). Only line S42IL-124 (long arm of chromosome 4H) was found under high CO_2 concentration with a negative effect (-27.6%) on this trait. Under control CO_2 the other three ILs revealed an increasing *Hsp* effect for this trait. In these lines shoot dry weigh was increased by 19.5% (S42IL-122, 6H) to 23.6% (S42IL-135, 7H). Across treatments no QTL was observed.

AC: For anthocyanin formation three QTLs were identified in the three lines S42IL-102, -124 and -176, carrying introgressions on chromosomes 1H, 4H and 5H, respectively. Regardless of the treatment level *Hsp* alleles reduced anthocyanin formation. The strongest *Hsp* effect was found in S42IL-176 (QAc.S42IL-5H) with an decrease by 80.0%, 90.9% and 71.4%, compared to 'Scarlett', across treatments, within high CO_2 and control CO_2, respectively.

Table 27: Results of Dunnett test of significant genotype effects for five morphological traits under two different CO_2 concentrations

						Effect across treatment			effect within stress CO_2			Effect within control CO_2			
Trait[a]	**QTL name**	**Treatment[b]**	**Genotype**	**chr[c]**	**Introgression position[c]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**LSmean IL[d]**	**Diff[e]**	**RP[f]**	**Candidate gene[g]**
TN	QTn.S42IL-2H.a	All	S42IL-107	2H	34.31-66.78	2.56	-0.4	-13.38							
HEI	QHei.S42IL-2H	All, N CO_2	S42IL-108	2H	34.31-104.81	356.09	-27.05	-7.06				347.87	-31.17	-8.22	Ppd-H1 & HvFT41
	QHei.S42IL-4H	N CO_2	S42IL-121	4H	74.11-119.06							408.79	29.75	7.85	
SFW	QSfw.S42IL-4H	N CO_2	S42IL-116	4H	5.42-47.80							1.98	0.34	21.05	
		N CO_2	S42IL-117	4H	27.52-64.77							1.95	0.31	19.15	
	QSfw.S42IL-6H	N CO_2	S42IL-122	6H	180.69-208.13							1.96	0.32	19.59	
		All, N CO_2	S42IL-131	6H	140.00-180.69	2.19	0.3	15.86				1.97	0.33	20.43	
	QSfw.S42IL-7H	N CO_2	S42IL-135	7H	101.23-152.29							2.08	0.44	27.1	
SDW	QSdw.S42IL-4H	H CO_2	S42IL-124	4H	171.25-183.54				0.16	-0.06	-27.63				
	QSdw.S42IL-6H	N CO_2	S42IL-122	6H	180.69-208.13							0.22	0.04	19.52	
	QSdw.S42IL-7H	N CO_2	S42IL-135	7H	101.23-152.29							0.22	0.04	23.61	
		N CO_2	S42IL-136	7H	134.43-152.29							0.22	0.04	22.47	
AC	Qac.S42IL-1H	All, H CO_2	S42IL-102	1H	1.10-98.23	0.48	-0.3	-37.94	0.19	-0.5	-72.73				
	Qac.S42IL-4H	H CO_2	S42IL-124	4H	171.25-183.54				0.25	-0.44	-63.64				HvC12
	Qac.S42IL-5H	All, H CO_2, N CO_2	S42IL-176	5H	154.37-234.98	0.16	-0.62	-80	0.06	-0.62	-90.91	0.25	-0.62	-71.43	

[a] Trait abbreviations are given in Table 7.
[b] Significant line by trait association which determined by Dunnett test, across treatments (All), stress (H CO_2) and control CO_2 (N CO_2)
[c] Location of chromosome and introgression target (cM) based on Schmalenbach et al. (2011)
[d] Least squares means of ILs across treatments, high: 760 ppm CO_2 and control: 380 ppm CO_2
[e] Difference between LSmeans [S42IL] - LSmeans [Scarlett] across, high: 760 ppm CO_2 and control: 380 ppm CO_2
[f] Relative performance in %: LSmeans [S42IL] - LSmeans [Scarlett] ×100 / LSmeans [Scarlett]
[g] Candidate gene: 1: Wang et al. (2010), 2: Himi and Taketa (2015)

3.4 Response of introgression lines

The investigated introgression lines responded differently to the three different stress treatments. In eight (17.0%) and 11 (23.4%) out of 47 introgression lines no exotic QTL allele could be detected in the P and K stress experiments, respectively (Tab. 28). This number was significantly higher with 35 lines (74.5%) under CO_2 stress (Table 27). This means that 39, 36 and 12 S42ILs under P, K and CO_2 stress, respectively, showed at least one significant trait effect compared to the control variety 'Scarlett' (Fig. 8). Introgression lines S42IL-101 and -103 were only identified for SRLR and S42IL-111 and -138 for HEI under K deficiency. Under phosphorus starvation introgression lines S42IL-134 and -161 were only significantly associated with SRWR and RL, respectively. In contrast, two (P) and seven (K) introgression lines revealed a maximum of 7 and 4 exotic QTL alleles, respectively (Table 15 (P deficiency) and Table 23 (K deficiency)). Under CO_2 stress a maximum of two exotic QTL alleles could be detected in three lines (Table 27). Especially the introgression lines S42IL-102, -109, -121, -140 and -176 are characterized by a high number of exotic QTL alleles in both nutrient deficiency analyses. For only one introgression line (S42IL-132) no exotic QTL allele could be found in all three experiments (Table 28, Fig. 8).

Table 28: List of introgression lines without exotic QTL alleles (X) compaired between P and K deficiency experiments

Introgression line	Phosphorus deficiency	Potassium deficiency
S42IL-101	X	
S42IL-103	X	
S42IL-106		X
S42IL-108		X
S42IL-111	X	
S42IL-113		X
S42IL-114		X
S42IL-115	X	
S42IL-117	X	
S42IL-124		X
S42IL-131		X
S42IL-132	X	X
S42IL-134		X
S42IL-138	X	
S42IL-141		X
S42IL-149		X
S42IL-153	X	
S42IL-161		X
Total	**8**	**11**

But 24 introgression lines were identified with the same *Hsp* allele effect for HEI, RL, SRLR and SRWR under phosphorus and potassium deficiency. Only S42IL-121 with an exotic *Hsp* introgression on the long arm of barley chromosome 4H was detected for HEI in all three experiments (P, K and CO_2 stress).

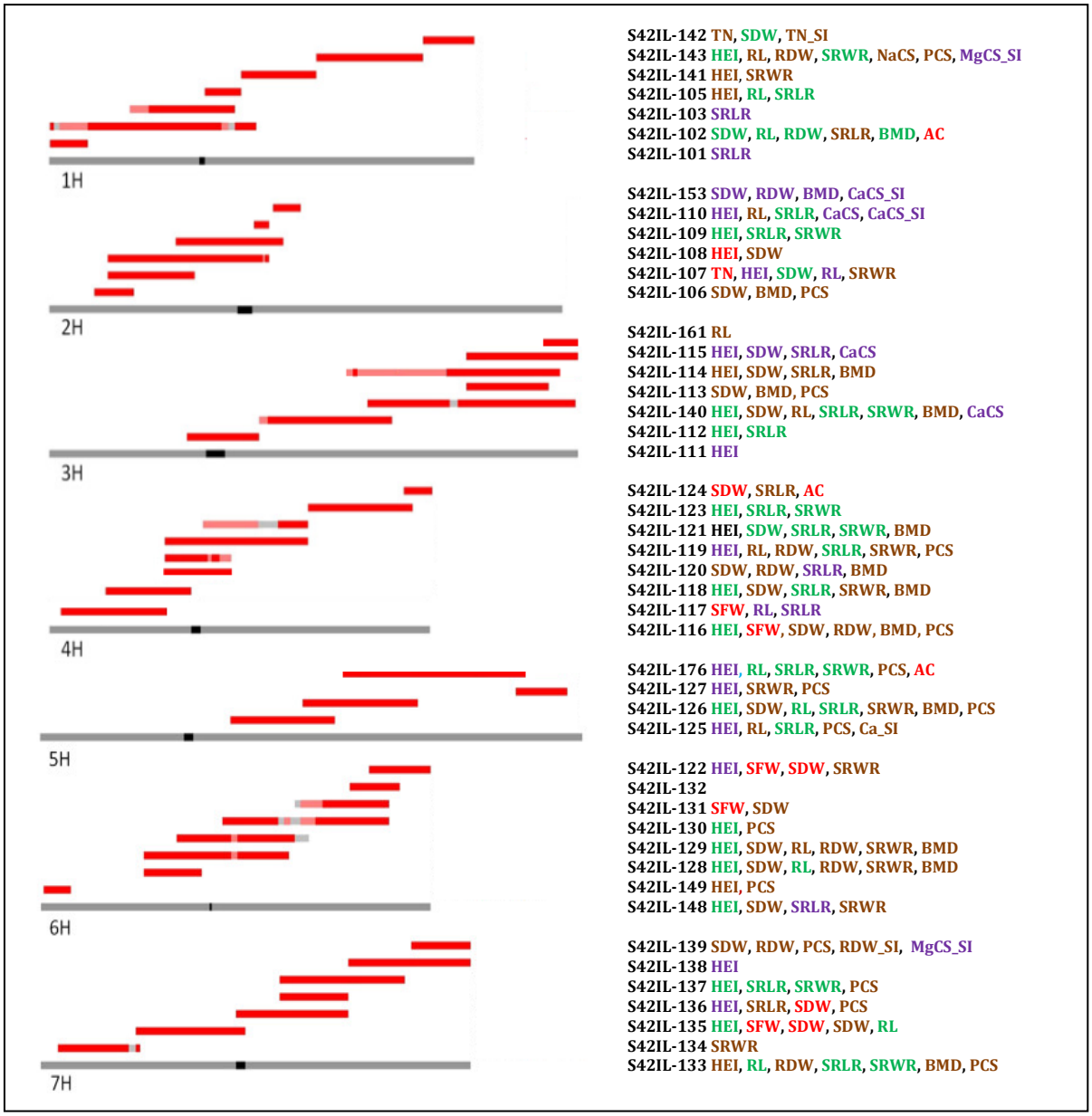

Figure 8: Response of introgression lines to different stress treatments (QTLs for: phosphorus (**P**), potassium (**K**), both nutrients (**PK**), carbon dioxide (**CO_2**) and all three stress events (**P**K**CO_2**), trait abbreviations are given in Tables 4 to 7)

4 General discussion

Many factors are influencing growth and development of plants. These include both the two essential macronutrients phosphorus (P) and potassium (K) as well as carbon dioxide (CO_2) in the atmosphere. As a model to utilize wild relatives for genetic improvement of cultivated plants both P and K deficiency as well as the increase of CO_2 concentration in the air were studied in the wild barley introgression library S42IL, possessing individual sub-chromosomal substitutions between the elite barley cultivar 'Scarlett' and the *Hsp* wild barley accession ISR42-8 from Israel. The main goal of this study was to identify S42ILs which show a significant decrease or increase in growth relative to 'Scarlett' and to locate genes, causing these effects, on sub-chromosomal *Hsp* introgressions of the respective S42IL line. For this purpose, four and five hydroponic experiments under two different P and K levels (control and low), respectively, were conducted with a total of 47 selected ILs from the S42IL population and the control cultivar 'Scarlett', following a protocol already applied in a hydroponic nitrogen study with the same S42IL library (Hoffmann et al. 2012). Using hydroponics 62 root and shoot traits (eight morphological traits, 24 nutrient concentration traits and 30 stress index traits) were examined during the seedling stage. In order to improve uniformity of seedling development across ILs, approximately 30 seeds per genotype and experiment were pre-germinated and the most vigorous plantlets were selected. The cultivation procedure turned out to be successful because only 47 (P) and 95 (K) seedlings out of 1,920 (2.4%) and 2,304 (4.1%), resepectively, failed to produce root and shoot data. The same 47 selected introgression lines as well as the control variety 'Scarlett' were examined in five growth chamber experiments under two different CO_2 concentrations (control: 380 ppm, increased: 760 ppm). Using these experiments five morphological traits and five stress index traits of seedling plants were measured. After pre-germination of the seeds in these experiments, too, only 5 seedlings out of 1,920 (0.26%) failed to produce morphological data.
Interactions between nutrients are positive (synergism), negative (antagonism) or nonexistent (Fageria 2001; Ranade-Malvi 2011). In case of synergism, the plant is physiologically promoted by the cooperation of two or more nutrient elements, whereas in antagonism the uptake of one nutrient is reduced by the excess of another. For example, an optimal calcium supply improves the uptake of phosphorus and potassium (Ranade-Malvi 2011).

The response of the 47 ILs studied is discussed in detail in the following paragraphs:

4.1 Phosphorus (P)

Plants and animals require phosphorus as an essential macronutrient, being involved in many physiological processes. However, phosphate resources are often limited in soils and, in future, phosphate fertilization of crops is expected to become limited and expensive. Thus, plant breeding is urged to provide new cultivars, achieving yield increases with a minimal requirement of P supply. Young plants of annual crops including barley respond extremely sensitive to P deficiencies. The quest for desirable genes or QTLs, which secure an improved phosphate use efficiency (PUE) is becoming increasingly important for both plant breeders and farmers. Improvements may be possible through variation of genes that determine appropriate features such as phosphate uptake from the soil and phosphate utilization or deposition in sink organs. Wild relatives of crop plants may assist in providing the requested genes since they exhibit a huge allelic variation, often much wider than within a crop gene pool.

The successful execution of the hydroponic study is further confirmed by the fact, that treatment effects could be observed for all investigated traits, except root length (Table S1, Supporting Information, Soleimani et al. 2017). In particular, significant reductions of shoot and root dry weight and P concentration in roots proved that barley seedlings were sensitive to the reduction of P supply. Interestingly, P concentration in shoots was higher under P10 than under P100. Nevertheless, the total amount of P was reduced in roots and in shoots with -41% in roots (from 208 to 122 µg P per plant) and -34% in shoots (from 362 to 236 µg P per plant), calculated as the product of mean dry weight and mean P concentration per treatment and plant organ. From this it can be concluded that in our barley seedling experiment P stress revealed more severe effects in roots than in shoots.

Genotype effects could be observed for all morphological traits, across and within both P treatments, increasing the odds to subsequently detect line-by-trait associations and QTLs, which are involved in controlling trait performances of the introgression lines (Table S1, Supporting Information). In contrast, genotype effects were only observed for two nutrient concentration traits (NaCS and ZnCR), indicating that the measurement of nutrients may not be as reproducible as morphological traits. This interpretation may be further supported through a clear difference in heritability. Whereas morphological traits revealed heritabilities between 41.9 and 85.8% (except TN across treatments and within P10 with 0.0%), heritabilities for nutrient concentration traits varied between 0.0 and 58.3% with 28 trait treatment combinations exhibiting a heritability of 0.0%. The lower chance to detect genotype effects and to find strong heritabilities may be explained by the smaller number of samples analyzed. Across four experiments morphological traits were analyzed in up to 1.874 observations

whereas nutrient concentration traits and stress index traits were only analyzed in up to 373 and 181 observations, respectively, resulting in a much smaller number of replicates to estimate the accurate phenotype of each S42IL.

Subsequently, line-by-trait association studies were conducted based on MIXED model ANOVA and Dunnett tests to locate a total of 91 QTLs controlling eight morphological traits, two nutrient concentration traits and three stress index traits. QTLs were not distributed evenly, neither across traits nor across S42ILs. The majority of QTLs (75) were detected for morphological traits, reflecting the higher odds to find genetic effects and higher heritability values for these traits (Table 15). No QTLs were detected for root nutrient concentration traits, whereas QTLs for two shoot nutrient concentration traits were detected. This finding may again contribute to the low heritability found among root nutrient concentration traits where only four trait-by-treatment combinations reached heritability values larger than 30%, whereas ten shoot nutrient concentration trait-by-treatment combinations were found with heritability values larger than 30%. Regarding the distribution of QTLs across ILs, the majority of QTLs where detected in S42IL-126 and -133, with seven QTLs on chromosomes 5H and 7H, respectively, followed by S42IL-128, -129, -140 and -143 (6 QTLs each) with *Hsp* introgressions on chromosome arms 5HL, 7HS, 6HL, 6HL, 3HL and 1HL, respectively (Table 15). These ILs might be ideal as donors in barley breeding to increase, for example, root length and root dry weight (S42IL-128 and -129), biomass (S42IL-126,-128, -129 and -140) and P concentration shoot (S42IL-126, -133 and -143). In the following, all traits, which were significantly controlled by QTLs are discussed individually.

Morphological traits

Tiller number

P deficiency decreased tiller number in barley plants under treatment P10 compared to adequate P supply (P100). However, genetic variation for tiller number was only observed under P100. Reduction of tiller emergence was also reported in wheat under phosphate starvation by Rodriguez et al. (1999) and Su et al. (2006). Only one IL (S42IL-142) revealed an increasing *Hsp* effect for this trait, compared against the recurrent parent 'Scarlett' (Table 15). This hidden wild barley *Hsp* gene, located on chromosome 1H, may be useful to increase tiller number in particular under adequate P supply.

Plant height

In most cases, plants respond to P deficiency by reducing shoot growth. This observation was also reported for *Arabidopsis* (Jiang et al. 2007) under P starvation. Also in this study barley seedlings were on average 8 cm shorter under P10 than under P100. Among 14 QTLs found, five exotic *Hsp* alleles were associated with a reduced plant height across P treatments, three QTLs independently on chromosome 1H and two further on chromosomes 6H and 7H (Table 15). Some of these QTL correspond to known candidate genes, which may explain the QTL effects on plant height, for example *HvFT3, Flt-2L, sdw1/denso, ari-e-GP and brh1* on chromosomes 1H, 2H, 3H, 5H and 7H (Table 15). Also, QTL effects on plant height in eleven S42ILs corresponded to previously reported effects of the same ILs in studies on nitrogen stress and drought stress, conducted by Hoffmann et al. (2012) and Arifuzzaman et al. (2014). This high degree of correspondence is striking since different growth stages (14-day old seedlings, six-week old young plantlets and adult plants), different cultivation systems (hydroponics and greenhouse cultivation) and different stresses (nitrogen and drought) were applied. This finding may indicate that wild barley introgression lines often react in similar ways, compared to the elite barley cultivar 'Scarlett', regardless, which growth stage, cultivation system and stress was applied.

Shoot dry weight and Shoot root weight ratio

Both phosphorus and nitrogen strongly affect photosynthesis and dry matter distribution between shoots and roots. Carbohydrates comprise the biggest part of dry matter in plants. The reduction of photosynthesis efficiency under P deficiency leads to a decrease of carbohydrates in plants. On the other hand, shoot carbohydrates are involved in plant growth and signaling. Under P deficiency more carbohydrates are transferred to roots than to shoots, which in turn leads to a reduced shoot root weight ratio (Fredeen et al. 1989; Nielsen et al. 2001). In this study, this effect was confirmed by a reduction of shoot root weight ratio from 2.3 to 1.6 (Table 8). This negative effect of P starvation on shoot dry weight was also reported in wheat (Su et al. 2006) and soybean (Fredeen et al. 1989). In total, twelve QTLs were detected for shoot dry weight and in all cases but one the exotic *Hsp* allele increased shoot dry weight. The largest effect was observed for S42IL-121 (chromosome 4H). The same IL was detected under nitrogen deficiency (Hoffmann et al. 2012) and also under drought stress (Honsdorf et al. 2014a). Likewise, 14 QTLs were detected to control shoot root weight ratio with trait-improving *Hsp* alleles at all but two loci. For this trait five QTLs were also reported in Hoffmann et al. (2012). Thus, it can be concluded that many exotic QTL alleles are present in the S42ILs, which have the potential to increase shoot dry weight and shoot root weight ratio

compared to the elite cultivar 'Scarlett'. These *Hsp* alleles may be advantageous for barley breeding, in particular under P starvation.

Root length

Generally, it is assumed that P deficiency causes a delay in root development and growth. Change of root morphology is one of the plant responses to P deficiency where primary root length may be reduced whereas growth of lateral roots and root hairs may be enhanced (Reymond et al. 2006; Svistoonoff et al. 2007). In this study, the average root length was shortened from 29.8 to 27.8 cm (Table 8). However, it must be admitted that the length of the longest root axis, as measured here, may not be indicative for the total plant root length, as it does not include branching and density parameters of roots. In total, nine QTLs were detected for root length with two showing increasing effects of the exotic *Hsp* alleles relative to the elite 'Scarlett' allele (Table 15). One QTL on chromosome 5H may be attributed to the semi-dwarf gene *ari-e.GP* (Chloupek et al. 2006) exhibiting a root length reducing *Hsp* effect. The root length effects of six introgression lines corresponded to the same effects previously detected in S42ILs tested under nitrogen deficiency in hydroponics (Hoffmann et al. 2012). Also Naz et al. (2014) reported root length increasing *Hsp* effects under drought in four S42ILs (S42IL-102, -119, -125 and -176). The *Hsp* effect under drought was also confirmed by Arifuzzaman et al. (2014). However, the *Hsp* effects under drought were contrary to the effects under P stress, increasing root length in the former experiments but decreasing root length in the latter. This may indicate, that the *Hsp* reaction on root length might be different under both stresses, although the same introgression lines and potentially the same genes may be involved in several line-by-trait associations.

Root dry weight

P deficiency induces sucrose accumulation in roots, causing root growth, dry matter accumulation and, finally, a decrease in shoot to root ratio (Amtmann et al. 2005). In this study, the average root dry weight was reduced under P deficiency, however, not as strong as shoot dry weight, resulting in an overall reduced shoot to root ratio (Table 8). In total, eight OTLs were detected with four increasing *Hsp* effects. The root dry weight increasing *Hsp* effects present in S42IL-102 and -176 were also reported under drought stress by Naz et al. (2012) and Naz et al. (2014). In addition, Naz et al. (2014) and Arifuzzaman et al. (2014) reported an increasing *Hsp* effect on root dry weight in S42IL-133, where a reducing effect in the same line under P stress was found here. Finally, the root dry weight *Hsp* effects present in S42IL-102 and -143 were also observed under nitrogen deficiency (Hoffmann et al. 2012) and under

drought stress (Arifuzzaman et al. 2014). Thus, it can be concluded, that a number of *Hsp* effects on root dry weight can be reproduced regardless which stress is applied.

Shoot root length ratio

Several studies reported that a lower shoot root length ratio occurs when macronutrients are deficient (Wilson 1988). This was also confirmed in this study. Under adequate and low P treatments, the average shoot root length ratio dropped from 1.0 to 0.8 (Table 8). In total, nine QTLs controlling shoot root length ratio in 18 ILs were detected. All exotic *Hsp* QTL alleles except one exhibited an increase in trait performance. One QTL effect, present in S42IL-126 was already reported in Hoffmann et al. (2012).

Biomass

P deficiency often results in reduced dry biomass, both in roots and in shoots (Manschadi et al. 2014). In order to maintain a balanced plant phosphorus status, the rate of P uptake must be proportional to the rate of dry matter accumulation (Koide 1991). Thus, plant biomass has a substantial effect on nutrient uptake, including phosphorus, where vigorous genotypes exhibit a higher nutrient uptake capacity. This observation was also confirmed in this study. Under low P treatment, the average dry biomass dropped from 175.90 to 119.30 mg (Table 8, Fig. 5). Across P treatments biomass revealed a positive correlation with P concentration root (r=0.36) and a negative correlation with P concentration shoot (r=-0.34, data not shown). These correlations varied between P treatments. Under P10 only the negative correlation between biomass and P concentration shoot was noteworthy (r=-0.37) whereas under P100 only the positive correlation between biomass and P concentration root was detected (r=-0.45) (data not shown). Thus it can be concluded that under P starvation phosphorus tends to be diluted in shoots of vigorous seedlings but to remain constant in roots. In contrast, under sufficient P supply, phosphorus concentration tends to remain stable in shoots but to increase in roots of vigorous seedlings (Fig. 9).

In total, eight QTLs controlling dry biomass in 13 ILs were detected (Table 15). All exotic *Hsp* QTL alleles except one (S42IL-133) exhibited an increase in biomass. Two trait-improving *Hsp* effects, present in S42IL-121 and -129 were already reported in Hoffmann et al. (2012) and Honsdorf et al. (2014b). It can be concluded that exotic QTL alleles are present in the S42ILs, which have the potential to increase biomass compared to the elite barley cultivar 'Scarlett'. These *Hsp* alleles may be advantageous for barley breeding, in particular under P starvation.

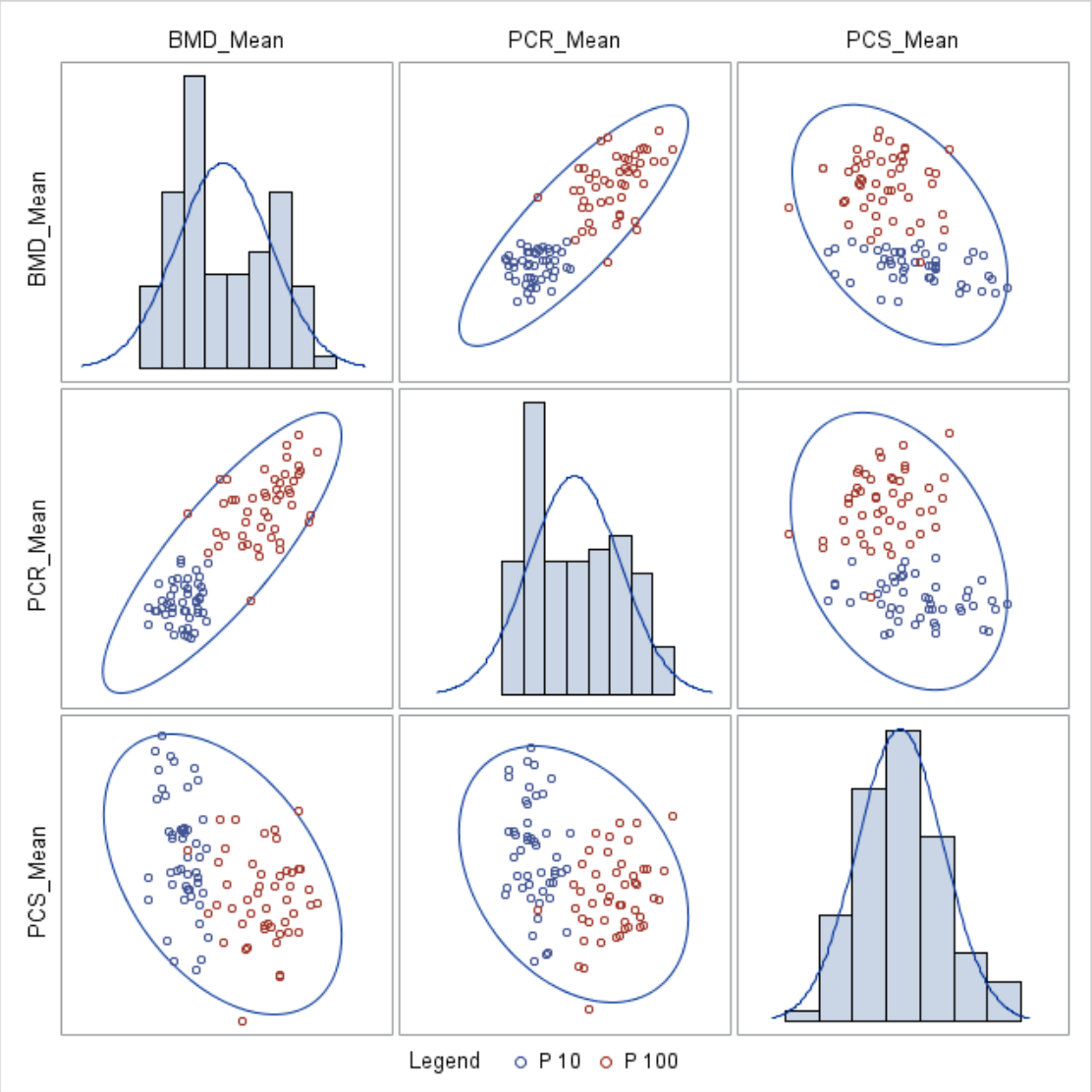

Figure 9: Scatter plot for biomass, phosphorus concentration root and phosphorus concentration shoot per genotype, grouped by P treatment.
Blue and red dots in scatter plots indicate mean performance of each genotype under P10 and P100, respectively. The prediction ellipse with confidence interval P<=95% is encircled in blue. Histograms for each trait are shown in the matrix diagonal including a normal density curve (blue line).

Nutrient concentration traits

A certain amount of macro- and micronutrients is essential for plant growth. The nutrient solution in hydroponics should, thus, contain all nutrients a plant needs for optimal growth. With the classical chemical plant analysis, the nutrient concentration in plant tissue can be determined and the diagnosis of the current nutritional status of plants is possible. For a practical application non-destructive methods for the detection of nutritional status are

becoming increasingly important, for example, by estimation through analysis of wavelength spectra. Hyperspectral imaging as a camera-based evaluation system is currently tested among others for this purpose (Dale et al. 2013). The hyperspectral imaging data need to be modelled using chemical plant analysis with a fraction of the full set of samples in order to subsequently allow a prediction of nutrient concentrations. Therefore, nutrient concentrations in leaf and root samples of our calibration set were first analyzed by ICP-OES. Comparing the correlation between the wet lab calibration data and the predicted values based on HSI modelling proved, that moderate to excellent predictions for all nutrients could be made with r values between 0.57 (Mg) and 0.92 (iron) (Table 14). Thus, the indirect prediction of nutrient concentrations, based on HSI analysis, may be a valuable and cost-effective option, useful to predict the concentration of plant nutrients in large sample sets in case a reduction of time and costs associated with the analysis is mandatory.

The synergistic effect of phosphorus with macronutrient and micronutrients has been documented very well. Some of these synergisms could be confirmed by finding significant positive correlations of P concentration in roots with root concentrations of iron, manganese, sodium, sulfur and zinc (Table 13). Likewise, we found significant negative correlations of P concentration in shoots with shoot concentrations of potassium, sodium and zinc.

Searching for introgression lines and QTLs, which control nutrient concentrations in shoots and roots, QTLs for two nutrient concentration traits in shoots and one nutrient stress index trait were detected (Table 15). Surprisingly, no QTLs controlling nutrient concentration traits directly in roots were found. This finding may reflect the generally lower heritability of root concentration traits compared to shoot concentration traits. In future studies, the number of replicates may be increased in particular for root concentration traits but also for other nutrient traits in order to increase heritability values and, as a consequence, the odds to detect significant line-by-trait associations. In the following, QTLs controlling nutrient concentration traits are discussed separately.

NaCS

One QTL controlling sodium concentration in shoots was located on chromosome 1H, revealing a trait reducing exotic *Hsp* effect (Table 15). The QTL showed a decreasing *Hsp* effect across treatments. So far, no sodium transporter genes or other genes could be identified as candidate genes to potentially explain the observed sodium effect.

PCS

Twelve QTLs controlling P concentration in shoots were located on all barley chromosomes. All effects were solely significant under P100 treatment and exotic *Hsp* alleles increased P concentration in shoots compared to 'Scarlett' (Table 15). The increase of phosphorus in shoots due to the action of *Hsp* alleles could be beneficial for barley cultivation, in particular, under P deficiency. Pacak et al. (2016) reported on four families of inorganic phosphate transporters (*PHT1, PHT2, PHT3* and *PHT4*), which are involved in uptake and transport of inorganic phosphate. *PHT1* is known as a plasma membrane located phosphate transporter, where ten homologues are present in barley. The homologue *HvPT1;6* is expressed in roots and, predominantly, in leaves and its up-regulation under phosphate deficiency has been reported by Preuss et al. (2010). Based on mapping information, the barley homologue *HvPT1;6* (i.e. MLOC_80912.2, (Pacak et al. 2016) is located inside the chromosome 5H introgression of S42IL-127. The phosphate increasing effect of the *Hsp* allele, present in S42IL-127, may, thus, be explained by *HvPT1;6*. So far, no further P transporter genes or other phosphorus-related genes could be identified as candidate genes to potentially explain P concentration shoots effects.

Stress index traits

A stress index, defined as the ratio of trait performance under P10 and P100, was calculated for each trait. In total, QTLs for three out of 24 stress index traits were detected (Table 15). This low number may be explained by the low degree of heritability found for the stress index traits. Whereas only one morphological trait showed a heritability equal zero (TN) across and within P10 treatments (Table 8). All seven nutrient concentration traits across and within P treatments (Table 11) and seven stress index traits revealed zero heritability across treatments, indicating an increased error variance in calculating these trait values. Similar findings were reported by (Honsdorf et al. 2014a) who found ten out of 14 stress index traits with heritabilities equal to zero. The argument is also supported by Wang et al. (2012). The authors pointed out that an increased complexity of the genetic architecture of derived traits (e.g. stress indices) may reduce the power of QTL detection. In the following the stress index traits are discussed in detail.

TN_SI

The introgression lines showed on average a ratio of 0.8 in tiller number between P10 and P100 treatments and a heritability of 36.9% (Table 11). Only one introgression line, S42IL-142 (chromosome 1H), revealed a decreasing *Hsp* effect of -30.09% on TN_SI compared to 'Scarlett'.

The same IL also revealed a QTL for tiller number across and within P100 treatments where the *Hsp* allele was associated with an increase in tiller number. The contrasting *Hsp* effects are caused by the fact that S42IL-142 revealed a stronger increase in tiller number than 'Scarlett', which resulted in a lower stress index. A direct utilization of the pleiotropic *Hsp* QTL allele may, thus, be possible since the disadvantage in stress tolerance is compensated by the increased tiller number. Of course, it has to be tested in field experiments, if the tiller number effect in S42IL-142 is associated with a yield increase under P stress compared to 'Scarlett'.

RDW_SI

The introgression lines showed on average a ratio of 0.9 in root dry weight between P10 and P100 treatments and a heritability of 33.1% (Table 11). Only one introgression line, S42IL-139 (chromosome 7H), revealed an increasing *Hsp* effect of 46.61% on RDW_SI (Table 15). The same IL also revealed a QTL for root dry weight across treatments where the *Hsp* allele was associated with a reduced root dry weight. Since the RDW_SI effect of the *Hsp* QTL allele in S42IL-139 is associated with a reduced root dry weight a utilization of the *Hsp* allele does not seem advisable in a barley breeding program.

CaS_SI

The introgression lines showed on average a ratio of 1.2 in calcium concentration shoot between P10 and P100 treatments and a heritability of 38.2% (Table 11). Only one introgression line, S42IL-125 (chromosome 5H), revealed an increasing *Hsp* effect of 148.18% on CaS_SI, but no direct effect on calcium concentration in shoots or roots. However, a *Hsp* allele in the same introgression line also increased phosphorus concentration shoot. It remains open if these effects are pleiotropic or caused by two linked genes, which are present on the same *Hsp* introgression.

4.2 Potassium (K)

Potassium is known as an important macronutrient for plant growth and development and plays a key role in important metabolic processes in plants, too. Already during early vegetative growth a high potassium concentration is important. This can counteract abiotic stress such as cold, frost, heat and salinity and ensure an optimal plant development amd yield performance. A high supply of available potassium from soil and fertilization is the prerequisite. Potassium improves water utilization efficiency and frost tolerance and affects

many quality traits. Before the appearance of visible damages under potassium deficiency, reductions in assimilation, water supply and, therefore, yield and quality may occur. In analogy to phosphorus, the search for desirable QTLs and genes that ensure improved potassium utilization efficiency (KUE), is therefore increasingly important for plant breeders and farmers. Regarding potassium, improvements should be possible by gene variations, too. This includes traits such as potassium uptake from the soil, optimal potassium utilization or storage in the vacuoles. Desirable genes can be found in exotic (wild) relatives of the corresponding crop plant in large allelic variations. In these wild accessions no narrowing of the gene pool took place in the past, comparable to the domestication process of our cultivated plants.

The hydroculture system is excellently suited for plant growth under defined nutrient solution composition in a short growth phase, such as the juvenile stage. Plant symptoms of potassium deficiency were not detectable after 15 days of plant cultivation except for mean root length (Tab. 16). Despite longer roots (+ 5.8 cm) under K0, the average root dry weight was reduced (- 1.6 mg) compared to K100. Large differences in SWD and biomass between both treatments (lower mean values under K0) might be explained by a negative effect of potassium starvation on biomass production. Zhao et al. (2014) reported similar results for wheat in hydroponic cultivation.

Treatment effects could be observed for half of the analysed morphological traits, namely tiller number, shoot dry weight, shoot root length ratio and biomass (Table S2, Supporting Information). Reductions of shoot and root dry weight and K concentration in shoots and roots proved that barley seedlings were sensitive to the reduction of K supply. The total amount of K was reduced with -14.29% in roots and -16.87% in shoots. From this, it can be concluded that in barley seedlings K stress in roots and shoots has approximately the same effect. Genotype effects could be observed for all morphological traits, across and within both K treatments, increasing the odds to subsequently detect line-by-trait associations and QTLs, which are involved in controlling trait performances of the introgression lines (Table S2, Supporting Information). In contrast, genotype effects were only observed for six shoot nutrient concentration traits (across: CaCS, KCS, MnCS, PCS, SCS, K100: FeCS), indicating that the measurement of nutrients may not be as reproducible as morphological traits. This interpretation may be further supported through a clear difference in heritability. Whereas morphological traits revealed heritabilities between 22.2 and 74.7% (except TN within K0 without estimation), heritabilities for nutrient concentration traits varied between 0.0 and 54.7%. For 22 trait treatment combinations no heritabilities could be estimated. Heritabilities for stress index traits could be found between 0.0 and 38.2% with 12 trait treatment

combinations exhibiting a heritability of 0.0%. The lower chance to detect genotype effects and to find strong heritabilities may be explained by the smaller number of samples analyzed. Across five experiments morphological traits were analyzed in up to 2,209 observations whereas nutrient concentration traits and stress index traits were only analyzed in up to 464 and 1,063 observations, respectively, resulting in a much smaller number of replicates to estimate the accurate phenotype of each S42IL.

As for phosphorus investigations, line-by-trait association studies were conducted based on MIXED model ANOVA and Dunnett tests to locate a total of 58 QTLs controlling seven morphological traits (without tiller number), one shoot nutrient concentration trait (CaCS) and two stress index traits (CaS_SI, MgS_SI). QTLs were not distributed evenly, neither across traits nor across S42ILs. The majority of the QTLs (52) were detected for morphological traits, reflecting the higher odds to find genetic effects and higher heritability values for these traits (Table 23). No QTLs were detected for root nutrient concentration traits, whereas two QTLs for at least one shoot nutrient concentration traits were found. This finding may again contribute to the lower heritability of the root nutrient concentration traits (maximum of 49.2% for CCR) compared to shoot nutrient concentration traits (maximum of 54.7% for KCS). Seven and ten trait-by-treatment combinations for root and shoot nutrient concentration traits were found with heritabilities larger than 30%. For 36 out of 47 S42ILs the Dunnett test revealed significant deviations from the recurrent parent 'Scarlett' for at least one trait studied. Regarding the distribution of QTLs across ILs, the majority was detected for seven ILs (S42IL-102, -110, -115, -121, -140, -153 and -176 (4 QTLs each) with *Hsp* introgressions on chromosome arms 1HL, 2HL, 3HL, 4HL, 3HL, 2HL and 5HL, respectively (Schmalenbach et al. 2011), followed by 8 S42ILs (3 QTLs each). *Hsp* alleles had positive effects on trait performance in most of the detected QTLs (47 QTLs or 81 %). Twenty one out of 58 detected QTLs were also reported with the same effect of *Hsp* alleles on plant height (9 QTL), shoot dry weight (3 QTL), root length (5 QTL), shoot root length ratio (1 QTL) and shoot root weight ratio (3 QTL) in previous field and green house studies (Hoffmann et al. 2012; Honsdorf et al. 2014a; Honsdorf et al. 2014b). Among 47 ILs, five introgression lines (S42IL-115, -140, -110, -121 and -176) revealed a high number of QTLs on the long arm of chromosome 3H, 4H and 6H (4 QTL) in this study. *Hsp* alleles on the long arm of chromosome 3H showed positive effects and improved HEI, SDW, SRLR, SRWR and CaCS. S42IL-115 overlapped with S42IL-140 revealing an increasing *Hsp* effect in 4 detected QTLs. These ILs might be ideal as donors in barley breeding. In the following, all traits, which were significantly controlled by QTLs, are discussed individually.

Plant height

According to Ruan et al. (2015) reduced K supply results in several negative effects on plants such as reduced growth. This observation confirms the results both under potassium as well as phosphorus deficiency in this study. Barley seedlings were on average 0.9 cm shorter under K0 compared to K100. In total 16 QTLs were found on all chromosomes in 26 introgression lines. Nine of them were already detected with the same *Hsp* effect on plant height by Hoffmann et al. (2012) under nitrogen stress. Fourteen out of 26 ILs were also identified with the same *Hsp* allele effect for this trait under phosphorous deficiency in this study (Soleimani et al. 2017, see Chapter 3.1). Only *Hsp* alleles on the long arm of chromosome 1H reduced plant height in S42IL-143 in this investigation, by Hoffmann et al. (2012) under nitrogen stress, Honsdorf et al. (2014a, 2014b) under drought stress and Soleimani et al. (2017) under phosphorus stress (see Chapter 3.1). Two QTLs on chromosome 2H were identified with a positive effect on plant height in S42IL-107 and -109, which correspond to known candidate genes, for example flowering time genes such as *Ppd-H1* and *HvFT4* (Wang et al. 2010). Line S42IL-109 was already detected with a same effect by Hoffmann et al. (2012). In addition QHei.S42IL-3H.c (S42IL-115, -140) was also observed in Hoffmann et al. (2012) and Honsdorf et al. (2014a, 2014b). The semi dwarfing gene *Sdw1* was mapped on chromosome 3H (Chloupek et al. 2006; Laurie et al. 1993), which coincided with line S42IL-140. A positive *Hsp* effect in S42IL-121 (short arm of chromosome 4H) was also obtained in studies of Hoffmann et al. (2012), Honsdorf et al. (2014a), Schmalenbach et al. (2009) and Soleimani et al. (2017). This line revealed the highest increase on HEI compared to 'Scarlett'. A further semi dwarfing gene, *ari-e.GP* on chromosome 5H, coincided with line S42IL-126 and was described by Chloupek et al. (2006). As already shown in the phosphorus study, this high correspondence is striking, since different growth stages, cultivation systems and stress conditions are used. It can also be inferred here that wild barley introgression lines often react in a similar way, compared to the elite barley cultivar 'Scarlett', regardless of the experimental conditions.

Shoot dry weight

Potassium as one of the three primary macronutrients is present in about 2 to 10 % of the total dry weight of a plant (Ruan et al. 2015). According to Mahmood et al. (2001) shoot dry weight is regarded as the most sensitive plant reaction parameter for nutrient deficiency and is used as a selection criterion for the assessment of genotypes for nutrient efficiency at juvenile plant stage. This is confirmed by a mean difference of 21.2 mg between both potassium levels, less SDW being established under K0. Mahmood et al. (2001), Kong et al. (2013) and Zhao et al. (2014) reported that K deficiency results in a reduction of SDW in wheat plants, which could be

confirmed in this study with the barley introgression lines (according to mean values in Table 16). In this analysis six QTLs for shoot dry weight in six ILs representing barley chromosomes 1H to 4H were found. Four of them increased SDW in four ILs. One QTL on 2H (S42IL-107) with a positive effect of the *Hsp* allele was detected under phosphorus deficiency (Soleimani et al. 2017) and also under drought stress in the greenhouse (Honsdorf et al. 2014a). Line S42IL-115 with positive *Hsp* alleles on chromosome 3H could also be found under nitrogen stress (Hoffmann et al. 2012). But under drought stress (Honsdorf et al. 2014a) this IL showed a negative *Hsp* effect for this trait. The detected QTL for S42IL-121 supported our results compared with three previous studies (Hoffmann et al. 2012; Honsdorf et al. 2014a; Soleimani et al. 2017). Mahmood et al. (2001) stated that one of the most efficient approaches to improving plant production in a resource-poor environment is the identification, selction, use and, therefore, breeding of nutrient-efficient varieties.

Root length

Changes in root architecture (root morphology) are one of the ways of plant adaption to nutrient deficiency. Høgh-Jensen and Pedersen (2003) reported an increase of root hair elongation in *Arabidopsis,* red clover, rye and barley in response to potassium starvation, which helps to uptake potassium in plants. In rice Jia et al. (2008) found that roots of more efficient genotypes can better tolerate K stress. At a low K nutrition root length, surface, volume and number were increased. The average root length under reduced K was also higher in the introgression lines examined. Seven QTLs in 9 introgression lines on all barley chromosomes except 3H were found. 57.1% of the detected QTLs (4 out of 7) exhibited a negative *Hsp* effect on root length. In addition, 5 QTLs with the same *Hsp* effect on RL have been reported by Hoffmann et al. (2012) under nitrogen stress and Soleimani et al. (2017) under phosphorus starvation. In both investigations the detected QTL on the short arm of 2H in S42IL-107 corresponded with an increased root length. Positive *Hsp* effects were also reported in 4 ILs (S42IL-102, -119, -125 and -176) under drought stress (Naz et al. 2014), two of which (S42IL-102, -176) with an opposite effect in the present study. A comparable situation was found under phosphorus starvation (Chapter 4.1). This indicates, that the *Hsp* reaction on root length might depend on the type of stress, although the same introgression lines and potentially the same genes may be involved in the line-by-trait associations. The genetic location of the dwarfing gene *ari-e.GP* (Chloupek et al. 2006) coincided with QRl.S42IL.5H, which reduced root length in two ILs (S42IL-126 and -176). On chromosome 7H two QTLs were identified for this trait in Hoffmann et al. (2012) and this study with reducing and increasing *Hsp* effects in S42IL-

133 and 135, respectively. Also, this may correspond to the presence of the dwarfing gene *Brh1* (Li et al. 2000) on the short arm of 7H with QRl.S42IL-7H.a.

Root dry weight

Under nutrient stress conditions, plants promote root development depending on plant species and nutrient (Mahmood et al. 2001). But no or even negative effects of potassium starvation on root biomass has been reported (Zhao et al. 2014). In contrast, Mahmood et al. (2001) showed RDW increases in wheat genotypes by 22% with control K supply compared to a deficient K level. This can be confirmed by a higher mean value of 1.6 mg under K100 compared to K0 in this study (Table 16). Two QTLs in two introgression lines (S42IL-102, -153, representing chromosomes 1H and 2H, respectively) were found. The lines significantly differed from the control variety 'Scarlett'. In both lines *Hsp* alleles decreased RDW, especially on 1H (S42IL-102) under K0. Positive *Hsp* effects were reported for S42IL-102 in previous studies under nitrogen (Hoffmann et al. 2012) and drought stress (Naz et al. 2014). These phenotypic differences on root formation are stress-dependent.

Shoot root length ratio

A lower shoot root length ratio may occur when macronutrients are only available to a limited extend for the plant (Wilson 1988). This could already be confirmed with regard to phosphorus (see Chapter 4.1). In the potassium study, the average shoot root length ratio dropped from 1.1 to 0.9 (Table 16) under control and low K treatments, respectively. Potassium deficiency decreased plant height (-0.9 cm) and increased root length (+5.8 cm) under K0. The mean value for SRLR was 0.20 lower under K0 compared to K100. In total, 11 SRLR QTLs in 20 introgressions lines representing all barley chromosomes were detected. All exotic *Hsp* alleles exhibited an increase in trait performance with the highest effect on chromosome 5H in S42IL-126 across treatments and control K.

Shoot root weight ratio

It is generally acknowledged that the macroelement nutrition can influence dry substance distribution between shoot and root in higher plants regardless of growth and development as well as nutrient treatment (Andrews et al. 1999). The shoot root dry weight ratio can decrease when plant growth is limited under N, S, or P supply. But the ratio can fall or rise with a decreased growth, caused by Mg, Ca or K deficiency. Andrews et al. (1999) noted that the reaction to potassium deficiency is not contradictory. Zhao et al. (2014) found a decrease of SRWR in wheat under potassium deficiency. This could be confirmed in our study. The mean

SRWR of the introgression lines was 0.40 lower under K0 compared to K100. This difference is mostly related to the high reduction of SDW (-21.2 mg) under reduced K compared to RDW (-1.6 mg). In seven out of eight QTLs in eight introgression lines an increasing *Hsp* effect was observed. Only S42IL-109 showed a negative effect on SRWR relative to 'Scarlett'. Under nitrogen deficiency five QTLs were detected (Hoffmann et al. 2012) and four of them were identified in the present study, too. In addition, five of the detected QTLs were also observed under P deficiency (Soleimani et al. 2017) with the same *Hsp* effects. For example, *Hsp* alleles reduced the SRWR in S42IL-109 for both phosphorus and potassium deficiencies.

Biomass

In order to improve the nutrient utilization efficiency (NUE) by breeding, defined as the amount of biomass, genes must be identified that influence plant growth at low internal nutrient concentrations (Moriconi and Santa-Maria 2013). The modification of biomass and nutrient distribution between roots and shoots is probably most significant. Plant roots as main organ of nutrient uptake have a direct influence on growth as well as biomass formation of shoots. Changes in root morphology and biomass distribution are adaptations of the plant to nutrient starvation (Jia et al. 2008). Positive correlations between potassium deficiency and the reduction of root and shoot biomass and, thus, a negative effect on shoot and root biomass has been documented very well by Zhao et al. (2014) and Asif et al. (2017) in wheat. This observation could also be made on barley seedlings of the introgression lines. The mean dry biomass dropped from 157.5 mg under K100 to 135.2 mg (-22.3 mg, Table 16) under low K. Across K treatments biomass is neither positively or negatively correlated with K concentration in roots (r=0.00) and biomass revealed a negative correlation with K concentration in shoots (r=-0.15, Tab. 21). Correlation between biomass and KCR was negative under two K treatment. KCS showed a positive and a negative correlation with BMD under K100 and K0, respectively (data not shown). Therefore, low availability of potassium leads to reduction of potassium concentration in root and shoot. Also low concentration of K in soil results in sodium (Na) to be easily absorbed by roots and to increase the Na concentration in plants.

Nutrient concentration traits

For optimal growth and development of plants, macro- and micronutrients are necessary in certain quantities. In a nutrient solution these elements are available for plants in a soil free medium. Hydroponic system facilitates root and shoot phenotyping. Determination of nutrient concentration in plants helps to understand the reaction of plants to varying nutrient

concentrations and various determination methods are available. Traditionally, classical chemical analysis has been used. Now hyperspectral imaging (HSI) is increasingly in focus. It allows the measurement of the nutrient concentration from non-destructive samples by estimation through analysis of wavelength spectra. Hyperspectral imaging as a camera-based evaluation system is currently tested among others for this purpose (Dale et al. 2013). The procedure has already been described in chapter 4.1 on phosphorus deficiency. Potassium correlations between wet lab calibration data and the predicted values based on HSI modelling proved, that weak to high predictions for all nutrients could be made with r values between 0.21 (Mn) and 0.84 (Cu). This indirect prediction of nutrient concentration is poorer compared to the phosphorus assay.

The synergistic/antagonistic effects of potassium with macro- and micronutrients have been documented very well. In plants the competition between potassium, calcium and magnesium is well described (Ranade-Malvi 2011), whereby the uptake of magnesium and calcium is reduced under excess of potassium. For K concentration in roots synergisms could be confirmed by finding significant positive correlations with root concentrations of manganese and phosphorus and a significant negative correlation with sulfur (Table 21). Likewise, significant positive correlations of K concentration in shoots with shoot concentrations of manganese and potassium and a negative correlation with shoot concentration of magnesium were found.

Searching for introgression lines and QTLs, which control nutrient concentrations in shoots and roots, two QTLs for one nutrient concentration trait in shoots were detected (Tables 23). Surprisingly, no QTL controlling nutrient concentration traits directly in roots was found. This finding may reflect the generally lower heritability of root concentration traits compared to shoot concentration traits. In future studies, the number of replicates may be increased in particular for root concentration traits but also for other nutrient traits in order to increase heritability values and, as a consequence, the odds to detect significant line-by-trait associations. In the following, QTLs controlling nutrient concentration traits are discussed separately.

CaCS

It is known that different interactions exist between individual nutrients. Similar properties as size and charge can cause competition, as for Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} (Fageria 2001). The mean calcium concentration in roots was higher under K100 compared to K0 (Table 19). On the contrary, the mean calcium concentration in shoots was higher under K0 compared to K100. A

week negative correlation was observed for K and Ca contens in shoots under K100 (Table 21). This was confirmed by Fageria (2001) in rice and Zhao et al. (2014) in wheat. This higher Ca concentration under K deficiency confirms the antagonistic interaction between both elements in plants (Fageria 2001). Ca uptake can be reduced due to a high mobility of potassium, especially in high K concentrations. Calcium sensor genes could be identified that contribute to improved potassium uptake under K starvation. In *Arabidopsis* the regulation of a K channel under calcium influence in response to K deficiency was described (Li et al. 2006).

Stress index traits

A stress index, defined as the ratio of trait performance under K0 and K100, was calculated for each trait. In total, QTLs for two out of 24 stress index traits were detected (Table 23). This low number may be explained by the low degree of heritability found for the stress index traits. Only for one morphological trait (TN within KO) heritability could not be estimated (Table 16). In addition, twelve stress index traits revealed zero heritability across treatments, indicating an increased error variance in calculating these trait values. Similar findings were reported by Honsdorf et al. (2014a) under drought stress who found ten out of 14 stress index traits with heritabilities equal to zero. The argument is also supported by Wang et al. (2012). The authors pointed out that an increased complexity of the genetic architecture of derived traits (e.g. stress indices) may reduce the power of QTL detection. In the following the two stress index traits are discussed in detail.

CaS_SI

The introgression lines showed on average a ratio of 2.9 in calcium concentration shoot between K0 and K100 treatments and a heritability of 36.0% (Table 19). Only two introgression lines, S42IL-110 and -153 (both chromosome 2H), revealed an increasing *Hsp* effect on CaS_SI (Table 23). However, a *Hsp* allele in the introgression line S42IL-110 also increased plant height, shoot root length ratio and calcium concentration shoot. In contrast, the *Hsp* allele in introgression line S42IL-153 decreased shoot dry weight, root dry weight and biomass.

MgS_SI

Magnesium as a further macronutrient in plants is decisively involved in photosynthesis and activation of various important enzymes and, therefore, in plant growth. Thus, an antagonistic effect could be observed, whereby the Mg uptake decreases with increasing K supply (Ding et al. 2006, Ranade-Malvi 2011). A reduction of Mg concentration by high K concentration in shoots of tall fescue was reported by Hannaway et al. (1982). In contrast a higher mean Mg concentration in roots and shoots was found in the barley introgression lines (Table 19) and in wheat (Zhao et al. 2014) under K.

4.3 Carbon dioxide (CO_2)

Carbon dioxide is the lifeblood of the plants par excellence. Plants use CO_2 for photosynthesis and provision of carbon for organic molecules in plant structures. Human activities to supply food and energy demands are one reason for the increase of atmospheric CO_2 concentration, which significantly increased since the pre-industrial level from 280 ppm to 379 ppm today (Craufurd and Wheeler 2009) and could increase to 750-1000 ppm without counter-measures in 2100 (Weigel and Manderscheid 2012). This increase may be associated with changing global temperature and annual precipitation which directly and indirectly affects growth and development of plants (Ainsworth et al. 2006; Drake and Gonzalez-Meler 1997; Hunsaker et al. 2000; Kleemola et al. 1994; Plessl et al. 2005). Lawson et al. (2001) found initial evidence of the impact of increased CO_2 on growth and yield over a century ago. It is assumed that plants can promote growth and productivity under increased carbon dioxide concentration because of a direkt influence on photosynthesis (Phothi et al. 2016). The rised CO_2 usually increases the growth of C3 plants (Clausen et al. 2011; Hager et al. 2016), whereby large variations can be observed both between and within species (Pleijel et al. 2000). The plant responses to rising CO_2 are accompanied with some physiological, morphological and developmental changes including a stimulated plant growth. Among the positive results of rising CO_2 are the increase of net photosynthesis rate, the reduction of photorespiration, the improvement of water use efficiency and the increase of biomass and yield components (Hager et al. 2016).

Under increased CO_2 concentration, some morphological changes such as significant increase of above-ground biomass, root biomass and tiller number were observed in the spring barley cultivar ‘Scarlett’ (Plessl et al. 2005), which was used as recipient parent for the production of the introgression lines studied here. In addition, exotic wild barley alleles for the improvement

of economically relevant traits (pre-breeding material) can make an important contribution to breeding new, adapted cultivars. Agricultural production with these cultivars is strongly dependent on weather and environment. The influence is complex and not always sufficient data exist for important agricultural crops, also with respect to possible interactions. Conclusions to effects and adaptation measures become more and more necessary for agricultural production worldwide. Extensive studies have to be performed for relevant crops under comparable conditions (e. g. in climate chambers). In practical terms, plants are exposed to various levels of different stress, which can have an inhibiting or promoting effect. If it is possible to elucidate the relationships between CO_2 concentration and growth performance of plants and to find QTLs which control the reaction of plants to increased CO_2 concentrations, this can be useful in the long-term for breeding. The effects of atmospheric CO_2 increase on development, growth and yield of our cultivated plants have been investigated for about 30 years. In addition to climate chambers and greenhouses, field tunnels as well as open and closed field chambers are increasingly being used (Weigel and Manderscheid 2012). More meaningful results are, however, possible under normal agronomic production conditions. At the Thünen Institute for Biodiversity in Braunschweig (Germany), research is being conducted on free-field conditions with increased CO_2 concentration and its influence on different crop plants (Weigel and Manderscheid 2012). It is to be clarified whether the CO_2 fertilizing effect leads to a higher yield (Manderscheid et al. 2014).

The set of 47 selected introgression lines (S42ILs) has been designed to evaluate the reaction of juvenile barley plants to high CO_2 concentration as a pre-breeding study. The aim was to detect S42ILs with a significant decrease or increase on trait performance compared to the control genotype 'Scarlett'. The highest increases under elevated CO_2 were observed for the three traits tiller number, shoot fresh weight and shoot dry weight with 0.24 more tillers (8.4%) and 0.32 g more SFW (18.1%) compared to the control condition. For SDW this effect was not so strong compared to SFW. Under high CO_2 SDW was only increased by 0.01 g (5.3%) in barley shoots. The heritability is used to quantify the precision in field trials by breeders (Piepho and Möhring (2007). The five traits studied had heritabilities between 35.4% (HEI) and 73.9% (AC) under increased CO_2 and were used to detect QTLs and line-by-trait associations. In total 12 QTLs in 12 different introgression lines were identified. The majority of the detected ILs was associated with one trait while three ILs were significantly associated with two traits. In lines S42IL-122 (chromosome 6H) and -135 (chromosome 7H) two QTLs increased SFW and SDW under control CO_2 and in line S42IL-124 (chromosome 4H) two QTLs reduced SDW and AC under elevated CO_2 conditions compared to the control. In the following each trait is discussed separately.

Tiller number

Plants respond to rising CO_2 by increasing tiller number. Mulholland et al. (1997) reported a small but non-significant increase in the number of ear-bearing tillers in wheat plants. Kim et al. (2001) found increased tiller number in own and further published experiments in rice. Fangmeier et al. (2000) pointed out that an increase in biomass and yield in barley plants is associated with a higher tiller production and tiller survival. On average the introgression lines and 'Scarlett' had 0.24 more tillers under high CO_2 (Table 24). For 'Scarlett' itself, 3.16 and 2.79 tillers were detected under elevated and control CO_2, a difference of 0.33 tillers. In the study of Plessl et al. (2005) with 'Scarlett' for four weeks at 400 and 700 ppm CO_2, starting at seedling emergence, a significantly higher tiller number as well as above-ground biomass and root biomass could also be found under increased CO_2 concentration. In addition, in fifteen out of 47 ILs tiller number was increased under high CO_2 compared to 'Scarlett', especially in S42IL-136 (12%) with an introgression on the long arm of chromosome 7H (data not shown). In contrast, a reduced tiller number betwenn 1.49 and 14.93% under control CO_2 was observed in eleven lines (S42IL-107, -108, -110, -121, -127, -129, -130, -137, -149, -153 and -161). For TN, only one QTL was identified in line S42IL-107 (chromosome 2H) which reduced tiller number compared to 'Scarlett' across treatments. This introgression line also revealed a reducing effect of the *Hsp* alleles for this trait under drought stress in a greenhouse experiment (Honsdorf et al. 2014a). An exotic *Ppd-H1* allele that affect early flowering have been localized in line S42IL-107 (Wang et al. 2010), which are also associated with reduced values for heading and plant height. The reduction of tiller number might be explained by a pleiotropic effect of this gene which reduces vegetative growth phase. Due to the positive correlation between rising atmospheric CO_2 concentration and global temperature increase, the use of lines with a short duration of the vegetative growth phase at elevated CO_2 concentration might be useful for plant breeding.

Plant height

As a rule, it is assumed that positive growth effects on C3 plants result from increased CO_2. Hence, it was unexpected that plant height of potatoes was unaffected (Lawson et al. 2001). An increase on plant height has been found, for example, in wheat (Mulholland et al. 1997), barley (Clausen et al. 2011) and tomato (Huang et al. 2012) under high CO_2. In this investigation mean plant height of juvenile barley plants under high CO_2 was reduced by 3.454 mm (Table 24). The reaction of many species to above-ground as well as underground biomass formation under elevated CO_2 is different (Madhu and Hatfield 2013). Often, a stronger increase in root growth and, therefore, better nutrient uptake is the consequence, which can influence other plant

traits and should be taken into account when interpreting the results. In this study, root biomass was not recorded. No QTL could be detected under the influence of elevated CO_2. However, two QTLs were identified at control CO_2 on chromosomes 2H and 4H with both a decreasing and an increasing effect on plant height, respectively. Plant height was reduced at QHei.S42IL-2H in line S42IL-108 across treatments and control CO_2. For this introgression line two flowering genes, *Ppd-H1* and *HvFT4,* were localized in this region of chromosome 2H (Wang et al. 2010). There, the exotic alleles were associated with QTL effects decreasing plant height. In contrast, S42IL-121 with a wild barley introgression on chromosome 4H showed a positive effect on plant height under control CO_2 which is also shown in hydroponic culture experiments both under reduced nitrogen nutrition (Hoffmann et al. 2012), phosphorus (Soleimani et al. 2017) and potassium deficiency (Soleimani et al., in prep). QTLs with a same effect on plant height in S42IL-121 were also found in a field study analyzing different agronomic traits (Schmalenbach et al. (2009) and greenhouse tests under drought stress (Honsdorf et al. 2014a). These results underline the observation that exotic *Hsp* alleles in this introgression line exert a stable increasing effect on plant height under different experimental stress conditions.

Shoot fresh weight and Shoot dry weight

Several studies with monocotyledonous (wheat, rice and barley) and dicotyledonous (potato, soybean) C3 plants repeatedly confirmed growth and yield improvements under elevated CO_2 conditions. An increase of photosynthetic capacity and a decrease of photorespiration are reactions of the plants to rising CO_2 (Asif et a. 2017). Already Farrar and Williams (1991) noted an increasing effect of rising CO_2 on carbon assimilation and carbohydrate accumulation. All photosynthetically active plants require the enzyme Rubisco for CO_2 fixation. Despite the reduction of the Rubisco activity by a long exposure to high CO_2, an increase of biomass was reported in C3 plants. This was also confirmed by Fangmeier et al. (2000), Plessl et al. (2005) and Clausen et al. (2011) on barley plants under the influence of rising CO_2 underlining our observations for shoot fresh and shoot dry weight with the barley introgression lines. The mean values for SFW and SDW under high CO_2 were higher with +18.1% and +5.3% compared to control CO_2 (Table 24).

The correlations between shoot fresh and shoot dry weight were high and positively significant (>0.9, Tables 25, 26). For SFW and SDW, each three QTLs were detected in five and four introgression lines, respectively. Only S42IL-122 (6H) and -135 (7H) were significantly associated with both SFW and SDW under control CO_2. For S42IL-135 under phosphorus

starvation (Soleimani et al. 2017) and for S42IL-136 under nitrogen deficiency (Hoffmann et al. 2012) an increasing effect on SDW was determined, too, which confirme our findings. Under high CO_2 concentration the only QTL with a negative *Hsp* effect was observed for shoot dry weight in line S42IL-124. After Wang et al. (2010) the gene *VRN-H2* is located on the long arm of chromosome 4H and revealed a significant reduction effect on plant height but an increase in heading. This gene might also influence the above-ground biomass reduction by a shortened vegetative growth phase in line S42IL-124, possibly in favour of the underground root biomass that was not determined (see plant height).

Anthocyanin formation

Tanaka et al. (2008) reported different major classes of plant pigments, including anthocyanins. Pigment biosynthesis in plants can be triggered by the influence of abiotic growth conditions (light, temperature) and stress (Albert et al. 2014). Martin et al. (2002) and Takatani et al. (2014) demonstrated the accumulation of anthocyanin *in Arabidopsis* in response to a changed C:N ratio due to a high CO_2 concentration which increases carbohydrate amount through an increased photosynthesis rate. Plant pigmentation is known as a protective mechanism and is involved in the regulation of plant hormonal balance, gene expression and enzyme activity (Tanaka et al. (2008). Paz-Ares et al. (1987) described several loci for enzymes, for example C1, involved in the anthocyanin biosynthesis in *Zea mays*. Based on a high homology, corresponding genes in barley and wheat were designated *HvC1* and *TaC1* (Himi and Taketa 2015). *HvC1,* located on chromosome 7HS, is considered a candidate gene for Ant1 (ant1 without pigmantion) due to a complete co-segregation of the anthocyanin pigmentation of the basal leaf sheath with the *HvC1* genotype. In addition, a *HvC1*-like gene was found on chromosome 4HL. The duplication of the *C1* gene in wild and cultivated barley can control pigmentation differently. The anthocyanin biosynthesis genes are activated via a complex of transcription factors (Albert et al. 2014). This acts through a hierarchical gene regulation network with enhancer and feedback mechanisms that enable gene regulation and, thus, anthocyanin accumulation. Anthocyanin synthesis occurs temporarily as a function of developmental stage and stress (Chalker-Scott 1999).

Under defined and reproducible conditions (eg. in climate chambers) plant genotypes can be studied comparatively. The two used experimental test types varied only in terms of CO_2 concentration. All other growth conditions such as light, temperature and irrigation were the same. In separate experiments under the same growth conditions (Sommer 2016) a chemical analysis confirmed that the introgression lines produced anthocyanin. Therefore, it could be assumed that anthocyanins were also produced in our experiments. The formation of

anthocyanins in plants involved in photoprotection varies strongly, also among species and seedlings produce abundant juvenile anthocyanins (Merzlyak et al. 2008). On average, plants grown under control CO_2 concentration produced slightly more anthocyanin (0.89) compared with plants under high CO_2 (0.72, Table 24). A total of three QTLs could be found in three ILs (S42IL-102, -124 and -176). All *Hsp* alleles reduced anthocyanin formation compared to 'Scarlett'. This reduction was highest in S42IL-176 with a wild introgression on the long arm of chromosome 5. Line S42IL-124 revealed a reducing effect of *Hsp* alleles under high CO_2. Possibly, this QTL in S42IL-124 corresponds to the *HvC1*-like gene decribed by Himi and Taketa (2015).

The impact of climate change on agricultural production is already apparent today, mainly due to changes in environmental conditions such as temperature, precipitation and atmospheric CO_2 concentration. With modern breeding methods, varieties have to be developed, which are adapted to the changing climate conditions. Crop yield prformance must be sustainably secured and increased. In the future, further knowledge in molecular biology will stimulate the development of new, adapted and improved varieties.

5 Summary

Nutrient deficiencies, but also a rising carbon dioxide concentration in the atmosphere are global stress factors that limit yield performance on today's crops. The most important task of plant breeding is the provision of suitable breeding material for plant production adapted to these stress conditions. In order to realize this goal, wild relatives of a corresponding crop plant, for example barley, are used. On their basis, wild barley introgression lines (ILs) are developed by introducing wild / exotic donor alleles into the elite barley gene pool. ILs are distinguished by small chromosomal segment of the exotic donor in the genetic background of adapted varieties. These introgressions can thus be analyzed without further negative effects typical for wild species and can then be used for the improvement of the variety trait expression.

In the present thesis, a same set of 47 wild barley introgression lines of the S42IL library and the control variety 'Scarlett' were examined for its reaction to both phosphorus and potassium deficiency as well as increased carbon dioxide concentration in the air during the first two weeks of the juvenile growth stage. Subsequently, quantitative trait loci (QTLs) were identified for selected morphological, nutrient concentration and stress index traits. For this reason, three different and independent studies in the greenhouse or a growth chamber were carried out at the Martin-Luther-University Halle-Wittenberg, Chair of Plant Breeding.

In the hydroculture experiments in the greenhouse the ILs and the control variety 'Scarlett' were analysed both under phosphorus or potassium deficiency compared to a standard nutrient solution as control. Twenty four introgression lines could be identified with the same *Hsp* allele effect for plant height, root length, shoot root length ratio and shoot root weight ratio under phosphorus and potassium deficiency. Both investigations revealed useful *Hsp* alleles for barley breeding.

A total of 91 independent QTLs were located among 39 ILs in the phosphorus deficiency studies, of which 64 QTLs displayed trait-improving *Hsp* effects. An unknown *Hsp* allele on barley chromosome 4H increased shoot dry weight under P deficiency in two overlapping ILs (S42IL-120, -121) by 25.9%. Likewise, an *Hsp* allele on barley chromosome 6H increased root dry weight under P deficiency in S42IL-129 by 27.6%.

Overall, 58 independent QTLs were detected among 36 ILs in the potassium deficiency experiments, of which 47 QTLs exhibited trait-improving *Hsp* effects. Here too, an unknown *Hsp* allele on barley chromosome 4H increased plant height in S42IL-121 by 20.9% and shoot root length ratio in further two overlapping ILs (S42IL-118, -119) by 22.6 and 20.7% under potassium deficiency. Finally, *Hsp* alleles in S42ILs-105 (chromosome 1H) and S42IL-126

(chromosome 5H) improved shoot root length ratio by 30.15 and 39.40% under control potassium treatment, respectively.

In growth chamber experiments the ILs were tested under 760 ppm (CO_2 stress) compared to 380 ppm (control treatment).
A total of twelve independent QTLs were located among 12 ILs, of which six QTLs showed trait-improving *Hsp* effects under control CO_2 concentration. No trait-improving effect could be detected under high CO_2 stress. QTLs were found for all five morphological traits but none for stress index traits. The most favorable *Hsp* allele effects (6 QTLs) were detected under control CO_2 treatment for shoot fresh and shoot dry weight. For both traits the greatest increase was observed for line S42IL-135 (chromosome 7H).

In eight (17.0%), eleven (23.4%) and 35 (74.5%) out of 47 introgression lines no exotic QTL allele could be detected in the phosphorus, potassium and CO_2 stress experiments, respectively. In introgression line S42IL-132 no exotic QTL allele was found in all three experiments. The introgression lines S42IL-102, -109, -121, -140 and -176 are characterized by a high number of exotic QTL alleles in both phosphorus (7) and potassium (4) deficiency studies. Under high CO_2 the highest number with two QTLs were found in three ILs (S42IL-122, -124 and -135). Only line S42IL-121 (introgression on 4H) was detected for plant height in all three experiments. This line has been observed several times by a similar behavior in previous greenhouse and field trials and could, therefore, be a valuable source for barley breeding.

In total, about 31 QTLs confirmed *Hsp* effects already identified in previous hydroculture, greenhouse and field experiments with the same set of introgression lines. Wild barley contains numerous trait-improving QTL alleles, which are active both under phosphorus and potassium deficiency and increased CO_2 concentration. Introgression lines with interesting traits should be investigated under field condition to determine whether these results can be confirmed under natural plant cultivation and production. If this is the case, these ILs and the underlying genes can be subjected to cloning /crossings and, simultaneously, used in elite barley breeding to improve new varieties.

6 Zusammenfassung

Nährstoffmangel, aber auch steigende Kohlendioxidkonzentrationen in der Atmosphäre sind weltweit wirkende Stressfaktoren, die die Ertragsleistung der heutigen Nutzpflanzen begrenzen. Die wichtigste Aufgabe der Pflanzenzüchtung ist die Bereitstellung von geeigneten Zuchtmaterial für die Pflanzenproduktion, welche an die entsprechenden Stressbedingungen angepasst sind. Um dieses Ziel zu erreichen, werden Wildarten aus dem Verwandschaftskreis einer Kulturart verwendet. Auf ihrer Basis werden Introgressionslinien (ILs) durch die Einführung von wilden / exotischen Spenderallelen in den Elitegenpool dieser Kulturpflanze entwickelt. ILs zeichnen sich durch ein kleines Chromosomenstück des exotischen Spenders im genetischen Hintergrund der angepassten Sorten aus. Diese Introgressionen können somit ohne weitere, für Wildarten typische negative Effekte analysiert und anschließend zur Verbesserung der Merkmalsexpression neuer Sorten verwendet werden.

In dieser Doktorarbeit wurde ein gleichbleibendes Set aus 47 Gersteintrogressionslinien der S42IL-Bibliothek und die Kontrollsorte ‚Scarlett' auf ihre Reaktion auf Phosphor- bzw. Kaliummangel, aber auch auf erhöhte Kohlendioxidkonzentration in der Luft während der ersten beiden Wochen der juvenilen Wachstumsphase untersucht. Anschließend wurde versucht, QTLs (quantitative trait loci) für ausgewählte morphologische, Nährstoffkonzentrations- und Stressindexmerkmale zu finden. Dazu wurden an der Martin-Luther-Universität Halle-Wittenberg an der Professur für Pflanzenzüchtung drei verschiedene und unabhängige Untersuchungen im Gewächshaus bzw. einer Klimakammer durchgeführt.

Die ILs und die Kontrollsorte "Scarlett" wurden gemeinsam sowohl unter Phosphor- als auch unter Kaliummangel im Vergleich zu einer Standardnährlösung als Kontrolle in Hydrokultur unter Gewächshausbedingungen kultiviert. Vierundzwanzig Introgressionslinien konnten mit dem gleichen *Hsp*-Alleleffekt für Pflanzenhöhe, Wurzellänge, Halmwurzellängenverhältnis und Halmwurzelgewichtsverhältnis sowhl unter Phosphor- als auch Kaliummangel identifiziert werden. In beiden Untersuchungen wurden vorteilhafte *Hsp*-Allele für die Gerstezüchtung gefunden.

In den Phosphormangel-Experimenten konnten insgesamt 91 unabhängige QTLs in 39 ILs ermittelt werden. 64 QTLs zeigten verbesserte *Hsp*-Alleleffekte. Ein unbekanntes *Hsp*-Allel auf dem Gerstenchromosom 4H erhöhte das oberirdische Trockengewicht unter Phorphormangel in zwei ILs mit sich überlappenden Introgressionen (S42IL-120, -121) um 25,9%. Außerdem ist ein *Hsp*-Allel auf dem Gerstenchromosom 6H für eine Erhöhung des Wurzeltrockengewichtes unter Phorphormangel in der Linie S42IL-129 um 27,6% verantwortlich.

In den Kaliummangel-Experimenten wurden insgesamt 58 unabhängige QTLs in 36 ILs erkannt, von denen 47 QTLs für verbesserte *Hsp*-Effekte verantwortlich sind. Wie beim Phosphormangel erhöhte ein unbekanntes *Hsp*-Allel auf dem Gerstenchromosom 4H auch beim Kaliummangel die Pflanzenlämge in S42IL-121 um 20,9% und das Halmwurzellängenverhältnis bei zwei weiteren ILs mit sich überlappenden Introgressionen (S42IL-118, -119) um 22,6 und 20,7%. Dagegen verbesserten *Hsp*-Allele in den Linien S42ILs-105 (Chromosom 1H) und S42IL-126 (Chromosom 5H) das Halmwurzellängenverhältnis um 30,15 bzw. 39,40% unter Kontrollbedingungen.

In den CO_2-Klimakammerversuchen wurden die ILs sowohl unter 760 ppm (CO_2 Stress) als auch unter 380 ppm (Kontrolle) untersucht. Insgesamt zwölf unabhängige QTLs konnten bei zwölf ILs festgestellt werden. Sechs dieser zwölf QTLs zeigten positive *Hsp*-Effekte unter Kontrolbedingungen. Unter Stressbedingungen war kein positver Effekt feststellbar. QTLs wurden für die fünf analysierten morphologischen Merkmale, nicht aber für die Stress-Index-Merkmale beobachtet. Die besten *Hsp*-Alleleffekte (6 QTLs) wurden beim Frisch- und Trockengewicht unter Kontrollbedingungen gefunden. Linie S42IL-135 (Chromosom 7H) zeigte die beste Merkmalsausprägung.

In acht (17,0%), elf (23,4%) und 35 (74,5%) von 47 Introgressionslinien konnte kein QTL in den Phosphor, Kalium- und CO_2-Stressexperimenten nachgewiesen werden. Die Introgressionslinien S42IL-102, -109, -121, -140 und -176 zeichneten sich durch eine hohe Anzahl exotischer QTLs aus. Bei den Phosphor- und Kaliumangeluntersuchungen waren das maximal sieben bzw. vier. Unter hohem CO_2 betrug die höchste Anzahl zwei QTLs in drei ILs (S42IL-122, -124 und -135). S42IL-121 (*Hsp*-Introgresion auf 4H) war die einzige Linie, die in allen drei Experimenten QTLs für die Pflanzenhöhe hatte. Diese Introgressionslinie fiel mehrfach durch ein ähnliches Verhalten in früheren Gewächshaus- und Feldversuchen auf und könnte daher eine wertvolle Quelle für die zukünftige Gerstezüchtung sein.

Es konnte gezeigt werden, dass 31 QTLs mit solchen aus früheren Hydrokultur-, Gewächshaus- und Feldversuchen auf Basis des gleichen Sets an Introgressionslinien übereinstimmen. Wildgerste enthält zahlreiche merkmalsverbessernde QTL-Allele, die sowohl unter Phosphor- und Kaliummangel als auch erhöhter CO_2-Konzentration aktiv sind. Introgressionslinien mit interessanten Merkmalen sollten unter Feldbedingungen geprüft werden, um zu klären, ob diese Ergebnisse unter Praxisbedingungen bestätigt werden können. Sollte dies der Fall sein, könnten deren QTLs, kloniert oder eingekreuzt, zur Züchtung neuer, verbesserter Sorten herangezogen werden.

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8 List of figures

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10 Abbreviations

Abbreviations	Explanation
AC	Anthocyanin
ALL	Effect across treatments
ANOVA	Analysis of variance
BAC	Bacterial artificial chromosome
BC_2DH	Backcross two, doubled haploid
BC_3S_4	Backcross three, selfing four
BMD	biomass
Btr - brt	Brittle rachis, shattering – non-brittle rachis
C	Carbon
Ca, Ca^{2+}	Calcium, calcium ion
CaCR / CaCS	Calcium concentration root / shoot
CCR / CCS	Carbon concentration root / shoot
cDNA	Copy desoxyribonucleic acid
Chr.	Chromosome
con	Control treatment
CO_2	Carbon dioxide
CuCR / CuCS	Copper concentration root / shoot
CV	Coefficient of variance
Diff.	Difference
DNA	Desoxyribonucleic acid
DW	Dry weight
EC	Electric conductivity
EL	Elemental analyzer
EST	Expressend sequence tags
FeCR / FeCS	Iron concentration root / shoot
Gb – Gbp	Giga base - giga base pairs
H	hydrogen
H.	*Hordeum*
H CO_2	Increased carbon dioxide, 760 ppm
HNO_3	Nitric acid
HPO_4^{2-}, $H_2PO_4^-$	Phosphorus ions
HSI	Hyperspectral imaging
HEI	Plant height
Hsp	*Hordeum vulgare* ssp. *spontaneum*
Hv	Hordeum vulgare ssp. *vulgare*
h^2	heritability
H_2O	Water
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
IBGSC	International Barley Genome Sequencing Consortium
ICP-OES	Inductively coupled plasma - optical emission spectrometry
IFF	Fraunhofer Institute for Factory Operation and Automation Magdeburg
IL	Introgression line
IPCC	Intergovernmental Panel on Climate Change
IPK	Leibniz-Institute of Plant Genetics and Crop Plant Research Gatersleben
K, K^+	Potassium, potassium ion
KCR (KCS	Potassium concentration root / shoot
KUE	Potassium utilization efficiency
K_2O	Potassium oxide
K0	Potassium reduction in nutrition solution
K100	Control potassium nutrition solution
L.	Linne
LSmeans	Least square means
MAS	Marker-assisted selection
Mg	Magnesium

MgCR / MgCS	Magnesium concentration root / shoot
Mlo	Resistance to barley powdery mildew
MnCR / MnCS	Manganese concentration root / shoot
mRNA	Messenger ribonucleic acid
MT	Morphological traits
Mt	Million tons
N	Number of observations
N	Nitrogen
NaCR / NaCS	Sodium concentration root / shoot
NAE	Nutrient acquisition efficiency
N CO_2	Control carbon dioxide, 380 ppm
NCR / NCS	Nitrogen concentration root / shoot
NCT	Nutrient concentration traits
NGS	Next generation sequencing
NIRS	Near-infrared spectroscopy
nm	nanometer
NUE	Nutrient use efficiency
NUtE	Nutrient utilization efficiency
O	Oxygen
P	Phosphorus
PCR / PCS	Phosphorus concentration root / shoot
Pi	Phosphate
PSR	Phosphate starvation responce
PUE	Phosphate use efficiency
PUP	Phosphate uptake
P10	Phosphorus reduction in nutrition solution, 10 µM phosphate
P100	Control phosphorus nutrition solution, 100 µM phosphate
ppm	Part per million
QTL	Quantitative trait locus
RL	Root length
RDW	Root dry weight
ROS	Reactive osygen species
RP	Relative performance
Rym	Resistance to yellow mosaic virus
S	Stress treatment
S	Sulphur
SCR / SCS	Sulphur concentration root / shoot
SD	Standard deviation
SDW	Shoot dry weight
SFP	Single-feature polymorphism
SFW	Shoot fresh weight
SI	Stress index of a certain trait
SNP	Single nucleotide polymorphisms
SRLR	Shoot root length ratio
SRWR	Shoot toot weight ratio
SSR	Simple sequence repeat or mircrosateliite
T_{con}	Mean trait performances of an IL under control condition
T_s	Mean trait performances of an IL under stress condition
TN	Tiller number
Vrn - vrn	Two-rowed barley – six-rowed barley
ZnCR / ZnCS	Zinc concentration root / shoot

11 Supporting Information

Table S1, Supporting Information: Results of Mixed model analysis for 8 morphological, 24 nutrient concentration and 30 stress index traits, calculated across (ALL) and within treatments (P10 or P100).

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
1	All	TN	Genotype	47.0	1621.0	2.16	0.000	<0.05
1	All	TN	Experiment	3.0	1621.0	16.49	0.000	<0.05
1	All	TN	Treatment	1.0	7.0	25.16	0.001	<0.05
1	All	TN	Experiment*Genotype	141.0	1621.0	1.00	0.487	
1	All	TN	Genotype*Treatment	47.0	1621.0	2.54	0.000	<0.05
1	All	HEI	Genotype	47.0	1566.0	11.45	0.000	<0.05
1	All	HEI	Experiment	3.0	1566.0	64.56	0.000	<0.05
1	All	HEI	Treatment	1.0	7.0	95.07	0.000	<0.05
1	All	HEI	Experiment*Genotype	141.0	1566.0	1.35	0.005	<0.05
1	All	HEI	Genotype*Treatment	47.0	1566.0	1.35	0.060	
1	All	SDW	Genotype	47.0	1579.0	5.33	0.000	<0.05
1	All	SDW	Experiment	3.0	1579.0	72.95	0.000	<0.05
1	All	SDW	Treatment	1.0	7.0	107.95	0.000	<0.05
1	All	SDW	Experiment*Genotype	141.0	1579.0	1.76	0.000	<0.05
1	All	SDW	Genotype*Treatment	47.0	1579.0	1.64	0.004	<0.05
1	All	RL	Genotype	47.0	1591.0	8.23	0.000	<0.05
1	All	RL	Experiment	3.0	1591.0	7.70	0.000	<0.05
1	All	RL	Treatment	1.0	7.0	1.95	0.205	
1	All	RL	Experiment*Genotype	141.0	1591.0	1.08	0.261	
1	All	RL	Genotype*Treatment	47.0	1591.0	1.23	0.140	
1	All	RDW	Genotype	47.0	1586.0	7.26	0.000	<0.05
1	All	RDW	Experiment	3.0	1586.0	133.01	0.000	<0.05
1	All	RDW	Treatment	1.0	7.0	6.31	0.040	<0.05
1	All	RDW	Experiment*Genotype	141.0	1586.0	2.02	0.000	<0.05
1	All	RDW	Genotype*Treatment	47.0	1586.0	1.44	0.028	<0.05
1	All	SRLR	Genotype	47.0	1419.0	7.52	0.000	<0.05
1	All	SRLR	Experiment	3.0	1419.0	16.28	0.000	<0.05
1	All	SRLR	Treatment	1.0	7.0	13.83	0.007	<0.05
1	All	SRLR	Experiment*Genotype	141.0	1419.0	1.16	0.106	
1	All	SRLR	Genotype*Treatment	47.0	1419.0	1.75	0.001	<0.05
1	All	SRWR	Genotype	47.0	1484.0	11.55	0.000	<0.05
1	All	SRWR	Experiment	3.0	1484.0	88.67	0.000	<0.05
1	All	SRWR	Treatment	1.0	7.0	40.13	0.000	<0.05
1	All	SRWR	Experiment*Genotype	141.0	1484.0	1.09	0.222	
1	All	SRWR	Genotype*Treatment	47.0	1484.0	2.16	0.000	<0.05
1	All	BMD	Genotype	47.0	1545.0	5.74	0.000	<0.05
1	All	BMD	Experiment	3.0	1545.0	94.09	0.000	<0.05
1	All	BMD	Treatment	1.0	7.0	58.12	0.00	<0.05

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
1	All	BMD	Experiment*Genotype	141.0	1545.0	1.89	0.000	<0.05
1	All	BMD	Genotype*Treatment	47.0	1545.0	1.65	0.004	<0.05
2	P10	TN	Experiment	3.0	708.0	6.77	0.000	<0.05
2	P10	TN	Genotype	47.0	708.0	8.12	0.000	<0.05
2	P10	TN	Experiment*Genotype	141.0	708.0	0.89	0.805	
2	P10	HEI	Experiment	3.0	708.0	6.77	0.000	<0.05
2	P10	HEI	Genotype	47.0	708.0	8.12	0.000	<0.05
2	P10	HEI	Experiment*Genotype	141.0	708.0	0.89	0.805	
2	P10	SDW	Experiment	3.0	716.0	159.92	0.000	<0.05
2	P10	SDW	Genotype	47.0	716.0	3.61	0.000	<0.05
2	P10	SDW	Experiment*Genotype	141.0	716.0	1.17	0.104	
2	P10	RL	Experiment	3.0	720.0	27.52	0.000	<0.05
2	P10	RL	Genotype	47.0	720.0	7.33	0.000	<0.05
2	P10	RL	Experiment*Genotype	141.0	720.0	1.07	0.290	
2	P10	RDW	Experiment	3.0	722.0	125.89	0.000	<0.05
2	P10	RDW	Genotype	47.0	722.0	4.30	0.000	<0.05
2	P10	RDW	Experiment*Genotype	141.0	722.0	1.27	0.026	<0.05
2	P10	SRLR	Experiment	3.0	632.0	27.34	0.000	<0.05
2	P10	SRLR	Genotype	47.0	632.0	6.25	0.000	<0.05
2	P10	SRLR	Experiment*Genotype	141.0	632.0	1.12	0.188	
2	P10	SRWR	Experiment	3.0	672.0	35.36	0.000	<0.05
2	P10	SRWR	Genotype	47.0	672.0	15.26	0.000	<0.05
2	P10	SRWR	Experiment*Genotype	141.0	672.0	0.94	0.671	
2	P10	BMD	Experiment	3.0	697.0	145.10	0.000	<0.05
2	P10	BMD	Genotype	47.0	697.0	3.41	0.000	<0.05
2	P10	BMD	Experiment*Genotype	141.0	697.0	1.20	0.076	
2	P100	TN	Experiment	3.0	717.0	17.14	0.000	<0.05
2	P100	TN	Genotype	47.0	717.0	2.09	0.000	<0.05
2	P100	TN	Experiment*Genotype	141.0	717.0	1.02	0.427	
2	P100	HEI	Experiment	3.0	711.0	70.70	0.000	<0.05
2	P100	HEI	Genotype	47.0	711.0	5.85	0.000	<0.05
2	P100	HEI	Experiment*Genotype	141.0	711.0	1.25	0.039	<0.05
2	P100	SDW	Experiment	3.0	716.0	15.31	0.000	<0.05
2	P100	SDW	Genotype	47.0	716.0	3.17	0.000	<0.05
2	P100	SDW	Experiment*Genotype	141.0	716.0	1.23	0.046	<0.05
2	P100	RL	Experiment	3.0	724.0	36.45	0.000	<0.05
2	P100	RL	Genotype	47.0	724.0	3.10	0.000	<0.05
2	P100	RL	Experiment*Genotype	141.0	724.0	0.87	0.849	
2	P100	RDW	Experiment	3.0	717.0	33.81	0.000	<0.05
2	P100	RDW	Genotype	47.0	717.0	3.90	0.000	<0.05
2	P100	RDW	Experiment*Genotype	141.0	717.0	1.25	0.034	<0.05
2	P100	SRLR	Experiment	3.0	640.0	10.09	0.000	<0.05
2	P100	SRLR	Genotype	47.0	640.0	3.65	0.000	<0.05
2	P100	SRLR	Experiment*Genotype	141.0	640.0	1.07	0.301	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
2	P100	SRWR	Experiment	3.0	665.0	64.86	0.000	<0.05
2	P100	SRWR	Genotype	47.0	665.0	4.37	0.000	<0.05
2	P100	SRWR	Experiment*Genotype	141.0	665.0	1.18	0.089	
2	P100	BMD	Experiment	3.0	701.0	17.97	0.000	<0.05
2	P100	BMD	Genotype	47.0	701.0	3.44	0.000	<0.05
2	P100	BMD	Experiment*Genotype	141.0	701.0	1.27	0.028	<0.05
3	ALL	CaCR	Genotype	47.0	274.0	0.94	0.590	
3	ALL	CaCR	Experiment	1.0	274.0	15.06	0.000	<0.05
3	ALL	CaCR	Treatment	1.0	274.0	18.76	0.000	<0.05
3	ALL	CaCR	Genotype*Treatment	47.0	274.0	0.80	0.826	
3	ALL	CCR	Genotype	47.0	255.0	0.95	0.564	
3	ALL	CCR	Experiment	1.0	255.0	30.65	0.000	<0.05
3	ALL	CCR	Treatment	1.0	255.0	17.13	0.000	<0.05
3	ALL	CCR	Genotype*Treatment	47.0	255.0	0.76	0.871	
3	ALL	CuCR	Genotype	47.0	273.0	0.89	0.677	
3	ALL	CuCR	Experiment	1.0	273.0	8.95	0.003	<0.05
3	ALL	CuCR	Treatment	1.0	273.0	4.42	0.036	<0.05
3	ALL	CuCR	Genotype*Treatment	47.0	273.0	0.63	0.972	
3	ALL	FeCR	Genotype	47.0	272.0	0.95	0.577	
3	ALL	FeCR	Experiment	1.0	272.0	176.50	0.000	<0.05
3	ALL	FeCR	Treatment	1.0	272.0	59.38	0.000	<0.05
3	ALL	FeCR	Genotype*Treatment	47.0	272.0	0.56	0.991	
3	ALL	KCR	Genotype	47.0	268.0	1.17	0.225	
3	ALL	KCR	Experiment	1.0	268.0	419.83	0.000	<0.05
3	ALL	KCR	Treatment	1.0	268.0	293.80	0.000	<0.05
3	ALL	KCR	Genotype*Treatment	47.0	268.0	1.07	0.363	
3	ALL	MgCR	Genotype	47.0	276.0	1.31	0.098	
3	ALL	MgCR	Experiment	1.0	276.0	0.05	0.831	
3	ALL	MgCR	Treatment	1.0	276.0	13.53	0.000	<0.05
3	ALL	MgCR	Genotype*Treatment	47.0	276.0	0.72	0.911	
3	ALL	MnCR	Genotype	47.0	269.0	1.22	0.170	
3	ALL	MnCR	Experiment	1.0	269.0	216.06	0.000	<0.05
3	ALL	MnCR	Treatment	1.0	269.0	286.93	0.000	<0.05
3	ALL	MnCR	Genotype*Treatment	47.0	269.0	0.80	0.814	
3	ALL	NaCR	Genotype	47.0	271.0	1.30	0.102	
3	ALL	NaCR	Experiment	1.0	271.0	99.37	0.000	<0.05
3	ALL	NaCR	Treatment	1.0	271.0	167.54	0.000	<0.05
3	ALL	NaCR	Genotype*Treatment	47.0	271.0	0.95	0.571	
3	ALL	NCR	Genotype	47.0	259.0	0.40	0.999	
3	ALL	NCR	Experiment	1.0	259.0	131.73	0.000	<0.05
3	ALL	NCR	Treatment	1.0	259.0	770.05	0.000	<0.05
3	ALL	NCR	Genotype*Treatment	47.0	259.0	0.39	0.999	
3	ALL	PCR	Genotype	47.0	273.0	1.35	0.073	
3	ALL	PCR	Experiment	1.0	273.0	121.55	0.000	<0.05

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
3	ALL	PCR	Treatment	1.0	273.0	344.52	0.000	<0.05
3	ALL	PCR	Genotype*Treatment	47.0	273.0	0.82	0.787	
3	ALL	SCR	Genotype	47.0	273.0	1.33	0.084	
3	ALL	SCR	Experiment	1.0	273.0	50.14	0.000	<0.05
3	ALL	SCR	Treatment	1.0	273.0	496.27	0.000	<0.05
3	ALL	SCR	Genotype*Treatment	47.0	273.0	0.96	0.557	
3	ALL	ZnCR	Genotype	47.0	272.0	1.41	0.050	<0.05
3	ALL	ZnCR	Experiment	1.0	272.0	0.01	0.932	
3	ALL	ZnCR	Treatment	1.0	272.0	8.57	0.003	<0.05
3	ALL	ZnCR	Genotype*Treatment	47.0	272.0	1.01	0.458	
3	ALL	CaCS	Genotype	47.0	269.0	1.00	0.479	
3	ALL	CaCS	Experiment	1.0	269.0	0.03	0.864	
3	ALL	CaCS	Treatment	1.0	269.0	16.47	0.000	<0.05
3	ALL	CaCS	Genotype*Treatment	47.0	269.0	1.10	0.319	
3	ALL	CCS	Genotype	47.0	265.0	1.29	0.109	
3	ALL	CCS	Experiment	1.0	265.0	85.52	0.000	<0.05
3	ALL	CCS	Treatment	1.0	265.0	2.95	0.087	
3	ALL	CCS	Genotype*Treatment	47.0	265.0	0.45	0.999	
3	ALL	CuCS	Genotype	47.0	271.0	1.18	0.215	
3	ALL	CuCS	Experiment	1.0	271.0	1.58	0.209	
3	ALL	CuCS	Treatment	1.0	271.0	685.38	0.000	<0.05
3	ALL	CuCS	Genotype*Treatment	47.0	271.0	0.68	0.942	
3	ALL	FeCS	Genotype	47.0	265.0	1.09	0.336	
3	ALL	FeCS	Experiment	1.0	265.0	12.57	0.000	<0.05
3	ALL	FeCS	Treatment	1.0	265.0	99.70	0.000	<0.05
3	ALL	FeCS	Genotype*Treatment	47.0	265.0	1.17	0.220	
3	ALL	KCS	Genotype	47.0	261.0	0.85	0.750	
3	ALL	KCS	Experiment	1.0	261.0	10.42	0.001	<0.05
3	ALL	KCS	Treatment	1.0	261.0	128.97	0.000	<0.05
3	ALL	KCS	Genotype*Treatment	47.0	261.0	0.87	0.712	
3	ALL	MgCS	Genotype	47.0	265.0	1.00	0.487	
3	ALL	MgCS	Experiment	1.0	265.0	0.01	0.907	
3	ALL	MgCS	Treatment	1.0	265.0	66.27	0.000	<0.05
3	ALL	MgCS	Genotype*Treatment	47.0	265.0	0.69	0.938	
3	ALL	MnCS	Genotype	47.0	266.0	1.10	0.317	
3	ALL	MnCS	Experiment	1.0	266.0	6.55	0.011	<0.05
3	ALL	MnCS	Treatment	1.0	266.0	19.79	0.000	<0.05
3	ALL	MnCS	Genotype*Treatment	47.0	266.0	1.25	0.145	
3	ALL	NaCS	Genotype	47.0	265.0	1.40	0.052	
3	ALL	NaCS	Experiment	1.0	265.0	1.44	0.231	
3	ALL	NaCS	Treatment	1.0	265.0	227.65	0.000	<0.05
3	ALL	NaCS	Genotype*Treatment	47.0	265.0	1.02	0.449	
3	ALL	NCS	Genotype	47.0	267.0	0.83	0.772	
3	ALL	NCS	Experiment	1.0	267.0	34.34	0.000	<0.05

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
3	ALL	NCS	Treatment	1.0	267.0	1344.87	0.000	<0.05
3	ALL	NCS	Genotype*Treatment	47.0	267.0	0.38	0.999	
3	ALL	PCS	Genotype	47.0	266.0	1.34	0.078	
3	ALL	PCS	Experiment	1.0	266.0	0.91	0.341	
3	ALL	PCS	Treatment	1.0	266.0	22.45	0.000	<0.05
3	ALL	PCS	Genotype*Treatment	47.0	266.0	1.06	0.374	
3	ALL	SCS	Genotype	47.0	270.0	0.84	0.758	
3	ALL	SCS	Experiment	1.0	270.0	48.58	0.000	<0.05
3	ALL	SCS	Treatment	1.0	270.0	71.96	0.000	<0.05
3	ALL	SCS	Genotype*Treatment	47.0	270.0	0.45	0.999	
3	ALL	ZnCS	Genotype	47.0	268.0	1.24	0.151	
3	ALL	ZnCS	Experiment	1.0	268.0	12.17	0.000	<0.05
3	ALL	ZnCS	Treatment	1.0	268.0	397.64	0.000	<0.05
3	ALL	ZnCS	Genotype*Treatment	47.0	268.0	1.01	0.454	
4	P10	CaCR	Genotype	47.0	137.0	0.82	0.777	
4	P10	CCR	Genotype	47.0	131.0	0.74	0.878	
4	P10	CuCR	Genotype	47.0	137.0	0.85	0.732	
4	P10	FeCR	Genotype	47.0	137.0	0.42	0.999	
4	P10	KCR	Genotype	47.0	135.0	0.40	0.999	
4	P10	MgCR	Genotype	47.0	140.0	0.96	0.557	
4	P10	MnCR	Genotype	47.0	135.0	0.44	0.999	
4	P10	NaCR	Genotype	47.0	134.0	0.50	0.996	
4	P10	NCR	Genotype	47.0	138.0	0.38	0.999	
4	P10	PCR	Genotype	47.0	137.0	0.45	0.998	
4	P10	SCR	Genotype	47.0	133.0	0.76	0.862	
4	P10	ZnCR	Genotype	47.0	135.0	0.82	0.785	
4	P10	CaCS	Genotype	47.0	137.0	1.16	0.252	
4	P10	CCS	Genotype	47.0	133.0	0.80	0.806	
4	P10	CuCS	Genotype	47.0	137.0	1.06	0.389	
4	P10	FeCS	Genotype	47.0	133.0	1.36	0.087	
4	P10	KCS	Genotype	47.0	128.0	1.02	0.448	
4	P10	MgCS	Genotype	47.0	132.0	0.91	0.636	
4	P10	MnCS	Genotype	47.0	134.0	1.19	0.218	
4	P10	NaCS	Genotype	47.0	131.0	1.72	0.009	<0.05
4	P10	NCS	Genotype	47.0	136.0	0.66	0.947	
4	P10	PCS	Genotype	47.0	135.0	1.29	0.129	
4	P10	SCS	Genotype	47.0	134.0	0.50	0.996	
4	P10	ZnCS	Genotype	47.0	137.0	1.09	0.346	
4	P100	CaCR	Genotype	47.0	138.0	0.85	0.739	
4	P100	CCR	Genotype	47.0	125.0	0.73	0.890	
4	P100	CuCR	Genotype	47.0	137.0	0.62	0.968	
4	P100	FeCR	Genotype	47.0	136.0	0.61	0.973	
4	P100	KCR	Genotype	47.0	134.0	0.54	0.992	
4	P100	MgCR	Genotype	47.0	137.0	1.11	0.318	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
4	P100	MnCR	Genotype	47.0	135.0	0.67	0.939	
4	P100	NaCR	Genotype	47.0	138.0	1.10	0.325	
4	P100	NCR	Genotype	47.0	122.0	0.25	1.000	
4	P100	PCR	Genotype	47.0	137.0	1.08	0.362	
4	P100	SCR	Genotype	47.0	141.0	1.10	0.324	
4	P100	ZnCR	Genotype	47.0	138.0	1.64	0.014	<0.05
4	P100	CaCS	Genotype	47.0	133.0	0.93	0.599	
4	P100	CCS	Genotype	47.0	133.0	0.63	0.962	
4	P100	CuCS	Genotype	47.0	135.0	0.82	0.782	
4	P100	FeCS	Genotype	47.0	133.0	0.73	0.889	
4	P100	KCS	Genotype	47.0	134.0	0.71	0.911	
4	P100	MgCS	Genotype	47.0	134.0	0.75	0.874	
4	P100	MnCS	Genotype	47.0	133.0	1.03	0.432	
4	P100	NaCS	Genotype	47.0	135.0	0.91	0.639	
4	P100	NCS	Genotype	47.0	132.0	0.42	0.999	
4	P100	PCS	Genotype	47.0	132.0	1.20	0.205	
4	P100	SCS	Genotype	47.0	137.0	0.71	0.914	
4	P100	ZnCS	Genotype	47.0	132.0	1.14	0.283	
2	ALL	TN_SI	Experiment	3.0	714.0	9.88	0.000	<0.05
2	ALL	TN_SI	Genotype	47.0	714.0	2.22	0.000	<0.05
2	ALL	TN_SI	Experiment*Genotype	141.0	714.0	1.18	0.093	
2	ALL	HEI_SI	Experiment	3.0	669.0	25.76	0.000	<0.05
2	ALL	HEI_SI	Genotype	47.0	669.0	1.35	0.061	
2	ALL	HEI_SI	Experiment*Genotype	141.0	669.0	0.87	0.839	
2	ALL	SDW_SI	Experiment	3.0	684.0	20.47	0.000	<0.05
2	ALL	SDW_SI	Genotype	47.0	684.0	1.76	0.001	<0.05
2	ALL	SDW_SI	Experiment*Genotype	141.0	684.0	0.86	0.869	
2	ALL	RL_SI	Experiment	3.0	688.0	39.86	0.000	<0.05
2	ALL	RL_SI	Genotype	47.0	688.0	1.46	0.025	<0.05
2	ALL	RL_SI	Experiment*Genotype	141.0	688.0	1.01	0.455	
2	ALL	RDW_SI	Experiment	3.0	686.0	9.71	0.000	<0.05
2	ALL	RDW_SI	Genotype	47.0	686.0	1.66	0.004	<0.05
2	ALL	RDW_SI	Experiment*Genotype	141.0	686.0	0.81	0.941	
2	ALL	BMD_SI	Experiment	3.0	653.0	17.42	0.000	<0.05
2	ALL	BMD_SI	Genotype	47.0	653.0	1.77	0.001	<0.05
2	ALL	BMD_SI	Experiment*Genotype	141.0	653.0	0.83	0.908	
4	ALL	CR_SI	Genotype	47.0	114.0	0.73	0.8923	
4	ALL	CS_SI	Genotype	47.0	123.0	0.67	0.943	
4	ALL	NR_SI	Genotype	47.0	117.0	0.43	0.999	
4	ALL	NS_SI	Genotype	47.0	124.0	0.86	0.725	
4	ALL	CaR_SI	Genotype	47.0	132.0	1.01	0.471	
4	ALL	CuR_SI	Genotype	47.0	130.0	0.73	0.889	
4	ALL	FeR_SI	Genotype	47.0	130.0	0.70	0.917	
4	ALL	KR_SI	Genotype	47.0	126.0	1.01	0.475	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
4	ALL	MgR_SI	Genotype	47.0	133.0	1.00	0.489	
4	ALL	MnR_SI	Genotype	47.0	126.0	0.67	0.944	
4	ALL	NaR_SI	Genotype	47.0	128.0	0.98	0.518	
4	ALL	PR_SI	Genotype	47.0	130.0	0.75	0.869	
4	ALL	SR_SI	Genotype	47.0	130.0	1.04	0.419	
4	ALL	ZnR_SI	Genotype	47.0	129.0	1.28	0.141	
4	ALL	CaS_SI	Genotype	47.0	126.0	1.40	0.074	
4	ALL	CuS_SI	Genotype	47.0	130.0	0.62	0.970	
4	ALL	FeS_SI	Genotype	47.0	122.0	0.98	0.513	
4	ALL	KS_SI	Genotype	47.0	119.0	0.69	0.923	
4	ALL	MgS_SI	Genotype	47.0	123.0	0.90	0.654	
4	ALL	MnS_SI	Genotype	47.0	123.0	1.21	0.204	
4	ALL	NaS_SI	Genotype	47.0	123.0	0.95	0.563	
4	ALL	PS_SI	Genotype	47.0	125.0	1.11	0.318	
4	ALL	SS_SI	Genotype	47.0	129.0	0.50	0.995	
4	ALL	ZnS_SI	Genotype	47.0	126.0	1.23	0.182	

Table S2, Supporting Information: Results of MIXED model analysis for 8 morphological, 24 nutrient concentration nd 30 stress index traits, calculated across (ALL) and within treatments (K0 or K100).

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
1	All	TN	Genotype	47.0	1900.0	3.58	0.000	<0.05
1	All	TN	Experiment	4.0	1900.0	179.25	0.000	<0.05
1	All	TN	Treatment	1.0	4.0	9.20	0.039	<0.05
1	All	TN	Experiment*Genotype	188.0	1900.0	2.08	0.000	<0.05
1	All	TN	Treatment*Genotype	47.0	1900.0	1.69	0.003	<0.05
1	All	HEI	Genotype	47.0	1897.0	8.94	0.000	<0.05
1	All	HEI	Experiment	4.0	1897.0	149.99	0.000	<0.05
1	All	HEI	Treatment	1.0	4.0	0.64	0.469	
1	All	HEI	Experiment*Genotype	188.0	1897.0	1.71	0.000	<0.05
1	All	HEI	Treatment*Genotype	47.0	1897.0	1.23	0.138	
1	All	SDW	Genotype	47.0	1877.0	4.98	0.000	<0.05
1	All	SDW	Experiment	4.0	1877.0	178.64	0.000	<0.05
1	All	SDW	Treatment	1.0	4.0	15.86	0.016	<0.05
1	All	SDW	Experiment*Genotype	188.0	1877.0	1.70	0.000	<0.05
1	All	SDW	Treatment*Genotype	47.0	1877.0	0.76	0.879	
1	All	RL	Genotype	47.0	1911.0	6.33	0.000	<0.05
1	All	RL	Experiment	4.0	1911.0	87.28	0.000	<0.05
1	All	RL	Treatment	1.0	4.0	5.53	0.078	
1	All	RL	Experiment*Genotype	188.0	1911.0	0.89	0.846	
1	All	RL	Treatment*Genotype	47.0	1911.0	0.77	0.875	
1	All	RDW	Genotype	47.0	1891.0	3.57	0.000	<0.05
1	All	RDW	Experiment	4.0	1891.0	81.76	0.000	<0.05
1	All	RDW	Treatment	1.0	4.0	0.24	0.649	
1	All	RDW	Experiment*Genotype	188.0	1891.0	1.87	0.000	<0.05
1	All	RDW	Treatment*Genotype	47.0	1891.0	0.81	0.822	
1	All	SRLR	Genotype	47.0	1786.0	5.69	0.000	<0.05
1	All	SRLR	Experiment	4.0	1786.0	67.86	0.000	<0.05
1	All	SRLR	Treatment	1.0	4.0	15.48	0.017	<0.05
1	All	SRLR	Experiment*Genotype	188.0	1786.0	1.01	0.450	
1	All	SRLR	Treatment*Genotype	47.0	1786.0	1.09	0.311	
1	All	SRWR	Genotype	47.0	1795.0	5.88	0.000	<0.05
1	All	SRWR	Experiment	4.0	1795.0	291.64	0.000	<0.05
1	All	SRWR	Treatment	1.0	4.0	13.75	0.021	<0.05
1	All	SRWR	Experiment*Genotype	188.0	1795.0	0.97	0.595	
1	All	SRWR	Treatment*Genotype	47.0	1795.0	1.43	0.030	<0.05
1	All	BMD	Genotype	47.0	1836.0	4.50	0.000	<0.05
1	All	BMD	Experiment	4.0	1836.0	137.98	0.000	<0.05
1	All	BMD	Treatment	1.0	4.0	6.23	0.067	
1	All	BMD	Experiment*Genotype	188.0	1836.0	1.81	0.000	<0.05
1	All	BMD	Treatment*Genotype	47.0	1836.0	0.83	0.791	
2	K0	TN	Experiment	4.0	856.0	283.46	0.000	<0.05

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
2	K0	TN	Genotype	47.0	856.0	3.39	0.000	<0.05
2	K0	TN	Experiment*Genotype	187.0	856.0	2.91	0.000	<0.05
2	K0	HEI	Experiment	4.0	857.0	151.09	0.000	<0.05
2	K0	HEI	Genotype	47.0	857.0	6.50	0.000	<0.05
2	K0	HEI	Experiment*Genotype	188.0	857.0	1.38	0.001	<0.05
2	K0	SDW	Experiment	4.0	851.0	169.89	0.000	<0.05
2	K0	SDW	Genotype	47.0	851.0	3.85	0.000	<0.05
2	K0	SDW	Experiment*Genotype	188.0	851.0	1.18	0.065	
2	K0	RL	Experiment	4.0	861.0	82.07	0.000	<0.05
2	K0	RL	Genotype	47.0	861.0	4.60	0.000	<0.05
2	K0	RL	Experiment*Genotype	188.0	861.0	0.90	0.824	
2	K0	RDW	Experiment	4.0	853.0	42.46	0.000	<0.05
2	K0	RDW	Genotype	47.0	853.0	2.29	0.000	<0.05
2	K0	RDW	Experiment*Genotype	188.0	853.0	1.21	0.040	<0.05
2	K0	SRLR	Experiment	4.0	806.0	44.50	0.000	<0.05
2	K0	SRLR	Genotype	47.0	806.0	6.03	0.000	<0.05
2	K0	SRLR	Experiment*Genotype	188.0	806.0	1.47	0.000	<0.05
2	K0	SRWR	Experiment	4.0	804.0	263.59	0.000	<0.05
2	K0	SRWR	Genotype	47.0	804.0	3.54	0.000	<0.05
2	K0	SRWR	Experiment*Genotype	188.0	804.0	1.08	0.232	
2	K0	BMD	Experiment	4.0	831.0	103.60	0.000	<0.05
2	K0	BMD	Genotype	47.0	831.0	3.13	0.000	<0.05
2	K0	BMD	Experiment*Genotype	188.0	831.0	1.20	0.049	<0.05
2	K100	TN	Experiment	4.0	853.0	65.15	0.000	<0.05
2	K100	TN	Genotype	47.0	853.0	2.80	0.000	<0.05
2	K100	TN	Experiment*Genotype	188.0	853.0	1.59	0.000	<0.05
2	K100	HEI	Experiment	4.0	848.0	50.38	0.000	<0.05
2	K100	HEI	Genotype	47.0	848.0	4.12	0.000	<0.05
2	K100	HEI	Experiment*Genotype	188.0	848.0	1.09	0.214	
2	K100	SDW	Experiment	4.0	834.0	59.81	0.000	<0.05
2	K100	SDW	Genotype	47.0	834.0	2.32	0.000	<0.05
2	K100	SDW	Experiment*Genotype	188.0	834.0	1.17	0.083	
2	K100	RL	Experiment	4.0	858.0	210.58	0.000	<0.05
2	K100	RL	Genotype	47.0	858.0	4.86	0.000	<0.05
2	K100	RL	Experiment*Genotype	188.0	858.0	1.04	0.357	
2	K100	RDW	Experiment	4.0	846.0	41.41	0.000	<0.05
2	K100	RDW	Genotype	47.0	846.0	1.93	0.000	<0.05
2	K100	RDW	Experiment*Genotype	188.0	846.0	1.25	0.023	<0.05
2	K100	SRLR	Experiment	4.0	788.0	162.93	0.000	<0.05
2	K100	SRLR	Genotype	47.0	788.0	4.29	0.000	<0.05
2	K100	SRLR	Experiment*Genotype	188.0	788.0	1.05	0.339	
2	K100	SRWR	Experiment	4.0	799.0	76.15	0.000	<0.05
2	K100	SRWR	Genotype	47.0	799.0	4.08	0.000	<0.05
2	K100	SRWR	Experiment*Genotype	188.0	799.0	0.98	0.573	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
2	K100	BMD	Experiment	4.0	813.0	54.22	0.000	<0.05
2	K100	BMD	Genotype	47.0	813.0	2.25	0.000	<0.05
2	K100	BMD	Experiment*Genotype	188.0	813.0	1.19	0.062	
3	ALL	CaCR	Genotype	47.0	358.0	0.90	0.666	
3	ALL	CaCR	Experiment	4.0	358.0	41.70	0.000	<0.05
3	ALL	CaCR	Treatment	1.0	358.0	1.77	0.184	
3	ALL	CaCR	Treatment*Genotype	47.0	358.0	0.66	0.957	
3	ALL	CCR	Genotype	47.0	362.0	1.12	0.282	
3	ALL	CCR	Experiment	4.0	362.0	47.35	0.000	<0.05
3	ALL	CCR	Treatment	1.0	362.0	34.61	0.000	<0.05
3	ALL	CCR	Treatment*Genotype	47.0	362.0	1.19	0.198	
3	ALL	CuCR	Genotype	47.0	355.0	1.01	0.452	
3	ALL	CuCR	Experiment	4.0	355.0	103.54	0.000	<0.05
3	ALL	CuCR	Treatment	1.0	355.0	30.18	0.000	<0.05
3	ALL	CuCR	Treatment*Genotype	47.0	355.0	0.68	0.944	
3	ALL	FeCR	Genotype	47.0	360.0	0.62	0.976	
3	ALL	FeCR	Experiment	4.0	360.0	230.54	0.000	<0.05
3	ALL	FeCR	Treatment	1.0	360.0	5.05	0.025	<0.05
3	ALL	FeCR	Treatment*Genotype	47.0	360.0	0.78	0.849	
3	ALL	KCR	Genotype	47.0	358.0	1.04	0.406	
3	ALL	KCR	Experiment	4.0	358.0	41.09	0.000	<0.05
3	ALL	KCR	Treatment	1.0	358.0	194.24	0.000	<0.05
3	ALL	KCR	Treatment*Genotype	47.0	358.0	0.49	0.998	
3	ALL	MgCR	Genotype	47.0	349.0	0.83	0.779	
3	ALL	MgCR	Experiment	4.0	349.0	49.81	0.000	<0.05
3	ALL	MgCR	Treatment	1.0	349.0	526.75	0.000	<0.05
3	ALL	MgCR	Treatment*Genotype	47.0	349.0	0.73	0.905	
3	ALL	MnCR	Genotype	47.0	360.0	1.05	0.393	
3	ALL	MnCR	Experiment	4.0	360.0	22.54	0.000	<0.05
3	ALL	MnCR	Treatment	1.0	360.0	61.72	0.000	<0.05
3	ALL	MnCR	Treatment*Genotype	47.0	360.0	0.46	0.999	
3	ALL	NaCR	Genotype	47.0	364.0	0.84	0.765	
3	ALL	NaCR	Experiment	4.0	364.0	119.11	0.000	<0.05
3	ALL	NaCR	Treatment	1.0	364.0	171.82	0.000	<0.05
3	ALL	NaCR	Treatment*Genotype	47.0	364.0	0.89	0.677	
3	ALL	NCR	Genotype	47.0	360.0	0.96	0.547	
3	ALL	NCR	Experiment	4.0	360.0	52.94	0.000	<0.05
3	ALL	NCR	Treatment	1.0	360.0	121.00	0.000	<0.05
3	ALL	NCR	Treatment*Genotype	47.0	360.0	1.08	0.348	
3	ALL	PCR	Genotype	47.0	358.0	1.18	0.202	
3	ALL	PCR	Experiment	4.0	358.0	65.91	0.000	<0.05
3	ALL	PCR	Treatment	1.0	358.0	9.51	0.002	<0.05
3	ALL	PCR	Treatment*Genotype	47.0	358.0	0.78	0.844	
3	ALL	SCR	Genotype	47.0	359.0	1.21	0.170	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
3	ALL	SCR	Experiment	4.0	359.0	42.13	0.000	<0.05
3	ALL	SCR	Treatment	1.0	359.0	2.05	0.153	
3	ALL	SCR	Treatment*Genotype	47.0	359.0	0.71	0.928	
3	ALL	ZnCR	Genotype	47.0	359.0	0.67	0.953	
3	ALL	ZnCR	Experiment	4.0	359.0	125.43	0.000	<0.05
3	ALL	ZnCR	Treatment	1.0	359.0	94.74	0.000	<0.05
3	ALL	ZnCR	Treatment*Genotype	47.0	359.0	0.66	0.959	
3	ALL	CaCS	Genotype	47.0	338.0	1.66	0.006	<0.05
3	ALL	CaCS	Experiment	4.0	338.0	20.16	0.000	<0.05
3	ALL	CaCS	Treatment	1.0	338.0	31.71	0.000	<0.05
3	ALL	CaCS	Treatment*Genotype	47.0	338.0	1.24	0.148	
3	ALL	CCS	Genotype	47.0	355.0	0.93	0.597	
3	ALL	CCS	Experiment	4.0	355.0	57.36	0.000	<0.05
3	ALL	CCS	Treatment	1.0	355.0	107.16	0.000	<0.05
3	ALL	CCS	Treatment*Genotype	47.0	355.0	0.81	0.815	
3	ALL	CuCS	Genotype	47.0	326.0	0.98	0.523	
3	ALL	CuCS	Experiment	4.0	326.0	10.04	0.000	<0.05
3	ALL	CuCS	Treatment	1.0	326.0	0.00	0.980	
3	ALL	CuCS	Treatment*Genotype	47.0	326.0	1.59	0.011	<0.05
3	ALL	FeCS	Genotype	47.0	362.0	0.93	0.603	
3	ALL	FeCS	Experiment	4.0	362.0	22.54	0.000	<0.05
3	ALL	FeCS	Treatment	1.0	362.0	39.52	0.000	<0.05
3	ALL	FeCS	Treatment*Genotype	47.0	362.0	0.93	0.606	
3	ALL	KCS	Genotype	47.0	355.0	1.79	0.002	<0.05
3	ALL	KCS	Experiment	4.0	355.0	85.67	0.000	<0.05
3	ALL	KCS	Treatment	1.0	355.0	314.73	0.000	<0.05
3	ALL	KCS	Treatment*Genotype	47.0	355.0	0.63	0.971	
3	ALL	MgCS	Genotype	47.0	352.0	1.16	0.230	
3	ALL	MgCS	Experiment	4.0	352.0	41.15	0.000	<0.05
3	ALL	MgCS	Treatment	1.0	352.0	792.20	0.000	<0.05
3	ALL	MgCS	Treatment*Genotype	47.0	352.0	1.01	0.468	
3	ALL	MnCS	Genotype	47.0	356.0	1.62	0.009	<0.05
3	ALL	MnCS	Experiment	4.0	356.0	74.91	0.000	<0.05
3	ALL	MnCS	Treatment	1.0	356.0	330.65	0.000	<0.05
3	ALL	MnCS	Treatment*Genotype	47.0	356.0	0.82	0.791	
3	ALL	NaCS	Genotype	47.0	340.0	0.94	0.590	
3	ALL	NaCS	Experiment	4.0	340.0	8.01	0.000	<0.05
3	ALL	NaCS	Treatment	1.0	340.0	9.63	0.002	<0.05
3	ALL	NaCS	Treatment*Genotype	47.0	340.0	0.84	0.760	
3	ALL	NCS	Genotype	47.0	362.0	0.96	0.542	
3	ALL	NCS	Experiment	4.0	362.0	42.32	0.000	<0.05
3	ALL	NCS	Treatment	1.0	362.0	205.48	0.000	<0.05
3	ALL	NCS	Treatment*Genotype	47.0	362.0	0.71	0.926	
3	ALL	PCS	Genotype	47.0	360.0	1.74	0.003	<0.05

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
3	ALL	PCS	Experiment	4.0	360.0	27.70	0.000	<0.05
3	ALL	PCS	Treatment	1.0	360.0	99.96	0.000	<0.05
3	ALL	PCS	Treatment*Genotype	47.0	360.0	0.71	0.926	
3	ALL	SCS	Genotype	47.0	364.0	1.99	0.000	<0.05
3	ALL	SCS	Experiment	4.0	364.0	32.92	0.000	<0.05
3	ALL	SCS	Treatment	1.0	364.0	75.70	0.000	<0.05
3	ALL	SCS	Treatment*Genotype	47.0	364.0	0.92	0.617	
3	ALL	ZnCS	Genotype	47.0	356.0	1.01	0.466	
3	ALL	ZnCS	Experiment	4.0	356.0	52.77	0.000	<0.05
3	ALL	ZnCS	Treatment	1.0	356.0	226.42	0.000	<0.05
3	ALL	ZnCS	Treatment*Genotype	47.0	356.0	0.72	0.912	
4	K0	CaCR	Genotype	47.0	183.0	1.08	0.353	
4	K0	CCR	Genotype	47.0	180.0	0.74	0.892	
4	K0	CuCR	Genotype	47.0	178.0	0.54	0.993	
4	K0	FeCR	Genotype	47.0	182.0	0.27	1.000	
4	K0	KCR	Genotype	47.0	182.0	0.38	1.000	
4	K0	MgCR	Genotype	47.0	184.0	0.61	0.976	
4	K0	MnCR	Genotype	47.0	183.0	0.45	0.999	
4	K0	NaCR	Genotype	47.0	183.0	0.40	1.000	
4	K0	NCR	Genotype	47.0	179.0	0.91	0.637	
4	K0	PCR	Genotype	47.0	182.0	0.87	0.716	
4	K0	SCR	Genotype	47.0	183.0	1.08	0.345	
4	K0	ZnCR	Genotype	47.0	184.0	0.45	0.999	
4	K0	CaCS	Genotype	47.0	172.0	1.31	0.107	
4	K0	CCS	Genotype	47.0	178.0	0.58	0.986	
4	K0	CuCS	Genotype	47.0	166.0	1.04	0.423	
4	K0	FeCS	Genotype	47.0	183.0	0.51	0.996	
4	K0	KCS	Genotype	47.0	180.0	0.70	0.924	
4	K0	MgCS	Genotype	47.0	176.0	0.72	0.904	
4	K0	MnCS	Genotype	47.0	180.0	0.74	0.892	
4	K0	NaCS	Genotype	47.0	176.0	0.80	0.817	
4	K0	NCS	Genotype	47.0	184.0	0.43	1.000	
4	K0	PCS	Genotype	47.0	185.0	1.06	0.377	
4	K0	SCS	Genotype	47.0	184.0	1.13	0.281	
4	K0	ZnCS	Genotype	47.0	181.0	0.55	0.991	
4	K100	CaCR	Genotype	47.0	179.0	0.27	1.000	
4	K100	CCR	Genotype	47.0	186.0	0.78	0.842	
4	K100	CuCR	Genotype	47.0	181.0	0.35	1.000	
4	K100	FeCR	Genotype	47.0	182.0	0.22	1.000	
4	K100	KCR	Genotype	47.0	180.0	0.81	0.798	
4	K100	MgCR	Genotype	47.0	169.0	0.50	0.997	
4	K100	MnCR	Genotype	47.0	181.0	0.87	0.704	
4	K100	NaCR	Genotype	47.0	185.0	0.38	1.000	
4	K100	NCR	Genotype	47.0	185.0	0.37	1.000	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
4	K100	PCR	Genotype	47.0	180.0	0.35	1.000	
4	K100	SCR	Genotype	47.0	180.0	0.35	1.000	
4	K100	ZnCR	Genotype	47.0	179.0	0.22	1.000	
4	K100	CaCS	Genotype	47.0	170.0	1.06	0.389	
4	K100	CCS	Genotype	47.0	181.0	0.50	0.997	
4	K100	CuCS	Genotype	47.0	164.0	1.40	0.066	
4	K100	FeCS	Genotype	47.0	183.0	1.48	0.037	<0.05
4	K100	KCS	Genotype	47.0	179.0	0.61	0.976	
4	K100	MgCS	Genotype	47.0	180.0	0.82	0.783	
4	K100	MnCS	Genotype	47.0	180.0	0.63	0.967	
4	K100	NaCS	Genotype	47.0	168.0	0.87	0.702	
4	K100	NCS	Genotype	47.0	182.0	0.83	0.776	
4	K100	PCS	Genotype	47.0	179.0	0.81	0.804	
4	K100	SCS	Genotype	47.0	184.0	1.05	0.405	
4	K100	ZnCS	Genotype	47.0	179.0	0.62	0.971	
2	ALL	TN_SI	Experiment	4.0	813.0	7.66	0.000	<0.05
2	ALL	TN_SI	Genotype	47.0	813.0	1.46	0.026	<0.05
2	ALL	TN_SI	Experiment*Genotype	187.0	813.0	1.40	0.001	<0.05
2	ALL	HEI_SI	Experiment	4.0	811.0	56.62	0.000	<0.05
2	ALL	HEI_SI	Genotype	47.0	811.0	1.87	0.000	<0.05
2	ALL	HEI_SI	Experiment*Genotype	187.0	811.0	0.97	0.602	
2	ALL	SDW_SI	Experiment	4.0	796.0	39.92	0.000	<0.05
2	ALL	SDW_SI	Genotype	47.0	796.0	1.42	0.035	<0.05
2	ALL	SDW_SI	Experiment*Genotype	188.0	796.0	0.97	0.598	
2	ALL	RL_SI	Experiment	4.0	818.0	248.05	0.000	<0.05
2	ALL	RL_SI	Genotype	47.0	818.0	2.35	0.000	<0.05
2	ALL	RL_SI	Experiment*Genotype	188.0	818.0	1.25	0.022	<0.05
2	ALL	RDW_SI	Experiment	4.0	803.0	10.24	0.000	<0.05
2	ALL	RDW_SI	Genotype	47.0	803.0	1.44	0.029	<0.05
2	ALL	RDW_SI	Experiment*Genotype	188.0	803.0	0.96	0.626	
2	ALL	BMD_SI	Experiment	4.0	760.0	26.25	0.000	<0.05
2	ALL	BMD_SI	Genotype	47.0	760.0	1.56	0.011	<0.05
2	ALL	BMD_SI	Experiment*Genotype	188.0	760.0	0.94	0.689	
4	ALL	CaR_SI	Genotype	47.0	170.0	0.56	0.990	
4	ALL	CR_SI	Genotype	47.0	174.0	0.85	0.738	
4	ALL	CuR_SI	Genotype	47.0	167.0	0.62	0.973	
4	ALL	FeR_SI	Genotype	47.0	173.0	0.63	0.969	
4	ALL	KR_SI	Genotype	47.0	170.0	0.41	1.000	
4	ALL	MgR_SI	Genotype	47.0	161.0	0.79	0.820	
4	ALL	MnR_SI	Genotype	47.0	172.0	0.41	1.000	
4	ALL	NaR_SI	Genotype	47.0	177.0	0.88	0.698	
4	ALL	NR_SI	Genotype	47.0	173.0	0.88	0.682	
4	ALL	PR_SI	Genotype	47.0	171.0	0.84	0.758	
4	ALL	SR_SI	Genotype	47.0	172.0	0.55	0.991	

Model	Treatment	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
4	ALL	ZnR_SI	Genotype	47.0	171.0	0.51	0.996	
4	ALL	CaS_SI	Genotype	47.0	154.0	1.48	0.040	<0.05
4	ALL	CS_SI	Genotype	47.0	169.0	0.79	0.822	
4	ALL	CuS_SI	Genotype	47.0	142.0	1.13	0.291	
4	ALL	FeS_SI	Genotype	47.0	175.0	0.84	0.754	
4	ALL	KS_SI	Genotype	47.0	168.0	0.84	0.759	
4	ALL	MgS_SI	Genotype	47.0	164.0	1.45	0.045	<0.05
4	ALL	MnS_SI	Genotype	47.0	168.0	0.98	0.517	
4	ALL	NaS_SI	Genotype	47.0	155.0	1.48	0.041	<0.05
4	ALL	NS_SI	Genotype	47.0	175.0	0.69	0.929	
4	ALL	PS_SI	Genotype	47.0	172.0	0.82	0.786	
4	ALL	SS_SI	Genotype	47.0	178.0	0.87	0.712	
4	ALL	ZnS_SI	Genotype	47.0	169.0	0.75	0.879	

Table S3, Supporting Information: Results of MIXED model analysis for 5 morphological and 5 stress index traits, calculated across (ALL) and within treatments (H CO_2 or N CO_2).

Model	**Treatment**	Trait	Effect	NumDF	DenDF	FValue	ProbF	ProbF<0.05
1	All	TN	Genotype	47.0	1809.0	3.04	0.00	<0.05
1	All	TN	Treatment	1.0	3.0	4.33	0.13	
1	All	TN	Treatment*Genotype	47.0	1809.0	1.36	0.05	
1	All	HEI	Genotype	47.0	1809.0	3.58	0.00	<0.05
1	All	HEI	Treatment	1.0	3.0	0.03	0.87	
1	All	HEI	Treatment*Genotype	47.0	1809.0	1.38	0.05	
1	All	SFW	Genotype	47.0	1803.0	3.40	0.00	<0.05
1	All	SFW	Treatment	1.0	3.0	1.20	0.35	
1	All	SFW	Treatment*Genotype	47.0	1803.0	1.80	0.00	<0.05
1	All	SDW	Genotype	47.0	1803.0	3.83	0.00	<0.05
1	All	SDW	Treatment	1.0	3.0	0.18	0.70	
1	All	SDW	Treatment*Genotype	47.0	1803.0	1.63	0.00	<0.05
1	All	AC	Genotype	47.0	1809.0	6.63	0.00	<0.05
1	All	AC	Treatment	1.0	3.0	10.99	0.05	
1	All	AC	Treatment*Genotype	47.0	1809.0	2.76	0.00	<0.05
2	N CO_2	TN	Genotype	47.0	1091.0	2.36	0.00	<0.05
2	N CO_2	HEI	Genotype	47.0	1091.0	2.98	0.00	<0.05
2	N CO_2	SFW	Genotype	47.0	1089.0	3.03	0.00	<0.05
2	N CO_2	SDW	Genotype	47.0	1089.0	3.20	0.00	<0.05
2	N CO_2	AC	Genotype	47.0	1091.0	4.61	0.00	<0.05
2	H CO_2	TN	Genotype	47.0	711.0	1.88	0.00	<0.05
2	H CO_2	HEI	Genotype	47.0	711.0	2.18	0.00	<0.05
2	H CO_2	SFW	Genotype	47.0	707.0	2.18	0.00	<0.05
2	H CO_2	SDW	Genotype	47.0	707.0	2.21	0.00	<0.05
2	H CO_2	AC	Genotype	47.0	711.0	4.03	0.00	<0.05
2	All	TN_SI	Genotype	47.0	708.0	1.67	0.00	<0.05
2	All	HEI_SI	Genotype	47.0	708.0	1.34	0.07	
2	All	SFW_SI	Genotype	47.0	704.0	1.15	0.23	
2	All	SDW_SI	Genotype	47.0	704.0	1.07	0.35	
2	All	AC_SI	Genotype	47.0	593.0	2.87	0.00	<0.05

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Declaration under oath

Eidesstattliche Erklärung / Declaration under Oath

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

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

Halle (Saale), October 2017 ----------

Datum / Date Unterschrift / Signature