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A global barley diversity panel uncovered novel drought inducible QTL and footprints of evolution

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„Nur ein Narr macht keine Experimente“

-Charles Darwin-

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List of Abbreviations

A/A	Adenine/Adenine
A/G	Adenine/Guanine
AB	Advanced backcross
ABA	Abscisic acid
ABRE	ABA-responsive elements
ADMIX	Admixture group
AA	Amino acid
ANOVA	Analysis of variance
BBA	Biologische Bundesanstalt
B.C.	before Christ
BBCH stages	BBA, BSA und CI
BC_iDH	Back cross in generation i double haploid
BC_iS_j	Back cross in generation i and selfing in generation j
BLASTn	Nucleotide basic local alignment search tool
bp	Base pair
BSA	Bundessortenamt
C	Barley modern cultivar (*Hordeum vulgare* ssp. *vulgare*)
CAPS	Cleaved amplified polymorphic sequence
CDS	Coding sequence
CEP	C-Terminally encoded peptide
Chr	Chromosome
CI	Chemische Industrie
CK	Cytokinin
CLUMPAK	Cluster Markov Packager Across K
cM	Centi Morgan
DARwin	Dissimilarity Analysis and Representation for Windows
DAS	Days after sowing
DPA	Days past floral anthesis
DRE/CRT	Drought-responsive element/C-repeat
DREB	Dehydration-responsive element binding factor
DRYM	Deviation of relative starch yield from the experimental median
DT	Drought treatment
DtG	Days to germination
ERF	Ethylene response factor
F_i	Filial generation in generation i

FDR	False discovery rate
G/G	Guanine/Guanine
GWAS	Genome-wide association study
H^2	Broad sense heritability
Hei	Plant height
IL	Isogenic line
IPK	Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung
L	Barley landrace (*Hordeum vulgar*e ssp. *vulgare*)
LD	Linkage disequilibrium
Lea	Leave number
Ll	Leaf length
LOD	Logarithm of the odds
MAF	Minor allele frequency
MCMC	Markov Chain Monte Carlo
MCo	Membership coefficient
NAM	Nested association mapping
NCBI	National Centre for Biotechnology Information
NIL	Near isogenic line
ORF	Open reading frame
PCA	Principal component analysis
PCR	Polymerase chain reaction
Phy	Phyllochron
Phyt	Phytomer
PIPs	Plasma membrane intrinsic proteins
PO	Posterior odds
PROC	Procedure
PROC CORR	Procedure correlation
PROC GLM	Procedure general linear model
PROC MIXED	Procedure mixed linear model
PROC VARCOMP	Procedure variance components
QTL	Quantitative trait locus
Rdw	Root dry weight
RIL	Recombinant inbred line
Rl	Root length
ROS	Reactive oxygen species
RP	Relative performance
RS	Root-shoot ratio

S42IL	Scarlett x ISR42-8 isogenic line
Sdw	Shoot dry weight
Sl	Seedling length
SNP	Single nucleotide polymorphism
SPOP	Sub-group
STD	Standard deviation
Til	Tiller number
tin	Tillering inhibition
TIPs	Tonoplast intrinsic proteins
VMC	Volumetric moisture content
WT	Barley wild form (*Hordeum vulgare* ssp. *spontaneum)*
WUE	Water use efficiency
WW	Well-watered / control conditions

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Abstract

The fibrous root system is a visible sign of ecological adaptation among barley natural populations. In the present study, we utilized rich barley diversity to dissect the genetic basis of root system variation and its link with shoot attributes under well-water and drought conditions. Therefore, we analyzed five root and related shoot traits: root dry weight, root length, root-shoot ratio, shoot dry weight and number of tillers. Genome-wide association mapping of phenotype data using a dense genetic map (5892 SNP markers) revealed 17 putative quantitative trait loci (QTL) for these root and shoot traits. Among these, at 14 loci the preeminence of exotic QTL alleles resulted in trait improvements. The most promising QTL were quantified using haplotype analysis at local and global genome levels. The strongest QTL was found on chromosome 1H which accounted for root dry weight and tiller number simultaneously. Candidate gene analysis across the targeted region detected a crucial amino acid substitution mutation in the conserved domain of a WRKY29 transcription factor among genotypes bearing major and minor QTL alleles. Similarly, the drought inducible QTL QRdw.5H (5H, 95.0 cM) seems to underlie 37 amino acid deletion and substitution mutations in the conserved domain of two related genes CBF10B and CBF10A, respectively. The identification and further characterization of these candidate genes will be essential to decipher genetics behind developmental and natural adaptation mechanisms of barley.

Further, we analyzed the population to detect evolutionary footprints within the global barley diversity set. Therefore, we performed a loci outlier analyzes using the outlier detection tool BayeScan with the dense genetic map (5892 SNP marker) on three detected subpopulations (SPOPs). The analysis resulted in one outlier locus among the three SPOPs on barley chromosome 2H. A sequence analysis of the candidate gene revealed a crucial point mutation within the coding sequence (CDS) which leads to a truncated protein. Moreover, the mutation showed a SPOP-based as well as subspecies-based pattern. Furthermore, a detailed phenotypic analysis of this mutation in near isogenic lines (NIL) revealed a putative function of the candidate gene in shoot development. Further characterization of this candidate gene will gain a better insight in the differences in shoot development between *Hordeum vulgare* ssp. *spontaneum* (wild barley) and *Hordeum vulgare* ssp. *vulgare* (cultivated barley).

Zusammenfassung

Ein Wurzelsystem mit feinen Wurzelhaaren ist ein sichtbares Zeichen für die Anpassung an verschiedene Ökosysteme in Wildpopulationen von Gerste. In der vorliegenden Arbeit wurde eine große Gerstendiversität untersucht, um die genetische Grundlage für die Wurzelsystemvariation sowie deren Verbindung zu Sprossmerkmalen unter bewässerten und unbewässerten Bedingungen zu erforschen. Hierfür analysierten wir fünf Wurzel- und zugehörige Sprossmerkmale (Wurzeltrockengewicht, Wurzellänge, Wurzel-Spross Verhältnis, Sprosstrockengewicht, Anzahl der Bestockungstriebe). Die genomweite Assoziationskartierung der phänotypischen Daten zusammen mit einer genetischen Karte, die 5892 SNP-Marker enthält, deckte 17 mögliche Regionen für quantitative Merkmale (QTL) für Wurzel- und Sprossmerkmale auf. Unter diesen 17 QTL trugen 14 Loci exotische Wildformallele, welche eine Merkmalsverbesserung zur Folge hatten. Die vielversprechendsten QTL wurden mittels einer Haplotypenanalyse auf lokaler und globaler genomischen Ebene untersucht. Das signifikanteste QTL wurde auf Chromosom 1H detektiert und weist einen gleichzeitigen Effekt für das Wurzeltrockengewicht sowie die Anzahl der Bestockungstriebe auf. Eine Kandidatengenanalyse über die Zielregion identifizierte eine Aminosäuremutation in der konservierten Domäne des WRKY29 Transkriptionsfaktors zwischen verschiedenen Genotypen, die das Haupt- bzw. das Nebenallele des QTL tragen. Ebenso zeigt das durch Trockenheit induzierte QTL / Allel QRdw.5H (5H, 95,0 cM) eine 37 Aminosäuren große Deletion in der konservierten Domäne des Gens CBF10B sowie eine Substitution in CBF10A. Die Identifizierung und weitere Charakterisierung dieser Gene ist essentiell für die genetische Entschlüsselung von Entwicklungs- und Adaptationsmechanismen in Gerste.

Des Weiteren wurde eine Evolutionsanalyse an der globalen Gerstenpopulation durchgeführt. Hierfür detektierten wir mittels Loci Outlier Analyse implementiert in BayeScan, mit Hilfe von 5892 SNP-Markern, Allele die unter Selektion stehen. Die Analyse deckte einen Outlier Locus auf Chromosom 2H auf. Eine Sequenzanalyse dieses Locuses identifizierte ein Kandidatengen, welches eine Punktmutation innerhalb der Kodierungssequenz (CDS) des Gens aufweist. Diese Mutation zeigt eine Subpopulation spezifische sowie eine Subspezies spezifische Verteilung auf. Die phänotypische Analyse der Mutation in nah-isogenen Linien (NIL) enthüllte die mögliche Funktion des Kandidatengenes in der Sprossentwicklung. Eine weitere Charakterisierung des Kandidatengenes könnte einen besseren Einblick in die Unterschiede der Sprossentwicklung zwischen *Hordeum vulgare* ssp. *spontaneum* (Wildgerste) und *Hordeum vulgare* ssp. *vulgare* (Kulturgerste) ermöglichen.

Introduction

1. Hordeum vulgare ssp. *vulgare (*Barley*)*

Domesticated barley (*Hordeum vulgare* ssp. *vulgare*) evolved from the progenitor *Hordeum vulgare* ssp. *spontaneum* and belongs to the family Poaceae in the tribe of Triticeae; the largest group of monocotyledonous plants (Payne, 1969). The genus *Hordeum* consists of around 32 species and 45 taxa including annual to perennial species. Most species within the genus are diploid but there exist tetraploid, hexaploid as well as autoploid plants (von Bothmer *et al.*, 2003). Barley is one of the most important plants in food production. It ranks under the fifteenth most important crops with maize, rice, wheat and soybean in the world (FAO 2013, http://faostat.fao.org). Nearly 75 % of the produced barley is used in animal livestock feed, in alcoholic and non-alcoholic beverages production 20 % of barley is malted; approximately 5% is used in human food production (Blake *et al.*, 2011).

2. Barley Origin and Diversity

Barley, as one of the founder crops of Old World agriculture is in addition to wheat, maize and rice one of the most important crops. Archeological remains of barley grains at different sites in the Fertile Crescent indicate the domestication about 8000 *B.C.* (Vallage and Hari, 1979) and revealed; one of the earliest sites of crop domestication is the Fertile Crescent; consequently, the center of origin of some wild cereals e.g. barley (Salamini *et al.*, 2002) (**Figure 1**). The wild ancestor of barley is known as *Hordeum vulgare* ssp. *spontaneum* (wild barley) and still colonizes the Fertile Crescent from Israel and Jordan to south east Turkey as well as Syria, Iraq and west Iran (Harlan and Zohary, 1966). Additional to the primary habitat in the Fertile Crescent, wild barley had been reported in Greece, Ethiopia, Egypt and Asia (Vallage and Hari, 1979; von Bothmer *et al.*, 1995). Until today, barley is globally cultivated but a lot of different theories exist, how barley started its spreading to populate nearly the whole globe. As a result, there has been extensive research regarding the evolution and domestication of barley. Different scientists clearly identified and proved the origin of barley in the Fertile Crescent by genetic and chromosomal studies (Stebbins and Yagil, 1966; Badr *et al.*, 2000; Badr and El-Shazly, 2012). Likewise, multiple sites of barley domestication east of the Fertile Crescent have been supported due to increasing evidence, like the fixation of non-brittle rachis by two closely linked genes (Azhaguvel and Komatsuda, 2007; Komatsuda *et al.*, 2007; Morrell and Clegg, 2007). The investigation of evolutionary changes due to natural selection and domestication is enabled by genomic diversity. The use of modern marker systems for genome-wide marker analysis is a powerful tool to analyze the genetic architecture and genomic regions of barley; like single nucleotide polymorphism (SNP) markers (Pourkheirandish and Komatsuda, 2007; Dai *et al.*, 2012). Hence, different traits and genes have been analyzed under the aspects of domestication and evolution in barley by

marker assisted genetic analysis (Kandemir *et al.*, 2004). Further, the use of “top-down” and “bottom-up” approaches uncovered several domestication related genes in barley. For instance, *BKn-3*, *Vrs1*, *Nud* and *Btr1Btr2* are the primary domestication-related genes in barley (Badr *et al.*, 2000; Azhaguvel and Komatsuda, 2007; Komatsuda *et al.*, 2007; Pourkheirandish and Komatsuda, 2007; Badr and El-Shazly, 2012). Badr *et al.* use the different alleles of the homeobox gene *BKn-3* as diagnostic markers to determine the origin of domestication of barley to the Fertile Crescent (Badr *et al.*, 2000). While, there are in addition to those genes mentioned above, unknown genes in barley which provide for the investigation of barley evolution and domestication.

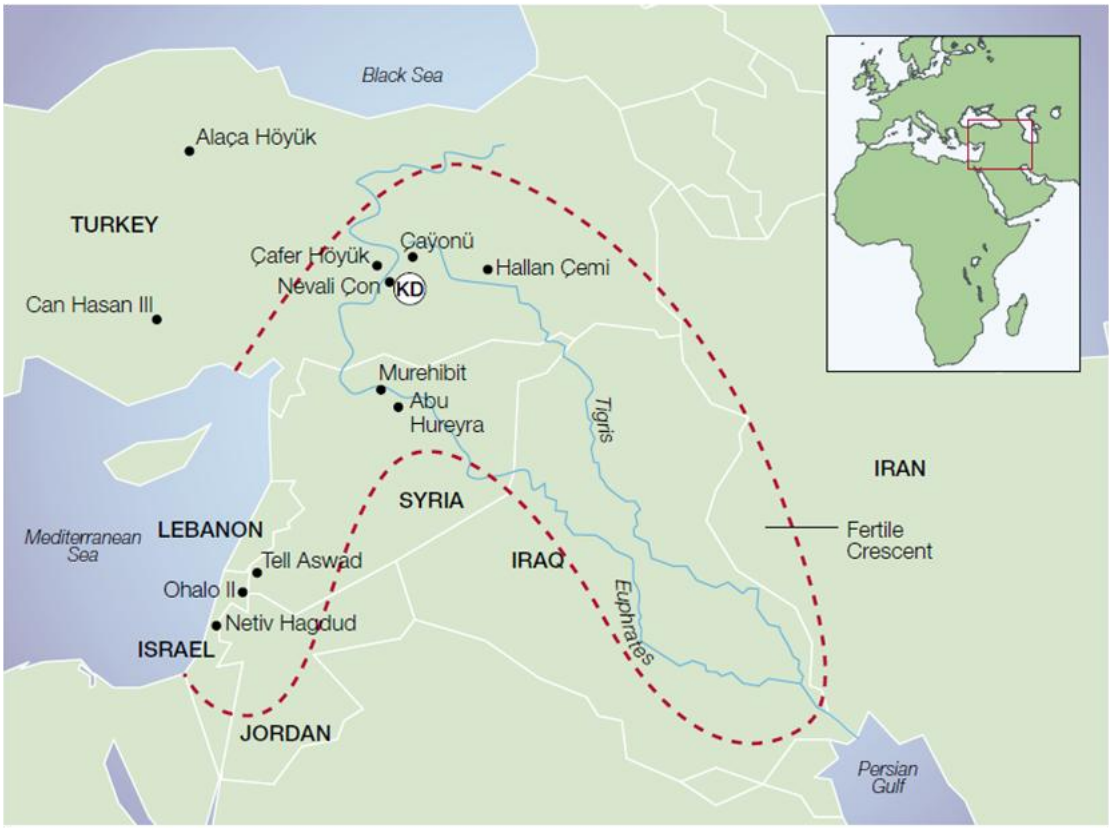

Figure 1: Fertile Crescent; site of barley origin in the Middle-East. The Fertile Crescent is indicated by a dashed red line ranging from south-east of Turkey to Israel and Jordan as well as western Iran and north-east Iraq (Euphrates and Tigris region) (Salamini *et al.*, 2002).

3. Abiotic Stress in Crop Production

Due to their sessile life plants are not able escape from abiotic stress situations. So some specialized plant species like succulents or grasses developed sensitive mechanisms to detect and strategies to survive such stress situations. The major abiotic stress factors are drought, high-salinity, cold and heat that reduce the yield of crop production. Particularly, water deficiency affects agronomical performance in important horticultural regions worldwide. By the 2080s, a yield loss for wheat is predicted by up to 80 % due to extreme

climatic events (Deryng *et al.*, 2014). Therefore, breeding programs have been developed to produce crops tolerant to several abiotic stresses. But traditional breeding approaches had limited success because of the multigenic nature of stress tolerance (Ahmad and Prasad, 2012). In the last decades, extensive research has been done on the physiological, morphological and genetic mechanisms of stress tolerance (Cramer *et al.*, 2011; Aroca, 2012). This knowledge led to new breeding techniques as well as the development of novel breeding programs to enhance abiotic tolerance in crop plants.

3.1. Drought Stress

Drought leading to water stress has an enormous impact on crop plants. Due to global warming the global temperature on Earth has increased since 1880 by about 0.8°C (Carlowicz, 2010). Consequently, increasing desertification and looming water shortages lead to more and longer drought periods, which affect the crop productivity especially in tropical, semi-arid and arid regions worldwide during grain-filling phase and results in yield losses dramatically (Samarah, 2005; Pennisi, 2008). Plant productivity depends on the amount of water available for the CO_2 fixation. This can be observed by the water use efficiency (WUE) in C_3 and C_4 plants. In C_3 plants, 1.3 to 2 g dry material is produced per 1 kg water and in C_4 this amount is twofold higher (Rao *et al.*, 2006). In the US water stress is the main factor for the loss of agriculture products (Boyer, 1982). Water deficits inside and outside of plants have different reasons and cause different results; water deficit outside of plants is a result from low rainfall and poor soil water storage (**Figure 2**).

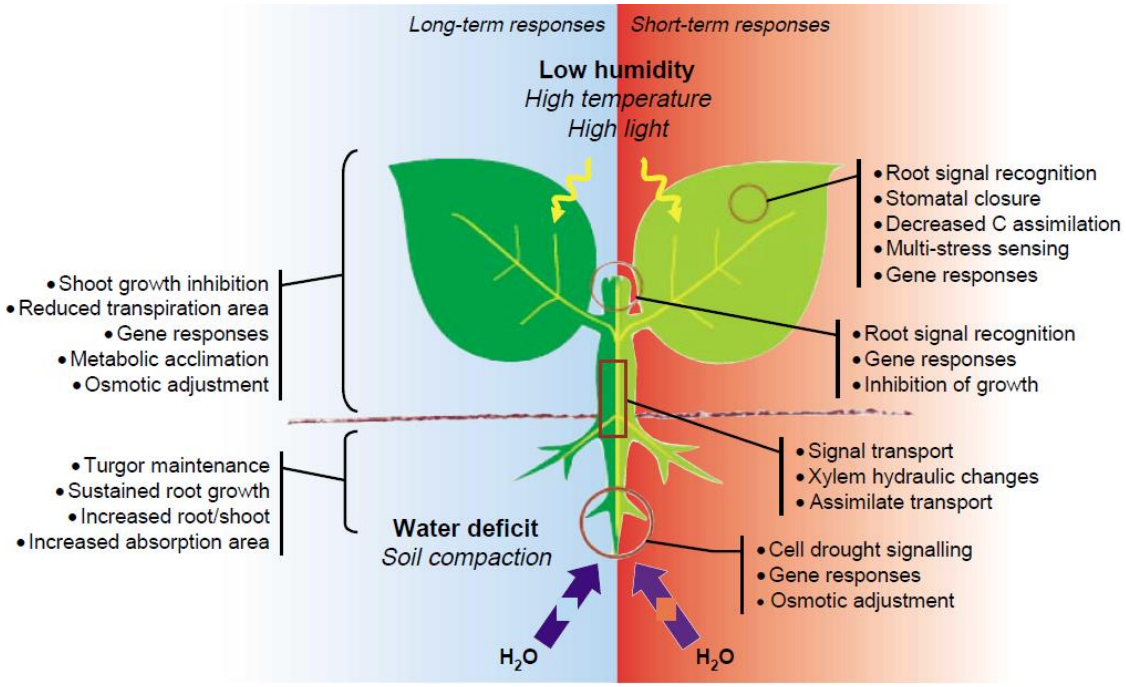

Figure 2: Long-term and short-term responses of plants to water deficiency stress (Oliveira *et al.*, 2013). Water stress induces different morphological, physiological and molecular responses influencing drought tolerance.

Water deficit inside of plants on cellular level results in loss of turgor, changes in cell volume and water potential gradients, change of membrane integrity and denaturation of proteins (Griffiths and Parry, 2002; Lawlor, 2002; Lawlor and Cornic, 2002; Parry, 2002; Raymond and Smirnoff, 2002; Bartels and Souer, 2004). The absence of irrigation causes drying of the atmosphere and soil which lead to different physiological and morphological reactions. In leaves a difference in leaf-air vapor pressure activates the stomata closing to decrease the loss of water (Mott and Parkhurst, 1991). Furthermore, the leaf area decreases as an early adaptive response to water deficit. The decrease is based on a decrease of the cell turgor which leads to a cell wall relaxation. In case of a strong water deficiency, the stress stimulates the leaf abscission which is enhanced by a strong ethylene synthesis (Taiz and Zeiger, 2010). Additionally, the loss of water reduces the photosynthesis which is ascribed to the dehydration of mesophyll cells and a destruction or organization of chlorophyll. A main problem of the decreasing photosynthesis based on water deficiency is a reduced transport of photosynthetic products. This transport depends on the turgor, which is directly influenced by the leaf water potential. Decreasing water potential leads to an inhibition of assimilate movement via phloem (Massacci *et al.*, 1996). In addition to minimizing the water loss, plants have to enhance their water absorbability in roots. Due to a low water concentration in soil, ascribed to aridness, the water potential in roots increases which hamper the water absorption. To counteract the loss of water, plants synthesize and accumulate small molecules called *compatible solutes* which decrease the water potential within plants (Rao *et al.*, 2006). Moreover, root growth increases to allow the assimilation of

water from deeper soil layers or to cover a greater area to get water from distant water storages (Hsiao and Xu, 2000). The effect of drought stress on plants depends on the duration of drought periods, the developmental stage of the plant, environmental interactions as well as the genotypic capacity of species. Extensive research was made to understand the recognition of water stress and the signal transduction of this stress (Bohnert *et al.*, 1995). The water stress leads to gene expression and increased hormone production to maintain cellular function. Important gene products under drought stress are proline and glycinebetaine which protect cellular structures and lead to osmotic adjustment (Bray, 1997, 2002). Moreover, the concentration of the plant hormone abscisic acid (ABA) increases under drought stress as well as other abiotic stresses because ABA is an important stress hormone which enables mechanisms to cope drought periods (Fujita *et al.*, 2013). Therefore, a detailed analysis of the physiological, morphological and metabolic reactions on drought stress is important to enable the tremendous effects of different mechanisms to develop drought tolerant cultivars that may cope with drought periods to deal with food shortages due to drought in the world (Pennisi, 2008; Naz *et al.*, 2012, 2014; Comas *et al.*, 2013).

3.2. Drought Tolerance

In both conditions, natural and agricultural, plants are exposed frequently to drought stress which is a major factor in decreased crop productivity (Lambers *et al.*, 2008). Therefore, plants had to evolve a wide range of reactions or mechanisms to survive in drought stress situations. Stress tolerance is defined as plant's fitness to cope with an unfavorable environment (Lichtenthaler, 1996). The drought stress tolerance can be distinguished in three parts:

1. Morphological adaptation
2. Physiological adaptation
3. Molecular adaptation

These three parts include a wide range of adaptations at subcellular, cellular and organ level; like cuticle thickness, stomatal regulation, root system, hormonal balances, antioxidant defense system, osmotic adjustment and maintenance of tissue water contents, etc. (Aroca, 2012).

Morphological Adaptation

Most important morphological adaptations to drought are drought escape and dehydration postponement. Drought escape is the ability to complete the life cycle during wet season before the onset of drought and a general phenomenon in many desert plants. This form of adaptation needs an extremely short life cycle, where seeds are produced during short rainy seasons (Levitt, 1980). Therefore, drought escape is associated with the time of flowering. In

environments where drought stress periods are likely early flowering is highly successful to avoid the stress but the plant has to pay for this ability with a yield loss (Turner *et al.*, 2001). Dehydration postponement is the ability to maintain hydration by preserve a high plant water status or cellular hydration (Blum, 2005). Plants create this hydration either by an increased water uptake or reduced water loss through transpiration. To maximize the water uptake, plants have to produce more root biomass or facilitate the water uptake which makes the root plasticity an important factor in dehydration postponement (Wasson *et al.*, 2012). Root plasticity is defined as the ability of a genotype to regulate its root growth according to prevailing circumstances (Kano *et al.*, 2011). Higher rooting depth, root length, root system size, etc. are considered as drought avoidance traits. Drought stress influences root growth negatively by reducing the root biomass, even in tolerant genotypes, but the effect is more prominent in drought susceptible genotypes. In 2003, Piro *et al.* revealed a higher effect of drought on root growth for drought sensitive wheat genotypes due to an overall reduction of newly synthesized cell wall polysaccharides such as pectins, hemicelluloses, and cellulose. Furthermore, rice near isogenic lines (NIL) carrying the deeper rooting 1 gene (*Dro1*) develop deeper roots which enhances the performance under drought stress by increasing the drought tolerance (Uga *et al.*, 2013). Moreover, more root dry weight and root length density in peanut (*Arachis hypogaea* L.) genotypes resulted in more yield compared to genotypes with less root dry weight and root length density (Jongrungklang *et al.*, 2013). Another important adaptation mechanism of dehydration postponement is the reduction of transpiration. A higher root / shoot ratio created by fewer and / or smaller leaves and a bigger root system leads to a higher water uptake and minimal loss to withstand water deficit conditions (Lei *et al.*, 2006). Furthermore, an enhanced stomatal and cuticular resistance, less small stomata, reduced leaf area and a change in leaf orientation are other important dehydration postponement traits to minimize water loss due to transpiration under drought stress conditions (Aroca, 2012).

Physiological Adaptation

Dehydration tolerance is defined as the ability to function while dehydration (Oliver *et al.*, 2010). Major physiological adaptations of dehydration tolerant plants are osmotic adjustment, antioxidant defense system and changes in phytohormone dynamics. The osmotic adjustment is the accumulation of organic and inorganic solutes under water deficiency stress to create a high water status. The increased concentration of these solutes helps to lower the water potential without decreasing the actual water content (Serraj and Sinclair, 2002). These solutes are also known as compatible solutes and they include soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids trehalose, etc. (Taiz and Zeiger, 2010). The compatible solutes synthesis is caused by various stresses like heat, salt and water (Chen and Murata, 2002). But the function of compatible solutes under water stress is not

only that of hygroscopic substances, they also act as stabilizers to protect the functional structure of a wide range of proteins and macro molecules (Akashi *et al.*, 2001; Kaushik and Bhat, 2003). Furthermore, they can protect the cellular membrane against damaging effects of reactive oxygen species (ROS) and ion leakage (Rao *et al.*, 2006). Osmotic adjustment is the major adaptation of plants on cellular level to reduce effects of drought (Blum, 2005). There are two points how osmotic adjustment helps plants under drought conditions: 1) it improves the stomatal conductance by maintaining the leaf turgor for an efficient assimilation of CO_2 (Kiani *et al.*, 2007), and 2) it increases the ability of water uptake in roots (Chimenti *et al.*, 2006). A limited water supply can cause the promotion of oxidative stress with an enhanced production of ROS. These substances are highly reactive and can negatively influence plant metabolism and causes oxidative damage to lipids, proteins and other macro molecules (Foyer and Shigeoka, 2011). To erase the ROS, plants developed an antioxidant system with enzymatic and non-enzymatic components like superoxidase dismutase, catalase, peroxidase, ascorbic acid, reduced glutathione etc. as well as the compatible solutes proline and glycinebetaine to avoid oxidative damage (Scandalios, 2005; Ozkur *et al.*, 2009). The production of antioxidants is enhanced in plants to minimize detrimental effects of ROS to normalize the metabolic activities under drought stress. Another important factor in physiological adaptation to drought is the regulation of phytohormones. Plants produce phytohormones like auxins, gibberellins, cytokinins, ethylene and ABA which regulate plant development. Especially ABA is known as an important regulator for plant growth and adaptation to drought. Drought alters the endogenous synthesis of ABA to enhance the concentration of ABA which helps to regulate the plant water budget (Rao *et al.*, 2006; Fujita *et al.*, 2013). The increased synthesis of ABA activates physiological short-term adaptations to drought like stomata closure as well as long term adaptation like root growth (Verma *et al.*, 2016). The analysis of ABA deficient mutants *aba1, aba2 and aba3* in *Arabidopsis thaliana* (*A. thaliana*) revealed a major function of ABA in osmotic stress tolerance (Tuteja, 2007). The drought tolerance induced by ABA is transmitted via the ABA-dependent pathway which enables the expression of drought tolerance genes. The ABA induced expression of genes relies on *cis*-acting elements also known as ABA-responsive elements (ABRE) (Uno *et al.*, 2000). Additional to the ABA-dependent pathway an ABA-independent pathway exists. Genetic analyses indicate that there is no clear line between both drought tolerance pathways and the components of other pathways. Moreover, a cross talk between ABA-dependent and ABA-independent is postulated. In some cases there even exists a convergence of reactions of the different pathways (Tuteja, 2007).

Molecular Adaptation

Plants affected by drought developed many adaptive processes at molecular levels to modulate water balance. The cascade of molecular responses to drought ranges from stress

perception, to signal transduction to cytoplasm and nucleus, to gene expression and finally metabolic changes (Ahmad and Prasad, 2012). Among these processes, the up- and downregulation of transcripts as well as the accumulation of stress proteins is very important (Kavar *et al.*, 2008). In 2002, Chen and Murata identified a group of genes including transcription factors of drought-responsive element / C-repeat (DRE/CRT) binding factor family as well as MYB proteins, bZIP/HD-ZIPs and AP2/EREBP domain proteins which were upregulated under drought stress. Moreover, Seki *et al.* (2002) revealed in a full-length cDNA microarray, containing 7000 *A. thaliana* cDNAs, several drought-induced target genes and stress-related transcription factors of different families like dehydration-responsive element binding factor (DREB), ethylene response factor (ERF), WRKY, MYB, bZIP, helix-loop-helix and NAC. Besides the expression of transcription factors, the accumulation of stress proteins like aquaporins increases the drought tolerance in plants. Water channels also known as aquaporins regulate the movement of water and other small molecules across plant vacuolar and plasma membranes; they are associated with plant tolerance to abiotic stresses (Li *et al.*, 2015). Plant's aquaporins comprise a large and highly diverse family with more than 150 proteins identified until today (Johansson *et al.*, 2000). Aquaporins are passive transporter were water moves down its water potential gradient. Aquaporins are localized in the plasma membrane and tonoplast so far. The plasma membrane aquaporins are called plasma membrane intrinsic proteins (PIPs). The tonoplast aquaporins are called tonoplast intrinsic proteins (TIPs). These two subfamilies form two distinct phylogenic groups within the family of aquaporins. Several studies demonstrated that the over-expression of aquaporins increases the abiotic stress tolerance in plants (Ayadi *et al.*, 2011; Hu *et al.*, 2012; Liu *et al.*, 2013). In 2015, Ding *et al.* observed an increased PIP aquaporin accumulation in rice plants under drought stress as well as root protoplast water permeability. Moreover, they detected a close correlation between the enhanced PIP accumulation and root protoplast permeability.

All in all, the drought tolerance in plants is a multi-factorial process. The above mentioned three mechanisms of adaptation (morphological, physiological, and molecular) cannot be seen as independent processes. Moreover, under drought all three mechanisms are activated and become blurred which makes a clear separation very difficult. Therefore, all three adaptive mechanisms have to be observed for a precise analysis of drought tolerance in plants (**Figure 3**).

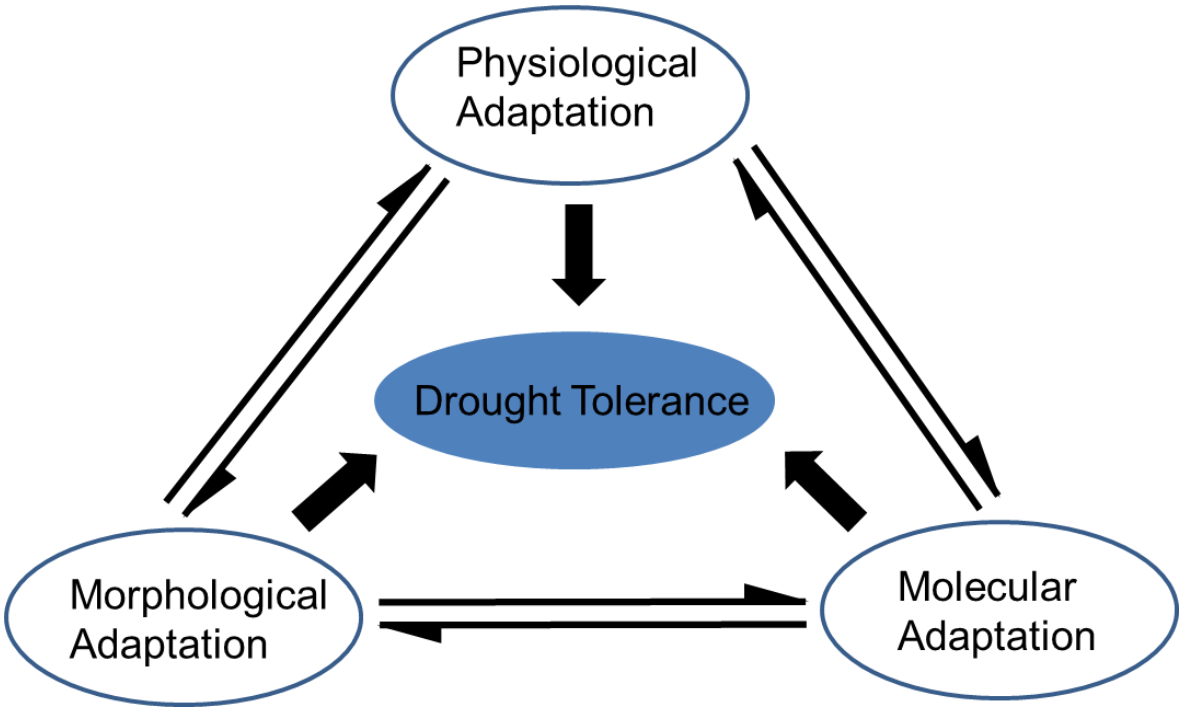

Figure 3: Pictorial representation of the three adaptive mechanisms (physiological, morphological and molecular) and their connection in drought tolerance. The double arrows represent the interaction among the different adaptive mechanisms.

4. Barley Development

Like every other plant, barley consists of a part above ground (shoot) and a part beneath ground (root). Each part has a system of development which is specific for the particular species as well as for the developed part. The development of root and shoot is closely related whereby an effect of root development and shoot development and vice versa is expected. Barley's development is divided into ten primary stages and 100 secondary stages (Ten within each primary stage). The ten primary stages are: 0) Germination, 1) Seedling growth, 2) Tillering, 3) Stem elongation, 4) Booting, 5) Inflorescence emergence, 6) Anthesis, 7) Milk development, 8) Dough development, 9) Ripening (Zadoks *et al.*, 1974).

Barley shoots comprising of two main components: leaves and tillers (Reid, 1985). The first leaf of barley emerges in secondary stage 11 within primary stage one (Seedling growth). During this stage all leaves of the main stem develop which can be nine or more than nine leaves. In the following stage (Stage 2: Tillering) side shoots (Tillers) emerge from the root shoot junction of the main stem. Depending on the biological status of the barley plants five to more than 40 tillers can be developed. The first root emerges from the seed in stage 0 at secondary stage five. This root will be the initiation for the root system development. Barley root system comprises of two components: seminal and nodal roots

(Wahbi, 1995). Seminal roots develop in the post-embryogenesis from embryo´s radical whereas nodal roots are initiated through the base of each established tiller later in plant development (Wahbi, 1995). This process continues for at least eight weeks depending upon the ability of nutrients and suitable environmental conditions (Lancashire *et al.,* 1991). The development of each tiller above ground consequently increases the number of nodal roots below ground because of their location close to soil. Both seminal and nodal roots develop lateral roots and water sucking organs, the root hairs (Naz *et al.,* 2012; Smith and De Smet, 2012). This peculiar developmental scheme is the rule in cereal crops like wheat and barley suggesting two parallel mechanisms influencing root system variation; 1) the inherent seminal rooting ability and 2) shoot dependent nodal root initiation. The latter mechanism seems more complex because it is still unclear if more tillering is the cause of more nodal rooting or if there exists positive feedback in which an increase in nodal rooting facilitates more shoot development by the acquisition of more water and nutrients. Several studies were made to find the interplay of root and shoot dependency in cereals. For instance, Narayanan and Prasad found a close relationship of root and shoot traits, especially for shoot dry weight and the tiller number to most root traits in a spring wheat association panel comprising 250 genotypes (Narayanan and Prasad, 2014). Moreover, Canè *et al.* detected in a genome-wide association study of 183 durum elite accessions 15 overlapping QTL for root and agronomic traits and/or grain yield in two or more environments (Canè *et al.,* 2014). Recently, Lou *et al.* performed in depths genetic analysis of deep rooting in rice and predicted the role of auxin associated genes in mediating different root attributes of rice (Lou *et al.,* 2015). Roots and their architecture are seen as the most important plant organ for crop productivity and adaptation to drought stress due to their versatile ability in capturing water and nutrients. Furthermore, roots are the prime organs that sense and respond to water deficit conditions (Naz *et al.,* 2012; Vadez, 2014). Especially, deeper and more profuse root systems increase the drought tolerance of crops like rice, wheat and barley (Chloupek *et al.,* 2010; Uga *et al.,* 2013). For instance, Uga *et al.* discovered DEEPER ROOTING 1 (*Dro1*) gene which mediates fibrous rooting in rice and established gene bearing near isogenic lines (NILs) (Uga *et al.,* 2013). *Dro1*-NIL exhibited a significant increase in yield performance under drought conditions due to increased drought avoidance by deep rooting compared to control genotype IR64.

5. Genome-Wide Association Study

QTL are quantitative trait loci; these loci are positions on chromosomes effecting phenotypic variations of quantitative traits due to genetic or environmental influences. These variations can consist of discrete values like numbers of tillers or can be continuous like plant height (The Complex Trait Consortium, 2003). Association studies are the effort to identify QTL through different kinds of populations. One kind of population is a natural diversity

panel. Linkage analysis and association mapping are the two most commonly used tools for dissecting complex traits. In contrast to linkage mapping, association mapping searches for functional variation in a much broader germplasm context (Zhu *et al.*, 2008). Moreover, association mapping offers three advantages: 1) increased mapping resolution, 2) reduced time cost and 3) greater allele number (Yu and Buckler, 2006). Based on the scale and focus of research association mapping can be separated into two categories: 1) candidate-gene association mapping and 2) genome-wide association studies (GWAS) (**Figure 4**). Candidate-gene association mapping analyzes polymorphisms within candidate genes which have a purported role in controlling phenotypic variations. In contrast, GWAS tries to identify signals of trait variations in the whole genome (Risch and Merikangas, 1996). The first whole genome QTL analysis in crop plants was performed in tomato in the late 1980s and early 1990s by Paterson (Paterson *et al.*, 1988, 1991). Hereupon, QTL analysis of several different other crops like soybean (Keim *et al.*, 1990) and maize (Beavis *et al.*, 1991) followed. In the early 1990s Heun (1992) as well as Hayes *et al.* (1993) performed the first QTL analysis in barley. Until today, a great number of QTL analyses have been conducted on barley, in different environments (drought, salinity, cold, etc.), on different traits (yield, resistance, tolerance, etc.) and on different populations like natural diversity, advanced backcross (AB), recombinant inbred lines (RILs), near isogenic lines (NILs). Further, advancements in genotyping and sequencing technologies have reduced the costs per molecular marker, especially single nucleotide polymorphisms (SNPs) (Hirschhorn and Daly, 2005; Syvanen, 2005). Moreover, increased availability of annotated genome sequences from several model as well as non-model species enabled a massive quantity of candidate sequences and whole genome sequences for different complex traits and species. These advances enabled the identification of hundreds of thousands of SNPs through resequencing of sequences of divers genotypes and species. For example, the tool HapMap of *A. thaliana* provides a powerful catalog of genetic diversity with millions of SNPs (Clark *et al.*, 2007). All in all, due to reduced cost and advances in genotyping, high-throughput phenotyping as well as sequencing: association analysis is a powerful tool, more than ever, to detect genes influencing QTL.

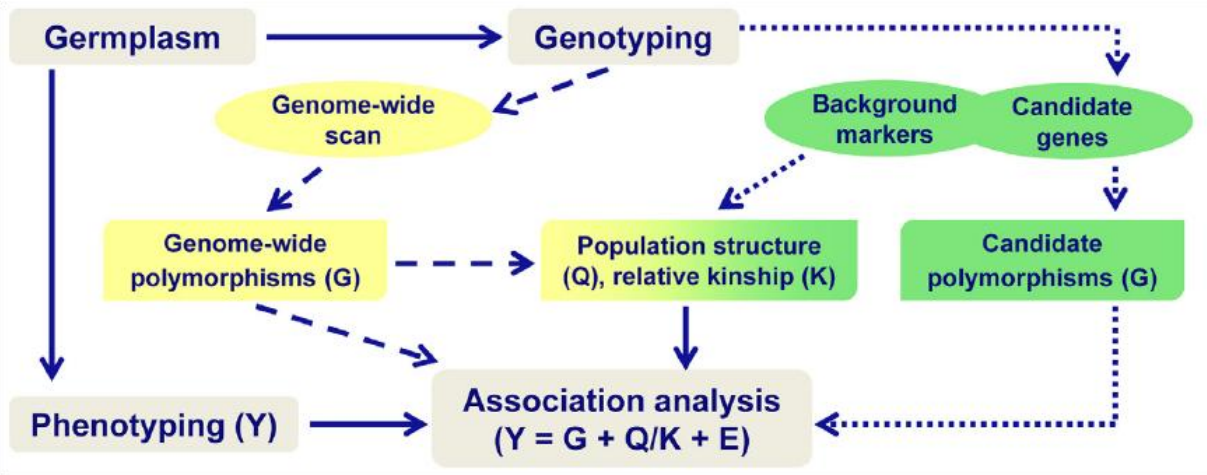

Figure 4: Scheme of contrast of GWAS and candidate-gene association mapping. The inclusion of population structure (Q), relative kinship (K), or both in final association analysis depends on the genetic relationship of the association mapping panel and the divergence of the trait examined. E: residual variance (Zhu *et al.*, 2008).

6. Aim of Study

Drought stress is a major factor limiting crop productivity, especially regarding of global warming. In this context, the development of cultivars which are tolerant to drought stress have a great importance. It is well known that a huge and / or deeper root system increases the water assimilation due to greater root-soil contact. But until today, the genetic potential of root system variations has not been utilized and breeding mainly focused on yield instead of supplying optimal water under drought conditions due to root variations. Consequentially, strong irrigation for crop cultivation is necessary which has a negative ecologic and economic impact. Hence, the aim of this study was to explore the significance of root system variations in the model grass barley in achieving sustainable supply of water under drought conditions. The ancestor of modern barley cultivars (*Hordeum vulgare* ssp. *vulgare*), *Hordeum vulgare* ssp. *spontaneum*, is well adapted to diverse environmental conditions and displays huge root system variations which makes this species, originated from the Fertile Crescent, a perfect candidate for root-related drought tolerance research. Further, a detailed genetic analysis of root traits enables the tremendous potential of wild germplasms for plant breeding to develop drought tolerant cultivars that may cope drought periods to deal with food shortages due to drought in the world (Pennisi, 2008; Naz *et al.*, 2012, 2014; Comas *et al.*, 2013). Thus, we developed a global barley diversity set comprising of wild barley, landraces and modern cultivars to survey the following hypotheses and objectives:

Hypotheses:

1. *It is possible to dissect the genetic variations of barley root system under drought and control conditions by using a global barley population.*
2. *The use of a global barley population in a "bottom-up" approach enables the detection of footprints of selection in barley.*

Objectives:

1. *Establish a state of the art genetic resources based on morphological novelties, geographic distribution and inherent environmental adaptation.*
2. *Identification of lines which are well adapted to drought stress due to their root system morphology.*
3. *Identification of root system variations under control and drought conditions in modern cultivars and wild barley.*
4. *Population analysis of global barley diversity set via loci outlier detection to identify marker linked to genes under selection.*

5. *Analysis of phenotypic and genotypic data via genome-wide association mapping to identify quantitative trait loci (QTL). These QTL provide the basis for marker assisted selection for drought tolerance breeding.*
6. *Candidate gene analysis of genes identified via genome-wide association mapping as well as loci outlier detection.*

Material and Methods

1. Plant Material

The studied germplasm panel contains 179 different genotypes that were collected in 38 countries across the globe (**Appendix Table 10**). It includes 48 *Hordeum vulgare* ssp. *spontaneum* (wild) accessions and 131 *Hordeum vulgare* L. ssp. *vulgare* (cultivar) accessions. The latter is made up of 72 landraces and 59 modern cultivars. The seeds were provided by Leibniz Institute for Plant Genetic and Crop Science (IPK, Gartersleben, Germany), Nordgen (NGB, Alnarp, Sweden) and the International Center for Agricultural Research in the Dry Areas (ICARDA, Beirut, Lebanon).

Additionally, a wild barley introgression library comprising 72 lines was used to analyze the phenotypic effect of candidate gene. The introgression lines derived from a cross between the Israeli wild barley accession ISR42-8 (*Hordeum vulgare* ssp. *spontaneum*) and the German spring barley cultivar Scarlett (*Hordeum vulgare* L. ssp. *vulgare*). A population of 301 BC_2DH lines resulting from a backcross of the F1 cross was produced from which the 72 introgression lines derived. This population is known as S42 population. 40 Lines of this population were selected through marker assisted selection, repeated backcrossing with Scarlett and several rounds of selfing were utilized to generate a BC_3S_6 population. Further details can be found in Schmalenbach *et al.* 2008 (Schmalenbach *et al.,* 2008).

2. Phenotypic Evaluation of Root and Shoot Related Traits

2.1. Phenotyping Experimental Setup in 2014 and 2015 for the Genome-Wide Association Study

The phenotypic evaluation in 2014 and 2015 was located in Bonn-Poppelsdorf and set up in a polytunnel which enables natural growth conditions under controlled water conditions. 179 different barley genotypes were phenotyped at terminal drought and well-watered conditions. In both years, the individuals were replicated four times and arranged in a split plot design with one treatment and two levels (control and drought) in sub-plots. The sub-plots were separated in lines in which they were arranged randomly in the polytunnel with close to ambient conditions. The randomization was limited to minimize the effect of the pot position within the tunnel. Therefore, the pot of the drought treated plant was placed next to the control pot of the same genotype. One seed of individual accession was sown in plastic pots (19.5 x 25.5 cm) containing a mixture of topsoil (40 %) and natural sand (60 %) (Cordel & Sohn, Salm, Germany). A drip water irrigation system (Netafilm, Adelaide, Australia) was installed to water the pots three times a day. Additional 0.066 g Kristalon™ fertilizer (Christoffel GmbH & Co.KG, Trier, Germany) per day and pot was given in solution

using the irrigation system. To determine the volumetric moisture content (VMC) the DL2e Data Logger soil moisture sensor was used. At plant development stage BBCH 31 – 34 (Lancashire *et al.,* 1991) the water supply was reduced until reaching the VMC of 5 % within two weeks. The soil moisture was kept at 5 % for another two weeks to conduct the drought stress treatment. Control plants were irrigated without interruption. Plants were harvested after ten weeks of growth. In 2014 and 2015 the mean average temperatures during the experimental period were 13.5°C and 17.7°C and the relative humidity was 68.8 % and 56.3 %, respectively. Five root and shoot related traits were evaluated as followed (**Table 1**).

Table 1: List of phenotypic traits, abbreviation, Unit and method of measurement measured in a pot experiment 2014 and 2015 under control and drought conditions in Bonn-Poppelsdorf.

Trait	**Abbr.**	**Unit**	**MoM**
Tiller Number	Til	no/plant	Count number of tillers one day before invasive measurement
Shoot Dry Weight	Sdw	g/plant	Amount of shoot mass after drying at 50°C for one week
Root Length	Rl	cm	Root length starting from nod
Root Dry Weight	Rdw	g/plant	Amount of root mass after drying at 50°C for one week
Root-Shoot Ratio	RS	-	Dividing Rdw by Sdw

Abbr. = Abbreviation, MoM = Method of measurement

Environmental factors, especially the water supply was observed in the foil tunnel experiment to create similar conditions in years 2014 and 2015 to allow the comparison of the phenotypic results of both years. Therefore, the soil moisture content was observed using the DL2e Data Logger soil moisture sensor. Moreover, the plants were automatically irrigated using a drip water irrigation system (Netafilm, Adelaide, Australia). To react on differences in VMC as quickly as possible the soil moisture was checked every day. VMC under control conditions was at 25 % in 2014 and 2015 during the whole experimental phase; with the exception of the days around the 10th of May 2014 and the 14th of May 2015. These days, the temperature was so high that the VMC dropped to below 25 % for a short period. For the drought treatment, the irrigation was reduced at 22th of April 2014 and 14th of May 2015 until the VMC reached 5%. These conditions were held for two weeks (**Figure 5**).

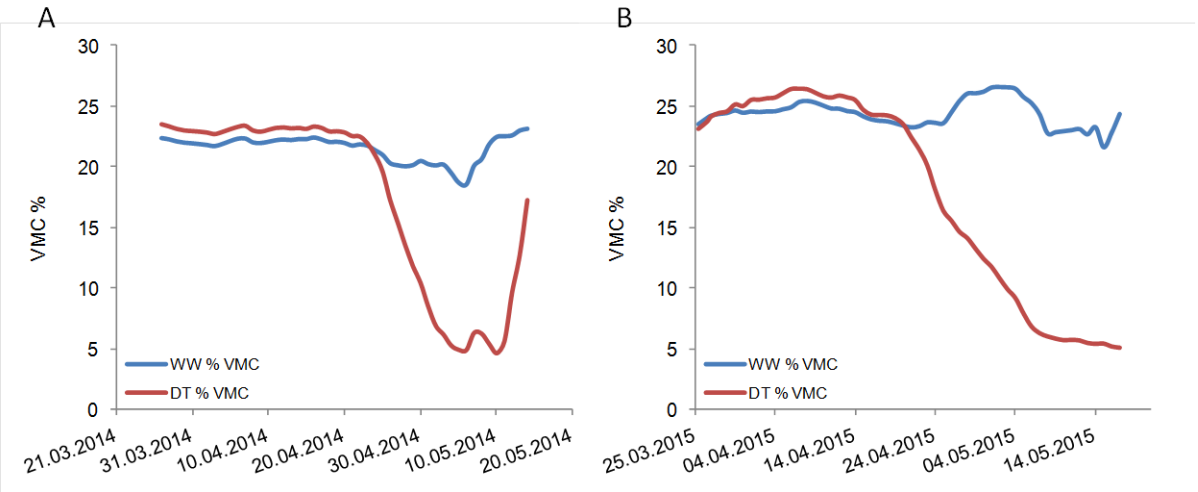

Figure 5: Volumetric soil moisture content (%) for a pot experiment under well-watered conditions (WW) in blue and drought treatment (DT) in red. A) VMC in 2014. B) VMC in 2015. The experiment was performed in polytunnel in Bonn-Poppelsdorf.

2.2. Drought Tolerance Rating

The drought tolerance of each accession was rated using the index calculation of the "Deviation of the relative starch yield from the experimental median" (DRYM) according to Sprenger *et al.* (2015). The DRYM was calculated for Rdw, Rl and Sdw.

$$DRYM_{Gx,Ei} = RelSY_{Gx,Ei} - median(RelSY_{Ei}) \quad (1)$$

$$RelSY = SY_{DT} - \bar{a}(SY_{Gx,WW}) \quad (2)$$

By dividing the phenotypic value under drought stress (SY_{DT}) by the average phenotypic value of the respective cultivar under control conditions ($\bar{a}(SY_{Gx,WW})$) in the same experiment the relative phenotypic value ($RelSY$) was calculated. The deviation of the relative phenotypic value from the experimental median (DRYM) was calculated for each cultivar G_x and Experiment E_i by subtracting the median of the relative phenotypic value of Experiment E_i from the relative starch yield for the respective cultivar and experiment (Sprenger *et al.*, 2015).

2.3. Phenotyping Experimental Setup in 2015 for the Detection of Signs for Evolution

The phenotypic evaluation was located in Bonn-Poppelsdorf in 2015 in a greenhouse and a climate chamber (Viessmann Kältetechnik AG, Hof bei Saale, Germany). The set up in a greenhouse and a climate chamber enables full controlled conditions.

Greenhouse Experiment

Five seeds of barley cultivar Scarlett as well as five seeds of barley NIL S42IL109 were phenotyped terminal drought and well-watered conditions in the greenhouse. The seeds were stratified for two days at 8 °C. One seed per plant was sown in plastic pots (19.5 x 25.5 cm) containing a mixture of topsoil (40 %) and natural sand (60 %) (Cordel & Sohn, Salm, Germany). The individuals were arranged in a split plot design with two treatments (control and drought) in sub-plots. The plants were automatically irrigated three times a day using a drip water irrigation system (Netafilm, Adelaide, Australia). The temperature was set to 20°C at day and 15°C at night. Standard fertilizer and pesticides were given during the whole development. At plant development stage BBCH 56 – 60 (Lancashire *et al.,* 1991) the water supply was reduced until reaching the VMC of 5 % within two weeks. The soil moisture was kept at 5 % for another two weeks to conduct the drought stress treatment. Control plants were irrigated without interruption. Six root and shoot related traits as well as the BBCH stages were evaluated as followed (**Table 2**).

Table 2: List of phenotypic traits, abbreviation, Unit and method of measurement measured in a pot experiment in a greenhouse in 2015 under control and drought conditions in Bonn-Poppelsdorf.

Trait	**Abbr.**	**Unit**	**MoM**
BBA, BSA and CI Stage	BBCH	-	Lancashire et al., 1991
Plant Height	Hei	cm	Distance between soil ground level and leaf tip
Tiller Number	Til	no/plant	Count number of tillers one day before invasive measurement
Leaf Number	Lea	no/plant	Count number of leaves
Shoot Dry Weight	Sdw	g/plant	Amount of shoot mass after drying at 50°C for one week
Root Length	Rl	cm	Root length starting from nod
Root Dry Weight	Rdw	g/plant	Amount of root mass after drying at 50°C for one week

Abbr. = Abbreviation, MoM = Method of measurement, BBA = Biologische Bundesanstalt, BSH = Bundessortenamt, CI = Chemische Industrie

Climate Chamber Experiment

40 seeds of barley cultivar Scarlett as well as 40 seeds of barley NIL S42IL109 were phenotyped under control conditions in a climate chamber (Viessmann Kältetechnik AG, Hof bei Saale, Germany). The seeds were stratified for two days at 8 °C. After stratification the seeds were sown in a 96 well plate (one seed per well) on compound soil (40% top soil and 60% silica sand) (Cordel & Sohn, Salm, Germany). The temperature was set to 24°C at day and 18°C at night with 16h day and 8h night conditions. The plants were irrigated three- to four-times a week by hand if necessary. The germination was checked each day. After emergence of the leaf tip of the first leaf nine traits were evaluated as followed (**Table 3**).

Table 3: List of phenotypic traits, abbreviation, Unit and method of measurement measured in a seedling experiment in a climate chamber in 2015 under control conditions in Bonn-Poppelsdorf.

Trait	**Abbr.**	**Unit**	**MoM**
Days to Germination	DtG	Days	Count days after sowing until germination
Phyllochron 1	Phyt1	Days	Measure first phyllochron according to Itoh et al., 2001
Phyllochron 2	Phyt2	Days	Measure second phyllochron according to Itoh et al., 2001
Phyllochron 3	Phyt3	Days	Measure third phyllochron according to Itoh et al., 2001
Phyllochron 4	Phyt4	Days	Measure forth phyllochron according to Itoh et al., 2001
Leaf Length	Ll	cm	Whole leaf length per plant one and two weeks after germination
Seedling Lenght	Sl	cm	Whole seedling length per plant one and two weeks after germination

Abbr. = Abbreviation, MoM = Method of measurement

3. Genotyping

3.1. SNP-based Genotyping

The germplasm panel was genotyped using the Illumina 9K iSelect SNP chip (Mayer *et al.,* 2012) and the analysis was performed at TraitGenetics (TraitGenetics GmbH, Seeland OT Gatersleben, Germany). The 7842 obtained markers were processed using the criteria as described by Miyagawa *et al.* 2008 (Miyagawa *et al.,* 2008): minor allele frequency (MAF) >0.05; <0.95 for SNP call rate; >0.05 missing values, removing the monomorphic ones were performed using SAS 9.3 (SAS Institute 2008, CARY, NC, USA). A total of 5892 polymorphic markers fulfilled the mentioned cleaning criteria and were used for further analysis.

3.2. CAPS-based Genotyping

Additional to chip based genotyping, a Cleaved Amplified Polymorphic Sequence (CAPS) genotyping was performed to analyze the gene distribution of candidate gene among genotypes of global population as well as S42ILs population (Konieczny and Ausubel, 1993). The CAPS marker derived from a SNP marker which was analyzed as marker under selection. To detect a restriction site polymorphism next to this marker the program DNAStar–SeqBuilder (DNASTAR® Inc., Madison, USA) was used. A specific flanking PCR primer pair (**Appendix Table 11**, *Hv*CAPS002) was designed which allowed the amplification of a 250-500 bp PCR fragment. These fragments possessed the CAPS polymorphism which can be detected in different genotypes by amplifying the specific fragment and subsequent restriction digest using the matching restriction enzyme.

4. Population Structure Analysis

A population structure analysis was performed with 5892 SNP marker using the software package STRUCTURE v2.3.4 with a Bayesian Markov Chain Monte Carlo (MCMC) approach. Settings of calculation are according to Morrell and Clegg (2007): Default admixture and independent allele frequency models were adapted; *K* was set from 1 to 20; burnin period was set to 100000 and the number of MCMC replications after each burnin to 300000. The iteration number was 10. Detection of the value of ΔK was performed with a Markov clustering algorithm implemented in CLUMPAK (Kopelman *et al.,* 2015).

The Kinship matrix was calculated with rrBLUP. FactoMineR was used to calculate the principal component analysis (PCA). We used 5892 SNP marker for the PCA. See marker distribution in **Table 4**. The linkage disequilibrium (LD) for the whole population and groups of genotypes with the same biological status (SPOP 1 = cultivars, SPOP 2 = landraces, SPOP 3 = wild barley) was performed with 5892 polymorphic SNP marker. The PCA (Package: FactoMineR), Kinship matrix (Package: rrBLUP) and the LD (Package: genetics)

were created by using the statistical software R (R Development Core Team (2008)), respectively.

Table 4: Distribution of 5892 polymorph SNP marker across all seven barley chromosomes, the biggest gap between two markers per chromosome and the average marker density per chromosome.

Chr	Chr size (cM)	No of Marker	Marker Gap	Ave Marker Density
1H	133.1	523	4.4	3.9
2H	149.5	925	4.9	6.2
3H	155.0	794	8.8	5.1
4H	115.2	568	7.5	4.9
5H	169.7	1043	5.0	6.1
6H	126.6	663	6.6	5.2
7H	141.4	699	4.8	4.9
Un		677		

Chr = Chromosome

The analysis of the genetic distance of randomly selected genotypes was determined by calculating the Rogers distance (PROC distance) using the software package SAS 9.3. The genetic relationship of those selected genotypes was compared locally and globally for the most significant marker for each trait.

- Local comparison: For the local comparison a 5 cM area left and right of the significant marker was chosen and the Rogers distance was calculated for all markers within this 10 cM region. The marker average over all traits within the 10 cM region was 64.
- Global comparison: For the global comparison, the Rogers distance was computed for all 5892 polymorphic SNP marker.

5. Phylogenetic Analysis

Based on a set of 5892 polymorphic SNP marker a genetic distances matrix was calculated. With this matrix, a phylogenetic tree was calculated with 1000 bootstraps using the neighbor-joining analysis implemented in DARwin 6 (Perrier and Jacquemoud-Collet, 2006). The calculation was performed with two sets of genotypes; a main set of 179 genotypes and a subset of 115 genotypes.

6. Statistical Analysis

A summary statistic was performed by using the software package SAS 9.3. The analysis of variance (ANOVA) was computed with the general linear model (PROC GLM) procedure:

$$Y_{ijk} = \mu + T_i + R_{j(Treatment)} + G_k + G_k\, x\, T_i + G_k\, x\, Y_l + G_k\, x\, T_i\, x\, Y_l + \varepsilon_{ijk} \qquad (3)$$

μ is the general mean, T_i the fixed effect of the *i*-th treatment, R_j fixed effect of the *j*-th replication, G_k the fixed effect of the *k*-th genotype, $G_k x\, T_i$ the fixed interaction effect of the *k*-th genotype with *i*-th treatment, $G_k\, x\, Y_l$ is the fixed interaction effect of the *k*-th genotype with *l*-th year and $G_k\, x\, T_i\, x\, Y_l$ is the fixed multiple interaction effect of the *k*-th genotype with *i*-th treatment and *l*-th year.

To calculate the coefficients for broad-sense heritability (H^2) (Falconer and Mackay, 1996; Holland *et al.,* 2003) variance components were estimated with PROC VARCOMP procedure in SAS: Variance of genotype (V_G), variance of genotype by treatment ($V_{G\,x\,T}$), the variance of genotype by year ($V_{G\,x\,Y}$) and the variance of the experimental error (V_E). Respectively, *t, y* and *r* are the number of treatments (*t* = 2), the number of years (*y* = 2) and the average number of replications (*r* = 3.8).

$$H^2 = \frac{V_G}{V_G + \frac{V_{G\,x\,T}}{t} + \frac{V_{G\,x\,Y}}{y} + \frac{V_{G\,x\,T\,x\,Y}}{ty} + \frac{V_E}{tyr}} \quad (4)$$

A Pearson correlation was performed by using the PROC CORR procedure in SAS. The correlation coefficient was calculated between the five different root and shoot traits: Rdw, Rl, Sdw, Til and RS, respectively.

7. Association Mapping Model

To determine the phenotype-genotype associations we used SNP marker, population structure and kinship matrix data mentioned above. The population structure and kinship matrix were calculated using 5892 polymorphic SNP in the statistical software R. The SNP markers were selected based on minor alleles frequency >0.05, a SNP call rate <0.95 and missing value >0.05. Genome-wide association mapping was performed following the GRAMMAR method described by (Aulchenko *et al.*, 2007), where the population structure was represented by the first principal components and the kinship matrix was included in the marker by trait analysis. A mixed model was used to calculate marker main and marker by treatment interaction effects by using the PROC MIXED procedure in SAS 9.3. For marker by treatment interactions, we first obtained the residual by including the principle components and kinship matrix and calculated the QTL using the residuals as new trait values in a linear mixed model as presented below:

$$Y_{ij} = \mu + M_i + L_i(M_i) + \varepsilon_{ij} \quad (5)$$

where Y_{ijk} is the phenotypic value; μ is the general mean; M_i is the fixed effect of *i*-th marker genotype/haplotype; T_j is the random effect of *j*-th treatment; $M_i * T_j$ is the interaction effect of *i*-th marker with *j*-th treatment; $L_k\ (M_i)$ is the random effect of *k*-th barley line nested within *i*-th marker genotype/haplotype and ε_{ijk} is the residual. To determine traits of interest in the

genome-wide detection analysis a log of odds (LOD) threshold with *p*-value ≤0.0001 and 1,000 permutations was determined. The QTL-model comprises an iterative multi-locus procedure. Therefore, the most informative SNP (QTL) was set as a fixed factor during each calculation iteration step. All remaining marker were again incorporated in the next iteration round and reanalyzed. The starting point of next calculation round was determined by the result of the previous iteration. *P*-values of significant markers were corrected using probability of false discovery rate (PFDR), implemented in the SAS procedure PROC MULTTEST according (Benjamini and Yekutieli, 2005). This procedure was repeated until no marker could be detected, which led to a reduction of significant marker and thereby a reduced number of false positive QTLs. A confidence interval of 5 cM was chosen on both sides of the most significant SNP and designated as putative QTL. SNPs were combined to one joint QTL depending on their estimated (significant) *p*-value from the first iteration of the multi-locus procedure. Therefore, the size of the genetic interval was dependent on the significance value of flanking SNPs. A “leave-20%-out” cross validation procedure was used to increase the validity of all significant SNPs (Sannemann *et al.,* 2015).

8. Detection of Evolutionary Trends

The genotypes were separated in different sub-cluster (SPOP 1, SPOP 2, SPOP 3) based on their genetic composition using the population structure result calculated with STRUCTURE v2.3.4 (Pritchard *et al.,* 2000; Falush *et al.,* 2003, 2007; Hubisz *et al.,* 2009) (**Appendix Table 10**).

SNP outlier analysis among different SPOPs was performed by using the loci outlier detection tool implemented in BayeScan 2.1 (Foll and Gaggiotti, 2008; Foll *et al.,* 2010; Fischer *et al.,* 2011). The detection of outlier loci in BayeScan is based on higher or lower levels of population divergence compared to neutral loci, which suggests diversifying or purifying selection. To reveal the degree of selection to a given SNP, the probability of that SNP under selection will be estimated by calculating the posterior odds (PO). The ratio of the posterior probabilities of the two models (selection/neutral) for each locus due to the allele frequency is defined as the PO. For the calculation, default parameters were adapted with prior odds for neutral model of 10. The membership coefficient (MCo) for each SPOP was set to 0.85. Genotypes with a lower genetic similarity of 0.85 were grouped as admixture and excluded from SNP outlier analysis. The loci outlier analysis among the different SPOPs was iterated 10 times.

9. Candidate Gene Analysis

9.1. Candidate Genes from Genome-Wide Association Study

Marker sequences of most significant QTL were blasted with BLASTn tool implemented in National Centre for Biotechnology Information (NCBI) and IPK Barley Blast Server. Moreover, markers were compared with marker and position of known genes on a Genome Zipper. The coding sequence (CDS) of putative candidate genes was amplified using different sets of primer (**Appendix Table 11,** *Hv*CBF10A, *Hv*CBF10B, *Hv*WRKY29) in four different genotypes (BCC906, HOR4206, ICB181160 and ICB180006). Hereupon, the gene coding sequences (CDS) were sequenced with the LIGHTRUN sequencing approach of GATC (GATC Biotech AG, Constance, Germany). The sequences of different genotypes were aligned by using the MUSCLE alignment approach implemented in MegAlign Pro (DNASTAR Inc., Madison, USA).

9.2. Candidate Genes from Signs for Evolution

The BLASTn tool implemented in National Centre for Biotechnology Information (NCBI) and IPK Barley Blast Server as well as a Genome Zipper was used to detect candidate genes which were identified as loci under selection. Moreover, a mutation bearing S42IL line carrying a small ISR42-8 fragment in Scarlett background was selected for detailed analysis of outlier loci. Hereupon, the CDS of selected S42IL, Scarlett and ISR42-8 was amplified in total using the two primer sets *Hv*RTrans1 as well as *Hv*RTrans2 (**Appendix Table 11**). Gene's CDS was then sequenced using LIGHTRUN sequencing approach of GATC (GATC Biotech AG, Constance, Germany). All sequences were aligned and compared by using the MUSCLE alignment approach implemented in MegAlign Pro (DNASTAR Inc., Madison, USA).

Results

First, the results of the population structure analysis of the highly diverse global barley population are depicted. Hereupon, the traits used in GWAS as well as loci outlier analysis and their variation are described. Detected significant QTL, their effect on each trait as well as a global and local comparison of the genomic regions surrounding each most significant QTL are specified. Likewise, the results from the loci outlier analysis are explained and graphical depicted. Finally, the genes which revealed as candidate genes in GWAS as well as in loci outlier analysis are characterized and analyzed.

1. Population Structure Analysis

Population structure was calculated in order to see the structural pattern of global barley population. The best K value detection implemented in CLUMPAK revealed three distinct sub-clusters (SPOPs) within the population (**Figure 6**). Therefore, kinship and PCA had to be included in association mapping analysis to reduce structural effects during GWAS.

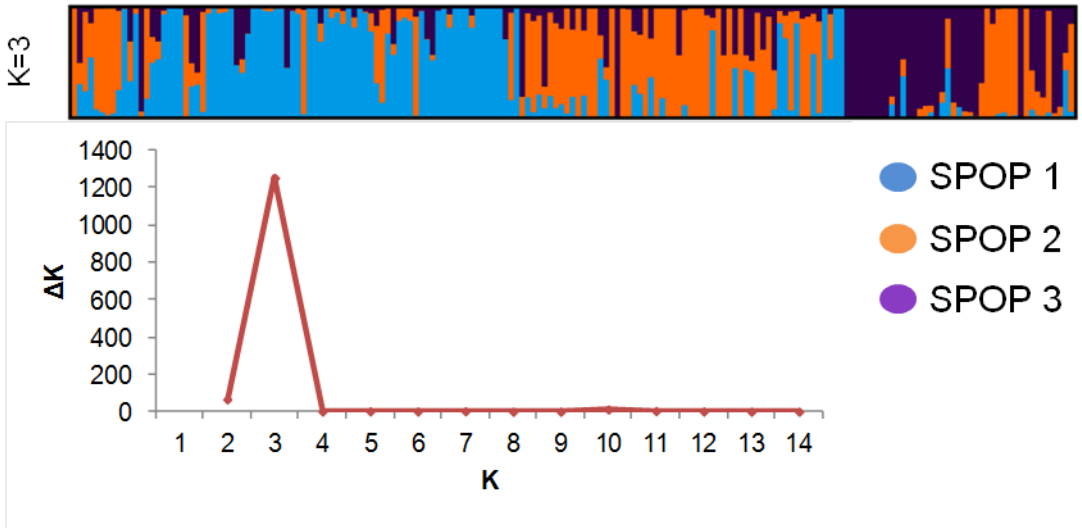

Figure 6: Population structure and genetic differentiation analysis for barley diversity panel. Population structure of 179 accessions calculated with 5892 polymorph SNP marker revealed three sub-groups (K=3). The genetic distribution within each accession is denoted as a colored vertical line. The three different colors represent different sub-groups. Blue: SPOP 1; Orange: SPOP 2; Purple: SPOP 3.

Moreover, based on the MCo the 179 barley accessions were grouped in three different sub-groups SPOP 1, SPOP 2 and SPOP 3. Genotypes which showed a MCo < 0.85 were divided into admixture group (ADMIX). Therefore, the main panel was separated into 115 genotypes within SPOP 1 - 3 and 64 genotypes in ADMIX. Due to high genetic variability in ADMIX the main panel was reduced by the 64 ADMIX genotypes to 115 genotypes for outlier analysis (**Figure 7**). Furthermore, a detailed observation of SPOP 1, SPOP 2 and SPOP 3 revealed a barley subspecies specific distribution (**Table 8**). SPOP 1 (blue) contained 48

genotypes comprising of 35 wild forms and 13 landraces. SPOP 2 (orange) is made of 42 accessions were 13 modern cultivars, 27 landraces and two wild forms are. SPOP 3 comprises of 22 modern cultivars and three landraces (**Figure 7**).

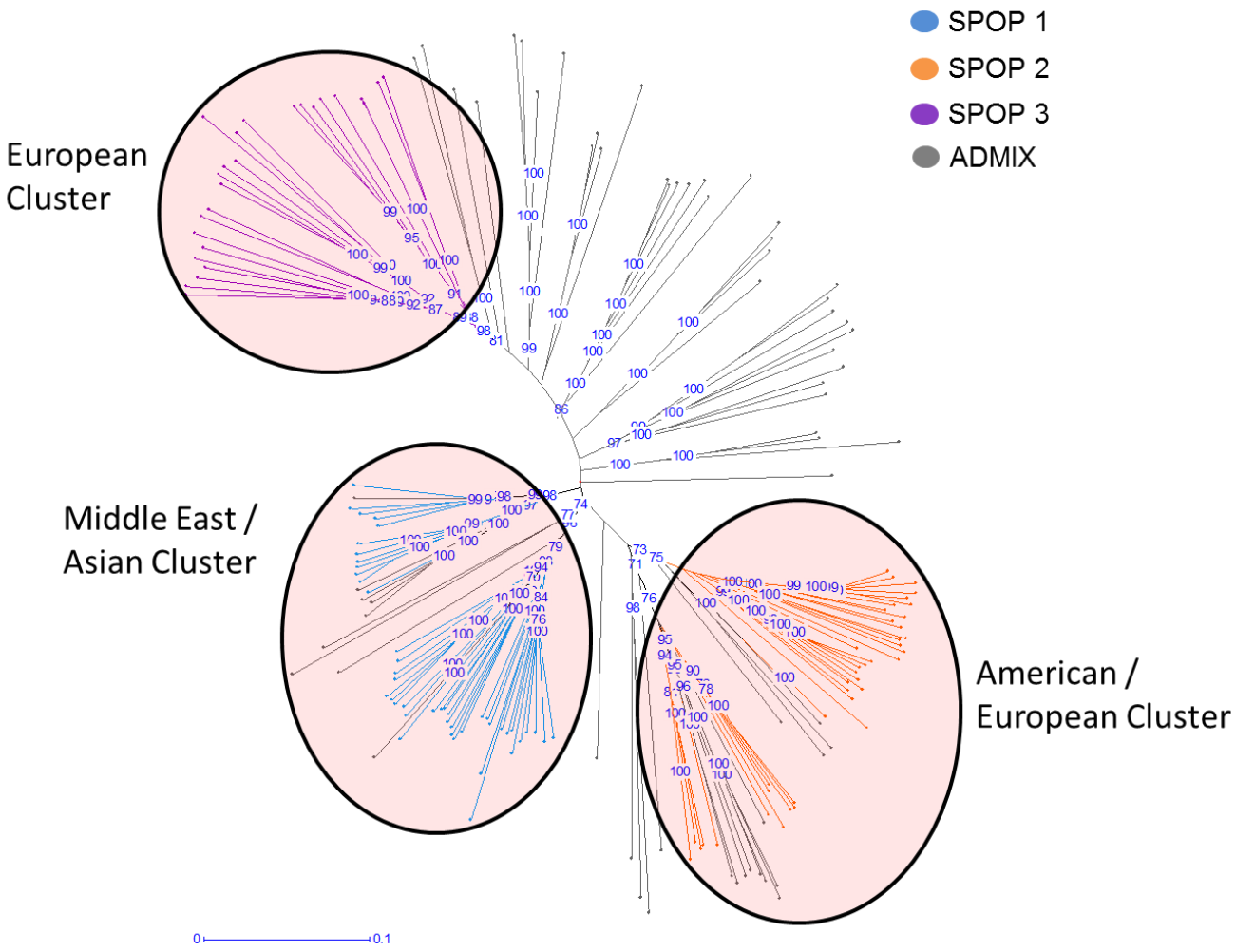

Figure 7: Phylogenetic analysis of 179 barley accessions. Phylogenetic tree (neighbor joining) based on 5892 SNP marker. Bootstraps were calculated with 1000 iterations, the threshold was set to ≥ 80%. The blue numbers represents the percentage of bootstrap. The classification of different accessions into sub-groups due to STRUCTURE analysis with MCo ≥ 0.85; Red transparent circles show the territorial distribution within each SPOP. Blue: SPOP 1 Middle East / Asian Cluster; Green: SPOP 2 American / European Cluster; Orange: SPOP 3 European Cluster; Grey: ADMIX

Moreover, a territorial distribution was detected for the different SPOPs with a threshold of ≥75 %. Based on the collecting site of the accessions and the composition of the SPOPs, SPOP 1 represented a Middle East / Asian Cluster, SPOP 2 an American / European Cluster and SPOP 3 a European Cluster within the reduced global barley panel (**Figure 7**).

For haplotype analysis and genetic distribution among different SPOPs, we computed the global genetic relatedness at the genome level. In order to see the genetic background of genotypes among and within different SPOPs we performed a global comparison of those haplotypic groups. The comparison revealed close genetic relatedness among genotypes within different SPOPs. Furthermore, SPOP 1 showed a high genetic diversity among genotypes of SPOP 2 and SPOP 3. In contrast, SPOP 2 and SPOP 3 possessed a high genetic similarity among different genotypes (**Figure 8**).

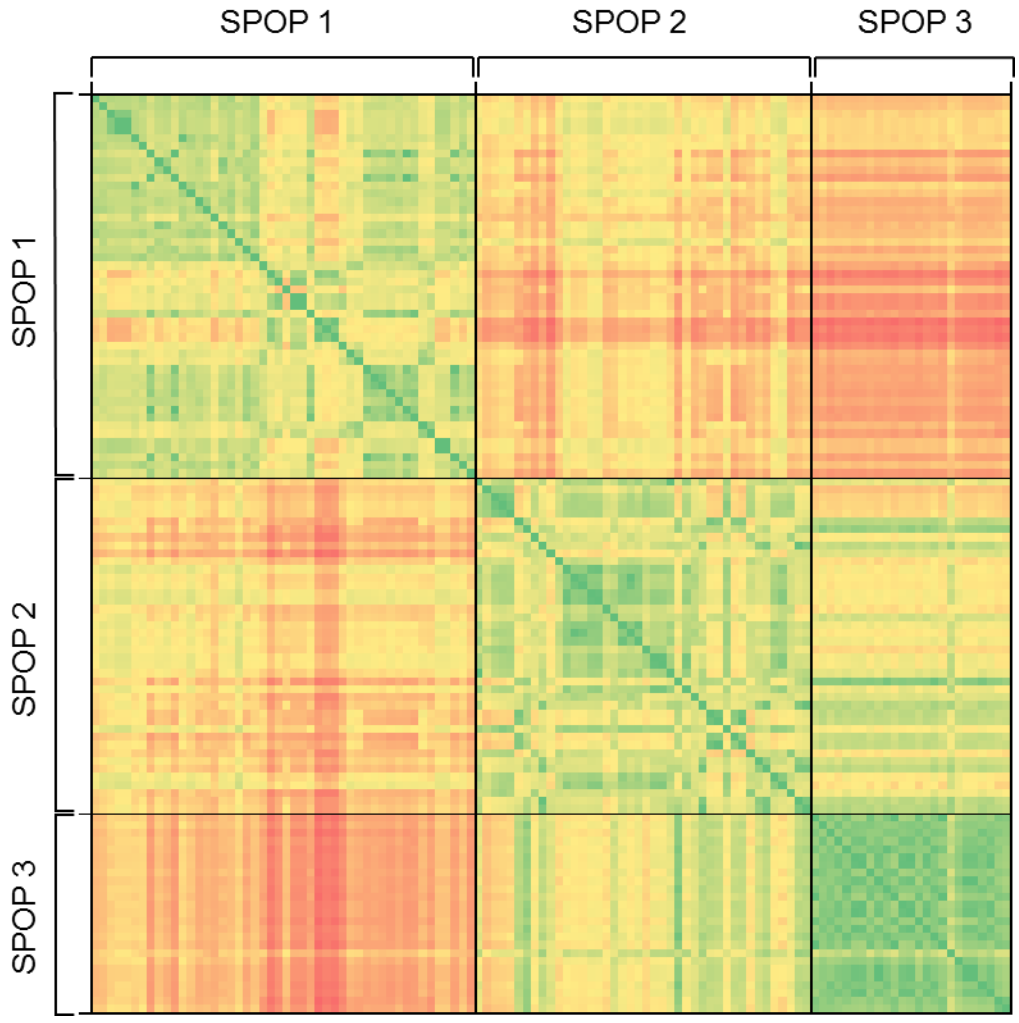

Figure 8: Genetic comparison of genomic groups for different sub-groups (SPOP) of reduced main panel due to MoC ≥ 0.85. Dark green: Rogers distance coefficient of 1.00, dark red: Rogers distance coefficient of 0.00.

Linkage disequilibrium (LD) was calculated to see the genetic recombination across the chromosomes. This revealed the LD-decay for all chromosomes among all genotypes (**Figure 9D**). The recombination fraction of chromosome 7H decreased from 0.17 to <0.1 within 6.7 cM, whereas chromosomes 1H to 6H exhibited r^2 below 0.1. For the purpose of showing differences in genetic recombination due to genomic background of genotypes, we calculated LD for three sub-species: modern cultivars, landraces and wild accessions. The cultivated barley revealed the highest recombination fraction across all chromosomes compared to barley landraces and wild types (**Figure 9A**). On the other hand, wild barley (**Figure 9C**) showed the lowest recombination fraction, whereas barley landraces (**Figure 9B**) possessed a recombination fraction between cultivated barley and wild barley. Overall, the subspecies show a clear pattern of LD-decay for all chromosomes. Furthermore, chromosome 7H revealed the highest recombination fraction compared to chromosomes 1H to 6H for cultivated barley and landraces. Whereas, wild barley SPOP exhibited equal recombination fraction for chromosomes 1H to 7H compared to cultivars and landraces.

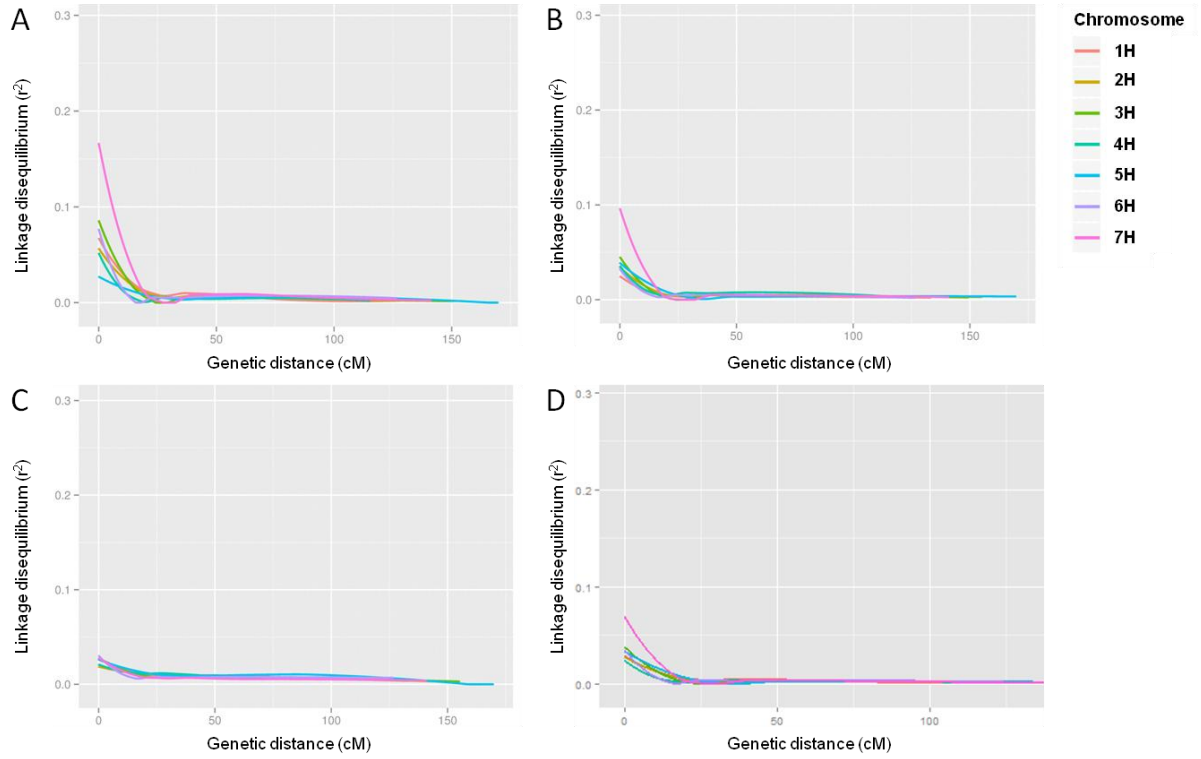

Figure 9: Plot of LD-decay for the global barley population with 5892 SNP marker. The colored lines represent the seven chromosomes (Chr) of barley. A) Plot of LD-decay for barley cultivars. B) Plot of LD-decay for landraces. C) Plot of LD-decay for wild barley lines. D) Plot of LD-decay for the whole global population.

2. Genome-Wide Association Study

2.1. Trait Variation

Five different traits were investigated in a split plot experiment in foil tunnels in 2014 and 2015.

The analysis of variance revealed a high diversity among genotypes within the global barley population. Moreover, the population showed highly significant differences between drought and control conditions for all traits. The effect for genotype by treatment was highly significant for most traits except RI. However, the interaction effect of genotype by year revealed highly significant variations for all five traits. Similarly, the genotype by treatment by year effect showed significant differences for Rdw, Sdw and Til. The broad-sense heritability (H^2) revealed high coefficients for Rdw (0.62), RI (0.48) Sdw (0.54), RS (0.66) and the highest heritability for Til (0.90) (**Table 5**).

Table 5: Variance analysis for five analyzed traits among 179 accessions in 2014 and 2015 under control and drought conditions; the experiment was performed in pots in polytunnel in Bonn-Poppelsdorf

Trait	SOV	DF	MS	F value	*p*-value	H^2
Rdw	Treatment	1	9584.51	800.31	<0.001	0.62
	$Replication_{(Treatment)}$	6	12.61	1.05	ns	
	Genotype	177	35.09	4.54	<0.001	
	Genotype x Treatment	177	11.44	1.48	<0.001	
	Genotype x Year	173	39.70	5.14	<0.001	
	Genotype x Treatment x Year	171	10.10	1.31	<0.01	
RI	Treatment	1	15975.35	315.94	<0.001	0.48
	$Replication_{(Treatment)}$	6	329.11	6.51	<0.001	
	Genotype	177	94.85	2.40	<0.001	
	Genotype x Treatment	177	38.14	0.97	ns	
	Genotype x Year	173	134.55	3.41	<0.001	
	Genotype x Treatment x Year	171	60.94	1.54	<0.001	
Sdw	Treatment	1	119560.89	2908.64	<0.001	0.54
	$Replication_{(Treatment)}$	6	359.57	8.75	<0.001	
	Genotype	177	115.48	5.35	<0.001	
	Genotype x Treatment	177	38.14	1.77	<0.001	
	Genotype x Year	173	206.48	9.57	<0.001	
	Genotype x Treatment x Year	171	29.19	1.35	<0.01	
Til	Treatment	1	18928.75	355.34	<0.001	0.90
	$Replication_{(Treatment)}$	6	54.65	1.03	<0.001	
	Genotype	177	395.32	41.91	<0.001	
	Genotype x Treatment	177	25.75	2.73	<0.001	
	Genotype x Year	173	275.03	29.16	<0.001	
	Genotype x Treatment x Year	171	11.50	1.22	<0.05	
RS	Treatment	1	84.24	284.03	<0.001	0.66

$Replication_{(Treatment)}$	6	0.25	0.83	ns
Genotype	177	1.18	5.78	<0.001
Genotype x Treatment	177	0.31	1.53	<0.001
Genotype x Year	173	0.44	2.17	<0.001
Genotype x Treatment x Year	171	0.30	1.48	<0.001

Trait Rdw = Root dry weight, Rl = Root length, Sdw = Shoot dry weight, Til = No of tiller, RS = Root-shoot ratio, SOV = Sources of variation, DF = Degrees of freedom, MS = Mean sum of squares, *p*-value = indicates the level of significance at 0.05, 0.01 and 0.001, ns: non-significant, H^2 = Heritability

In order to see the relationship of root and shoot traits, Pearson correlation was calculated for Rdw, Rl, Sdw, Til and RS under control and drought conditions (**Table 6**). For Rdw and RS (0.80), the correlation revealed the highest significant positive correlation among all traits under control conditions. Furthermore, Sdw and RS revealed the highest negative correlation under control conditions (-0.53). Rdw and Rl (0.11) showed no correlation under control conditions. Under drought conditions, Rdw and Til showed the strongest positive correlation (0.49). Moreover, the strongest negative correlation under drought conditions was observed for RS and Sdw (-0.47).

Table 6: Pearson correlation coefficients of phenotypic mean values between root and shoot traits under control and drought conditions in 2014 and 2015. The phenotyping was performed in Bonn-Poppelsdorf in pots in a polytunnel

	Trait	**Rdw**	**Rl**	**RS**	**Sdw**	**Til**
Control	**Rdw**	1				
	Rl	0.11**	1			
	RS	0.80***	0.18***	1		
	Sdw	ns	-0.13***	-0.53***	1	
	Til	0.39***	0.14***	0.54***	0.45***	1
Stress	**Rdw**	1				
	Rl	0.14***	1			
	RS	0.42***	0.13***	1		
	Sdw	0.16***	ns	-0.47***	1	
	Til	0.49***	0.25***	0.46***	0.23***	1

Trait: Rdw = Root dry weight, Rl = Root length, RS = Root-shoot ratio, Sdw = Shoot dry weight, Til = No of tiller; *, **, *** = indicates the level of significance at 0.05 (*), 0.01 (**) and 0.001 (***), ns: non-significant

Mean comparison of trait values showed significant variation in the different environments like control and drought conditions as well as in years 2014 and 2015 (**Appendix Figure 31 to 40**). Overall, the trait values were reduced significantly under drought stress conditions as compared to control. The population wide mean comparison showed strong differences for Rdw under control and drought conditions with 9.7 g under control and 5.1 g under drought conditions in 2014 (**Appendix Figure 31**) as well as 6.2 g under control and 3.3 g under drought conditions in 2015 (**Appendix Figure 32**). Similarly, we observed strong differences for Sdw, Til and RS under drought and control conditions (**Appendix Figure 35 to Appendix Figure 40**). The trait Rl revealed least mean differences across drought stress and control blocks (**Appendix Figure 33 and Appendix Figure 34**).

2.2. QTL Detection and Quantification

GWAS analysis revealed 17 significant marker by trait associations for five analyzed root and shoot traits within the global barley population. A total of nine marker among five traits were detected which only showed a significant main marker effect. Three out of 17 significant marker by trait associations revealed only a significant marker by treatment effect, and only five significant marker exhibited a significant main marker and marker by treatment effect (**Table 7**). A QTL map showing the associated and flanking SNP markers across the chromosomes is presented **Figure 10**. For the following quantification is the allele displayed by the nucleotide.

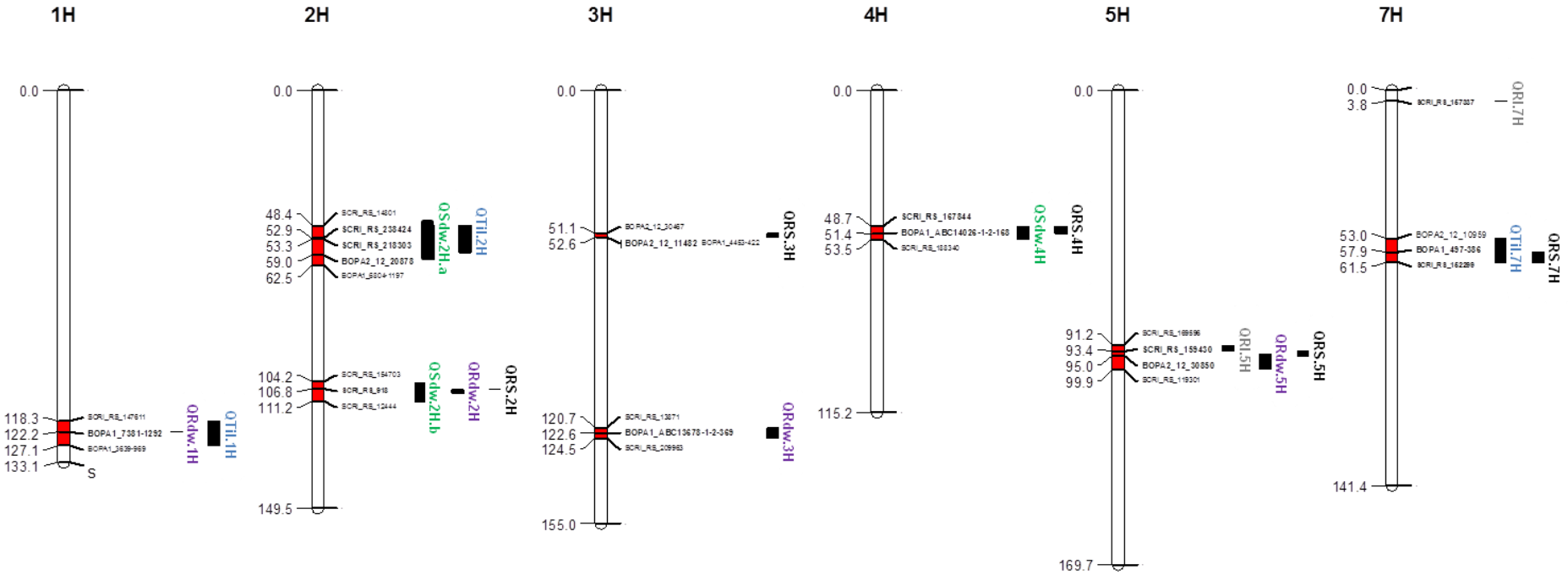

Figure 10: Association mapping for five different root and shoot traits on global diversity panel in molecular linkage map. 17 QTL located on six different chromosomes (1H, 2H, 3H, 4H, 5H and 7H). Purple: Root dry weight (Rdw); Grey: Root length (Rl); Green: Shoot dry weight (Sdw); Blue: Tiller number (Til); Black: Root-shoot ratio (RS). Flaking regions of QTL indicated in red and black bars.

Table 7: List of significant QTL regions for root and shoot traits with marker information and trait effect of particular allele analyzed in the global population.

Trait	QTL	Marker	Effect	Pos (cM)	Flanking region	LOD	Var (%)	Major/Minor	Major	Het	Minor	RP (%)
Rdw	QRdw.1H	BOPA1_7381-1292	M	1H (122.17)	122.09 - 122.17	11.57	13.81	G/A	5.84	4.09	7.48	82.76
	QRdw.2H	SCRI_RS_918	MxT	2H (106.80)	106.79 - 107.97	17.77	18.59	T/C	7.85	7.15	5.60	40.18
	QRdw.3H	BOPA1_ABC13678-1-2-369	M	3H (122.59)	120.68 - 124.54	14.54	20.76	A/G	5.90	6.70	8.39	42.20
	QRdw.5H	BOPA2_12_30850	M / MxT	5H (95.00)	94.44 - 99.93	26.20	24.93	G/A	6.96	7.52	9.48	36.16
Rl	QRl.5H	SCRI_RS_159430	M	5H (93.40)	91.16 - 93.40	16.14	14.29	T/C	46.00	47.50	48.67	5.80
	QRl.7H	SCRI_RS_157337	M	7H (3.82)	3.82 - 3.82	15.37	10.12	C/T	47.33	45.75	44.00	7.57
Sdw	QSdw.2H.a	BOPA2_12_20878	M	2H (58.99)	54.32 - 62.46	40.70	33.66	A/G	20.80	26.62	15.88	67.65
	QSdw.2H.b	SCRI_RS_918	MxT	2H (106.80)	104.15 - 111.26	32.05	27.85	T/C	17.61	19.56	22.85	29.76
	QSdw.4H	SCRI_RS_167844	M	4H (48.65)	48.65 - 53.47	22.66	19.86	G/A	21.24	16.83	16.42	29.32
Til	QTil.1H	BOPA1_7381-1292	M	1H (122.17)	118.34 - 127.09	102.61	53.20	G/A	10.00	14.50	15.00	50.00
	QTil.2H	SCRI_RS_218303	M	2H (53.26)	48.44 - 58.05	39.55	35.91	C/T	11.00	19.50	14.00	77.27
	QTil.7H.	BOPA1_497-386	M / MxT	7H (57.93)	52.97 - 61.47	35.99	28.84	G/A	11.00	13.00	20.00	81.82
RS	QRS.2H	SCRI_RS_918	MxT	2H (106.80)	106.80 - 106.80	14.15	15.08	T/C	0.42	0.38	0.30	38.33
	QRS.3H	BOPA2_12_11482	M / MxT	3H (52.62)	51.14 - 52.62	17.76	13.54	A/C	0.36	0.81	0.37	125.00
	QRS.4H	BOPA1_ABC14026-1-2-168	M	4H (51.40)	48.65 - 51.40	13.94	15.88	A/G	0.34	0.36	0.42	23.53
	QRS.5H	BOPA2_12_30850	M / MxT	5H (95.00)	93.40 - 95.00	66.09	29.30	G/A	0.35	0.38	0.55	57.14
	QRS.7H	SCRI_RS_152299	M / MxT	7H (61.47)	57.93 - 61.47	11.19	12.86	C/T	0.34	0.39	0.39	14.71

Trait: Rdw = Root dry weight, Rl = Root length, Sdw = Shoot dry weight, Til = No of tiller, RS = Root-shoot ratio; M = main effect; MxT = marker by treatment effect; Pos = cM position on chromosome; LOD = LOD score; Var (%) = genetic variation explained by a single QTL; Major/Minor = Major allele and minor allele; Major/Het/Minor = Phenotypic effect of the homozygous major allele, heterozygous allele and minor allele; RP (%)= Relative performance of positive allele compared to negative allele

Root Dry Weight

We detected four putative QTL for Rdw located on chromosomes 1H, 2H, 3H and 5H. Two of them revealed a main marker effect, a marker by treatment effect and a main marker as well as a marker by treatment effect. The summary statistics as well as the relative performance (RP) for all QTL is presented in **Table 7**. Among these, the strongest QTL was QRdw.5H located on chromosome 5H between 94.44 and 99.93 cM, where the minor allele affects the relative performance (RP) by about 36.16%. Another notable QTL was QRdw.1H on chromosome 1H between 122.09 and 122.17 cM which influenced the relative performance positively by 82.76%. The effect of the strongest QTL (QRdw.5H) was visualized in a pin plot to see the allele-wise differences of the phenotype among the whole population. The genotypes carrying the homozygous allele Adenine/Adenine (A/A) of QRdw.5H exhibited the maximum phenotypic effect. On the other hand genotypes bearing the homozygous allele Guanine/Guanine (G/G) showed a moderate phenotypic effect (**Figure 11A**) compared to homozygous A/A allele. Later on, we analyzed the allele-wise distribution of QRdw.5H to detect the major (G/G) and minor (A/A) allele (**Figure 11B**). Genotypes carrying the minor allele are mostly wild barley accessions. Homozygous A/A allele is revealing the highest Rdw (average 13 g) whereas the mean of the homozygous major G/G allele is 5 g. The heterozygous allele showed an average effect in between the homozygous alleles (**Figure 11C**)**.**

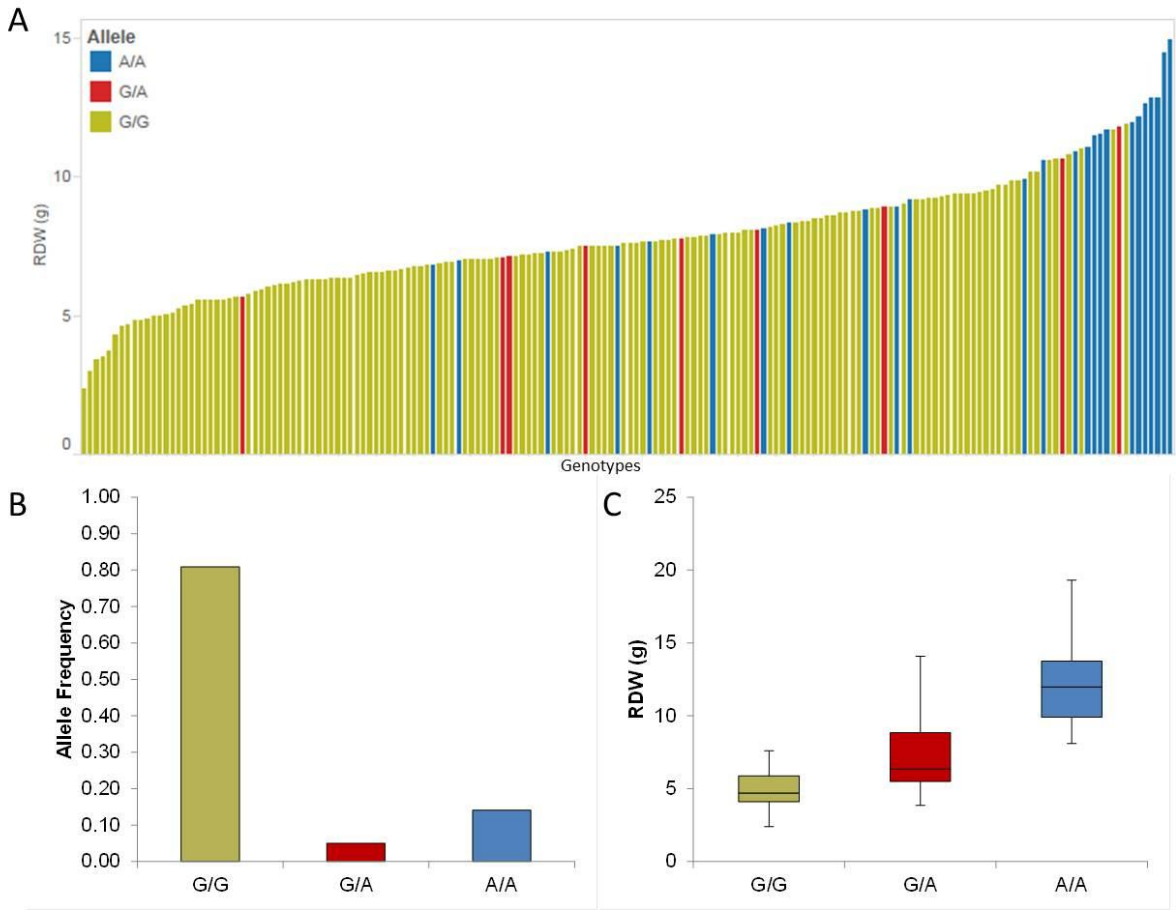

Figure 11: Quantification of allele based trait effect of QRdw.5H. A) Pin plot analysis based on allelic effects for Rdw across the whole population. Genotypes are ordered based on their average Rdw in 2014 and 2015. B) Allele frequency at QTL QRdw.5H. C) Whisker plot for ten randomly selected genotypes per allele to quantify the trait effect of the particular allele, except heterozygous allele / heterogenic line. Yellow: Major allele; Red: Heterozygous allele / Heterogenic line; Blue: Minor allele.

For haplotype analysis, we randomly selected 30 genotypes of most promising QTL regions and computed the local and global genetic relatedness at genome level. Based on the LD analysis we chose a 5 cM area left and right from the particular significant marker for the local comparison. In order to see the genetic background of genotypes possessing homozygous G/G allele and A/A allele we performed the local and global comparison of those haplotypic groups. The local genetic comparison of QRdw.5H for a region between 90.18 cM and 98.89 cM revealed a SPOP based relationship of genotypes for the minor allele A/A. A marginal genetic similarity was observed between SPOP 1 and SPOP 2 after comparing the local genetic composition of both sub-pops. Similarly, the comparison of haplotypic SPOP 2 and SPOP 3 exhibited a moderate overall genetic relatedness like SPOP 1 and SPOP 2. Furthermore, SPOP 1 and SPOP 2 showed a high genetic diversity among genotypes within each haplotypic sub-pop. In contrast, SPOP 3 possessed a high

genetic similarity among genotypes within this SPOP (**Figure 12A**). Like the local comparison, the global comparison of SPOP 1, 2 and 3 displayed a marginal similarity among the genotypes of the different sub-pops. But, the individuals in SPOP 3 revealed a strong genetic relatedness where all individuals carrying the minor A/A allele accounted for higher trait performance (**Figure 12A**). Equally to the local genetic similitude among genotypes within each SPOP and among sub-pops, the global comparison revealed a high genetic similarity among individuals within SPOP 3 but low genetic relatedness among genotypes of other sub-pops and among other sub-pops (**Figure 12B**).

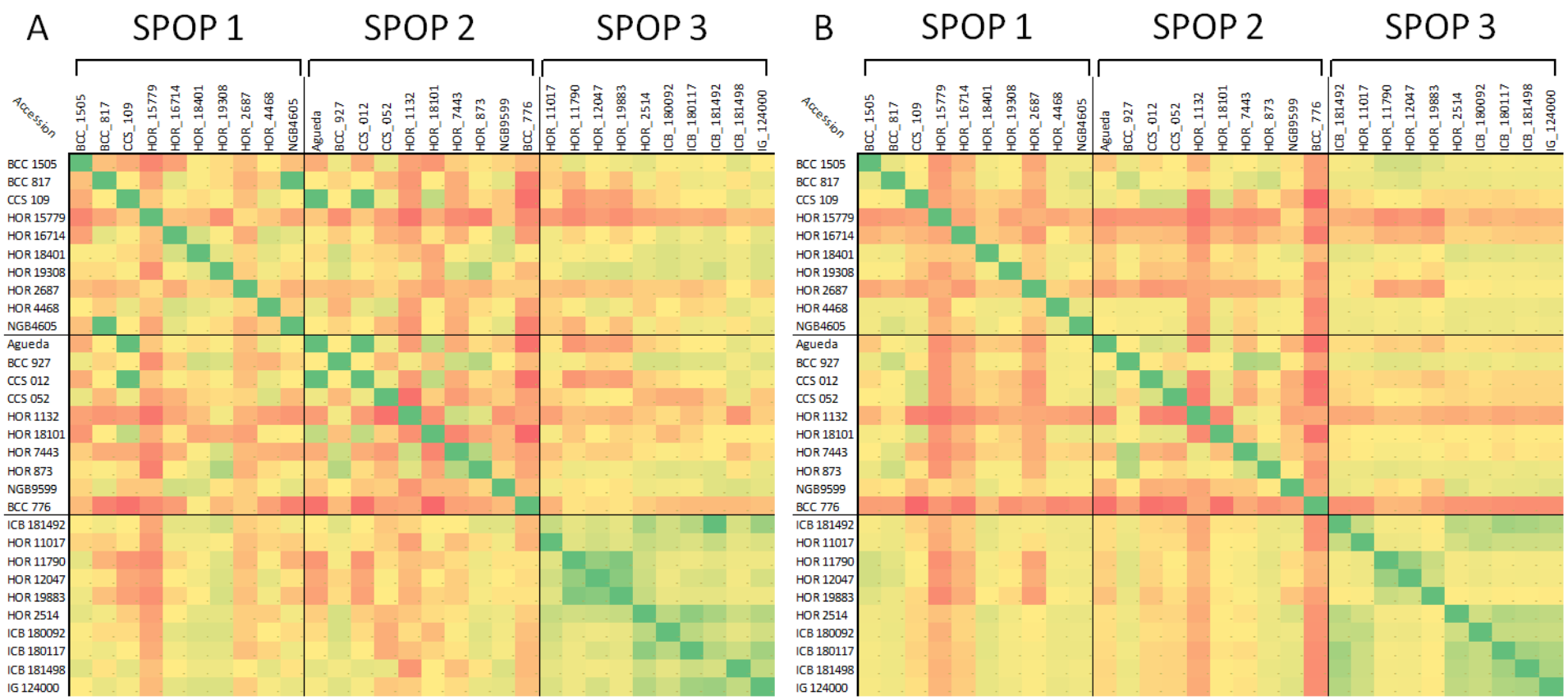

Figure 12: Genetic comparison of local and global genomic groups for QRdw.5H. Each group contains 30 randomly selected genotypes from the global barley population. A) Comparison of local genomic groups. B) Comparison of global genomic groups. Dark green: Rogers distance coefficient of 1.00, dark red: Rogers distance coefficient of 0.00

Root Length

We identified two putative QTL located on chromosomes 5H and 7H. Both significant markers revealed only one main marker effect. According to LOD, chromosome 7H exhibited the strongest QTL at 3.82 cM, QRl.7H (RP: 7.57%), where the homozygous major allele C/C revealed the highest effect on the phenotype. Genotypes carrying the homozygous minor allele were mostly wild accession from the Middle East. The lowest QTL effect was located on chromosome 5H between 91.16 and 93.40 cM (QRl.5H). QRl.5H affected the RP by about 5.8% (**Table 7**).

Shoot Dry Weight

The association mapping for Sdw revealed three significant QTL on chromosomes 2H and 4H (**Table 7**). Two out of these three significant marker exhibited a main marker effect and a marker by treatment effect. Chromosome 2H carried the strongest QTL (QSdw.2H.a) between 54.32 and 62.46 cM which affected the RP by 67.65% (**Table 7**). To see the allele-wise differences of the phenotype among the whole population we visualized the strongest QTL effect in a pin plot analysis. Genotypes carrying the heterozygous allele A/G of QSdw.2H.a exhibited the maximum phenotypic effect compared to other allelic variants. By contrast, genotypes bearing the homozygous G/G allele possessed the moderate phenotypic effect (**Figure 13A**). Hereupon, the analysis of the allele-wise distribution for QSdw.2H.a displayed homozygous A/A as major allele and homozygous G/G allele as minor allele (**Figure 13B**). Genotypes featuring heterozygous/heterogenic Adenine/Guanine (A/G) allele showed the strongest phenotype (average 27 g) while homozygous minor allele G/G revealed moderate phenotype (average 10 g) (**Figure 13C**).

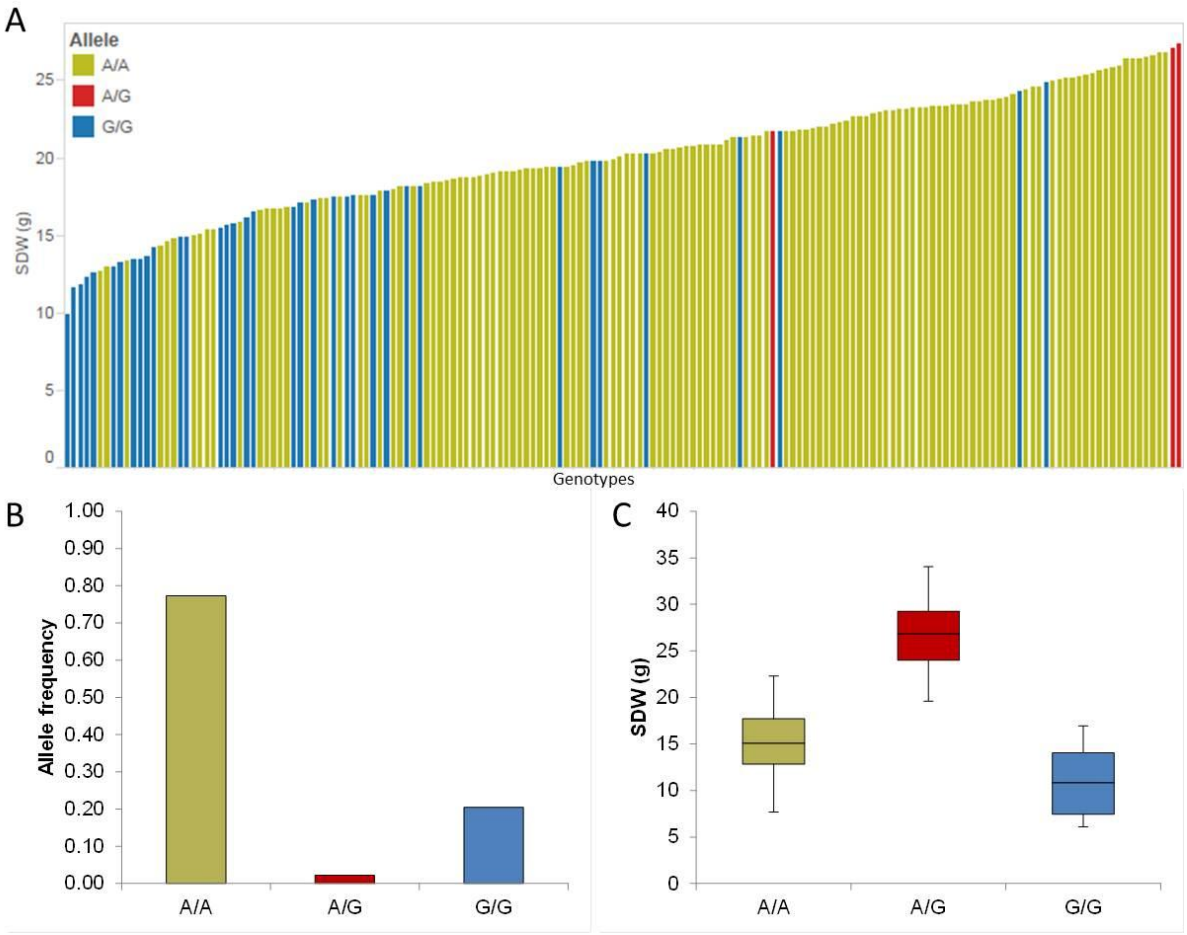

Figure 13: Quantification of allele based trait effect of QSdw.2H.b. A) Pin plot analysis based on allelic effects for Sdw across the whole population. Genotypes are ordered based on their Sdw in 2014 and 2015. B) Allele frequency at QSdw.2H.b. C) Whisker plot for ten randomly selected genotypes per allele to quantify the trait effect of the particular allele, except heterozygous allele / heterogenic line. Yellow: Major allele; Red: Heterozygous allele / Heterogenic line; Blue: Minor allele.

We compared the genetic relatedness of haplotypic groups to see the genetic background at the local and global genomic level of genotypes bearing homozygous A/A allele and G/G allele. For the local comparison the region between 53.26 cM and 63.54 cM on chromosome 2H was chosen. The local overall genetic relatedness of SPOP 1 and SPOP 2 revealed to be distinct due to a high genetic diversity. Similarly, the local comparison of SPOP 1 and 3 and SPOP 2 and 3 showed marginal genetic similarities. Nevertheless, the comparison of genotypes within SPOP 1 revealed a high genetic similarity among those genotypes. By contrast, individuals within SPOP 2 and 3 exhibited a high genetic diversity compared to individuals in SPOP 1 (**Figure 14A**). Likewise to the local genetic similarity among genotypes within each SPOP and the genetic similarity among sub-pops, the global comparison revealed a high genetic similarity among genotypes within SPOP 1 but low genetic similarities among genotypes of other sub-pops (**Figure 14B**).

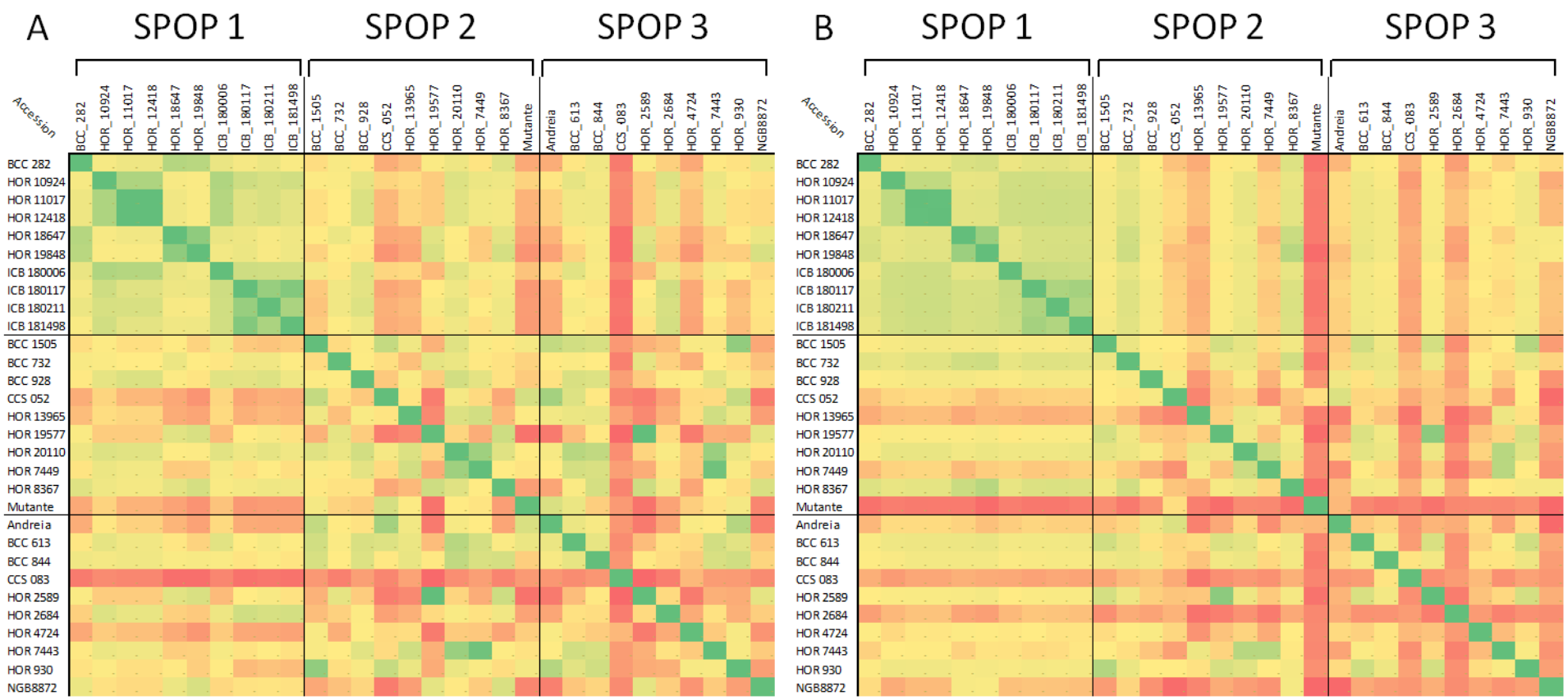

Figure 14: Genetic comparison of local and global genomic groups for QSdw.2H.b. Each group contains 30 randomly selected genotypes from the global barley population. A) Comparison of local genomic groups. B) Comparison of global genomic groups. Dark green: Rogers distance coefficient of 1.00, dark red: Rogers distance coefficient of 0.00

Tiller Number

We identified four significant QTL on chromosomes 1H, 2H and 7H (**Table 7**). The marker on chromosomes 1H and 2H revealed a main marker effect whereas the marker on 7H showed a main marker effect as well as a marker by treatment effect. The strongest QTL (QTil.1H) was on chromosome 1H between 118.34 and 127.09 cM where the minor allele increased the RP by 50%. The allele-wise differences of the phenotype of all genotypes among the whole population for the most promising QTL (QTil.1H) were visualized in a pin plot diagram. Genotypes bearing the homozygous A/A allele revealed the highest phenotypic effect compared to homozygous G/G allele. While, genotypes possessing the homozygous G/G allele showed marginal phenotypes (**Figure 15A**). The homozygous major allele G/G and homozygous minor allele A/A was revealed by an analysis of the allele-wise distribution. Genotypes carrying the minor allele were mostly wild barley accessions (**Figure 15B**). The strongest QTL effect with an average of 28 tillers per plant was shown by genotypes bearing the homozygous minor allele A/A. On the other hand, genotypes possessing the homozygous major allele G/G exhibited the lowest phenotypic effect (average 8 tillers per plant) (**Figure 15C**).

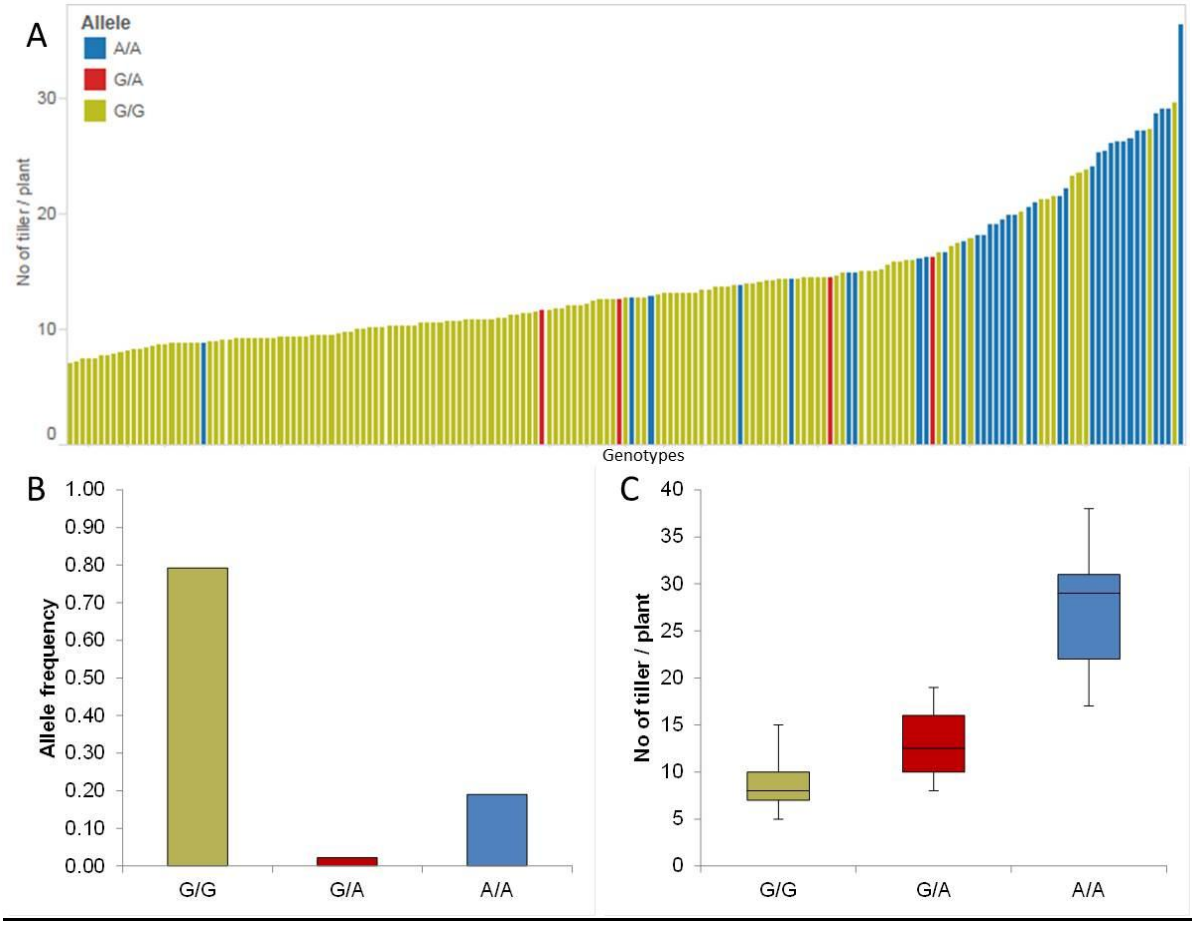

Figure 15: Quantification of allele based trait effect of QTil.1H. A) Pin plot analysis based on allelic effects for Til across the whole population. Genotypes are ordered based on their average tiller number per plant in 2014 and 2015. B) Allele frequency at QTil.1H. C) Whisker plot for ten randomly selected genotypes per allele to quantify the trait effect of the particular allele, except heterozygous allele / heterogenic line. Yellow: Major allele; Red: Heterozygous allele / Heterogenic line; Blue: Minor allele.

To analyze the genetic background of genotypes carrying homozygous G/G allele and A/A allele we computed the local and global comparison of genomic groups. The local comparison was performed for the genomic region of QTil.1H between 117.49 cM and 127.06 cM. The local comparison of SPOP 1 and SPOP 2 displayed a marginal genetic similarity between these sub-pops. Furthermore, SPOP 1 and SPOP 3 and SPOP 2 and SPOP 3 revealed a moderate genetic similarity after comparing their local genetic composition. Moreover, the local comparison of individuals of SPOP 1 just showed a low genetic similarity among those genotypes. Additionally, genotypes of SPOP 2 exhibited negligible similarity among each other. Contrary, the genotypes within SPOP 3 revealed a high genetic similarity to each other but a low genetic similarity to genotypes from other SPOPs (**Figure 16A**).Moreover, individuals in SPOP 3 carrying the homozygous minor allele A/A exhibited the highest trait performance. The global comparison of haplotypic groups at genome level revealed a high genetic similarity among genotypes within SPOP 3 but low genetic similarities among genotypes of other SPOPs and among other SPOPs, likewise the local comparison (**Figure 16B**).

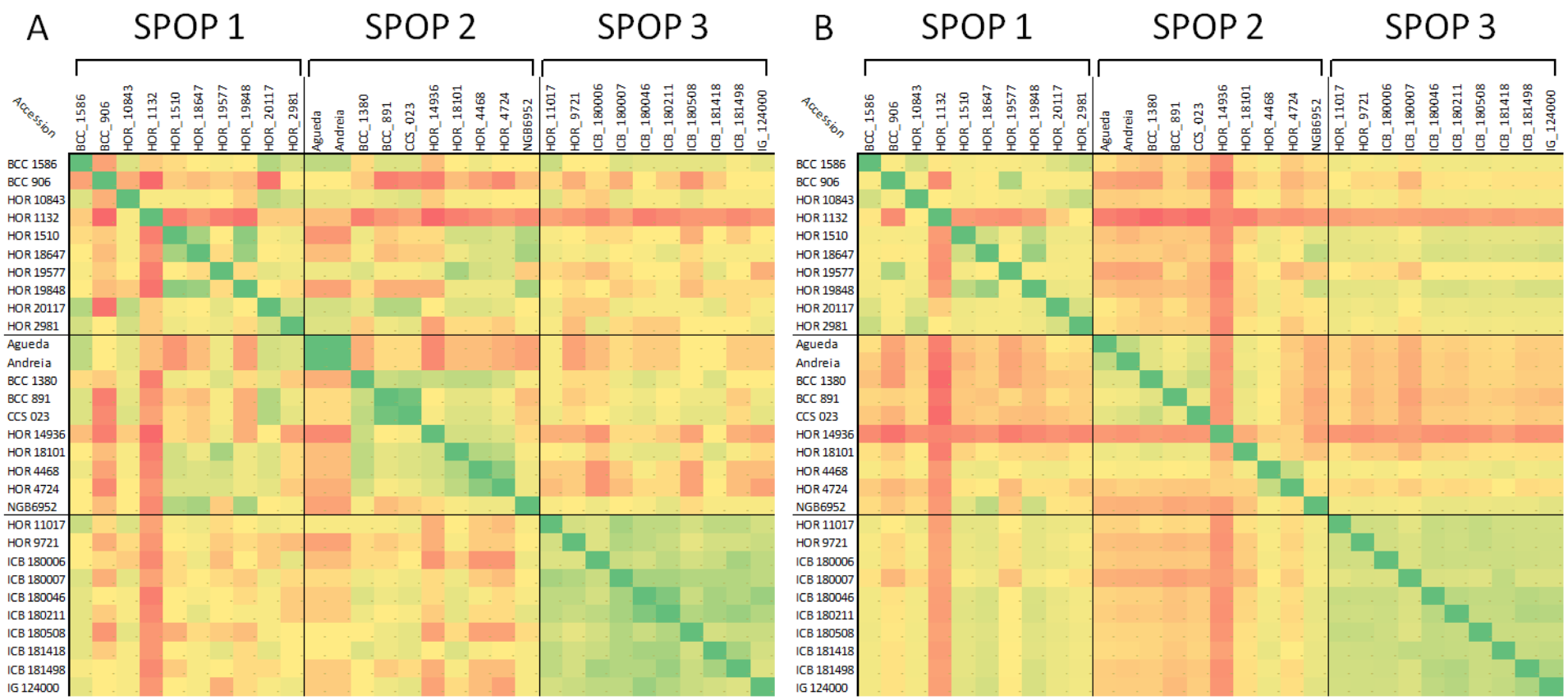

Figure 16: Genetic comparison of local and global genomic groups for QTil.1H. Each group contains 30 randomly selected genotypes from the global barley population. A) Comparison of local genomic groups. B) Comparison of global genomic groups. Dark green: Rogers distance coefficient of 1.00, dark red: Rogers distance coefficient of 0.00

Root-Shoot Ratio

Five putative QTL were detected on chromosomes 2H, 3H, 4H, 5H and 7H (**Table 7**). The marker on chromosome 4H showed a significant main marker effect. On chromosome 2H was a marker located with a significant marker by treatment effect and three marker with significant main marker and marker by treatment effects were assigned to chromosomes 3H, 5H and 7H. The strongest QTL (QRS.5H) lays on chromosome 5H in the region between 93.40 and 95.00 cM, where the effect of QRS.5H minor allele A/A increased the RP up to 57.14%. To analyze the most promising QTL (QRS.5H), we visualized the allele-wise differences of the phenotype among the whole population in a pin plot (**Figure 17A**). Genotypes carrying the homozygous A/A allele featured the strongest phenotypic effect, while genotypes possessing the homozygous G/G allele revealed the lowest phenotype (**Figure 17A**). By analyzing the allele-wise distribution the homozygous G/G allele revealed as major allele and the homozygous A/A allele displayed as minor allele (**Figure 17B**). The homozygous minor allele bearing genotypes showed the strongest phenotype (average 0.9). By contrast, genotypes possessing homozygous major allele G/G exhibited moderate phenotypic effects (average 0.3) (**Figure 17C**).

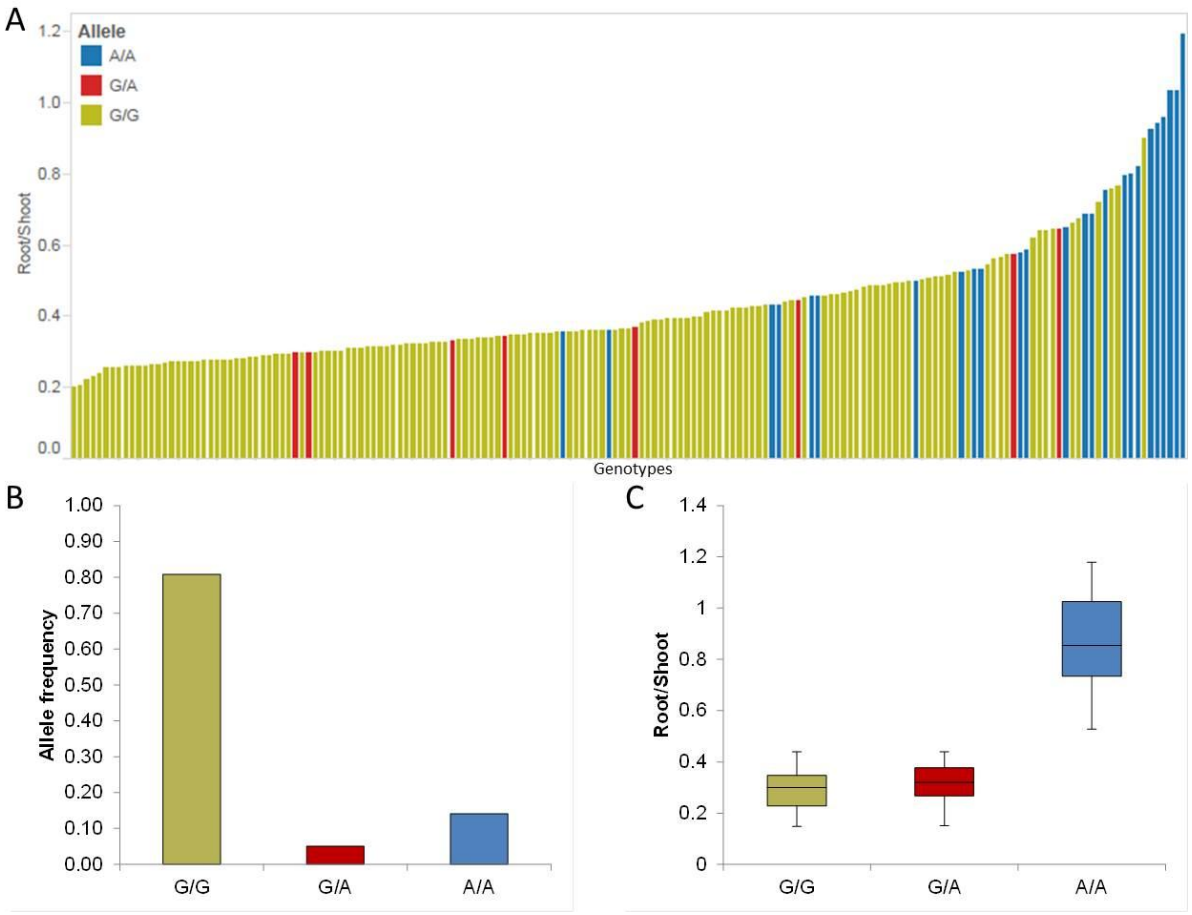

Figure 17: Quantification of allele based trait effect of QRS.5H. A) Pin plot analysis based on allelic effects for RS across the whole population. Genotypes are ordered based on their average root-shoot ratio in 2014 and 2015. B) Allele frequency of QRS.5H. C) Whisker plot for ten randomly selected genotypes per allele to quantify the trait effect of the particular allele, except heterozygous allele / heterogenic line. Yellow: Major allele; Red: Heterozygous allele / Heterogenic line; Blue: Minor allele.

We analyzed the genetic background of genotypes carrying the homozygous major (G/G) allele and homozygous minor allele (A/A) by comparing local and global haplotypic groups at genome level. The local genetic comparison of QRS.5H was done at a region of 90.18 cM to 98.89 cM and revealed low genetic similarities among SPOP 1, SPOP 2 and SPOP 3. On the other hand, the local comparison of individuals within SPOP 3 showed a high genetic similarity among genotypes, except BCC776. While, comparing genotypes within SPOP 2 revealed a low genetic similitude among those genotypes. Equally, genotypes of SPOP 1 possessed a moderate genetic similarity to each other, compared to genotypes within SPOP 3 (**Figure 18A**). The global comparison of selected haplotypic groups displayed a high overall genetic diversity between SPOP 1, 2 and 3, likewise local comparison of haplotypic groups. The global comparison among genotypes within haplotypic groups revealed a high genetic similarity among individuals of SPOP 3 also seen for the local comparison of genotypes in SPOP 3. On the other hand, individuals of SPOP 1 and SPOP 2 showed a low genetic similarity among each other compared to genotypes within SPOP 3 (**Figure 18B**).

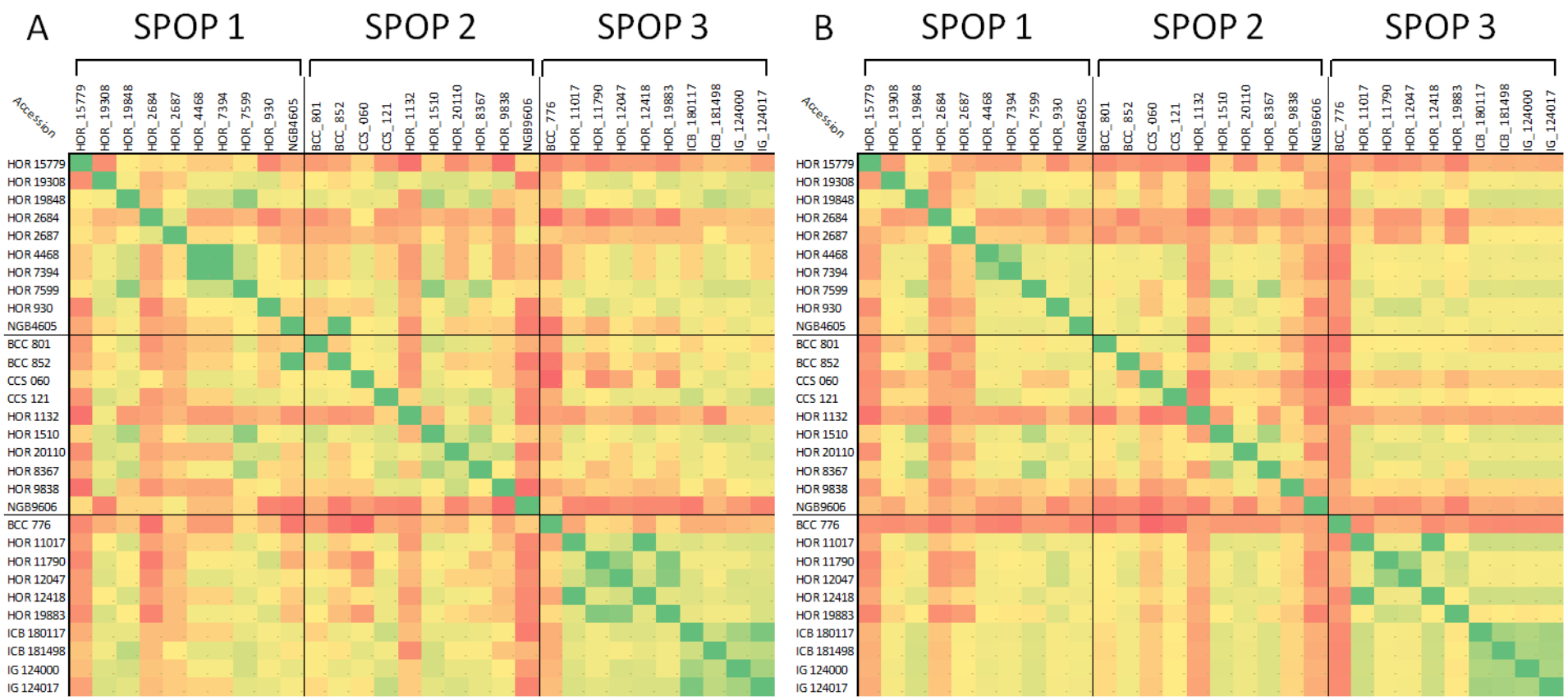

Figure 18: Genetic comparison of local and global genomic groups for QRS.5H. Each group contains 30 randomly selected genotypes from the global barley population. A) Comparison of local genomic groups. B) Comparison of global genomic groups. Dark green: Rogers distance coefficient of 1.00, dark red: Rogers distance coefficient of 0.00

2.3. Drought Tolerance Rating

Due to the agronomic importance of drought tolerant lines for plant breeding genotypes within the global barley population were rated based on their drought resistance and drought susceptibility according to Sprenger *et al.* (2015). The index calculated by equations one and two (Chapter 2.2. Drought Tolerance Rating) includes the median which allows a better differentiation between drought tolerant and susceptible lines. Moreover, the index value is centered to 0 whereby genotypes above 0 are tolerant to drought and genotypes below 0 are susceptible.

In order to see the ability of drought tolerance and susceptibility for lines within the global barley population for three important traits Rdw, Rl and Sdw we calculated the DRYM according to Sprenger *et al.* (2015) to select genotypes favorable for plant breeding. For **Figure 19** we selected the three most tolerant and most susceptible genotypes for Rdw, Rl and Sdw. The most tolerant genotype for DRYM Rdw was HOR19848 a landrace from Japan (0.5). A slightly lower tolerance value showed genotype HOR18401 a wild barley line from Pakistan (0.45). Genotype HOR18101 revealed the lowest tolerance value compared to HOR19848 and HOR18401. HOR18101 is a landrace from Great Britain which showed a tolerance value of 0.4. In contrast to genotypes HOR19848, HOR18401 and HOR181801, the genotypes CCS141 (Modern cultivar from German), HOR1479 (Landrace from China) and HOR2687 (Wild barley from Iran) showed DRYM indices in a range from -0.25 to -0.35. Therefore, those genotypes are drought susceptible. For Sdw, the genotypes CCS041 (Modern cultivar from Germany), NGB4668 (Landrace from Afghanistan) and HOR19848 (Landrace from Japan) revealed index values above 0. The most tolerant genotype was CCS041 with 0.2 but genotypes NGB4668 and HOR19848 showed just slightly lower values (NGB4668: 0.19 and HOR19848 0.18). Moreover, the differences between these tolerant genotypes were much slighter compared to the differences between the susceptible genotypes. Genotypes CCS141 (Modern cultivar from German), HOR1479 (Landrace from China) and HOR2687 (Wild barley from Iran) were rated as susceptible for Sdw. The index values were ranging from -0.2 (HOR1479) to -0.25 (CCS141). Likewise the differences between tolerant genotypes for Sdw, the tolerant genotypes for Rl exhibited just small differences compared to each other. Genotype HOR9565, a landrace from Peru, showed the highest tolerance index with 0.19. Moreover, genotypes ICB181162 and ICB180013, a wild form from Iran and a wild form from Jordan, revealed just slightly lower indices (0.18 and 0.17) compared to HOR9565. In contrast, the susceptible genotypes CCS141 (Modern cultivar from German), HOR1479 (Landrace from China) and HOR2687 (Wild barley from Iran) possessed stronger differences compared to the tolerant genotypes. HOR1479 showed the highest index for susceptible lines with -0.4. The index value of HOR2687 was a little lower to HOR1479 with -0.6. The most susceptible genotype for Rl was the modern cultivar

from Germany CCS141 which had an index of -0.8. Interestingly, for each trait genotypes CCS141 (Modern cultivar from German), HOR1479 (Landrace from China) and HOR2687 (Wild barley from Iran) were rated as susceptible. On the other hand, Genotype HOR19848, a landrace from Japan, was ranked as tolerant genotype for Rdw as well as Sdw (**Figure 19**).

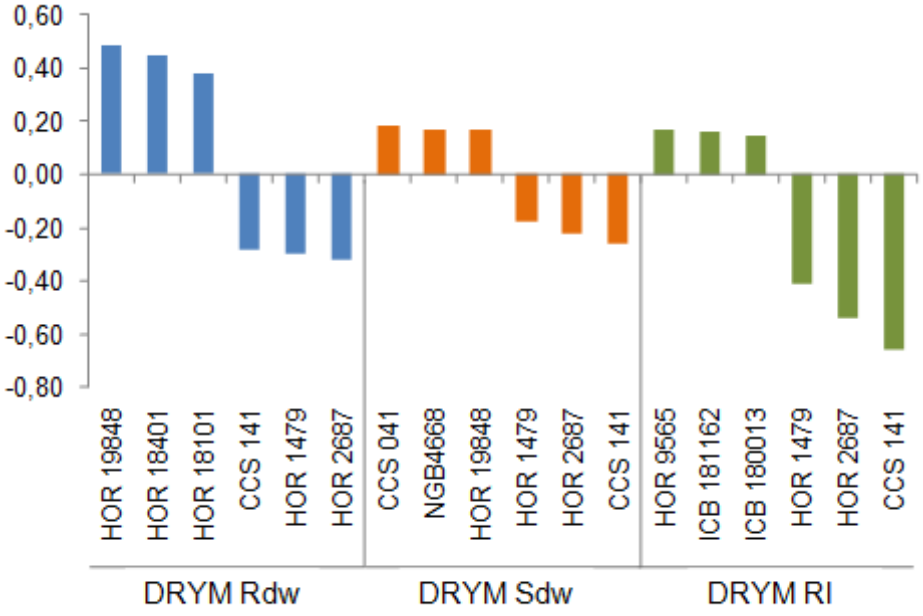

Figure 19: Rating of drought tolerance based on Sprenger *et al.* (2015) deviation of the relative starch yield from the experimental median (DRYM) for three most tolerant and three most susceptible accessions out of global barley population for traits root dry weight (Rdw), shoot dry weight (Sdw) and root length (Rl).

2.4. Candidate Gene Analysis

Putative QTL effects were localized on barley genetic and physical maps to uncover the underlying candidate genes. For this, we focused a hot spot QTL region on chromosome 1H (122.17 cM) associated commonly with shoot and root variation which accounted the highest LOD score for Til. *In silico* analysis of the associated marker BOPA1_7381_1292 with barley Genome Zipper found an essential WRKY transcription factor (WRKY29) gene known for its role in the development of shoot and root (Bakshi and Oelmüller, 2014). Hence, we made full length sequencing of WRKY29 gene in selected genotypes having minor and major QTL alleles for QRdw.1H and QTil.1H. Sequence comparison of selected genotypes along with the reference genotypes revealed two important SNP at positions (+451) and (+515) from ATG (**Figure 20**). The first SNP caused an amino acid substitution of valine 51 (V) to leucine 51 (L) in the conserved domain of WRKY29 protein. The second mutation resulted in the

substation of proline 72 (P) to leucine 72 (L) at the position next to conserved domain (**Figure 21**).

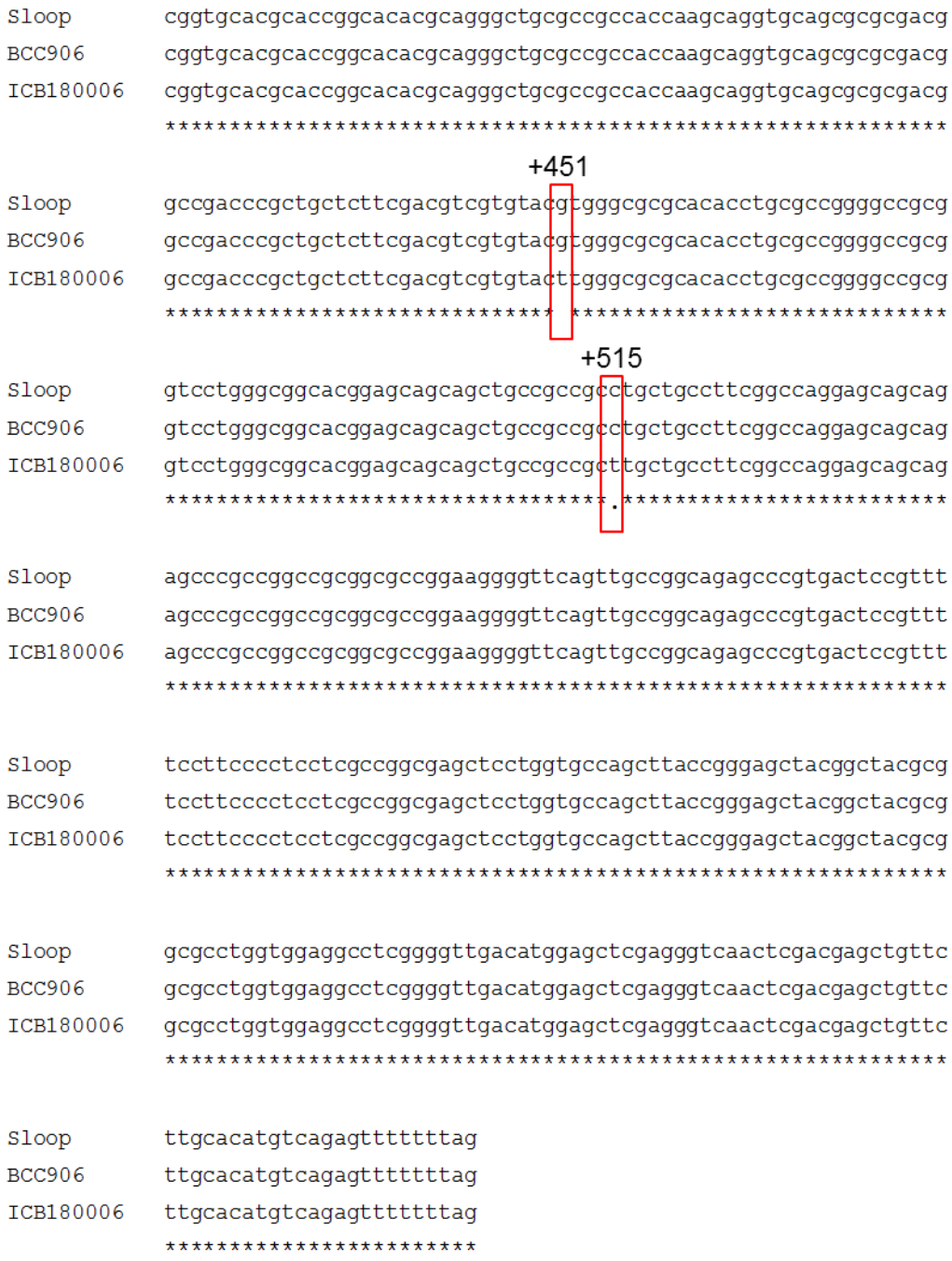

Figure 20: DNA alignment of WRKY29 transcription factor in cultivated barley Sloop (DQ863113, reference sequence) as well as Morex (BCC 906) and wild barley ICB180006 was made using MAFFT alignment software (Nuin *et al.*, 2006). The red boxes indicate nucleotide exchanges. "*" indicates the identical nucleotide in all sequences. ":" indicates conserved substitutions. " " indicates non-conserved substitutions.

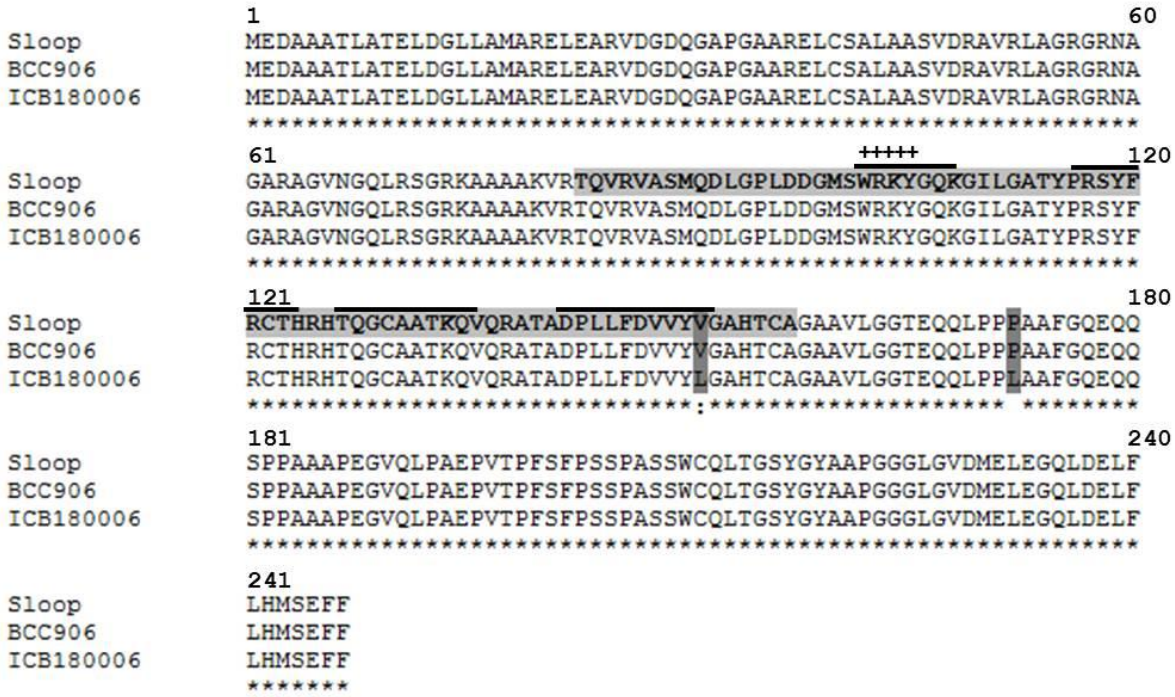

Figure 21: Protein alignment of WRKY29 transcription factor in cultivated barley Sloop (DQ863113, reference sequence) as well as Morex (BCC 906) and wild barley ICB180006 was made using MAFFT alignment software (Nuin *et al.*, 2006). The DNA-binding WRKY domain is indicated by a light gray tag. Amino acid exchanges are indicated by a dark grey tag. "+" indicates the WRKY signature motif. The solid over line indicates an anti-parallel beta-sheet. "*" indicates the identical amino acids in all sequences. ":" indicates conserved substitutions. " " indicates non-conserved substitutions.

The second candidate region we focused, harbor a major QTL affect (QRdw.5H) that accounted for the highest genetic variance for Rdw. This QTL effect was found to be drought inducible as it showed significant M and M x T interaction effects simultaneously. We found drought related regulatory genes CBF10B/CBF10A around 5089 bp away from associated marker BOPA2_12_30850. Sequence analysis of CBF10B among selected genotypes having major and minor QTL alleles of QRdw.5H revealed a major deletion of 111 bp at position +162 (**Figure 22**). This mutation resulted in 37 amino acids deletions in the conserved domain of CBF10B allele originating from wild accession ICB180006 (**Figure 24A**). Sequence analysis of CBF10A in the similar genotypes resulted in seven SNP at positions +53, +168, +177, +219, +252, +294 and +304 from ATG (**Figure 23**). These SNP resulted in amino acid substitutions of which the change of thymine (T) to cytosine (C) at position +304 caused a substitution of serine (S) to proline (P) in the conserved domain of CBF10A gene between major and minor QTL alleles (**Figure 24B**).

Figure 22: DNA alignment of transcription factor CBF10B in different barley accessions using MAFFT alignment (Nuin *et al.*, 2006). Alignment of cultivated barley Optic (Reference sequence) and Cape (HOR 4206) as well as wild barley ICB180006. The red boxes indicate major mutations. "*" indicates identical nucleotide in all sequences. ":" indicates conserved substitutions. "." indicates semi-conserved substitutions. " " indicates non-conserved substitutions.

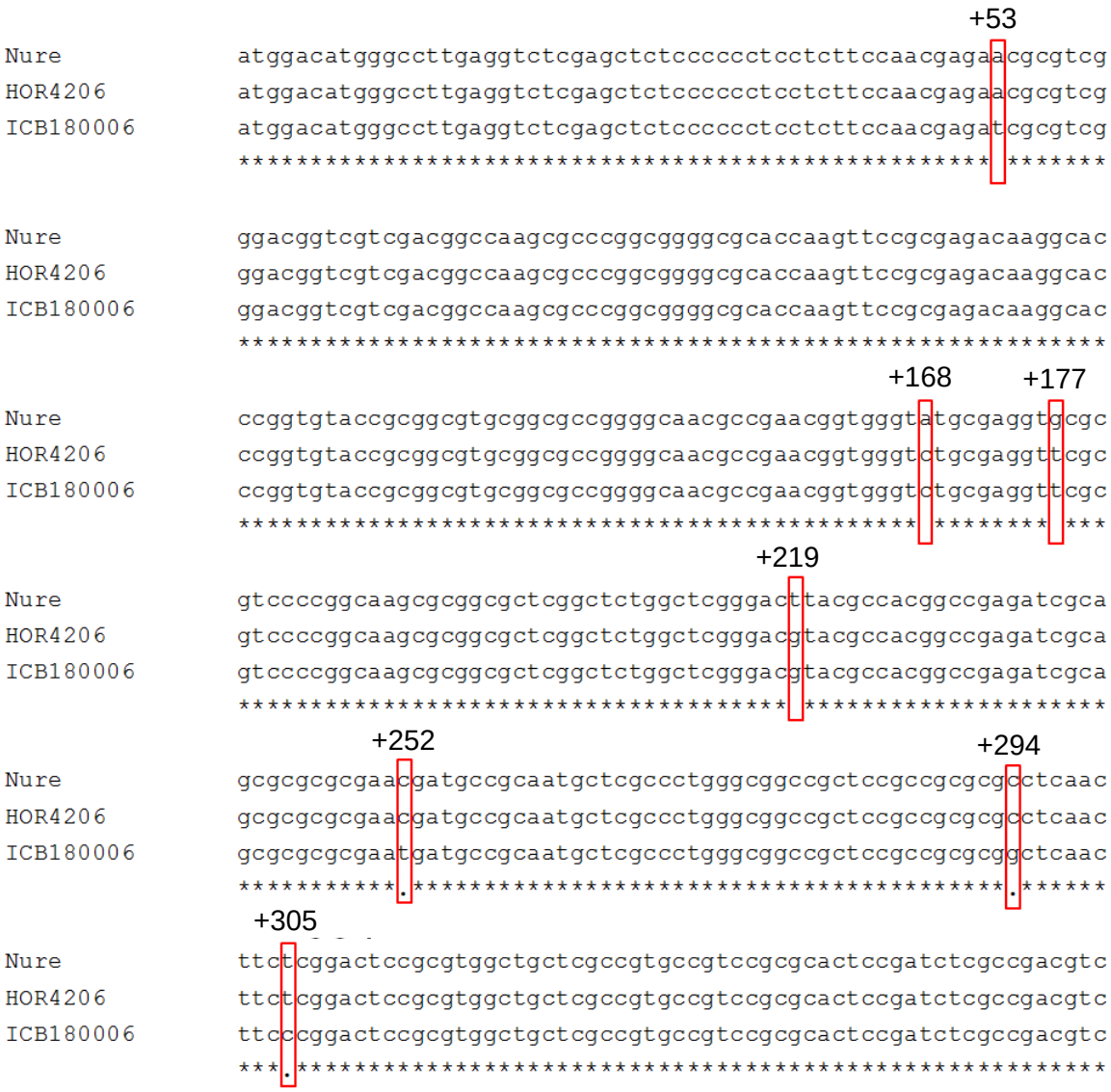

Figure 23: DNA alignment of transcription factor CBF10A in different barley accessions using MAFFT alignment (Nuin *et al.*, 2006). Alignment of CBF10A cultivated barley Nure (Reference sequence) and Cape (HOR4206) as well as wild barley ICB180006. The red boxes indicate nucleotide exchanges. "*" indicates identical nucleotide in all sequences. ":" indicates conserved substitutions. "." indicates semi-conserved substitutions. " " indicates non-conserved substitutions.

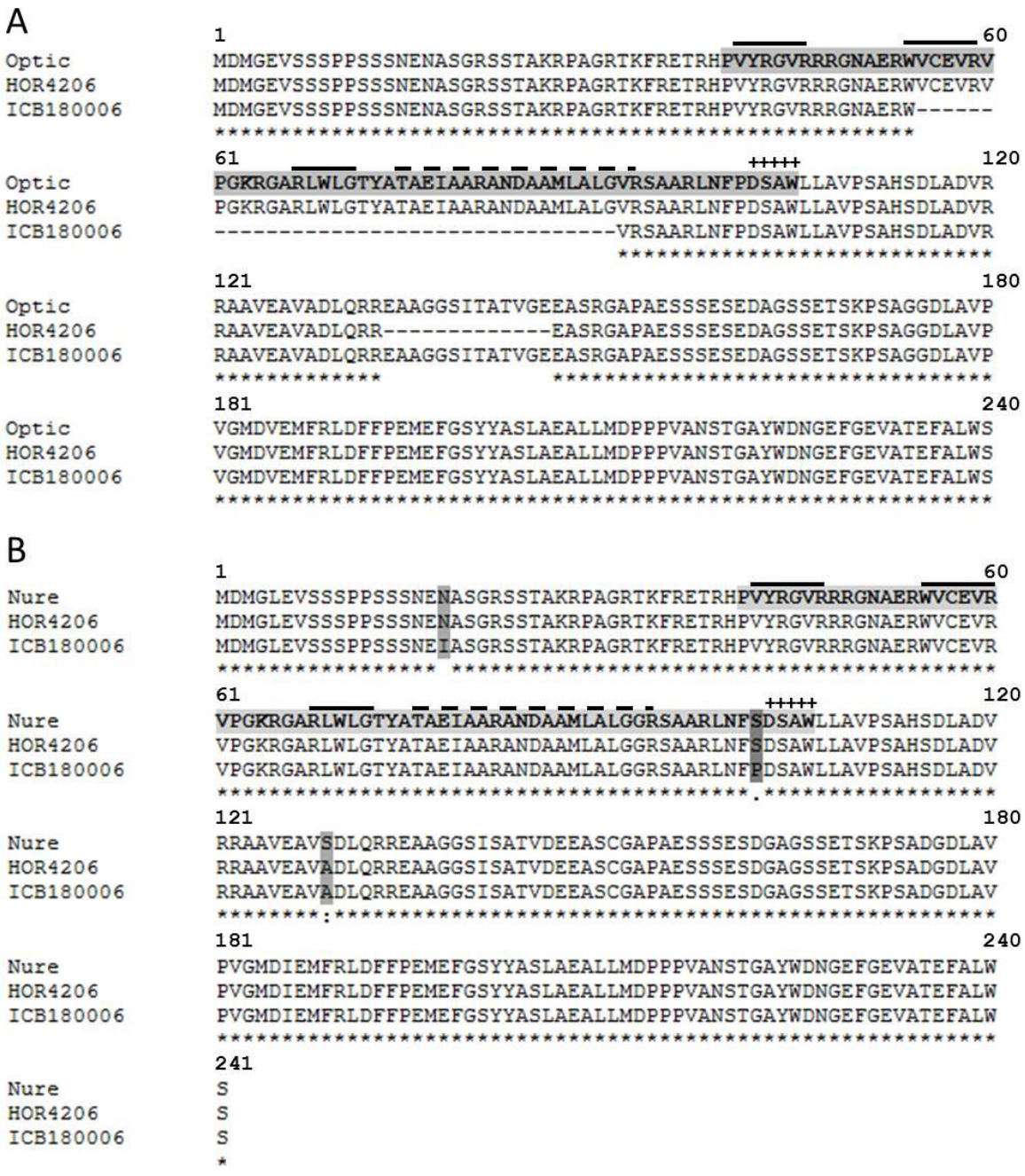

Figure 24: Protein alignment of transcription factors CBF10B and CBF10A in different barley accessions using MAFFT alignment. The DNA (CRT/DRE) binding AP2/ERF domain is indicated by a light gray tag. The dark gray tag indicates amino acid exchanges. “+” indicates the CBF signature motif DSAW signature motif (Jaglo *et al.,* 2001). The solid over line indicates an anti-parallel beta-sheet (Allen *et al.,* 1998). The dashed over line indicates an amphipathic alpha-helix. “*” indicates the identical amino acids in all sequences. “:” indicates conserved substitutions. “.” indicates semi-conserved substitutions. “ “ indicates non-conserved substitutions. A) Alignment of CBF10B in cultivated barley Optic (AAX28956, reference sequence) and Cape (HOR 4206) as well as wild barley ICB180006. B) Alignment of CBF10A cultivated barley Nure (DQ445241, reference sequence) and Cape (HOR4206) as well as wild barley ICB180006.

3. Evolutionary Analysis of Global Barley Population

3.1. Detection of Signs for Evolution

We used the loci outlier detection tool BayeScan to identify footprints of selection among SPOPs within the global barley population. Five outlier loci were detected among different SPOPs, one outlier locus between SPOP 1 / SPOP 2 (**Figure 25A**)and four outlier loci between SPOP 1 / SPOP 3 (**Figure 25B**), but we did not detect any outlier loci between SPOP 2 / SPOP 3 (**Figure 25C**). Each identified outlier showed a positive alpha value which indicated directional selection. One of the five detected loci was detected between SPOP 1 / SPOP 2 as well as SPOP 1 / SPOP 3. The investigated locus between SPOP 1 and SPOP 2 showed the highest FST-value of all five identified loci (FST = 0.41). Moreover, the four loci between SPOP 1 and SPOP 3 showed FST-values ranging from 0.27 to 0.37. The strongest outlier locus between SPOP 1 and SPOP 2 as well as SPOP 1 and SPOP 3 shared the same SNP marker: SCRI_RS_170235.

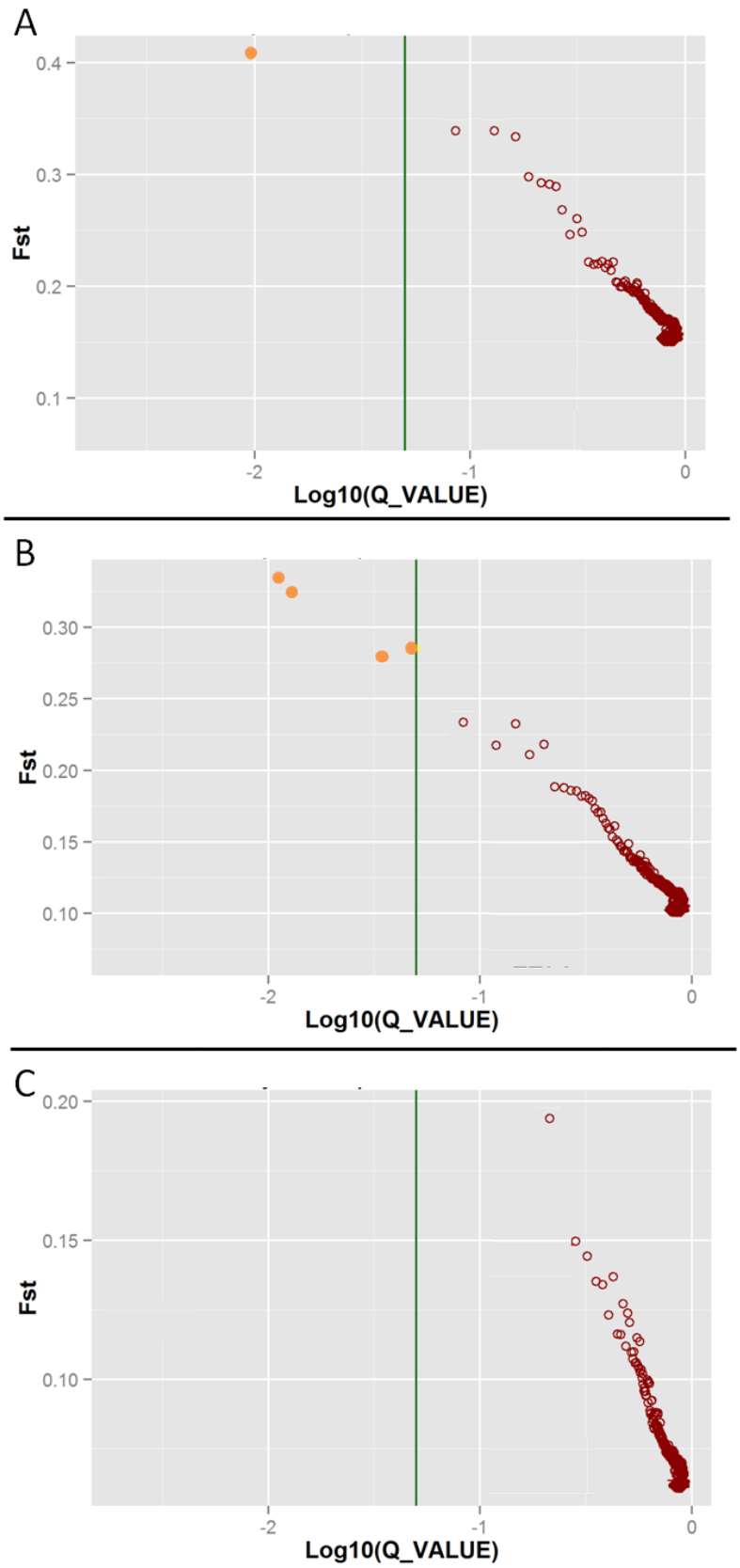

Figure 25: SNP outlier analysis among different SPOPs calculated with 5892 SNP marker. Green vertical line indicates threshold (FDR ≤ 0.05). Orange dots are significant outlier loci. Red circles are non-significant marker. A) Outlier analysis between SPOP 1/2 showed one significant outlier. B) Outlier locus analysis between SPOP 1/3 revealed four significant outlier loci. C) BayeScan outlier analysis between SPOP 2/3 showed no significant outlier.

3.2. Candidate Gene Detection and Analysis

To evaluate the evolutionary potential of SNP marker SCRI_RS_170235 we performed a BLASTn analysis of the SNP marker sequence. The analysis revealed AK366024 as a candidate gene which we fully amplified with a set of primer, sequenced and aligned. Further, we designed a CAPS marker to genotype the whole global barley population. Moreover, we investigated the S42IL NIL-library for a detailed evaluation of the point mutation within AK366024 CDS.

CAPS Marker Analysis

Eight genotypes, four modern cultivars (RBC170, RBC171, RBC173, Scarlett) and four wild barley accessions (RBC039, RBC040, RBC045, ISR42-8) were amplified, sequenced and aligned to detect any differences among those genotypes. The sequence alignment revealed a 1 bp deletion in modern cultivars at position 224 bp after the ATG compared to wild barley accessions (**Figure 26A**). This additional thymine at position 224 bp in wild barley led to an *Ava*II restriction site within AK366024. The restriction site enabled the development of CAPS derived marker to genotype the whole global barley population for this 1 bp deletion. Genotypes carrying the additional thymine should exhibit two PCR fragments, a small 75 bp fragment and a large 227 bp fragment. Genotypes missing this thymine should show one large 302 bp fragment (**Figure 26B**).

Figure 26: CAPS marker digest and sequence alignment of genotypes from global barley population. A) Genomic sequence alignment of 67 nucleotides of AK366024 among two barley wild accessions (RBC040, RBC045) and two barley cultivar accessions (RBC170, RBC171). The green marked nucleotides show matches to consensus sequence of wild barley accessions whereas cultivars revealed a 1 bp deletion. B) Agarose gel showing *Ava*II restriction digest of 48 genotypes from global barley population. The fragment reveals the presence or absence of a thymine at position 224 bp after ATG of AK366024 sequence. Fragment sizes are 302 for genotypes missing the thymine and 227 bp for genotypes carrying the thymine.

Population Distribution

Based on the CAPS marker analysis we evaluated the distribution of this mutation within the reduced barley panel, it revealed that 100 % of the genotypes within SPOP 1 possessed the additional thymine. Moreover, this SPOP included 35 wild barley accessions as well as 13 landraces. On the other hand, in 52.38 % of SPOP 2's genotypes the thymine is missing which resulted in the large 302 bp fragment. Further, 38.10 % genotypes are carrying the additional thymine. Moreover, 9.52 % of the genotypes within SPOP 2 were heterozygous / heterogenic and showed the 302 bp fragment as well as the 227 bp and 75 bp fragments. SPOP 2 was made of 42 genotypes which included 24 landraces and 18 modern cultivars. In contrast to SPOP 1, SPOP 3 comprised 100 % genotypes which are missing the thymine at position 224 bp after ATG. This SPOP is made of 25 genotypes whereas three are landraces and 22 are modern cultivars (**Table 8**). Based on the mentioned findings we identified a SPOP specific pattern of the distribution of this mutation. Besides this SPOP specific distribution, we discovered a distribution based on the biological status of each genotype. 100 % of the wild accessions within the reduced panel possessed the AK366024 sequence with the additional thymine. On the other hand, only 55 % of the landraces carried the additional thymine whereas 40 % missing the thymine and 5 % were heterozygous. For modern cultivars, the amount of genotypes carrying the additional thymine is 17.5 %. Moreover, 77.5 % of all modern cultivars within the reduced germplasm panel showed the sequence with the missing thymine and 5 % were heterozygous (**Table 8**).

Table 8: Subspecies-based and sub-groups based distribution of 115 barley accessions. The table read from left to right shows the distribution of WT, L and C as well as the distribution of the point mutation within the different SPOPs. The table read top down shows the distribution of the point mutation within each barley subspecies.

	WT	L	C	**Total**	% -T	% T	% T/-T
SPOP 1	35	13	0	**48**	0.00	100.00	0.00
SPOP 2	0	24	18	**42**	52.38	38.10	9.52
SPOP 3	0	3	22	**25**	100.00	0.00	0.00
Total	**35**	**40**	**40**	**115**			
% -T	0.00	40.00	77.50	55.65			
% T	100.00	55.00	17.50	40.87			
% T/-T	0.00	5.00	5.00	3.48			

SPOP 1: sub-group 1, SPOP 2: sub-group 2, SPOP 3: sub-group 3, WT: Wild form, L: Landrace, C: Modern cultivar, % -T: Percentage of genotypes missing thymine within CDS of AK366024, % T: Percentage of genotypes carrying an additional thymine within CDS of AK366024. % T/-T: Percentage of heterozygous / heterogenic genotypes

Further, we detected a geographical distribution of the different within for the reduced global panel. Based on the accession composition of each SPOP we grouped each SPOP into different geographical locations. In SPOP 1, 90 % of the genotypes came from the Middle East and Asia. In detail, the collection site of 48 % of the accessions is the Middle East, 42 % were collected in Asia and 10 % in South Europe as well as North Africa. In contrast to SPOP 1, SPOP 2 was more admixed due to geographical position of collection site. Within this SPOP 71 % of all accessions were collected in America (North and South America) and Europe. Moreover, 29 % of the accessions within SPOP 2 came from North Africa and Asia. The geographical distribution within SPOP 3 was less various than in SPOP 1 and SPOP 2. Nearly all accessions (92 %) within SPOP 3 were collected in Europe. Only 8 % of the accessions came from regions outside Europe. Based on the results of the geographical distribution we ordered SPOP 1 into a Middle East / Asian cluster, SPOP 2 in a European / American cluster and SPOP 3 in a European cluster (**Figure 7**).

Gene Characterization

Additional to the analysis of the global diversity panel, we surveyed NILs created by several crossings of German modern cultivar Scarlett and Israel wild barley ISR42-8. Further, we performed a sequence analysis and expression analysis of candidate gene AK366024 to detected structural differences and expression differences of wild type and cultivar AK366024 due to the point mutation within CDS. All genotypes of NIL library carrying a ISR42-8 fragment on chromosome 2H were genotyped with the CAPS marker to detect a line which showed the 1 bp insertion. Further, we selected one line which exhibited the smallest ISR42-8 introgression to reduce background mutation for later comparison. **Figure 27** showed the Agarose gel of the *Ava*II digest of Scarlett (Sca), ISR42-8 (ISR), S42IL102 (102), S42IL106 (106), S42IL107 (107) and S42IL109 (109). Scarlett and ISR42-8 revealed different fragments after restriction digest due to the present *Ava*II restriction site in ISR42-8 genotype. Scarlett showed a large 302 bp fragment. In contrast, ISR42-8 exhibited a 227 bp fragment and a small 75 bp fragment due to the *Ava*II restriction site resulting from an additional thymine within AK366024. Furthermore, NILs S42IL102, S42IL106 and S42IL107 showed an identical 302 bp fragment like Scarlett. On the other hand S42IL109 revealed two small fragments (227 bp and 75 bp) equally to ISR42-8. The two fragments in S42IL109, equal to ISR42-8's restriction fragments, uncovered this genotype as a candidate for further phenotypic analysis of point mutation in AK366024.

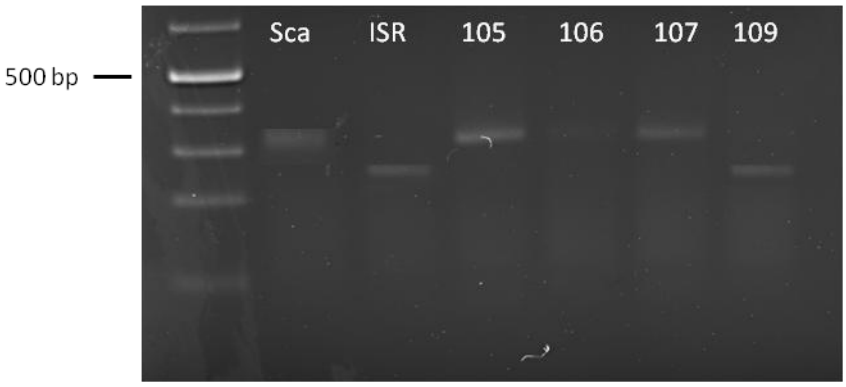

Figure 27: CAPS marker digest of Scarlett (Sca), ISR42-8 (ISR), S42IL102 (102), S42IL106 (106), S42IL107 (108) and S42IL109 (109). Scarlett showed the 302 bp fragment because of the missing cleavage site of *Ava*II due to the missing thymine. ISR42-8 showed the 227 bp fragment resulting from an *Ava*II cleavage site. S42ILs 102, 106 and 107 were showing the 302 bp fragment due to the Scarlett DNA. S42IL109 revealed the 227 fragment because of an ISR42-8 fragment on 2H between 37.82 cM and 63.53 cM within the Scarlett background.

In order to see effects of point mutation on protein sequence we calculated based on DNA sequence the protein sequence of AK366024 for S42IL109 and Scarlett. A comparison of open reading frames (ORF) of both proteins revealed a frame shift in S42IL109's AK366024 protein. Further, this frame shift led to an early stop codon in S42IL109 which resulted in a reduced protein sequence of 84 amino acids compared to 317 amino acids in Scarlett.

We analyzed the expression of AK366024 from BARLEX database. The gene expression of AK366024 was performed at different developmental stages in cultivar Morex: 4-days embryo, root from seedlings (10 cm shoot stage), shoot from seedling (10 cm shoot stage), young developing inflorescence (5 mm), developing inflorescence (1- 1.5 cm), developing tillers at six-leaf stage (3rd internode), developing grain (5 DPA) and developing grain (15 DPA). The expression analysis possessed an increased expression of AK366024 during tiller development at six-leaf stage. The expression at this stage is with 22 FPKM nearly 10-fold higher compared to the other stages, but a small peak was also detected in roots from seedlings (10 cm shoot stage) (**Figure 28**).

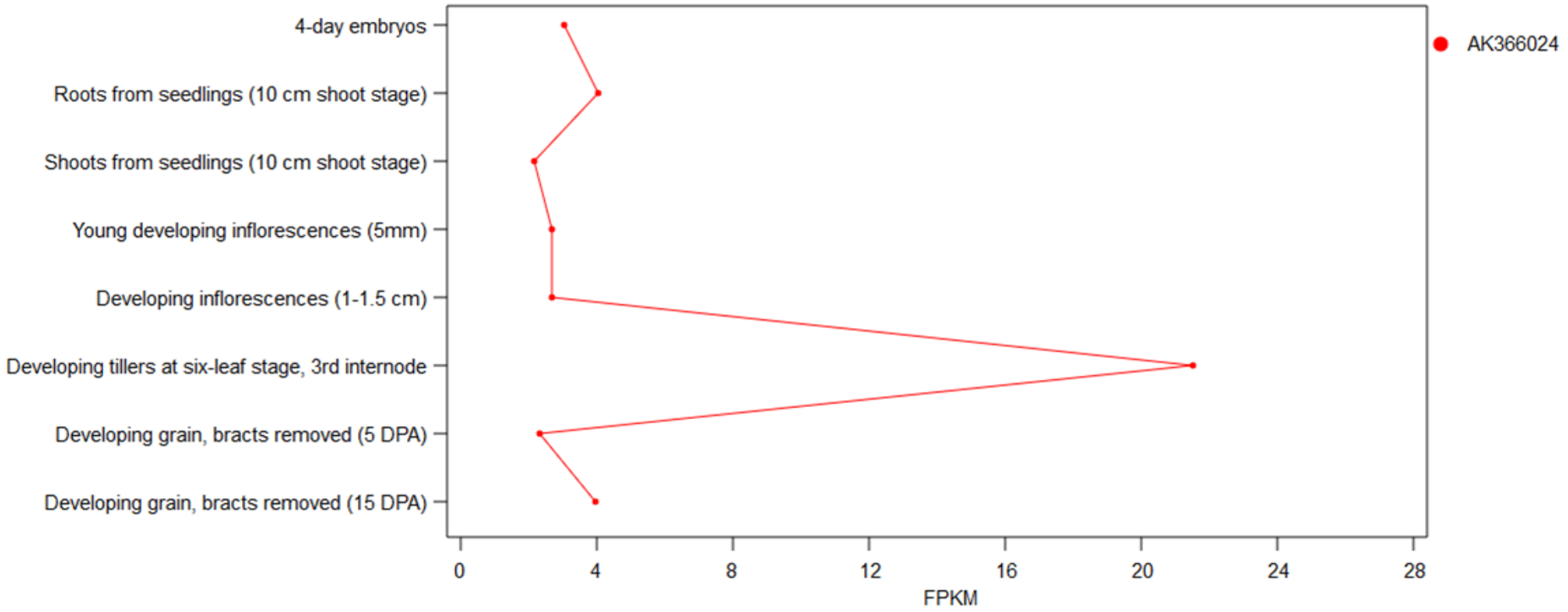

Figure 28: Expression profile of AK366024 from BARLEX (The Barley Genome Explorer). Expression analysis revealed a high expression of AK366024 during tiller development at six-leaf stage. FPKM = fragments per kilobase of exon per million fragments mapped.

3.3. Phenotypic Evaluation of AK366024

A total of 11 different traits were analyzed to detect differences between Scarlett and S42IL109. Seven out of the 11 traits were tested in a pot experiment in the greenhouse and four were analyzed in a seedling experiment in climate chamber.

Mean comparison of trait values for Scarlett and S42IL109 in the greenhouse experiment showed significant variation in the different treatments, control (WW) and drought (DT) conditions, (**Figure 29**) between both genotypes. Especially, the BBCH revealed highly significant differences between Scarlett and S42IL109 under control as well as drought conditions. Under control conditions Scarlett showed a lower mean BBCH of 56 compared to S42IL109 mean BBCH of 60. Equal results were observed for BBCH under drought conditions. Scarlett showed a lower mean BBCH (58) compared to S42IL109 (61) (**Figure 29A**). The differences of the developmental trend observed for BBCH between Scarlett and S42IL109 were similar under control and drought conditions. Furthermore, significant differences were detected for Rdw and Til. Rdw showed just significant differences under control conditions whereas Til showed just significant differences under drought treatment conditions. S42IL109 revealed a higher Rdw compared to Scarlett with 4.8 g in contrast to 3.2 g. On the other hand, under drought conditions the Rdw of S42IL109 and Scarlett were nearly equal with 3.2 g (S42IL109) and 3.0 g (Scarlett) (**Figure 29D**). In contrast to Rdw, Til revealed significant differences under drought conditions between Scarlett and S42IL109 whereas S42IL109 showed a larger average tiller number per plant (13) compared to Scarlett (10). Furthermore, we observed no significant differences between Scarlett (9 tiller per plant) and S42IL109 (9 tiller per plant) under control conditions (**Figure 29G**). The traits Hei, Lea, Rl and Sdw, revealed no significant differences across drought stress and control treatment between Scarlett and S42IL109 (**Figure 29B, 29C, 29E, 29F**).

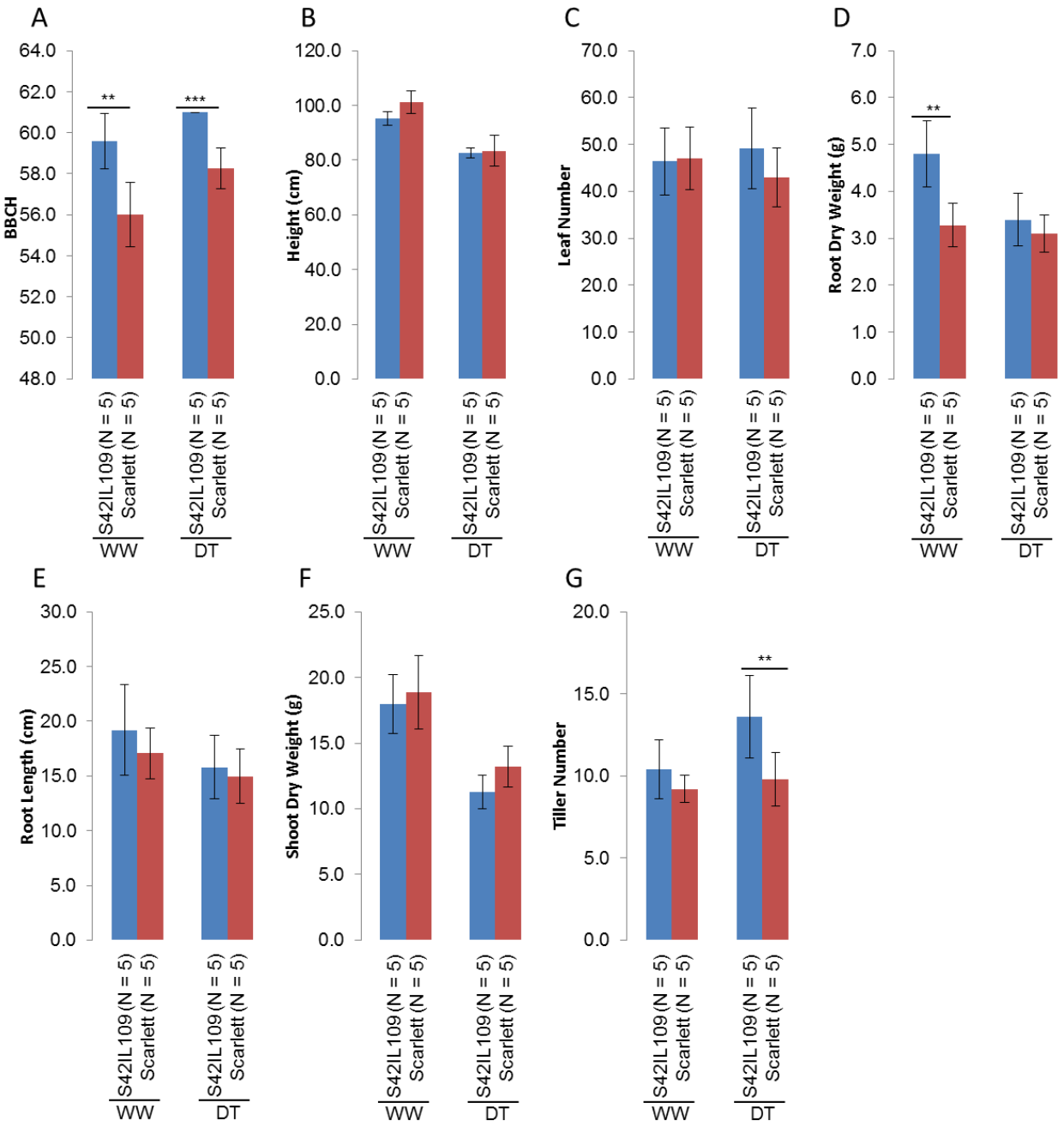

Figure 29: Mean comparison of trait variation under control (WW) and drought (DT) conditions between S42IL109 (red) and Scarlett (blue) in a greenhouse experiment. For each trait five plants per genotype were analyzed (N = 5). A) BBCH scale, B) Height (Hei), C) Leaves (Lea), D) Root dry weight (Rdw), E) Root length (Rl), F) Shoot dry weight (Sdw), G) Tiller number (Til). *, **, *** = indicates the level of significance at 0.05 (*), 0.01 (**) and 0.001 (***). Error bar = standard deviation (STD).

Additionally to the greenhouse experiment, we performed a seedling experiment with Scarlett and S42IL109 in a growth chamber to analyze developmental differences between both genotypes. The mean comparison of trait values for Scarlett and S42IL109 of this experiment showed significant differences between Scarlett and S42IL109 in the development of phytomer two (Phyt 2), phytomer three (Phyt 3) and phytomer four (Phyt 4). On the other hand, no significant differences were detected between Scarlett and S42IL109 for analyzed traits DtG, Phyt 1, Ll1, Sl1, Ll2 and Sl2 (**Figure 30A, 30E, 30F**). Especially Phyt 2 showed highly significant differences between Scarlett and S42IL109. With seven days to develop the third leaf, Scarlett revealed a slower development of new leaves compared to S42IL109 with five days of total appearance of the third leaf (**Figure 30B**). Moreover, the development of Phyt 3 (fourth leaf) is slightly increased in Scarlett but not in S42IL109 (**Figure 30C**). In Scarlett the development of Phyt 3 required six days whereas S42IL109 needed again five days for the full appearance of Phyt 3. In contrast to the development of Phyt 3 as well as Phyt 2, the development of Phyt 4 is increased in Scarlett and S42IL109. The fifth leaf (Phyt 4) was fully developed after five days in Scarlett but in S42IL109 the fifth leaf appeared one day earlier compared to Scarlett, after four days (**Figure 30D**). In contrast to Phyt 2, Phyt 3 and Phyt 4 the development of Phyt 1 showed no significant differences as well as the DtG were similar in both genotypes (**Figure 30E**). Moreover, we detected no significant differences between Scarlett and S42IL109 for Ll1, Sl1, Ll2 and Sl2 (**Figure 30F**).

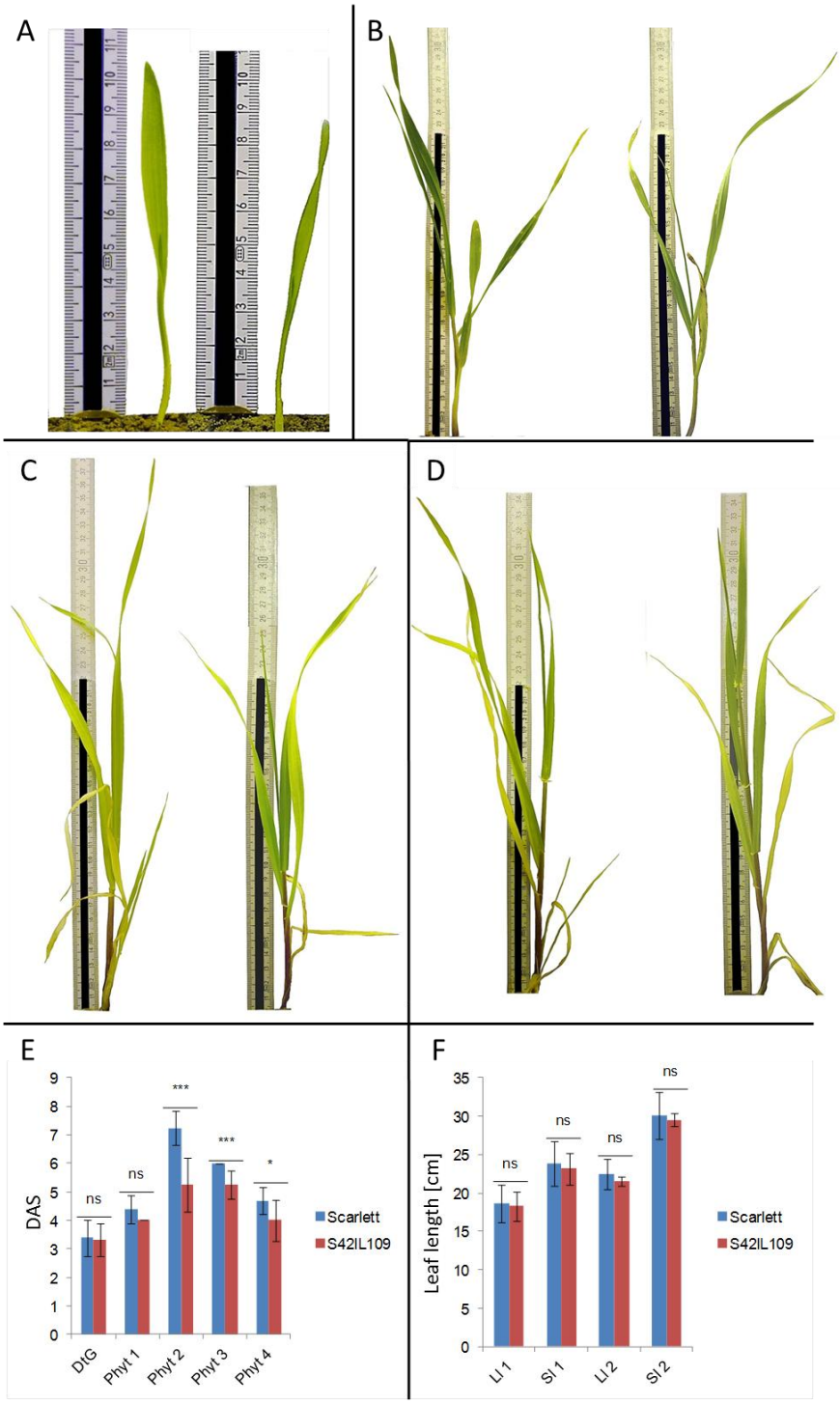

Figure 30: Comparison of plant development at different phytomer stages: A) Phytomer 1, B) Phytomer 2, C) Phytomer 3, D) Phytomer 4, E and F) Mean comparison of trait variation under control (WW) conditions between S42IL109 (red) and Scarlett (blue) in seedling experiment. For each trait minimum five plants per genotype were analyzed (N ≥ 10). Traits: Days to germination (DtG), Phytomer 1 (Phyt 1), Phytomer 2 (Phyt 2), Phytomer 3 (Phyt 3), Phytomer 4 (Phyt 4), days after sowing (DAS); Leaf length after one week (Ll 1), Seedling length after one week (Sl 1), Leaf length after two weeks (Ll 2), Seedling length after two weeks (Sl 2). *, **, ***, ns = indicates the level of significance at 0.05 (*), 0.01 (**), 0.001 (***) and not significant (ns). Error bar = standard deviation (STD).

In order to see the relationship of root and shoot traits as well as developmental stages, Pearson correlation was calculated for BBCH, Hei, Lea, Rdw, Rl, Sdw and Til under control and drought conditions (**Table 9**). Under control conditions, correlations were detected among BBCH, Hei and Rdw as well as Lea, Sdw and Til. The highest significant positive correlation revealed BBCH / Hei (0.73) and Lea / Sdw (0.73), respectively. Furthermore, strong significant correlations were detected under control conditions among BBCH / Rdw (0.69) and Lea / Til (0.68). Under drought conditions, Lea and Til showed the strongest positive correlation (0.78). Moreover, we identified significant correlations among Rdw / Til (0.67), Lea / Rdw (0.64) as well as BBCH / Til (0.63).

Table 9: Pearson correlation coefficients of phenotypic mean values (N = 5) of root and shoot traits under control and drought conditions in a greenhouse experiment between Scarlett and S42IL109.

	Trait	BBCH	Hei	Lea	Rdw	Rl	Sdw	Til
Control	**BBCH**	1						
	Hei	0.73*	1					
	Lea	ns	ns	1				
	Rdw	0.69**	ns	ns	1			
	Rl	ns	ns	ns	ns	1		
	Sdw	ns	ns	0.73*	ns	ns	1	
	Til	ns	ns	0.68*	ns	ns	ns	1
Stress	**BBCH**	1						
	Hei	ns	1					
	Lea	ns	ns	1				
	Rdw	ns	ns	0.64*	1			
	Rl	ns	ns	ns	ns	1		
	Sdw	ns	ns	ns	ns	ns	1	
	Til	0.63*	ns	0.78**	0.67*	ns	ns	1

Trait: BBCH = Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie stage, Hei = Height, Lea = Leaves, Rdw = Root dry weight, Rl = Root length, Sdw = Shoot dry weight, Til = No of tiller; *, **, *** = indicates the level of significance at 0.05 (*), 0.01 (**) and 0.001 (***), ns: non-significant

Discussion

Genetic diversity of barley natural population is known for its inherent morphological novelties, geographic and environmental adaptations. These features enable barley genotypes to grow from boreal to equatorial regions world-wide. Overall, this trait diversity is the product of plant evolution and related forces like natural selection. The first objective of the present work was to establish a state of the art genetic resources based on morphological novelties, geographic distribution and inherent environmental adaptation. Secondly, we employed genome-wide association approach using a dense genetic map to dissect the genetic basis of root and shoot traits as well as their putative role in drought adaptation. Thirdly, we performed a population and evolution analysis with the developed global diversity set using different approaches to detect evolutionary footprints within this population. For this, we focused primarily on root trait variation as well as shoot trait variations, to find major genetic players contributing to different root systems in barley and secondly to dissect the putative genetic interplay of root and shoot traits. It has been reported that the root architecture takes major role in plant adaptation to drought (Chloupek *et al.,* 2010; Wasson *et al.,* 2012; Barati *et al.,* 2015). Although, numerous GWAS studies have been made on barley diversity analysis by Nandha *et al.* (2014) and Russell *et al.* (2014), but genetic dissection of root traits remained fragmented due to its difficulty for phenotypic evaluations. Furthermore, the evolutionary basis of trait differences is still barely investigated in barley. To our knowledge, the current work presents the first study of its kind that utilized world-wide germplasm of barley to investigate the essential root and related shoot trait variations using a high resolution SNP map through GWAS.

1. Global Diversity Set Characterization

Population Structure

The population structure was conducted with STRUCTURE calculation program to determine the variability within the global diversity set (Pritchard *et al.*, 2000; Falush *et al.*, 2003, 2007; Hubisz *et al.*, 2009). A high degree of sub-structures was expected due to the composition of this population made of modern cultivars, landraces and wild accessions. With ΔK value at K = 3, performed with a Markov clustering algorithm implemented in CLUMPAK (Kopelman *et al.,* 2015) three sub-clusters were detected within the population. Due to these different sub-groups, a high variability within the global diversity set was supposed which caused to further corrections for the GWAS. To reduce the occurring structural effects a PCA as well as a Kinship matrix was included in the GWAS. Interestingly, two of the three sub-clusters showed a subspecies specific distribution including one sub-group (SPOP 1) made of wild barley accessions and one sub-group (SPOP 3) made of

modern cultivars with the exception of three landraces (**Figure 7**). Recently, Hübner *et al.* (2012) discovered structural distribution of barley landraces on Sardinia compared to other landraces and modern cultivars. Because of the global selection of genotypes for this barley population a high variability was expected and intended. Moreover, we detected a geographical dependent distribution within this population. This distribution, especially within SPOP 1 underlined the current findings of Allaby (2015). He supposed that the high degree of adaptation in barley occurs because of several centers of origin in the Middle East and Asia which is supported by the results of Morrell and Clegg (2007) and Dai *et al.,* (2012). Further, our finding of the geographical composition of the wild barley sub-group (SPOP 1) mainly from the Middle East and Asia is in line with Allaby (2015).

Linkage Disequilibrium

Caldwell *et al.* (2006) considered a precise knowledge of linkage disequilibrium (LD) as essential for an effective and correct population-based genome-wide association mapping. The population history (number of generations), the breeding system as well as the species of interest affect the decay of LD. The analysis of LD decay in the global diversity set revealed a rapid decrease of LD within the first 1 cM. This rapid decay can be explained by the composition of the global barley diversity set which comprised of modern cultivars, landraces and wild accession. The combination of these different barley forms led to a rapid decay due to long population history of wild accessions as well as landraces. In contrast to this rapid LD decay for the whole population, the LD decrease for groups separated due to their biological status is less rapid in modern cultivars. The LD decrease for modern cultivars is slow with 6.7 cM compared to the rapid decay for the whole population. But, Zhou *et al.* (2012) reported a decay of LD in an elite barley population from the United States from 4.0 to 19.0 cM which fits to the LD decay for the modern cultivar group. On the other hand, the LD decay for the landrace and wild accession groups is more rapid compared to modern cultivars as well as whole population. This low LD, especially for wild accessions, had been known for barley and reported. Our findings corresponded to the theoretical expectation of self-pollinating plants. Morrell *et al.* (2005) demonstrated a rapid LD decay rate for wild barley similar to that observed for outcrossing species like *Zea mays*. Moreover, the LD decay for the whole population was close to the LD decrease (2.5 – 3.5 cM) recently reported by Comadran *et al.* (2009) in an association panel of 192 barley accessions. The low overall LD as well as high genetic variability in the global diversity set enabled a great basis for genome-wide association studies.

2. Genome-Wide Association Study

2.1. Trait Variation

Root traits and traits related to root were and still are important for the enhancement of drought tolerance in plants (Comas *et al.*, 2013). Therefore, root traits and related shoot traits were investigated in a 179 genotypes comprising global diversity association panel in 2014 and 2015 under control and drought stress treatment.

Phenotypic evaluation showed significant variations for Rdw, Sdw, Til and RS under control and drought conditions between various genotypes indicating a broad genetic and phenotypic variance within the global barley population. Particularly, wild barley accessions showed higher values for Rdw, Til and RS as compared to cultivated varieties. Nandha *et al.* (2014) studied 27 wild accessions originating from the Middle East as well as 20 cultivars and found the presence of vital exotic alleles in determining root trait variation. Tyagi *et al.* (2011) reported significant environmental adaptation among the wild accessions from the Fertile Crescent. These present data also showed high correlation of Rdw and Til indicating the presence of common genetic components influencing root and shoot traits. These results are in line with Anderson-Taylor and Marshall (1983) as well as Narayanan and Prasad (2014), who also found close relationship of root traits and tiller number per plant in barley and other crops. Phenotypic evaluations were made across the years 2014 and 2015 but we found significant heritability of most of the root and shoot traits except RI suggesting the genetic control of these traits (**Table 5**). Heritability is the most important criteria for selecting traits in plant breeding and hence, traits possessing higher heritability across different environments could be prime leads for breeding.

2.2. QTL Detection and Quantification

The present GWAS detected 17 QTL for five root and shoot traits. The number of QTL was relatively low because we employed a highly stringent criteria of backward forward selection of significant SNP markers using higher threshold of probability and FDR (Miyagawa *et al.,* 2008). A major reason of this strict threshold was to get rid of the false positive QTL effect. A total of nine QTL with a marker effect, three with a marker by treatment interaction as well as five QTL with a marker effect and marker by treatment interaction were identified, respectively. Among the detected QTL at 14 loci (78%) the preeminence of exotic alleles from the wild barley accessions was associated with increase in trait values. Likewise, at 7 loci (39%) the exotic alleles showed significant interaction with drought treatment. These data indicate the presence of valuable alleles in the exotic germplasm for the improvement of root-shoot attributes and drought stress tolerance. Quantification of these QTL alleles is always a challenge in association panels due to their heterogeneous background. Therefore,

we made a pin plot analysis of the most promising QTL to visualize distribution of trait values population wide. Later, we selected extreme groups of the homozygous major and minor alleles for the quantification of allelic effects on a given trait. In order to confirm the haplotype relationship of genotypes contributing to individual QTL effect, we selected 30 genotypes randomly for each QTL effect and analyzed their genetic relatedness at local and global genome levels. This analysis showed that the wild accessions contributing to a given QTL effect revealed higher genetic similarities at both local and global genome levels. Zhao *et al.* analyzed genotype relatedness by calculating the identity by state (IBS) in GWAS analysis for QTL quantification to explain phenotypic variations among genotypes of a rice association panel (Zhao *et al.,* 2011). They also detected phenotypic similarities among genotypes from same geographical locations.

Root Dry Weight

Root dry weight is an important trait for adaptation to different environments especially drought. Moreover, a broad root system increases the ability in nutrition assimilation. The root biomass is mostly influenced by the number of tillers which leads to genotypes with huge root systems, particularly in wild accessions. Therefore, the global barley diversity set includes additional to modern cultivars, landraces and wild barley accessions. GWAS for Rdw revealed a total of four highly significant QTL across the barley genome. The different QTL were located on chromosomes 1H, 2H, 3H and 5H. Recently, Naz *et al.* (2014) as well as Arifuzzaman *et al.* (2014) detected QTL for Rdw under drought conditions on chromosomes 1H, 2H ,3H and 5H in a barley introgression library. The QTL detected on chromosome 5H correspond with the position mentioned by Cockram *et al.* (2007) for *VRN-H1*. But a detailed analysis of candidate genes in this region, by using barley Genome Zipper, led us to surmise CBF10A and CBF10B as candidate genes.

Root Length

Similar to Rdw, the root length is a critical trait for drought adaptation. Deep rooting leads to drought avoidance because of the ability to extract water from deep soil water layers (Wasson *et al.*, 2012). In 2013, Uga *et al.* revealed an increased drought tolerance in rice NILs which carried a *Dro1* allele of a deep rooting rice variety. We located a total of two QTL, one on 5H and one on 7H for Rl. Chen *et al.* (2010), Sayed (2011) and Naz *et al.* (2014) identified QTL for Rl on 5H for marker by treatment effects. However, we detected Rl QTL only for main effects. Moreover, just slight differences in RP were detected between drought and control conditions for both QTL among all genotypes. These circumstances can be explained by the difficulty of root length experiments in pots as well as effects of pots on root development (Poorter *et al.*, 2012). Therefore, an experiment to determine an exact analysis of Rl should be performed on field.

Shoot Dry Weight

Additional to root parameter, shoot parameter like shoot dry weight are important traits for drought resistance and adaptation. Moreover, a negative correlation of shoot and root parameter is known especially under drought conditions due to an increased root growth and decreased shoot growth. In our experiment, we identified two QTL on chromosome 2H and one QTL on chromosome 4H. In 2015, Wehner *et al.* detected on barley chromosomes 2H and 4H QTL for Sdw in GWAS experiment using 156 winter barley genotypes in pots and greenhouse. Interestingly, marker SCRI_RS_918 on chromosome 2H was detected as significant marker with a marker by treatment effect for Sdw as well as Rdw but with the opposite allele for a positive trait effect. This result is in line with the negative correlation detected under drought conditions for Sdw and Rdw. Furthermore, Carvalho *et al.* (2014) revealed a shoot/root relationship for barley as well as wheat under drought conditions.

Tiller Number

The number of tillers is in contrast to Sdw positively correlated with Rdw. The positive correlation of Til and Rdw is an effect of adventitious rooting as well as the initiation of nodal rooting. More tillers led to an higher Rdw because of increased Til (Hockett, 1986). We revealed a total of three significant QTL for Til located on chromosomes 1H, 2H and 7H, respectively. QTL on 1H and 2H showed similar positions with QTL identified by Naz *et al.* (2014) in wild barley introgression lines, but QTL on chromosome 7H seems to be a unique QTL for Til. Furthermore, the marker BOPA1_7381-1292 used to detect QTL QTil.1H also identified a significant QTL for Rdw (QRdw.1H) with a main marker effect. Additional, we revealed a positive correlation between these traits. This finding leads to the conclusion of a close relationship of shoot and root development.

Root-Shoot Ratio

The Root-shoot ratio is an index calculated by dividing the root dry weight by shoot dry weight which increases with an increasing Rdw and decreases with a decreasing Sdw under drought conditions. This opposing trend is a result of plant hormones abscisic acid (ABA) and cytokinin (CK) which lead to adaptation to drought e.g. an enhanced root development (O'Brien and Benková, 2013). Therefore, RS is a key trait of interest related to acquisition of soil resources for drought adaptation experiments (Comas *et al.*, 2013). Our GWAS revealed five significant QTL located on chromosomes 2H, 3H, 4H, 5H and 7H. Arifuzzaman *et al.* (2014) already detected QTL for RS on barley chromosomes 3H, 5H and 7H with similar positions in a 301 BC_2DH barley lines comprising IL population; but QTL identified on chromosomes 2H and 4H are novel QTL for RS in barley. Furthermore, RS exhibited highly significant positive correlation to Rdw under drought and control conditions as well as highly significant negative correlation to Sdw. The positive correlation to Rdw as well the negative

correlation to Sdw occurred because of the calculation of RS. Interestingly, marker SCI_RS_918 revealed significant for RS, Rdw and Sdw; whereas the major allele is the beneficial allele for Rdw and RS but the minor allele showed an increase RP for Sdw. These findings suggest a close inverse relationship of root and shoot development. Recently, Hendriks *et al.* (2015) showed an increase of root biomass, root length as well as root-shoot ratio due to reduced shoot development in a tillering inhibition (*tin*) mutant NIL wheat line.

2.3. Drought Tolerance Rating

Drought tolerance is an ability of agronomic importance due to global warming. Therefore a precise and adequate technique to distinguish between resistant / tolerant and susceptible genotypes is crucial. A reliable method to select resistant / tolerant genotypes for breeding is the use of drought tolerance indices (Khalili *et al.*, 2013). Common indices are based just on mean values; the DRYM, used in the present study, includes the median which allows a better differentiation between drought resistant / tolerant and susceptible lines. Moreover, the index is centered to 0 whereby genotypes above 0 are resistant to drought and genotypes below 0 are susceptible.

In order to see the ability of drought tolerance and susceptibility for lines within the global barley population we calculated the DRYM for three important traits Rdw, RI and Sdw according to Sprenger *et al.* (2015) to select genotypes favorable for plant breeding. These traits, especially Rdw and RI, are crucial traits for drought resistance breeding (Wasson *et al.*, 2012; Comas *et al.*, 2013). In total, 11 different genotypes were selected as tolerant and susceptible genotypes; whereas eight of these 11 genotypes are resistant genotypes and only three are susceptible for the given traits. Interestingly, the three susceptible genotypes CCS141 (Modern cultivar from Germany), HOR1479 (Landrace from China) and HOR2687 (Wild barley from Iran) are susceptible for all three traits indicating an overall drought susceptibility. On the other hand, seven different genotypes showed a high drought resistance for one of the three traits with the exception of HOR19848, a landrace from Japan, which exhibited a high index for Rdw as well as Sdw. The indicated resistance of HOR19848 led to the conclusion of two distinct processes of drought resistance in this genotype. Moreover, five out of the eight resistant genotypes were from the Middle East and Asia (HOR19848 Japan, HOR18401 Pakistan, NGB4668 Afghanistan, ICB181162 Iran, ICB180013 Jordan); furthermore three lines were wild barley accessions, four were landraces and only one genotype was a modern cultivar. Especially, the high number of resistant genotypes from the Middle East and Asia are in line with findings of Nandha and Singh (2014) and Narwal *et al.* (2015). They postulated a high drought stress resistance in barley accessions from Middle East and Asia. Moreover, Tyagi *et al.* (2011) revealed a

strong drought resistance in wild accessions from the Fertile Crescent which supports our results of drought resistance ranking.

2.4. Candidate Gene Analysis

The strongest QTL detected in the present study was localized on chromosome 1H (122.17 cM) where a unique exotic allele influenced root and shoot variation. The highest LOD score (102.61) at QTil.1H indicates the role of a major gene controlling tiller number. Similar marker (BOPA1_7381-1292) showed a significant association with QTL QRdw.1H but at relatively lower LOD score (11.57). These data suggest that this locus may underlie a major gene that controls primarily the tiller number. However, excessive tillering resulted in the initiation of more nodal roots suggested the dependence of shoot and root development. Similar results were reported earlier by Naz *et al.,* (2014) and Arifuzzaman *et al.*, (2014) where a putative QTL region was found for Rdw and related shoot traits on chromosome 1H in barley. To find the putative candidate gene underlying this variation, we identified 10 putative genes of different categories in the targeted QTL interval using barley genome sequence (Mayer *et al.,* 2012). Among these, based on the functional relevance and existing literature we suspect the role of a WRKY transcription factor, WRKY29 in this major trait variation (Rushton *et al.,* 2010; Bakshi and Oelmüller, 2014). Due to sequence comparison of the genotypes carrying major and minor QTL alleles, we found a crucial amino acid substitution mutation, from V51 (Valine) to L51 (Leucine) in the conserved WRKY DNA-binding domain (**Figure 21**). Therefore, we suppose this substitution mutation may change DNA-binding affinity among the selected haplotypes. However, further experiments are needed to test its role in a more isogenic background. According to Betts and Russell a substitution to L (Leucine) is crucial for secondary structures because of leucine's properties (Betts and Russell, 2007). Hydrophobic leucine prefers to bury in hydrophobic protein cores and being in alpha-helices in contrast to valine which prefers to be in beta-sheets. Therefore, it seems possible that the exchange from V51 to L51 leads to a wrongly folded beta-sheet because of the involvement of V51 in the fourth beta-sheet of WRKY DNA-binding domain (Zhu *et al.,* 1993).

The second promising QTL was identified on chromosome 5H that showed marker main as well as marker x treatment effects indicating the role of an exotic QTL allele in root system variation under control and drought stress conditions. There are a lot of reports that advocate the patterning of root under stress conditions (Chloupek *et al.,* 2010; Naz *et al.,* 2012; Narayanan *et al.,* 2014). To find genetic component behind this novel adaptation under drought, we searched candidate genes in the targeted QTL region using Genome Zipper of barley (Mayer *et al.,* 2012). We found altogether 12 putative candidate genes of which only two were related (C-repeat binding factor, CBF10B/CBF10A) transcription factors having a

regulatory function under drought conditions. The function of CBF transcription factors in drought stress tolerance has been reported in many cases (Akhtar *et al.,* 2012; Nakashima *et al.,* 2014). Notably, both genes CBF10B/CBF10A and associated SNP marker were lying on the same genomic contig on the physical map. Therefore, we sequenced both genes in selected genotypes harboring major and minor QTL alleles for QRdw.5H. Sequence comparison of full length CBF10B gene among the selected genotypes revealed a macro mutation in term of large deletion of 37 amino acids of the conserved domain in the wild barley accession as compared to cultivated genotypes (Figure 22A). Moreover, we found a vital amino acid substitution from S102 (Serine) to P102 (Proline) within the AP2/ERF DNA-binding domain (Figure 22B). The shift of serine to proline was suggested as crucial by Betts and Russell because of structural properties of proline. Although, there exists qualitative gene polymorphism among barley genotypes, we hypothesize there may be a complex and redundant regulation of this gene in root patterning under control and drought stress conditions (Betts and Russell, 2007). Previously, Naz *et al.* (2012) mapped a large QTL region for root system variation using introgression line on the long arm of chromosome 5H which putatively underlie Vrn-H1 locus. However, the above mentioned QTL effect does not correspond to Vrn-H1 region suggesting the novelty of this putative QTL allele in root system determination under drought stress conditions.

The present GWAS analyses also identified a major QTL QSdw.2H.a for shoot dry weight that explained the highest genetic variance (33.7 %) on chromosome 2H (58.99 cM). Notably, this QTL effect appeared as prominent effect where the heterozygous / heterogenic alleles resulted in a major increase in shoot dry weight as compared to homozygous alleles. Wang *et al.,* (2010) as well as Arifuzzaman *et al.,* (2014) mapped a QTL region on chromosome 2H which seems to underlie major circadian clock gene Ppd-H1 that controls plant development and early heading in barley under long day conditions. But they mapped Ppd-H1 between 19.9 cM to 23 cM. However, here we identified two unique haplotypes HOR2692 (Iranian wild accession) and NGB4673 (Landrace from Afghanistan) having heterozygous / heterogenic alleles at QTL QSdw.2H.a. The effect of this QTL on enhanced shoot dry weight led us to surmise that these genotypes may underlie novel candidate genes. Within the detected QTL region we detected more than 200 putative candidate genes on a Genome Zipper. Therefore, a detailed analysis of this region using near-isogenic lines could be of great potential to detect another player for shoot parameter under drought and control conditions in barley.

3. Evolutionary Analysis of Global Barley Population

3.1. Trait Variation

Greenhouse Experiment

The comparison of NILs with the parents is an important method for the detailed analysis and investigation of phenotypic differences due to mutations in organisms which are difficult to mutate. Therefore, we compared the NIL S42IL109 with the parent Scarlett to investigate a 1 bp point mutation within the CDS of the unknown gene AK366024.

The trait-wise mean comparison of Scarlett and S42IL109 grown in greenhouse showed significant variations under control (WW) and drought conditions (DT) for trait BBCH as well as significant differences under control conditions for Rdw. Moreover, we detected significant differences under drought conditions for Til. These variations indicating an effect of the point mutation on processes which are important for shoot and root development. Delay *et al.* (2013) reported significant variations in root and shoot development due to mutation in a *C-TERMINALLY ENCODED PEPTIDE* (CEP) gene. Furthermore, we detected a correlation between the developmental stage of the plant and Til as well as Rdw indicating a connection of identified phenotypic variations to developmental processes (**Table 9**). Recently, Maurer *et al.* (2016) showed the connection of developmental processes to trait variation during flowering in a nested association mapping (NAM) population. Until now, there is no publication were the BBCH system to coding the phenological growth stages of plants was used for the analysis of changes in plant development.

Climate Chamber Experiment

Additional to the greenhouse experiment, we performed a climate chamber experiment to dissect the developmental differences between Scarlett and S42IL109 more in detail during seedling development and growth. The development of plants is a multifactorial process; therefore, a detailed analysis of several traits and different growth stages is important to detect the phenotypic effect of a given mutation. In plants, by contrast to animals, the pleiotropic effects of genes are less important and quantitative differences are often caused by single or view genes (Coyne and Lande, 1985). The climate chamber experiment revealed significant differences between Scarlett and S42IL109 for the phytomer development. Especially, phytomer 2, phytomer 3 and phytomer 4 showed significant variations between both genotypes (**Figure 30B – 30E**). Based on the model proposed by Rutishauser and Sattler (1985), phytomer units contain a specific arrangement of meristematic regions that give rise to an ordered development of organs. Different organs, e.g. flowers, can arise by variation, but the phytomer structure provides a degree of rigidity and predictability to the morphological development of the plant. Therefore, the phytomer is

of fundamental importance in plant development. In 2001, Itoh *et al.* analyzed the phytomer development in rice and showed a close synchronization as well as equal intervals between the development of different phytomers. Forster *et al.* (2007) converted and proposed a new model of elucidating the phytomeric structure of barley. Similar to rice, the phytomer development from one to another revealed a close synchronization. The present data showed slight differences between the intervals of phytomer development within one genotype which can be explained by the difficulty of phyllochron measurement in early plant development. However, we observed significant differences of phytomer 2, phytomer 3 and phytomer 4 development between Scarlett and S42IL109. Therefore, we hypothesized an effect of the 1 bp point mutation within AK366024 on developmental processes.

3.2. Candidate Gene Detection and Analysis

Natural selection is a major factor in creating the genetic variation of a population thereby determines local adaptations (Kawecki and Ebert, 2004). Particularly, high genetic variations can be observed among populations of modern cultivars and their ancestors (wild accessions as well as landraces) (Hübner *et al.*, 2012, 2013; Bellucci *et al.*, 2013). In our study we used a Bayesian based method to detect loci effecting selection. Therefore, the three SPOPs were compared with each other revealing a total of five outlier loci. Four outlier loci were detected between SPOP 1 and SPOP 3, one outlier locus between SPOP 1 and SPOP 2 but no outlier locus between SPOP 2 and 3. These findings are in line considering the composition of each SPOP (**Table 8**), with a high percentage of barley wild accessions in SPOP 1 (73 %) as well as a high percentage of modern cultivars in SPOP 3 (88 %). Therefore, we expected a higher number of outlier loci between SPOP 1 and SPOP 3 compared to SPOP 1 and SPOP 2. The lack of outlier loci between SPOP 2 and SPOP 3 is a result of the high amount of modern cultivars in SPOP 2 (43 %) as well as a lower genetic diversity of modern cultivars and landraces (Chen *et al.*, 2012; Bellucci *et al.*, 2013). Further, the detection of more outlier loci between SPOP 1 / SPOP 3 instead of SPOP 1 / SPOP 2 or SPOP 2 / SPOP 3 is in line with the geographical distribution detected within the reduced germplasm panel (**Figure 7**). A large variability among barley cultivars, landraces and wild barley exists in the primary gene pool. Especially, wild barley shows a high genetic diversity compared to landraces and modern cultivars due to morphological, physiological and functional adaptation of wild barley, which facilitated colonization of the Fertile Crescent in a range of most diverse environments (von Bothmer *et al.*, 2003). Moreover, we identified the same outlier locus between SPOP 1/SPOP 2 and SPOP 1/SPOP 3. This result indicates an evolutionary force led to the differentiation of SPOP1 to SPOP 2 and SPOP3.

3.3. Phenotypic Evaluation of AK366024

In barley, several genes were used for the analysis of evolution and diversity. Especially, *BKn-3*, *Vrs1*, *Nud* and *Btr1Btr2* are the primary domestication-related genes in barley (Badr *et al.*, 2000; Azhaguvel and Komatsuda, 2007; Komatsuda *et al.*, 2007; Pourkheirandish and Komatsuda, 2007; Badr and El-Shazly, 2012). We used a Bayesian based approach to detect new genes which can explain the barley diversity. In our study we identified a highly significant outlier locus between three different SPOPs made of barley wild accessions, landraces and modern cultivars. This locus revealed an unknown gene (AK366024) which possessed a barley subspecies specific point mutation. All of the wild barley accession used in this analysis exhibited no mutation in AK366024, whereas 77.5 % of modern cultivars revealed the point mutation. Moreover, SPOP 1 (including 73 % wild barley lines and 27 % landraces) comprises to 100 % of lines lacking this point mutation. On the other hand, SPOP 3 (including 88 % modern cultivars and 12 % landraces) possessed to 100 % lines carrying the mutation. The *in vitro* analysis of the outlier locus suggested an influence of this mutation on developmental processes. This result is in line with the finding of the phenotypic analysis of S42IL109 in the climate chamber experiment which also showed significant differences in development compared to Scarlett. Wild barley accessions reveal a high genetic diversity compared to modern cultivars due to adaptations to a wide range of environments (Kawecki and Ebert, 2004; Dai *et al.*, 2012). These adaptations originated from mutational differences between wild and cultivated barley (Orr, 2005). Therefore, we suppose a function of AK366024 in developmental processes which led to natural selection of wild and cultivated barley as well as the evolutionary separation of genotypes within SPOP 1 and SPOP 3. Based on the geographical distribution of SPOP 1, we surmise the origin of this mutation at different locations, one origin in the Middle East and a second in Asia but more experiments are necessary to dissect the origin and the exact effect of this mutation.

Conclusion

Taken together, the present GWAS has successfully screened natural diversity of barley to identify novel variants for root and shoot attributes that seem beneficial for improving the inferior rooting system of cultivated varieties. Further, the genetic determination of these phenotypes revealed important QTL/candidate genes which provide an opportunity for continuing research to characterize the role of these genes more precisely and to understand the genetic mechanisms of barley drought resistance as well as root and shoot development across diverse climatic and geographic conditions. Furthermore, this study discovered the potency of global association panels in abiotic stress studies and provides a basis for

following research on drought tolerance in barley. Moreover, the Bayesian loci outlier approach used in this study enabled the investigation of barley evolution and led to the detection of a novel gene which provides the opportunity to distinguish between wild barley accessions and modern cultivars. Further, this gene is a novel candidate for the analysis of barley evolution and domestication in addition to known genes like *BKn-3, Vrs1, Nud and Btr1Btr2*. The global diversity barley germplasm panel is, besides the immense potency in association studies, a powerful tool in evolution and domestication studies and facilitates a basis of following research.

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Appendix

Table 10: Genotype list of global barley population. List of all Genotypes which are included in the global barley population with accession number, ID, sub-group membership, SNP allele of marker SCRI_RS_170235, CAPS allele for gene AK366024, country of collection, the biological status, Altitude, Longitude and Latitude.

No	Accession	ID	Sub-group	SNP	CAPS	Country	Biological status	Altitude	Longitude	Latitude
1	HOR 9721	RBC010	SPOP 1	G	T	Libya	Wild type	NN 590	32°44'54'' N	21°45'38'' E
2	HOR 9840	RBC012	SPOP 1	G	T	Libya	Wild type	NN 656	32°47'46'' N	22°7'18'' E
3	ICB 180006	RBC017	SPOP 1	G	T	Syria	Wild type	NN 250	35°49'42'' N	036°18'28''E
4	ICB 180862	RBC018	SPOP 1	G	T	Syria	Wild type			
5	ICB 180902	RBC019	SPOP 1	G	T	Syria	Wild type			
6	IG 121857	RBC020	SPOP 1	G	T	Syria	Wild type	NN 1059	32°33'00''N	036°35'42''E
7	ICB 180092	RBC025	SPOP 1	G	T	Palestine	Wild type			
8	ICB 180117	RBC026	SPOP 1	G	T	Palestine	Wild type			
9	ICB 180410	RBC027	SPOP 1	G	T	Palestine	Wild type			
10	ICB 180994	RBC028	SPOP 1	G	T	Palestine	Wild type	NN 54	31°40'00''N	034°34'00''E
11	ICB 181160	RBC029	SPOP 1	G	T	Iran	Wild type			
12	ICB 181442	RBC033	SPOP 1	G	T	Jordan	Wild type	NN 782	31°17'51''N	035°50'41''E
13	ICB 181418	RBC034	SPOP 1	G	T	Jordan	Wild type	NN 812	31°46'47''N	035°48'00''E
14	ICB 180013	RBC035	SPOP 1	G	T	Jordan	Wild type	NN 480	32°14'25''N	035°51'55''E
15	ICB 181268	RBC036	SPOP 1	G	T	Jordan	Wild type	NN750	32°18'6''N	035°55'17''E
16	ICB 180007	RBC037	SPOP 1	G	T	Jordan	Wild type	NN 591	32°29'15''N	035°55'39''E
17	ICB 180260	RBC038	SPOP 1	G	T	Israel	Wild type	NN 36	33°00'00''N	035°08'00''E
18	ICB 180329	RBC040	SPOP 1	G	T	Israel	Wild type	NN 83	31°26'00'' N	34°29'00'' E
19	ICB 180508	RBC041	SPOP 1	G	T	Israel	Wild type			
20	ICB 180046	RBC043	SPOP 1	G	T	Iraq	Wild type	NN 323	36°00'00''N	043°31'00''E
21	ICB 180069	RBC044	SPOP 1	G	T	Iraq	Wild type	NN 470	34°48'00''N	045°36'00''E
22	HOR 2514	RBC046	SPOP 1	G	T	India	Wild type			
23	HOR 11421	RBC047	SPOP 1	G	T	India	Landrace	NN 2880	31°41'31'' N	77°31'35'' E
24	HOR 8367	RBC048	SPOP 1	G	T	India	Landrace			
25	HOR 8372	RBC049	SPOP 1	G	T	India	Landrace			
26	HOR 7603	RBC050	SPOP 1	G	T	Pakistan	Landrace	NN 2830	36°05'40'' N	074°04'35'' E
27	HOR 7599	RBC052	SPOP 1	G	T	Pakistan	Landrace	NN 2100	36°17'40'' N	073°46'57'' E
28	ICB 181243	RBC053	SPOP 1	G	T	Pakistan	Wild type	NN 1560	30°18'00'' N	066°54'00'' E
29	HOR 1479	RBC059	SPOP 1	G	T	China	Landrace	NN 3685	29°21'00'' N	090°39'00'' E
30	HOR 1510	RBC060	SPOP 1	G	T	China	Landrace	NN 3650	29°38'59'' N	091°05'59'' E
31	HOR 1566	RBC061	SPOP 1	G	T	China	Landrace	NN 4076	29°15'19'' N	090°49'59'' E
32	NGB4668	RBC063	SPOP 1	G	T	Afghanistan	Landrace			
33	NGB6952	RBC066	SPOP 1	G	T	Afghanistan	Landrace			
34	NGB9599	RBC067	SPOP 1	G	T	Afghanistan	Landrace			
35	ICB 181498	RBC069	SPOP 1	G	T	Uzbekistan	Wild type	NN 350	41°9'58''N	069°02'00''E
36	IG 124000	RBC070	SPOP 1	G	T	Uzbekistan	Wild type	NN 1450	39°42'00''N	068°02'45''E
37	IG 124017	RBC071	SPOP 1	G	T	Uzbekistan	Wild type	NN 700	40°00'00''N	067°05'15''E
38	ICB 180211	RBC073	SPOP 1	G	T	Turkmenistan	Wild type	NN 1530	37°42'59'' N	058°24'50'' E
39	ICB 180215	RBC074	SPOP 1	G	T	Turkmenistan	Wild type			
40	ICB 180217	RBC075	SPOP 1	G	T	Turkmenistan	Wild type	NN 250	37°40'00''N	065°35'00'' E

41	ICB 181492	RBC076	SPOP 1	G	T	Turkmenistan	Wild type	NN 456	38°02'00"N	058°00'00"E
42	HOR 18647	RBC077	SPOP 1	G	T	Japan	Landrace			
43	HOR 19848	RBC080	SPOP 1	G	T	Japan	Landrace			
44	HOR 11017	RBC127	SPOP 1	G	T	Greece	Wild type	NN 20	35°30'59'' N	024°01'59'' E
45	HOR 12418	RBC130	SPOP 1	G	T	Greece	Wild type	NN 20	35°30'59'' N	024°01'59'' E
46	ICB 181500	RBC135	SPOP 1	G	T	Tadjikistan	Wild type	NN 1030	39°28'25"N	067°30'1"E
47	ICB 180070	RBC137	SPOP 1	G	T	Turkey	Wild type	NN 840	39°39'52"N	031°9'40"E
48	ICB 181162	RBC138	SPOP 1	G	T	Iran	Wild type			
49	HOR 16097	RBC001	SPOP 2	G	T	Egypt	Cultivar			
50	BCC 126	RBC005	SPOP 2	G	T	Marocco	Landrace			
51	BCC 149	RBC006	SPOP 2	G	T	Marocco	Landrace			
52	HOR 13412	RBC007	SPOP 2	G	T	Marocco	Landrace	NN 596	31°22'00'' N	008°31'00'' W
53	BCC 131	RBC008	SPOP 2	G	T	Marocco	Landrace			
54	HOR 930	RBC021	SPOP 2	A	-T	Turkey	Landrace			
55	HOR 19883	RBC024	SPOP 2	A	-T	Turkey	Landrace			
56	HOR 20921	RBC042	SPOP 2	A	-T	Israel	Landrace			
57	HOR 17616	RBC056	SPOP 2	A	-T	Nepal	Landrace			
58	HOR 56	RBC062	SPOP 2	A	-T	China	Landrace			
59	HOR 4124	RBC089	SPOP 2	A	-T	Mexico	Landrace			
60	BCC 848	RBC091	SPOP 2	G	T	Mexico	Cultivar			
61	HOR 7443	RBC093	SPOP 2	A	-T	Bolivia	Landrace			
62	HOR 7446	RBC094	SPOP 2	R	T/-T	Bolivia	Landrace			
63	HOR 2981	RBC097	SPOP 2	G	T	Chile	Cultivar			
64	HOR 20110	RBC099	SPOP 2	G	T	Chile	Landrace			
65	HOR 14485	RBC100	SPOP 2	G	T	Chile	Landrace			
66	HOR 10843	RBC103	SPOP 2	G	T	Colombia	Landrace	NN 2000	5°45'15" N	73°34'37" W
67	BCC 927	RBC105	SPOP 2	A	-T	Peru	Cultivar			
68	HOR 7449	RBC107	SPOP 2	A	-T	Peru	Landrace			
69	HOR 9565	RBC108	SPOP 2	A	-T	Peru	Landrace			
70	HOR 17307	RBC110	SPOP 2	G	T	Uruguay	Landrace			
71	BCC 862	RBC111	SPOP 2	G	T	Uruguay	Cultivar			
72	BCC 896	RBC112	SPOP 2	G	T	Uruguay	Cultivar			
73	HOR 35	RBC113	SPOP 2	G	T	Australia	Cultivar			
74	HOR 4206	RBC114	SPOP 2	A	-T	Australia	Cultivar			
75	HOR 18209	RBC116	SPOP 2	G	T	Australia	Cultivar			
76	HOR 13965	RBC118	SPOP 2	A	-T	Australia	Cultivar			
77	BCC 1551	RBC120	SPOP 2	A	-T	Armenia	Cultivar			
78	BCC 1474	RBC123	SPOP 2	A	-T	Ukraine	Cultivar			
79	BCC 1505	RBC125	SPOP 2	A	-T	Ukraine	Cultivar			
80	HOR 1131	RBC129	SPOP 2	G	T	Greece	Landrace	NN 226	35°27'52'' N	023°46'17'' E
81	HOR 199	RBC131	SPOP 2	A	-T	Russia	Landrace			
82	HOR 3372	RBC132	SPOP 2	A	-T	Russia	Landrace			
83	BCC 1348	RBC164	SPOP 2	G	T/-T	Spain	Cultivar			
84	BCC 1523	RBC165	SPOP 2	A	-T	Spain	Cultivar			
85	HOR 19267	RBC166	SPOP 2	A	-T	Spain	Landrace			
86	BCC 1586	RBC167	SPOP 2	G	T/-T	Spain	Cultivar			
87	HOR 873	RBC168	SPOP 2	G	-T	France	Cultivar			
88	HOR 1132	RBC169	SPOP 2	G	T/-T	France	Landrace	NN 650m	42°27'55'' N	2°54'49'' E
89	HOR 11790	RBC171	SPOP 2	A	-T	France	Cultivar			
90	HOR 12047	RBC175	SPOP 2	A	-T	GB/Irland	Landrace	NN 157	52°24'28'' N	001°56'41'' W
91	HOR 16287	RBC013	SPOP 3	A	-T	Sudan	Landrace			

92	BCC 871	RBC098	SPOP 3	A	-T	Chile	Cultivar			
93	CCS 004	RBC139	SPOP 3	A	-T	Germany	Cultivar			
94	CCS 010	RBC140	SPOP 3	A	-T	Germany	Cultivar			
95	CCS 012	RBC141	SPOP 3	A	-T	Germany	Cultivar			
96	CCS 018	RBC142	SPOP 3	A	-T	Germany	Cultivar			
97	CCS 023	RBC143	SPOP 3	A	-T	Germany	Cultivar			
98	CCS 041	RBC144	SPOP 3	A	-T	Germany	Cultivar			
99	CCS 052	RBC145	SPOP 3	A	-T	Germany	Cultivar			
100	CCS 060	RBC146	SPOP 3	A	-T	Germany	Cultivar			
101	CCS 081	RBC148	SPOP 3	A	-T	Germany	Cultivar			
102	CCS 084	RBC150	SPOP 3	A	-T	Germany	Cultivar			
103	CCS 086	RBC151	SPOP 3	A	-T	Germany	Cultivar			
104	CCS 089	RBC152	SPOP 3	A	-T	Germany	Cultivar			
105	CCS 095	RBC153	SPOP 3	A	-T	Germany	Cultivar			
106	CCS 096	RBC154	SPOP 3	A	-T	Germany	Cultivar			
107	CCS 109	RBC155	SPOP 3	A	-T	Germany	Cultivar			
108	Agueda	RBC158	SPOP 3	A	-T	Germany	Cultivar			
109	Montoya	RBC159	SPOP 3	A	-T	Germany	Cultivar			
110	Danielle	RBC160	SPOP 3	A	-T	Germany	Cultivar			
111	Britney	RBC161	SPOP 3	A	-T	Germany	Cultivar			
112	Andreia	RBC162	SPOP 3	A	-T	Germany	Cultivar			
113	BCC 1380	RBC170	SPOP 3	A	-T	France	Cultivar			
114	NGB8822	RBC176	SPOP 3	A	-T	GB/Irland	Landrace			
115	NGB9480	RBC177	SPOP 3	A	-T	GB/Irland	Landrace			
116	HOR 19027	RBC002	ADMIX	A	-	Egypt	Landrace			
117	HOR 20117	RBC003	ADMIX	G	-	Egypt	Landrace			
118	HOR 19308	RBC004	ADMIX	G	-	Egypt	Landrace			
119	HOR 9838	RBC009	ADMIX	G	-	Libya	Wild type	NN 424	27°2'16'' N	14°25'36'' E
120	HOR 10164	RBC011	ADMIX	G	-	Libya	Wild type	NN 300	32°6'34'' N	21°10'9'' E
121	HOR 2589	RBC014	ADMIX	G	-	Sudan	Landrace			
122	HOR 16359	RBC015	ADMIX	G	-	Sudan	Landrace			
123	HOR 15956	RBC016	ADMIX	G	-	Sudan	Landrace			
124	HOR 14953	RBC022	ADMIX	A	-	Turkey	Landrace			
125	HOR 14936	RBC023	ADMIX	A	-	Turkey	Landrace			
126	HOR 2684	RBC030	ADMIX	G	-	Iran	Wild type	NN 110	32°6'21'' N	048°50'2'' E
127	HOR 2692	RBC031	ADMIX	-	-	Iran	Wild type	NN 120	32°23'36'' N	047°38'25'' E
128	HOR 2687	RBC032	ADMIX	G	-	Iran	Wild type	NN 90	31°35'29'' N	049°5'20'' E
129	HOR 9470	RBC039	ADMIX	-	-	Israel	Wild type			
130	HOR 11106	RBC045	ADMIX	G	-	Iraq	Wild type			
131	HOR 18401	RBC051	ADMIX	G	-	Pakistan	Wild type			
132	BCC 732	RBC054	ADMIX	G	-	Nepal	Landrace			
133	BCC 776	RBC055	ADMIX	A	-	Nepal	Landrace			
134	HOR 18945	RBC057	ADMIX	G	-	Nepal	Landrace			
135	HOR 16714	RBC058	ADMIX	G	-	China	Landrace			
136	NGB9606	RBC064	ADMIX	A	-	Afghanistan	Landrace			
137	NGB4673	RBC065	ADMIX	A	-	Afghanistan	Landrace			
138	NGB8872	RBC068	ADMIX	G	-	Afghanistan	Landrace			
139	BCC 282	RBC072	ADMIX	G	-	Uzbekistan	Landrace			
140	HOR 15779	RBC078	ADMIX	G	-	Japan	Landrace			
141	BCC 613	RBC079	ADMIX	A	-	Japan	Cultivar			
142	BCC 891	RBC081	ADMIX	A	-	USA	Cultivar			

143	BCC 906	RBC082	ADMIX	G	-	USA	Cultivar			
144	BCC 817	RBC083	ADMIX	G	-	USA	Cultivar			
145	BCC 875	RBC084	ADMIX	G	-	USA	Cultivar			
146	BCC 801	RBC085	ADMIX	A	-	Canada	Cultivar			
147	BCC 852	RBC086	ADMIX	A	-	Canada	Cultivar			
148	BCC 881	RBC087	ADMIX	A	-	Canada	Cultivar			
149	BCC 888	RBC088	ADMIX	A	-	Canada	Cultivar			
150	HOR 13597	RBC090	ADMIX	G	-	Mexico	Wild type			
151	BCC 900	RBC092	ADMIX	G	-	Mexico	Cultivar			
152	BCC 882	RBC095	ADMIX	G	-	Bolivia	Cultivar			
153	BCC 928	RBC096	ADMIX	G	-	Bolivia	Cultivar			
154	BCC 844	RBC101	ADMIX	G	-	Colombia	Cultivar			
155	BCC 921	RBC102	ADMIX	-	-	Colombia	Cultivar			
156	HOR 10845	RBC104	ADMIX	G	-	Colombia	Landrace	NN 2790	1°12'59'' N	77°23'30'' W
157	HOR 19577	RBC106	ADMIX	G	-	Peru	Landrace			
158	HOR 16345	RBC109	ADMIX	A	-	Uruguay	Landrace			
159	HOR 4278	RBC115	ADMIX	G	-	Australia	Cultivar			
160	HOR 20173	RBC117	ADMIX	A	-	Australia	Cultivar			
161	HOR 4724	RBC119	ADMIX	A	-	Armenia	Landrace			
162	HOR 4468	RBC121	ADMIX	A	-	Armenia	Landrace			
163	HOR 7394	RBC122	ADMIX	A	-	Armenia	Landrace			
164	BCC 1493	RBC124	ADMIX	A	-	Ukraine	Cultivar			
165	BCC 1533	RBC126	ADMIX	A	-	Ukraine	Cultivar			
166	HOR 10924	RBC128	ADMIX	G	-	Greece	Wild type	NN 32	28°58'54'' N	26°23'53'' E
167	HOR 2448	RBC133	ADMIX	G	-	Russia	Landrace			
168	BCC 1491	RBC134	ADMIX	A	-	Russia	Landrace			
169	ICB 180063	RBC136	ADMIX	G	-	Turkey	Wild type			
170	CCS 067	RBC147	ADMIX	A	-	Germany	Cultivar			
171	CCS 083	RBC149	ADMIX	A	-	Germany	Cultivar			
172	CCS 121	RBC156	ADMIX	A	-	Germany	Cultivar			
173	CCS 141	RBC157	ADMIX	G	-	Germany	Cultivar			
174	Mutante	RBC163	ADMIX	A	-	Germany	Cultivar			
175	BCC 829	RBC172	ADMIX	A	-	GB/Irland	Cultivar			
176	HOR 16665	RBC173	ADMIX	A	-	GB/Irland	Cultivar			
177	HOR 18101	RBC174	ADMIX	A	-	GB/Irland	Landrace			
178	NGB4605	RBC178	ADMIX	A	-	Romania	Landrace			
179	NGB9312	RBC179	ADMIX	A	-	Romania	Landrace			

Sub-group = Membership to a sub-group (SPOP 1, SPOP 2, SPOP 3) based on the membership coefficient of ≥0.85, otherwise grouped in ADMIX. SNP = Allele of marker SCRI_RS_170235 for particular genotype; Alleles: G = guanine (Major allele), A = adenine (Minor allele), R = heterozygous / heterogeneous, "-" = missing value. CAPS = Allele of CAPS marker for particular genotype; Alleles: T = thymine present, -T = thymine deletion, T/-T = heterozygous / heterogeneous, "-" = missing value.

Table 11: List of primer for candidate gene analysis. TA: Annealing temperature in degrees Celcius

Primer	TA°C	Sequence	Fragment (bp)	Experiment	Note
*Hv*CBF10A-fwd	63.0	TCACACTCCTCACTAAGCTCA	825	GWAS	
*Hv*CBF10A-rev	63.0	AGTCAAAACAAAGCAGAGTCCA			
*Hv*CBF10B-fwd	63.0	ACGTCTTCACACACTCCACA	852	GWAS	
*Hv*CBF10B-rev	63.0	AGGCTGCAGAATCAAAACGA			
*Hv*WRKY29-fwd	60.0	GAGTGTGAGAGTGAGACCCG	957	GWAS	
*Hv*WRKY29-rev	60.0	GGACCGAATTCAGCCATCAC			
*Hv*RTrans1-fwd	60.0	CACCAACCATCCAACAGG	1658	Outlier	1st part of gene
*Hv*RTrans1-rev	60.0	TGTTCCTTGAAGCGGTCT			
*Hv*RTrans2-fwd	56.0	TCCTAGTCCACGTCCCAT	1263	Outlier	2nd part of gene
*Hv*RTrans2-rev	56.0	CTAGTTGGTCACCCGTGT			
*Hv*CAPS002-fwd	56.0	AACCGATGACAAACGCCAC	302	Outlier	Frag 1: 75 bp, Frag 2: 227 bp
*Hv*CAPS002-rev	56.0	CCACGCCAAGCCTCTAAAG			

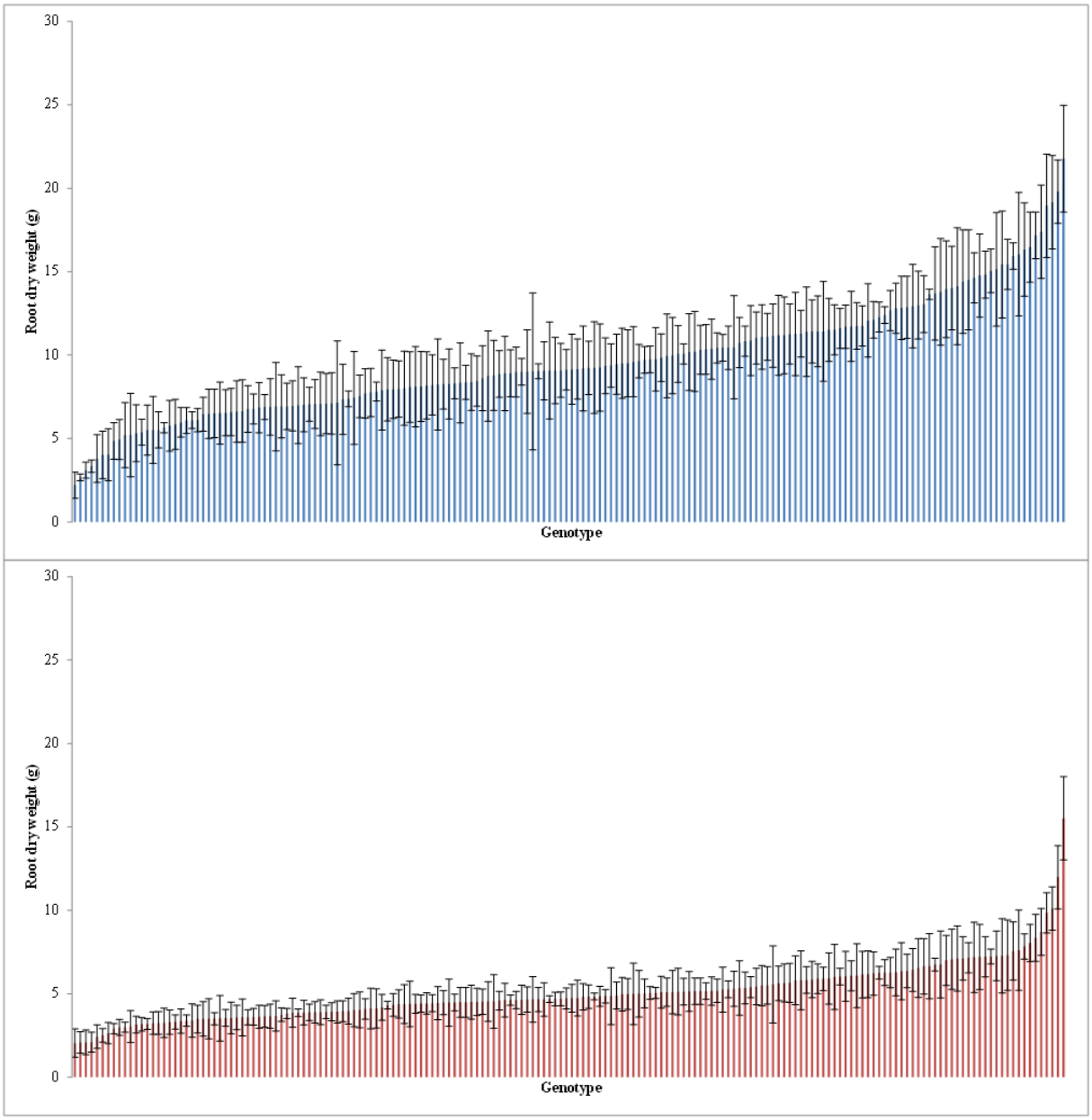

Figure 31: Phenotypic variation of root dry weight (Rdw) in 2014 of global population under control and drought conditions. A) Rdw (g) of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) Rdw (g) of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

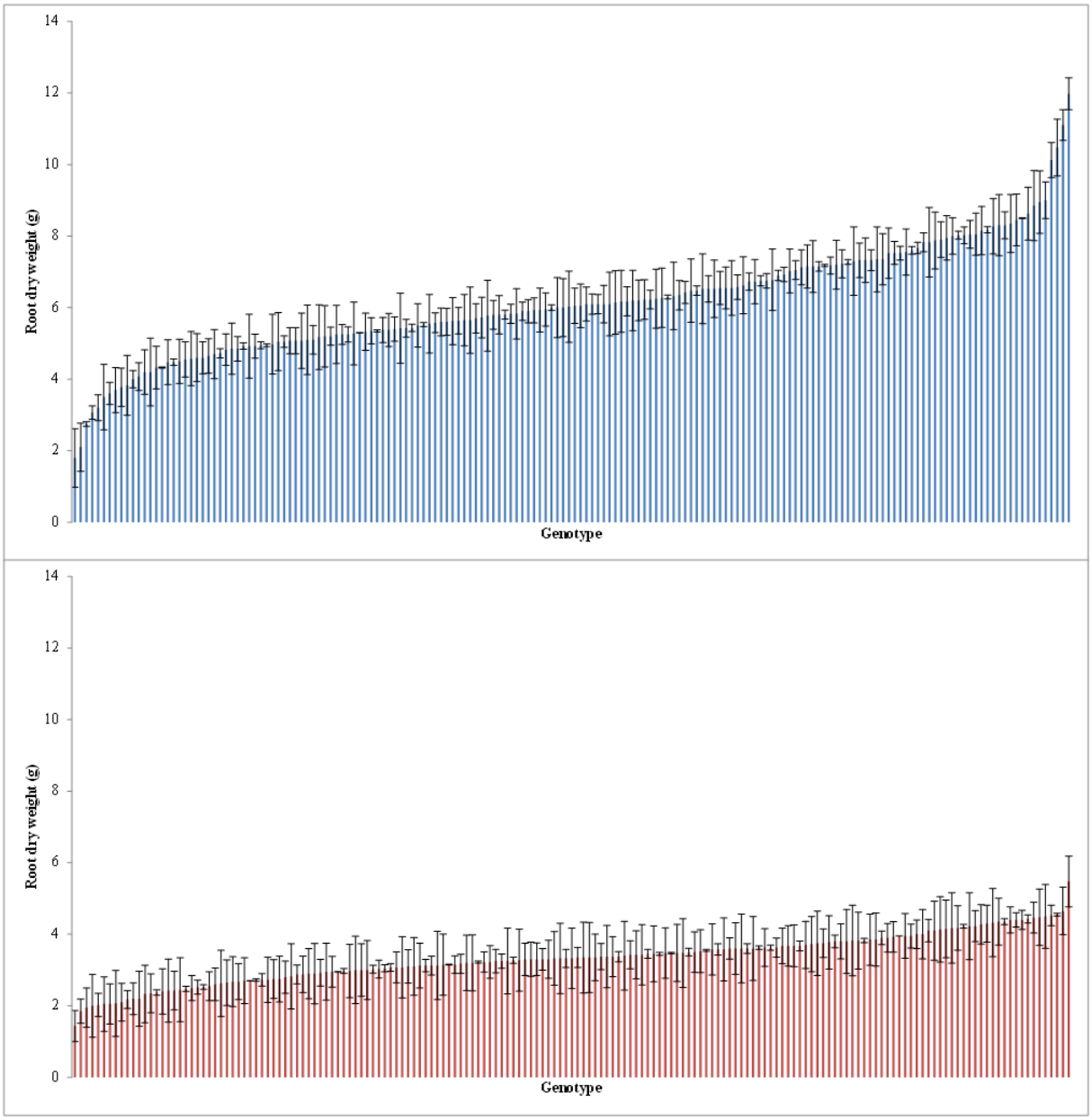

Figure 32: Phenotypic variation of root dry weight (Rdw) in 2015 of global population under control and drought conditions. A) Rdw (g) of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) Rdw (g) of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

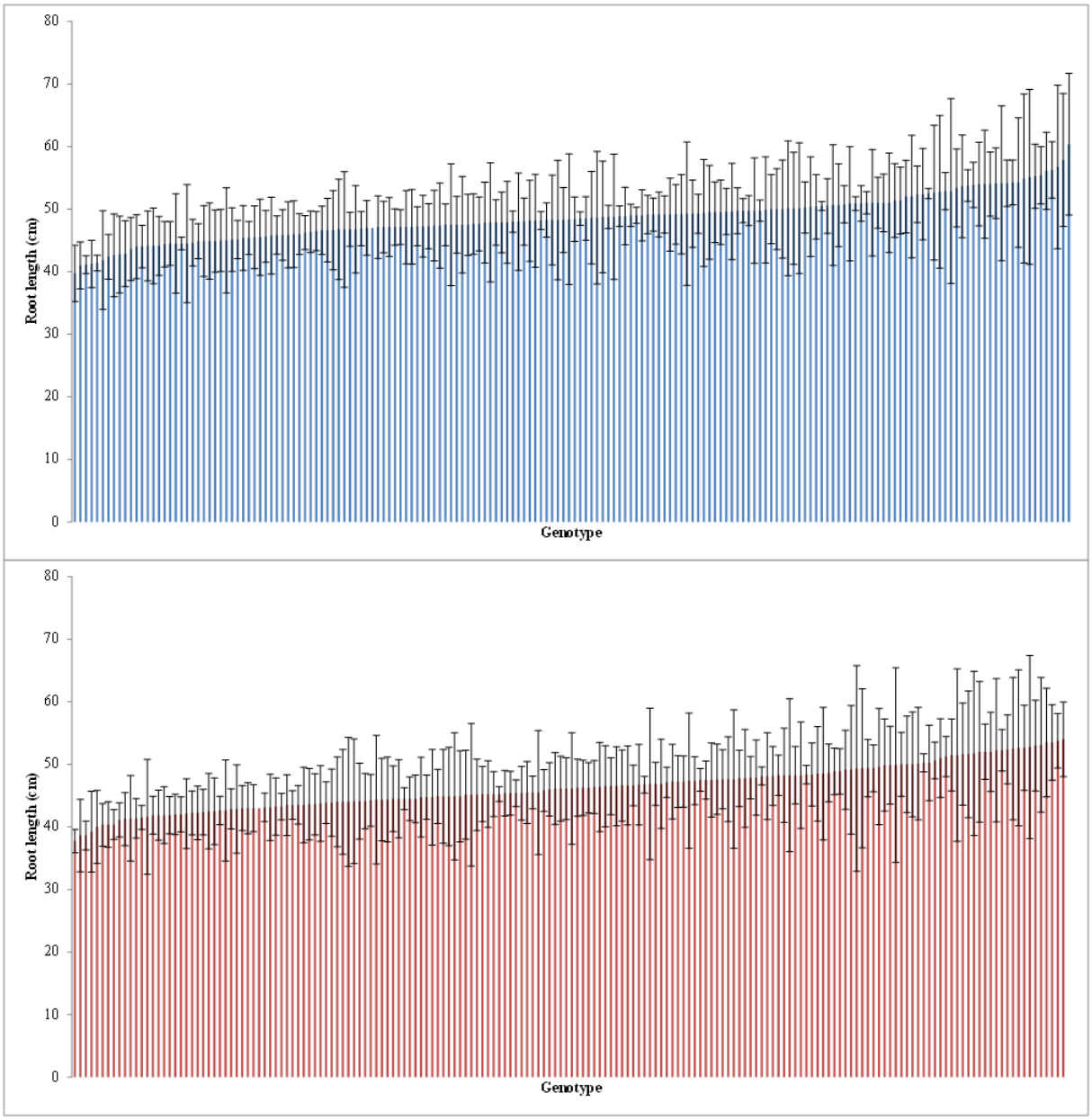

Figure 33: Phenotypic variation of root length (RI) in 2014 of global population under control and drought conditions. A) RI (cm) of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) RI (cm) of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

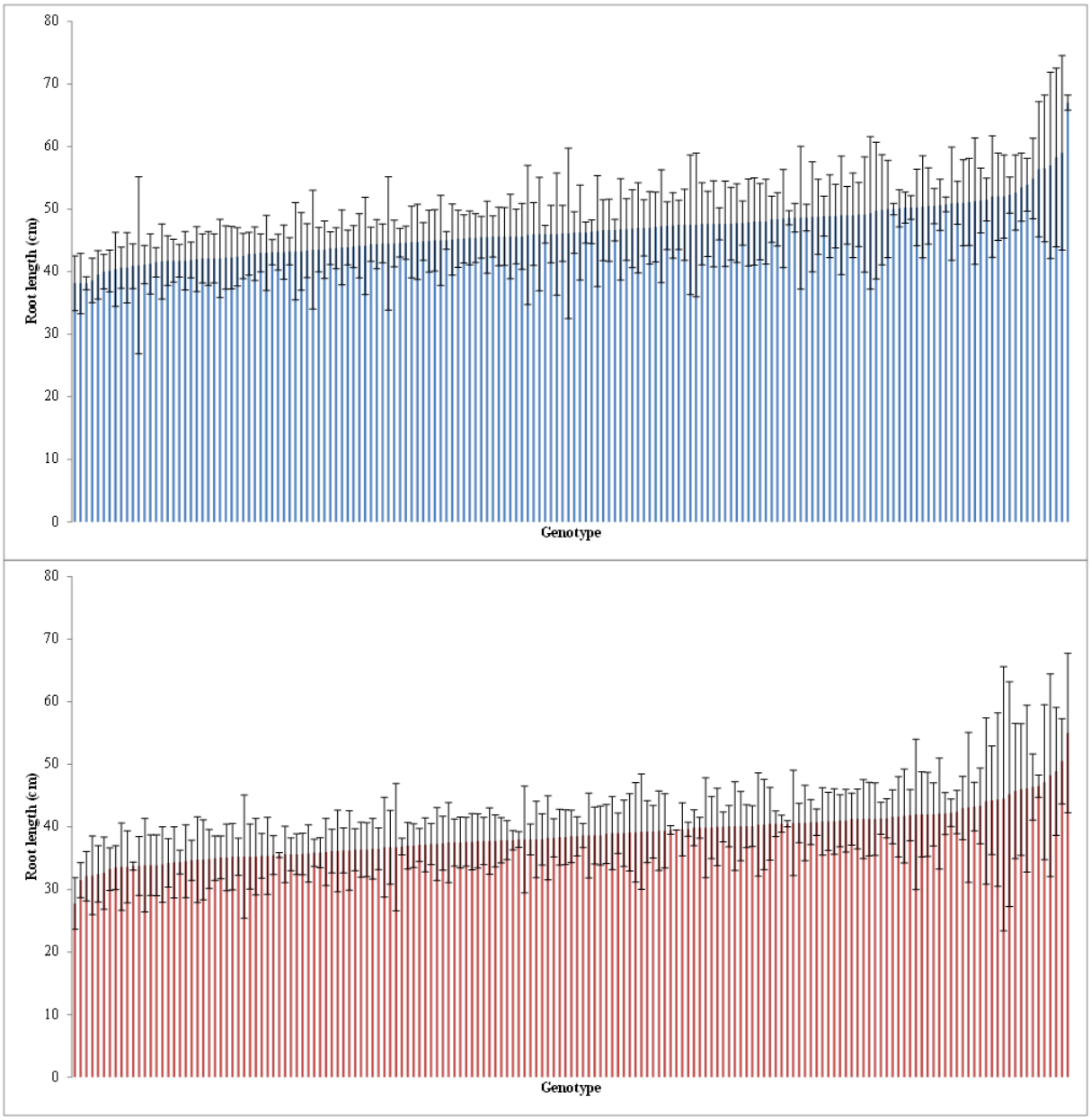

Figure 34: Phenotypic variation of root length (RI) in 2015 of global population under control and drought conditions. A) RI (cm) of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) RI (cm) of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

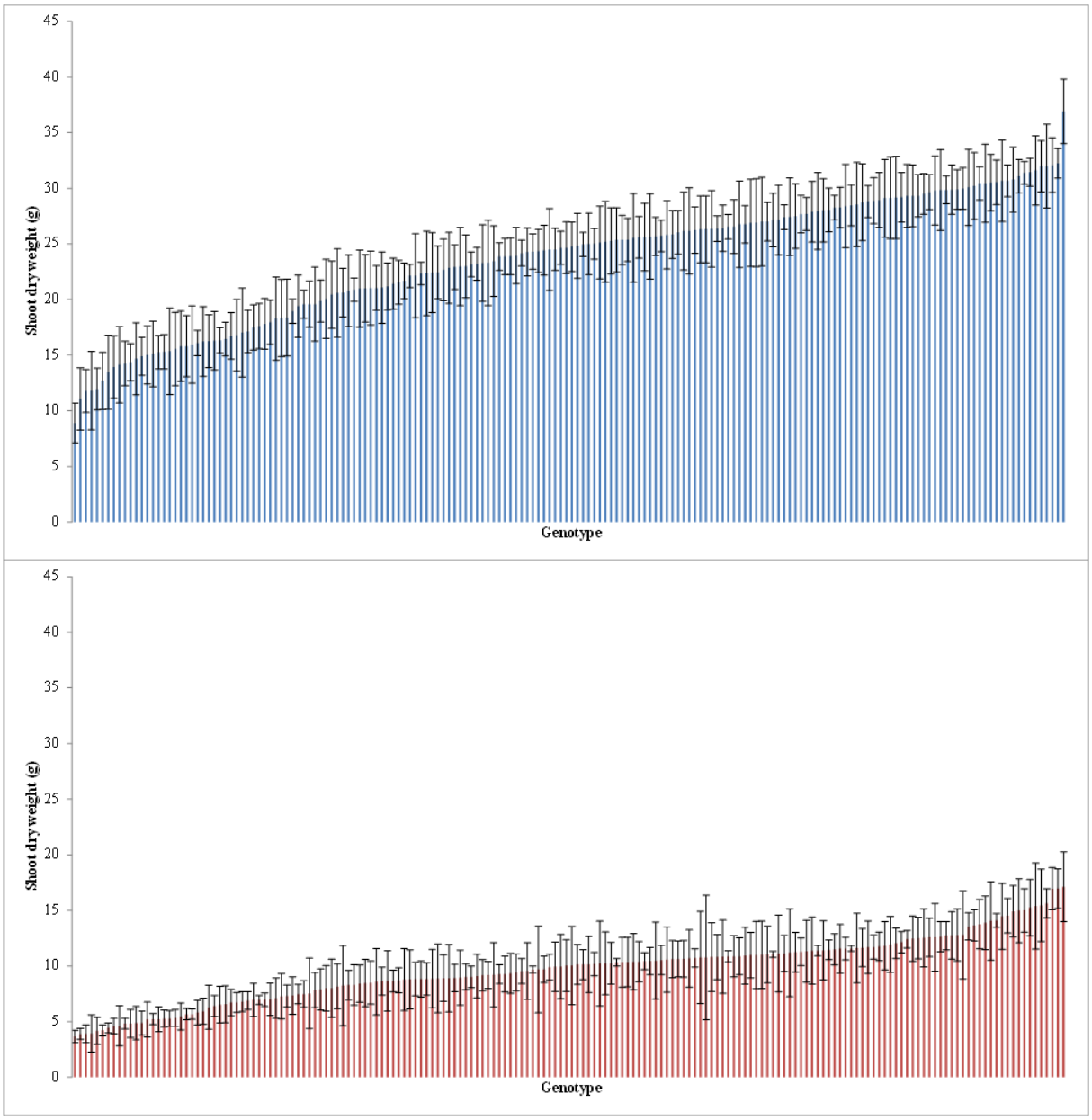

Figure 35: Phenotypic variation of shoot dry weight (Sdw) in 2014 of global population under control and drought conditions. A) Sdw (g) of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) Sdw (g) of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

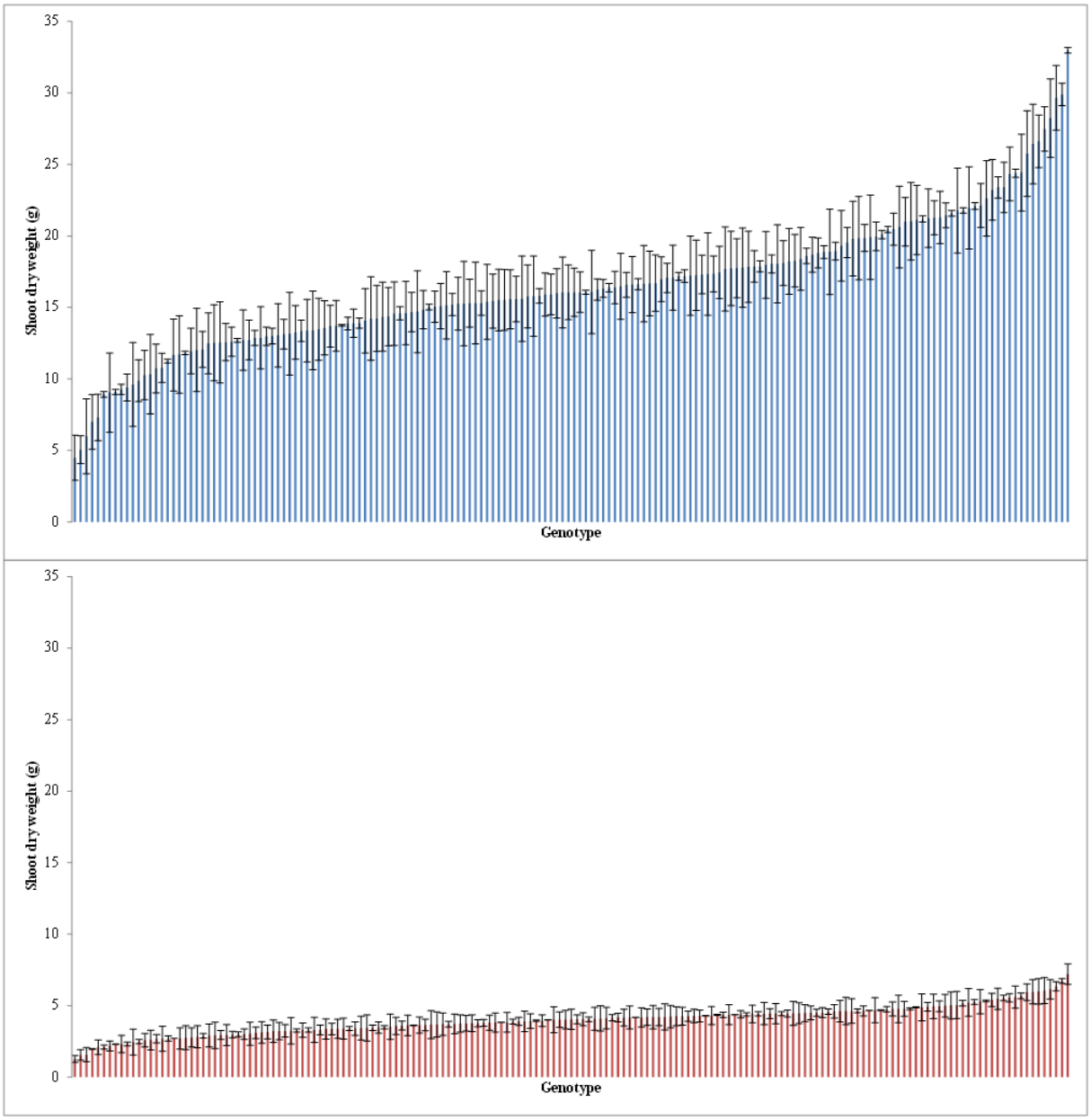

Figure 36: Phenotypic variation of shoot dry weight (Sdw) in 2015 of global population under control and drought conditions. A) Sdw (g) of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) Sdw (g) of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

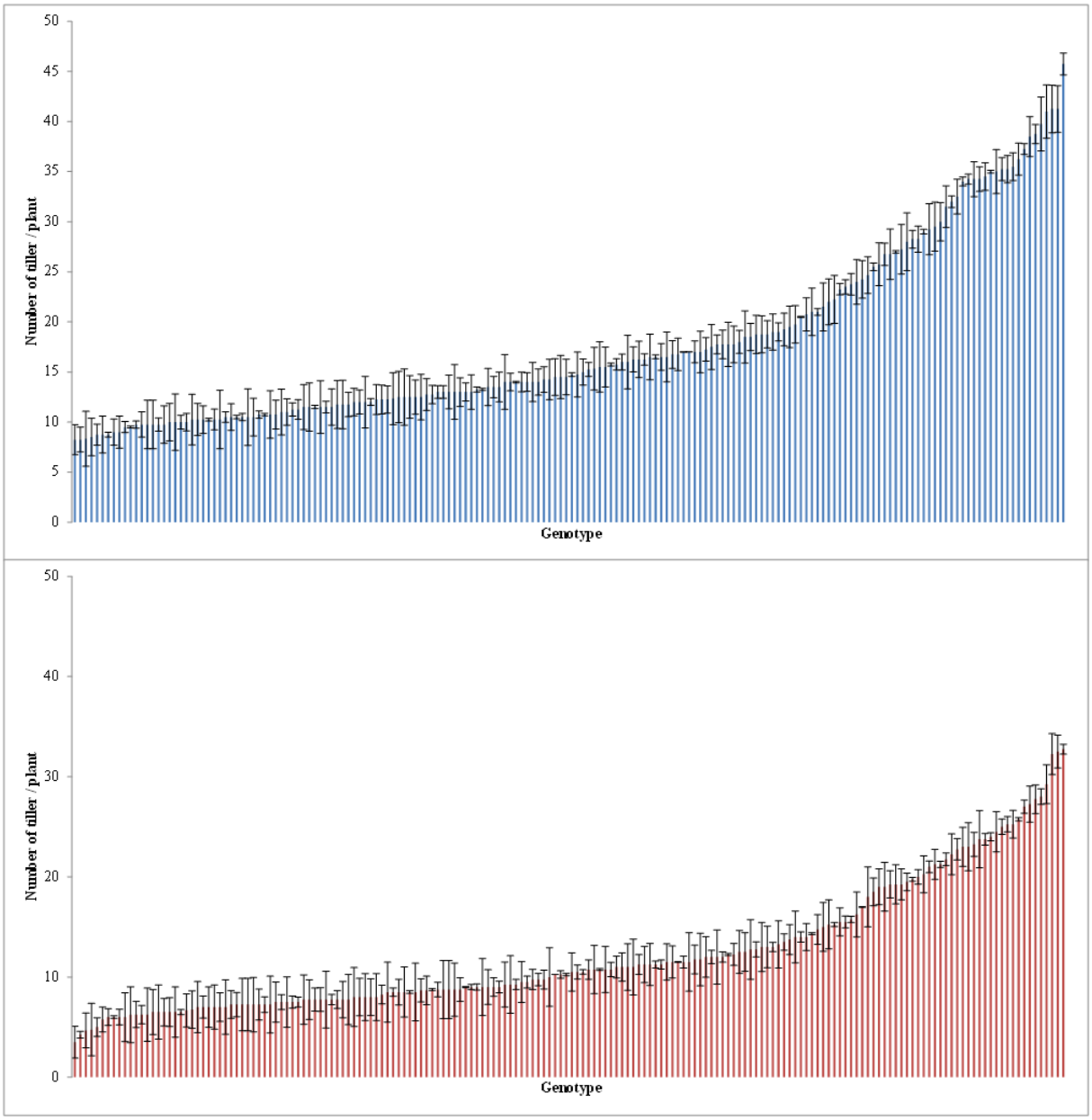

Figure 37: Phenotypic variation of tiller number (Til) in 2014 of global population under control and drought conditions. A) Til of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) Til of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

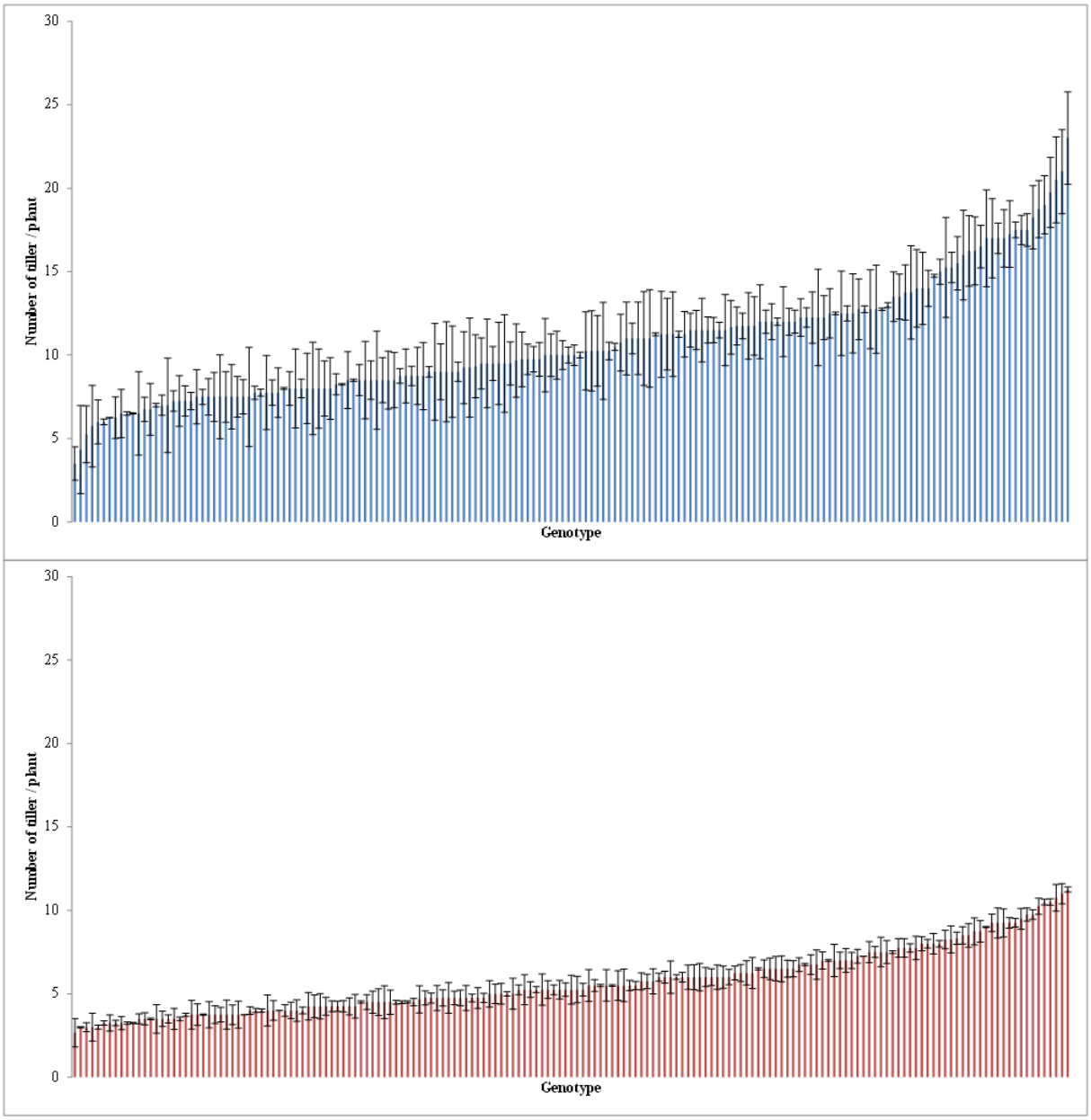

Figure 38: Phenotypic variation of tiller number (Til) in 2015 of global population under control and drought conditions. A) Til of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) Til of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

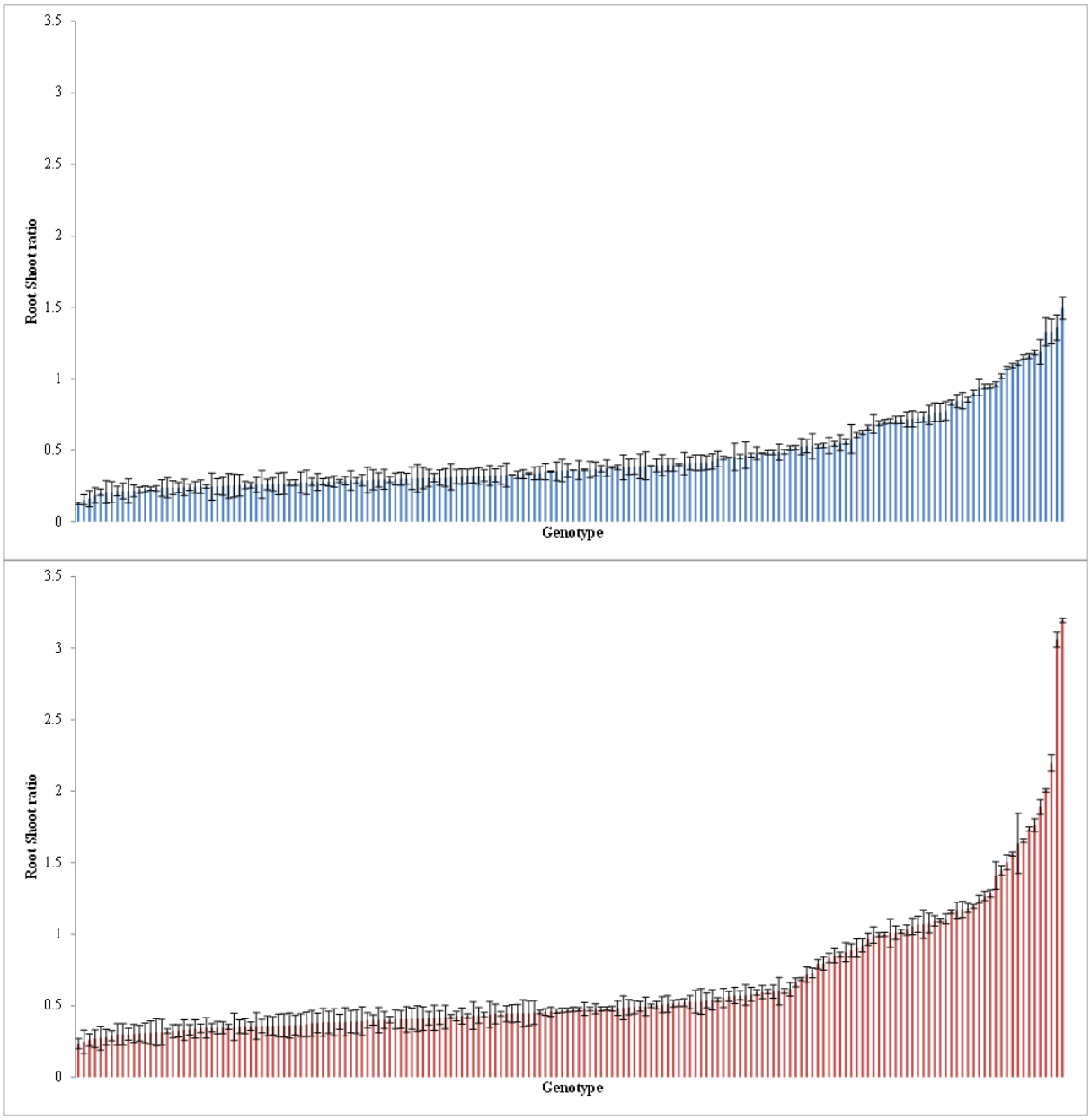

Figure 39: Phenotypic variation of root-shoot ratio (RS) in 2014 of global population under control and drought conditions. A) RS of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) RS of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4

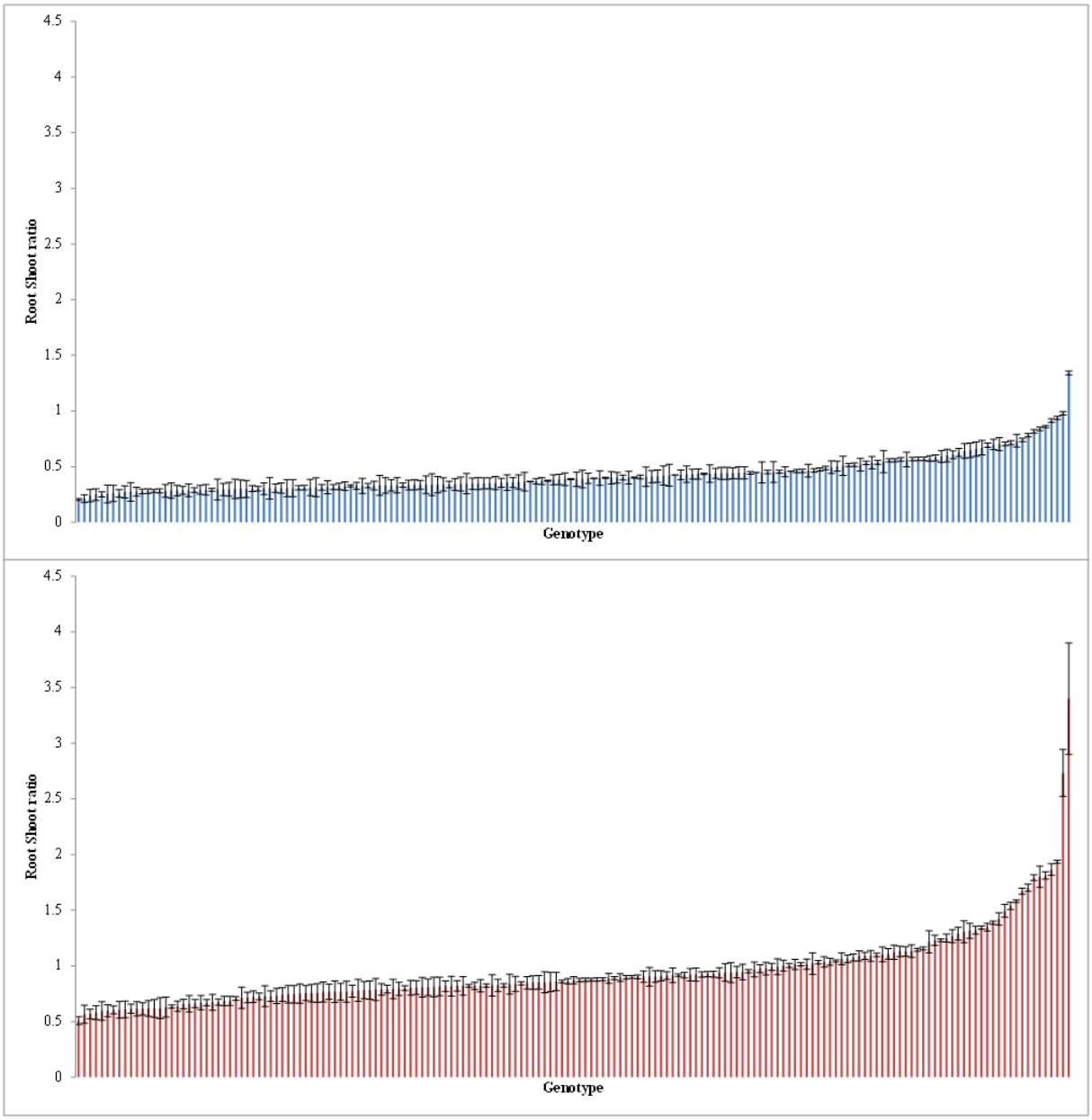

Figure 40: Phenotypic variation of root-shoot ratio (RS) in 2015 of global population under control and drought conditions. A) RS of genotypes of barley population under control conditions as well as population mean, max, min and standard deviation (STD). B) RS of genotypes of barley population under drought conditions as well as population mean, max, min and standard deviation (STD). N = 4