

Article

QTL for Water Use Related Traits in Juvenile Barley

Gwendolin Wehner¹, Christiane Balko¹ and Frank Ordon^{2,*}

¹ Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Rudolf-Schick-Platz 3, 18190 Sanitz, Germany;

gwendolin.wehner@julius-kuehn.de (G.W.); christiane.balko@julius-kuehn.de (C.B.)

² Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

* Correspondence: frank.ordon@julius-kuehn.de; Tel.: +49-3946-47-601

Academic Editor: Silvio Salvi

Received: 28 September 2016; Accepted: 1 December 2016; Published: 8 December 2016

Abstract: Water use efficiency (WUE) is a trait of prime interest in cases of drought stress because it provides information on biomass production in limited water conditions. In order to get information on WUE and additional water use related traits, i.e., dry weight (DW), fresh weight (FW), total leaf water (LW) and leaf water content (WC), greenhouse pot experiments were conducted on 156 barley genotypes (*Hordeum vulgare* L.) for control (70% maximal water capacity of soil) and drought stress conditions (20% of the maximal water capacity of soil). Significant correlations between WUE and the other water use related traits ($r \leq 0.65$) were determined in juvenile barley, and genotypes suited for improving drought stress tolerance in early developmental stages were identified. Furthermore, based on the significant effects of genotypes and treatments, as well as their interaction, data were used for genome wide association studies (GWAS) resulting in the identification of 14 marker trait associations (MTAs) corresponding to four quantitative trait loci (QTL). For WUE, four MTAs were detected mostly located on barley chromosome 4H. For four MTAs, functional annotations related to the involvement in response to abiotic stress were found. These markers may be of special interest for breeding purposes in cases when they will be validated and also detected in later growth stages.

Keywords: barley; drought stress; water use efficiency; water content; GWAS; QTL

1. Introduction

Drought stress is one of the most important abiotic stress factors in crop production worldwide and therefore of prime importance in plant breeding [1]. Drought stress response is regulated by many factors on the molecular, biochemical and physiological level [2–4] and is inherited in a quantitative manner. For instance, increased root growth, shoot growth inhibition, reduced transpiration and inhibition of photosynthesis are physiological responses to water deficit [3,5]. Drought stress may appear at different developmental stages, as a lack of water can occur pre-flowering, during grain-filling, or continuously [6]. Already in the juvenile stages, from sowing to tillering, drought can severely influence yield development [7]. At all stages, plants try to maintain water content in leaves in order to avoid drought stress [8,9], e.g., by stomatal closure, which, like most of the drought stress response mechanisms, is regulated by the phytohormone abscisic acid (ABA) [2,10].

Plant water uptake is conducted via aquaporin protein channels of root hair cells and is regulated by a gradient in water potential between the ambient soil and the root hair cell cytoplasm [11,12]. The actual amount of absorbed water, i.e., total leaf water (LW), can be measured by comparing fresh weight (FW) and dry weight (DW) of above ground biomass at a defined time point [13,14]. Other parameters are the leaf water content (WC), which is estimated in relation to dry weight, or the relative water content (RWC), based on the full turgid weight of leaves [15]. In the present study, the water

use related traits FW, DW and LW as well as WC and water use efficiency (WUE) based on DW were determined.

Flow of water through plants is regulated by transpiration, which is driven by differences in the water potential between the leaf cell cytoplasm and the ambient air [12]. Transpiration mainly takes place at the stomata and can be measured by gravimetric screening [16]. Leaf stomata are able to balance transpiration and CO₂ uptake, which has a direct impact on photosynthesis and carbon assimilation [5]. Carbon isotope discrimination ($\Delta^{13}\text{C}$) is positively correlated to the ratio of internal to ambient CO₂ levels and negatively correlated to transpiration efficiency [17]. Studies on plant photosynthesis showed that C discrimination, which occurs during carbon assimilation, is closely related to WUE [18] and can be used as an indirect parameter to get information on WUE, which is defined as the amount of water needed for the production of one unit plant dry matter or crop yield [12,19]. However, WUE is not always specifically correlated to crop yield [20], and, besides this, differences in terminology concerning units and scales have to be taken into account [21]. Additionally, the term “effective use of water” was introduced by Blum [22] as a major target for crop production, which is calculated out of soil moisture and stomatal transpiration. Nevertheless, high WUE is an advantage in managing unfavourable environmental conditions, such as drought, especially in critical reproductive growth stages [16], and, thus, its improvement is a major goal in plant breeding [23,24].

Barley (*Hordeum vulgare* L.) is a crop of worldwide importance [25], which is adapted to growing areas ranging from the polar circle to the tropics [26]. In barley, a lot of studies on water use related traits were conducted. For instance, juvenile barley was analysed for biomass parameters and water content, as well as WUE under limited water conditions [13,27]. Furthermore, the influence of osmotic adjustment to RWC [28] and the relationship between dry matter and WUE [29] were determined. For the analysis of overall drought response mechanisms, a lot of tools are developed in the phenotyping of water use related traits e.g., infrared, in addition to hyper spectral thermography and digital growth analysis [30–32].

To combine phenotypic information with genotypic data, genome wide association studies (GWAS) are commonly used today [33]. Genotypic data can be generated applying e.g., next generation sequencing (NGS) [34,35], high throughput array technologies like the Infinium iSelect assay by Illumina [36], the Axiom technology by Affymetrix [37] or genotyping by sequencing (GBS) [38,39], resulting in high density genetic maps, e.g., published by Silvar, et al. [40]. Quantitative trait loci (QTL) for water use related traits under drought stress treatment in barley were identified e.g., by Varshney, et al. [41] for biomass on barley chromosome 3H, by Teulat, et al. [42] and Honsdorf, et al. [13] for WC on chromosomes 1H, 6H and 7H and by Diab, et al. [43] and Chen, et al. [44] for WUE on chromosomes 3H, 5H, 6H and 7H.

Therefore, based on genotypic data already published by Wehner, et al. [45] and biomass yield obtained in this study, the present study aimed at (i) the determination of above ground water use related traits under control and drought stress conditions in juvenile barley, to get information on genotypic differences concerning these traits in early developmental stages; and (ii) the identification of QTL using a whole genome association mapping approach.

2. Results

2.1. Phenotyping

Two years of pot experiments revealed significant variation for all water use related traits (Table 1). For DW of above ground biomass published already in Wehner, et al. [45], as well as for FW, total LW and leaf WC, means were lower in the stress treatment than in the well watered control variant. In contrast, the WUE increased under drought stress. Furthermore, for all traits, the coefficient of variation (CV) is higher in the control treatment, except for WC. The same holds true for the least significant differences (LSD) for all traits except WC and WUE (Table 1).

Presuming a normal distribution, tested by Shapiro–Wilk test (data not shown), analysis of variance (ANOVA) revealed significant effects of genotype, treatment, year and interactions of genotype and treatment as well as genotype and year for all traits analysed (Table 2). Results for DW can be found in Wehner, et al. [45].

Table 1. Descriptive statistics for water use related traits under control and drought stress conditions estimated on 156 barley genotypes.

Trait ^a	Description	Calculation	Treat. ^b	Unit	Min ^c	Max ^c	Mean ^c	SD ^c	CV ^c	LSD ^c
FW	Fresh weight of shoot biomass per pot		Control	g	67.96	188.00	120.92	19.18	0.16	27.86
			Stress	g	26.33	45.33	36.62	3.35	0.09	7.23
LW	Total leaf water	FW-DW	Control	g	64.20	177.83	111.81	17.66	0.16	26.11
			Stress	g	23.27	39.68	32.32	3.03	0.09	6.73
WC	Leaf water content	LW/DW	Control	unit free	10.06	17.15	12.24	1.02	0.08	2.03
			Stress	unit free	5.69	12.17	7.60	1.06	0.14	2.20
WUE	Water use efficiency	DW/total water added	Control	mg/g water	1.48	3.13	2.42	0.34	0.14	0.58
			Stress	mg/g water	2.51	4.31	3.42	0.31	0.09	1.11

^a FW: fresh weight, DW: dry weight, LW: total leaf water, WC: leaf water content, WUE: water use efficiency based on one year of results; ^b Control and drought stress treatment; ^c Minimum, maximum, mean, standard deviation in % (SD), coefficient of variation (CV) (standard deviation divided by mean) and least significant difference (LSD).

Table 2. Analysis of variance (ANOVA) of the water use related traits showing *F* and *p* values.

Trait ^a	Effect ^b	<i>F</i> Value	<i>p</i> Value
FW	Genotype	5.12	<0.0001
	Treatment	18,938.63	<0.0001
	Year	39.38	<0.0001
	G × T	4.01	<0.0001
	G × Y	1.51	0.0002
LW	Genotype	5.11	<0.0001
	Treatment	20,443.78	<0.0001
	Year	41.61	<0.0001
	G × T	4.21	<0.0001
	G × Y	1.50	0.0002
WC	Genotype	4.71	<0.0001
	Treatment	8631.12	<0.0001
	Year	245.29	<0.0001
	G × T	1.35	0.0048
	G × Y	2.39	<0.0001
WUE	Genotype	3.89	<0.0001
	Treatment	1928.18	<0.0001
	G × T	1.35	0.0076

^a FW: fresh weight, DW: dry weight, LW: total leaf water, WC: leaf water content, WUE: water use efficiency based on one year of results; ^b Genotype, Treatment (T), Year (Y) and G × Y or T: genotype × year or treatment interaction effect.

Correlations between the traits analysed are shown in Table 3. The highest correlations ($p < 0.001$) were estimated between FW and LW with $r = 1$ for control and $r = 0.98$ for the drought stress treatment (Table 3). The strong relationship of both traits resulted from the calculation of LW out of FW and DW. Thus, also between LW and DW, highly significant correlations were calculated with $r = 0.91$ for control and $r = 0.48$ for drought stress treatment. Furthermore, DW and FW were also significantly correlated for control ($r = 0.92$) and drought stress treatment ($r = 0.59$). The same holds true for WC and WUE, which were related on a dry weight basis, which explains the correlation between WC and DW as well as between WUE and DW of $r = -0.54$ and $r = 0.64$ for control and $r = -0.60$ and $r = 0.65$ for drought stress treatment, respectively. Between LW and WC, a significant ($p < 0.01$) but rather low correlation was calculated, i.e., $r = -0.22$ for control and $r = 0.31$ for drought stress. Both traits were significantly correlated to the WUE with $r = 0.54$ and $r = -0.44$ for control as well as $r = 0.54$ and $r = -0.22$, respectively, for the drought stress variant (Table 3).

All water use related traits analysed were significantly correlated to WUE (Table 3) and are therefore of special interest with respect to drought tolerance mechanisms. The variability of these traits in relation to WUE is shown in Figure 1. Results show a larger variability under control conditions than under drought stress. In this respect, genotypes with e.g., high biomass production (FW or DW) and high WUE are of special interest.

Table 3. Coefficients of correlation (Spearman) for control and drought stress treatment based on one year of results.

Trait ^a	Treatment	DW	LW	WC	WUE
FW	Control	0.92 ***	1 ***	−0.26 **	0.55 ***
	Drought stress	0.59 ***	0.98 ***	0.18 *	0.59 ***
DW	Control		0.91 ***	−0.54 ***	0.64 ***
	Drought stress		0.48 ***	−0.60 ***	0.65 ***
LW	Control			−0.22 **	0.54 ***
	Drought stress			0.31 **	0.54 **
WC	Control				−0.44 ***
	Drought stress				−0.22 **

^a FW: fresh weight, DW: dry weight, LW: total leaf water, WC: leaf water content, WUE: water use efficiency; r is significant with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

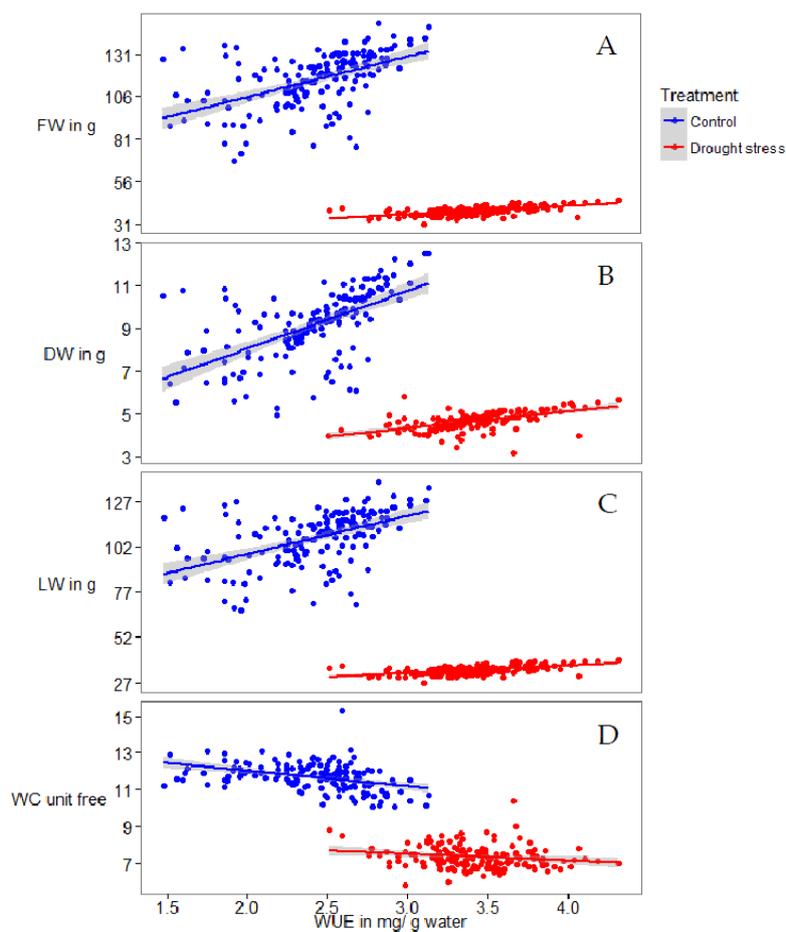


Figure 1. Linear regression for (A) fresh weight (FW), (B) dry weight (DW), (C) total leaf water (LW) and (D) leaf water content (WC) to water use efficiency (WUE) under control (blue) and drought stress (red) treatment based on one year of results.

2.2. Genome Wide Association Study

Associated genomic regions with regard to drought stress were identified for all water use related traits (Table 4). Out of these, QTL for FW are not shown because of the autocorrelation to LW (Table 3), and QTL for DW were published already in Wehner, et al. [45]. For the other traits analysed, i.e., LW, WC and WUE, 14 marker trait associations (MTAs) ($p < 0.001$) were detected (Table 4). Out of these, 10 MTAs were found for control and four MTAs for drought stress conditions. Identified MTAs were located on barley chromosomes 3H to 5H and 7H. MTAs were assigned to four QTL regions by the linkage disequilibrium (LD) decay, which was calculated for this set of genotypes at an average of 2.52 cM [45].

For LW, two MTAs were detected under control and drought stress conditions each, resulting in two QTL. One QTL was located on chromosome 5H at 124.52 cM and the other QTLs on chromosome 7H at 48.49 cM, also comprising an MTA for WUE at 47.14 cM for the well watered treatment (Table 4).

Six MTAs for WC were found mostly on chromosome 3H under control conditions, except one MTA on chromosome 3H at 21.38 cM and another MTA on chromosome 5H at 36.18 cM in the drought stress treatment (Table 4). Two of these MTAs on chromosome 3H were associated to markers located at 113.27 cM with functional annotations of Leucine rich repeat receptor like protein kinases.

For WUE, four MTAs were identified on chromosome 4H, of which one was located at 14.74 cM and three formed one QTL at 61 cM (Table 4). While the QTL was found under well watered conditions, the MTA was detected under drought stress treatment. Another QTL for WUE determined under control conditions was located on chromosome 7H at 47.14 cM. All of these explained a rather high portion of the phenotypic variance (R^2 values ranges from 10.5% to 12.89%).

For all significantly associated markers, a corresponding Morex contig, as well as high or low confidential genes, were identified. Functional annotations are listed for the high confidential genes (Table 4).

Table 4. Significant marker trait associations ($p < 0.001$) with positions in the barley genome as well as corresponding Morex contigs and functional annotations.

Trait ^a	Treat. ^b	SNP Marker ^c	Chr. ^c	Pos. in cM ^c	F Value	p Value	$-\log p$ (LOD) ^d	R ² in %	Morex Contig ^e	Conf. ^e	Functional Annotation ^e	Gene Name ^e
WC	S	i_SCRI_RS_210101	3H	21.38	7.10	0.0010	3.01	3.46	morex_contig_46157	HC	Eukaryotic translation initiation factor 3 subunit, putative	MLOC_62056.2
WC	C	i_SCRI_RS_150063	3H	113.27	8.03	0.0004	3.40	3.73	morex_contig_6173	HC	Leucine-rich repeat receptor-like protein kinase	MLOC_72476.1
WC	C	i_SCRI_RS_203905	3H	113.27	7.39	0.0007	3.13	3.43	morex_contig_2551243	HC	Leucine-rich repeat receptor-like protein kinase	MLOC_38740.7
WC	C	i_11_20527	3H	124.89	7.39	0.0007	3.13	3.43	morex_contig_44198	HC	Hydroxyproline-rich glycoprotein-like	MLOC_60122.1
WC	C	i_12_30921	3H	147.34	12.04	0.0006	3.22	2.79	morex_contig_36914	HC	RNA-dependent RNA polymerase	MLOC_51409.1
WUE	S	i_11_10319	4H	14.74	7.98	0.0005	3.29	10.50	morex_contig_2553017	HC	Pathogenesis-related thaumatin-like protein	MLOC_39318.1
WUE	C	i_12_31385	4H	61.21	6.35	0.0004	3.35	12.62	morex_contig_2548269	HC	Homocysteine S-methyltransferase 1, putative, expressed	MLOC_37381.1
WUE	C	i_SCRI_RS_151213	4H	61.56	5.72	0.0010	3.00	11.36	morex_contig_276516	LC	-	MLOC_45156.1
WC	C	i_SCRI_RS_203117	5H	36.18	7.23	0.0009	3.06	3.36	morex_contig_1581968	LC	-	MLOC_18779.2
LW	C	i_SCRI_RS_220136	5H	80.33	13.60	0.0003	3.57	3.84	morex_contig_1565092	HC	unknown protein; involved in: N-terminal protein myristoylation	MLOC_13193.1
LW	S	i_11_21247	5H	124.52	12.62	0.0004	3.35	2.82	morex_contig_135323	HC	Protein kinase, putative	MLOC_4611.1
										HC	Aluminum-induced protein-like	MLOC_51340.2
LW	S	i_12_21471	5H	124.52	12.62	0.0004	3.35	2.82	morex_contig_36899	HC	Transcription factor, putative	AK373628
WUE	C	i_11_21528	7H	47.14	9.74	0.0001	3.97	12.89	morex_contig_1582615	HC	WD repeat-containing protein, putative	AK366046
LW	C	i_SCRI_RS_202061	7H	48.49	8.08	0.0004	3.41	4.56	morex_contig_1567807	HC	unknown protein;	AK372377
										HC	AT hook motif DNA-binding family protein	MLOC_14326.3

^a LW: total leaf water, WC: leaf water content, WUE: water use efficiency based on one year of results; ^b Control (C) and drought stress (S) treatment; ^c Single nucleotide polymorphism (SNP) markers and chromosome positions are based on Silvar et al. [40]; ^d LOD: likelihood of odds; ^e Morex contigs, high confidence (HC) and low confidence (LC) levels, as well as functional annotation and gene names were imported from the Barley genome explorer (BARLEX).

3. Discussion

Effects of drought stress on juvenile barley genotypes were estimated primarily as the reduction in FW (Table 1 and Figure 1) and DW [45]. Limited biomass production under drought stress was detected in a lot of studies in barley in field experiments [41], greenhouse experiments [13,45] and hydroponics [14], and was also observed in different developmental stages [46]. In our studies, a significant effect of the treatments and genotypes and also significant interactions of genotypes and treatments were detected (Table 2), which are due to the large variability of the 156 genotypes analysed, and makes this set highly suitable for GWAS. The variability for all traits analysed was higher in the control than in the stress variant (Table 1 and Figure 1). This may be due to the severe drought stress at 20% of maximal water capacity of soil and a stress period of four weeks. Nevertheless, this severe drought application allowed to differentiate treatment effects for all traits determined in the present study and in Wehner, et al. [45].

The total LW and leaf WC indicate the total amount of water in the above ground biomass and the relation to DW, respectively. Under drought stress conditions, the LW and WC in the leaves decreased with a decrease in biomass production (Table 1 and [45]), which was also shown in other studies in barley [13,47]. A high, significant negative correlation was estimated between WC and DW in the control ($r = -54$) and drought stress treatment ($r = -60$) (Table 3). High DW is an indicator for large plants transpiring more water than smaller plants because transpiration depends to a large extent on leaf area [48]. Water content in the leaves also depends on osmolality because the more solutes are accumulated in the leaves, the more water will be absorbed and retained [28]. With a defined soil volume in the experimental design used, the amount of water uptake is limited, but by individual watering of each pot until a defined soil water capacity of control and drought stress treatment, genotypic differences with respect to adaptability could be detected. For the control treatment, a high correlation was detected between LW and DW ($r = 0.91$), whereas, under drought stress conditions, this correlation was much lower ($r = 0.48$), indicating a different response of genotypes under water limiting conditions (Table 3). The same holds true for correlations of DW and FW (Table 3) and is also supported by significant genotypic effects and a significant interaction of genotype and treatment (Table 2).

A trait of special interest in breeding for drought tolerance is WUE [49]. In several studies, as well as in the present study (Table 1), an increase of WUE under drought stress treatment compared to the control was found [50,51]. WUE describes the amount of biomass production per unit water supplied, whereas Blum [22] introduced a trait named effective use of water for yield prediction. Thus, WUE is not highly suitable for yield improvement but gives valuable information on drought tolerance [52]. In the present study, the FW and DW for biomass production and LW and WC for water uptake were determined. Therefore, all parameters for identifying drought tolerant genotypes were analysed and turned out to be suited, because of correlations to WUE ranging from $r = -0.44$ to $r = 0.64$ and $r = -0.22$ to $r = 0.65$, for control and drought stress treatment, respectively (Table 3). For instance, with a combination of high biomass production, presented by high FW and DW, and a high WUE under water limiting conditions, most efficient genotypes can be identified [53] because the higher the usage of water for producing dry matter, the higher the efficiency [19]. The same holds true for the combination of high water availability, described by high LW, and WUE, as well as for low WC and high WUE under drought stress conditions. As a low WC is often linked to a high WUE, a negative correlation of these traits (Table 3) was observed, i.e., plants with a low WC, relative to DW, were able to use water more efficiently. In Figure 1, genotypes are grouped by WUE in relation to FW, DW, and LW, as well as WC and different groups of adaptability, can be identified applying the criteria described above. However, as the calculation of WUE is based on one year of data, only, results have to be validated in additional studies in order to identify genotypes with adaptability to drought stress.

In addition to GWAS conducted on this set of genotypes for physiological data already by Wehner et al. [45], GWAS for water related traits and functional annotations of some marker trait associations (MTA) were carried out in this study.

An interesting QTL region on chromosome 5H was located at 124.52 cM, where two MTAs were found for LW under drought stress. Nevertheless, no special functional annotation with regard to abiotic stress was found (Table 4). For the QTL on chromosome 7H (Table 4), and for the marker associated to LW e.g., an AT hook protein was detected. These proteins are acting as chromatin remodelling factors that delay leaf senescence in leaves of *Arabidopsis thaliana* [54]. Leaf senescence itself is closely linked to drought, as nutrients and metabolites are relocated from leaves to ears, and, therefore, chlorophyll is prematurely degraded [55,56].

Within the QTL for LW on chromosome 7H, an MTA for WUE was also located at 47.14 cM, which was observed under control conditions, too (Table 4). For the function of this MTA, a WD repeat-containing protein was identified, which is not related to abiotic stress tolerance in plants [57]. In addition, a QTL for WUE under control conditions was located on chromosome 4H around 61 cM. The functional annotation of one of the markers identified a Homocysteine S-methyltransferase 1 (Table 4). This protein is included in the methionine synthase [58] and may be activated in response to abiotic stresses, such as salt stress [59]. Most MTAs for WUE were located on chromosome 4H in this study (Table 4). Moreover, in a comparable other study in juvenile barley, a QTL for WUE was also found on chromosome 4H [27]. This chromosome seems to be one of the main regions for the regulation of WUE in barley [60,61].

For WC in the control treatment, a QTL was identified on barley chromosome 3H at 113.27 cM, including two different Morex contigs for the two co-segregating markers. For both, Leucine-rich repeat receptor kinases were identified, which are located in the plasma membrane and are involved in a plant disease resistance pathway [62], as well as in ABA signalling and in salt tolerance mechanisms in roots of legumes [63,64]. Moreover, at 124.89 cM on chromosome 3H, an MTA for WC was detected for control conditions (Table 4), for which functional annotation again gives a hint for plant defence mechanisms [65]. The link of abiotic and biotic stresses is the focus of research today [66].

The present study contributes to elucidating drought stress response in juvenile barley with regard to water use related traits and to identifying markers that may be the basis for the selection of drought tolerant genotypes. In particular, those with functional annotation in relation to abiotic stress are of special interest. These are i_SCRI_RS_202061, i_12_31385 and i_SCRI_RS_150063 as well as i_SCRI_RS_203905, associated with LW, WUE and WC, respectively (Table 4).

4. Materials and Methods

For analyzing drought stress, a set of barley genotypes [45,67–69] was used consisting of 113 German winter barley cultivars and 43 accessions of the Spanish Barley Core Collection (SBCC). The materials were listed in detail in Additional file 1 in Wehner, et al. [45].

Investigations were carried out in pot experiments in greenhouses of the Julius Kühn-Institut in Groß Lüsewitz, northern Germany for two years of trials. The experimental design used for drought stress application was described in [45], Wehner, et al. [70]. The barley genotypes were characterized in the juvenile stage (phenological growth stage (BBCH) 33, according to Stauss [71]). Drought stress application started at the primary leaf stage seven days after sowing (das). After a four-week stress period, the whole above ground biomass was harvested at 36 das and weighted for determination of FW under drought stress and well watered conditions. Furthermore, DW was analysed for the drought stress and control treatment according to Wehner, et al. [70]. Out of FW and DW of the biomass per pot, the parameters total leaf water ($LW = FW - DW$) and leaf water content ($WC = LW/DW$) were calculated (Table 1). Furthermore, for one experiment, the total amount of added water per pot was assessed by summing up the amount of daily watering in control and stress conditions. Based on these data, water use efficiency ($WUE = DW/\text{total water added}$) was calculated (Table 1).

The Shapiro–Wilk test for normal distribution (package stats, function shapiro.test) followed by an outlier test [72] and ANOVA using a mixed linear model were carried out using RStudio 3.2.5 (R, Boston, MA, USA) [73] (package nlme, function lme) to test effects of genotype, year and treatment, as well as interaction of genotype and treatment (GxT), in addition to genotype and year

(G×Y) (Table 2). Replication was set as a random effect in the model. Additionally, last square means (LSMeans) were generated in RStudio for the two years (package lsmeans, function lsmeans), and descriptive statistics (package pastecs, function stat.desc and package agricolae, function LSD.test) were conducted (Table 1). Using LSMeans, coefficients of correlation (Spearman) were calculated in RStudio (package Hmisc, function rcorr) between the parameters determined (Table 3). The graphical scatter plots for the correlations between all parameters and water use efficiency (Figure 1) were also generated in RStudio (package ggplot2, function ggplot). Because the factor “year” had a significant effect (Table 2), correlations (Table 3) as well as the scatter plots (Figure 1) in this paper are shown only for the year in which WUE was analysed.

Furthermore, LSMeans were used for GWAS. The whole set of genotypes was analysed with the Infinium 9 k iSelect Chip (Illumina, San Diego, CA, USA). For mapping, a recent consensus map of single nucleotide polymorphism (SNP) markers by Silvar, et al. [40] was used. After filtering for >5% minor allele frequency (MAF), <10% missing values and <5% heterozygous markers, 4438 SNP markers were used to construct a HapMap and to calculate a kinship of Roger’s distances [74] in the open, web-based platform Galaxy [75–77]. Population structure was already calculated in Wehner, et al. [45] and the respective q-matrix was also used in this study. Applying a mixed linear model (MLM) in Tassel 5 [78,79], taking into account kinship and q-matrix, GWAS was conducted based on two years of data for most traits but one year only for WUE. Because of significant effects of the years (Table 2), the effect of the year was included as a random effect into the GWAS model for all traits except WUE.

All associations with p -values < 0.001 (likelihood of odds, LOD = 3) were considered as significant (Table 4). In a final step, the contigs of the reference barley Morex genome [80] including the significantly associated markers (iSelect Markers) as well as the genes rendering these genomic regions (Gene List) were taken from the Barley genome explorer (BARLEX) [81] to get an idea of the possible function of the MTAs (Table 4).

5. Conclusions

Since drought stress is a quantitative trait, many characteristics have to be taken into account including increasing time and labour needed for phenotyping. Nevertheless, with this study and a previous one [45], a huge phenotypic and marker dataset has been published for a defined set of winter barley genotypes with respect to drought stress in the juvenile stage. This increases the chance for a pre-selection of favourable traits and alleles for drought tolerance in early developmental stages of barley. Using respective markers, a pre-selection of genotypes can be conducted, thereby reducing the number of genotypes to be tested for drought stress tolerance in the adult plant stage. In this respect, detailed analyses on the correlation between the reaction to drought stress in the juvenile and the adult plant stage have to be conducted.

Acknowledgments: The authors would like to thank Heike de Lahr for the excellent technical assistance, Ernesto Igartua of the Spanish National Research Council (CSIC), Spain for providing seeds from the Spanish Barley Core Collection (SBCC), and the Interdisciplinary Center for Crop Plant Research (IZN) of the Martin-Luther-University of Halle-Wittenberg for funding this project.

Author Contributions: G.W. conducted all experiments, including statistical and bioinformatics analyses and was the main writer of the manuscript; C.B. and F.O. designed the research, supervised the experimental design, conducted data analysis and participated in writing the manuscript. All authors approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shao, H.-B.; Chu, L.-Y.; Jaleel, C.A.; Manivannan, P.; Panneerselvam, R.; Shao, M.-A. Understanding water deficit stress-induced changes in the basic metabolism of higher plants—biotechnologically and sustainably improving agriculture and the environment in arid regions of the globe. *Crit. Rev. Biotechnol.* **2009**, *29*, 131–151. [[CrossRef](#)] [[PubMed](#)]

2. Bhargava, S.; Sawant, K. Drought stress adaptation: Metabolic adjustment and regulation of gene expression. *Plant Breed.* **2013**, *132*, 21–32. [[CrossRef](#)]
3. Barnabas, B.; Jager, K.; Feher, A. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* **2008**, *31*, 11–38. [[CrossRef](#)] [[PubMed](#)]
4. Anjum, S.A.; Xie, X.-Y.; Wang, L.-C.; Saleem, M.F.; Man, C.; Lei, W. Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.* **2011**, *6*, 2026–2032.
5. Chaves, M.M.; Maroco, J.P.; Pereira, J.S. Understanding plant responses to drought—From genes to the whole plant. *Funct. Plant Biol.* **2003**, *30*, 239–264. [[CrossRef](#)]
6. Reynolds, M.; Mujeeb-Kazi, A.; Sawkins, M. Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought-and salinity-prone environments. *Ann. Appl. Biol.* **2005**, *146*, 239–259. [[CrossRef](#)]
7. El Hafid, R.; Smith, D.H.; Karrou, M.; Samir, K. Physiological responses of spring durum wheat cultivars to early-season drought in a mediterranean environment. *Ann. Bot.* **1998**, *81*, 363–370. [[CrossRef](#)]
8. Blum, A. Osmotic adjustment and growth of barley genotypes under drought stress. *Crop Sci.* **1989**, *29*, 230–233. [[CrossRef](#)]
9. Fricke, W.; Pritchard, J.; Leigh, R.A.; Tomos, A.D. Cells of the upper and lower epidermis of barley (*Hordeum vulgare* L.) leaves exhibit distinct patterns of vacuolar solutes. *Plant Physiol.* **1994**, *104*, 1201–1208. [[CrossRef](#)] [[PubMed](#)]
10. Bray, E.A. Plant responses to water deficit. *Trends Plant Sci.* **1997**, *2*, 48–54. [[CrossRef](#)]
11. Tyerman, S.; Niemietz, C.; Bramley, H. Plant aquaporins: Multifunctional water and solute channels with expanding roles. *Plant Cell Environ.* **2002**, *25*, 173–194. [[CrossRef](#)] [[PubMed](#)]
12. Kosova, K.; Vitamvas, P.; Urban, M.O.; Kholova, J.; Prášil, I.T. Breeding for enhanced drought resistance in barley and wheat-drought-associated traits, genetic resources and their potential utilization in breeding programmes. *Czech J. Genet. Plant Breed.* **2014**, *50*, 247–261.
13. Honsdorf, N.; March, T.J.; Hecht, A.; Eglinton, J.; Pillen, K. Evaluation of juvenile drought stress tolerance and genotyping by sequencing with wild barley introgression lines. *Mol. Breed.* **2014**, *34*, 1475–1495. [[CrossRef](#)]
14. Zhao, J.; Sun, H.; Dai, H.; Zhang, G.; Wu, F. Difference in response to drought stress among tibet wild barley genotypes. *Euphytica* **2010**, *172*, 395–403. [[CrossRef](#)]
15. Weatherley, P. Studies in the water relations of the cotton plant. *New Phytol.* **1950**, *49*, 81–97. [[CrossRef](#)]
16. Vadez, V.; Kholova, J.; Medina, S.; Kakkera, A.; Anderberg, H. Transpiration efficiency: New insights into an old story. *J. Exp. Bot.* **2014**, *65*, 6141–6153. [[CrossRef](#)] [[PubMed](#)]
17. Farquhar, G.; Richards, R. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct. Plant Biol.* **1984**, *11*, 539–552. [[CrossRef](#)]
18. Farquhar, G.D.; Ehleringer, J.E.; Hubick, K.T. Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 503–537. [[CrossRef](#)]
19. Boyer, J.S. Advances in drought tolerance in plants. *Adv. Agron.* **1996**, *56*, 187–219.
20. Tuberosa, R. Phenotyping for drought tolerance of crops in the genomics era. *Front. Physiol.* **2012**, *3*. [[CrossRef](#)] [[PubMed](#)]
21. Morison, J.I.; Baker, N.R.; Mullineaux, P.M.; Davies, W.J. Improving water use in crop production. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 639–658. [[CrossRef](#)] [[PubMed](#)]
22. Blum, A. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crop Res* **2009**, *112*, 119–123. [[CrossRef](#)]
23. Condon, A.G.; Richards, R.A.; Rebetzke, G.J.; Farquhar, G.D. Improving intrinsic water-use efficiency and crop yield. *Crop Sci.* **2002**, *42*, 122–131. [[CrossRef](#)] [[PubMed](#)]
24. Condon, A.G.; Richards, R.; Rebetzke, G.; Farquhar, G. Breeding for high water-use efficiency. *J. Exp. Bot.* **2004**, *55*, 2447–2460. [[CrossRef](#)] [[PubMed](#)]
25. FAOSTAT. Available online: <http://faostat3.Fao.Org/faostat-gateway/go/to/home/e> (accessed on 13 November 2015).
26. Von Bothmer, R.; van Hintum, T.; Knüpfper, H.; Sato, K. *Diversity in Barley (Hordeum vulgare)*; Elsevier: Amsterdam, The Netherlands, 2003.
27. Honsdorf, N.; March, T.J.; Berger, B.; Tester, M.; Pillen, K. High-throughput phenotyping to detect drought tolerance qtl in wild barley introgression lines. *PLoS ONE* **2014**, *9*, e97047. [[CrossRef](#)] [[PubMed](#)]

28. Boyer, J.S.; James, R.A.; Munns, R.; Condon, T.A.; Passioura, J.B. Osmotic adjustment leads to anomalously low estimates of relative water content in wheat and barley. *Funct. Plant Biol.* **2008**, *35*, 1172–1182. [[CrossRef](#)]
29. Hubick, K.; Farquhar, G. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant Cell Environ.* **1989**, *12*, 795–804. [[CrossRef](#)]
30. Fahlgren, N.; Gehan, M.A.; Baxter, I. Lights, camera, action: High-throughput plant phenotyping is ready for a close-up. *Curr. Opin. Plant Biol.* **2015**, *24*, 93–99. [[CrossRef](#)] [[PubMed](#)]
31. Furbank, R.T.; Tester, M. Phenomics—technologies to relieve the phenotyping bottleneck. *Trends Plant Sci.* **2011**, *16*, 635–644. [[CrossRef](#)] [[PubMed](#)]
32. McAusland, L.; Davey, P.; Kanwal, N.; Baker, N.R.; Lawson, T. A novel system for spatial and temporal imaging of intrinsic plant water use efficiency. *J. Exp. Bot.* **2013**, *64*, 4993–5007. [[CrossRef](#)] [[PubMed](#)]
33. Mackay, T.F.; Stone, E.A.; Ayroles, J.F. The genetics of quantitative traits: Challenges and prospects. *Nat. Rev. Genet.* **2009**, *10*, 565–577. [[CrossRef](#)] [[PubMed](#)]
34. Ganal, M.W.; Polley, A.; Graner, E.-M.; Plieske, J.; Wieseke, R.; Luerssen, H.; Durstewitz, G. Large SNP arrays for genotyping in crop plants. *J. Biosci.* **2012**, *37*, 821–828. [[CrossRef](#)] [[PubMed](#)]
35. Varshney, R.K.; Nayak, S.N.; May, G.D.; Jackson, S.A. Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends Biotechnol.* **2009**, *27*, 522–530. [[CrossRef](#)] [[PubMed](#)]
36. Comadran, J.; Kilian, B.; Russell, J.; Ramsay, L.; Stein, N.; Ganal, M.; Shaw, P.; Bayer, M.; Thomas, W.; Marshall, D.; et al. Natural variation in a homolog of *antirrhinum centroradialis* contributed to spring growth habit and environmental adaptation in cultivated barley. *Nat. Genet.* **2012**, *44*, 1388–1392. [[CrossRef](#)] [[PubMed](#)]
37. Thomson, M.J. High-throughput SNP genotyping to accelerate crop improvement. *Plant Breed. Biotechnol.* **2014**, *2*, 195–212. [[CrossRef](#)]
38. Elshire, R.J.; Glaubitz, J.C.; Sun, Q.; Poland, J.A.; Kawamoto, K.; Buckler, E.S.; Mitchell, S.E. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* **2011**, *6*, e19379. [[CrossRef](#)] [[PubMed](#)]
39. Poland, J.A.; Brown, P.J.; Sorrells, M.E.; Jannink, J.-L. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE* **2012**, *7*, e32253. [[CrossRef](#)] [[PubMed](#)]
40. Silvar, C.; Martis, M.M.; Nussbaumer, T.; Haag, N.; Rauser, R.; Keilwagen, J.; Korzun, V.; Mayer, K.F.; Ordon, F.; Perovic, D. Assessing the barley genome zipper and genomic resources for breeding purposes. *Plant Genome J.* **2015**, *8*. [[CrossRef](#)]
41. Varshney, R.K.; Paulo, M.J.; Grando, S.; van Eeuwijk, F.A.; Keizer, L.C.P.; Guo, P.; Ceccarelli, S.; Kilian, A.; Baum, M.; Graner, A. Genome wide association analyses for drought tolerance related traits in barley (*Hordeum vulgare* L.). *Field Crop Res.* **2012**, *126*, 171–180. [[CrossRef](#)]
42. Teulat, B.; Borries, C.; This, D. New qtls identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. *Theor. Appl. Genet.* **2001**, *103*, 161–170. [[CrossRef](#)]
43. Diab, A.A.; Teulat-Merah, B.; This, D.; Ozturk, N.Z.; Benscher, D.; Sorrells, M.E. Identification of drought-inducible genes and differentially expressed sequence tags in barley. *Theor. Appl. Genet.* **2004**, *109*, 1417–1425. [[CrossRef](#)] [[PubMed](#)]
44. Chen, J.; Chang, S.X.; Anyia, A.O. Quantitative trait loci for water-use efficiency in barley (*Hordeum vulgare* L.) measured by carbon isotope discrimination under rain-fed conditions on the canadian prairies. *Theor. Appl. Genet.* **2012**, *125*, 71–90. [[CrossRef](#)] [[PubMed](#)]
45. Wehner, G.; Balko, C.; Enders, M.; Humbeck, K.; Ordon, F. Identification of genomic regions involved in tolerance to drought stress and drought stress induced leaf senescence in juvenile barley. *BMC Plant Biol.* **2015**, *15*. [[CrossRef](#)] [[PubMed](#)]
46. Jamieson, P.D.; Martin, R.J.; Francis, G.S.; Wilson, D.R. Drought effects on biomass production and radiation-use efficiency in barley. *Field Crop Res.* **1995**, *43*, 77–86. [[CrossRef](#)]
47. Samarah, N.H. Effects of drought stress on growth and yield of barley. *Agron. Sustain. Dev.* **2005**, *25*, 145–149. [[CrossRef](#)]
48. Richards, R.; Rebetzke, G.; Condon, A.; Van Herwaarden, A. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop Sci.* **2002**, *42*, 111–121. [[CrossRef](#)] [[PubMed](#)]

49. Blum, A. Selection for sustained production in water-deficit environments. In *International Crop Science I*; Buxton, D., Ed.; CSSA: Madison, WI, USA, 1993; pp. 343–347.
50. Ahmed, I.M.; Dai, H.; Zheng, W.; Cao, F.; Zhang, G.; Sun, D.; Wu, F. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between tibetan wild and cultivated barley. *Plant Physiol. Biochem.* **2013**, *63*, 49–60. [[CrossRef](#)] [[PubMed](#)]
51. Robredo, A.; Pérez-López, U.; de la Maza, H.S.; González-Moro, B.; Lacuesta, M.; Mena-Petite, A.; Muñoz-Rueda, A. Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environ. Exp. Bot.* **2007**, *59*, 252–263. [[CrossRef](#)]
52. Blum, A. Drought resistance, water-use efficiency, and yield potential—Are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* **2005**, *56*, 1159–1168. [[CrossRef](#)]
53. Sivamani, E.; Bahieldin, A.; Wraith, J.M.; Al-Niemi, T.; Dyer, W.E.; Ho, T.-H.D.; Qu, R. Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley hva1 gene. *Plant Sci.* **2000**, *155*, 1–9. [[CrossRef](#)]
54. Lim, P.O.; Kim, Y.; Breeze, E.; Koo, J.C.; Woo, H.R.; Ryu, J.S.; Park, D.H.; Beynon, J.; Tabrett, A.; Buchanan-Wollaston, V. Overexpression of a chromatin architecture-controlling at-hook protein extends leaf longevity and increases the post-harvest storage life of plants. *Plant J.* **2007**, *52*, 1140–1153. [[CrossRef](#)] [[PubMed](#)]
55. Gregersen, P.L.; Culetic, A.; Boschian, L.; Krupinska, K. Plant senescence and crop productivity. *Plant Mol. Biol.* **2013**, *82*, 603–622. [[CrossRef](#)] [[PubMed](#)]
56. Munne-Bosch, S.; Alegre, L. Die and let live: Leaf senescence contributes to plant survival under drought stress. *Funct. Plant Biol.* **2004**, *31*, 203–216. [[CrossRef](#)]
57. Neer, E.J.; Schmidt, C.J.; Nambudripad, R.; Smith, T.F. The ancient regulatory-protein family of WD-repeat proteins. *Nature* **1994**, *371*, 297–300. [[CrossRef](#)] [[PubMed](#)]
58. Ranocha, P.; Bourgis, F.; Ziemak, M.J.; Rhodes, D.; Gage, D.A.; Hanson, A.D. Characterization and functional expression of cDNAs encoding methionine-sensitive and-insensitive homocysteine S-methyltransferases from arabidopsis. *J. Biol. Chem.* **2000**, *275*, 15962–15968. [[CrossRef](#)] [[PubMed](#)]
59. Nohzadeh Malakshah, S.; Habibi Rezaei, M.; Heidari, M.; Hosseini Salekdeh, G. Proteomics reveals new salt responsive proteins associated with rice plasma membrane. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 2144–2154. [[CrossRef](#)] [[PubMed](#)]
60. Handley, L.L.; Nevo, E.; Raven, J.A.; MartÍnez-Carrasco, R.; Scrimgeour, C.M.; Pakniyat, H.; Forster, B.P. Chromosome 4 controls potential water use efficiency ($\delta 13c$) in barley. *J. Exp. Bot.* **1994**, *45*, 1661–1663. [[CrossRef](#)]
61. Molnár, I.; Linc, G.; Dulai, S.; Nagy, E.; Molnár-Láng, M. Ability of chromosome 4h to compensate for 4D in response to drought stress in a newly developed and identified wheat–barley 4H (4D) disomic substitution line. *Plant Breed.* **2007**, *126*, 369–374. [[CrossRef](#)]
62. Afzal, A.J.; Wood, A.J.; Lightfoot, D.A. Plant receptor-like serine threonine kinases: Roles in signaling and plant defense. *Mol. Plant Microbe Interact.* **2008**, *21*, 507–517. [[CrossRef](#)] [[PubMed](#)]
63. Osakabe, Y.; Maruyama, K.; Seki, M.; Satou, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signaling in arabidopsis. *Plant Cell* **2005**, *17*, 1105–1119. [[CrossRef](#)] [[PubMed](#)]
64. De Lorenzo, L.; Merchan, F.; Laporte, P.; Thompson, R.; Clarke, J.; Sousa, C.; Crespi, M. A novel plant leucine-rich repeat receptor kinase regulates the response of medicago truncatula roots to salt stress. *Plant Cell* **2009**, *21*, 668–680. [[CrossRef](#)] [[PubMed](#)]
65. Deepak, S.; Shailasree, S.; Kini, R.K.; Muck, A.; Mithöfer, A.; Shetty, S.H. Hydroxyproline-rich glycoproteins and plant defence. *J. Phytopathol.* **2010**, *158*, 585–593. [[CrossRef](#)]
66. Eastburn, D.; McElrone, A.; Bilgin, D. Influence of atmospheric and climatic change on plant–pathogen interactions. *Plant Pathol.* **2011**, *60*, 54–69. [[CrossRef](#)]
67. Wehner, G.; Balko, C.; Humbeck, K.; Zyprian, E.; Ordon, F. Expression profiling of genes involved in drought stress and leaf senescence in juvenile barley. *BMC Plant Biol.* **2016**, *16*. [[CrossRef](#)] [[PubMed](#)]
68. Rode, J.; Ahlemeyer, J.; Friedt, W.; Ordon, F. Identification of marker-trait associations in the german winter barley breeding gene pool (*Hordeum vulgare* L.). *Mol. Breed.* **2012**, *30*, 831–843. [[CrossRef](#)]

69. Igartua, E.; Gracia, M.P.; Lasa, J.M.; Medina, B.; Molina-Cano, J.L.; Montoya, J.L.; Romagosa, I. The spanish barley core collection. *Genet. Resour. Crop Evolut.* **1998**, *45*, 475–481. [[CrossRef](#)]
70. Wehner, G.; Balko, C.; Ordon, F. Experimental design to determine drought stress response and early leaf senescence in barley (*Hordeum vulgare* L.). *Bio-Protocol* **2016**, *6*. [[CrossRef](#)]
71. Stauss, R. *Compendium of Growth Stage Identification Keys for Mono-and Dicotyledonous Plants: Extended bbch Scale*; Ciba-Geigy AG: Basel, Switzerland, 1994.
72. Grubbs, F.E. Procedures for detecting outlying observations in samples. *Technometrics* **1969**, *11*, 1–21. [[CrossRef](#)]
73. RStudio Team. Rstudio: Integrated development for r. Available online: <http://www.rstudio.com/> (accessed on 13 June 2016).
74. Reif, J.C.; Melchinger, A.E.; Frisch, M. Genetical and mathematical properties of similarity and dissimilarity coefficients applied in plant breeding and seed bank management. *Crop Sci.* **2005**, *45*, 1–7. [[CrossRef](#)]
75. Goecks, J.; Nekrutenko, A.; Taylor, J. Galaxy: A comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.* **2010**, *11*. [[CrossRef](#)] [[PubMed](#)]
76. Afgan, E.; Baker, D.; van den Beek, M.; Blankenberg, D.; Bouvier, D.; Čech, M.; Chilton, J.; Clements, D.; Coraor, N.; Eberhard, C. The galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Res.* **2016**, *44*, W3–W10. [[CrossRef](#)] [[PubMed](#)]
77. Galaxy. Available online: <https://galaxyproject.org/> (accessed on 11 July 2016).
78. Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. Tassel: Software for association mapping of complex traits in diverse samples. *Bioinformatics* **2007**, *23*, 2633–2635. [[CrossRef](#)] [[PubMed](#)]
79. Tassel 5.0. Available online: <http://www.maizegenetics.net/tassel> (accessed on 25 July 2016).
80. Mayer, K.F.; Waugh, R.; Brown, J.W.; Schulman, A.; Langridge, P.; Platzer, M.; Fincher, G.B.; Muehlbauer, G.J.; Sato, K.; Close, T.J.; et al. A physical, genetic and functional sequence assembly of the barley genome. *Nature* **2012**, *491*, 711–716. [[CrossRef](#)] [[PubMed](#)]
81. Colmsee, C.; Beier, S.; Himmelbach, A.; Schmutzer, T.; Stein, N.; Scholz, U.; Mascher, M. Barlex—The barley draft genome explorer. *Mol. Plant* **2015**, *8*, 964–966. [[CrossRef](#)] [[PubMed](#)]



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).