Mol Breeding (2010) 26:583–593
DOI 10.1007/s11032-010-9393-5

Stability parameter and genotype mean estimates for drought stress effects on root and shoot growth of wild barley pre-introgression lines

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Received: 5 October 2009 / Accepted: 18 January 2010 / Published online: 5 February 2010

Abstract The goal of the present study was to select BC_2-lines from a cross between *Hordeum vulgare* and *H. vulgare* ssp. *spontaneum* and to identify introgressed candidate regions responsible for a superior pre-flowering development across environments including drought stress conditions by using stability parameter and genotype mean estimates. Three experiments were carried out under controlled environmental conditions. Drought stress was induced by permanent suboptimal water supply, stress cycles in continuously drying soils, and increased transpiration demands by reducing relative humidity of the air. The environmental effects on shoot dry weight, leaf area, tiller number, and root lengths of 36 lines and the recurrent parent, the spring barley cultivar 'Scarlett' was tested in ten different conditions. Results showed that 11 genotypes responded significantly ($P = 0.05$) different from the recurrent parent in at least one of the measured traits. The introgressions of those lines were assigned to five genome regions, which have been suggested as QTL regions for related traits before. Regions on chromosome 4H influence tillering and one region each on 2H, 5H, and 7H probably has effects on shoot dry weight and leaf area. Introgressions on the mentioned regions increased trait values in every case. Leaf area was highly correlated to shoot dry weight and tiller number while the correlation between shoot dry weight and tiller number was not significant. A weak correlation was observed between tiller number and root lengths. Slopes of response curves of lines to increasing water shortage did not significantly differ from the population mean and from the recurrent parent. Results give hint that superior genotypes within the population develop well under both well-watered and drought stress conditions.

Keywords Drought stress · Wild barley · Introgressed genome regions · Leaf area · Shoot dry weight · Tillering · Root lengths

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Introduction

The lack of water availability and growing cereal markets increase the demand for water efficient crops. Progress in plant breeding highly depends on the occurrence of desirable genetic variation for crop improvement and the availability of precise methods for the transfer of favorable genes and selection strategies. Wild barley and Middle Eastern landraces have shown to be fruitful sources of genes for crop development (Ellis et al. 2000). Pre-flowering drought stress is a randomly occurring environmental factor in temperate climates. Situations of extreme stress may require strong adaptation processes, e.g., by excessively reducing leaf growth. In contrast, under conditions of moderate abiotic stress, good plant vigor and maintenance of growth and leaf area development may be advantageous for crops in a way that high assimilation rates are guaranteed after periods of water shortage (Ben Haj Salah and Tardieu 1997; Sambatti and Caylor 2007). Linear regressions carried out on crop responses to environmental conditions have been suggested to assist in finding the best compromise between maximizing potential and stability of growth and yield (Uptmoor et al. 2009).

Root growth may increase crop productivity in deep soils especially under drought stress conditions (Zheng et al. 2003). Extensive root systems enable plants to explore deep soil layers for water and nutrient uptake. In *Lolium perenne*, regrowth rates after drought were closely associated with root amounts (Turner et al. 2008). The very old concept of morphogenetic equilibrium assumed that plant height and rooting depth as well as above ground and root biomass are positively correlated (Feil 1992). While hypotheses about relations between plant height and rooting depth were rejected, theories about strong associations between root and shoot growth still exist. Chloupek et al. (2006) speculated that root development from tiller nodes leads to a correlation between tillering and root growth in barley. On the contrary, Palta et al. (2008) suggested that restricted tillering in wheat could result in an investment of assimilate surpluses into the rooting system. However, the latter study observed no relation between root biomass and tiller number and no genotype differences in root to total biomass ratio. Tillering is an important character for phenotypic plasticity to drought (Baum et al. 2003). A study on low-tillering barley mutants suggests that there is a step in the tillering pathway that interacts with stress response genes (Dabbert et al. 2009).

Similar to stability parameters used to describe the performance of varieties in multiple environments (Eberhart and Russell 1966; Pipho 1999), the present study uses a linear regression model in order to compare progenies of a biparental backcross population to the recurrent parent. Only a few other studies used stability parameters, e.g., for the detection of QTL for phenotypic plasticity of several traits including heading date (Lacaze et al. 2009; Emebiri and Moody 2006). The objectives of the present study were (1) to reveal the effects of pre-flowering drought stress on shoot dry weight, leaf area, tiller number, and root lengths in BC_2-lines from a cross between *H. vulgare* × *H. vulgare* ssp. *spontaneum*, (2) to select lines with superior performance in the mentioned traits under well-watered and drought stress conditions, (3) to identify chromosomal candidate regions influencing the traits of interest, and (4) to detect correlations between traits.

Materials and methods

Plant material

The present study was carried out on 36 selected lines of a BC_2DH population derived from a cross between the spring barley cultivar 'Scarlett' as recurrent parent and the wild barley accession ISR42-8 (*H. vulgare* ssp. *spontaneum*) as donor (von Korff et al. 2004). The lines were selected according to genome coverage and seed availability. They were genotyped with 97 SSR markers as previously published by von Korff et al. (2004) and carry on average three donor introgressions accounting for ~14% of the genome. The population of selected lines covers more than 90% of the total donor genome.

Phenotyping

Three experiments were carried out in order to estimate responses of vegetative growth to water shortage induced by high transpiration demands due to low relative humidity (RH) and by limited soil water availabilities. In the first experiment, seven

plants per genotype and replication were sown in polyvinyl boxes containing a peat sand mixture as growing media. The boxes were placed in two replications in growth chambers. Drip irrigation was used to provide constant water supply. A 0.15% nutrient solution with 16% N, 6% P, and 26% K was used for fertigation. RH in the three growth chambers was 42, 55, and 70%. There was a constant temperature of 24.3°C for the 42 and 55% RH chambers. Temperature of the 70% RH chamber was 22.5°C. The second and third experiments were carried out in a greenhouse. Single plants were cultivated in 12.5 × 50 cm polyvinyl pots with two replications per treatment and BC_2-line. The pots were filled with 9.4 kg homogenized sun-dried soil. Soil water status of the four different treatments of the second experiment were 35, 50, 65, and 80% of the water holding capacity (WHC). Each pot contained 1.1 kg of water at 80% WHC. Plants were watered every second day to the required percentage WHC using the same nutrient solution as in the first experiment. Total nutrient supply of the four treatments was different but the method protects plants from nutrient deficiency due to leaching during early growth stages or else the well-watered treatment could not have been fulfilled due to absolute nutrient deficiencies in the soil. The mean daily air temperature was 20°C during the second experiment. Mean daily maximum and minimum temperature was 24.6 and 16.5°C. Growing conditions in the third experiment were similar to those in the second experiment with the main difference that all treatments were irrigated to 80% WHC and irrigation was stopped 3 weeks after sowing for the drought stress treatments. Plants were harvested after stress cycles of 9 and 15 days. At the same time a fully irrigated control was harvested. The control included 14 random lines for the 9 days stress cycle and the whole set of genotypes for the 15 days stress cycle. Plant available water was fully consumed during the 15 days stress cycle. Plants to which the drought stress cycle was induced were cultivated in three replications while the well-watered control was cultivated in two replications. The mean daily air temperature was 19.7°C, daily maximum temperature was 24.1°C and daily minimum was 15.1°C.

Plants were harvested before flowering in all three experiments. The number of tillers was counted for each plant, and leaf area including leaf blades and leaf sheaths was measured using a leaf area meter (Licor 3100, Licor Inc., Lincoln, NE, USA). Shoots were dried at 105°C in order to obtain shoot dry matter. Roots of the second and third experiment were carefully separated from the growing media and washed. Roots of single plants were placed in a water bath in order to scan the roots for measuring total root lengths with WinRhizo (Regent Inc., Canada).

Data analysis

In order to account for the different growing conditions and differences in the experimental durations, the relative performance of every BC_2-line in all the experiments and treatments was estimated by equalizing the population means of the 70% RH, 80% WHC, and well-watered controls of the third experiment to 100. All the following data analyses were carried out on the means of the replications for the ten environments including the optima of the three experiments and the different stress levels. Pearson's correlation coefficients were calculated between the genotype means over all environments using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Prior to analysis of covariance, deviations from the normal distribution were analyzed on the residuals of the data set using the Shapiro–Wilk-test procedure of SAS 9.1. Equality of variances was tested with a modified Levene-test (Brown and Forsythe 1974). The Shapiro–Wilk-test revealed that leaf area, shoot dry matter, and tiller number data deviated significantly from the normal distribution ($P = 0.1$). However, box plots of the residuals showed that carrying out further tests requiring normal distribution is acceptable since the test procedures used in the present study are assumed to be relatively robust against deviations from the normal distribution. The modified Levene-test showed no significant deviations from the assumption of homogeneity of variances ($P = 0.1$).

In order to identify genotypes with regression parameters, describing the responses to stress environments, differing significantly from the recurrent parent, the following model with $i = 1, 2, \ldots, k$ genotypes and $j = 1, 2, \ldots, n$ environments was used:

$$y_{ij} = \mu + \tau_i + \beta a_{ij} + \gamma_i a_{ij} + \varepsilon_{ij},$$

where $\mu + \tau_i$ is the fixed unknown regression parameter 'intercept' describing the across environments

mean $\mu_i + \beta\bar{a}_i$ of the ith genotype, $\beta a_{ij} + \gamma_i a_{ij}$ is the unknown regression parameter 'slope' of the co-variable a, which is described by the population mean, with γ_1, γ_2, …, γ_k interactions between the k genotypes and the main factor 'slope' and $\gamma_k = 0$. ε_{ij} is the error of the ith genotype in the jth environment. In case the global H_0: $\gamma, = \gamma, = \ldots = \gamma_k$ was not rejected, the general form would be

$$y_{ij} = \mu + \tau_i + \beta a_{ij} + \varepsilon_{ij},$$

assuming the same constant slope for all genotypes (cf. Blaersild and Granfeldt 2003). Pairwise genotype comparisons to the recurrent parent were carried out on the multiple $\alpha = 0.05$ using Bonferroni adjustment. Variance over environments was estimated according to Lin et al. (1986). Broad sense heritability for trait means over environments was estimated as follows

$$h^2 = \sigma_g^2/(\sigma_g^2 + 1/e \times \sigma_{ge}^2 + \sigma_\varepsilon^2),$$

where σ_g^2 is the genotypic variance, e is the number of environments, σ_{ge}^2 is the genotype × environment interaction variance, and σ_ε^2 is the error variance.

Results

Both increasing leaf to air vapor pressure differences induced by decreasing RH and low soil water contents reduced vegetative growth (Table 1). The most striking reduction in vegetative growth was induced by the 15 days stress cycle. On average leaf area was reduced by 75% after 15 days without water supply. Leaf area was reduced to 52% comparing the 35% WHC environment to the 80% WHC environment. At a RH of 42%, leaf area was 77% of the 70% RH environment. Shoot dry matter was reduced to a

Table 1 Population means, ranges, and standard deviations (SD) of environmental effects on relative shoot dry weight, leaf area, tiller number, and root lengths

	Relative humidity (%)			Soil water holding capacity (%) (WHC)				Stress cycle (days)		Well watered control
	42	55	70	35	50	65	80	9	15	
Leaf area										
Mean	77	95	100	52	71	87	100	90	25	100
SD	18	22	25	8	10	12	12	11	4	19
Max	110	132	142	69	94	108	140	117	36	164
Min	30	30	33	34	46	58	74	70	18	55
Shoot dry weight										
Mean	78	83	100	55	73	90	100	103	59	100
SD	13	17	24	11	14	16	14	15	4	16
Max	104	118	150	76	126	134	145	137	68	136
Min	54	24	44	23	39	52	71	75	50	68
Tiller number										
Mean	82	86	100	65	81	93	100	91	69	100
SD	14	19	24	14	15	18	17	14	11	16
Max	118	141	143	102	117	135	142	125	101	137
Min	53	46	34	40	58	66	66	75	54	62
Root lengths										
Mean				56	62	65	100	88	80	100
SD				11	10	12	14	12	7	12
Max				76	80	92	132	115	99	119
Min				37	44	43	64	65	66	75

similar level at 35% WHC and after the 15 days stress cycle. The reduction was less pronounced by cultivating plants at 42% RH. Reduction in the number of tillers was less intensive especially under the most intensive stress conditions. Roots responded differently to the different methods used to produce a low soil water status. After the 15 days stress cycle, total root lengths were reduced to 80% of the well-watered plants, while shoot dry weight was reduced to 59%. At a constant water supply of about 35% WHC root lengths were reduced to 56% of the 80% WHC environment while shoot dry weight was only reduced to 65%.

Correlations between the genotype means over all environments showed significant coefficients between leaf area and tiller number and between leaf area and shoot dry weight ($P = 0.05$). Tiller number was significantly correlated to total root lengths. Correlation between shoot dry weight and tiller number was statistically not significant (Table 2). Heritability estimates were nearly the same for leaf area, shoot dry weight and root lengths. Highest heritability was found for tiller number (Table 3).

Regression analyses carried out on the data showed an overall acceptable fit to the linear model. Mean coefficients of regression were comparatively low for tiller number ($r^2 = 0.54$) but high for leaf area ($r^2 = 0.79$) and root lengths ($r^2 = 0.76$, Table 3). The slope of the regressions, which describes the genotype plasticity in response to environmental conditions, shows high ranges for all traits. However, deviations from the recurrent parent were statistically not different for all the barley lines and traits. None of the lines had a slope statistically different from '1' ($P = 0.05$, data not shown). Variance over environments (σ^2) was highest for leaf area and lowest for tiller number and root lengths (Table 3).

In contrast to that, significant differences between lines and the recurrent parent were observed for leaf area, shoot dry weight, and tiller number means across all environments. Examples of lines with significantly higher shoot dry weight and leaf area or tiller number are given in Fig. 1. Figure 2 shows all lines, which perform significantly different from the recurrent parent in at least one of the traits analyzed at $P = 0.05$ for the experimentalwise error rate. BC_2-lines were added to Fig. 2 if comparisons to the recurrent parent were statistically significant at $P = 0.05$ for the unadjusted comparisonwise error rate and introgressed genome regions were shared with lines performing significantly different on the experimentalwise error rate. Accentuated chromosomal regions of Fig. 2 signalize that all genotypes, which carry an introgression on the respective region, perform statistically different from the recurrent parent for the relevant trait at least at the comparisonwise error rate $P = 0.05$.

Mean leaf area of Sca073 and Sca229 was significantly higher than that of the recurrent parent. The two lines and Sca042, Sca062, and Sca175

Table 2 Correlation coefficients of trait means across all environments, slopes of regression analyses, and variances over environments (σ^2)

		Leaf area		Shoot dry weight			Tiller number			Root lengths		
		Slope	σ^2	Mean	Slope	σ^2	Mean	Slope	σ^2	Mean	Slope	σ^2
Leaf area	Mean	0.88***	−0.07	0.52***	0.53***	−0.10	0.57***	0.59***	0.11	0.30	0.05	−0.09
	Slope		0.00	0.42*	0.60***	0.01	0.45**	0.71***	0.08	0.18	0.06	−0.10
	σ^2			0.19	−0.01	0.63***	−0.23	−0.05	0.39*	−0.46**	0.19	−0.18
Shoot dry weight	Mean				0.55***	0.03	0.21	0.37*	0.20	−0.17	0.36*	−0.24
	Slope					0.15	0.12	0.61***	0.29	−0.02	0.21	−0.17
	σ^2						−0.26	0.00	0.47**	−0.26	0.21	0.16
Tiller number	Mean							0.49**	0.09	0.35*	−0.10	0.15
	Slope								0.23	0.11	0.00	−0.22
	σ^2									−0.17	0.22	0.00
Root lengths	Mean										−0.13	0.00
	Slope											0.08

*, **, *** Represent the level of significance ($P = 0.05$, $P = 0.01$, $P = 0.001$, respectively)

Table 3 Across environment means, slopes of regression analyses, variances over environments (σ^2), and r^2 for relative shoot dry weight, leaf area, tiller number, and root lengths of the recurrent parent 'Scarlett' and 36 BC_2-lines

	Leaf area				Shoot dry weight				Tiller number				Root lengths			
	Mean	Slope	σ^2	r^2	Mean	Slope	σ^2	r^2	Mean	Slope	σ^2	r^2	Mean	Slope	σ^2	r^2
'Scarlett'	69	0.93	154	0.81	71	1.03	140	0.75	76	1.02	27	0.81	72	0.59	24	0.58
Mean	80	1.00	264	0.79	84	1.00	200	0.68	87	1.00	168	0.54	79	1.00	157	0.76
SD	8	0.19	337	0.18	8	0.23	199	0.16	11	0.32	127	0.20	5	0.23	158	0.14
Max	93	1.32	1,848	0.95	105	1.49	768	0.94	114	1.51	420	0.85	88	1.51	562	0.98
Min	53	0.33	1	0.13	68	0.49	6	0.25	65	0.23	5	0.07	71	0.50	8	0.28
h^2	0.71				0.71				0.90				0.71			

SD standard deviation, h^2 heritability

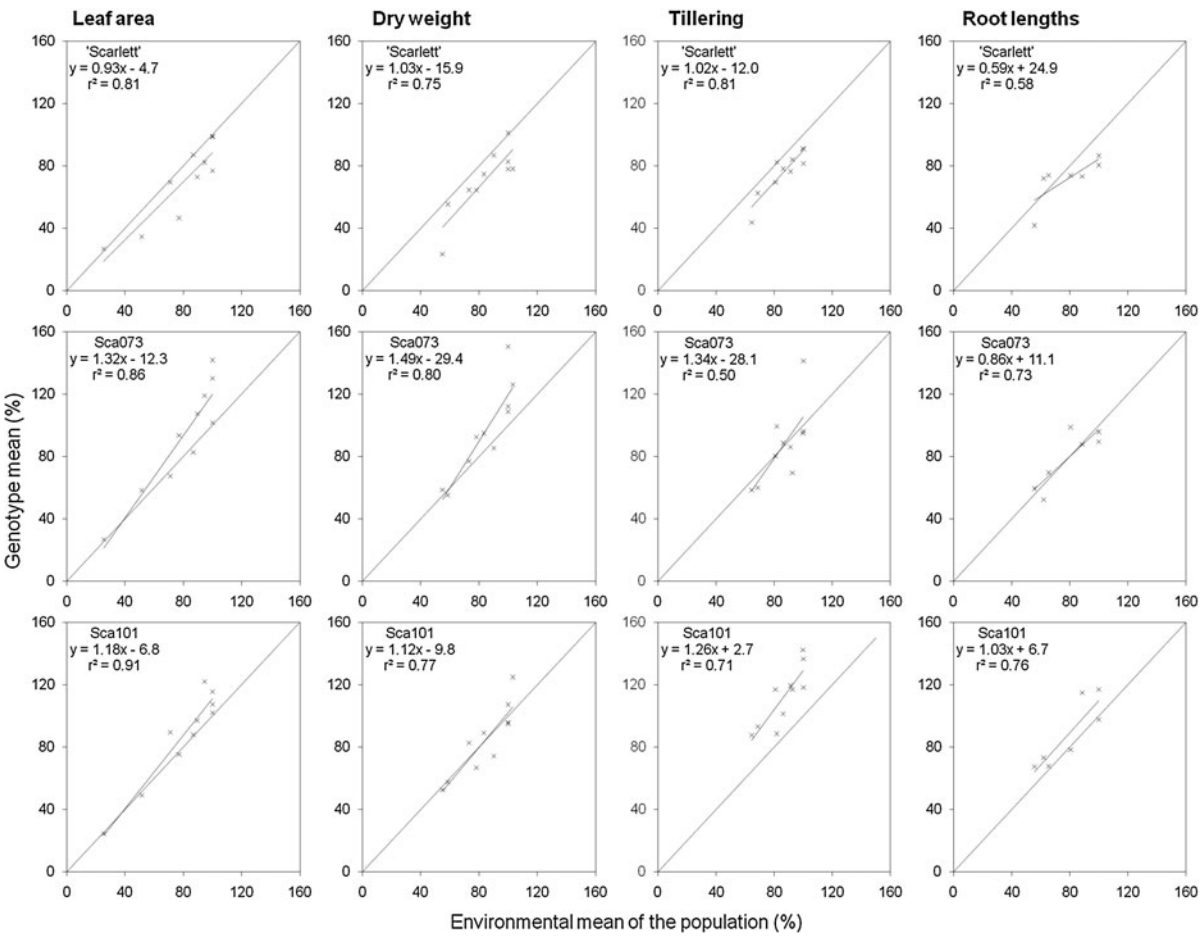

Fig. 1 Environmental effects on relative shoot dry weight, leaf area, tiller number, and root lengths of the recurrent parent 'Scarlett' in comparison to BC_2-lines with significantly higher shoot dry weight and leaf area (Sca073) or tiller number (Sca101)

produced significantly higher shoot dry matter. All lines carrying the donor allele on 2H between 17 and 27 cM, on 5H at Bmag223, and on 7H at HVA22S produced a higher shoot dry mass than the recurrent parent at least at the comparisonwise error rate $P = 0.05$.

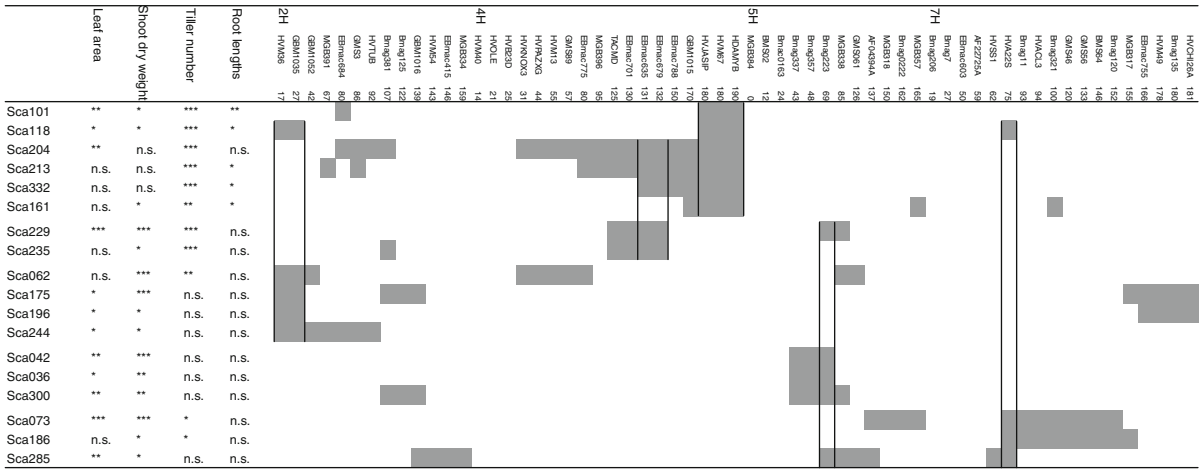

Fig. 2 Introgressions (*grey*) on chromosomes 2H, 4H, 5H, and 7H of BC_2-lines performing significantly different from the recurrent parent 'Scarlett' for the traits leaf area, shoot dry weight, tiller number, and root lengths. Marker names and positions in cM are taken from von Korff et al. (2004). Line trait combinations marked '*' or '**' are statistically significant different from 'Scarlett' at $P = 0.05$ or $P = 0.01$ for the comparisonwise error rate. Lines marked '***' are significantly different from 'Scarlett' according to Bonferroni ($P = 0.05$). Line trait combinations marked 'n.s.' are statistically not different from 'Scarlett'. Accentuated chromosomal regions indicate putative QTL regions

Tiller number of seven lines was significantly higher compared to the recurrent parent. All seven lines have an introgression on chromosome 4H. Five of them share an introgressed region between 180 and 190 cM, whereas Sca229 and Sca235 and additional three lines from the first group share an introgression between 131 and 132 cM. None of the lines with no significant increase in tiller number have introgressions on that marker position except Sca161, carrying the donor allele between 170 and 190 cM. However, tiller number of Sca161 differed significantly from 'Scarlett' at the comparisonwise error rate $P = 0.01$. Root lengths of four lines with the donor allele between 180 and 190 cM on 4H was significantly higher than root lengths of the recurrent parent at the comparisonwise error rate $P = 0.05$.

Discussion

Results of the present study indicate that introgressions from wild barley may increase leaf area and pre-flowering dry matter production. Early plant vigor and leaf area development could increase crop productivity and reduces water losses due to evaporation from unshaded soils (Borràs et al. 2009). The present study revealed significant correlations between leaf area and both shoot dry weight and tiller number while no significant correlation was observed between tiller number and above ground dry matter. Ellis et al. (2002) conducted a study on barley and have also reported that there is no association between tiller number and shoot weight. Locations close to the three chromosomal regions on 2H, 5H, and 7H, probably influencing shoot dry weight, and the two regions on 5H and 7H, which may have impacts on leaf area development, were described as putative QTL regions for related traits previously: Elberse et al. (2004) mapped a QTL for leaf length and leaf width on 2H between 0 and 15 cM. A QTL for harvest index was significantly associated to the marker HVM36 on 2H and a QTL for yield to GBM1035 by carrying out AB-QTL analysis on a population derived from the same cross used in the present study (von Korff et al. 2006). QTL for plant size were mapped on 5H (Turner et al. 2008). Teulat et al. (1997) mapped QTL for the number of leaves under water stress on 5H and 7H. Von Korff et al. (2006) detected a QTL for yield on chromosome 5H at Bmag337 and MGB338. QTL for above ground biomass and plant height were detected at Bmag337, a QTL for harvest index at Bmag357. Forster et al. (2004) detected QTL for plant height on 7H between 89 and 120 cM. Since some QTL co-localize to previously detected QTL for grain yield, further research is needed to reveal the effects of vegetative

growth under stress on yield determining processes. If positive relations between traits under study and yield become evident, screening methods can be devised and the trait of interest could be evaluated in advanced plant material (e.g., in a series of overlapping introgression lines) by the principles of indirect selection (Fukai and Cooper 1995).

Our study suggests that tillering in the *H. vulgare* × *H. vulgare* ssp. *spontaneum* cross is mainly regulated by regions on chromosome 4H. However, it becomes not clear if both accentuated regions on 4H (Fig. 2) affect tiller number or if tillering is influenced by only one of the regions. In the latter case additional undetected regions may be involved in the genetic control of the trait. A QTL for the number of fertile tillers on 4H at HVM67 was detected previously by Teulat et al. (2001). Baum et al. (2003) detected a QTL for tiller number on 4H 27 cM upwards of HVM67 in a *H. vulgare* × *H. vulgare* ssp. *spontaneum* cross. The *H. vulgare* ssp. *spontaneum* allele increased tiller number. Several QTL for tillering have been described in rice. Amongst them, QTL for tiller number were detected on chromosome 03 (Cairns et al. 2009; Liu et al. 2009; Quarry et al. 1997). Syntheny between the rice chromosome 03 and barley chromosome 4H are described in Thiel et al. (2009). Rice tillering occurs in a two-stage process: the development of axillary buds and bud outgrowth. Single stemmed mutants do not produce tiller buds, e.g., due to a mutation in the *MONOCULM1* gene on chromosome 06 (Li et al. 2003). A homolog of the wheat tiller inhibition gene *tin3* was mapped on chromosome 01 in rice (Kuraparthy et al. 2008). *Tin3* is located on chromosome 3A in *T. monococcum* and the mutant is responsible for monoculm growth habit (Kuraparthy et al. 2007). The *HIGH-TILLERING DWARF1* (*HTD1*) and *DWARF10* (*D10*) genes were mapped on rice chromosomes 04 and 01, respectively, and are orthologs of the *Arabidopsis MAX3* and *MAX4* genes. *D10* controls lateral bud outgrowth and is upregulated in high tillering mutants (Arite et al. 2007) while *HTD1* negatively regulates tiller bud outgrowth (Zou et al. 2006). Another gene, *FINE CULM 1*, a homolog of *teosinte branched 1* (*tb1*), controlling lateral bud outgrowth, was mapped on chromosome 03 (Takeda et al. 2003). *Tb1* is responsible for tillering suppression during maize domestication (Doebley et al. 1997). A sorghum ortholog of *Tb1* (*SbTb1*) showed an altered expression in response to light quality and plant density, which was mediated by phytochrome-B (Kebrom et al. 2006). The latter shows that loci, which were involved in changing tiller number during domestication processes, may also regulate tillering in response to environmental signals. However, our study gave no evidence that those wild barley introgressions, which generally increased tiller number, regulated tillering in response to abiotic stress in a different way, i.e., we did not observe significant changes in the phenotype plasticity due to wild barley introgressions.

We found a weak but positive correlation between tiller number and root lengths. Growth maintenance under stress conditions is assumed to require an extensive rooting system for acquiring water from deep soil layers. Co-localization of QTL for tillering and root system size was reported by Chloupek et al. (2006). A direct relation between root system size and tillering was suggested because nodal roots, which may dominate root system size, emerge directly from stem bases. However, Anderson-Taylor and Marshall (1983) reported that most nodal roots in barley were attached to primary tillers, while 50% of secondary tillers did not produce nodal roots. The majority of unrooted tillers died prematurely. In rice, genotypes with high tiller numbers have more but short roots, which mainly develop from late appearing tillers, while genotypes with few tillers have higher root length densities in deeper soil layers (Fukai and Cooper 1995). In contrast, seminal roots, which arise from primordial, developed during the embryonal stage, grow until anthesis, and generally reach deeper layers of the soil profile (Belford et al. 1987; Whabi and Gregory 1995). Deep root per tiller QTL were detected in rice (Yadav et al. 1997), however, the study did not distinguish between seminal and nodal roots. Further investigations are needed to reveal the role of both seminal and nodal roots in water uptake under drought stress.

In the present study, total root lengths decreased with decreasing constant soil water holding capacities. Similar to our results, Sahnoune et al. (2004) found that under severe water deficit both root and shoot dry mass production were reduced and that there was no root growth in soil layers deeper than 40 cm. Severe water deficit reduced the size of piliferous layer cells in durum wheat and may limit root growth by causing the death of apices (Adda

et al. 2005). Other root growth liming factors under field conditions are increasing soil penetration resistances with decreasing soil water contents (cf. Cairns et al. 2009). This can be ruled out here, since the sandy growing medium does not produce significant soil compaction. We observed a notable change in the root-shoot ratio only after the 15 days stress cycle while the cited studies showed an increase in the root-shoot ratio for barley and durum wheat also at low to moderate stress levels. This may result from frequently watering plants from the top of the soil in the second experiment. Frequent watering in companion with the pot height could not emulate the typical vertical water content pattern of a continuously drying natural soil. Results imply that significant changes in the root to above ground biomass are induced only if the soil dries from the surface to deeper layers and not if reduced amounts of water are applied to the soil surface constantly. However, measurements of root lengths remain to be approximative if carried out as in the present study since root hairs may get lost during the washing process and differences in root hair thickness between treatments may result in different percentages of losses.

Occasionally strong deviations were found from the response to environmental conditions of the population mean, described by the slope of the regression analysis. All slopes of leaf area, shoot dry weight, and tiller number were significantly correlated to each other and to all three genotype means across environments, which gives hint that superior performing genotypes react stronger on drought stress. However, slopes were statistically not different from the recurrent parent, which had a slope close to '1' for all traits except root lengths. Extreme environments with low potential may have been underrepresented in the present study for detecting differences in the plasticity of genotypes. The majority of sub-optimal environments produced only moderate stress levels. For leaf area, shoot dry weight, and tiller number, trait values below two thirds of the population mean of optimal growing conditions were only observed in the 35% WHC environment and after the 15 days stress cycle. Zheng et al. (2009) found 10 to 12 environments to be sufficient for estimating slopes of joint regression analyses but only if extreme environments with high and low potentials were sampled. As a consequence, increasing variances under extreme stress situations may reduce the accurate predictability of slopes if these environments are sampled only sporadically.

Deviations from regressions are frequently used as stability parameters (Kraakman et al. 2004) since high variances are assumed to occur predominantly in unstable genotypes. We followed that path but did not compare environmental variance of lines to the recurrent parent since low evidence for the genetic control of variance based stability parameters was found (Emebiri and Moody 2006). Significant correlations between variances may give hint that the parameter is to a certain extent under genetic control as suggested by Lacaze et al. (2009). However, our data did not show any relation to superior performing genotypes across environments or to average performers. Extreme instability was occasionally observed in overall low performing genotypes. A significant negative correlation was found between mean root lengths and environmental variance of leaf area. Low root growth may have led to high variances in leaf area development.

We conclude from the present study that at least one region on 4H controls tillering under both well-watered and drought stress conditions. Introgressions from *H. spontaneum* increased tiller number and tillering probably promotes root growth. The combination of increased tillering and root growth may be advantageous for agriculture in extreme regions with high drought stress probabilities. Introgressions on chromosomes 2H, 5H, and 7H led to an increased dry matter production and leaf area development across all environments. This was widely independent from tillering and root growth. However, pre-introgression line libraries consisting of lines with more than one donor segment provide no ideal plant population for unequivocal QTL detection.

We did not find any negative impacts of high potential biomass and leaf area development on crop growth under moderate to severe drought stress for the duration of the study. Barley genotypes showing above average productivity under well-watered conditions, reduced shoot biomass and leaf area at the uppermost to the population mean under drought stress. However, further studies including anthesis and grain filling period are needed to explore the resulting effects of vegetative growth under drought stress on yield performance.

Acknowledgments We thank Katharina Meyer and Ilona Napp for excellent technical assistance. The Ministry for Science and Culture of Lower Saxony funded parts of the work presented here (Project 51100016 ZN 1947).

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