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ORIGINAL PAPER

Quantitative trait loci associated with adaptation to Mediterranean dryland conditions in barley

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Abstract The objective of the present study was to identify quantitative trait loci (QTL) influencing agronomic performance across rain fed Mediterranean environments in a recombinant inbred line (RIL) population derived from the barley cultivars ER/Apm and Tadmor. The population was tested in four locations (two in Syria and two in Lebanon) during four consecutive years. This allowed the analysis of marker main effects as well as of marker by location and marker by year within location interactions. The analysis demonstrated the significance of crossover interactions in environments with large differences between locations and between years within locations. Alleles from the parent with the higher yield potential, ER/Apm, were associated with improved performance at all markers exhibiting main effects for grain yield. The coincidence of main effect QTL for plant height and yield indicated that average yield was mainly determined by plant height, where Tadmor's taller plants, being susceptible to lodging, yielded less. However, a number of crossover interactions were detected, in particular for yield, where the Tadmor allele improved yield in the locations with more severe drought stress. The marker with the highest number of cross-over interactions for yield and yield component traits mapped close to the flowering gene *Ppd-H2* and a candidate gene for drought tolerance *HVA1* on chromosome 1H. Effects of these candidate genes and QTL may be involved in adaptation to severe drought as frequently occurring in the driest regions in the Mediterranean countries. Identification of QTL and genes affecting field performance of barley under drought stress is a first step towards the understanding of the genetics behind drought tolerance.

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Introduction

Drought is the single abiotic stress causing the major crop losses worldwide and continues to be a challenge to scientists (Ceccarelli et al. 2004). In the face of climatic changes, drought is receiving increasing attention, as shown by the growing number of studies on drought tolerance in plants. New genomics platforms, transcriptome, metabolome analyses and bioinformatics have advanced the deciphering and manipulation of the genetic basis of drought tolerance (Shinozaki et al. 2003; Kawaguchi et al. 2004; Riccardi et al. 2004; Hazen et al. 2005; Shinozaki and Yamaguchi-Shinozaki 2007). However, despite the recent technological advances, the overall contribution of genomics to the release of drought resistant cultivars has so far been marginal (Tuberosa and Salvi 2006). This may be explained by the variability in frequency, timing and severity of drought, and by its co-occurrence with other biotic and abiotic stresses, as well as by the quantitative

genetic basis of drought tolerance in plants. Indeed, compared with the large number of drought analyses at plant or cell level, only few studies have combined genetics with comprehensive field trials in the target environments. In barley, numerous QTL studies conducted in non-drought stressed environments have allowed the detection of quantitative trait loci of importance to practical barley breeding (Han et al. 1997; Sayed et al. 2004; Reinheimer et al 2004; Prada et al. 2004; Schmierer et al. 2005). By contrast only few QTL studies have attempted to dissect the quantitative nature of drought tolerance by analysing field trials in dry environments. In barley, QTL analyses for agronomic performance under Mediterranean environments have been conducted by Teulat et al. (2001b), Baum et al. (2003) and Talamé et al. (2004). Baum et al. (2003) and Talamé et al. (2004) calculated QTL effects for single environments and focussed in their discussions on QTL that were consistently identified across different environments, although these environments were characterised by differences in average rainfall and yield levels. The majority of these effects coincided with QTL also detected in moderate to high rainfall environments (Forster et al. 2000, 2004). The allele effects may thus have only reflected genes that generally improve the yield potential, but not necessarily drought tolerance. It has been shown that only under strong pressure of environmental stress, varieties with high yield potential produce less than cultivars which have lower yield potential, but are better adapted to stress (Blum 2005). These crossover interactions occur at yield levels of around 2.5–3 t/ha (Ceccarelli and Grando 1991). When searching for genotypes or loci responsible for adaptation to drought, agronomic performance has to be examined in environments where the yield level is below the crossover point. Unfortunately, the occurrence of this severe stress is unpredictable and large climatic differences are seen among years and locations (Ceccarelli et al. 1991). Therefore, when conducting QTL analyses for drought it is important to also take into consideration marker by location interactions (M × L), as well as marker by year within location interactions [M × Y(L)]. Markers showing repeatable interactions with location can be used for breeding for specific adaptation and may together with M × Y(L) interactions give insight into the genetics of adaptation to drought stressed environments.

In the present study, a RIL population derived from the landrace selection Tadmor and the breeding line ER/Apm was evaluated for agronomic performance in 4 years of rain fed field trials conducted at four locations (two in Syria and two in Lebanon). The parents were selected for combining high yield potential under moderate stress (ER/Apm), good adaptation to the driest locations in Syria (Tadmor), and for showing only marginal differences in development. The RIL progeny thus allows studying segregation of genes for yield potential and drought tolerance. The objective of the study was to identify marker–trait associations for agronomic performance in rain fed environments characterised by moderate to severe drought stress. Empirical breeding for drought stressed environments is difficult and will therefore considerably benefit from the possibility of controlling more precisely the accumulation of alleles for drought tolerance while preserving the alleles for high yield potential as long as the two are not mutually exclusive.

Materials and methods

Phenotyping

The mapping population with 158 recombinant inbred lines (RILs) obtained from the cross Tadmor and ER/Apm, and the two parents were phenotyped in four locations (Tel Hadya and Breda in Syria and Terbol and Kfardane in Lebanon) during four consecutive cropping seasons from 2002 to 2005.

The population of RILs has been described in detail and used by Teulat et al. (1998, 2001a, b, 2002) and therefore will be only briefly described. Tadmor is a pure line selection from the black-seeded landrace Arabi Aswad, grown on about 75% of the barley growing area in Syria and particularly in the driest part of the country (Weltzien 1988). ER/Apm is a breeding line with a yield potential of about 6 t/ha in favourable conditions. It is short, lodging resistant and shows a particularly good adaptation to North Africa where it has been released in Morocco, Tunisia and Libya with the names of Aglou, Faiz and Iraween, respectively.

The two parents differ for a number of simply inherited traits such as seed colour (Tadmor has black seed and ER/Apm has white seed), vernalisation requirement (ER/Apm does not require vernalisation, Tadmor does), growth habit (ER/Apm is erect while Tadmor is more prostrate), reaction to powdery mildew (Tadmor is very susceptible and ER/Apm is moderately resistant), and seed dormancy (higher in Tadmor, absent in ER/Apm). ER/Apm is slightly earlier heading and more lodging resistant than Tadmor. The cross was made at ICARDA in 1987, advanced as bulk to the F_4 when the RILs started to be developed by single seed descent.

The experimental design was as an α-lattice with two replications and with 4 m^2 plots (8 rows at 0.20 m distance, 2.5 m long), and with a different randomisation in each combination of location and year. The sowing density was 12 g/m^2 for all the entries and planting was done with a plot drill. A plot combine was used for harvesting, and the seeds were subsequently ventilated before taking the weight. The

two locations in Syria were Tel Hadya (TH, 36_01°N; 36_56°E, elevation 284 m a.s.l.) and Breda (BR, 35_56°N; 37_10°E, elevation 300 m a.s.l.) with a long term average rainfall of 340 (30 seasons) and 275 mm (25 seasons), respectively. The two locations in Lebanon were Terbol (TR, 33_49°N; 35_59°E, elevation 890 m a.s.l.) and Kfardane (KF, 34_01°N; 36_03°E, elevation 1,080 m a.s.l.) with a long term average rainfall of 539 (25 seasons) and 461 mm (11 seasons). In 2002, and because of unavailability of land, Terbol was replaced by an early planting at Kfardane. All tested environments have been used by both the winter barley and winter wheat breeding programs at ICARDA and therefore had vernalisation conditions. Sowing and emergence dates in the different environments are given in Supplementary Table 1.

Water use efficiency (WUE) as an indicator of the amount of water available to the plant was calculated as grain yield divided by the growing season rainfall. Estimates of WUE did not include stored soil water because the soil profile is virtually empty at the time of planting. In fact planting follows 4 months with no rainfall, high temperatures and an average evaporation during June, July, August and September of 10–13 mm/day (average of the last 3 years).

In order to compare mean WUE across environments the relative WUE was calculated as

$$\mathrm{WUE}r = \mathrm{WUE} \times \mathrm{mm}_i/\mathrm{mm}_u$$

Where mm_i is the growing season rainfall in *i*th location by year combination and mm_u is the average growing season rainfall across all location by year combinations.

The following traits were recorded on a plot basis: days from emergence to heading (DH), days from emergence to maturity (DM) as the date when the peduncle was completely discoloured, grain filling period (FP) calculated as the difference between DM and DH, early growth vigour (GV) as a visual score from 0 = poor vigour to 5 = good vigour, grain yield (GY) in kg/ha measured on 3 m^2 (6 rows 2.5 m long), 1,000 kernel weight (KW) in g, lodging (LDG) evaluated visually on a scale from 0 = no lodging to 9 = all plot flat on the ground, peduncle length (PED) in cm from the last node to the bottom of the spike, peduncle extrusion (PEDEX) in cm from the ligule of the flag leaf to the bottom of the spike (this measure can be negative in the case all or part of spike remains in the boot), plant height (PH) in cm to the bottom of the spike, spike length (SL) in cm excluding the awns, seed dormancy (DOR) as the arcsine transformed percentage of germinated seeds in 2 replicates of 25 seeds, leaf fluorescence (SPD) determined using a chlorophyll meter (SPAD-502, Minolta, Japan) in the middle of two random leaves (the average was used for the analysis) at full heading stage before any symptoms of senescence were visible, wilting (WILT) evaluated visually on a scale from 0 = no wilting to 5 = maximum wilting when most of the plots were in the flag leaf stage. Traits were measured in 16 location by year combinations with the exception of DH (measured only in KF, TH, and TR), DM and FP (measured only in KF, TR), PED and PEDEX (measured only in BR, KF, TH, TR, 2004–2005), GV (measured only in BR02, BR04, KF04, TH02, TH03, TH04, TH05, and TR04), LDG (measured only in KF03, TH02 TH03, TH04, TH05, and TR03), SPD (measured only in TH02), DOR (measured only in BR04) and WILT (measured only in BR04).

Genotyping

The RIL population was genotyped with a total of 165 markers, including 76 RFLPs, 15 AFLPs, 29 SSRs, 1 RAPD and 10 genes as described in Teulat et al. (2002), and 31 polymorphic loci derived from cDNA constructed from drought stressed barley (Diab et al. 2004). The markers were mapped in seven linkage groups with an average distance between markers of 12.1 cM (Diab et al. 2004; Fig. 2).

Statistical analyses

Statistical analyses were carried out with SAS version 9.1 (SAS Institute 2003). The procedure MEANS was used to calculate means and standard deviations for each trait in the RIL population, ER/Apm and Tadmor for each location, separately. Significant differences between means were identified with the Tukey–Kramer test for multiple comparisons. In order to determine the proportion of genetic variation of the traits a 3-way ANOVA was conducted using the following mixed model in the SAS general linear model (GLM) procedure:

$$\begin{aligned} Y_{ijkm} = {} & \mu + G_i + L_j + Y_k(L_j) \\ & + G_i \times L_j + G_i \times Y_k(L_j) + \varepsilon_{jikm} \end{aligned} \quad (1)$$

where G_i is the fixed effect of the *i*th RIL genotype, L_j is the fixed effect of the *j*th location, $Y_k(L_j)$ is the random effect of the *k*th year nested in the *j*th location, $G_i \times L_j$ is the fixed interaction effect of the *i*th RIL genotype with the *j*th location, and $G_i \times Y_k(L_j)$ is the random interaction effect of the *i*th RIL genotype with the *k*th year nested within the *j*th location, ε_{jikm} is the error of Y_{ijkm}.

The model allows a subdivision of genotype × environment (GE) interactions in the two components G × L and G × Y(L) which have a great relevance in discussing breeding strategies related to wide and specific adaptation. Transgressive segregation for yield was calculated for the two macroenvironments Breda with Terbol, and Tel Hadya with Kfardane separately. A Dunnett multiple comparison of least square means differences of the RILs with the higher yielding parent was implemented.

Differences between environments (location by year effects) were investigated by calculating best linear unbiased estimates (BLUEs) of the genotype effects for yield (Singh et al. 2003) and using the environmentally standardised BLUEs to compute genotype × environment interactions in the GGEbiplot software (Yan et al. 2000).

Correlations between grain yield and plant height were calculated for each environment separately to test for crossover interactions indicative of different environmental conditions and the occurrence of abiotic stress. Based on these correlations, on yield and relative WUE, the locations were classified into those with moderate stress (Kfardane and Tel Hadya) and those with more severe stress (Breda and Terbol).

Genetic correlations between all traits were calculated with the least square means of RIL genotypes averaged across Breda and Terbol, and Kfardane and Tel Hadya, separately.

The detection of QTL for the 11 traits measured in multiple environments was carried out using the following mixed hierarchical model in the GLM procedure:

$$Y_{ijkmn} = \mu + M_i + L_j + Y_k(L_j) + G_m(M_i) + M_i \times L_j + M_i \times Y_k(L_j) + \varepsilon_{n(jikm)} \quad (2)$$

where μ is the general mean, M_i is the fixed effect of the ith marker genotype, L_j is the fixed effect of the jth location, $Y_k(L_j)$ is the random effect of the jth year nested in the jth location, $G_m(M_i)$ is the random effect of the mth RIL genotype nested in the ith marker genotype, $M_i \times L_j$ is the interaction effect of the ith marker with the jth location, $M_i \times Y_k(L_j)$ is the random interaction effect of the ith marker with the kth year nested in the jth location, $\varepsilon_{n(jikm)}$ is the error of Y_{ijkmn}. Marker main effects, M × L and M × Y(L) interactions with an FDR of 0.05 (false discovery rate, Benjamini and Yekutieli 2005) were interpreted as putative QTL and/or interaction effects. Crossover interaction effects were defined when the least square means of significant markers involved a change of sign in the different M × L or M × Y(L) combinations. Linked significant markers with a distance of ≤20 cM and showing the same effect were summarized to a single effect, and only the most significant marker from each group of linked loci is recorded.

The genetic variance explained by a marker (R^2_M), and by the respective M × L ($R^2_{M\times L}$) and M × Y(L) ($R^2_{M\times Y(L)}$) interactions was calculated as follows:

$$R^2_M = SQ_M/SQ_G, \quad R^2_{M\times L} = SQ_{M\times L}/SQ_{G\times L},$$

$$R^2_{M\times Y(L)} = SQ_{M\times Y(L)}/SQ_{G\times Y(L)}$$

SQ_M, $SQ_{M\times L}$, $SQ_{M\times Y(L)}$ correspond to the sums of squares of M and M × L and M × Y(L). SQ_G, $SQ_{G\times L}$, $SQ_{G\times Y(L)}$ was calculated as the type III sums of square in the ANOVA model 1.

Simple interval mapping (SIM) based on best linear unbiased estimates (BLUEs) was performed for a more precise localisation of QTL main effects within marker intervals using the software package MQTL (Tinker and Mather 1995). In addition, simplified composite interval mapping (sCIM) integrating interval mapping with information from multiple markers outside the interval was conducted to account for phenotypic variation due to the effect of additional QTL. The likelihood ratio described by Haley and Knott (1992) was used for the test statistic (TS) by MQTL both for QTL main effects and QTL × E (environment) interactions (Tinker and Mather 1995). The TS threshold for SIM QTL main effects and QTL × E interactions was determined by a permutation test with 2,000 replications to keep the probability of a type I error below 5% (Tinker and Mather 1995). The calculated threshold of the TS for SIM was used to infer the presence of QTL, while sCIM was used primarily to verify the QTL, to infer their locations and to estimate their effects. For sCIM, 26 background markers well distributed throughout the genome and at QTL detected in the single marker regression were used as cofactors to control the effect of the genetic background (printed in bold in Fig. 2). The interval mapping was carried out for 14 traits including those recorded only in a single environment.

Results

Relative water use efficiency, mean yield levels as well as correlations between yield and plant height per environment were used as indicators of drought stress in the different locations and environments (Table 1, Supplementary Table 3). Yield and relative water use efficiency were lowest in Breda and Terbol with an average yield of 2,973 and 2,922 kg/ha and an average relative water use efficiency of 6.2 and 6.1, respectively. Plants at Tel Hadya exhibited the highest yield (5,006 kg/ha) and relative WUE (10.4 kg/mm), respectively. However, rainfall was highest at Terbol followed by Kfardane, Tel Hadya and Breda, and the correlation coefficient between growing season rainfall and water use efficiency was −0.2. Water use efficiency varied between the years within locations with the highest variation recorded for Breda (from 3.7 kg/mm in 2004 to 8.8 kg/mm in 2003), which was also the location with the lowest average rainfall (324 mm). The correlation analysis showed significant and positive correlations between grain yield and plant height in Breda and Terbol in all years (with the exception of BR02), and significant and negative correlations in Tel Hadya in all years (Supplementary Table 3). At Kfardane, only in 2003 we found a significant negative

Table 1 Mean grain yield (kg/ha), growing season rainfall (mm) and relative water use efficiency in the 16 location–year combinations

Year	Location				Mean
	BR	KFE/KFL[a]	TH	TR	
2002					
Yield (kg/ha)	3,494	4,508/4,684	5,162		4,462
Rainfall (mm)	339	361/452	405		389
WUE*r* (kg/mm)	7.2	9.3/9.7	10.7		9.2
2003					
Yield (kg/ha)	4,267	3,651	5,401	3,420	4,185
Rainfall (mm)	386	867	492	995	685
WUE*r* (kg/mm)	8.8	7.6	11.2	7.1	8.7
2004					
Yield (kg/ha)	1,772	4,028	4,325	2,549	3,169
Rainfall (mm)	303	539	400	551	448
WUE*r* (kg/mm)	3.7	8.3	9.0	5.3	6.6
2005					
Yield (kg/ha)	2,357	3,739	5,134	2,798	3,507
Rainfall (mm)	267	499	303	571	410
WUE*r* (kg/mm)	4.9	7.7	10.6	5.8	7.3
Mean					
Yield (kg/ha)	2,973	4,122	5,006	2,922	
Rainfall (mm)	324	544	400	706	
WUE*r* (kg/mm)	6.2	8.3	10.4	6.1	

BR Breda (Syria), *KF* Kfardane (Lebanon), *TH* Tel Hadya (Syria), *TR* Terbol (Lebanon)

[a] In 2002 early and late planting in KF, KFE Kfardane early planting, *KFL* Kfardane late planting

correlation between yield and plant height, while in KF02 (early planting), KF04 and KF05 there were no significant correlations between grain yield and plant height.

The significance of the differences between the four locations was tested for the RIL population and the two parents (Table 2). ER/Apm flowered earlier than Tadmor (not significant), but showed a significantly longer grain filling period than Tadmor, and the RIL performance was intermediate between the parents. Tadmor yielded significantly less in KF and TH than ER/Apm, but showed an improved yield in BR and TR (not significant) as compared to ER/Apm. Peduncle extrusion was significantly higher in Tadmor than in Er/Apm at the locations BR and TR.

The total phenotypic variation of traits measured in multiple environments was subdivided into genotype, location and years within locations as well as into variation due to interactions with the genotype (G × L, G × Y(L), Supplementary Table 2). The genetic variation ranged between 0.8% for DH and 25.0% for GV. The factor location explained most of the variance for DH, GY, GV and PEDEX, while years within locations contributed most to the total variation for DM, FP, KW, LDG, PED, PH and SL. The variance of the G × Y(L) interaction was for all traits higher than the variance of the G × L interaction.

The principal component analysis for environments (or GGE biplot) clustered Tel Hadya with Kfardane and Breda with Terbol (Fig. 1). The biplot also demonstrates that variation of yield in Breda and Terbol varied more from year to year than in Tel Hadya and Kfardane. The WUEr values, the results from the correlations between yield and plant height, and the GGE biplot indicated two different macro-environments: two locations with moderate drought stress including Kfardane and Tel Hadya, and two locations with more severe stress, Breda and Terbol.

Genetic correlations were calculated for the two different macro-environments, Breda/Terbol (BR/TR), and

Table 2 Recorded traits, abbreviations and number of environments in which the 14 traits were measured

Abbr	Trait	#env[a]	Means BR			Means KF			Means TH			Means TR		
			RILs	ER/Apm	Tadmor	RILs	ER/Apm	Tadmor	RILs	ER/Apm	Tadmor	RILs	ER/Apm	Tadmor
DH	Days to heading (d)	12				128±1.6a	127a	129a	99±1.4b	98b	100b	133±2.1c	132c	137d
DM	Days to maturity	8				170±1.3a	171a	168a				175±2.1b	177b	175b
FP	Grain filling period	8				42±1.6a	43a	39b				42±2.1a	45c	37b
GV	Early growth vigour	8	2.4±0.3a	2.75ab	2.5ab	1.9±0.4b	2.3ab	1.35b	2.3±0.5a	2.8a	2.7a	2.5±0.7a	3.3a	2.3ab
GY	Grain yield (kg/ha)	16	2975±214a	2699a	2795a	4110±368b	4663bc	3520a	5012±393c	5560bc	3511a	2922±362a	2683a	3156a
KW	Kernel weight (g)	16	42±2.7a	43a	40a	40±2.3a	39a	39a	44±2.7ab	46b	38a	43±2.5ab	42ab	43ab
LDG	Lodging	6				1.7±0.3a	1.5a	1.5a	2.6±1.1b	1.0a	5.1c	2.3±0.4d	2.5abd	2.5abd
PED	Peduncle length (cm)	8	18±2.8a	15ab	22b	17±2.4a	14a	17ab	22±3.3b	20ab	19ab	18±2.7ab	16ab	22b
PEDEX	Peduncle extrusion (cm)	8	-5.2±1.9a	-6.0a	-3.3b	-0.8±1.7b	-2.8b	0.0bcd	2.8±2.7c	0.8bcd	0.3bcd	1.3±2.4d	-2.3ab	4.5cd
PH	Plant height (cm)	16	51±5.9a	43b	57a	68±4.1c	64c	67c	76±5.5d	70d	77d	49±5.2b	40b	57a
SL	Spike length (cm)	13	7.0± 0.6a	6.9abc	6.4abc	7.1±0.6a	6.9abc	6.8abc	7.7±0.6b	8.3ab	7.0abc	6.7±0.9c	6.0abc	6.5abc
DOR*	Dormancy	BR04	0.97±0.4a	1.25a	0.61a									
SPD	Chlorophyll fluorescence	TH02							39±2.5a	44a	36a			
WILT	Wilting	BR04	2.1±0.5a	2.8a	2.4a									

Means and standard deviations are calculated for the RIL population, and means for ER/Apm and Tadmor, for the four locations, separately

Significant differences between means of RILs and parents and between the different locations are indicated with different letters according to the Tukey–Kramer test for multiple comparisons ($P < 0.05$). Standard deviations (sd) are given for RILs (Means)

[a] Number of environments in which the trait was measured (see "Materials and methods")

[b] The germination rate was arcsine transformed and high values indicate a low dormancy

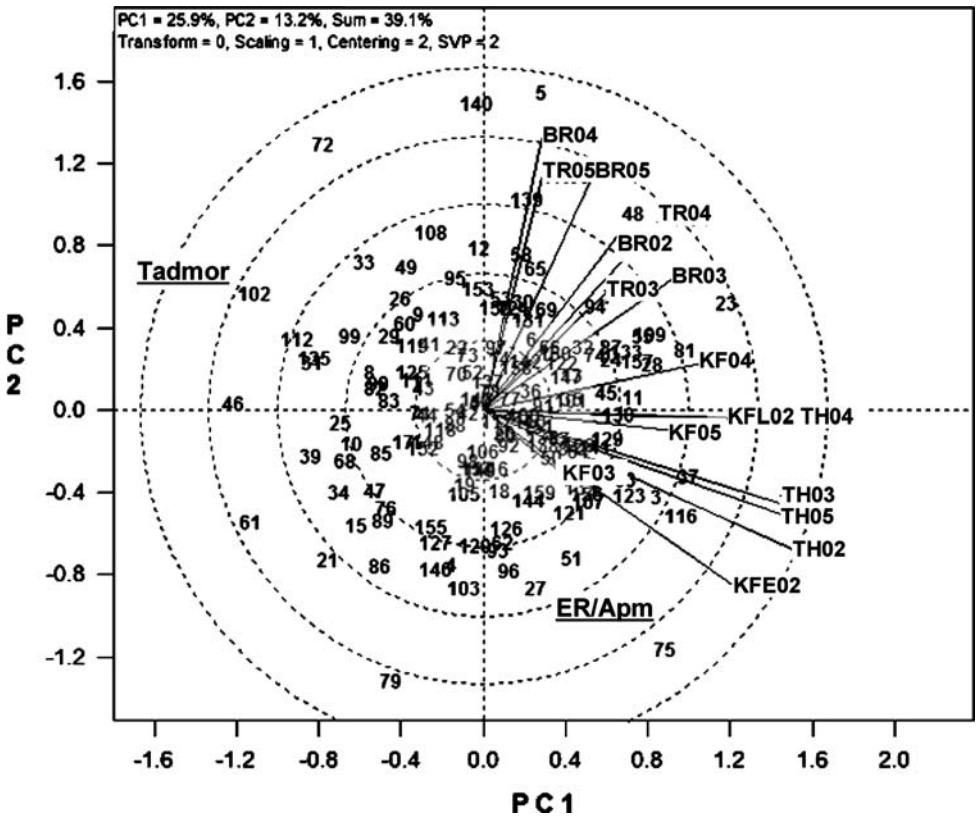

Fig. 1 GGE biplot based on best linear unbiased estimates for yield. *TH* Tel Hadya, *BR* Breda, *TR* Terbol, *KF* Kfardane, *KFE* Kfardane early planting, *KFL* Kfardane late planting, *02* 2002, *03* 2003, *04* 2004, *05* 2005

Kfardane/Tel Hadya (KF/TH), separately, in order to test whether different ideotypes yielded better in different stress conditions (Table 3). In Tel Hadya and Kfardane yield was negatively correlated with PH, LDG, PED and PEDEX and positively correlated with DM, FP, GV and SPD. In Breda and Terbol only LDG and GV showed a significant negative correlation to yield. KW showed a positive correlation with PH in BR/TR and no significant

Table 3 Correlation coefficients (*r*) according to Pearson between 14 agronomic traits

Trait	GY	PH	DH	DM	FP	KW	SL	PED	PEDEX	GV	LDG	SPD	WILT
GY		**−0.55**	0.05	**0.34**	**0.19**	0.05	−0.09	**−0.39**	**−0.30**	**0.17**	**−0.70**	**0.20**	nd
PH	0.14		**−0.27**	**−0.28**	0.03	0.14	**0.21**	**0.67**	**0.48**	**−0.24**	**0.57**	0.01	nd
DH	−0.04	**−0.25**		**0.47**	**−0.75**	−0.06	**0.22**	**−0.30**	**−0.40**	**0.18**	−0.09	**−0.23**	nd
DM	0.02	**−0.16**	**0.47**		0.06	**0.20**	−0.08	−0.16	0.11	**0.37**	**−0.50**	−0.04	nd
FP	0.04	0.07	**−0.51**	**0.50**		**0.18**	0.04	**0.18**	**0.31**	0.08	**−0.18**	**0.28**	nd
KW	−0.02	**0.44**	0.04	0.03	0.01		**0.22**	**0.22**	0.07	0.07	−0.08	0.12	nd
SL	−0.04	**0.23**	−0.04	0.15	**0.24**	**0.24**		**0.22**	0.01	**−0.18**	0.09	0.15	nd
PED	0.04	**0.83**	**−0.19**	0.01	**0.17**	**0.38**	**0.32**		**0.82**	0.1	**0.27**	0.06	nd
PEDEX	−0.05	**0.50**	**−0.16**	0.13	**0.27**	**0.18**	0.15	**0.71**		**0.20**	0.08	−0.04	nd
GV	**−0.20**	**−0.41**	**0.40**	**0.57**	**0.17**	−0.09	−0.04	**−0.2**	−0.00		**−0.18**	0.14	nd
LDG	**−0.30**	−0.12	0.03	0.01	−0.03	−0.06	0.12	−0.09	−0.08	−0.02		−0.04	nd
WILT	−0.12	**−0.48**	**0.27**	**0.40**	0.13	**0.36**	−0.08	**−0.34**	**−0.16**	**0.57**	−0.04	nd	
DOR[a]	−0.01	**−0.19**	−0.15	0.01	**0.15**	**−0.27**	0.09	−0.14	−0.06	0.07	−0.08	nd	0.15

For calculating correlations the least square means of the trait performance of each RIL averaged across locations were used. In the upper triangular correlations based on least square means averaged across Kfardane and Tel Hadya (moderate stress) and in the lower triangular least square means averaged across environments in Breda and Terbol (more severe stress) are depicted. Significant correlations ($P < 0.05$) are printed in bold

nd not determined

[a] Values for dormancy were arcsine transformed, so that negative coefficients indicate a positive correlation and vice versa

correlation in KF/TH. GV was negatively correlated with yield in the BR/TR and positively correlated with yield in KF/TH, possibly due to the strong negative correlation with plant height in BR/TR and the moderate positive correlation with plant height in KF/TH. At the same time GV was characterised by a positive correlation with DH of 0.4 in BR/TR and 0.18 in KF/TH. SPD only measured in TH02 showed a significant positive correlation to grain yield and grain filling period ($r = 0.2$ and 0.28, respectively). Wilting (BR04) showed negative correlations with plant height and PED and positive correlation with DH, DM, KW and GV. Seed dormancy (BR04) correlated positively with wilting ($r = 0.15$) and grain filling period (0.15) and negatively with PH ($r = -0.19$) and KW ($r = -0.27$). Significant transgressive segregation for yield was only detected in the macroenvironment BR/TR, where the RILs 5, 48 and 58 out yielded the higher yielding parent Tadmor with 3,719, 3,627 and 3,499 kg/ha, respectively, as compared to the mean yield of Tadmor of 3,038 kg/ha.

For the QTL analysis of eleven traits analysed in multiple environments a 4-factorial ANOVA was calculated to identify markers with a main effect and marker interactions with either the location (M × L) or with years within locations [M × Y(L)] (Table 5).

The ANOVA revealed 71 significant marker main effects for eleven traits. Due to linkage between markers, these effects were summarised to 38 putative QTL for eleven agronomic traits (Table 4; Fig. 2). In Tables 5 and 6 only significant interaction effects are recorded, if they showed a crossover effect. Markers, which were also significant as a main effect and only exhibited a difference in magnitude were not considered, but are indicated in Table 4. Altogether nine M × L interactions were found, of which one locus was also significant as a marker main effect and only exhibited a quantitative difference between environments (Table 4), and eight loci showed a cross-over interaction between the four locations (Table 5). Totally 39 loci revealed M × Y(L) interactions of which 19 showed only quantitative interaction effects and were also significant as marker main effect (Table 4), two loci were also significant as marker main effect and exhibited a cross-over interaction (Qgy-tera_3H.a, Qgy-tera_3H.b), and 18 markers showed only a cross-over interaction effect between different environments (Table 6).

SIM and sCIM analysis for 14 traits revealed 42 QTL main effects of which 25 were also significant as a QTL × E interaction effect (Table 7). In addition, 12 loci exhibited only a QTL × E interaction effect. Out of the 38 main effects detected in the single marker analysis, 33 were also revealed with the sCIM mapping procedure. Five marker main effects were only detected in the single marker regression. Five additional QTL main effects were only found with the SIM/sCIM procedure together with four QTL for DOR, SPD and WILT only analysed with MQTL. Of the 48 loci showing M × L or M × Y(L) interactions 29 were also detected as a QTL × E interaction in the sCIM analysis. In the following the results will be presented for the different traits separately.

Days to heading

The ANOVA detected six QTL for days to heading on chromosomes 1H, 2H, 3H and 5H. The strongest effect was measured at Qdh-tera_1H.b, which explained 15.8% of the genetic variance, and lines with the Tadmor allele flowered 1.3 days earlier on average. No significant interactions were found between marker and locations, but three interactions between marker and year within locations [M × Y(L)] were detected. These were located on chromosomes 3H and 5H and explained between 2.3 and 3.4% of the G × Y(L) interaction variance. SCIM confirmed five of the marker main effects (Qdh-tera_1H.b to Qdh-tera_5H.a) and revealed in addition a QTL × E interaction effect at marker $CDO475_{(7H)}$.

Days to maturity

Two QTL were detected for days to maturity on 1H and 6H coinciding between the ANOVA and the sCIM analysis. ER/Apm increased days to maturity at both loci with the strongest effect at Qdm-tera_1H.a, which explained 31.4% of the genetic variance.

Grain filling period

The ANOVA revealed two marker main effects for grain filling period on chromosomes 1H and 2H. The ER/Apm allele increased the grain filling period at both loci explaining 25.7 and 12.2% of the genetic variance, respectively. In addition, an M × Y(L) interaction effect was detected at marker $wg908_{(5H)}$. The sCIM analysis confirmed the QTL on 2H and the M × Y(L) interaction effect on 5H, and identified an additional QTL at $cMWG653_{(6H)}$ at which ER/Apm increased the grain filling period.

Early growth vigour

The ANOVA identified one QTL Qgv-tera_1H.a for early growth vigour, which explained 20.8% of the genetic variance, and ER/Apm improved early growth vigour at this locus. The sCIM analysis confirmed the QTL on 1H and identified a QTL × E interaction effect at marker $cdo1396A_{(3H)}$.

Table 4 List of 38 marker main effects for 11 agronomic traits

QTL[a]	Marker	Chr[b]	Pos[c]	Range[d]	Bin[e]	Effect[f]	F-stat	R^2%[g]	LSM ER/Apm[h]	LSM Tadmor[h]	ER/Apm – Tadmor[i]	Teulat et al. 2001b[j]
DH												
Qdh-tera_1H.a	C1B11b	1H	135.1	135	7	M	12.1	9.3	120.8	119.8	1.0	
Qdh-tera_1H.b	wg983	1H	195.7	192-196	8	M+M×Y(L)	20.8	15.8	120.9	119.6	1.3	ER/Apm
Qdh-tera_2H.a	Bmac684	2H	108	103-130	7	M	17.7	11.1	120.3	121.4	-1.1	
Qdh-tera_2H.b	cdo1417	2H	187.8	188	13	M	12.1	8.0	120.6	119.7	0.9	ER/Apm
Qdh-tera_3H.a	ABG495	3H	320.5	321-326	16	M	12.7	7.4	120.6	119.7	0.9	ER/Apm
Qdh-tera_5H.a	C1F9a	5H	242	242	14	M+M×Y(L)	9.1	8.7	119.9	120.9	-0.9	Tadmor
DM												
Qdm-tera_1H.a	pHva1	1H	206.5	191-227	12	M+M×Y(L)	31.4	36.9	173.4	171.5	1.9	
Qdm-tera_6H.a	cdo497	6H	109.4	109	6	M	10.3	6.8	172.8	171.9	0.9	
FP												
Qfp-tera_1H.a	CDO202	1H	240.2	227-244		M	25.7	25.3	42.6	41.0	1.6	
Qfp-tera_2H.a	Bcd1069	2H	102.7	103-108	7	M	12.2	6.5	42.2	41.4	0.8	
GV												
Qgv-tera_1H.a	pHva1	1H	206.5	191-206	12	M+M×Y(L)	19.5	20.8	2.4	2.1	0.3	
GY												
Qgy-tera_3H.a	Bmag136	3H	101	101	4	M+M×Y(L)	8.6	8.7	3817.6	3684.3	133.3	
Qgy-tera_3H.b	bcd1127	3H	203	203	6	M+M×Y(L)	10.1	10.6	3831.9	3684.2	147.7	
Qgy-tera_3H.c	Bmag013	3H	287.4	287	14	M	12.8	9.6	3818.8	3673.8	145.0	
Qgy-tera_6H.a	Bmag173	6H	71.8	63-72	5	M	21.8	13.0	3843.0	3678.9	164.1	
Qgy-tera_6H.b	cdo497	6H	109.4	109-145	6	M+M×Y(L)	19.2	17.6	3843.9	3651.6	192.3	
Qgy-tera_7H.a	bss1B	7H	246.6	247	12	M	12.3	11.6	3831.0	3660.7	170.3	ER/Apm
KW												
Qkw-tera_5H.a	wg889	5H	129.8	130	6	M	15.6	9.2	41.7	43.1	-1.4	
Qkw-tera_5H.b	cdo400	5H	260.8	261	14	M	12.0	8.0	41.7	43.0	-1.3	Tadmor
Qkw-tera_6H.a	Bmag173	6H	71.8	72	6	M+M×Y(L)	24.2	13.6	41.4	43.0	-1.7	Tadmor
Qkw-tera_6H.b	cttaccA	6H	216.4	216	9	M+M×L	11.8	7.8	43.0	41.7	1.3	ER/Apm
LDG												
Qldg-tera_3H.a	bcd1127	3H	203.3	203.3	6	M	7.8	10.4	2.1	2.3	-0.2	
Qldg-tera_6H.a	Bmag173	6H	71.8	72	5	M+M×Y(L)	8.1	18.9	2.0	2.4	-0.3	
Qldg-tera_6H.b	cdo497	6H	109.4	109	6	M+M×Y(L)	8.5	24.5	2.0	2.4	-0.3	
PED												
Qped-tera_3H.a	Bmag136	3H	101.1	101-111	4	M	31.5	19.0	17.8	19.7	-1.9	
Qped-tera_3H.b	bcd1127	3H	203.3	203-212	6	M	28.3	13.5	17.9	19.5	-1.6	
Qped-tera_3H.c	PSR170	3H	264.8	265	11	M+M×Y(L)	14.3	16.5	18.0	19.6	-1.6	
PEDEX												
Qpedex-tera_3H.a	C2D5b	3H	111.3	111-118	4	M+M×Y(L)	19.2	12.3	-1.1	0.2	-1.3	
Qpedex-tera_3H.b	bcd1127	3H	203.3	190-203	6	M+M×Y(L)	20.6	16.3	-1.2	0.2	-1.4	
Qpedex-tera_3H.c	Bmag013	3H	287.4	287	14	M+M×Y(L)	16.6	14.2	-1.0	0.4	-1.3	
PH												
Qph-tera_3H.a	Bmag136	3H	101.1	101-118	4	M+M×Y(L)	22.2	19.4	59.4	63.7	-4.3	Tadmor
Qph-tera_3H.b	bcd1127	3H	203.3	185-213	6	M+M×Y(L)	23.9	18.6	59.3	63.5	-4.2	Tadmor
Qph-tera_3H.c	cdo1396A	3H	256.2	256-287	11	M+M×Y(L)	17.3	13.6	59.8	63.4	-3.6	
Qph-tera_4H.a	C1F8a	4H	218.2	218	13	M	11.4	8.2	62.7	59.9	2.8	ER/Apm
Qph-tera_6H.a	Bmag173	6H	71.8	63-72	5	M+M×Y(L)	23.2	16.4	59.3	63.3	-3.9	Tadmor
Qph-tera_6H.b	cdo497	6H	109.4	109.4	6	M+M×Y(L)	21.4	17.5	59.6	63.6	-4.1	Tadmor
SL												
Qsl-tera_1H.a	C1A6	1H	156.9	145-158	9	M+M×Y(L)	15.7	10.6	7.3	6.9	0.4	
Qsl-tera_2H.a	bcd1069	2H	102.7	103	7	M+M×Y(L)	11.7	6.5	7.3	7.0	0.3	

[a] A putative QTL was assumed in the vicinity of a marker locus, if the marker main effect (M) was significant in the 4-factorial ANOVA with an FDR of 0.05 (Benjamini and Yekutieli 2005)

[b] Chromosomal localisation of the markers

[c] Position of the listed marker in cM, based on Diab et al. (2004)

[d] CentiMorgan position from the first to the last significant marker in a linkage group

[e] Genotyped markers were assigned to bins using the GrainGenes data base (http://grain.jouy.inra.fr/cgi-bin/graingenes/browse.cgi)

[f] A putative QTL was assumed in the vicinity of a marker locus, if the marker main effect (M) was significant in the 4-factorial ANOVA with an FDR < 0.05, some marker main effects did also show a significant M × L or M × Y(L) interaction effect

[g] R^2 is proportion of the genetic variance, which is explained by the marker main effect

[h] Least square means of trait values across all tested environments for RILs carrying the ER/Apm genotype (LSM ER/Apm) or the Tadmor allele (LSM Tadmor) at the given marker locus

[i] LSM ER/Apm–LSM Tadmor, positive values show that the ER/Apm allele increases the trait value, negative values show that the Tadmor allele increases the trait value (in grey)

[j] QTL that coincided with QTL detected in Teulat et al. (2001b) are indicated with the parent allele that increased the trait value

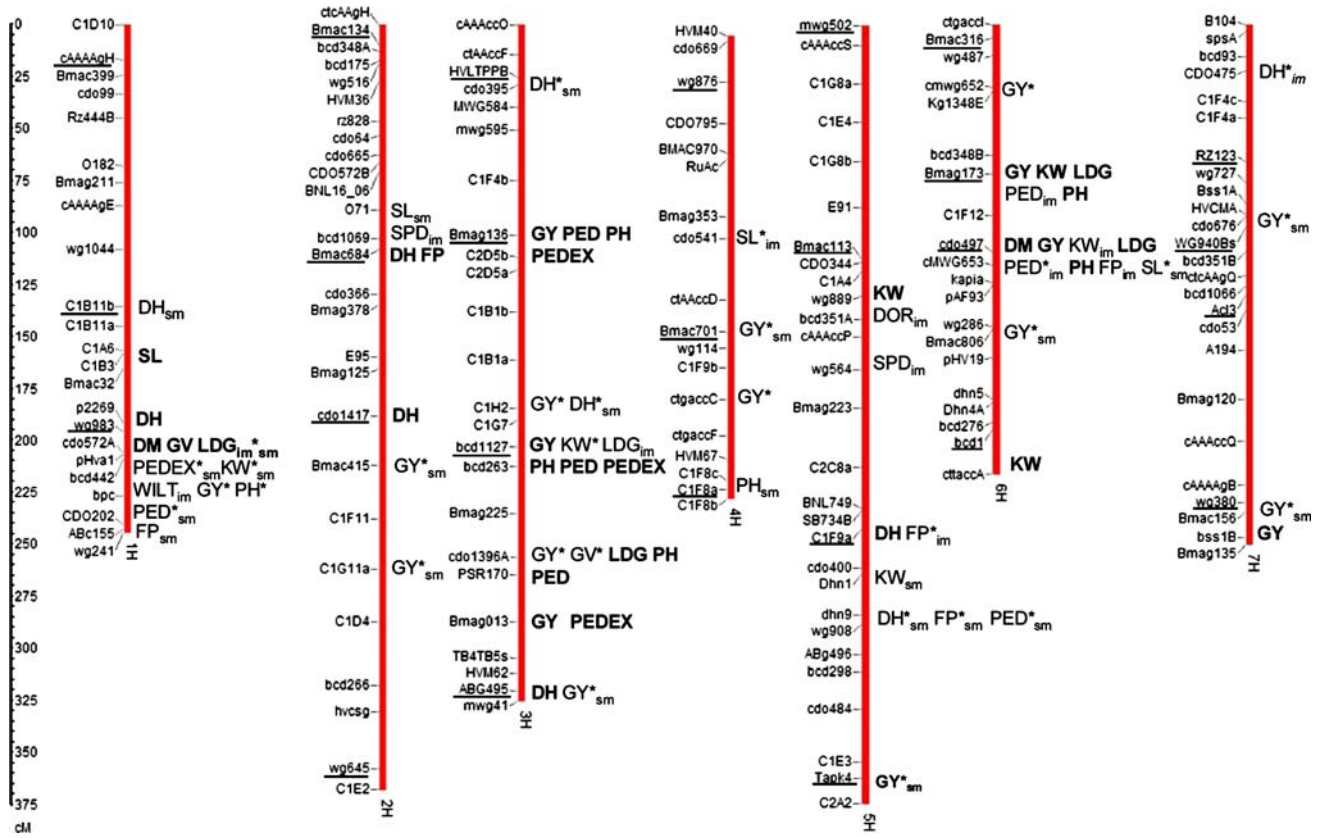

Fig. 2 QTL map of the population Tadmor × ER/Apm showing marker main effects and M × L or M × Y(L) interaction effects for 14 traits. Results of the single marker regression and the simplified composite interval mapping (MQTL) are shown. Main effects detected in both analyses are printed in *bold*, effects only significant as an interaction effect are shown with an *asterisk*. Main and interaction effects only found in the ANOVA are indicated with a lower *sm* (single marker analysis), QTL and QTL × E interaction effects only identified with MQTL are indicated with a lower *im* (interval mapping) (see Tables 4, 5, 6, 7). Markers used as cofactors in the sCIM procedure are underlined. Trait abbreviations of QTL follow Table 2. The marker map is based on Diab et al. (2004)

Table 5 Eight significant M × L interaction effects for three traits

Marker	Chr[a]	Pos[b]	Range[c]	Bin[d]	Fstat	R^2%[e]	LSM ER/Apm-Tadmor Br[f]	LSM ER/Apm-Tadmor KF[f]	LSM ER/Apm-Tadmor Th[f]	LSM ER/Apm-Tadmor TR[f]	Candidate genes[g]
GY											
pHva1	1H	206.5	205.5-208.5	12	19.6	7.2	-92.7	103.0	220.6	-116.3	HVA1
C1H2	3H	184.7	184.7	5	8	1.1	-56.9	187.2	172.8	-97.1	Aminomethyltransferase
Bmac701	4H	142.8	142.8	10	10	2.1	-77.7	39.5	112.7	75.7	
Tapk4	5H	361.7	361.7	14	11.0	6.5	-49.2	131.2	178.5	-152.8	Tapk4
Bmac806	6H	146	146	7	14.5	3.3	-27.5	177.3	199.1	128.2	
cdo676	7H	91.8	91.8	6	7.0	3.2	33.6	-75.4	-83.8	147.8	HvA22
KW											
bcd1127	3H	203.3	203	6	7.0	8.2	-0.8	0.47	0.24	-0.47	
SL											
cdo497	6H	109.4	109.4	6	12.0	3.0	-0.2	-0.3	-0.1	0.1	

BR Breda, *KF* Kfardane, *TH* Tel Hadya, *TR* Terbol

[a] Chromosomal localisation of the markers

[b] Position of the listed marker in cM, based on Diab et al. (2004)

[c] CentiMorgan position from the first to the last significant marker in a linkage group

[d] Genotyped markers were assigned to bins using the GrainGenes data base (http://grain.jouy.inra.fr/cgi-bin/graingenes/browse.cgi)

[e] R^2 is proportion of the G × L variance, which is explained by the interaction effect between marker and location

[f] Differences in least square means between the RILs ± sd[c] with the ER/Apm allele and the Tadmor allele for each location separately. In grey are the least square means differences which show that the Tadmor allele increased the trait value

[g] Candidate genes: Xu et al. (1996), Diab et al. (2004), Guo et al. (2002), Shen et al. (2001)

Table 6 Marker by year within location interaction effects for nine traits

Marker	Chr[a]	Pos[b]	Range[c]	R^2%[d]	LSM_ER/Apm - LSM_Tadmor [e]															
					BR02	BR03	BR04	BR05	KFE 02	KFL 02	KF03	KF04	KF05	TH02	TH03	TH04	TH05	TR03	TR04	TR05
DH																				
HVLTPPB	3H	25	25	2.3					-0.5	-0.6	-1.1	-0.2	0.1	-0.3	-0.1	-0.5	-0.2	-0.6	-0.9	-0.1
C1H2	3H	185	185	3.0					-0.4	-0.4	-0.1	0.1	0.0	-0.5	0.0	0.1	-0.1	0.1	-1.0	0.3
wg908	5H	288	288	3.4					-0.6	-0.6	-0.5	-0.2	0.1	-0.5	0.1	0.1	-0.1	-0.1	-1.4	-0.1
FP																				
wg908	5H	288	261-288	3.6					-0.17	0.57	0.51	-0.47	-0.26					-0.13	0.59	-1.2
GV																				
cdo1396A	3H	256	256	1.8			0.1					0.2		-0.1	0.1	-0.1		0.4		
GY																				
Bmac415	2H	212	212	2.1	-81	-15	-66	30	-175	126	-356	-70	54	70	-30	-68	74	-83	-83	-262
C1G11a	2H	262	262	3.8	-68	-219	-33	8	-549	-125	-492	-2	13	-146	-21	-139	-88	-12	-136	-212
Bmag136	3H	101	101.1	2.1	-54	6	-82	-3	420	175	562	238	100	260	252	140	286	-113	88	124
bcd1127	3H	203	203.3	2.6	-137	77	-44	-17	568	208	538	214	169	269	341	93	364	-69	43	72
PSR170	3H	265	265	3.2	57	75	-45	-66	181	74	507	-106	-1	180	399	67	167	-72	-176	-187
mwg41	3H	325	325.1	2.2	67	61	6	-17	206	-84	335	-89	-17	142	-76	106	8	-80	-55	117
CtgaccF	4H	192	192	2.1	-135	-80	-30	31	191	-99	-253	26	-21	40	26	-29	5	40	57	-250
cmwg652	6H	30	30	2.5	-259	-221	-129	-164	345	-18	192	-120	-114	13	-203	-67	160	48	-56	-8
wg380	7H	230	230	1.9	38	31	53	93	-106	248	-155	135	106	26	115	224	3	77	129	30
KW																				
Bpc	1H	227	227	2.0	0.0	-0.1	-0.5	-0.4	0.6	2.5	0.2	0.3	0.5	0.5	0.4	0.6	0.6	-0.6	-0.4	0.2
LDG																				
cdo572A	1H	207	192-209	3.9							-0.1			0.6	0.3	0.8	0.5		-0.03	1.8
PED																				
pHva1	1H	207	192-209	8.7			-3.4	0.9				0.0	0.2			0.5	0.2		0.3	1.8
wg908	5H	288	288	3.1			0.5	-1.4				-0.8	0.3			-0.2	-1.7		-0.6	-0.4
PEDEX																				
cdo572A	1H	206	192-207	3.6			0.3	1.3				0.1	0.1			0.7	0.3		-0.4	1.5
PH																				
pHva1	1H	207	192-209	8.7	-2.8	-3.1	-2.9	-1.0	1.1	-1.3	-3.9	-2.6	-1.1	-1.6	-0.3	-2.0	-1.9	-3.1	-2.9	0.3

BR Breda, *KF* Kfardane, *KFE* Kfardane early planting, *KFL* Kfardane late planting, *TH* Tel Hadya, *TR* Terbol, *02* 2002, *03* 2003, *04* 2004, *05* 2005

[a] Chromosomal localisation of the markers

[b] Position of the listed marker in cM, based on Diab et al. (2004). CentiMorgan position from the first to the last significant marker in a linkage group

[c] If several linked markers are significant, the cM range is given

[d] R^2 is proportion of the interaction variance between the genotype and year within location, which is explained by the M × Y(L) interaction effect

[e] Differences in least square means between the RILs with the ER/Apm allele and the Tadmor allele for each environment separately. In grey are the least square differences which show that the Tadmor allele increased the trait value

Grain yield

For grain yield the ANOVA identified six QTL on 3H, 6H and 7H. The ER/Apm allele increased yield at all loci with the strongest effect at Qgy-tera_6H.b. This locus explained 17.6% of the genetic variance, and the ER/Apm allele increased yield by, on average, 192.3 kg/ha. Six loci showed significant interactions with the location with the strongest effects at pHva1$_{(1H)}$ and Tapk4$_{(5H)}$, explaining 7.2 and 6.5% of the G × L interaction variance. At these loci the Tadmor allele increased yield in Breda and Terbol, and the ER/Apm allele increased yield in Kfardane and Tel Hadya. Nine M × Y(L) interaction effects were identified explaining between 1.9 and 3.8% of the interaction variance G × Y(L). The sCIM analysis confirmed the six marker main effects and identified five QTL × E interaction effects which coincided with two M × L and three M × Y(L) effects.

Kernel weight

The analysis identified four QTL for kernel weight on chromosomes 5H and 6H. The Tadmor allele increased KW at three loci by a maximum 1.7 g at Qkw-tera_6H.a, which explained 13.6% of the genetic variance. Marker bcd1127$_{(3H)}$ showed a significant M × L interaction with positive effects of the Tadmor allele in BR/TR and negative effects in KF/TH. Marker bpc$_{(1H)}$ showed a significant M × Y(L) interaction. At this marker the Tadmor allele increased KW in three Breda environments and two Terbol environments, but decreased KW in Kfardane and Tel Hadya. The interval mapping analysis supported the QTL Qkw-tera_5H.a, −6Ha and −6H.b and the M × Y(L) interaction effect at marker bcd1127$_{(3H)}$.

Lodging

Three QTL were detected on 3H and 6H with the strongest effect at Qldg-tera_3H.a which explained a maximum of 24.5% of the genetic variance. The Tadmor allele increased lodging susceptibility at all three loci. One significant crossover M × Y(L) interaction was detected at cdo572A$_{(1H)}$. At this locus the Tadmor allele decreased lodging in Kfardane and Terbol 2003 and increased lodging in the four Tel Hadya environments. SCIM confirmed the QTL Qldg-tera_3H.a and _6H.a, and detected three additional QTL at cdo572A$_{(1H)}$, cdo1396A$_{(3H)}$ and cMWG653$_{(6H)}$ at which Tadmor increased lodging susceptibility.

Table 7 QTL and QTL by environment interactions for 14 traits analysed in one to 16 environments

Trait[a]	Marker[b]	Chr	Pos (cM)[c]	Bin	TS SIM[d]	TS SIM QTLxE[d]	TS sCIM[d]	TS sCIM QTLxE[d]	add sCIM[e]
DH	wg983+0	1H	175-200.7	8-12	159.2	31.4	163.4	41.9	0.84
	Bmac684+0	2H	102.7-123	7	129.3	ns	134.3	ns	-0.74
	cdo1417+5	2H	187.8-192.8	13	90.9	ns	93.4	ns	0.63
	ABG495-5	3H	297.4-320.5	16	100.1	ns	98.3	ns	0.86
	C1F9a+5	5H	237.9-252	14	115.8	34.3	152.5	52.0	-1.12
	CDO475+5	7H	20-32	3	ns	30.1	ns	48.4	-0.28
DM	wg983+5	1H	191.6-218	8-12	318.9	ns	355.5	ns	1.44
	cdo497+5	6H	106.7-114.4	6	63.1	ns	96.3	ns	0.75
FP	Bmac684	2H	98.9-108	7	74.3	ns	99.3	ns	0.84
	C1F9a+10	5H	242-260.8	6	ns	23.9	39.8	29.7	0.78
	cMWG653-1	6H	114.4-115.9	14	66.0	ns	104.5	ns	1.02
GV	cdo572A-5	1H	195.7-208.9	8-12	144.1	61.4	173.1	66.1	0.35
	cdo1396A+5	3H	256.2-261.2	11	ns	29.4	ns	21.2	-0.01
GY	pHva1+0	1H	200.7-206.5	8-12	ns	78.9	ns	77.0	16.5
	Bmag136+0	3H	101.1-106.1	4	68.8	70.2	59.6	90.2	68.9
	C1H2+0	3H	184.7	5	ns	57.4	ns	64.6	51.6
	bcd1127+0	3H	199.7-208.3	6	80.1	105.0	108.2	140.8	89.5
	cdo1396A+0	3H	250.1-261.2	11	ns	78.9	ns	80.8	57.4
	Bmag013-5	3H	274.8-284.8	14	55.4	ns	56.9	52.7	80.3
	ctgaccF+0	4H	192	11	ns	26.7	ns	56.5	9.6
	cmwg652+0	6H	24.7-30	?	ns	63.9	ns	43.8	-36.4
	Bmag173+5	6H	71.8-81.8	5	69.4	ns	93.7	50.7	109.1
	cdo497+0	6H	106.7-109.4	6	92.6	83.2	100.4	88.6	86.7
	bss1B+0	7H	246.6	12	48.0	ns	41.2	16.6	55.9
KW	bcd1127+0	3H	203.3	6	ns	44.0	ns	56.8	0.18
	wg889+0	5H	129.8	6	100.2	ns	107.7	ns	-0.85
	Bmag173+0	6H	71.8	5	197.6	38.2	301.5	51.6	-1.44
	cdo497+0	6H	109.4	6	104.4	34.2	189.5	38.8	-1.18
	cttaccA-7	6H	209.5-216.5	?	105.6	31.0	67.3	25.9	0.96
LDG	cdo572A+0	1H	195.7-213.9	8-12	92.4	82.8	85.9	77.6	-0.46
	bcd1127+0	3H	199.7-203.3	6	38.2	27.2	56.7	30.7	-0.38
	cdo1396A+0	3H	250-256.2	11	50.1	48.2	42.7	39.0	-0.33
	Bmag173+0	6H	71.8-96.8	5	76.1	51.6	78.7	52.3	-0.47
	cMWG653-1	6H	114.4-123.9	6	101.6	79.1	79.9	81.1	-0.55
PED	Bmag136+0	3H	94.8-106.1	4	75.5	ns	76.5	ns	-0.78
	bcd1127+0	3H	194.7-208.3	6	51.8	ns	44.8	ns	-0.58
	PSR170+5	3H	250.1-279.8	11	68.2	37.1	77.2	42.3	-0.89
	Bmag173+10	6H	71.8-81.8	5	44.5	30.9	43.4	34.9	-0.87
	cdo497+0	6H	109.4	6	ns	43.9	27.3	44.0	-0.47
PEDEX	Bmag136+0	3H	101.1-106.1	4	78.7	34.0	96.4	48.6	-0.69
	bcd1127-4	3H	194.7-203.3	6	78.2	36.6	83.9	44.5	-0.62
	PSR170+10	3H	261.8-292	11	66.2	36.8	85.1	45.0	-0.73
PH	pHva1+0	1H	206.5	8-12	ns	45.4	45.3	42.1	-1.01
	Bmag136+5	3H	101.1-116.3	4	256.7	79.5	243.0	86.6	-2.55
	bcd1127-5	3H	175.9-203.3	6	244.3	90.6	259.5	119.0	-3.1
	PSR170+5	3H	255.2-279.8	11	222.8	67.2	207.2	70.9	-3.39
	Bmag173+0	6H	67.8-86.8	5	261.0	67.5	315.9	87.4	-2.81
	cdo497-5	6H	101.7-109.4	6	293.9	94.9	244.1	99.3	-2.55
SL	C1A6-2+0	1H	149.5-157.8	8	66.1	43.4	91.5	67.7	0.22
	cdo541+5	4H	91.8-107.3	7	ns	53.9	ns	44.7	0.11
DOR	wg889+5	5H	134.8	6	127.8	nd	94.7	nd	0.55
SPD	bcd1069+0	2H	102.7	7	14.1	nd	24.3	nd	1.67
	wg564+0	5H	165.2	7	16.9	nd	14.9	nd	1.27
WILT	pHva1+0	1H	195.7-206.5	8-12	18.5	nd	16.0	nd	0.14

Simple interval mapping and simplified composite interval mapping were performed with MQTL (Tinker and Mather 1995)

[a] Trait abbreviations are explained in Table 2

[b] Closest significant marker and distance to peak TS in cM is given

[c] Significant marker interval in cM

[d] Significant test statistics for each trait and QTL main effect and interaction effects were established by calculating 2,000 permutations of the data (experimentwise $P = 0.05$) during SIM. SCIM was carried out verify the QTL, infer their locations and to estimate their effects

[e] Additive genetic effect expressed as the change in the value of each trait due to the contribution of an allele from Tadmor compared to Er/Apm (positive value: ER/Apm increases trait value)

Peduncle length and peduncle extrusion

For both traits three QTL were detected on chromosome 3H. The Tadmor allele increased peduncle length and peduncle extrusion at all three loci by a maximum of 1.9 and 1.4 cm, respectively. Qped-tera_3H.a had the strongest effect on PED explaining 19.0% of the genetic variance, while Qpedex-tera_3H.b was the major locus for PEDEX with 16.3% of the genetic variance explained. Two and one M × Y(L) interaction effects were found for PED and

PEDEX, respectively. The marker pHva1(1H) explained 8.7 (PED) and 3.6% (PEDEX) of the interaction variance G × Y(L). SCIM supported all three main effects for PED and PEDEX and detected an additional QTL for PED at Bmag173$_{(6H)}$, where Tadmor increased the trait value and a QTL × E interaction effect at marker cdo497$_{(6H)}$.

Plant height

Six QTL were detected for plant height on chromosomes 3H, 4H and 6H. The Tadmor allele increased height at five out of six loci by maximal 4.3 cm at Qph-tera_3H.a, which explained 19.4% of the genetic variance. No significant interaction effects were recorded for plant height.

SCIM analysis corroborated all main effects detected on 3H and 6H and detected a QTL × E interaction effect at pHva1$_{(1H)}$.

Spike length

Two QTL for spike length were detected on 1H and 2H. Qsl-tera_1H.a explained 10.6% of the genetic variance, and the ER/Apm allele increased spike length by on average 0.4 cm. Marker cdo497$_{(6H)}$ showed an M × L interaction effect at which the Tadmor allele increased spike length in all locations except for Terbol. The identification of Qsl-tera_1H.a was supported by sCIM analysis, which detected in addition a QTL × E interaction effect at marker cdo541$_{(4H)}$.

Seed dormancy

A single QTL was identified for seed dormancy at marker wg889$_{(5H)}$ and the allele from Tadmor increased seed dormancy at this locus.

Leaf chlorophyll content

SCIM detected two QTL for SPD on chromosomes 2H and 5H at which the ER/Apm allele increased the trait value.

Wilting

A QTL for wilting was located at the marker pHva1$_{(1H)}$ where the allele from ER/Apm increased the susceptibility to wilting.

Discussion

Differences in yield and relative WUE among locations and within locations, and the high contribution of G × L and G × Y(L) to the phenotypic variance confirmed the variability of rain fed environments. This explains the difficulties plant breeders have historically faced when breeding for these environments. The GGE biplot showed that the locations with the higher yield and WUE, like Tel Hadya, are characterised by more stable conditions across years as compared to Breda with large differences between years (Fig. 1). High variation in extreme environments makes standardization of field experiments for drought difficult. Experiments in non-controlled environments may be a better reflection of the true nature of the phenomenon drought, and may also explain why the results of experiments conducted under controlled conditions in the greenhouse are usually difficult to extrapolate to natural field conditions. On the other hand, natural field conditions, which are undoubtedly more relevant, are often less precise because the phenomenon itself appears in a range of different ways. The growing season rainfall was not a good predictor of yield, as available water may have been also determined by the seasonal rainfall distribution, soil characteristics, temperature and other abiotic factors. The low yield at the location with the highest rainfall (Terbol) can probably be explained by water logging due to high rainfall in January and February followed by a low rainfall period during grain filling. It has been shown that an excess of water in the soil may paradoxically cause water stress symptoms in plants (Lambers et al. 1998) due to a lowered conductivity to water uptake that typifies oxygen-deficient roots (Tournaire-Roux et al. 2003). These effects of water logging may explain why plants in Terbol with the highest amount of rainfall and plants in Breda with the lowest rainfall showed very similar behaviour and more symptoms of drought stress as compared to the other two locations (Fig. 1).

The mean yield of the parental genotypes shows that ER/Apm was better adapted to Kfardane and Tel Hadya, the locations with moderate drought stress, and Tadmor was better adapted to Breda and Terbol, characterised by more severe drought (Fig. 1; Table 2). These results confirmed the higher yield potential of ER/Apm under moderate stress and the better adaptation to more severe stress of Tadmor. The two parental genotypes were characterised by different adaptive developmental strategies, low seed dormancy, earlier flowering and a longer grain filling period in ER/Apm and high seed dormancy, later flowering and a shorter grain filling period in Tadmor. Three RILs showed transgressive segregation for yield in the locations with more severe stress Breda/Terbol, presumably because they combined the genes for high yield potential from ER/Apm and the genes for better adaptation to abiotic stress from Tadmor. The transgressive RILs were also characterised by high seed dormancy, intermediate grain filling period and plant height, large kernels and low wilting (data not shown). However, the test for

transgression was based on a small number of replicates per environment and G × E interaction is considerable between environments measured at Breda and Terbol. Therefore, putatively transgressive lines are currently studied in more extensive field trials with a large number of replicates per environment.

Correlations

To test if different ideotypes are the highest yielding in different locations, correlations were calculated separately for the higher (KF/TH) and lower (BR/TR) yielding locations. In Tel Hadya and Kfardane, peduncle length and peduncle extrusion were negatively correlated with yield and positively correlated with plant height. The negative relationship between yield and PED may be due to the positive correlations of PED and PEDEX with plant height. Plant height in turn was negatively related to yield because of its positive association with lodging susceptibility. It has been shown in wheat that under fully irrigated conditions peduncle extrusion and plant height are negatively correlated with yield; under late drought conditions, however, peduncle extrusion shows a positive correlation to yield (van Ginkel et al. 1998). Despite these findings that peduncle length is positively correlated with yield under water limited conditions there was no clear evidence for a significant correlation between peduncle length and yield in the macro-environment BR/TR. However, positive correlations between yield and PED, PEDEX were observed in the environments with the lowest yield (BR04 and TR05, data not shown). Again, the absence of clear correlations with yield may be explained by the larger genotype x year interactions in the low-yielding locations, where correlations in one year may not be repeatable in the next (Ceccarelli et al. 1991). Early growth vigour was negatively correlated with yield in the lower yielding locations and positively correlated with yield in the higher yielding locations. This switch in correlations was probably due to the negative correlations between GV and PH, while PH affected grain yield negatively in the higher yielding locations and positively in the lower yielding locations.

QTL analysis

Two different approaches for QTL mapping, a mixed model analysis and composite interval mapping were used. QTL results of both approaches showed a good agreement in terms of position and effect, 87% of the marker main effects coincided in both analyses. Out of the 39 M × Y(L) effects (Tables 4, 6) 30 (77%) were also detected as a QTL by environment interaction in the MQTL procedure. However, out of the nine M × L (Tables 4, 5) only three (33%) were also detected as a QTL by environment interaction effect in the sCIM mapping. Differences in the detected QTL and QTL by environment interactions between the two mapping procedures may be explained by the use of single marker regression versus interval mapping, by different definitions of error thresholds, resampling method and FDR, by the incorporation of cofactors in the MQTL procedure and by the different test statistics. However, the positions of the majority of marker main effects agreed well between the single marker regression and the composite interval mapping, presumably because background genetic effects were also controlled for in the ANOVA by incorporating G(M) in the model. The largest differences between the two analysis methods were found in the detection of M × L interaction effects. The ANOVA model was more suitable to detect marker alleles providing adaptation to specific locations and macroenvironments. The identification of markers and alleles adapted to specific locations or macroenvironments will be useful for marker assisted selection in breeding programs for yield improvement in stress locations. The separation of interaction effects into M × L and M × Y(L) in this study bridges the molecular analysis with the phenotypic analysis of multi-environment trials where, by dissecting genotype by environment (GE) into genotype by location (GL) and genotype by year within location (GY(L)) interactions, the breeder is able to identify repeatable GL interaction and hence subdivide the target population of environments in sub sets within which selection is expected to be more efficient because of the lower GE.

The QTL analysis revealed significant marker main effects (ANOVA) or QTL effects (MQTL) for all analysed traits with the explained genetic variance ranging from 6.5 to 24.5% in the ANOVA (Tables 4, 7; Fig. 2). The majority of main effects were detected on chromosome 3H, and affected primarily PH, PED, PEDEX, LDG and GY. The Tadmor allele increased PH, PED and PEDEX and decreased GY at these loci. On chromosome 6H, two loci in bin 5 and 6 influenced GY, KW, PH and LDG, with the locus in bin 6 explaining 17.6, 13.6, 17.5 and 20.8% of the genetic variance for yield, kernel weight, plant height and lodging, respectively. The coincidence of QTL for plant height and yield indicated that average yield was mainly determined by plant height, where Tadmor's taller plants, being susceptible to lodging, yielded less. However, M × Y(L) interaction effects for yield coinciding with the three QTL for height on 3H showed that an increase in plant height caused by the Tadmor allele also improved yield in some environments (Table 6). The locus at cdo1396A (3H, bin 11), for example, influenced PED and PH and mapped close to the dwarfing gene *sdw1*, suggesting that differences in PED may be linked to plant height and influenced by the dwarfing gene. Baum et al.

(2003) detected a strong QTL for plant height and yield at the *sdw1* locus in a RIL population derived from the adapted Syrian variety Arta and a wild barley accession. The population was tested in Tel Hadya and Breda where the taller phenotype of the wild barley affected yield negatively. In this study no marker main effect for GY mapped to the *sdw1* locus, but an M × Y(L) interaction effect did with favourable effects of the Tadmor allele, in particular in BR04, BR05 and in the three years at Terbol. This interaction effect suggests that increased plant height had a favourable effect on yield in these environments and a negative effect on yield in all years in Tel Hadya, in KF02, KF03, BR02 and BR03. In addition, at five and three markers with M × L interaction effects the Tadmor allele increased yield in Breda and Terbol, respectively and decreased yield in Kfardane and Tel Hadya. These findings suggest that some of the Tadmor alleles, which confer tolerance to drought and thus improve yield under more severe stress, result in a yield reduction under moderate stress as in Kfardane and Tel Hadya. It is interesting to note, that four of the markers with M × L interaction effects are located close to or in genes which are known to confer drought tolerance (Table 5). The major M × L interaction effects were detected at pHva1 (1H, bin 12) and Tapk4 (5H, bin 14). Tapk4, for example, encodes a protein kinase, which has been shown to be involved in stress signalling (Guo et al. 2002) and the regulation of stomatal aperture by abscicic acid (Mustilli et al. 2002). Differences in the Tapk4 gene or its expression pattern may have affected drought tolerance and ultimately the yield of the RIL progeny.

Six marker main effects for DH were detected, but none of them coincided with a QTL for yield. This indicates that loci for flowering time have not influenced yield, which is also reflected in the low correlation between flowering time and yield. However, days to maturity and grain filling period showed positive correlations with yield in KF/TH ($r = 0.34$ and 0.19, respectively). The QTL Qdm-tera_6H.a coincided with the major QTL for yield where the allele of ER/Apm increased days to maturity and improved yield.

The major QTL for flowering time Qdh-tera_1H.b explaining 15.8% of the genetic variance maps close to the location of the *Ppd-H2* gene on chromosome 1H. *Ppd-H2* promotes flowering time under short day conditions and has been shown to affect flowering time in Mediterranean environments when trials were sown in autumn under short day conditions (Igartua et al. 1999). Indeed, when flowering time was analysed in every location separately (data not shown), the effect of the QTL Qdh-tera_1H.b was stronger at the two locations Kfardane and Terbol with earlier sowing dates suggesting a possible response to short day conditions (*Ppd-H2*) at this locus. The QTL Qdh-tera_2H.a matches the position of the flowering gene *Eam6* that confers early flowering under both long and short day conditions (Horsley et al. 2006). The genomic region harbouring *Eam6* has also been detected as a major locus for flowering time in the population Beka x Logan tested in Northern Spain (Moraleja et al. 2004) and in populations involving Australian germplasm, where the locus was associated with variation in the duration of the basic vegetative period (Boyd et al. 2003). Qdh-tera_1H.a and Qdh-tera_2H.a mapped close to the two main effects for spike length. The ER/Apm allele increased spike length at both loci, but postponed flowering at the locus on 1H and caused earlier flowering at the locus on 2H, which may explain the low correlation between SL and DH.

The major QTL for days to maturity Qdm-tera_1H.a mapped close to the major QTL for grain filling period and early growth vigour, where the ER/Apm allele increased all three trait values. The same locus affected also wilting, and lines with the ER/Apm allele were more prone to wilting. It has already been shown that the genomic region on 1HL, originally associated with the vernalisation gene *Vrn-H3*, has an effect on seedling growth habit (Boyd et al. 2003). The authors suggested that genes for plant growth have also a pleiotropic effect on flowering time, a finding that is supported by the coincidence of QTL for early growth vigour and flowering time in this study. Interestingly, this locus was also detected as the major interaction effect for GY, LDG, PED, PEDEX, KW and PH with crossover interactions for all six traits (Tables 5, 6; Fig. 2). These interaction effects were possibly affected by the co-located QTL for days to maturity and grain filling period, which showed a positive correlation with yield in KF/TH and no correlation with yield in BR/TR. In addition, the QTL for flowering time Qdh-tera.1H.a with *Ppd-H2* as candidate gene maps close to this genomic region, and therefore may have been the causal gene behind the interaction effects. Different periods of exposure to short day conditions prior to vernalization in the different environments, may have caused differences in development and thus in PH and yield, as seen by the switch of rank for yield in the two different macro-environments BR/TR and KF/TH. The significant marker for this interaction effect, pHva1, with a favourable effect of the Tadmor allele on yield at the drier locations BR/TR, is located in the gene *HVA1* (1H, bin 12), a late embryogenesis abundant (*LEA*) protein gene. It has been shown that transformation of wheat, oat and rice with the barley *HVA1* gene improved drought and salt resistance (Bahieldin et al. 2005; Oraby et al. 2005; Xu et al. 1996). Bahieldin et al. (2005) demonstrated that improved expression of *HVA1* increased yield under drought, but not under normal conditions. In addition, Teulat et al. (1998, 2002) and detected QTL for osmotic potential and osmotic potential at full turgor close to the marker pHva1.

Three traits, leaf chlorophyll content (SPD), wilting (WILT) and seed dormancy (DOR) were only measured in

one environment and were therefore only analysed in the sCIM procedure. SPD showed positive correlations with grain yield and negative correlations with heading date. The QTL at bcd1069$_{(2H)}$ coincided with a QTL for flowering time, where the earlier genotype ER/Apm increased SPD. Teulat et al. (2002) identified QTL for carbon isotope discrimination, osmotic adjustment and thousand kernel weight in the same genomic region. In addition, QTL for leaf osmotic potential, relative water content, water soluble carbohydrates and chlorophyll content were located close to the two QTL for SPD on 1H and 5H by Teulat et al. (2002) and This et al. (2000). These findings suggest a common genetic control and a physiological link between plant development, water status and photosynthesis. The influence of different water regimes on plant photosynthesis cannot be analysed in this study, as SPD was only measured in TH02, an environment with moderate drought stress.

Seed dormancy was significantly and positively correlated with PH and KW, and the only seed dormancy QTL matched the position of a QTL for KW, where the Tadmor allele increased both seed dormancy and KW. The QTL Qdor-tera_5H.a coincides with a major seed dormancy QTL detected in the Steptoe/Morex doubled haploid population (Ullrich et al. 1993) and mapped as the SD1 locus to the centromeric region of the long arm of chromosome arm 5H (Han et al. 1999). Hori et al. (2007) confirmed the effect of this locus on dormancy in seven recombinant inbred lines and one doubled haploid population derived from crosses including 11 cultivated and one wild barley strains with a wide range of seed dormancy levels. Zhang et al. (2005) analysed germination in a cross between wild and cultivated barley and found a close correlation between seed dormancy and seedling desiccation tolerance. In addition, these authors found a coincidence of QTL for seed dormancy, and abiotic stress tolerance and suggested a common physiological basis of seed dormancy, seedling desiccation tolerance, salt, drought and cold tolerance. Similarly, the Tadmor allele with high seed dormancy may confer better adaptation to drought. Indeed, it has already been shown that plants adapted to more extreme environmental conditions produce many more dormant seeds, than plants adapted to less extreme environments (Baskin and Baskin 1998). However, further studies on seed dormancy in different environments and under different water regimes need to be conducted to establish a relationship of seed dormancy and drought tolerance in this population.

Interaction effects and stability of QTL

Teulat et al. (2001b) conducted a QTL analysis with the same population used in this study and tested agronomic performance in irrigated and rain fed environments in France and Spain. QTL detected in Teulat et al. (2001b) were compared to the main effects found in the present study for the traits in common, i.e. flowering time, yield, kernel weight and plant height. For DH, KW and PH the majority of main effects were also identified in Teulat et al. (2001b) with the same direction of effects as shown in Table 4. However, for grain yield only one QTL Qgy-tera_7H.a coincided with a yield QTL in the study by Teulat et al. (2001b). These findings suggest that genetic effects for DH, PH and KW are relatively stable across different environments, whereas these genetic effects may influence yield in different ways in different locations and years as found by Ceccarelli et al. (1991). This assumption is also supported by the analysis of interaction effects in the present study. The largest number of M × L and M × Y(L) interactions effects were found for yield. Teulat et al. (2001b) also analysed QTL × environment interaction, but concentrated on G × E effects in only one location with two contrasting treatments, namely irrigated and rain fed. The authors detected only a single QTL × E interaction effect for yield which did not coincide with any interaction effect in this study. This may be taken as a further indication that it is not easy to simulate the real drought in field conditions by using controlled conditions in the greenhouse.

Baum et al. (2003) conducted the QTL analysis for every environment separately and did not test for interaction effects. However, considerable differences in the detection of QTL in the different environments suggested the relevance of marker environment interactions for the expression of agronomic traits in the tested environments.

For breeding purposes the detection of QTL with adaptation to specific locations is of particular interest. Despite the strong effect of G × Y(L) interactions on yield, the biplot based on yield showed a clustering of the different years within locations, indicating that breeding for specific adaptation to locations may be possible even for locations affected by strong year-to-year fluctuations and abiotic stress. In this context, it is important to characterize genotype/QTL by environment interactions and to identify genotypes/QTL that are "stable" across years within locations or across macro-environments with similar climatic conditions. At the same time, a thorough assessment of environmental factors which affect the genotype/QTL stability will allow in future to make predictions of genetic effects in new environments with comparable climatological patterns. Molecular breeding, using information on relevant QTLs combined with phenotypic selection in target environments, will allow a more targeted and faster breeding of varieties with improved adaptation. Different correlation coefficients between the two different macro-environments in this study and the occurrence of crossover interactions indicated that different trait expressions and

different QTL alleles improved yield in the different locations. For example, the Tadmor alleles, conferring a taller phenotype and a shorter grain filling period, improved yield in Breda and Terbol with more severe stress and decreased yield in Kfardane and Tel Hadya, characterised by moderate drought stress. The present study demonstrates thus the importance of breeding for adaptation to specific locations, as beneficial alleles in one location may be associated with a yield penalty in others. The coincidence of main effects for plant height and developmental traits with crossover interaction effects for yield, indicate that differences in plant stature and development influenced yield.

Conclusions

The analysis of 16 different environments with moderate to severe stress revealed a number of putative QTL for 14 agronomic traits in the RIL population Tadmor × ER/Apm. At all marker main effects the allele from ER/Apm improved yield according to the higher yield potential of ER/Apm. The analysis of M × L and M × Y(L) interaction effects allowed the detection of loci for specific adaptation to a location or macro-environment. The interaction effects demonstrated that the Tadmor allele did improve yield primarily in the locations with more severe drought stress and the lowest grain yield, Breda and Terbol. This may indicate that in the presence of severe stress, genes for drought tolerance, with the beneficial allele from Tadmor, influenced yield. These findings are in line with the initial hypothesis that ER/Apm carries alleles for high yield potential, whereas Tadmor carries alleles for better adaptation to severe stress.

Effects of the detected QTLs and the discussed candidate genes will be verified by analysing a wider range of drought adapted germplasm and mapping populations available at ICARDA. In addition, differences in expression and genetic variation in these genes will be tested to identify functional variants and link expression levels with drought tolerance.

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