Theor Appl Genet (2003) 107:1215–1225
DOI 10.1007/s00122-003-1357-2

M. Baum · S. Grando · G. Backes · A. Jahoor · A. Sabbagh · S. Ceccarelli

QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' × *H. spontaneum* 41-1

Received: 23 March 2003 / Accepted: 23 May 2003 / Published online: 26 July 2003

Abstract A genetic linkage map has been developed for recombinant inbred lines (RILs) of the cross 'Arta' × *Hordeum spontaneum* 41-1. One hundred and ninety four RILs, randomly chosen from a population of 494 RILs, were mapped with 189 markers including one morphological trait (*btr* = brittle rachis locus). The linkage map extended to 890 cM. Agronomic traits such as grain yield, biological yield, days to heading, plant height, cold tolerance and others were evaluated at the ICARDA research stations Tel Hadya and Breda during the years 1996–97 and 1997–98. QTLs for agronomic traits related to drought resistance were localized. For the most-important character 'plant height under drought stress', QTLs on 2H, 3H and 7H were detected. The 'plant height' QTLs, specially the one on 3H, showed pleiotropic effects on traits such as days to heading, grain yield and biological yield. QTLs were also identified for other traits associated with adaptation to the Mediterranean environment such as cold tolerance, days to heading and tiller number. The identification of QTLs for agronomic traits is a first step to analyze and to dissect complex characters such as adaptation to drought tolerance.

Keywords QTL · Drought stress · Mediterranean environment · *Hordeum spontaneum* · Barley

Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop in the Near East, Asia, Central Africa, Latin and North America, and Europe. It covers more than 40 million hectares in developing countries where often it is the only possible rainfed crop that farmers can grow (Ceccarelli 1994). Its importance derives from the ability to grow and produce in marginal environments that are often characterized by drought and low-temperature conditions. These stresses reduce yield in areas where barley provides most of the daily sustenance. Therefore, the development of lines with increased drought and cold tolerance becomes increasingly important (Ceccarelli and Grando 1996).

The wild progenitor of cultivated barley, *H. vulgare* ssp. *spontaneum* (hereafter referred to as *Hordeum spontaneum*) can contribute useful genes for several characters. Resistance to powdery mildew (Moseman et al. 1983; Jahoor and Fischbeck 1987; Gustafsson and Claesson 1988; Nevo 1992; Lehman et al. 1998), leaf rust (Moseman et al. 1990; Nevo 1992), and other diseases (Nevo 1992) have been identified in *H. spontaneum*, and its use in breeding for disease resistance has been reported by several authors (Backes and Jahoor 2001). The species showed variation for important agronomic traits such as seminal root morphology (Grando and Ceccarelli 1995), floral structure (Giles and Bengtsson 1988), salt tolerance (Nevo et al. 1993), grain size (Giles 1990), milling energy (Ellis et al. 1993), grain protein content (Jaradat 1991), earliness, biomass, grain yield, plant height under drought and drought tolerance (Nevo 1992; Grando et al. 2001; Ellis 2002). This vast potential store of genetic resources is as yet largely unexploited. *H. spontaneum* is thus expected to have a potential in contributing useful genes in barley breeding as a donor of adaptive traits to extreme stress conditions, as suggested by its distribution in the driest areas of the West Asia region.

One of the most useful traits of *H. spontaneum* in relation to stress tolerance is its plant height under drought. This is important because one of the most-evident effects of drought is a reduction in plant height, making combine harvesting difficult or impossible (Grando et al. 2001). Therefore, the introgression of this trait into cultivated barley will contribute to stabilize the income of rural poor farmers. In addition, not only grain

Comunicated by R. Hagemann

M. Baum (✉) · S. Grando · A. Sabbagh · S. Ceccarelli
Germplasm Program, ICARDA, P.O. Box 5466, Aleppo, Syria,
e-mail: M.BAUM@cgiar.org
Tel.: +963-21-2243433
Fax: +963-21-2243490

G. Backes · A. Jahoor
Plant Research Department, Risø National Laboratory,
P.O. Box 49, 4000 Roskilde, Denmark,

but also straw has a value to farmers in West Asia and North Africa (WANA). Gene introgression from *H. spontaneum* into cultivated barley is a long and difficult process despite the fact that crosses between the two are easy to make and are fully fertile. This is because *H. spontaneum* has a number of undesirable traits such as brittle rachis, low kernel weight, asynchronous tillering and rough awns. An additional difficulty is that the improvement of plant height under drought often causes a reduction in tillering, and hence in both grain and straw yield.

It is evident that in order to fully exploit the potential of crosses between cultivated barley and *H. spontaneum*, a large number of recombinant lines derived from each cross has to be evaluated. The use of DNA molecular-marker techniques, which are now sufficiently well developed to be exploited in breeding programs, can considerably reduce the complexity of combining a number of desirable traits in the same line. These techniques make it feasible to develop linkage maps for barley (Graner et al. 1991; Qi et al. 1996; Ramsey et al. 2000). Together with statistical techniques, these linkage maps are used to locate and estimate phenotypic effects of quantitative trait loci (QTLs) (Backes et al. 1995; Tinker et al. 1996; Bezant et al.1997; Teulat et al. 1998; Yin et al. 1999; Zhu et al. 1999; Foster et al. 2000; Hayes et al. 2000; Teulat et al. 2001a, b). QTL analysis provides a powerful tool to locate genes on chromosomes. Therefore, the identification of molecular markers closely linked to QTLs of agronomic interest, or to negative traits, will allow the use of marker-assisted selection and thus increase the efficiency in the exploitation of *H. spontaneum*.

The objective of this study was to identify trait-marker linkages in a population of recombinant inbred lines (RILs) of a cross between 'Arta' and *H. spontaneum* 41-1 using the QTL approach.

Materials and methods

Plant material and growth conditions

A population of 494 F_7 RILs derived by single-seed descent from the cross 'Arta'/*H. spontaneum* 41-1 was developed at ICARDA. 'Arta', a high yielding pure line selected from the Syrian white-seeded landrace 'Arabi Abiad', is well-adapted to Syrian conditions, and combines high number of tillers and high kernel weight, but becomes very short under dry conditions. *H. spontaneum* 41-1, a pure line selected for its adaptation to severe drought stress conditions, combines earliness with acceptable cold tolerance, and with the ability to maintain plant height under severe drought. At least six characters are expected to segregate from this cross: rachis brittleness, awn roughness, peduncle extrusion, plant height, tillering and kernel size. The main objective of this cross was to develop lines combining the grain yield and tillering ability of 'Arta' with plant height and the adaptation to severe drought stress conditions of *H. spontaneum* 41-1 (Grando et al. 2001).

In the cropping seasons 1996–97 and 1997–98, the 494 lines and the parental lines were planted at ICARDA's research stations located near Tel Hadya (36°01′N; 37°20′E, elevation 300 m asl.) and near Breda (35°56′N; 37°10′E, elevation 354 m asl.) in Syria. The experimental design was a 20 × 25 α-lattice, with two replicates. Plots were 1.8-m wide (8 rows 20-cm apart) and 6-m long in 1996–97, and 1.6-m wide (6 rows at 20-cm apart) and 6-m long in 1997–98. In both years and locations the trials were grown under rainfed conditions. The mean maximum and minimum monthly temperature and monthly cumulative rainfall are reported in Table 1. Total rainfall in Tel Hadya 1996–97 (referred as 'Th97') and Tel Hadya 1997–98 ('Th98') was 433.7 and 410.5 mm respectively, whereas in Breda 1996–97 ('Br97') and Breda 1997–98 ('Br98') it was 230.8 and 227.4 mm respectively. The mean maximum temperature was 24.4 °C in Th97, 25.5 °C in Th98, 23.1 °C in Br97 and 24.5 °C in Br98. The mean minimum temperature was 9.5 °C and 10.7 °C in Th97 and Th98 respectively, and 10.4 °C and 10.7 °C in Br97 and Br98.

Agronomic traits

The following agronomic traits, measured as described by Ceccarelli et al. (1991) with minor modifications, were recorded. Early growth vigour (GrV) as a visual score at the 5–6 leaf stage, using a scale from 1 = good vigor to 5 = poor vigor; growth habit (GrH) as a visual score at the 5–6 leaf stage, using a scale from 1 = erect to 5 = prostrate; cold damage (CD) as a visual score, using a scale from 1 = absence of damage (all leaves completely green) to 5 = leaf blades and sheaths yellow; days to heading (DH) as the number of

Table 1 Maximum and minimum monthly temperature and rainfall in 1996–97 and 1997–98 in Tel Hadya and Breda research stations

Item	Month	S	O	N	D	J	F	M	A	M	J	J	A	Total/avrg.
Breda														
Max. Temp.	1996–97	31.1	24.3	18.0	13.1	9.0	10.1	14.0	18.6	32.0	35.6	37.2	34.1	23.1
	1997–98	31.3	25.7	18.5	12.7	9.3	12.9	15.8	24.0	28.7	35.8	39.0	40.5	24.5
Min. Temp.	1996–97	15.8	10.0	7.1	6.1	0.7	–1.7	1.9	5.1	11.9	17.6	20.6	19.0	9.5
	1997–98	14.2	12.8	5.7	4.0	2.0	1.8	4.2	8.8	13.2	17.0	22.0	23.2	10.7
Rainfall	1996–97	5.4	29.8	8.8	57.8	25.6	11.2	35.6	54.4	2.2	0.0	0.0	0.0	230.8
	1997–98	17.8	18.0	10.8	29.4	47.2	17.0	38.6	39.2	9.4	0.0	0.0	0.0	227.4
Tel Hadya														
Max. Temp.	1996–97	33.1	26.1	20.1	15.2	12.0	12.9	15.3	20.0	31.9	34.8	36.8	34.5	24.4
	1997–98	33.1	27.3	20.2	13.6	11.0	14.4	17.0	24.6	28.9	36.0	39.1	40.6	25.5
Min. Temp.	1996–97	17.1	11.0	7.1	7.3	2.6	–1.0	2.2	6.3	11.6	17.7	21.4	21.4	10.4
	1997–98	14.8	13.4	7.1	4.4	2.2	0.9	3.9	7.8	11.9	17.5	21.8	21.4	10.6
Rainfall	1996–97	22.9	35.3	17.4	92.5	46.8	24.8	76.3	111.7	6.0	0.0	0.0	0.0	433.7
	1997–98	18.1	36.3	37.9	62.3	83.6	37.6	59.3	63.7	11.7	0.0	0.0	0.0	410.5

days from emergence to awns appearance in 50% of the plants in a plot; total chlorophyll content (Col) measured in intact leaves in the field using a portable chlorophyll meter (SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan) between 09:00 and 12:00 h (solar time); plant height (PH) measured in cm from ground level to the base of the spike, at maturity; number of tillers per m^2 (TN), calculated from the number of tillers on two rows of 1-m each 20 cm apart; biological yield (BY) in kg/ha, measured by hand harvesting the six central rows in 1996–97 and the four central rows in 1997–98 of each plot for the entire plot length; grain yield (GY) in kg/ha, measured after threshing the harvested sample; 1,000-kernel weight (KW) in g, measured as the average of three samples of 100 kernels per plot; kernel protein content (PrC) evaluated by near-infrared reflectance spectroscopy (NIRS) calibrated against the Kjeldahl method; and β-glucan content (GlC) evaluated by NIRS. All traits were measured or scored in all 194 lines; for GY, BY and KW, we report the value of the entire population, and the value of the sub-population of the 116 lines with a non-brittle rachis only (GYnb, BYnb, KWnb).

DNA extraction

One hundred and ninety four RILs were used to construct a genetic linkage map. Total genomic DNA was extracted following the procedure described by Saghai-Maroof et al. (1984), with minor modifications. Fresh above-ground parts from seedlings (4 to 6 weeks old) of the parents and a bulk of 10–20 individuals from each of the RILs were collected for DNA isolation. The DNA was RNase treated and quantified using a spectrophotometer (Beckman DU-65). The quality of the extracted DNA was visually checked on a 1% agarose gel.

Analysis of SSRs and AFLPs

Genetic mapping was carried out using amplified fragment length polymorphic (AFLP) markers and microsatellite-based markers. Simple sequence repeat (SSR) markers were amplified on a PE-9600 or 9700 system using the published protocols for the respective markers (Ramsay et al. 2000). Products were then electrophoretically separated on 6% polyacrylamide gels using standard sequencing gels with silver staining (Bassam et al. 1991) at ICARDA, or with fluorescence dyes on an ABI377 sequencer at Risø National Laboratory, Denmark. The protocol for the AFLP assay was carried out as described by Zabeau and Vos (1993) with minor modifications using combinations of *Pst*I and *Mse*I, or *Eco*RI and *Mse*I restriction enzymes and respective adapters. Pre-amplification was carried out using one base-pair extension primers. Selective amplification of restriction fragments was conducted using primers with two or three selective nucleotides. PCR-amplifications were separated on 6% denaturing polyacrylamide gels and stained with silver nitrate stain at ICARDA, while fluorescent dyes were used on an ABI377 sequencer at Risø.

Linkage mapping

Segregation analysis was performed according to Lander et al. (1987) with the MAPMAKER computer software program, and the Join Map (v.2.0, Stam and van Ooijen 1995) software package was employed for map construction. Recombination fractions were converted to centiMorgans (cM) according to the Kosambi mapping function (Kosambi 1944).

QTL analysis

The QTL analysis was performed using PLABQTL v. 1.1 (Utz and Melchinger 1996). This program uses an interval mapping approach by multiple regression with flanking markers according to the procedures described by Haley and Knott (1992). Markers to be included as cofactors in the regression, to increase the power of the detection and to reduce the bias in the estimated QTL positions and effects (Utz and Melchinger 1994), were selected through stepwise regression by the program. The LOD thresholds for the respective traits are empirical thresholds obtained by 500 permutations also performed by the program (Churchill and Doerge 1994). The monogenic and digenic QTL effects to be included in the model were calculated by stepwise multiple regression by PLABQTL. The variance explained by the whole model is indicated as the adjusted estimator according to Hospital et al. (1997). The significance of QTL × environment interactions results from a final ANOVA step in the program. Traits with classification instead of measurement, and/or strongly skewed distribution, were analyzed by the non-parametric Kruskal-Wallis test provided by the MapQTL software (v. 3.0, Van Ooijen and Maliepaard 1996). For the case of an interval analysis, a QTL position was accepted, if either the LOD surpassed the threshold determined by a 500-fold permutation or the LOD was higher than 2.5, and another QTL – either for another trait or for the same trait in another environment – was located at the same chromosomal position. For the case of a non-parametric analysis, a QTL position was accepted, if either the error probability was lower than 0.0001 or the error probability was lower than 0.001, and another QTL was located at the same chromosomal position. To determine the positional coincidence of QTLs, the LOD curves generated from scans with 1-cM step width were overlaid in the STATISTICA software package (StatSoft Incorporated, Tulsa, USA) and evaluated visually.

Results

Linkage map

The linkage map based on the 'Arta'/*H. spontaneum* 41-1 (A×H) population originally contained 189 marker loci including one morphological marker locus (*btr* = brittle rachis), 158 AFLP loci and 30 SSR loci, the latter mostly with a known location (Ramsey et al. 2000) that served as anchor markers. For the purpose of the QTL analysis, a reduced map was constructed, containing 129 marker loci (one morphological marker, 106 AFLPs and 22 SSRs). The map is shown in Fig. 1. It spans over 890 cM and has an average interval length of 7.3. Significant distortion-segregation was observed with 10 of the 129 loci.

Trait variations and correlations

The RIL population showed a large variation for the traits examined between the four environments (Table 2), particularly between the two locations. The population mean for grain yield (non-brittle) was 1,014 kg/ha in Br97 and 1,344 kg/ha in Br98, while 3,581 kg/ha were harvested in Th97 and 3,148 kg/ha in Th98. Similar relations were observed for the more favorable location (Tel Hadya) compared to the more drought-stressed location (Breda) for biological yield, kernel weight, tiller number and for plant height. The differences between the entire population and the non-brittle sub-population were generally higher in Tel Hadya than in Breda.

Table 3 shows the correlations between the traits in the different environments. As expected, significant positive correlations were observed between BY and GY in all environments. Significant positive correlations between

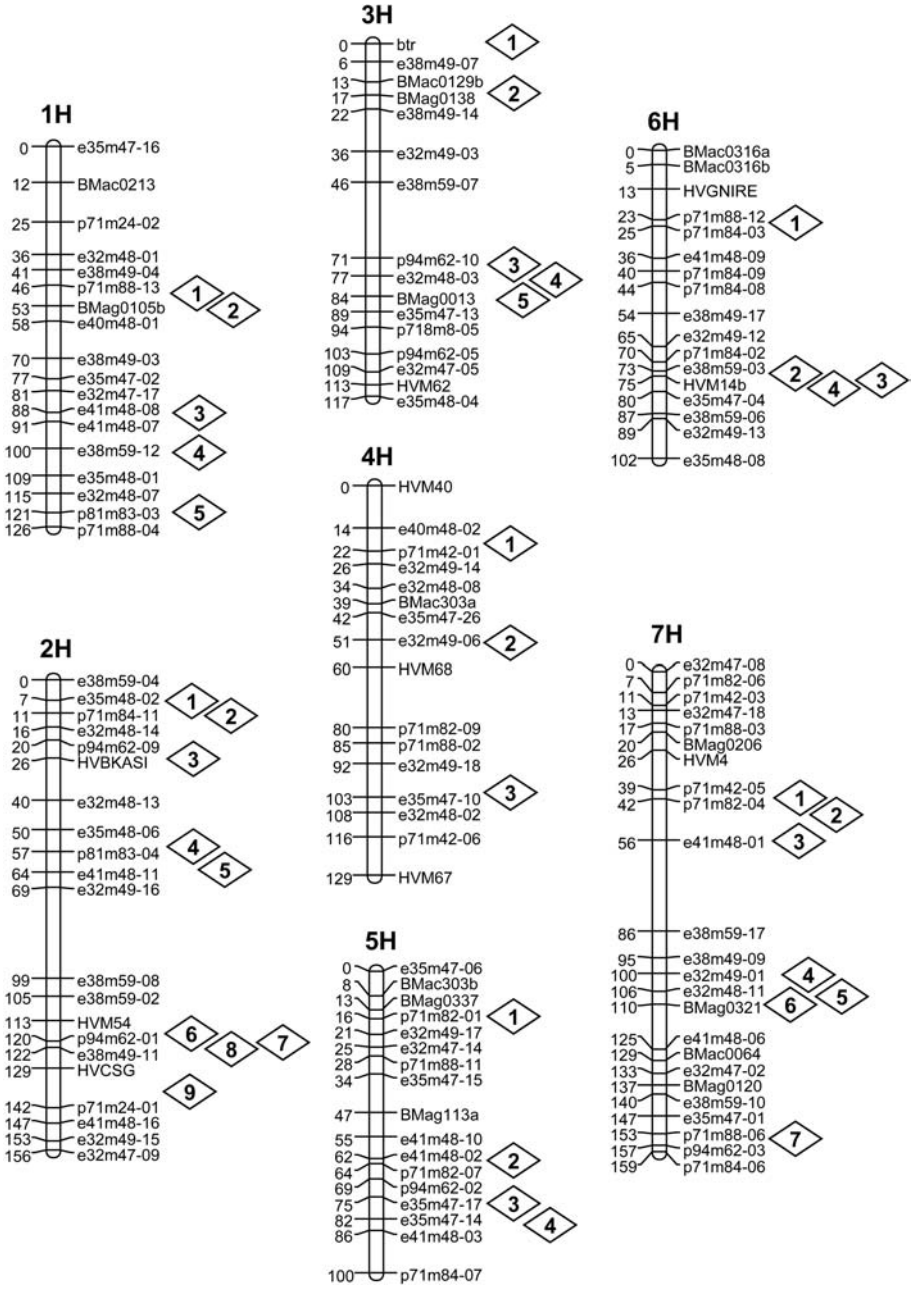

Fig. 1 Linkage map of the cross 'Arta' × *H. spontaneum* 41-1 with positions of QTLs (as indicated in Table 4) on the right side of the chromosomes

KW and GY were observed in Th97, Th98 and Br98, but not in Br97. Significant positive correlations between TN and GY were observed in Br98, but not in Th97 and Br97.

PH showed significant negative correlations with GY in Th97, but significant positive correlations with GY and BY in Br97. Significant positive correlations were observed between DH and KW and GY, and negative correlations with TN in Br97, while none of these traits showed significant correlations in Th97. For GrV no significant correlation was detected in Th97, while in Th98 and in Br97 there was a strong negative correlation between this trait and BY, as well as GY. Additionally, there was a significant positive correlation between GrV and CD in Th98, but not in Th97. There was no cold damage in Breda.

QTL analysis

For the traits CD (Th97, Th98), GrH (Th97, Th98) and GrV (Br98, Th97, Th98) a non-parametric QTL analysis was carried out, as those traits were characterized by an extremely skewed distribution or were determined by categories instead of measurements or both (Table 2). Table 4 and Fig. 1 show the QTLs detected in the 'Arta'/*H. spontaneum* 41-1 population. In Table 4, the QTLs are ordered by chromosomal position. The numbers at the

Table 2 Units and means of the traits analyzed and the kind of QTL analysis performed

Trait	Unit	Br97	Br98	Th97	Th98	QTL analysis
BY	kg/ha	5,625	3,745	9,310	7,047	MQM*
BYnb	kg/ha	5,804	3,828	10,695	7,803	MQM
CD	1–5	–	–	2.57	2.33	Non-param.
Col	SPAD	–	–	33.8	–	MQM
DH	Days	123.3	–	122.8	–	MQM
GlC	%	4.38	–	–	–	MQM
GrH	1–5	–	–	3.03	3.08	Non-param.
GrV	1–5	–	3.03	2.86	2.85	Non-param.
GY	kg/ha	936	1,251	2,608	2,536	MQM
GYnb	kg/ha	1,014	1,344	3,581	3,148	MQM
KW	g	27.09	25.44	28.50	30.37	MQM
KWnb	g	27.49	25.39	29.00	30.19	MQM
PH	cm	49.1	43.9	100.0	–	MQM
PrC	%	12.58	–	11.38	–	MQM
TN	n/m^2	525	348	766	–	MQM

MQM = marker-QTL-marker interval analysis with co-factors

Table 3 Correlations between the traits analyzed (correlations with significances <0.0001 in **bold**)

Trait	PH	GrH	GrV	TN	KW	KWnb	BY	BYnb	GY	GYnb	CD	Col	PrC
a) Tel Hadya (upper right part: 1997; lower left part: 1998)													
DH	–0.17	+0.03	+0.07	–0.06	–0.19	–0.16	+0.02	+0.08	+0.04	+0.19	+0.12	–0.12	–0.10
PH		–0.13	+0.01	–0.06	**–0.24**	**–0.28**	–0.13	–0.18	–0.19	**–0.25**	+0.13	–0.01	**+0.28**
GrH	–		+0.08	+0.03	+0.03	+0.10	+0.03	+0.11	+0.05	+0.14	–0.08	+0.04	–0.07
GrV	–	**+0.29**		**–0.04**	–0.14	–0.09	–0.05	–0.09	–0.02	–0.04	+0.09	+0.14	+0.z03
TN	–	–	–		–0.13	–0.20	+0.04	–0.05	+0.01	–0.15	–0.01	–0.11	+0.08
KW	–	–0.17	–0.09	–		**+1.00**	**+0.24**	**+0.30**	**+0.29**	**+0.33**	–0.17	+0.14	**–0.41**
KWnb	–	–0.11	–0.06	–	**+1.00**		+0.22	**+0.30**	**+0.39**	**+0.36**	–0.21	+0.10	**–0.50**
BY	–	–0.13	**–0.27**	–	+0.12	**+0.24**		**+1.00**	**+0.94**	**+0.77**	–0.19	–0.11	–0.01
BYnb	–	–0.11	**–0.28**	–	+0.22	+0.22	**+1.00**		**+0.71**	**+0.71**	**–0.32**	+0.09	–0.10
GY	–	–0.09	–0.20	–	+0.13	**+0.36**	**+0.91**	**+0.85**		**+1.00**	–0.14	–0.144	–0.15
GYnb	–	–0.08	**–0.24**	–	**+0.34**	**+0.34**	**+0.85**	**+0.85**	**+1.00**		–0.14	+0.10	**–0.29**
CD	–	**+0.35**	**+0.30**	–	+0.05	+0.10	–0.14	–0.12	–0.11	–0.13		–0.12	+0.14
Col	–	–	–	–	–	–	–	–	–	–	–		–0.02

Trait	PH	GrV	TN	KW	KWnb	BY	BYnb	GY	GYnb
b) Breda (upper right part: 1997; lower left part: 1998)									
DH	–0.23	–	**–0.29**	**+0.30**	**+0.31**	+0.10	+0.04	**+0.32**	**+0.33**
PH		–	**+0.23**	–0.03	–0.08	**+0.42**	**+0.54**	+0.17	**+0.24**
GrV	**–0.27**		–	–	–	–	–	–	–
TN	+0.09	**–0.33**		–0.15	–0.19	**+0.32**	**+0.24**	+0.14	+0.10
KW	–0.12	–0.05	–0.04		**+1.00**	+0.14	+0.10	+0.16	+0.20
KWnb	**–0.23**	–0.06	–0.05	**+1.00**		+0.11	+0.10	+0.20	+0.20
BY	+0.11	**–0.49**	**+0.29**	+0.18	**+0.24**		**+1.00**	**+0.71**	**+0.66**
BYnb	+0.09	**–0.53**	**+0.28**	**+0.24**	**+0.24**	**+1.00**		**+0.65**	**+0.65**
GY	–0.09	**–0.34**	**+0.32**	**+0.26**	**+0.38**	**+0.76**	**+0.75**		**+1.00**
GYnb	–0.06	**–0.43**	**+0.25**	**+0.38**	**+0.38**	**+0.75**	**+0.75**	**+1.00**	

right side of the chromosomes in Fig. 1 correspond to the running number of the chromosomal position in Table 4 (e.g. 1H-4 corresponds to the diamond symbol with the number '4' at the right side of chromosome 1H).

Ten QTLs were detected in total for BY and BYnb, and nine of them were detected in only one of the four environments (on 1H-1, 1H-3, 1H-5, 2H-1, 3H-2, 3H-4, 5H-4, 7H-4 and 7H-7). The QTL (3H-1) at the position of the *btr* locus was valid in Br97, Th97 and Th98. With the exception of the QTL on 1H-1, 'Arta' was always contributing the allele with the higher biological yield. The two QTLs on 3H (3H-1 and 3H-4) showed the highest effect. Six QTLs were detected for grain yield (GY and GYnb); three of them were environment-specific (4H3, 5H-4 and 7H-1) and three were common for more than one environment (1H-5, 3H-1 and 3H-4). For all detected QTLs, the higher grain yield was linked with the allele from 'Arta'. Twenty one QTLs were identified for kernel weight (KW and KWnb), 15 were linked to single environments and six to more than one environment (2H-5, 4H-2, 4H-3, 5H-4, 6H-1 and 6H-2). Eight out of the 15 specific QTLs were detected in Th97 (1H-2, 2H-1, 2H-9, 3H-2, 3H-5, 7H-1, 7H-2 and 7H-6). The allele with the heavier kernels for seven of the QTLs for KW (3H-1, 5H-3, 6H-2, 6H-3, 7H-1, 7H-2 and 7H-6) originated from the

Table 4 Position, statistics, effects and explained phenotypic variance of the QTLs detected. Position: The number connected to the chromosome is the number indicated in Fig. 1

Trait	Env	LOD/K^a	Effect[b]	$Var._{expl.}$[c]
1H-1: 47 (C.I.: 45–50)				
BYnb	Br97	3.1	2.93 [Hs]	4.7%
PH	Br97	3.0	1.39 [Hs]	1.4%
1H-2: 53 (C.I.: 45–56)				
GrH	Th97	*27.4*	0.65 [Hs]	10.2%
GrH	Th98	*13.3*	0.21 [Hs]	6.7%
KW	Th97	3.3	1.08 [Ar]	7.4%
KWnb	Th97	6.0	1.69 [Ar]	14.9%
1H-3: 88 (C.I.: 82–92)				
BYnb	Th97	3.0	256 [Ar]	2.3%
TN	Br97	2.6	36.6 [Ar]	6.3%
1H-4: 100 (C.I.: 81–109)				
GrH	Th97	*15.9*	0.49 [Hs]	7.0%
1H-5: 120 (C.I.: 115–123)				
GY	Br97	4.5	108 [Ar]	5.6%
GY	Br98	3.6	91 [Ar]	5.5%
BY	Br98	4.0	220 [Ar]	7.6%
2H-1: 7 (C.I.: 0-11)				
KW	Th97	9.0	1.79 [Ar]	17.7%
KWnb	Th97	4.6	1.52 [Ar]	11.7%
BY	Br98	2.6	219 [Ar]	7.1%
TN	Br97	4.0	36.8 [Ar]	6.6%
2H-2: 12 (C.I.: 9-16)				
DH	Th97	6.7	1.9 [Hs]	8.5%
2H-3: 26 (C.I.: 20–38)				
GlC	Br97	3.8	0.22 [Ar]	10.5%
Col	Th97	3.8	1.14 [Ar]	6.6%
CD	Th97	*15.8*	0.41 [Ar]	2.0%
2H-4: 55 (C.I.: 52–62)				
KW	Th97	6.9	1.69 [Ar]	14.8%
2H-5: 63 (C.I.: 58–62)				
KW	Br97	*10.7*	2.52 [Ar]	24.3%
KW	Br98	7.4	1.68 [Ar]	15.4%
KWnb	Br98	4.3	1.65 [Ar]	16.5%
KWnb	Th97	5.5	1.61 [Ar]	14.2%
2H-6: 115 (C.I.: 109–119)				
PH	Br98	5.0	3.84 [Hs]	8.9%
2H-7: 119 (C.I.: 114–122)				
KW	Th98	4.8	1.60 [Ar]	9.8%
2H-8: 122 (C.I.: 120–126)				
DH	Th97	5.6	2.12 [Ar]	11.0%
2H-9: 136 (C.I.: 125–143)				
KW	Th97	4.8	1.36 [Ar]	9.4%
3H-1: 0 (C.I.: 0–3)				
BY	Br97	5.1	406 [Ar]	8.3%
GY	Br97	8.6	186 [Ar]	16.2%
KW	Br97	2.5	0.86 [Ar]	3.6%
KWnb	Br97	2.7	11.38 [Hs]	5.1%
GY	Br98	8.1	234 [Ar]	29.8%
BY	Th97	54.9	3284 [Ar]	72.6%
GY	Th97	76.7	2545 [Ar]	84.8%
Col	Th97	2.6	0.64 [Ar]	2.3%
BY	Th98	25.1	1916 [Ar]	44.1%
GY	Th98	49.4	1565 [Ar]	67.5%
3H-2: 16 (C.I.: 13–21)				
TN	Br97	5.1	49.3 [Ar]	49.3%
BY	Br98	4.0	223 [Ar]	8.1%
DH	Th97	3.3	1.79 [Hs]	7.6%
KW	Th97	5.7	1.20 [Ar]	9.2%
3H-3: 73 (C.I.: 67–76)				
PrC	Th97	7.1	0.69 [Hs]	13.9%
3H-4: 77 (C.I.: 71–83)				
PH	Br97	8.4	5.16 [Hs]	11.8%
PH	Br98	16.7	7.4 [Hs]	29.1%
GY	Br98	2.7	97 [Ar]	6.1%
PH	Th97	4.4	3.92 [Hs]	4.8%
DH	Th97	9.8	3.14 [Ar]	20.0%
BYnb	Th97	5.1	656 [Ar]	13.3%
GY	Th97	3.6	245 [Ar]	4.9%
GYnb	Th97	4.1	457 [Ar]	12.8%
3H-5: 85 (C.I.: 80–90)				
KW	Th97	5.9	1.30 [Ar]	10.5%
KWnb	Th97	2.7	1.38 [Ar]	11.1%
4H-1: 16 (C.I.: 6–25)				
PH	Br97	4.4	3.97 [Hs]	11.5%
PH	Br98	2.6	3.12 [Hs]	6.3%
4H-2: 52 (C.I.: 43–59)				
KW	Th97	4.9	1.18 [Ar]	8.9%
KWnb	Th97	2.7	1.76 [Ar]	8.9%
KW	Th98	5.7	1.79 [Ar]	11.2%
KWnb	Th98	5.1	2.94 [Ar]	25.3%
GlC	Br97	2.6	0.14 [Hs]	4.3%
CD	Th98	*14.0*	0.14 [Ar]	8.0%
4H-3: 103 (C.I.: 94–107)				
DH	Br97	2.6	1.02 [Ar]	5.1%
KW	Br97	8.2	2.14 [Ar]	17.1%
TN	Br97	4.8	35.0 [Hs]	5.5%
KW	Br98	8.0	1.65 [Ar]	14.9%
KWnb	Br98	5.6	1.95 [Ar]	33.7%
KW	Th97	2.6	1.06 [Ar]	7.3%
GY	Th98	3.0	170 [Ar]	2.4%
CD	Th98	*14.0*	0.14 [Ar]	2.8%
5H-1: 15 (C.I.: 12–20)				
DH	Br97	3.6	1.23 [Hs]	5.8%
DH	Th97	5.2	2.24 [Hs]	10.7%
KW	Th98	3.1	1.24 [Ar]	6.0%
5H-2: 52 (C.I.: 48–69)				
CD	Th97	*53.3*	0.72 [Hs]	8.4%
CD	Th98	*34.4*	0.23 [Hs]	4.0%
5H-3: 78 (C.I.: 64–82)				
KW	Br97	3.3	0.48 [Hs]	7.1%
CD	Th97	*65.0*	0.77 [Hs]	
PH	Th97	4.4	2.90 [Hs]	5.5%
5H-4: 84 (C.I.: 76–95)				
PrC	Br97	2.9	0.37 [Hs]	4.5%
KWnb	Br98	6.4	1.16 [Ar]	8.5%
KW	Th97	2.6	1.03 [Ar]	7.1%
KWnb	Th97	5.5	0.81 [Ar]	10.0%
BYnb	Th97	3.1	557 [Ar]	10.2%
GY	Th97	2.6	244 [Ar]	5.0%
CD	Th98	44.6	0.26 [Hs]	11.4%
6H-1: 23 (C.I.: 18–29)				
KW	Th97	3.9	1.30 [Ar]	11.0%
KW	Th97	4.6	1.17 [Ar]	8.7%
PrC	Th97	4.4	0.55 [Hs]	9.0%

Table 4 (continued)

Trait	Env	LOD/*K*[a]	Effect[b]	Var.$_{expl.}$[c]
KW	Th98	4.8	1.31 [Ar]	7.2%
KWnb	Th98	3.0	1.06 [Ar]	4.5%
6H-2: 72 (C.I.: 69–75)				
KW	Br98	3.1	1.12 [Hs]	7.8%
KW	Th98	4.4	1.15 [Hs]	5.8%
6H-3: 75 (C.I.: 72–79)				
KW	Br97	2.7	0.89 [Hs]	3.9%
PH	Th97	3.8	3.78 [Hs]	9.9%
6H-4: 78 (C.I.: 75–83)				
GlC	Br97	5.9	0.22 [Hs]	10.2%
GrH	Th97	*23.4*	0.61 [Ar]	17.8%
GrH	Th98	*27.5*	0.29 [Ar]	12.2%
GrV	Th98	*14.9*	0.29 [Ar]	12.4%
CD	Th98	*5.9*	0.10 [Ar]	3.1%
7H-1: 40 (C.I.: 34–43)				
GY	Br97	3.0	107 [Ar]	5.2%
KW	Th97	6.6	1.71 [Hs]	16.6%
7H-2: 47 (C.I.: 42–52)				
KWnb	Th97	6.4	2.11 [Hs]	18.3%
7H-3: 56 (C.I.: 45–73)				
DH	Br97	9.4	3.66 [Ar]	35.7%
PH	Br98	2.9	2.57 [Hs]	3.1%
GlC	Br97	5.0	0.20 [Hs]	7.9%
7H-4: 100: (C.I.: 95–104)				
BY	Br97	3.8	440 [Ar]	6.4%
KWnb	Br98	3.6	1.06 [Ar]	4.4%
7H-5: 106 (C.I.: 106–110)				
CD	Th97	*29.4*	0.55 [Hs]	15.8%
CD	Th98	*9.3*	0.13 [Hs]	5.3%
7H-6: 109 (C.I.: 105–116)				
KW	Th97	5.0	18.0 [Hs]	14.5%
7H-7: 152 (C.I.: 146–157)				
DH	Th97	2.6	1.35 [Hs]	4.4%
BYnb	Br98	2.7	129 [Hs]	2.9%

[a] LOD/*K*: for the case of MQM analysis, the LOD-score is given, for the case of non-parametric analysis, the K-value is from the Kruskal-Wallis ANOVA (K-value *italics* in the Table)
[b] Effect: difference of the genotype with 2 alleles of the one parent compared to the genotype with 2 alleles from the other parent (in parenthesis: the parent with the higher value trait expression, [Ar] = Arta, [Hs] = *H. spontaneum*
[c] Var. expl: phenotypic variance explained by the trait

H. spontaneum line. In general, the level of explained variance was high for the QTLs for this trait.

All four QTLs for tiller number (TN) were detected in Br97 (1H-3, 2H-1, 3H-2 and 4H-3). The *H. spontaneum* line shows the allele for higher number of tillers for the QTL on 4H-3, 'Arta' for the other three QTLs. Seven QTLs were detected for plant height (PH), the allele for taller plants originated always from the *H. spontaneum* line. One QTL was detected in all three environments (3H-4), but showed a much higher effect in Breda than in Tel Hadya, one QTL in Breda in both years (4H-1) and five QTLs in individual environments (1H-1, 2H-6, 5H-3, 6H-3 and 7H-3). 'Days to heading' was recorded in Th97 and Br97. Five QTLs were detected in Th97 (2H-2, 2H-8, 3H-2, 3H-4 and 7H-7), two in Br97 (4H-3 and 7H-3) and one in both environments (5H-1). 'Arta' contributed the allele with late heading in four of the eight QTLs (2H-8, 3H-4, 4H-3 and 7H-3). The QTL on 7H-3 showed by far the largest effect (3.5-days difference and 36 % explained the variance). Growth habit (GrH) was observed in Th97 and Th98, three QTLs were found, two common in both years (1H-2 and 6H-4) and one only in Th97 (1H-4). For the two common QTLs, the largest effect was found in Th97. For the QTL on 6H-4, the 'Arta'-allele caused the more-prostrate growth type; for the two other QTLs, it was the allele from the *H. spontaneum* line. Only one QTL was found for the growth vigor (GrV) at 6H-4, together with the QTL for GrH mentioned before. The 'Arta'-allele caused the more-vigorous growth.

Cold damage (CD) was evaluated in Th97 and Th98, resulting in the detection of eight QTLs. Two of them were detected in Th97 only (2H-3 and 5H-3), four in Th98 (4H-2, 4H-3, 5H-4 and 6H-4), and two in both environments (5H-2 and 7H-5). Only for the QTLs with minor effects (2H-3, 4H-3 and 6H-4), the allele from the *H. spontaneum* line showed the better protection. For all other QTLs, the allele of 'Arta' showed better ability to protect the plant from cold damage.Three of the four alleles with a high effect on CD (5H-2, 5H-3 and 5H-5) were localized on chromosome 5H. Protein content in the kernel was measured in Br97 and Th97. Three QTLs were detected, one in Br97 (5H-4), two in Th97 (3H-3 and 6H-1). For all of them, the allele with the higher protein content originated from the *H. spontaneum* line. On 5H-4, as well as on 6H-1 on the same chromosomal position, a QTL for kernel weight was localized with 'Arta' as the parent with the heavier kernels. Measurements of glucan content (GlC) were carried out only in Br97. Nevertheless, four QTLs were detected (2H-3, 4H-2, 6H-4 and 7H-3). For one of them 'Arta' contributed the allele with the higher glucan content, for the other three the *H. spontaneum* line contributed the positive allele. For the total chlorophyll content (Col), which was only determined in Th97, two QTLs were localized (2H-3 and 3H-1). For both of them the allele of 'Arta' mediated the higher chlorophyll content.

Discussion

Comparison of QTLs with other studies

The population tested here is specifically adapted to the stressful Mediterranean environment. It is interesting to examine whether the major QTLs identified in this study are unique or common to other barley populations. Except for the studies of Teulat et al. (1998, 2001b), barley populations or lines were tested in more-favorable environments (Hayes et al. 1993; Pan et al. 1994; Backes et al. 1995; Thomas et al. 1995; Tinker et al. 1996; Bezant et al. 1997; Yin et al. 1999; Forster et al. 2000; Marquez-Cedillo et al. 2001; Ivandic et al. 2002).

QTLs for GY and BY were identified at the *btr* location on 3H, which very likely are due to the variation caused by the *btr* locus itself. A further grain yield QTL was identified on 3H-4 in Th97 and Br98, explaining 12.8% of the additive variation in the non-brittle lines in Th97 and with the high yield allele originating from 'Arta'. Grain yield QTLs near the *sdw1* locus were already identified in earlier studies (Tinker et al. 1996; Bezant et al. 1997; Yin et al. 1999). Other grain yield QTLs were identified on 3H in more proximal positions by Bezant et al. (1997) and Teulat et al. (2001b)

We have identified a major QTL for KW on 3H-2 that could correspond to the grain yield QTL identified by Marquez-Cedillo et al. (2001) on 3H. The major QTL for KW on 2H-5 might coincide with the grain yield QTLs and other traits that were mapped at the *vrs* locus on 2H (Hayes et al. 1993; Marquez-Cedillo et al. 2001; Tanno et al. 2002). The KW QTL on 4H-3 might match the kernel weight QTL of Bezant et al. (1997).

The major QTL for PH on 3H-4 is located proximal to the Bmac0013 SSR marker and most likely positioned at the *sdw1* locus (Ellis et al. 2002) or *denso* locus (Laurie et al. 1993), one of the dwarfing genes with commercial significance in barley breeding. The *H. spontaneum* allele at this position has a positive effect on stem elongation and increased the plant height under drought stress. Similarly to our findings for the increased plant-height alleles, the *sdw1* dwarfing gene has shown linkage to-date of heading, a number of agronomic traits (Barua et al. 1993) and a number of physiological traits (Yin et al. 1999). In other crosses not having the *denso* gene, the same region showed linkage to grain yield (Thomas et al. 1995) or earliness per se (Ivandic et al. 2002). The other PH-QTLs on 2H-6, 4H-1 and 6H-3 (the favorable allele from *H. spontaneum*) could correspond to the QTLs found in the Mediterranean environment by Teulat et al. (2001b).

Three QTLs for cold tolerance were identified on chromosomes 5H, with 'Arta' being the more cold-tolerant line. Vernalisation requirement in barley has been identified by comparative studies with wheat, and located as *Sh2* on chromosome 5H at around 125 cM (Laurie et al. 1995; Ivandic et al. 2002). In addition, Pan et al. (1994) reported a frost tolerance QTL on 5H, around the same location. This locus on 5H has been revealed with a *DHn* probe and named *Dhn9* (Choi et al. 2000). Recently, Choi et al. (2002) mapped the barley *HvCbf3* gene, an ortholog of the *Arabidopsis CBF*/*DREB1* gene, to a position more proximal to the *Dhn1*/*Dhn2* – *DHn9* interval (at about 80 cM). In our study we identified QTLs on 5H distal to Bmac113a with a QTL peak at 5H-2 and 5H-3. Based on the current map it seems that the cold-tolerance QTL location in the A×H map would be more proximal than the *Sh2* location but could correspond to the LT50 QTL identified by Hayes et al. (1993) in the marker interval *Rrn2-apADH*. These results emphasize the important role of chromosome 5H for abiotic stress resistance (Cattivelli et al. 2002).

A locus for vernalisation requirement (s*gh1*) has been identified on 4H (Laurie et al. 1995). This is likely to correspond to the cold damage QTL on 4H-3. The cold damage QTLs on 7H-5 have, to our knowledge, not been reported before.

The two major QTLs for days to heading were mapped on 7H-3 and 3H-4. The location on 7H-3 with the allele for the earlier heading originating from the *H. spontaneum* line may correspond to the one found by Hayes et al. (1993); Backes et al. (1995) and Teulat et al. (2001b). The QTL on 3H-4 coincides with that of Bezant et al. (1997). An additional QTL for DH was identified on 2H-2. This might correspond to the *Eam1* or Ppd-H1 location on 2H (Hayes et al. 1993; Backes et al. 1995; Laurie et al. 1995; Cattivelli et al. 2002), but the one on 2H-8 seems to be unique to this population. The QTL on 5H-1 might match the QTL identified by Marquez-Cedillo et al. (2001).

The 'brittle rachis' is possibly caused by complementary genes at two tightly linked loci *btr1* and *btr2* as suggested by Komatsuda and Mano (2002). Komatsuda and Mano (2002) mapped the brittle rachis trait to 3H but to a more-distal location on 3HS, which is in agreement with the position suggested by Franckowiak et al. (1997). Kandemir et al. (2000) mapped *Btr2* of *H. spontaneum* in the interval MWG798B – MWG014 on 3H. In the A×H population, 'brittle rachis' was treated as a qualitative trait and was mapped to 3H at the proximal end of the linkage group.

QTLs specific to this study

Proximal to the *btr* location at 3H-2 a QTL for TN was mapped (49% explained the variance). Increased plant height, asynchronous tillering and reduced tillering are co-segregating traits introgressed from *H. spontaneum*. High tillering is an important character in relation to phenotypic plasticity in response to drought. The higher tillering capacity originated from 'Arta'. Additionally, QTLs for tillering capacity were identified on 4H-3 originating from *H. spontaneum* and on 1H-3 and 2H-1 from 'Arta'.

QTLs for growth habit, with the alleles for the prostrate habit originating from *H. spontaneum*, were located on 1H (1H-2, 1H-4). The prostrate growth habit results in good ground cover in winter, and hence reduces loss of water by soil evaporation. Ceccarelli et al. (1991) reported high ground cover in winter, associated with higher yield under drought conditions. The QTL with the strongest effect was found on 6H (6H-4), and the allele for the prostrate growth originated from 'Arta'. It is interesting to note that a QTL for GrV was located on 6H at the same position with the allele for more-vigorous growth coming from 'Arta'. Early growth vigour, which describes the ability to grow at low temperature, has been shown to be positively correlated with water-use efficiency and dry matter accumulation before anthesis (Fischer 1979, 1981), and is considered to be a beneficial trait in Mediterranean-type drought-prone environments (Passioura 1986). The 1H and 6H locations seem to be important for plant-phenology traits.

QTLs for GY and BY in Br97 and Br98 were identified on 1H-5 with the more favourable allele originating from 'Arta'. These QTLs were not identified in TH and might be important QTLs for yield under dryland environments.

Correlations and co-localisations

Breeding for yield stability in the stressful Mediterranean environments has been slow due to the high variability in timing, duration and the severity of a number of climatic stresses. In Northwest Syria, the most-important abiotic stresses affecting rainfed barley are low temperatures in winter, terminal drought and terminal heat in spring (Ceccarelli et al. 1991). The combination and variation of these stresses lead to large variation within and between sites, and between years. In the present study, plant height was in general negatively correlated with yield (BYnb, BY, GYnb and GY) in Tel Hadya, but positively correlated with yield in Br97 and Br98. Under favourable conditions, increased plant height may cause lodging with consequent reduction of biomass and grain yield. Under drought conditions increased plant height and correlated increased root length is beneficial to exploit soil moisture at depth. Increased plant height also enables mechanical harvesting and therefore leads to increased biological as well as grain yield. In the present study the major QTL for PH has been located on 3H at the *sdw1* locus (Ellis et al. 2002) explaining 11.8% (Br97) and 29.1% (Br98) of the variation, but only 4.8% in Th97. The allele for increased plant height at this locus, as well as at the other loci at 2H-6, 4H-1, 6H-3 and 7H-3, originated from *H. spontaneum*. A QTL for GY was also mapped to the 3H location in Br98.

Days to heading was positively correlated with yield traits (KW, KWnb, GY and GYnb) in Br97 but not always significantly correlated with the same traits in Th97. Earliness is a drought escape mechanism (of *H. spontaneum*) effective to avoid the severe heat and drought stress at the end of the season. Two QTLs were detected in Br97 (4H-3 and 7H-3) and one in both environments (5H-1). At the 4H-3 location, a co-localization of QTLs for KW and TN was observed, while at the 7H-3 location only a QTL was detected for GlC. At the 5H-1 location no further QTLs were located.

Tiller number was positively correlated with yield-related traits in Breda, but not in Tel Hadya. Under stress conditions, the number of productive tillers contributes largely to grain yield, as a minimum number of kernels and a minimum kernel weight are mostly produced. Co-localization of QTLs at 1H-3 showed BYnb and TN, at 2H-1 KW and KWnb; BY and TN, at 3H-2 BY, DH and KW; and TN at 4H-3 DH, KW, GY, CD and TN.

Kernel weight (KW) was positively correlated with grain yield at all sites indicating that the trait was the major contributing factor to grain yield. Major QTL locations for KW were independent from other traits such at 2H-5, 2H-7, 2H-9 and 3H-5, or co-located with GlC and CD at 4H-2, with DH, TN, GY and CD at 4H-3, with PrC, BYnb, GY and CD at 5H-4 and with GY at 7H-1. Interesting to note is the co-localization of protein content, with BYnb, GY and CD at 5H-4 since a negative correlation between kernel protein content and kernel weight was observed in Th97.

The strongest correlations were found, as expected, between grain yield (GY, GYnb) and biological yield (BY, BYnb) in all the environments. A number of traits such as PH (Th97), DH (Th97), BYnb (Th97), GYnb (Th97), and possibly PrC (Th97) and KW (Th97), were co-localized at the 3H-3,4,5 positions in Th97 which may suggest a pleiotropic effect of the locus. Another locus at which several traits were co-localized was the 3H-1 locus, and the position of the *btr* locus: these were BY, GY, KW, KWnb in Br97, and BY, GY, Col in Th97.

Cold damage was positively correlated with GrH (more erect plants, which are generally earlier, were more damaged by cold than prostrate-growing plants) and GrV (poorly vigorous plants are more damaged by cold than vigorous-growing plants) but negatively correlated with yield (GYnb, BY, BYnb in TH97 and TH98). The ability of barley to grow under cold temperatures in winter is one of the key characters of the well-adapted genotypes because of the well-known positive relationship between dry matter accumulation before anthesis and grain yield (Turner 1982). The major QTL locations for cold damage were on 4H-2, 4H-3, 5H-2, 5H-3, 5H-4, 6H-4 and 7H-5. Only the 4H-2 location shows a co-localisation of CD with KWnb in Th98, and the 6H-4 location a co-localisation of CD with GrH, GrV in Th98.

Specific adaptation to Mediterranean environments

Plant height under drought stress was the primary character to be characterised in this population and its increase was the main objective of the cross. The plant height alleles originating from *H. spontaneum* and introgressed into the adapted line 'Arta' might provide useful recombinants exhibiting more drought tolerance under rain-fed conditions. Although no strong positive correlations between PH and GYnb or GY could be found, a number of tall, non-brittle lines were identified that produced high yield under stress. The ten best lines of the RIL population produced an average grain yield over the 2 years in Breda of 1,634 kg/ha with a plant height of 48.3 cm, as compared to 976 kg/ha and 52.4 cm for *H. spontaneum* 41-1 and 1,185.8 kg/ha and 29 cm for 'Arta'.

Adaptation to drought-stress conditions in barley is of a complex nature. Several authors have measured osmotic adjustment (OA) and carbon isotope discrimination (Δ) as traits that could be indicative for an increased drought tolerance (Morgan 1983; Blum 1988; Farquahr et al. 1989; Araus et al. 1997; Bort et al. 1998; Teulat et al. 1998, 2001a; Ellis et al. 2002; Teulat et al. 2002). Although only preliminary results for OA adjustment are available for 'Arta' × *H. spontaneum* 41-1, OA does not seem to play a major role for an increased yield potential or yield stability of *H. spontaneum* derivates (Ceccarelli

et al., unpublished). There are a number of morphological and physiological characters leading to adaptation in marginal environments and to drought escape, such as the prostrate growth habit, early vigour, good ground cover leading to a reduction of water loss by soil evaporation in the early growing season, cold tolerance for the adaptation to frost in winter, the ability of the roots to exploit deep soil moisture, a late flowering time to escape late frosts and a short grain-filling period to escape terminal drought stress (Ceccarelli et al. 1991). *H. spontaneum* contributes plant height (and possibly root length) and earliness; 'Arta' contributes cold tolerance, intermediate growth habit, good ground cover, high tiller capacity, late-flowering time and high kernel weight. *H. spontaneum* 41-1 is very early and has short grain filling as a true escape mechanism (120.6 DT in Br97 as compared to 127.4 for 'Arta'). Its increased plant height might also have a number of correlated root characteristics that play an important role under drought conditions (Ceccarelli et al., unpublished).

Acknowledgements This research was funded by BMZ grant 96.7860.8-001.00, by a grant of the Arab Fund for Economic and Social Development, and by the grant of the Danish Research Council for Developmental Research (Proj.-No. 90978). We are grateful to Dr. W. Powell, SCRI, for providing barley microsatellite primer sequences prior to publication.

References

Araus JL, Bort J, Ceccarelli S, Grando S (1997) Relationship between leaf structure and carbon isotope discrimination in field-grown barley. Plant Physiol Biochem 35:533–541

Backes G, Jahoor A (2001) Wege zur Nutzung des genetischen Potentials der Wildgerste – mit molekularen Markern zu neuen Resistenzgenen. Bericht der Arbeitstagung der 52. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreich, Gumpenstein: 81–86

Backes G, Graner A, Foroughi-Wehr B, Fischbeck G, Wenzel G, Jahoor A (1995) Localization of quantitative trait loci (QTLs) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.). Theor Appl Genet 90:294–302

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Barua UM, Chalmers KJ, Thomas WTB, Hackett CA, Lea V, Jack P, Forster BP, Waugh R, Powell W (1993) Molecular mapping of genes determining height, time to heading, and growth habit in barley (*Hordeum vulgare*). Genome 36:1080–087

Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey M (1997) Mapping QTLs controlling yield and yield components in a spring barley (*Hordeum vulgare* L.) cross using marker regression. Mol Breed 3:29–38

Blum A (1988) Drought resistance. In: Plant breeding for stress environments. CRC Press, Boca Raton, Florida, pp 43–76

Bort J, Araus JL, Hazzam H, Grando S, Ceccarelli S (1998) Relationships between early vigour, grain yield, leaf structure and stable isotope composition in field-grown barley. Plant Physiol Biochem 36:889–897

Cattivelli L, Baldi P, Crosatti C, Di Fonzo N, Faccioli P, Grossi M, Mastrangelo AM, Pecchioni N, Stanca AM (2002) Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*. Plant Mol Biol 48:649–665

Ceccarelli S (1994) Specific adaptation and breeding for marginal conditions. Euphytica 77:205–219

Ceccarelli S, Grando S (1996) Drought as a challenge for the plant breeder. Plant Growth Reg 20:149–155

Ceccarelli S, Acevedo E, Grando S (1991) Breeding for yield stability in unpredictable environments: single traits, interaction between traits, and architecture of genotypes. Euphytica 56:169–185

Choi DW, Koag MC, Close TJ (2000) Map locations of barley *Dhn* genes determined by gene-specific PCR. Theor Appl Genet 101:350–354

Choi DW, Rodriguez EM, Close TJ (2002) Barley *Cbf3* gene identification, expression pattern, and map location. Plant Physiol 129:1781–1787

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Ellis RP (2002) Wild barley as a source of genes for crop improvement. In: Slafer GA, Molin-Cano JL, Savin R, Araus JL, Ramagosa I (eds) Barley science: recent advances from molecular biology to agronomy of yield and quality. Food Products Press, The Haworth Press Inc, pp 65–83

Ellis RP, Nevo E, Beiles A (1993) Milling energy polymorphism in *Hordeum spontaneum* Koch in Israel and its potential utilization in breeding for malting quality. Plant Breed 111:78–81

Ellis RP, Forster BP, Gordon DC, Handley LL, Keith RP, Lawrence P, Meyer R, Powell W, Robinson D, Scrimgeour CM, Young G, Thomas WT (2002) Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. J Exp Bot 2002:1163–1176

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Fischer RA (1979) Growth and water limitations to dryland wheat yield in Australia: a physiological framework. J Aust Inst Agric Sci 45:83–94

Fischer RA (1981) Optimizing the use of water and nitrogen through breeding of crops. Plant and Soil 58:249–278

Forster BP, Ellis RP, Thomas WTB, Newton AC, Tuberosa R, This D, El- Enein RA, Bahri H, Ben Salem M (2000) The development and application of molecular markers for abiotic stress tolerance in barley. J Exp Bot 51:19–27

Franckowiak JD, Lundqvist U, Konishi T (1997) New and revised names for barley genes. Barley Genet Newslett pp 26:4–8

Giles BE (1990) The effects of variation in seed size on growth and reproduction in the wild barley *Hordeum vulgare* ssp. *spontaneum*. Heredity 64:239–250

Giles BE, Bengtsson BO (1988) Variation in anther size in wild barley (*Hordeum vulgare* ssp. *spontaneum*). Hereditas 108:199–205

Grando S, Ceccarelli S (1995) Seminal root morphology and coleoptile length in wild (*Hordeum vulgare* ssp. *spontaneum*) and cultivated (*Hordeum vulgare* ssp. *vulgare*) barley. Euphytica 86:73–80

Grando S, von Bothmer R, Ceccarelli S (2001) Genetic diversity of barley: use of locally adapted germplasm to enhance yield and yield stability of barley in dry areas. In: Cooper HD, Spillane C, Hodgink T (eds) Broadening the genetic base of crop production. CABI/FAO/IPGRI, pp 351–372

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Gustafsson M, Claesson L (1988) Resistance to powdery mildew in wild species of barley. Hereditas 108:231–237

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theor Appl Genet 87:392–401

Hayes PM, Castro A, Corey A, Marquez-Cedillo L, Jones B, Mather D, Matus I, Rossi C, Sato K (2000) A summary of

published barley QTL reports. http://www.css.orst.edu/barley/NABGMP/qtlsum.htm
Hospital F, Moreau L, Lacoudre F, Charcosset A, Gallais A (1997) More on the efficiency of marker-assisted selection. Theor Appl Genet 95:1181–1189
Ivandic V, Forster BP, Hackett CA, Nevo N, Keith R, Thomas TBW (2002) Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. Plant Mol Biol 48:511–527
Jahoor A, Fischbeck G (1987) Sources of resistance to powdery mildew in barley lines derived from *Hordeum spontaneum* collected in Israel. Plant Breed 99:274–281
Jaradat AA (1991) Grain protein variability among populations of wild barley (*Hordeum spontaneum* C Koch) from Jordan. Theor Appl Genet 83:164–168
Kandemir N, Kudrna DA, Ullrich SE, Kleinhofs A (2000) Molecular-marker-assisted genetic analysis of head shattering in six-rowed barley. Theor Appl Genet 101:203–210
Komatsuda T, Mano Y (2002) Molecular mapping of the intermedium spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). Theor Appl Genet 105:85–90
Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175
Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Laurie DA, Pratchett N, Romero C, Simpson E, Snape JW (1993) Assignment of the *denso* dwarfing gene to the long arm of chromosome 3 (3H) of barley by the use of RFLP markers. Plant Breed 111:198–203
Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of 5 major genes and 8 quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585
Lehmann LC, Jönsson R, Gustafsson M (1998) Identification of resistance genes to powdery mildew isolated from *Hordeum vulgare* ssp. *spontaneum* and landraces of barley. J Swedish Seed Assoc 108:94–101
Marquez-Cedillo LA, Hayes PM, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2001) The North American Barley Genome Mapping Project: QTL analysis of agronomic traits in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet 103:625–637
Morgan JM (1983) Osmoregulation as a selection criterion for drought tolerance in wheat. Aust J Agric Res 34:607–614
Moseman JG, Nevo E, Zohary D (1983) Resistance of *Hordeum spontaneum* collected in Israel to infection with *Erysiphe graminis hordei*. Crop Sci 23:1115–1119
Moseman JG, Nevo E, El-Morsidy MA (1990) Reactions of *Hordeum spontaneum* to infection with two cultures of *Puccinia hordei* from Israel and United States. Euphytica 49:169–175
Nevo E (1992) Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology, C.A.B. International, pp 19–43
Nevo E, Krugman T, Beiles A (1993) Genetic resources for salt tolerance in the wild progenitors of wheat (*Triticum dicoccoides*) and barley (*Hordeum spontaneum*) in Israel. Plant Breed 110:338–341
Pan A, Hayes PM, Chen F, Blake THH, Wright TKS, Karsai I, Bedö Z (1994) Genetic analysis of the components of winter hardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89:900–910
Passioura JB (1986) Resistance to drought and salinity: avenues for improvement. Aust J Plant Physiol 13:191–201
Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39:379–394
Ramsay L, Macaulay M, Degli Ivanissevich S, Maclean K, Carsle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005
Saghai-Maroof MA, Soliman KM, Gorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018
Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen
Tanno K, Taketa S, Takeda K, Komatsuda T (2002) A DNA marker closely linked to the *vrs1* locus (row-type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theor Appl Genet 104:54–60
Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). Theor Appl Genet 96:688–698
Teulat B, Borries C, This D (2001a) New QTLs identified for plant water status, water-soluble carbohydrates, osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. Theor Appl Genet 103:161–170
Teulat B, Merah O, Souyris I, This D (2001b) QTLs for agronomic traits from Mediterranean barley progeny grown in several environments. Theor Appl Genet 103:774–787
Teulat B, Merah O, Sirault X, Borries C, Waugh R, This D (2002) QTLs for grain carbon isotope discrimination in field-grown barley. Theor Appl Genet 106:118–126
Thomas WTB, Powell W, Waugh R, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Swanston JS, Ellis RP, Hanson PR, Lance RCM (1995) Detection of quantitative trait loci for agronomic yield, grain, and disease characters in spring barley (*Hordeum vulgare* L.). Theor Appl Genet 91:1037–1047
Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugar LP, Legge WG, Irvine RB, Choo TM, Briggs KG, Ullrich SE, Franckowik JD, Blake TK, Graf RJ, Dofing SM, Saghai Maroof MA, Scoles GJ, Hoffman D, Dahleen LS, Kilian, Chen F, Biyashev RM, Kudrna DA, Steffenson BJ (1996) Regions of the genome that affect agronomic performance in two-row barley. Crop Sci 36:1053–1062
Turner NC (1982) The role of shoot characteristics in drought resistance of crop plants. In: Drought resistance in crops with emphasis on rice. IRRI, Los Banos, The Philippines, pp 115–134
Utz HF, Melchinger AE (1994) Comparison of different approaches to interval mapping of quantitative trait loci. In: van Oojen JW, Jansen J (eds) Biometrics in plant breeding: application of molecular markers. Wageningen, 195–204
Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTLs. J Quant Trait Loci 2, http://www.ncgr.org/jag/papers96/paper196/indexp196.html
Van Ooijen JW, Maliepaard C (1996) MapQTL version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen
Yin X, Stam P, Johan Dourleijn C, Kropff MJ (1999) AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. Theor Appl Genet 99:244–253
Zhu H, Briceño G, Dovel R, Hayes PM, Liu BH, Liu CT, Toojinda T, Ullrich SE (1999) Molecular breeding for grain yield in barley: an evaluation of QTL effects in a spring barley cross. Theor Appl Genet 98:772–779
Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. Eur Pat App 92402629.7 (Publ Number 0 534858 A1)