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QTLs for grain carbon isotope discrimination in field-grown barley

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Abstract In several crops including cereals, carbon isotope discrimination (Δ) has been associated with drought tolerance in terms of water-use efficiency and yield stability in drought-prone environments. By using a complete genetic map generated from 167 recombinant inbred lines from a cross between Tadmor and Er/Apm, QTLs associated with grain Δ have been detected in barley grown in three Mediterranean field environments, two differing only in water availability. Ten QTLs were identified: one was specific to one environment, two presented interaction with the environment, six presented main effects across three or two environments and one presented both effects. Heading date did not contribute to the environment (E) and G × E effects acting on Δ. Seasonal rainfall and the ratio of rainfall to evapo-transpiration made large contributions to the environmental effect, but their influence on G × E was weaker. Eight QTLs for Δ co-located with QTLs for physiological traits related to plant water status and/or osmotic adjustment, and/or for agronomic traits previously measured on the same population. Some perspectives in terms of characterising drought tolerance are evoked.

Keywords Carbon isotope discrimination · QTL · Mediterranean conditions · Drought · Barley

Introduction

The Mediterranean basin is one of the regions where drought leads to substantial yield reductions (Loss and Siddique 1994). Moreover, major changes in agronomic practices such as irrigation or other inputs, is unlikely to happen in the forseeable future. In this region, barley (*Hordeum vulgare* L.) is cultivated in arid zones under rainfed conditions without irrigation, and with 200–300 mm of rainfall (Srivastava 1987). Drought tolerance and yield stability is therefore an important aim for breeders in these regions.

Direct selection for grain yield under water-stressed conditions is difficult due to low heritability and significant genotype × environment (G × E) interactions (Ceccarelli et al. 1991). As an alternative, a multitude of morpho-physiological characters have been suggested as indicators for increasing grain yield under drought conditions. Amongst these, transpiration efficiency (TE: the ratio of dry matter produced to water transpired) is considered as an important drought-adaptive trait in cereals. However, direct measurements of TE are difficult, slow, expensive and need uniform weather conditions on large populations of plants. Carbon isotope discrimination (Δ) provides an integrated measurement of TE of C3 crop species (Farquhar and Richards 1984). During photosynthesis, plants discriminate against the heavy isotope of carbon (^{13}C). And, as a result, in several C3 species including wheat and barley, Δ is positively correlated with the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (Ci/Ca) and negatively associated with TE (Farquhar and Richards 1984; Ehdaie et al. 1991; Johnson and Bassett 1991). Thus, a high Ci/Ca leads to a higher Δ and a lower TE (Farquhar and Richards 1984). The major advantage of using Δ in selection is its high heritability, which is primarily due to small genotype × environment interactions in dryland areas (Hall et al. 1990; Richards et al. 1999; Merah et al. 2001b). Δ has been found to be positively correlated with grain yield in cereals within and across contrasting environments (Acevedo 1993; Araus et al. 1997; Voltas et al. 1998; Merah et al. 2001a, b; Teulat et al. 2001b). While the choice of which part of the plant to use for Δ measurements is still being debated, for cereals grown under Mediterranean conditions the grain is considered

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most appropriate (Voltas et al. 1998; Merah et al. 2001b). Measuring Δ by mass spectrometry remains expensive. As a result, a number of alternative criteria for Δ have been suggested including stomatal conductance (Rebetzke et al. 2001), leaf structural traits such as dry weight per unit leaf area (Araus et al. 1997; Merah et al. 2001a) and ash content (Araus et al. 1997; Voltas et al. 1998; Merah et al. 2001a). Overall these have been shown to be less-effective measures. Δ is therefore a good example of a trait which could be efficiently tracked by molecular markers through the identification of quantitative trait loci (QTLs). Markers diagnostic of individual QTLs represent an important surrogate for physiological trait measurements (Price and Courtois 1999), and may ultimately improve selection efficiency through marker-assisted breeding.

There is currently limited insight into the genetic control of TE and Δ. Martin et al. (1989) found that 70% of the genetic variation for Δ in populations derived from a cultivated and a wild tomato was associated with three RFLP loci, mapped on three different chromosomes. In soybean, several QTLs for Δ were identified under favourable plant growth conditions (Mian et al. 1996). Surprisingly, the identification of QTLs involved in Δ variation under drought conditions is undocumented in cereals. The available studies have shown that Δ, measured on whole shoots, is largely controlled by chromosome 4H in wheat/barley addition lines (Handley et al. 1994). In addition, Ellis et al. (1997) identified nine anonymous genetic markers associated with Δ variation in barley seedlings grown under salt-stressed conditions. These studies were carried out using a limited number of genotypes under controlled environmental conditions.

The overall aim of this study was an attempt to identify chromosomal regions involved in grain Δ variation measured under Mediterranean field conditions in a recombinant inbred line population derived from a cross between two Mediterranean barley parents.

Materials and methods

Plant material, site and crop Management

A progeny of 167 two-row barley recombinant inbred lines (RILs) and the two parents Tadmor (selected by ICARDA from Arabi Aswad, a Syrian landrace) and Er/Apm (a selected line, released in Tunisia) were grown in rainfed field conditions with one replicate of RILs at CIDA (Centro de Investigación y Desarrollo Agrario, Granada, South of Spain) in 1996 (G96), and under two water treatments in 1999 (M99rain: rainfed conditions, and M99ir: with a supplemental irrigation) in the experimental field at ENSAM (Montpellier, South of France) in a random complete block design with two blocks per treatment. The plants were sown on December 5th (1995) for G96 and on November 20th (1998) for M99rain and M99ir. In G96, plots of 3 m^2 with a seeding rate of 220 seeds/m^2 were sown, and fertiliser was added in a single application (147 kg/ha Nitrogen Urea 46.5%). In M99, two rows per line were grown for each block, 101 kg/ha of PK was supplied before sowing, and then 124 kg/ha of Nitrogen (Ammonitrate) was added twice.

Climatic conditions

G96, M99rain and M99ir were considered as three different environments (E). The agronomic practices and meteorological conditions were described in detail in Teulat et al. (2001c). The three environments differed in total cumulative rainfall (and water supply for M99ir) as well as rainfall distribution. In G96, the total rainfall was high (590 mm) with more than 86% falling during the 3 first months after sowing, but the last 4 months were characterised by a terminal water stress. In M99rain, plants received 390 mm of rainfall. For this environment, only a third of the cumulative rainfall fell during the 3 first months. The main drought period was in February 1999 with 0 mm rainfall. The plants of the irrigated blocks (M99ir) received 98 mm of additional water in six applications so that the two environments M99rain and M99ir differed in plant water availability (Teulat et al. 2001c).

Measurements

In G96 and in one block for M99rain and M99ir, carbon isotope discrimination (Δ) was measured on a bulk of grains from several plants of each RIL ground into a fine powder and dried for 48 h at 80 °C. The carbon isotope composition was determined using an isotope mass spectrometer (Optima, Micromass, Villeurbanne, France): $\delta^{13}C(‰) = [(^{13}C/^{12}C)\ \text{sample}/(^{13}C/^{12}C)\ \text{reference-1}] \times 1000$. The carbon isotope discrimination values were obtained from δa and δp according to the formula (Farquhar and Richards 1984): $\Delta(‰) = (\delta a - \delta p)/(1+\delta p)$, where a and p refer to air and plant, and the carbon isotope composition of air was taken as –8‰. All Δ measurements were performed at the laboratory of Structure et Métabolisme des Plantes, CNRS Université Paris-Sud (France).

Statistical analysis

The data were first subjected to variance and correlation analyses using the GLM and CORR procedures of the SAS program respectively (SAS Institute 1987). As rainfall (R) and evapotranspiration (ET) were reported as being the main limiting factors of yield in the Mediterranean basin, R and R/ET were used as environmental factors in a factorial regression analysis performed using the REGFAM-function set of the software package INTERA (Decoux and Denis 1991). The relative contribution of the environmental factors to the environment and the G × E interaction sum of squares for Δ were determined by computing the ratio of their corresponding sum of squares. The analyses were carried out by considering the total cumulative rainfall and the mean R/ET of the whole growth cycle, and for the heading to the harvest period only. Factorial regression was also performed to determine the impact of heading date (HD) on Δ variation. HD was measured in each of the three environments (Teulat et al. 2001c).

Genetic map

A partial version of the map was described in Teulat et al. (2001a, c). Forty seven of the 48 SSR-based genetic markers recently described by Macaulay et al. (2001), and one additional SSR named Bmag0007 provided by the Scottish Crop Research Institute (SCRI), were used to complete the map, as well as six SSRs described by Liu et al. (1996) (HVM60, HVCSG, HVM2, HVM6, HVM30 and HVM50). Rice RFLP markers (clones provided by the S. Mc Couch laboratory, Cornell University, USA), barley dehydrin genes (*dhn5*, *dhn9* provided by T. Close, Riverside University, USA), a barley high pI α-amylase (pHv19, Chandler et al. 1984), a complete wheat γ-type gliadin (pKAp1a, Sabelli and Shewry 1991) and a rice sucrose phosphate synthase (YK754 kindly made available by H. Uchimiya, Insitute of molecular and cellular biosciences, University of Tokyo, Japan) were also added to the map. Six RAPDs were added giving a total of 170 markers (99 RFLPs including 17 genes, 47 SSRs, 15 AFLPs, 6 RAPDs, two markers derived from specific amplification and one morphological marker (bpc, for black pericarp color). These were

arranged into a linkage map using MAPMAKER (Lander et al. 1987) with Haldane (1919) distances (see Fig. 1).

QTL detection

For QTL detection, 133 of the 170 markers were used. QTLs from each environment separately were assessed first. These analyses were performed with PLABQTL (Utz and Melchinger 1995). Two methods were employed. First, simple interval mapping (SIM) (Lander and Botstein 1989) using multiple regression of phenotypic data on marker genotypic data (Haley and Knott 1992) was performed with 1,000 permutations to identify the minimum significant LOD score to be considered per trait, yielding an experiment-wise risk of 5%. Then composite interval mapping (CIM) (Zeng 1994) was performed using markers pre-selected by stepwise regression by the software as cofactors (Utz and Melchinger 1995). Putative QTLs were declared significant for CIM when the LOD score exceeded that obtained with SIM. As suggested by Tinker and Mather (1995a, b), two types of QTLs were declared significant, the primary QTLs are those obtained by both SIM and CIM, and secondary QTLs those obtained by SIM or CIM.

In a second step, SIM and simplified composite interval mapping (sCIM) analyses were performed using the software package MQTL, adapted for the evaluation of progeny in multiple environments to identify QTLs that either do, or do not, exhibit QTL × E interaction (Tinker and Mather 1995a, b). The software allows QTL identification based on approximately the same approach as that described by Zeng (1994). For sCIM, 40 background markers well-scattered along the chromosomes, and chosen near QTLs detected by PLABQTL for the traits studied, were used as cofactors to control the effect of the genetic background. They are indicated in Fig. 1. A test statistic (TS) value (Haley and Knott 1992) was produced for both the QTL main effect across E, and QTL × E interaction at each marker locus for both SIM and sCIM. To estimate significance thresholds, the generated TSs were divided by those obtained by 1,000 random permutations of the data performed during SIM analysis. Corrected TSs (cTSs) were obtained. When cTS was higher than 1, the QTL was accepted (type-I error rate below 5). As in Tinker et al. (1996), when evidence for a QTL main effect and a QTL × E interaction were found near the same position, a single QTL was inferred based on the strongest effect. Primary QTLs were those obtained with both methods. Secondary QTLs were those obtained when only SIM or sCIM gave evidence of a QTL (type-I error rate below 5%). For primary QTLs, the positions indicated correspond to the maximum cTS obtained by sCIM analysis. For secondary QTLs, the positions are those corresponding to the maximum cTS with SIM or sCIM.

Results

Genetic map of the Tadmor by Er/Apm population

Of the 48 SSRs described by Macaulay et al. (2001), 41 revealed polymorphism between the two parents and segregated in the progeny (i.e. 85.5%). Of the seven remaining, five were monomorphic (Bmag0218, HvHVA1, WMC1E8, Bmag0222 and HvLOX) and two did not amplify under any PCR conditions used (Bmag0382 and Bmag0021). The 41 polymorphic SSRs revealed 43 markers that were mapped (Fig. 1). These SSRs were well-distributed along the chromosomes. However, few clusters were observed on chromosomes 3H (Bmac0067, Bmac0209 and Bmag0136), 4H (Bmag0384, HVM3 and one locus of EBmac0970) and 6H (Bmag0009 and Bmac0018). These clusters generated problems in identifying the correct marker order and, as a result, some are only indicated in Fig. 1 for comparative mapping purposes. For the other six SSRs (Liu et al. 1996), HVCSG, HVM60 and HVM2A were mapped on chromosomes 2H, 3H and 5H. The other three did not present polymorphism. The rice markers used were mapped on chromosomes 7H (RZ123), 2H (RZ828), 1H (RZ444B) and 5H (RZ404A and RZ404B). Finally, the candidate genes pHv19, pKAP1a, dhn5, dhn9 and YK754 were mapped on chromosomes 6H for the first three, and 5H and 7H for the last two, respectively. The map represents the whole barley genome with seven chromosomes and a total cumulative genetic distance of 1,766 cM (Fig. 1).

Variation of carbon isotope discrimination

Table 1 describes Δ values for each environment for the RILs and the two parents Tadmor and Er/Apm. Significant genetic and environmental effects were detected. Variation across the progeny was high within each environment. The range of variation was particularly high in G96 where the extreme values differed by 4.5‰. The range within M99rain was higher (3.1‰) compared to M99ir (2.5‰). Δ was lower for the environment that expressed the lowest R/ET, and thus lower water availability during the grain-filling period (G96). The highest mean value of Δ was observed in the environment characterised by the highest R/ET. Despite a significant correlation between M99rain and M99ir (r = 0.359**), the environmental effect was significant when the analysis was performed with only M99rain and M99ir. Finally, Tadmor had lower Δ values in the three environments compared to Er/Apm.

Table 1 Trait means, standard deviation, minimum and maximum values obtained from each environment for carbon isotope discrimination (Δ) for the recombinant inbred line progeny. The values of the two parents Tadmor and Er/Apm are also indicated. G96: Granada 1996; M99rain and M99ir: Montpellier 1999, rainfed and with an irrigation supply. The environment and genotype effects are noted for all environments as well as for only M99ir and M99rain (#)

Trait	Environment	Mean	SD	Min	Max	Tadmor	Er/Apm	Environment effect	Genotype effect
Δ (‰)	G96	17.56	0.76	14.89	19.38	15.96	16.96	0.0001***	0.0051**
	M99rain	19.58	0.49	17.99	21.13	19.92	20.33	(0.0001***)#	(0.0001***)#
	M99ir	20.11	0.46	18.59	21.08	19.95	20.29		

, * Significant at the 0.01, 0.001 probability level

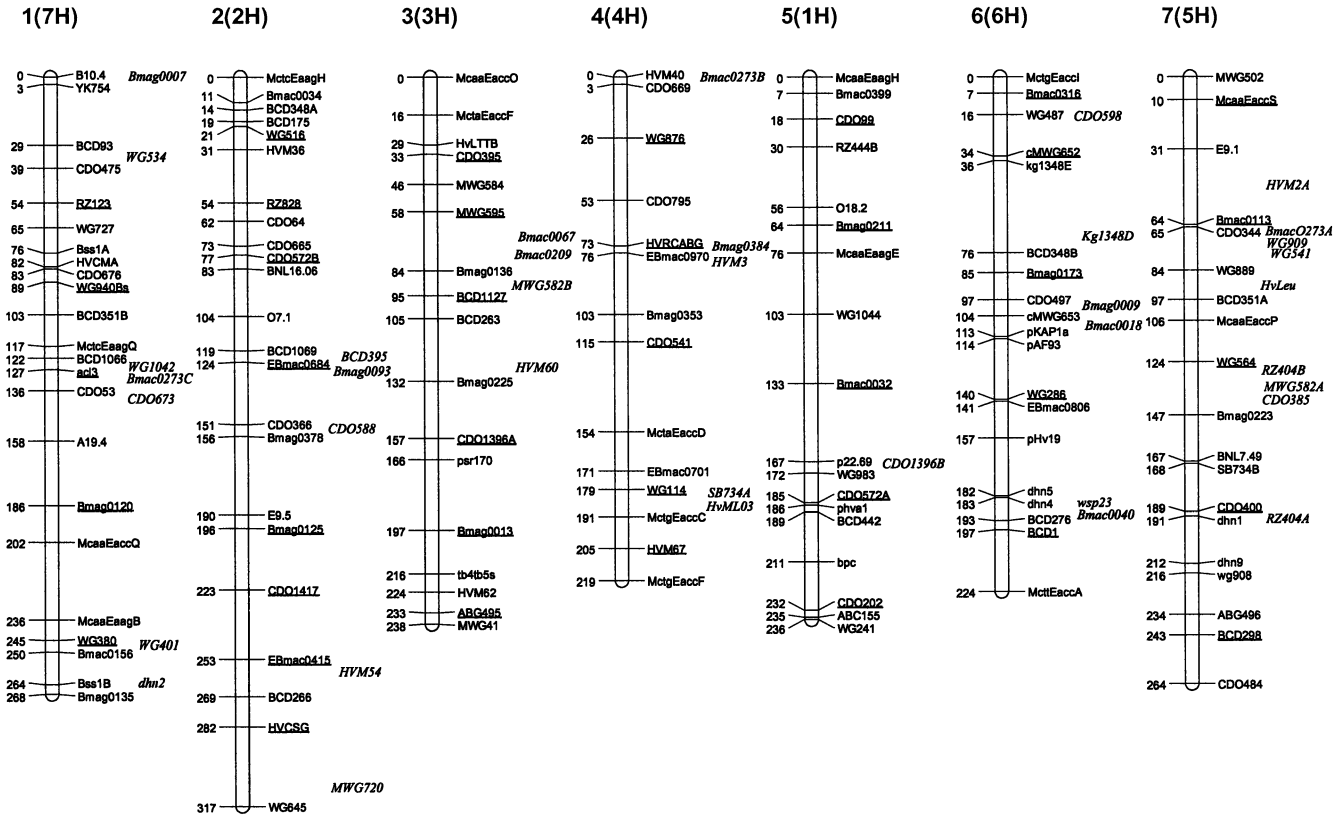

Fig. 1 Linkage map of the barley recombinant inbred line progeny Tadmor × Er/Apm, constructed with 170 molecular markers corresponding to 99 RFLPs, 47 SSRs, 15 AFLPs, 6 RAPDs, two products of specific amplification (followed by s) and one morphological marker (bpc). The 133 markers used for QTL analyses are indicated near the chromosome skeleton and 37 additional markers (20 RFLPs and 17 SSRs), useful for comparative aspects, are noted in *italics*. *Numbers* on the left of chromosomes indicate the cumulative distance in cM to the first marker at the top of chromosome. Markers are specified to the right of chromosomes. Those *underlined* were used as cofactors in the QTL analysis using MQTL software package

Table 2 QTLs identified for carbon isotope discrimination (Δ) with PLABQTL in each environment by simple interval mapping (SIM) and by composite interval mapping (CIM). The primary QTL (P) is detected by both methods. The other QTL is a secondary QTL, detected by only CIM. G96: Granada 1996; M99ir: Montpellier 1999, more irrigated. The allele effect is indicated (T: allele effect contributed by Tadmor). QTLs identified previously at the same loci on the same population are also indicated by the abbreviation of corresponding traits TGW: thousand-grain weight; OA: osmotic adjustment, WSC: water soluble carbohydrates, WSC100: WSC at full turgor, CWC and SA: contribution of a change in water content and of solute accumulation to OA. Except for OA**, information on those QTLs can be obtained in Teulat et al. (2001a, c)

Environment	Marker + distance to the maximum LOD value (cM)	Chromosome	LOD#	R^2%*	Allele effect	QTLs co-localised
G96	EBmac0684+6 (P)	2H	3.25	8.9	0.269 (T)	TGW, WSC, OA**, CWC, SA
M99ir	CDO669+23	4H	3.51	9.6	0.129 (T)	WSC100

Maximum likelihood odds ratio
* Coefficient of determination
** New QTL identified with the complete map presented in the paper

QTLs identified for carbon isotope discrimination

Table 2 describes the QTLs identified for Δ in each environment separately. The significant thresholds determined after 1,000 permutations were 2.80 for M99rain and M99ir, and 2.90 for G96. By the SIM analysis, one QTL was detected in G96. This QTL was located on chromosome 2H near EBmac0684. The use of cofactors in CIM allowed the identification of two QTLs: in G96, one was detected at the same locus as on chromosomes 2H by SIM, and in M99ir one was identified close to WG876 on chromosome 4H (Table 2 and Fig. 1). At both loci, positive alleles were provided by Tadmor. No significant QTL was identified for the rainfed conditions M99rain.

Table 3A describes the QTLs conferring main effects across all environments and QTLs interacting with the environment identified by SIM and/or sCIM with the

Table 3 QTLs for carbon isotope discrimination (Δ) obtained from simple interval mapping (SIM) and simplified composite interval mapping (sCIM) analyses performed with the software package MQTL (Tinker and Mather 1995b) for the three environments (A) and for M99rain and M99ir (B). The corrected test statistic value (cTS) was produced by dividing the TS obtained at each locus and for each trait for both simple main effect and QTL × E effect, by the significant TS produced for each trait for both effects by analysing 1,000 random permutations of the data during SIM (for experimentwise $P = 0.05$). Primary QTLs (P) are those obtained when peaks are significant with SIM and when sCIM peaks are also strong. All secondary QTLs (S) are QTLs only obtained with sCIM. For primary QTLs, the positions indicated correspond to the maximum cTS obtained by sCIM analysis. The allele effects are also given (T: Tadmor; E: Er/Apm). Non significant effects are indicated by dashes. QTLs identified previously at the same loci on the same population are also indicated by the abbreviation of corresponding traits (PH: plant height, HI: harvest index; RWC: relative water content; ψ_π: osmotic potential; $\psi_\pi 100$: osmotic potential at full turgor; TGW: thousand-grain weight; OA: osmotic adjustment, WSC: water soluble carbohydrates; CWC and SA: contribution of a change in water content and of solute accumulation to OA; HD: heading date; NFT: number of fertile tillers; DAB: dry aerian biomass). Except for OA*, information on those QTLs can be obtained in Teulat et al. (2001a, c)

	Marker + distance to the maximum cTS (cM)	Chromosome	Main effect	QTL × E effect	Allele effect	QTLs co-localised
A	acl3+0	7H	–	1.22 (S)	0.035 (T)	PH, HI, RWC, ψ_π
	Bmag0120+16	7H	1.07 (S)	–	0.110 (E)	
	EBmac0684+0	2H	1.49 (P)	1.02 (S)	0.120 (T)	TGW, OA[a], WSC, CWC, SA
	BCD266+8	2H	–	1.36 (S)	0.050 (E)	PH
	Bmag0013+0	3H	1.12 (S)	–	0.100 (E)	HD
	Bmag0173+4	6H	1.01 (S)	–	0.100 (E)	PH, TGW, NFT, DAB
B	RZ444B+8	1H	1.10 (S)	–	0.115 (T)	
	ABC155+0	1H	1.13 (P)	–	0.095 (E)	ψ_π, $\psi_\pi 100$
	BCD348B+8	6H	1.22 (S)	–	0.105 (E)	PH, TGW, NFT, DAB
	Bmag0223+4	5H	1.25 (S)	–	0.125 (T)	HD

[a] New QTL identified with the complete map presented in the paper

Table 4 Percentage contribution of heading date (HD), cumulated rainfalls (R) and ratio of R and evapotranspiration (ET) in the environment (E) and genotype by environment (G × E) total sum of squares for grain carbon isotope discrimination measured in three Mediterranean environments, obtained by factorial regression. For R and R/ET, two periods are considered: total growth cycle and period from heading to harvest

Effect	HD	R total	R/ET total	R [heading-harvest]	R/ET [heading-harvest]
E	2.00	55.40	96.70	99.60	100
G × E	0.92	67.30	20.00	23.40	25.7

software package MQTL. Four QTLs were detected as presenting main effects across environments: one primary QTL located on chromosome 2H, and three secondary QTLs detected by sCIM and located on chromosomes 7H, 3H and 6H. The primary QTL on chromosome 2H also presented a part of QTL by environment interaction. Two secondary QTLs were additionally found by sCIM as interacting with environment. They were detected on chromosomes 7H and 2H. Finally, positive effects on Δ were provided by both parents.

Impact of environmental parameters and heading date on carbon isotope discrimination

Heading date was not significantly correlated with carbon isotope discrimination in any studied environment (data not shown). In addition, the factorial regression analysis has shown that the genotypic factor HD did not explain the environment or the genotype by environment (G × E) effects on Δ for the set of environments considered (Table 4). Concerning the environmental covariates, total R contributed 55.4% and 67.3% of the environmental and G × E effects on Δ respectively. R/ET contributed 96.7% and 20% of the same effects respectively when considering all the growth cycle (Table 4). For the period from heading to harvest, R explained 99.6% and 23.4%, and R/ET 100% and 25.7%, of the environmental and G × E effects on Δ respectively. For this particular period of the growth cycle, the environmental factors considered did not significantly explain the G × E interaction effect whereas they significantly explained the environmental effect.

The influence of water availability was also studied through a QTL analysis performed with MQTL using only M99rain and M99ir environments. This allowed the identification of four QTLs (Table 3B). These only revealed main effects across environments, i.e. across the two water regimes. One primary QTL was located on the long arm of chromosome 1H and the secondary QTLs were located on chromosomes 1H, 5H and 6H. Except for the QTL mapped on chromosome 6H between BCD348B and Bmag0173, these QTLs were not identified when G96 was considered in the analysis (Table 3A and B).

Discussion

The barley Tadmor by Er/Apm genetic map

An updated genetic map of the Tadmor × Er/Apm population is presented in this paper. The markers used were mostly co-dominant (RFLPs and SSRs) and will be useful for comparative studies (genetic maps and QTLs) in barley and other cereals. For the SSRs from Macaulay et al. (2001), a high level of polymorphism was observed (85.5%). The markers were well spread along the seven chromosomes showing their value for rapidly generating barley genetic maps. The clusters observed on chromosomes 3H, 4H and 6H generated some problems in identifying the correct marker order. Two SSRs, EBmac0970 and EBmac0684, both expected to map on chromosome 5H, mapped to chromosomes 4H and 2H respectively. Vales et al. (2000) also mapped EBmac0684 on chromosome 2H. In general, few differences in marker order were observed when compared to the map from the Lina × *H. spontaneum* population (Ramsay et al. 2000). For chromosome 1H, Bmac0032 and Bmag0211 were inverted as also observed by Vales et al. (2000). When comparisons were extended, two other inversions were observed: HVM54 and EBmac0415 on chromosome 2H, and Bmag0223 and HvLeu on chromosome 5H. For the candidate genes, the position of the dehydrins was as expected. The α-amylase (pHv19), the γ-type gliadin (pKAP1) and sucrose phosphate synthase (YK754) were mapped for the first time. With the exception of the telomeres, the Tadmor by Er/Apm map covers the whole barley genome and is useful for QTL investigation, particularly for traits related to adaptation in Mediterranean conditions.

QTLs identified for grain Δ measured under field conditions

QTLs for traits involved in drought tolerance have been reported in several crops. For example, QTLs for osmotic adjustment were identified in rice (Lilley et al. 1996; Zhang et al. 2001), barley (Teulat et al. 1998, 2001a) and wheat (Morgan and Tan 1996). Similarly, QTLs for the anthesis-silking interval were detected in maize (Ribaut et al. 1996) and QTLs for "stay green" were detected in sorghum (Kebede et al. 2001). The present paper described the first QTL for grain Δ measured in Mediterranean field conditions and related to drought stress. A total of 10 QTLs were identified scattered along the chromosomes (Tables 2, 3). Of those, one was specific to one environment, two presented interaction with the environment, six presented main effects across two or three environments and one presented both effects. Only two reports have previously investigated marker associations with Δ (both in barley). In these, Δ was measured on different organs and experiments conducted under different conditions. In the first, Handley et al. (1994) reported that chromosome 4H controls potential water-use efficiency evaluated through whole-shoot carbon isotope composition ($\delta^{13}C$). This study used both barley and wheat/barley disomic chromosome addition lines grown under well-watered conditions in a polyethylene tunnel. Subsequently, Ellis et al. (1997) measured whole-shoot $\delta^{13}C$ on 57 double-haploids, from a cross between *H. vulgare* L. cv Lina and *H. spontaneum* HS92, grown in a hydroponic system under control and salt-stress conditions. In this work, one association between an AFLP marker and $\delta^{13}C$ was detected for the control conditions and nine AFLP markers were found associated with $\delta^{13}C$ measured on salt-stressed plants. The markers identified were located on chromosomes 7H, 2H, 4H, 6H and 5H. In the current study, we have also identified several regions associated with carbon isotope discrimination measured on mature grains. Despite common chromosomes associated with this trait in the two studies, we unfortunately can not compare the QTL positions precisely because of the lack of common markers.

Variation of Δ across environments: relationship with seasonal rainfall and water availability

As carbon isotope discrimination provides an integrative measure of photosynthetic activity, mainly regulated by stomatal aperture (Morgan et al. 1993; Reynolds et al. 2000), strong variations in climatic conditions are expected to have a significant influence on Δ values (Merah et al. 2001a). This was the case in the present study (Table 1). As two of the three environments were at the same site (M99rain and M99ir), the environmental factors that could be effectively compared were cumulative rainfall (including irrigation supplies), and water availability through R/ET. Ketata (1987) pointed out that seasonal rainfall in Syria accounted for up to 82% of cereal grain yield variation. The factorial regression performed in the present work, indicated that both total rainfall and R/ET had a significant impact on Δ, explaining mainly the environmental effect (Table 4). Considering the total growth cycle, seasonal rainfall contributed relatively more to the G × E interaction than to the environmental effect, whereas the contribution of R/ET in the interaction was weak. From heading to harvest, the two factors contributed highly to the environmental effect, confirming the impact of water moisture and water availability on traits measured under low-input conditions. The differences between the three environments influenced the QTLs detected in each (Table 2) and QTLs interacting with the environment were identified when all environments were considered together (Table 3A). A significant correlation was obtained between Δ values from M99rain and M99ir, but a highly significant environmental effect was still noticed (Table 1) between these two environments; while one QTL was found on chromosome 4H for the more-irrigated conditions, no QTL was significant for the rainfed environment (Table 2). In the same genomic region,

a peak which failed to meet the significance TS was however observed for the QTL × E effect, when the analysis with MQTL was performed (data not shown). These results underlined the impact of total rainfall and R/ET on the environmental effect, whereas the genotype by water availability interaction was present but less consistent. The influence of total rainfall and R/ET factors on Δ, particularly in the G × E interaction, must however be further studied with a larger number of environments.

Eight QTLs for Δ co-located with QTLs for agronomic and other adaptive traits

Eight regions controlling Δ variation identified in this study were congruent to QTLs previously identified in the same population, either for agronomic traits (Teulat et al. 2001c), or for traits related to plant water status and/or osmotic adjustment (OA) (Teulat et al. 2001a) (Tables 2 and 3).

Six regions controlling agronomic traits were co-located with QTLs for Δ. For example, one QTL previously associated with thousand-grain weight (Teulat et al. 2001c), on chromosome 2H, mapped near EBmac0684 which is linked to QTLs for Δ in the present study (Tables 2 and 3). On the same chromosome, close to BCD266, the QTLs interacting with environment for Δ co-located with a QTL controlling plant height across six environments. The QTLs for Δ located on chromosome 7H (nearby *acl3*) co-located with QTLs contributing to plant height across environments and the harvest index of M99rain. Two regions co-located with QTLs controlling heading date on chromosomes 3H and 5H, nearby Bmag0013 and Bmag0223 respectively. Finally, the region of chromosome 6H between BCD348B and CDO497, co-located with QTLs for several traits. In this area, excepted for the dry aerian biomass only identified in M99ir, the QTLs identified for plant height, thousand-grain weight and the number of fertile tillers presented main effects across environments (Teulat et al. 2001c).

Four QTLs controlling Δ also co-located with chromosomal regions where QTLs for physiological traits related to plant water status and/or OA, measured in controlled conditions, have been previously mapped (Teulat et al. 2001a). The first was the *acl3* area on chromosome 7H, where QTLs controlling relative water content (RWC) and leaf osmotic potential (ψ_π) measured under water stress conditions were identified (Teulat et al. 1998, 2001a). A large region of 2H (between BCD1069 and Bmag0125) where a QTL for Δ was mapped, corresponded to the area where several overlapping QTLs for characters such as factors affecting OA have been identified (Teulat et al. 2001a). Using the present map (Fig. 1), an additional QTL involved in OA variation was mapped (between O7.1 and EBmac0684) in the same area (Teulat, unpublished results). On chromosome 4H, the QTL identified for Δ for the irrigated conditions of M99 co-located with a QTL previously found for water-soluble carbohydrates (WSC) at full turgor, measured under well-watered conditions. In addition, the QTL mapped on the long arm of chromosome 1H (Table 3B) co-located with QTLs for ψ_π and ψ_π at full turgor, previously identified under well-watered conditions (Teulat et al. 2001a). Finally, two regions on chromosomes 7H (nearby *acl3*) and 2H (between O7.1 and Bmag0125), co-located with QTLs for Δ, traits related to plant water status and/or osmotic adjustment and agronomic traits. These regions are of interest in terms of plant breeding as they control both important drought-adaptive traits for cereals and yield components. Confirmation of the influence of these genomic regions by refining the map or observing similar effects in different populations, could help to elucidate the biological processes underlying complex traits such as yield or yield stability. For example, the three genomic areas controlling both plant height and Δ suggest a possible relationship between Δ and growth. Furthermore, the region of chromosome 2H between O7.1 and Bmag0125 containing overlapping QTLs for Δ, OA and WSC, could be involved in carbohydrate metabolism.

Conclusion

Interest in carbon isotope discrimination (Δ) as a selection criterion for drought-tolerance improvement has been largely documented in cereals, where it has been argued that molecular markers linked to genetic factors controlling Δ could enhance selection in breeding programs. The present work reports the first QTL study for carbon isotope discrimination measured in mature grains from plants grown in Mediterranean field conditions. The first results indicate that several chromosomal regions are involved in Δ variation. Total rainfall and R/ET have a high significant impact on the environmental effect acting on Δ, influencing QTL detection. Several QTLs for Δ overlap with QTLs for other physiological traits related to plant water status, osmotic adjustment and/or for yield components. The genetic and physiological basis of this co-localisation merits further investigation. In relation to this, field experiments conducted in North African countries are currently being analysed to identify QTL × E for grain yield and yield components, and for yield stability. Comparison of these regions with those involved in Δ variation could reinforce their potential value as targets for marker-assisted selection and could help to interpret complex relationships between physiological traits and yield stability in Mediterranean cereals.

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