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ORIGINAL PAPER

Quantitative trait loci for water-use efficiency in barley (*Hordeum vulgare* L.) measured by carbon isotope discrimination under rain-fed conditions on the Canadian Prairies

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Abstract Barley (*Hordeum vulgare* L.) yield is commonly limited by low rainfall and high temperature during the growing season on the Canadian Prairies. Empirical knowledge suggests that carbon isotope discrimination ($\Delta^{13}C$), through its negative relationship with water-use efficiency (WUE), is a good index for selecting stable yielding crops in some rain-fed environments. Identification of quantitative trait loci (QTL) and linked markers for $\Delta^{13}C$ will enhance its use efficiency in breeding programs. In the present study, two barley populations (W89001002003 × I60049 or W × I, six-row type, and Merit × H93174006 or M × H, two-row type), containing 200 and 127 recombinant inbred lines (RILs), were phenotyped for leaf $\Delta^{13}C$ and agronomic traits under rain-fed environments in Alberta, Canada. A transgressive segregation pattern for leaf $\Delta^{13}C$ was observed among RILs. The broad-sense heritability (H^2) of leaf $\Delta^{13}C$ was 0.8, and there was no significant interaction between genotype and environment for leaf $\Delta^{13}C$ in the W × I RILs. A total of 12 QTL for leaf $\Delta^{13}C$ were detected in the W × I RILs and 5 QTL in the M × H RILs. For the W × I RILs, a major QTL located on chromosome 3H near marker Bmag606 (9.3, 9.4 and 10.7 cM interval) was identified. This major QTL overlapped with several agronomic traits, with W89001002003 alleles favoring lower leaf $\Delta^{13}C$, increased plant height, and reduced leaf area index, grain yield, harvest index and days to maturity at this locus or loci. This major QTL and its associated marker, when validated, maybe useful in breeding programs aimed at improving WUE and yield stability of barley on the Canadian Prairies.

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Introduction

Drought continues to be a major constraint on the productivity of cereal crops and water deficit will increase in most arid and semi-arid regions under future climate-change scenarios (IPCC 2007; Wassmann et al. 2009). Agricultural production in Canada is mostly concentrated on the Prairies of western Canada (Canadian International Grains Institute (CIGI) 2004). The production of barley, one of the main crops grown in Canada, is centered in the three Prairie Provinces (Alberta, Saskatchewan and Manitoba), with an average of 12.3 million tonnes produced annually during 1986–2006 (FAOSTAT 2008). Both two- and six-row ear types of barley are commonly grown under rain-fed conditions for malting, livestock feed and food. During the short and dry growing seasons, sometimes with terminal heat stress (Anyia et al. 2008), barley relies on stored soil moisture and limited rainfall within the growing season. The unpredictable occurrence of drought can cause highly unstable barley yield across years. Therefore, breeding for drought-tolerant and water-use efficient

varieties has been a critical area of agricultural research in Canada.

Water-use efficiency (WUE) has been proposed as a criterion for yield improvement under drought (Condon and Richards 1992; Condon et al. 2002; Rebetzke et al. 2002; Richards et al. 2002), and improved WUE can increase yield in certain environments. However, the application of WUE in breeding programs has been largely limited by the time-consuming and expensive screening process under field conditions for large populations. Carbon isotope discrimination ($\Delta^{13}C$), through its negative relationship with transpiration efficiency has been demonstrated to be a simple but reliable measure of WUE (Farquhar et al. 1982; Farquhar and Richards 1984), and their negative correlation has been used for indirect selection of WUE under selected environments (Cattivelli et al. 2008).

Selection efficiency of leaf $\Delta^{13}C$ could be enhanced with a better understanding of its genetic control. The advancement of DNA-based molecular markers and computational methods in the late 1980s and 1990s has greatly revolutionized the dissection of quantitative trait inheritance and genetic improvement of yield in dry environments (Baum et al. 2007). The first quantitative trait loci (QTL) identified for $\Delta^{13}C$ was reported in tomato (*Lycopersicon esculentum* and *L. pennellii*) by Martin and Nienhuis (1989) and subsequently QTL for $\Delta^{13}C$ have been reported in *Arabidopsis thaliana* (Hausmann et al. 2005; Juenger et al. 2005), barley (grain) (Teulat et al. 2002), cotton (*Gossypium hirsutum* and *G. barbadense*) (Saranga et al. 2001), rice (*Oryza sativa* L.) (Laza et al. 2006; Takai et al. 2006; This et al. 2010; Xu et al. 2009), soybean (*Glycine max* L.) (Specht et al. 2001) and bread wheat (*Triticum aestivum* L.) (Rebetzke et al. 2008). Ellis et al. (1997) discovered several AFLP (amplified fragment length polymorphisms) markers associated with whole-shoot $\Delta^{13}C$ of barley measured on 57 doubled haploids (DHs) from a cross between *H. vulgare* L. cv Lina and *H. vulgare* ssp. *spontaneum* HS92 in a hydroponic system under controlled and salt-stress conditions. The first QTL study for grain $\Delta^{13}C$ in barley was by Teulat et al. (2002) using 167 recombinant inbred lines (RILs) derived from two-row barley cultivars Tadmor and Er/Apm in three Mediterranean field environments, with a total of ten QTL identified in the study.

Plant parts sampled for $\Delta^{13}C$ analysis can be awn, grain, leaf, sheath, stem or root, each with its own characteristic $\Delta^{13}C$ value and potential advantages for evaluating WUE under different environments. Leaf $\Delta^{13}C$ during the stem elongation stage, which is regarded as the formation of yield potential (Anyia et al. 2008), could reflect the integrated WUE and the vegetative establishment. Condon and Richards (1992) proposed that it would be most effective to assess $\Delta^{13}C$ at early stages in plant development under well-watered conditions. In addition, leaf $\Delta^{13}C$ measured before anthesis can enable selection and crosses of varieties to be made within the same season, thereby speeding up the breeding process. In our study penultimate leaf was sampled for $\Delta^{13}C$ analysis to avoid damaging of the flag leaf (Sicher 1993; Thorne 1965; Xue et al. 2008). According to Jiang et al. (2006), values of $\Delta^{13}C$ from different plant parts (flag leaf, awn and grain) were on average higher for six-row barley than those of two-row barley, suggesting a higher WUE of two-row barley compared with six-row barley under both irrigated and non-irrigated field conditions. So far, there has been no report on QTL analysis of $\Delta^{13}C$ on barley leaves or on two- and six-row barley examined under the same rain-fed environments.

In this study, two- and six-row barley RIL populations were grown under rain-fed conditions, and the penultimate leaves at stem elongation stage were sampled for $\Delta^{13}C$ analysis and QTL mapping. The objectives of the present study were to: (1) to determine the chromosomal regions and phenotypic effects of QTL associated with variations in leaf $\Delta^{13}C$ as well as agronomic traits; (2) to identify the common QTL regions across environments and populations; and (3) to dissect the genetic control of leaf $\Delta^{13}C$ and potential alleles for further WUE improvement in barley.

Materials and methods

Field experiments and climatic conditions

The experiments were conducted at Lacombe (52°28′N, 113°45′W, 847.3 m altitude), having an Orthic Black Chernozemic soil (Canadian system of soil classification), Vegreville (53°31′N, 112°6′W, 639.3 m altitude), having the Malmo series of an Eluviated Black Chernozemic soil, and Castor (52°8′N, 111°54′W, 807.7 m altitude), with a Dark Brown Chernozemic soil, in Alberta, Canada, under rain-fed conditions. The three sites were considered three different environments characterized by distinct soil moisture conditions with Castor as the driest site and Lacombe as the wettest site. The average annual precipitation and within-season rainfall (June–August) from 1977 to 2007 was 340 ± 89 and 172 ± 67 mm at Castor compared with Vegreville which had 382 ± 62 and 193 ± 52 mm, and Lacombe which had 440 ± 84 and 230 ± 63 mm, respectively (AgroClimatic Information Service (ACIS) 2009; Environment Canada 2009).

Mapping populations

One hundred and six diverse genotypes (advanced lines and commercial varieties) of spring barley were screened for variation in leaf $\Delta^{13}C$ at two field locations in Vegreville

and Lacombe in 2005. Among these genotypes, two-row genotypes (Merit, released by Busch Agricultural Resources Incorporation in 1997 and H93174006, developed at the Field Crop Development Centre, Lacombe, Alberta, and released in 2008 as TR05671) and six-row barley (W89001002003, hereafter referred to as W89 and I60049) showing contrasting levels (low vs. high) of leaf $\Delta^{13}C$ were selected as parental materials for mapping population development after additional greenhouse and field experiments confirmed their leaf $\Delta^{13}C$ rank stability (Fig. 1). Two RIL mapping populations were developed, with one cross between W89 and I60049 (hereafter referred to as W × I) and the other cross between Merit and H93174006 (hereafter referred to as M × H) in the field during the summer of 2006. The parents with lowleaf-$\Delta^{13}C$ (Merit and W89) were used as females since previous experience indicated that maternal inheritance may have an influence on $\Delta^{13}C$ and WUE traits (Dr. Richard Richards, CSIRO, personal communication). All F_1 seeds from each cross were planted in bulk in the field (F_2 populations) at Lacombe in 2007 and evaluated for segregation. The single seed descent (SSD) approach was used to advance the populations from F_3 to F_4 generation in the greenhouse at Lacombe during 2007 and 2008. The F_5 seeds from each F_4 plant were bulked and advanced to produce enough seeds for $F_{5:6}$ generation field trials.

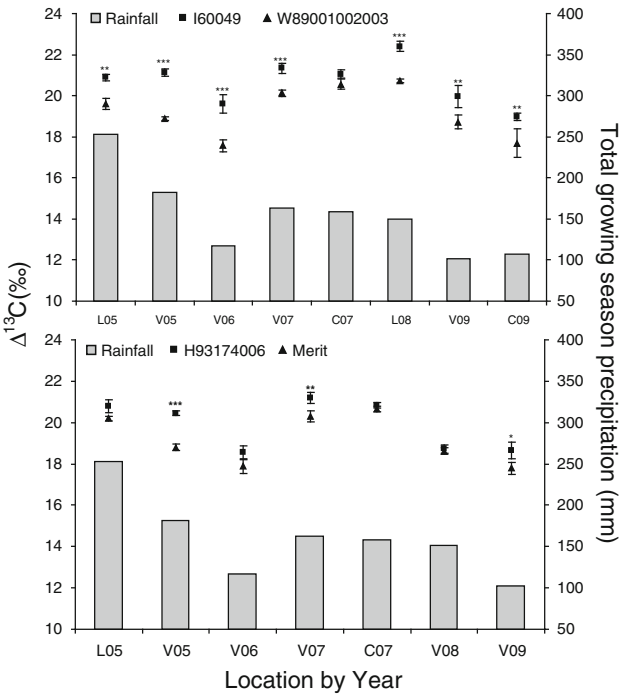

Fig. 1 Leaf carbon isotope discrimination ($\Delta^{13}C$) of parental lines W89001002003 and I60049 (*top*), and Merit and H93174006 (*bottom*) across locations and years that were characterized by different total growing season rainfall. Significant differences of leaf $\Delta^{13}C$ between parental lines are indicated by $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. L05, L08, C07, C09, V05, V06, V07, V08 and V09 stand for location by year, for Lacombe-2005, Lacombe-2008, Castor-2007, Castor-2009, Vegreville-2005, Vegreville-2006, Vegreville-2007, Vegreville-2008 and Vegreville-2009, respectively

Experiment design

The W × I mapping population

Two hundred F_5 RILs and two parental lines (W89 and I60049) were planted at Lacombe in 2008 (L08), Castor in 2009 (C09) and Vegreville in 2009 (V09).

For L08, the experiment used a completely randomized design with one replicate of RILs and four replicates of parental lines. Seeds of each line were planted in two rows in 0.3 × 2 m plots with a spacing of 15 cm within rows and 35 cm between plots. Winter triticale 'Pika' was seeded as guard row between plots. Synthetic fertilizer mix (6-25-30) was applied at 112.5 kg ha^{-1} prior to seeding. Plots were seeded on May 15 (Table 1), and harvested on September 5. Herbicides were applied at the rate of 29.7 g ha^{-1} for 'Refine' (33.35% thifensulfuron methyl, 16.65% tribenuron methyl) and 988.4 mL ha^{-1} for 'MCPA' (500 g L^{-1} amine, 500 g L^{-1} ester, 400 g L^{-1} K-salt and 300 g L^{-1} Na-salt) during the vegetative stage to control weed species.

For C09 and V09, the experiments used a randomized complete block design with three replicates of each $F_{5:6}$ RILs and parental lines. Seeds of each line were planted in four rows in 1 × 2 m plots with a spacing of 20 cm within rows and 20 cm between plots. Spring wheat 'AC Crystal' was seeded as a guard row between plots. Fertilizer was applied prior to seeding at a rate of 22.5 kg ha^{-1} P_2O_5 at Vegreville, and 31.4 kg ha^{-1} N and 18 kg ha^{-1} P_2O_5 at Castor based on results of soil test. Seeds were sown on May 13 at Castor and on May 22 at Vegreville, and both sites were harvested during the last week of September. Weeds were controlled manually at the 3-leaf stage (BBCH 13) (Zadoks et al. 1974) at Castor. For Vegreville, 'Round-up Weathermax' (540 g L^{-1} glyphosate) was sprayed at 1.66 L ha^{-1} before emergence on May 24.

Table 1 Monthly precipitation (mm) over the growing season for the three field locations during 2008 and 2009

Location, year	Sowing date	Precipitation (mm)			
		June	July	August	Total
Lacombe, 2008	May 15	45.8	48.8	55.5	150.1
Vegreville, 2008	May 16	42.4	44.6	64.3	151.3
Vegreville, 2009	May 22	32.2	44.6	25.2	102.0
Castor, 2009	May 13	21.5	33.8	51.9	107.2

The M × H mapping population

One hundred and twenty-seven F_5 RILs and two parental lines (Merit and H93174006) were planted at Vegreville in 2008 (V08), as well as C09, and V09.

For V08, the experiment was a completely randomized design with one replicate of RILs and four replicates of parental lines. 200 seeds of each line were sown in 2 rows of a 2-m-long plot with a spacing of 20 cm between rows and 60 cm between plots. Fertilizer was applied at seeding by adding 38.25 kg P_2O_5 ha^{-1}, and 61.88 kg N ha^{-1}. Seeds were sown on May 16 and plants were harvested on September 18. Herbicide 'Round-up Weathermax' was sprayed at 1.66 L ha^{-1} before seeding, and 'Achieve 40DG' and 'Buctril M' were applied on June 4 at 494.2 g ha^{-1} and 988.4 mL ha^{-1}, respectively.

For C09 and V09, the experiment design was the same as for the W × I population described above. Seeds were sown on May 15 at Castor and on May 25 at Vegreville, and both sites were harvested during the last week of September.

Phenotypic data collection

Carbon isotope discrimination analysis

At the stem elongation stage (BBCH 36–39), five fully expanded penultimate leaves per plot of each mapping population at each environment (L08, V08, C09 and V09) were randomly collected, bulked, and dried in an oven at 70°C for 48 h. Dried leaf samples were ground with a ball mill (Spex SamplePrep 8000D Mixer, Metuchen, NJ, USA) to fine powder and analyzed for carbon isotope composition ($\delta^{13}C$) using a continuous-flow stable isotope ratio mass spectrometer (Thermo Finnigan Mat Gmbh, Bremen, Germany). The $\delta^{13}C$ was calculated as:

$$\delta^{13}C\ (‰) = \left(R_{sample}/R_{reference} - 1\right) \times 1,000$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ measured in the plant material and the reference, respectively, and the reference material used was the belemnite carbonate standard (PDB) from the Pee Dee Formation. The $\Delta^{13}C$ was calculated according to Farquhar et al. (1989) as:

$$\Delta^{13}C(‰) = \left[\left(\delta^{13}C_a - \delta^{13}C_p\right)/\left(1 + \delta^{13}C_p/1,000\right)\right] \times 1,000$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ refer to the C isotope ratios of atmospheric CO_2 (−8.0 ‰) and plant, respectively.

Leaf area index (LAI)

LAI was measured for the W × I population in the 2009 field trials using a LAI-2000 Plant Canopy Analyzer (Li-Cor, Lincoln, NE, USA). A measurement cycle consisted of four below-canopy readings and one reference measurement. Reference measurements were collected above canopy level of each experimental plot (between 1 and 1.5 m above ground) at the beginning of each cycle. The below-canopy measurements were carried out at a diagonal transect between rows in each plot to improve the spatial average. The fish-eye lens of the instrument was covered with a view cap with a 45° opening, so that the reference measurements were not influenced by the operator and the angle of the sun (Li-Cor 1992). Measurements were taken at the stem elongation stage (BBCH 36–39) and grain filling stage (BBCH 71–75) under cloudy conditions.

Plant height, days to maturity, aerial biomass and grain yield

Plant height was measured from the ground level to the tip of the spike on five randomly selected plants per plot at the physiological maturity stage (BBCH 89) in the 2009 field trials for the W × I population. Days to maturity was recorded as the number of days from sowing till the stage that 90% of plants in the plot reached maturity (BBCH 91–92), this trait was only recorded at V09 for the W × I population. Biomass and grain yield were determined from 1 m^2 sub-plots. Grain was threshed, cleaned and air-dried for 2 weeks before weighing, with all grain yields expressed as oven-dried values, after the moisture content of the air-dried grains was determined. Harvest index (HI) was calculated as HI = grain yield/total aboveground biomass.

Molecular marker assay

Assay for the W × I population

Parents (W89 and I60049) were screened for polymorphism with a total of 516 simple sequence repeat (SSR) markers from the published literature (Chaabane et al. 2009; Karakousis et al. 2003; Li et al. 2003; Liu et al. 1996; Ramsay et al. 2000; Thiel et al. 2003; Varshney et al. 2007). Polymorphic SSR markers were chosen for scoring the entire W × I population.

Young leaf tissues from each plot in the field trials were collected for DNA extraction. Genomic DNA was isolated as described by Saghai-Maroof et al. (1984), and quantified using a spectrophotometer (NanoDrop ND-1000 Spectrophotometer, Wilmington, Delaware USA), and qualified by electrophoresis. Regular PCR (polymerase chain reaction) was carried out in 20-μL reactions containing 5× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM forward prime, 0.2 μM reverse primer, 1 U of *Go Taq* Flexi DNA polymerase (Promega Corporation, Wisconsin, USA),

deionized water, and 40 ng genomic DNA in 96-well plates using a Eppendorf Mastercyler (Hamburg, Germany). The SSRs amplification was performed using three PCR conditions: (1) a "touchdown" PCR consisting of 18 cycles of 1 min denaturing at 94°C, 30 s annealing at 62°C or 69°C and 1 min extension at 72°C. Annealing temperatures were decreased by 0.5°C per cycle, depending on the melting temperature (T_m) of primer pairs used, either from 62 to 53°C or from 69 to 60°C. The reaction continued for 30 additional cycles with 1 min at 94°C, 1 min at 53 or 60°C, 1 min at 72°C, and eventually 5 min at 72°C as the final extension step; (2) a regular PCR was performed with 2 min at 94°C, followed by 35 cycles at 94°C for 30 s, 30 s at optimal annealing temperature (ranging from 53 to 58°C), 1 min at 72°C, and ended with 5 min at 72°C, and (3) condition was the same as those described by Röder et al. (1995), 35 cycles were performed with 1 min at 96°C, 1 min at 60°C (varies with the T_m of primers) and 2 min at 72°C, and a final elongation step of 10 min at 72°C, in some cases the $MgCl_2$ concentration was increased to 2.5 mM.

Primers with similar annealing temperatures and non-overlapping ranges of amplified fragment sizes were combined in the same reaction. Amplified PCR products were separated on 2.5% agarose gels in 1× TAE buffer, stained with ethidium bromide and photographed. The entire population was genotyped for SSR markers using a multicapillary (12-channel) electrophoresis system (HAD-GT12, eGene, Irvine, CA, USA).

Assay for the M × H population

A total of 373 SSR and 72 STS (sequence-tagged site) markers were screened for polymorphism between parental lines (Merit and H93174006). To get additional genomic coverage, extracted DNA of the RILs and parental lines were sent to Triticarte Pty Ltd, Canberra, Australia (http://www.triticarte.com.au/) for Diversity Arrays Technology (DArT®) genotyping, which is a novel genotyping method and hybridization-based technology as described by Wenzl et al. (2004).

QTL analysis and map construction

The segregating patterns of SSR amplifications at polymorphic loci in all lines were compared to parental lines, and scored as maternal (1), paternal (2) or heterozygous/missing (3) of an allele. Segregation markers in the RILs were examined by the Chi-square (χ^2) test for goodness of fit. The expected ratio of individuals in each genotypic class (maternal or paternal) was 1:1 in the RILs. Mapmaker v3.0 (Lander et al. 1987) was used to assign markers to linkage groups at a LOD (logarithm of odds ratio) threshold of 3.0 with a maximum Kosambi distance of 30 centiMorgans (cM), and markers were assembled to initial linkage groups, then the LOD score was reduced to bridge some intervals. The position and order of markers in linkage groups were compared with published barley consensus map (Varshney et al. 2007; Wenzl et al. 2006). Genetic linkage maps were imported into Windows QTL Cartographer V2.5 (Wang et al. 2010) to locate putative QTL for all traits using composite interval mapping method (CIM) (Zeng 1994). A threshold score for the QTL was determined to be 2.0 by 1,000 time permutations using the Zmapqtl program (Churchill and Doerge 1994). A confidence interval for QTL location was determined by a one-LOD drop from the peak position (Flint-Garcia et al. 2003), and overlapping confidence intervals were used to determine common QTL across environments. The final linkage maps were drawn using the MapChart software (Voorrips 2002).

Data analysis

Data were analyzed using version 9.1 of the SAS software (SAS Institute, Inc., Cary, NC). Each location–year combination of the field trials (L08, V08, C09, and V09) was treated as a single environment. Analysis of variance (ANOVA) was performed for each environment as well as combined environments. Variance components were estimated using the restricted maximum likelihood (REML) method of PROC MIXED.

According to Hanson et al. (1956), the broad-sense heritability (H^2) for leaf $\Delta^{13}C$ across environments was estimated as:

$$H^2 = \sigma_g^2 / \left[\sigma_g^2 + \sigma_{g\times e}^2 / e + \sigma_r^2 / (r \times e) \right]$$

where variances were due to genotype (σ_g^2), genotype × environment ($\sigma_{g\times e}^2$), and residual (σ_r^2), e is the number of environments, and r is the number of replicates.

Homogeneity of variance and normality of distribution were tested before analysis of variance. Correlation analyses (PROC CORR) were performed to detect the relationships among traits, with means of each RIL combined over the environments. Pearson correlation coefficients were calculated between environments for leaf $\Delta^{13}C$, with means of each RIL based on each environment. An α value of 0.05 was chosen to indicate statistical significance.

Results

Environments and leaf $\Delta^{13}C$

Across eight different environments (location–year combinations) in this study, the leaf $\Delta^{13}C$ of W89 was consistently lower than that of I60049, except at Castor-2007 (Fig. 1).

Although the leaf $\Delta^{13}C$ of Merit was only significantly lower than that of H93174006 at three out of seven environments, trends of difference were still observed across environments (Fig. 1). The correlation analysis showed that leaf $\Delta^{13}C$ was significantly related to rainfall in June (data not shown, $r = 0.48$, $n = 122$, $p < 0.001$) and total within-season precipitation (from June to August) (data not shown, $r = 0.42$, $n = 122$, $p < 0.001$). Significant differences were observed for leaf $\Delta^{13}C$ between environments for both populations (Tables 2, 3).

For the W × I population, leaf $\Delta^{13}C$ of RILs were significantly higher in L08, followed by V09 and then C09 (Table 2). For the M × H population, leaf $\Delta^{13}C$ for the experiments conducted in Vegreville was significantly higher in 2008 than in 2009. Positive and significant correlations of leaf $\Delta^{13}C$ were observed between C09 and V09 (Fig. 2a, $r = 0.32$, $n = 200$, $p < 0.001$), C09 and L08 (Fig. 2a, $r = 0.32$, $n = 200$, $p < 0.001$), L08 and V09 (Fig. 2a, $r = 0.21$, $n = 200$, $p = 0.002$). The extreme genotypes differed by 3.38‰ at L08, 3.82‰ at C09 and 4.25‰ at V09. For the M × H population, a positive correlation of leaf $\Delta^{13}C$ was observed between V08 and V09 (Fig. 2b, $r = 0.16$, $n = 127$, $p = 0.08$). The extreme genotypes differed by 3‰ at V08 and 3.14‰ at V09.

Leaf $\Delta^{13}C$ and agronomic traits

The two populations differed in leaf $\Delta^{13}C$, with the M × H population producing a significantly lower leaf $\Delta^{13}C$ than the W × I population at V09 (data not shown, $p < 0.001$). Leaf $\Delta^{13}C$ of progeny within each population were significantly different among the environments studied (Tables 2, 3). The Shapiro–Wilk test showed that leaf $\Delta^{13}C$ of both populations had typically normal distributions under all environments (Fig. 3). Most of the leaf $\Delta^{13}C$ values for the RILs were between those of the parents in each field trial, but transgressive segregation was also noted as some progeny extremes for leaf $\Delta^{13}C$ exceeded parental values. Some of the extreme RILs that showed transgressive segregation for leaf $\Delta^{13}C$ from the W × I population (such as '176' and '191') and the M × H population (such as '43', '110' and '162') were consistent across all locations. The H^2 for leaf $\Delta^{13}C$ was high (0.80) for the W × I population across all environments. Under a single environment (C09 or V09), the H^2 for both populations reached 0.80 (data not shown).

Biomass, yield, HI, plant height, LAI, and days to maturity all showed continuous variation (Tables 2, 3). For the W × I population, ANOVA revealed significant variability among the 200 RILs for a range of agronomic traits (Table 2) except LAI measured at grain filling stage (LAI-G). Interactions between genotype and environment (G × E) were not significant for leaf $\Delta^{13}C$, LAI-G, and HI. For the M × H population, genotypic variation was not significant for biomass, yield and HI (Table 3), and G × E was only significant for leaf $\Delta^{13}C$.

For the W × I population, leaf $\Delta^{13}C$ showed significant positive relationships with LAI at stem elongation stage (LAI-S), biomass, yield and HI at both C09 and V09 (Table 4). For the M × H population, there was also significant positive correlation between $\Delta^{13}C$ and biomass at V08 and V09 (Table 5), and significant positive relationship between $\Delta^{13}C$ and grain yield was only observed at V08.

Linkage map construction

Screening of the parental lines with SSR markers revealed a low level of polymorphism between parental lines. For the W × I population, only 148 (28.7%) out of 516 screened markers could be used for scoring the 200 RILs. For the M × H population, 55 (12.9%) out of 373 SSR markers and 196 (39.3%) out of 499 DArT markers were polymorphic between Merit and H93174006. In total, 104 SSR markers were mapped for the W × I population, and 209 loci were mapped for the M × H population. In general, there was good agreement for the marker order between maps from this study and maps published in the literature.

For the W × I population, the 104 SSR loci were grouped into ten linkage groups which were assigned to seven chromosomes (Fig. 4a) based on alignments from previously published barley consensus maps (Karakousis et al. 2003; Varshney et al. 2007). Chromosome (Chr.) 1H, 2H, 3H, 4H, and 6H were each represented by a single linkage group, whereas Chr. 5H and 7H were split into two and three linkage groups, respectively.

For the M × H population, the 209 loci, including 21 SSR and 188 DArT loci, were grouped into 17 linkage groups which were assigned to 7 chromosomes (Fig. 4b) according to previously published high-density consensus maps of barley (Varshney et al. 2007; Wenzl et al. 2006). Chr. 1H, 2H and 3H were split into three linkage groups, whereas Chr. 6H and 7H were split into four and two linkage groups, respectively, and Chr. 4H and 5H were each represented by a single linkage group.

QTL identified for leaf $\Delta^{13}C$

Table 6 describes the QTL identified for leaf $\Delta^{13}C$ in each environment separately. For the W × I population, a total of 12 QTL clustering in nine chromosomal regions and explaining from 3.6 to 22% of the phenotypic variation were identified for leaf $\Delta^{13}C$, with 5 QTL detected at L08, 3 QTL at C09 and 4 QTL at V09 by the CIM analysis. Eight of these 12 QTL reduced leaf $\Delta^{13}C$ and the W89 allele was

Table 2 Means, standard deviations (SD), range (minimum and maximum values) of the 200 recombinant inbred lines (RILs) and their parental lines from the six-row (W89001002003 × I60049) barley population for traits measured in three field trials

Trait	Environment	Parents				RILs						
		W89001002003		I60049								
		Mean	SD	Mean	SD	Mean	SD	Range	*E*	*G*	*G* × *E*	*H*
Leaf $\Delta^{13}C$ (‰)	L08	20.71	0.23	22.40	0.48	21.35	0.61	19.84–23.22	***	***	ns	0.80
	C09	17.69	0.98	18.99	0.26	18.53	0.61	16.48–20.30				
	V09	18.72	0.56	19.97	0.95	19.71	0.65	17.94–22.19				
LAI-S (m^2 m^{-2})	C09	1.39	0.30	1.37	0.55	1.37	0.46	0.40–2.84	***	*	*	0.61
	V09	4.52	0.79	4.60	1.26	3.96	0.96	1.95–6.96				
LAI-G (m^2 m^{-2})	C09	1.34	0.19	1.71	0.14	1.63	0.33	0.82–2.74	***	ns	ns	0.63
	V09	4.54	0.59	4.89	1.02	4.34	0.89	2.30–7.04				
Plant height (cm)	C09	38	2.1	47	2.6	47	7.0	29–69	***	***	***	0.82
	V09	67	7.2	73	6.5	76	13.0	42–105				
Days to maturity	V09	88	2.7	91	1.0	87	3.7	73–92	–	***	–	–
Biomass (g m^{-2})	L08	1,512	100.5	1,229	60.4	1,650	354.5	1,072–3,243	***	***	***	0.75
	C09	236	22.9	265	43.3	261	56.1	143–520				
	V09	1,038	120.3	987	190.6	828	195.1	410–1518				
Grain yield (g m^{-2})	L08	701	39.6	513	36.0	708	153.7	412–408	***	***	***	0.74
	C09	113	21.6	128	6.1	129	35.2	60–294				
	V09	529	69.5	521	90.7	424	114.0	180–834				
HI	L08	0.46	0.01	0.42	0.02	0.43	0.02	0.35–0.49	***	**	ns	0.75
	C09	0.48	0.04	0.49	0.06	0.49	0.06	0.30–0.66				
	V09	0.51	0.02	0.53	0.01	0.51	0.04	0.35–0.60				

E environment effect, *G* genotype effect, *G* × *E* interaction between environment and genotype, H^2 broad-sense heritability, *L08* Lacombe-2008, *C09* Castor-2009, *V09* Vegreville-2009, *LAI-S* leaf area index measured at stem elongation stage, *LAI-G* leaf area index measured at grain filling stage, – not available, *ns* not significant

*, **, *** indicate 5, 1, 0.1% significance level, respectively

Table 3 Means, standard deviations (SD), range (minimum and maximum values) of the 127 recombinant inbred lines (RILs) and their parental lines from the two-row (Merit × H93174006) barley population for traits measured in three field trials

Trait	Environment	Parents				RILs						
		Merit		H93174006								
		Mean	SD	Mean	SD	Mean	SD	Range	*E*	*G*	*G* × *E*	H^2
Leaf $\Delta^{13}C$ (‰)	V08	18.62	0.35	18.73	0.46	18.93	0.63	17.53–20.53	***	***	*	0.66
	V09	17.80	0.50	18.67	0.70	18.46	0.52	17.19–20.33				
Biomass (g m^{-2})	V08	362	16.6	401	36.2	495	131.6	292–896	***	ns	ns	0.69
	C09	366	100.7	170	9.1	273	80.2	92–595				
	V09	572	117.5	662	38.2	665	132.4	305–1,155				
Grain yield (g m^{-2})	V08	99	11.6	104	7.4	137	43.9	65–305	***	ns	ns	0.63
	C09	194	59.0	68	6.5	127	47.6	51–307				
	V09	282	62.8	331	32.8	324	63.0	151–566				
HI	V08	0.28	0.02	0.26	0.01	0.27	0.03	0.19–0.35	***	ns	*	0.66
	C09	0.53	0.04	0.40	0.04	0.46	0.07	0.23–0.59				
	V09	0.49	0.01	0.50	0.03	0.49	0.03	0.34–0.66				

E environment effect, *G* genotype effect, *G* × *E* interaction between environment and genotype, *V08* Vegreville-2008, *C09* Castor-2009, *V09* Vegreville-2009, *ns* not significant

*, **, *** indicate 5, 1, 0.1% significance level, respectively

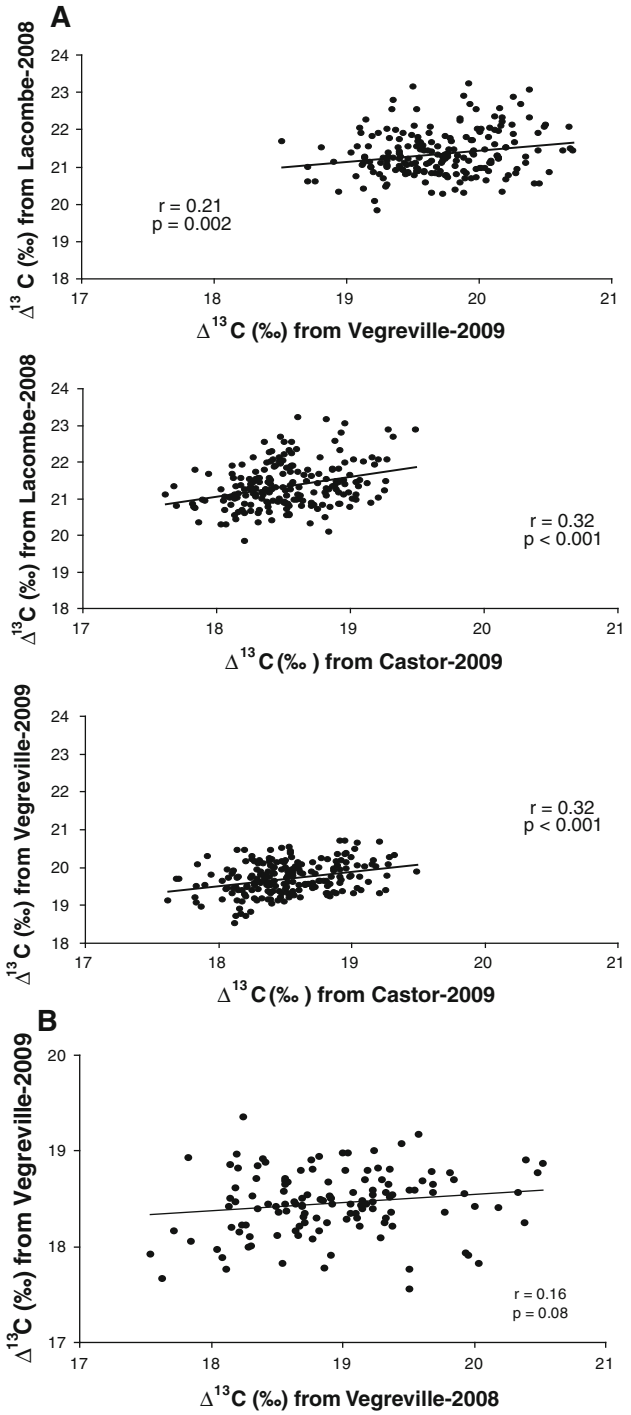

Fig. 2 **a** Correlation between leaf carbon isotope discrimination ($\Delta^{13}C$) of 200 recombinant inbred lines (RILs) from the six-row (W89001002003 × I60049) population measured at Lacombe in 2008, Castor in 2009 and Vegreville in 2009. **b** Correlation between leaf carbon isotope discrimination ($\Delta^{13}C$) of 127 recombinant inbred lines (RILs) from the two-row (Merit × H93174006) population measured at Vegreville in 2008 and 2009

associated with low leaf $\Delta^{13}C$. One QTL located on Chr. 3H near SSR marker Bmag606 (designated as ΔC3.1-WI) was detected consistently in all three environments. This QTL conferred main effect across all environments, with LOD values of 10.81, 5.86 and 5.27, explaining 22, 14.4 and 11% of the phenotypic variance in leaf $\Delta^{13}C$ at L08, C09 and V09, respectively. At this locus, leaf $\Delta^{13}C$ value was reduced by the W89 allele (Table 6).

For the M × H population, a total of 5 QTL clustering in three chromosomal regions and explaining from 8.6 to 11.7% of the phenotypic variation were detected by the CIM analysis for leaf $\Delta^{13}C$, with 3 QTL at V08 and 2 QTL at V09. Two QTL were detected consistently in two environments with one QTL located at Chr. 6H near DArT marker bPb1212 (designated as ΔC6.1-MH), explaining 11.2 and 10.8% of the phenotypic variance in leaf $\Delta^{13}C$ at V08 and V09, respectively, and the other QTL on Chr. 7H close to DArT marker bPb9898 (designated as ΔC7.1-MH), explaining 9.9 and 8.6% of leaf $\Delta^{13}C$ at V08 and V09, respectively. All of the five QTL increased leaf $\Delta^{13}C$ value with allele from Merit (Table 6).

QTL identified for agronomic traits

As listed in Table 7; Fig. 4a, b, a total of 38 QTL were identified by CIM analysis for six agronomic traits under each environment.

Leaf area index

LAI was measured only in the W × I population at C09 and V09, and the QTL for LAI were only found at V09. Two QTL for LAI-S and four QTL for LAI-G were detected. One QTL for LAI-S was co-located with QTL for LAI-G which was positioned on Chr. 3H near marker GBM1405. One QTL for LAI-G co-located with ΔC3.1-WI, with W89 allele responsible for reduced LAI at this locus (Table 7).

Plant height

Plant height was measured only in the W × I population. Eleven QTL were associated with plant height. Eight QTL affecting plant height were detected at C09, among those, three QTL were also identified at V09. The QTL with the largest effect on plant height was co-located with ΔC3.1-WI, explaining 27.4% of the phenotypic variance in plant height at V09, and this QTL was detected at C09 as well. Increased plant height was associated with the W89 allele at this locus (Table 7).

Days to maturity

Days to maturity was recorded in the W × I population only at V09. Five QTL were detected for days to maturity. One QTL located on Chr. 3H, explaining 16% of the

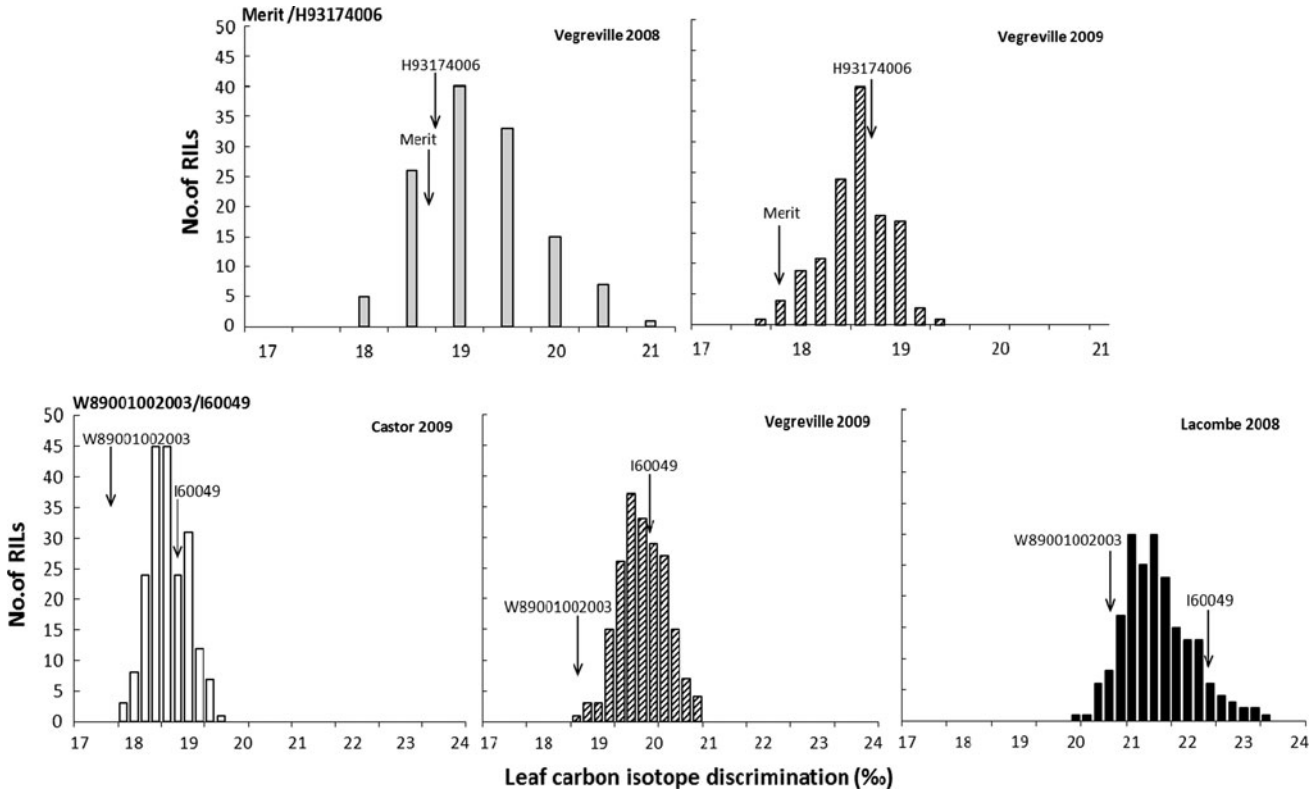

Fig. 3 Frequency distribution of mean leaf carbon isotope discrimination ($\Delta^{13}C$) measured on the recombinant inbred lines (RILs) from the two-row (Merit × H93174006) and the six-row (W89001002003 × I60049) mapping populations grown at Lacombe and Vegreville in 2008, and Castor and Vegreville in 2009. Parental means are indicated for each environment

Table 4 Correlations among leaf carbon isotope discrimination ($\Delta^{13}C$), leaf area index at stem elongation stage (LAI-S), leaf area index at grain filling stage (LAI-G), plant height (PH), biomass, yield, harvest index (HI) and days to maturity at Castor and Vegreville in 2009 for the six-row (W89001002003 × I60049) barley population

	Leaf $\Delta^{13}C$	LAI-S	LAI-G	PH	Biomass	Yield	HI
Castor-2009							
LAI-S	0.20**						
LAI-G	0.13ns	0.41***					
PH	–0.002ns	0.26***	0.30***				
Biomass	0.31***	0.45***	0.49***	0.29***			
Yield	0.28***	0.45***	0.40***	0.21**	0.78***		
HI	0.20**	0.24***	0.07ns	–0.12ns	0.28***	0.59***	
Vegreville-2009							
LAI-S	0.28***						
LAI-G	0.35***	0.77***					
PH	0.11ns	0.40***	0.50***				
Biomass	0.44***	0.62***	0.63***	0.52***			
Yield	0.46***	0.56****	0.58***	0.38***	0.96***		
HI	0.30***	0.11ns	0.13ns	–0.26***	0.36***	0.58***	
Days to maturity	0.11ns	0.29***	0.35***	0.06ns	0.28***	0.29***	0.13ns

ns not significant, – no records for days to maturity for Castor-2009

*, **, *** indicate 5, 1, 0.1% significance level, respectively

Table 5 Correlations among leaf carbon isotope discrimination ($\Delta^{13}C$), biomass, yield and harvest index (HI) at Vegreville in 2008 and 2009 for the two-row (Merit × H93174006) barley population

	Leaf $\Delta^{13}C$	Biomass	Yield
2008			
Biomass	0.65***		
Yield	0.61***	0.95***	
HI	0.20*	0.31***	0.58***
2009			
Biomass	0.28**		
Yield	0.02ns	0.34***	
HI	−0.08ns	0.001ns	0.94***

ns not significant

*, **, *** indicate 5, 1, 0.1% significance level, respectively

phenotypic variance in days to maturity with a LOD score of 10.78, and this QTL was also co-located with ΔC3.1-WI. Another QTL on Chr. 5H near marker Bmac113 was co-located with QTL for leaf $\Delta^{13}C$ (designated as ΔC5.1-WI), biomass and grain yield. The W89 allele of all QTL detected at V09 contributed to early maturity (Table 7).

Total aboveground biomass

For the W × I population, four QTL associated with total aboveground biomass were found. Only one QTL was detected at C09. The three QTL detected at V09 explained from 3.7 to 6.3% of the phenotypic variance in total aboveground biomass, with LOD scores ranging from 2.21 to 3.76. For the M × H population, only two QTL were detected, with one QTL at V08 and one at C09. Both loci increased total biomass with allele from Merit (Table 7).

Grain yield

For the W × I population, variation for grain yield was associated with six QTL that clustered into five chromosomal regions. On Chr. 5H near marker GBM5008, overlapping QTL from both locations were detected. This QTL interval included QTL for biomass, HI, and LAI. The W89 allele was associated with increased grain yield, biomass, HI and LAI at this locus (Table 7). One QTL on Chr. 5H near marker Bmag323 was co-located with QTL for leaf $\Delta^{13}C$ and biomass, with increased grain yield from the I60049 allele (Table 7). One QTL detected at C09 was co-located with ΔC3.1-WI, explaining 11.4% of the phenotypic variance in grain yield at a LOD value of 4.42, with the W89 allele responsible for reduced grain yield at this locus (Table 7).

For the M × H population, three QTL associated with grain yield were identified. One QTL was co-located with ΔC6.1-MH. Another QTL on Chr. 7H near marker bPb6821 overlapped with QTL for grain yield, biomass and ΔC7.1-MH. Both of these QTL (near bPb1212 and bPb682) increased the grain yield by the Merit allele (Table 7).

Fig. 4 **a** A linkage map of barley based on 200 $F_{5:6}$ recombinant inbred lines (RILs) from the cross between W89001002003 and I60049 constructed with 104 SSR markers. *Numbers on the left* of linkage groups indicate the cumulative map distances in cM (Kosambi). Marker loci are shown on the *right* of linkage groups. Co-segregating markers are listed next to each other in a *vertical line* on the *right side* of the linkage group. Markers with segregation distortion are indicated by $*p < 0.05$, $**p < 0.01$ and $p < ***0.001$. QTL confidence intervals (peak LOD scores minus one) are shown to the *right* of the linkage group *bar* with QTL for carbon isotope discrimination (ΔC) indicated by *black rectangles* and all others indicated by *white rectangles* as follows: leaf area index during stem elongation stage (*LS*), leaf area index during grain filling stage (*LG*), plant height (*PH*), days to maturity (*DM*), aboveground biomass (*AB*), grain yield (*GY*) and harvest index (*HI*). *L08*, *C09* and *V09* stand for location by year, for Lacombe-2008, Castor-2009 and Vegreville-2009, respectively. **b** A linkage map of barley based on 127 $F_{5:6}$ recombinant inbred lines (RILs) from the cross between Merit and H93174006 constructed with 21 SSR and 156 DArT markers. *Numbers on the left* of linkage groups indicate the cumulative map distances in cM (Kosambi). Marker loci are shown to the right of linkage groups. Co-segregating markers are listed next to each other in a *vertical line on the right side* of the linkage group. Markers with segregation distortion are indicated by with $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. QTL confidence intervals (peak LOD scores minus one) are shown to the *right* of the linkage group bar with QTL for carbon isotope discrimination (Δ*C*) indicated by *black rectangles* and all others indicated by *white rectangles* as follows: aboveground biomass (*AB*), grain yield (*GY*) and harvest index (*HI*). *C09*, *V08* and *V09* stand for location by year, for Castor-2009, Vegreville-2008 and Vegreville-2009, respectively

Harvest index

For the W × I population, only two QTL were detected for HI. One QTL on Chr. 5H was co-located with QTL for LAI-G, biomass and grain yield with increased HI from the W89 allele. The other QTL for HI on Chr. 3H was mapped around the same position with QTL for LAI-G, grain yield, plant height and ΔC3.1-WI.

For the M × H population, four QTL for HI were identified on Chr. 2H. One QTL near marker bPb4293 was co-located with the QTL for grain yield, which reduced the HI value by the Merit allele (Table 7).

Discussion

Leaf $\Delta^{13}C$ and rainfall across environments

Changes in the leaf $\Delta^{13}C$ of parental lines across years and locations were consistent with trends of the total growing

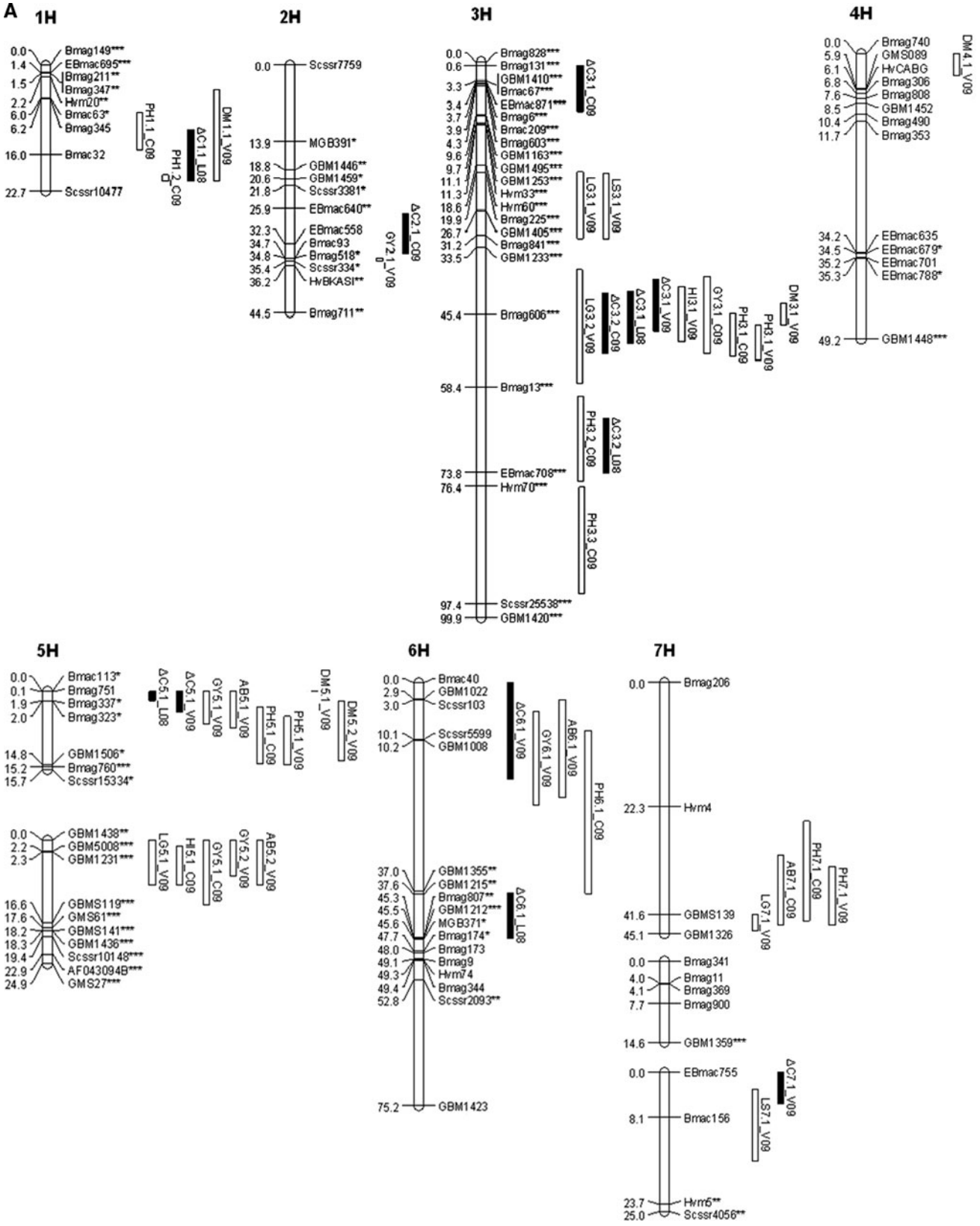
A
1H
0.0 Bmag149***
1.4 EBmac695***
1.5 Bmag211**
Bmag347**
2.2 Hvm20**
6.0 Bmac63*
6.2 Bmag345
16.0 Bmac32
22.7 Scssr10477
PH1.1_C09
ΔC1.1_L08
PH1.2_C09
DM1.1_V09
2H
0.0 Scssr7759
13.9 MGB391*
18.8 GBM1446**
20.6 GBM1459*
21.8 Scssr3381*
25.9 EBmac640**
32.3 EBmac558
34.7 Bmac93
34.8 Bmag518*
35.4 Scssr334*
36.2 HvBKASI**
44.5 Bmag711**
ΔC2.1_C09
GY2.1_V09
3H
0.0 Bmag828***
0.6 Bmag131***
GBM1410***
3.3 Bmac67***
3.4 EBmac871***
3.7 Bmag6***
3.9 Bmac209***
4.3 Bmag603***
9.6 GBM1163***
9.7 GBM1495***
11.1 GBM1253***
11.3 Hvm33***
18.6 Hvm60***
19.9 Bmag225***
26.7 GBM1405***
31.2 Bmag841***
33.5 GBM1233***
45.4 Bmag606***
58.4 Bmag13***
73.8 EBmac708***
76.4 Hvm70***
97.4 Scssr25538***
99.9 GBM1420***
ΔC3.1_C09
LG3.1_V09
LS3.1_V09
LG3.2_V09
ΔC3.2_C09
ΔC3.1_L08
ΔC31_V09
HI3.1_V09
GY31_C09
PH3.1_C09
PH3.1_V09
DM3.1_V09
PH3.2_C09
ΔC3.2_L08
PH3.3_C09
4H
0.0 Bmag740
5.9 GMS089
6.1 HvCABG
6.8 Bmag306
7.6 Bmag808
8.5 GBM1452
10.4 Bmag490
11.7 Bmag353
34.2 EBmac635
34.5 EBmac679*
35.2 EBmac701
35.3 EBmac788*
49.2 GBM1448***
DM4.1_V09
5H
0.0 Bmac113*
0.1 Bmag751
1.9 Bmag337*
2.0 Bmag323*
14.8 GBM1506*
15.2 Bmag760***
15.7 Scssr15334*
0.0 GBM1438**
2.2 GBM5008***
2.3 GBM1231***
16.6 GBMS119***
17.6 GMS61***
18.2 GBMS141***
18.3 GBM1436***
19.4 Scssr10148***
22.9 AF043094B***
24.9 GMS27***
ΔC5.1_L08
ΔC5.1_V09
GY5.1_V09
AB5.1_V09
PH5.1_C09
PH5.1_V09
DM5.1_V09
DM5.2_V09
LG5.1_V09
HI5.1_C09
GY5.1_C09
GY5.2_V09
AB5.2_V09
6H
0.0 Bmac40
2.9 GBM1022
3.0 Scssr103
10.1 Scssr5599
10.2 GBM1008
37.0 GBM1355**
37.6 GBM1215**
45.3 Bmag807**
45.5 GBM1212***
45.6 MGB371*
47.7 Bmag174*
48.0 Bmag173
49.1 Bmag9
49.3 Hvm74
49.4 Bmag344
52.8 Scssr2093**
75.2 GBM1423
ΔC6.1_V09
GY6.1_V09
AB6.1_V09
PH6.1_C09
ΔC6.1_L08
7H
0.0 Bmag206
22.3 Hvm4
41.6 GBMS139
45.1 GBM1326
0.0 Bmag341
4.0 Bmag11
4.1 Bmag369
7.7 Bmag900
14.6 GBM1359***
0.0 EBmac755
8.1 Bmac156
23.7 Hvm6**
25.0 Scssr4056**
PH7.1_C09
AB7.1_C09
LG7.1_V09
PH7.1_V09
ΔC7.1_V09
LS7.1_V09

Fig. 4 continued

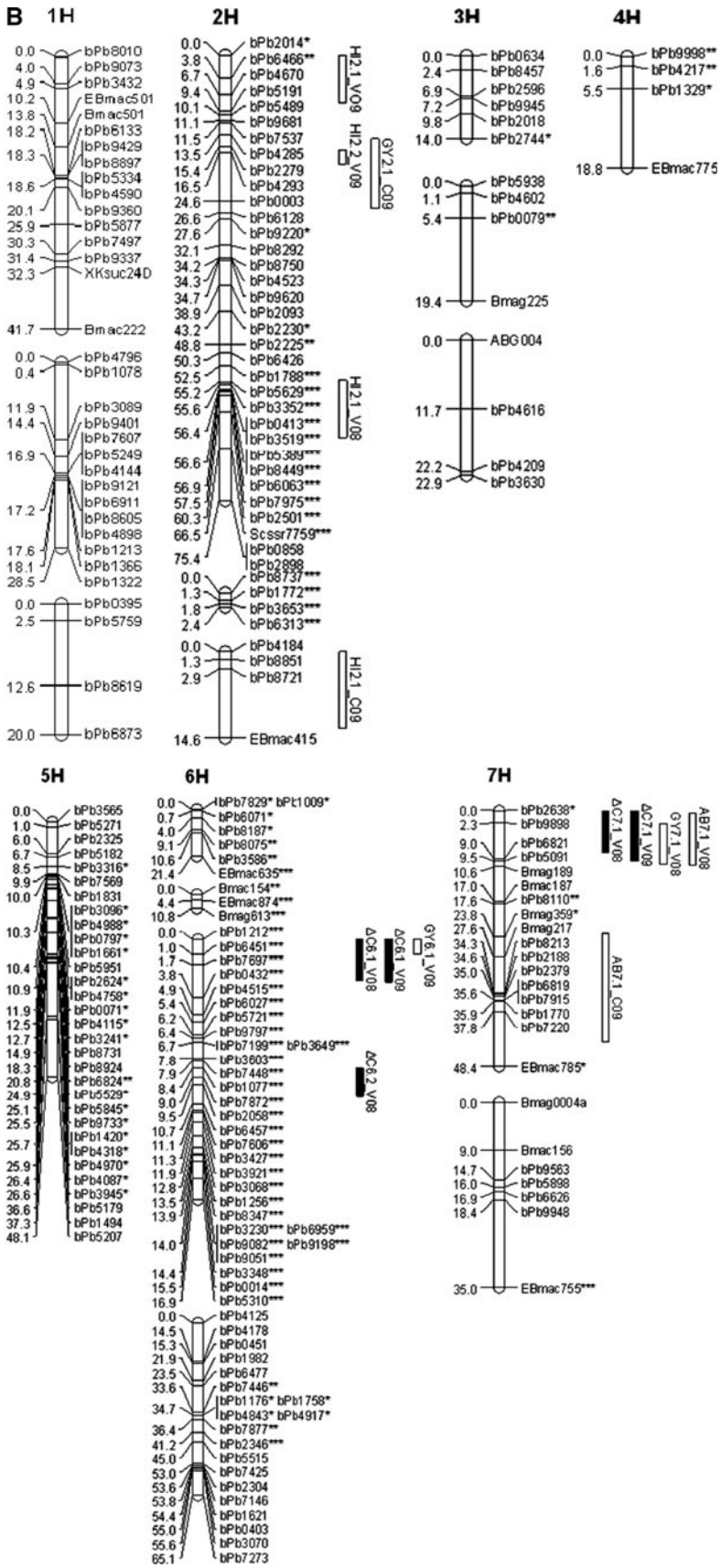

Table 6 QTL identified for carbon isotope discrimination ($\Delta^{13}C$) with QTL Cartographer 2.5 by the composite interval mapping (CIM) method using data from three locations (Castor, Lacombe and Vegreville) for the six-row (W89001002003 × I60049) barley population and data from Vegreville for the two-row (Merit × H93174006) barley population in 2 years (2008–2009)

Population	Environment	Position (marker + distance to the maximum LOD value) (cM)	Chromosome	LOD	R^2 (%)	Additive effect
Six-row	L08	Bmac32 + 2.04	1H	2.13	3.9	0.13
		Bmag606 + 2.03	3H	10.81	22	−0.36
		EBmac708 − 2.37	3H	3.78	7.3	−0.20
		Bmag751 + 0.06	5H	4.77	7.3	−0.18
		Bmag807 − 1.72	6H	2.18	3.6	0.12
	C09	EBmac558 − 0.36	2H	2.34	4.1	−0.08
		Bmag603 + 0.04	3H	2.44	4.1	−0.08
		Bmag606 + 3.03	3H	5.86	14.4	−0.17
	V09	Bmag606 − 0.87	3H	5.27	11	−0.18
		Bmac113 + 0	5H	2.93	5.3	−0.11
		GBM1022 + 0.04	6H	2.12	3.8	0.09
		EBmac755 + 0	7H	2.38	4.3	0.09
Two-row	V08	bPb1212 + 0	6H	3.48	11.2	0.29
		bPb7872 − 0.04	6H	3.65	11.7	0.32
		bPb9898 + 0.98	7H	3.11	9.9	0.20
	V09	bPb1212 + 0	6H	3.60	10.8	0.16
		bPb9898 + 1.98	7H	2.54	8.6	0.10

R^2 proportion of the phenotypic variance explained by each QTL, *Additive effect* additive effect of the allele from W89001002003 compared with I60049, *L08* Lacombe-2008, *C09* Castor-2009, *V08* Vegreville-2008, *V09* Vegreville-2009

season rainfall, suggesting environmental effects on leaf $\Delta^{13}C$ (Fig. 1). Teulat et al. (2002) reported that both total rainfall and the ratio of rainfall to evapo-transpiration had a significant impact on $\Delta^{13}C$, explaining mainly the environmental effects in their study. For water-limited environments such as the Canadian Prairies where crops rely on soil-stored moisture (Anyia et al. 2008), the temporal distribution of rainfall is critical for crop growth (Bonsal et al. 1999; Chakravartia 1972). In this study, leaf $\Delta^{13}C$ was significantly related to total within-season precipitation ($r = 0.42$, $n = 122$, $p < 0.001$), especially the amount of rainfall during June ($r = 0.48$, $n = 122$, $p < 0.001$). Ivlev and Voronin (2007) reported that the dynamics of $\delta^{13}C$ in the annual rings of trees were found to be related positively with air temperature in June but negatively with precipitation in June from 1980 to 2005; even though trees are very different plant species from the barley, their biological response to water availability should be similar.

Leaf $\Delta^{13}C$ was significantly reduced under drought conditions such as C09, which is in agreement with most reports on $\Delta^{13}C$ in C_3 crops (Craufurd et al. 1991; Hall et al. 1990; Virgona et al. 1990). Leaf $\Delta^{13}C$ of extreme RILs differed more in the low-rainfall environment (such as C09) than the high rainfall environment (such as L08). This result is in agreement with previous reports, which suggests that $\Delta^{13}C$ can be used as a sensitive indicator for plant water status or water availability during the growing period (Bloch et al. 2006; Craufurd et al. 1991; Merah et al. 2001). Significant correlations between leaf $\Delta^{13}C$ across environments were observed for both populations, demonstrating the stability of leaf $\Delta^{13}C$ across the tested environments.

Relationship between leaf $\Delta^{13}C$ and agronomic traits

Positive or neutral relationships are frequently reported between $\Delta^{13}C$ and grain yield and/or biomass in environments characterized with plentiful within-season rainfall or supplemental irrigation (Araus et al. 1998, 2003; Condon et al. 1987, 1993; Jiang et al. 2006; Merah et al. 1999, 2001; Monneveux et al. 2006; Morgan et al. 1993; Teulat et al. 2001c; Voltas et al. 1999). The correlations between leaf and/or grain $\Delta^{13}C$ and grain yield have been reported to be positive, negative or neutral, depending on the target environment and row type on the Canadian Prairies (Anyia et al. 2007). In this study, leaf $\Delta^{13}C$ was found to be positively correlated with aboveground biomass and grain yield for both populations under field conditions (Tables 4, 5; $p < 0.05$). One possible explanation of such relationship is the positive association between leaf $\Delta^{13}C$ and g_s (Condon et al. 1987), because more carbon could be fixed early in the season when stomatal opening was less limited by water deficit (therefore a high $\Delta^{13}C$).

Positive relationship between leaf $\Delta^{13}C$ and g_s in barley under field and greenhouse (well-watered) conditions was

Table 7 QTL identified for leaf area index measured during stem elongation stage (LAI-S), leaf area index measured during grain filling stage (LAI-G), plant height (PH), days to maturity, biomass, yield and harvest index (HI) with QTL Cartographer 2.5 by the composite interval mapping (CIM) method using data from two locations (Castor and Vegreville) for the six-row (W89001002003 × I60049) and the two-row (Merit × H93174006) barley populations in 2 years (2008–2009)

Population	Trait	*E*	Position (marker + distance to the maximum LOD value) (cM)	Chromosome	LOD	R^2 (%)	Additive effect
Six-row	LAI-S	V09	GBM1405 + 2.04	3H	2.61	5.4	−0.15
			Bmac156 + 0.01	7H	3.02	5.6	−0.14
	LAI-G	V09	GBM1405 + 1.04	3H	2.41	4.4	−0.13
			Bmag606 − 0.87	3H	2.11	4.2	−0.13
			GBM5008 + 0	5H	3.83	6.9	0.17
			GBMS139 + 2.02	7H	6.82	13.6	0.20
	PH	C09	Bmac32 − 3.76	1H	7.53	15.0	−2.15
			Bmac32 + 4.04	1H	7.96	14.5	−2.10
			Bmag606 + 4.03	3H	5.73	13.6	2.48
			EBmac708 − 5.37	3H	2.94	6.6	1.58
			Scssr25538 − 10.05	3H	3.23	8.4	1.68
			GBM1506 − 5.82	5H	5.18	10.7	−1.80
			GBM1355 − 11.79	6H	2.09	5.8	−1.32
			GBMS139 − 6.28	7H	2.98	6.9	1.42
		V09	Bmag606 + 5.03	3H	11.94	27.4	6.62
			GBM1506 − 3.82	5H	6.24	11.0	−3.42
			GBMS139 − 2.28	7H	5.00	9.3	3.22
	Biomass	C09	GBMS139 + 0.02	7H	2.73	5.6	8.90
		V09	Bmag337 − 0.01	5H	3.76	6.3	−32.16
			GBM1231 + 0.03	5H	3.55	6.3	34.08
			Scssr5599 − 0.01	6H	2.21	3.7	24.23
	Yield	C09	Bmag606 + 2.03	3H	4.42	11.4	−9.79
			GBM5008 + 0	5H	2.06	4.1	5.04
		V09	Bmag518 + 0.03	2H	2.91	5.0	−16.81
			Bmag323 + 0.05	5H	2.93	5.0	−17.07
			GBM1231 + 0.03	5H	5.62	10.2	25.63
			Scssr5599 − 0.01	6H	2.76	4.7	16.19
	HI	C09	GBM5008 + 0	5H	5.47	10.5	0.01
		V09	Bmag606 + 0.03	3H	5.18	10.5	−0.01
	Days to maturity	V09	Bmac32 − 4.76	1H	3.29	5.0	−0.68
			Bmag606 + 0.03	3H	10.78	16.0	−1.50
			Bmag740 + 0	4H	3.33	4.7	−0.65
			Bmac113 + 0	5H	4.06	5.5	−0.71
			Bmag323 + 4.05	5H	4.42	7.2	−0.80
Two-row	Biomass	V08	bPb6821 − 2.65	7H	2.32	9.7	41.79
		C09	bPb8213 − 1.67	7H	2.74	9.0	14.01
	Yield	V08	bPb6821 − 2.65	7H	2.06	9.2	13.57
		C09	bPb2279 + 0.04	2H	2.38	7.7	−12.22
		V09	bPb1212 + 0	6H	2.88	9.1	17.65
	HI	V08	bPb2501 − 0.77	2H	3.43	10.7	0.01
		C09	bPb8721 + 1.98	2H	2.04	7.4	0.01
		V09	bPb6466 − 0.83	2H	2.94	9.8	0.08
			bPb4293 + 0.01	2H	2.30	7.3	−0.07

E environment, R^2 proportion of the phenotypic variance explained by each QTL, *Additive effect* additive effect of the allele from W89001002003 compared with I60049, *C09* Castor-2009, *V08* Vegreville-2008, *V09* Vegreville-2009

observed in a previous study (Chen et al. 2011), and similar results have been reported in common bean (*Phaseolus vulgaris* L.) (Ehleringer 1990), rice (Kondo et al. 2004; Takai et al. 2009) and wheat (Monneveux et al. 2006). Takai et al. (2009) found that a QTL controlling leaf $\Delta^{13}C$ on the long arm of chromosome 3 in rice was associated with g_s. Another hypothesis is that genotypes differ in their abilities to translocate stem carbohydrate reserves for grain filling, since high leaf $\Delta^{13}C$ genotypes tend to grow faster and convert more assimilates to grain than low $\Delta^{13}C$ genotypes (Monneveux et al. 2005). The positive relationship between leaf $\Delta^{13}C$ and HI (Tables 4, 5) also suggested that genotypes with high leaf $\Delta^{13}C$ genotypes were more efficient in dry matter partitioning to grain (Teulat et al. 2001c).

The $\Delta^{13}C$ provides a long-term average estimate of cumulative WUE integrated over time and space (Condon et al. 2002). Leaves sampled for $\Delta^{13}C$ at the stem elongation stage, when there is usually little drought stress and low vapor-pressure deficit, could reflect the integrated WUE during vegetative development and formation of yield potential, and evaluate vegetative establishment (Anyia et al. 2008; Chen et al. 2011; Condon and Richards 1992). There was also a positive relationship between leaf $\Delta^{13}C$ and LAI-S across locations in this study (Table 4). LAI is usually used to estimate early vigor (the early growth of leaf area and biomass, Richards (1996)), canopy photosynthesis, evapo-transpiration and final yield since it represents the percentage of incident solar radiation intercepted (Dale et al. 1980). The significant positive correlations among LAI-S, leaf $\Delta^{13}C$, biomass and grain yield in this study suggest that both leaf $\Delta^{13}C$ and LAI at the stem elongation stage could be used to estimate final yield.

Leaf $\Delta^{13}C$ and row type

Jiang et al. (2006) reported that values of $\Delta^{13}C$ from different plant parts (flag leaf, awn and grain) were on average higher for six-row than for two-row barley, suggesting a higher WUE of two-row than six-row barley under irrigated and non-irrigated field conditions. A proposed explanation for the difference in $\Delta^{13}C$ values between barley ear types is the difference in days to maturity, as low $\Delta^{13}C$ values appeared in late-maturing genetic lines (Craufurd et al. 1991; Sayrea et al. 1995). Another possible explanation suggested by Jiang et al. (2006) is that the intrinsic difference may exist in water/carbon metabolism at the whole tiller, flag leaf and ear levels, since flag leaves of two-row barley are generally much smaller than six-row barley. The six-row barley may fix more carbohydrates from flag leaf blades and stems, and therefore they were enriched in ^{13}C. We did not find any significant difference in leaf $\Delta^{13}C$ between two-row and six-row barley in previous studies (Anyia et al. 2007; Chen et al. 2011). In the present study, the two populations differed in leaf $\Delta^{13}C$, with the M × H RILs producing a significantly lower leaf $\Delta^{13}C$ than the W × I RILs at V09 (data not shown, $p < 0.001$), which needs to be verified under multiple environments.

Six-row barley has more florets and therefore it is usually considered to be more fertile than two-row barley. In this study, the W × I RILs produced more biomass and grain yield than the M × H RILs at V09 ($p < 0.01$), but there was no significant difference in grain yield between these two types of barley population when soil moisture was not sufficient at C09. According to Forster et al. (2004), six-row barley has an advantage over two-row barley since six-row type generally matures earlier and therefore it is valuable in breeding for drought escape for countries in North Africa such as Morocco. It is also possible that the differences in tiller number between six-row and two-row barley may explain their leaf $\Delta^{13}C$ differences; however, this may need to be investigated further.

Heritability of leaf $\Delta^{13}C$

In the present study, the H^2 for leaf $\Delta^{13}C$ was high (0.80) for the six-row barley population across all environments. Under a single environment (C09 or V09), the H^2 for both populations all reached 0.80. These results suggest that leaf $\Delta^{13}C$ is under strong genetic control and can be reliably used as a measure of WUE under field conditions. For many complex traits (such as biomass, HI and yield components), genetic gain is slow under water-limited environments due to a large interaction between genotype and environment and a low heritability (Rebetzke et al. 2008). The high H^2 of physiological or secondary traits that are correlated with yield, such as $\Delta^{13}C$, presents a good opportunity for plant breeding in drought-prone regions (Stiller et al. 2005). Although the expression of $\Delta^{13}C$ in leaf and other plant tissues varies with water supply, and the spatial variability in soil water availability can reduce genetic variance of $\Delta^{13}C$ (Condon et al. 1992; Rebetzke et al. 2008), the heritability for $\Delta^{13}C$ is still high compared with other complex traits under drought conditions. Several studies have previously reported high H^2 values for $\Delta^{13}C$. Hubick et al. (1988) observed that the H^2 of $\Delta^{13}C$ (whole plant, excluding the pods and roots) in field-grown peanut cultivars was 0.81, and there was no significant interaction between genotype and environment for $\Delta^{13}C$. Condon and Richards (1992) found that the H^2 of $\Delta^{13}C$ in wheat was greatest for plant material sampled before or during early stem elongation (0.95 on genotype basis or 0.88 on a single-plot basis) as compared with plant parts formed near anthesis (flag leaf or plants tops at ear emergence). In another study on wheat, the narrow-sense heritability of

leaf $\Delta^{13}C$ ranged from 0.37 to 0.91 on a single environment basis and from 0.76 to 0.86 on genotype-mean basis in a QTL analysis using three wheat mapping populations across 3 years (Rebetzke et al. 2008).

QTL for $\Delta^{13}C$ across environments and populations

The CIM method revealed 12 putative QTL associated with leaf $\Delta^{13}C$ for the W × I population, and 5 QTL for leaf $\Delta^{13}C$ from the M × H population in the current study. Common QTL across environments and populations can provide validation of the QTL and identify robust markers across different gene pools for marker-assisted selection. The significant low level of polymorphism between parental lines (especially in the M × H population) resulted in the low density and uneven distribution of markers in the QTL maps obtained in the present study. For example, Chr. 3H of the M × H map was split into three linkage groups and was only covered by 14 markers, which may have hindered the detection of putative QTL for this chromosome. The low marker density and the lack of common markers prevented us from identifying and comparing common QTL across the two populations evaluated in the present study. However, it may be possible to identify a few common QTL for leaf $\Delta^{13}C$ across the two mapping populations if more markers are placed to saturate the maps. Since the order and not the distance between markers is usually considered conserved between populations (This et al. 2010), 7 of the 12 QTL regions detected in the W × I population in this study (region on Chr. 2H around marker EBmac558, Chr. 3H between marker Bmag606 and Bmag13, Chr. 5H near marker Bmac113 and on Chr. 6H near marker Bmag173) were identified to be similar to those previously reported for grain $\Delta^{13}C$ in the Tadmor × Er/Apm population under three Mediterranean field environments by Teulat et al. (2002). Specifically, the ΔC5.1-WI in our study overlapped with grain $\Delta^{13}C$ in the Tadmor × Er/Apm population under irrigated field conditions (Diab et al. 2004). Ellis et al. (2002) reported similar chromosome regions for shoot $\Delta^{13}C$ (3H and 5H) in their Derkado × B83-12/21/5 DH population.

Although ΔC3.1-WI on the W × I linkage map was detected consistently in all three field locations, its effect on leaf $\Delta^{13}C$ varied across environments, and this QTL explained 22% of the phenotypic value at L08, followed by C09 (14.4%) and then V09 (11%), with no significant G × E for leaf $\Delta^{13}C$ in the W × I population. In agreement with Teulat et al. (2002), the observed differential effects of this QTL across the different environments studied may be attributable to the total growing season rainfall. Condon et al. (1992) also pointed out that the environmental effects on $\Delta^{13}C$ can be attributed to stomatal closure in response to declining soil water and/or increasing vapor-pressure deficit. Overall, the QTL identified in this study varied in size and accounted for small to modest amounts of the phenotypic variance, which is consistent with most of the previous studies in QTL mapping of $\Delta^{13}C$ across a range of plant species.

Effect of parental alleles

It is imperative to understand the inheritance of $\Delta^{13}C$ and the mode of gene action to develop cultivars with high WUE via selection for low $\Delta^{13}C$ lines. There was one report on cytoplasmic inheritance of $\Delta^{13}C$ in cultivated sunflower by Lambrides et al. (2004), but most studies provided strong evidence for nuclear genetic control of $\Delta^{13}C$, with QTL for $\Delta^{13}C$ assigned to specific chromosomes in several different species, such as *Arabidopsis thaliana* (Hausmann et al. 2005; Juenger et al. 2005), barley (Diab et al. 2004; Ellis et al. 2002; Ellis et al. 1997; Handley et al. 1994; Teulat et al. 2002), cotton (Saranga et al. 2001), rice (Laza et al. 2006; Price et al. 2002; Takai et al. 2006; This et al. 2010; Xu et al. 2009), soybean (Specht et al. 2001), tomato (Xu et al. 2008) and wheat (Rebetzke et al. 2008). Predominantly additive gene action of $\Delta^{13}C$ has been reported in alfalfa (*Medicago sativa* L.) (Johnson and Rumbaugh 1995), *Arabidopsis thaliana* (Hausmann et al. 2005; Juenger et al. 2005), common bean (White 1993), wheat (Ehdaie and Waines 1994; Rebetzke et al. 2006) and rice (Takai et al. 2006).

In the present study, the low leaf $\Delta^{13}C$ parent W89 had favorable alleles for WUE at loci on Chr. 2H, 3H and 5H, and the high leaf $\Delta^{13}C$ parent I60049 contributed favorable alleles for WUE at loci on Chr. 1H, 6H and 7H. For the M × H population, the high leaf $\Delta^{13}C$ parent H93174006 had favorable alleles for WUE at loci on Chr. 6H and 7H. The complementary alleles at multiple loci contributed by the parents provided the most plausible explanation for transgression observed among the progeny (Tanksley 1993). The additive gene action underlying $\Delta^{13}C$ suggests that replacement and fixation of desirable alleles within a locus could be achieved by selecting lines with high or low $\Delta^{13}C$ (Rebetzke et al. 2003, 2008). Independent alleles at multiple loci could also be pyramided to develop lines for further altered $\Delta^{13}C$.

Clusters of QTL

Multiple regions controlling leaf $\Delta^{13}C$ co-located with the QTL for agronomic traits (such as aboveground biomass and grain yield) were identified in both RIL populations in the present study, which is consistent with previous reports. Teulat et al. (2001a, b, 2002) reported eight QTL for grain $\Delta^{13}C$ co-located with QTL for agronomic traits and traits related to plant water status and/or osmotic adjustment.

Most QTL for $\Delta^{13}C$ have previously been reported to be co-located with QTL for yield components and heading date and/or plant height (Forster et al. 2004; Juenger et al. 2005).

Correlations (Tables 4, 5) between leaf $\Delta^{13}C$ and LAI-S, plant height, days to maturity, biomass, grain yield and HI were partially explained at the level of co-localized QTL (Fig. 3a, b). One of the genomic regions with the most overlapping traits was on Chr. 3H near marker Bmag606, with W89 alleles favoring lower leaf $\Delta^{13}C$, increased plant height, and reduced LAI, grain yield, HI and days to maturity. Around the same region, QTL for $\Delta^{13}C$ was identified near the semi-dwarf gene *sdw1* in different barley mapping populations (Ellis et al. 2002; Teulat et al. 2002), which is homoeologous to rice Chr. 1 where $\Delta^{13}C$ is located. Another noteworthy region was on Chr. 5H near marker Bmac113, with W89 alleles favoring lower leaf $\Delta^{13}C$, reduced biomass and yield. In the same region, QTL for shoot $\delta^{13}C$ and grain yield were associated with another semi-dwarf gene *ari-e*.GP (Ellis et al. 2002).

The association between $\Delta^{13}C$ and plant height and/or heading date could confound and compromise the relationship between $\Delta^{13}C$ and grain yield (Rebetzke et al. 2008). In this study, there were also QTL (such as ΔC6.1-MH and ΔC7.1-MH) that explained 8.6–11.2% of the phenotypic variation but only showed co-localization with aboveground biomass and grain yield, which may be more attractive to plant breeders. Rebetzke et al. (2008) suggested that the effect of $\Delta^{13}C$ on yield should be separated from plant height and development effects (such as heading date and flowering time) through covariance analysis. The co-localization of QTL may result from pleiotropic relationships between these traits or due to genetic linkage among the traits. However, clustering regions are of interest in terms of plant breeding as they control both drought-adaptive traits such as $\Delta^{13}C$ and yield components.

Conclusions

The temporal variation of within-season rainfall distribution on the Canadian Prairies has a significant impact on barley leaf $\Delta^{13}C$. Sufficient genotypic variation, stability across environments, and a high H^2 indicate that leaf $\Delta^{13}C$ is a good surrogate for improved WUE in breeding for barley varieties under rain-fed conditions. The transgressive variation of leaf $\Delta^{13}C$ observed in our mapping population and results from previous studies (Anyia et al. 2007; Chen et al. 2011) suggest that it is possible to select progeny lines with low $\Delta^{13}C$ or high WUE that contribute favorable alleles of value for improvement of WUE. This study has confirmed the polygenic inheritance of leaf $\Delta^{13}C$ in barley by detecting multiple QTL controlling leaf $\Delta^{13}C$. Several QTL for leaf $\Delta^{13}C$ were found to overlap with QTL for important agronomic traits that would need further validation. A major QTL for leaf $\Delta^{13}C$ (ΔC3.1-WI) was identified across all locations that overlapped with several agronomic traits, with W89 alleles favoring lower leaf $\Delta^{13}C$, increased plant height, and reduced LAI, grain yield, HI and days to maturity. Since overlapping or co-location of QTL suggests pleiotropic relationship or genetic linkage, care must be taken to ensure that low $\Delta^{13}C$ does not impose penalty on grain yield under favorable conditions. More research is needed to further dissect this major QTL region for low $\Delta^{13}C$ towards the identification of candidate genes as well as to understand the genetic mechanisms underlying these co-located traits.

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References

AgroClimatic Information Service (ACIS) (2009) http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/cl12944. Agriculture and Rural Development, Government of Alberta

Anyia AO, Slaski JJ, Nyachiro JM, Archambault DJ, Juskiw P (2007) Relationship of carbon isotope discrimination to water use efficiency and productivity of barley under field and greenhouse conditions. J Agron Crop Sci 193:313–323

Anyia AO, Slaski JJ, Capo-Chichi L, Chen J, Chang SX (2008) Physiological traits contributing to water productivity and yield stability of barley on the Canadian Prairies. In: The 5th International Crop Science Congress, Jeju Island, South Korea. April 13–18

Araus JL, Amaro T, Casadesús J, Asbati A, Nachit MM (1998) Relationships between ash content, carbon isotope discrimination and yield in durum wheat. Aust J Plant Physiol 25:835–842

Araus JL, Villegas D, Aparicio N, García del Moral LF, El Hani S, Rharrabti Y, Ferrio JP, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci 43:170–180

Baum M, Von Korff M, Guo P, Lakew B, Hamwieh A, Lababidi S, Udupa SM, Sayed H, Choumane W, Grando S, Ceccarelli S (2007) Molecular approaches and breeding strategies for drought tolerance in barley. In: Varshney R, Tuberosa R (eds) Genomics-assisted crop improvement, vol 2: genomics applications in crops. Springer, Dordrecht, pp 51–79

Bloch D, Hoffmann CM, Märländer B (2006) Impact of water supply on photosynthesis, water use and carbon isotope discrimination of sugar beet genotypes. Eur J Agron 24:218–225

Bonsal BR, Zhang X, Hogg WD (1999) Canadian Prairie growing season precipitation variability and associated atmospheric circulation. Clim Res 11:191–208

Canadian International Grains Institute (CIGI) (2004) Canada: crop production, consumption and exports. In: Agriculture and Agri-

Food Canada Market Analysis Division (ed) Grains and oilseed textbook, 5th edn.
Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1–14
Chaabane R, Felah ME, Salah HB, Naceur MBB, Abdelly C, Ramla D, Nada A, Saker M (2009) Molecular characterization of Tunisian barley (*Hordeum vulgare* L.) genotypes using microsatellites (SSRs) markers. Eur J Sci Res 36:6–15
Chakravartia AK (1972) The June–July precipitation pattern in the Prairie Provinces of Canada. J Geogr 71:155–160
Chen J, Chang SX, Anyia AO (2011) The physiology and stability of leaf carbon isotope discrimination as a measure of water-use efficiency in barley on the Canadian Prairies. J Agron Crop Sci 197:1–11
Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971
Condon AG, Richards RA (1992) Broad sense heritability and genotype × environment interaction for carbon isotope discrimination in field-grown wheat. Aust J Agric Res 43:921–934
Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Sci 27:996–1001
Condon A, Richards R, Farquhar G (1992) The effect of variation in soil water availability, vapour pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. Aust J Agric Res 43:935–947
Condon AG, Richards RA, Farquhar GD (1993) Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. Aust J Agric Res 44:1693–1711
Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. Crop Sci 42:122–131
Craufurd PQ, Austin RB, Acevedo E, Hall MA (1991) Carbon isotope discrimination and grain-yield in barley. Field Crops Res 27:301–313
Dale RF, Coelho DT, Gallo KP (1980) Prediction of daily green leaf area index for corn. Agron J 72:999–1005
Diab AA, Teulat-Merah B, This D, Ozturk NZ, Benscher D, Sorrells ME (2004) Identification of drought-inducible genes and differentially expressed sequence tags in barley. Theor Appl Genet 109:1417–1425
Ehdaie B, Waines JG (1994) Genetic analysis of carbon isotope discrimination and agronomic characters in a bread wheat cross. Theor Appl Genet 88:1023–1028
Ehleringer JR (1990) Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans. Plant Physiol 93:1422–1425
Ellis RP, Forster BP, Waugh R, Bonar N, Handley LL, Robinson D, Gordon DC, Powell W (1997) Mapping physiological traits in barley. New Phytol 137:149–157
Ellis RP, Forster BP, Gordon DC, Handley LL, Keith RP, Lawrence P, Meyer R, Powell W, Robinson D, Scrimgeour CM, Young G, Thomas WTB (2002) Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. J Exp Bot 53:1163–1176
Environment Canada (2009) http://www.climate.weatheroffice.gc.ca. National Climate Data and Information Archive
FAOSTAT (2008) Food and Agricultural Organization (FAO). http://faostat.fao.org/site/291/default.aspx
Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11:539–552
Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137
Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537
Flint-Garcia SA, Jampatong C, Darrah LL, Mcmullen MD (2003) Quantitative trait locus analysis of stalk strength in four maize populations. Crop Sci 43:13–22
Forster BP, Ellis RP, Moir J, Talamè V, Sanguineti MC, Tuberosa R, This D, Teulat-Merah B, Ahmed I, Mariy SAEE, Bahri H, El Ouahabi M, Zoumarou-Wallis N, El-Fellah M, Salem MB (2004) Genotype and phenotype associations with drought tolerance in barley tested in North Africa. Ann Appl Biol 144:157–168
Hall AE, Mutters RG, Hubick KT, Farquhar GD (1990) Genotypic differences in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci 30:300–305
Handley LL, Nevo E, Raven JA, Carrasco RM, Scrimgeour CM, Pakniyat H, Forster BP (1994) Chromosome 4 controls potential water use efficiency (^{13}C) in barley. J Exp Bot 45:1661–1663
Hanson CH, Robinson HF, Comstock RE (1956) Biometrical studies of yield in segregating populations of Korean *Lespedeza*. Agron J 48:268–272
Hausmann NJ, Juenger TE, Sen S, Stowe K, Dawson TE, Simms EL (2005) Quantitative trait loci affecting δ^{13}C and response to differential water availability in *Arabidopsis thaliana*. Evolution 59:81–96
Hubick KT, Shorter R, Farquhar GD (1988) Heritability and genotype × environment interactions of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogaea* L.). Aust J Plant Physiol 15:799–813
IPCC (2007) Climate change 2007: impacts, adaptation and vulnerability: contribution of Working Group II to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press
Ivlev AA, Voronin VI (2007) The mechanism of carbon isotope fractionation in photosynthesis and carbon dioxide component of the greenhouse effect. Biol Bull Russ Acad Sci 34:603–609
Jiang QZ, Roche D, Hole DJ (2006) Carbon isotope discrimination of two-rowed and six-rowed barley genotypes under irrigated and non-irrigated field conditions. Can J Plant Sci 86:433–441
Johnson DA, Rumbaugh MD (1995) Genetic variation and inheritance characteristics for carbon isotope discrimination in Alfalfa. J Range Manage 48:126–131
Juenger TE, McKay JK, Hausmann N, Keurentjes JJB, Sen S, Stowe KA, Dawson TE, Simms EL, Richards JH (2005) Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: δ^{13}C, stomatal conductance and transpiration efficiency. Plant Cell Environ 28:697–708
Karakousis A, Gustafson JP, Chalmers KJ, Barr AR, Langridge P (2003) A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust J Agric Res 54:1173–1185
Kondo M, Pablico P, Aragones D, Agbisit R (2004) Genotypic variations in carbon isotope discrimination, transpiration efficiency, and biomass production in rice as affected by soil water conditions and N. Plant Soil 267:165–177
Lambrides CJ, Chapman SC, Shorter R (2004) Genetic variation for carbon isotope discrimination in sunflower: association with transpiration efficiency and evidence for cytoplasmic inheritance. Crop Sci 44:1642–1653
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

Laza MR, Kondo M, Ideta O, Barlaan E, Imbe T (2006) Identification of quantitative trait loci for ^{13}C and productivity in irrigated lowland rice. Crop Sci 46:763–773

Li JZ, Sjakste TG, Röder MS, Ganal MW (2003) Development and genetic mapping of 127 new microsatellite markers in barley. Theor Appl Genet 107:1021–1027

Li-Cor (1992) LAI-2000 plant canopy analyzer operating manual. Li-Cor Inc., Lincoln, NE, USA

Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Martin B, Nienhuis J (1989) Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science 243:1725–1728

Merah O, Deléens E, Monneveux P (1999) Grain yield, carbon isotope discrimination, mineral and silicon content in durum wheat under different precipitation regimes. Physiol Plant 107: 387–394

Merah O, Monneveux P, Deléens E (2001) Relationships between flag leaf carbon isotope discrimination and several morpho-physiological traits in durum wheat genotypes under Mediterranean conditions. Environ Exp Bot 45:63–71

Monneveux P, Reynolds MP, Trethowan R, González-Santoyo H, Peña RJ, Zapata F (2005) Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes. Eur J Agron 22:231–242

Monneveux P, Rekika D, Acevedo E, Merah O (2006) Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. Plant Sci 170:867–872

Morgan JA, LeCain DR, McCaig TN, Quick JS (1993) Gas exchange, carbon isotope discrimination, and productivity in winter wheat. Crop Sci 33:178–186

Price AH, Cairns JE, Horton P, Jones HG, Griffiths H (2002) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. J Exp Bot 53: 989–1004

Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle 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

Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rain-fed bread wheat. Crop Sci 42:739–745

Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2003) Gene action for leaf conductance in three wheat crosses. Aust J Agric Res 54:381–387

Rebetzke GJ, Richards RA, Condon AG, Farquhar GD (2006) Inheritance of carbon isotope discrimination in bread wheat (*Triticum Aestivum* L.). Euphytica 150:97–106

Rebetzke GJ, Condon AG, Farquhar GD, Appels R, Richards RA (2008) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. Theor Appl Genet 118:123–137

Richards RA (1996) Defining selection criteria to improve yield under drought. Plant Growth Regul 20:157–166

Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci 42:111–121

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246: 327–333

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Saranga Y, Menz M, Jiang C, Wright RJ, Yakir D, Paterson AH (2001) Genomic dissection of genotype × environment interactions conferring adaptation of cotton to arid conditions. Genome Res 11:1988–1995

Sayrea KD, Acevedob E, Austinc RB (1995) Carbon isotope discrimination and grain yield for three bread wheat germplasm groups grown at different levels of water stress. Field Crops Res 41:45–54

Sicher R (1993) Assimilate partitioning within leaves of small grain cereals. In: Abrol YP, Mohanty P, Govindjee (eds) Photosynthesis: photoreactions to plant productivity. Kluwer, Dordrecht, pp 351–360

Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Germann M, Orf JH, Lark KG (2001) Soybean response to water—a QTL analysis of drought tolerance. Crop Sci 41:493–509

Stiller WN, Read JJ, Constable GA, Reid PE (2005) Selection for water use efficiency traits in a cotton breeding program: cultivar differences. Crop Sci 45:1107–1113

Takai T, Fukuta Y, Sugimoto A, Shiraiwa T, Horie T (2006) Mapping of QTLs controlling carbon isotope discrimination in the photosynthetic system using recombinant inbred lines derived from a cross between two different rice (*Oryza sativa* L.) cultivars. Plant Prod Sci 9:271–280

Takai T, Ohsumi A, San-oh Y, Laza MRC, Kondo M, Yamamoto T, Yano M (2009) Detection of a quantitative trait locus controlling carbon isotope discrimination and its contribution to stomatal conductance in *japonica* rice. Theor Appl Genet 118:1401–1410

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Teulat B, Borries C, This D (2001a) New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. Theor Appl Genet 103:161–170

Teulat B, Merah O, Souyris I, This D (2001b) QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments. Theor Appl Genet 103:774–787

Teulat B, Merah O, This D (2001c) Carbon isotope discrimination and productivity in field-grown barley genotypes. J Agron Crop Sci 187:33–39

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

Thiel T, Michalek W, Varshney R, Graner A (2003) Exploiting EST databases for the development of cDNA derived microsatellite markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422

This D, Comstock J, Courtois B, Xu Y, Ahmadi N, Vonhof WM, Fleet C, Setter T, McCouch S (2010) Genetic analysis of water use efficiency in rice (*Oryza sativa* L.) at the leaf level. Rice 3:72–86

Thorne GN (1965) Photosynthesis of ears and flag leaves of wheat and barley. Ann Bot 29:317–329

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114:1091–1103

Virgona JM, Hubick KT, Rawson HM, Farquhar GD, Downes RW (1990) Genotypic variation in transpiration efficiency, carbon-isotope discrimination and carbon allocation during early growth in sunflower. Aust J Plant Physiol 17:207–214

Voltas J, Romagosa I, Lafarga A, Armesto AP, Sombrero A, Araus JL (1999) Genotype by environment interaction for grain yield and

carbon isotope discrimination of barley in Mediterranean Spain. Aust J Agric Res 50:1263–1271

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang S, Basten CJ, Zeng Z-B (2010) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh RK, Howell G, Pathak H, Sumfleth K (2009) Chapter 2 Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. Adv Agron 101:59–122

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci USA 101: 9915–9920

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206–228

White JW (1993) Implications of carbon isotope discrimination studies for breeding common bean under water deficits. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon–water relations. Academic Press, San Diego, pp 387–398

Xu X, Martin B, Comstock JP, Vision TJ, Tauer CG, Zhao B, Pausch RC, Steven K (2008) Fine mapping a QTL for carbon isotope composition in tomato. Theor Appl Genet 117:221–233

Xu Y, This D, Pausch RC, Vonhof WM, Coburn JR, Comstock JP, McCouch SR (2009) Leaf-level water use efficiency determined by carbon isotope discrimination in rice seedlings: genetic variation associated with population structure and QTL mapping. Theor Appl Genet 118:1065–1081

Xue D, Chen M, Zhou M, Chen S, Mao Y, Zhang G (2008) QTL analysis of flag leaf in barley (*Hordeum vulgare* L.) for morphological traits and chlorophyll content. J Zhejiang Univ Sci B 9:938–943

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468