

# Physiological characterization of recombinant inbred lines of barley with contrasting levels of carbon isotope discrimination

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## Abstract

**Background and Aims** Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) in  $\text{C}_3$  plants used as an indirect measure of water-use efficiency (WUE) provides a tool for selecting crops with high WUE under dry environments.

**Methods** We evaluated the physiology and  $\Delta^{13}\text{C}$  of a set of 8  $\text{F}_5$  recombinant inbred lines (RILs) with contrasting levels of leaf  $\Delta^{13}\text{C}$  derived from two parents, ‘W89001002003’ (low  $\Delta^{13}\text{C}$ ) and ‘I60049’ (high  $\Delta^{13}\text{C}$ ) of six-row barley (*Hordeum vulgare* L.) in a greenhouse and under field conditions in three locations (Lacombe, Vegreville and Castor). In the greenhouse experiment, seven days of water deficit was

imposed at the stem elongation stage followed by re-watering to pre-deficit level.

**Results** A significant negative relationship between WUE and leaf  $\Delta^{13}\text{C}$  was observed. Under water-deficit conditions, both photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ) were significantly reduced with a strong positive correlation ( $r = 0.89$ ) between the two, and the variation in  $g_s$  was proportionally greater than  $A$ . The low leaf- $\Delta^{13}\text{C}$  RIL ‘147’ maintained the highest  $A$  and  $g_s$  among ten genotypes (RILs and parents) under water-deficit conditions. Leaf  $\Delta^{13}\text{C}$  was positively correlated with biomass and grain yield in the field trials. Multivariate analysis of leaf  $\Delta^{13}\text{C}$ , harvest index and plant height discriminated genotypes into three clusters: drought sensitive, drought tolerant and an intermediate type.

**Conclusions** The study suggests that it is possible to select low  $\Delta^{13}\text{C}$  lines such as RIL ‘147’, which is able to maintain or produce high yields under low moisture conditions on the Canadian Prairies

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**Keywords** Canonical discriminant analysis (CDA) · Drought tolerance · Genotypic variation · The Canadian Prairies · Water-use efficiency (WUE)

## Introduction

Canada is one of the world’s largest barley (*Hordeum vulgare* L.) producers and exporters, with an average of 12.3 million tonnes produced annually between

1986 and 2006 (FAOSTAT 2008). Barley production is mostly concentrated on the Prairies in western Canada, which is characterized by short and dry growing seasons with frequent drought and heat stress later in the growing season (Anyia et al. 2008). As a consequence of seasonal and temporal moisture deficits, barley yields and production are highly variable across locations and years. As drought continues to be one of the main abiotic constraints on agricultural productivity worldwide, and water deficit is likely to increase in most arid and semi-arid regions under future climate change scenarios (Barnabás et al. 2008), the development of barley varieties with high water-use efficiency (WUE, the ratio of dry matter production to transpired water) and drought tolerance that can maintain yield under water-limited conditions has been a key area of agricultural research around the world.

Plant physiological research has provided new insights to yield improvement in drought-prone environments. Considerable research and substantial breeding efforts have been devoted to identifying and selecting for morpho-physiological traits that increase WUE and yield under water-limited conditions (Blum 1996; Richards 1996; Richards et al. 2002). Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ), a measure of the  $^{13}\text{C}/^{12}\text{C}$  ratio in plant material relative to the ratio in atmospheric  $\text{CO}_2$  (Hall et al. 1994), has been intensively exploited and demonstrated to be a simple but reliable indicator of WUE for many  $\text{C}_3$  crops, and their negative correlation has been used for indirect selection of  $\text{C}_3$  crops with improved WUE under selected environments (Cattivelli et al. 2008). Genotypic variations of WUE and  $\Delta^{13}\text{C}$  have long been reported in  $\text{C}_3$  crops, but the complexity underlying drought resistance mechanisms and our limited knowledge of the genetic and physiological basis of yield have hindered the breeding process in drought environments (Passioura 2002; Tuberosa and Salvi 2006). Improved WUE and drought tolerance without a yield penalty offers a promising way to sustain agricultural production and land use in semi-arid regions (Karaba et al. 2007).

Although the relationship between  $\Delta^{13}\text{C}$  and WUE in  $\text{C}_3$  crops has been well described and verified by many researchers, there are questions regarding the type of relationship between  $\Delta^{13}\text{C}$  and biomass or grain yield (Anyia et al. 2007). Significant correlations between  $\Delta^{13}\text{C}$  and biomass or grain yield have been reported, which were either positive or negative,

depending on the plant tissue analyzed and the type of environment sampled (Anyia et al. 2007; Condon et al. 2002). In Australia, selection for low leaf  $\Delta^{13}\text{C}$  resulted in improved wheat (*Triticum aestivum* L.) yield (Rebetzke et al. 2002). Under terminal-drought in a Mediterranean-type environment, the correlation between  $\Delta^{13}\text{C}$  and grain yield has been mostly positive and selection for high  $\Delta^{13}\text{C}$  or low WUE was thought to be most appropriate in that region (Condon et al. 2004; Merah et al. 2001; Voltas et al. 1999). However, for stored-moisture environments such as eastern Australia and the Canadian Prairies, yield improvements may be optimized through a combination of low  $\Delta^{13}\text{C}$  or high WUE and greater early vigor (Anyia et al. 2008; Condon et al. 2002).

Multivariate procedures have been largely used in the assessment of genetic divergence in crop and grass species based on morphological and agronomic characteristics (Capo-chichi et al. 2005; Loos 1993; Mathewa et al. 1994; Riggs 1973; Vaylay and van Santen 2002). When each trait is considered separately in a univariate analysis, considerable overlap of genetic variation may occur (Vaylay and van Santen 2002). During multivariate canonical discriminant analysis (CDA), all independent traits are viewed simultaneously to differentiate target groups (e.g., genotypes). The resulting differentiation extracts maximum genetic variability (between groups) compared with the environmental variability (within groups) (Riggs 1973). However, there were few reports on discriminating genotypes with different levels of WUE (measured as  $\Delta^{13}\text{C}$ ) using the CDA method.

The objectives of this study were: (1) to examine the relationships among grain yield, leaf  $\Delta^{13}\text{C}$  (WUE) and leaf gas exchange parameters of selected recombinant inbred lines (RILs) of barley with contrasting levels of leaf  $\Delta^{13}\text{C}$ ; and (2) to classify the drought tolerance patterns of the RILs and their parents using CDA based on agronomic and physiological traits.

## Materials and methods

### Plant materials

Ten six-row barley breeding lines (8 RILs and their parents) with contrasting levels of leaf  $\Delta^{13}\text{C}$  (Table 1) were used in this study, including both field- and greenhouse-based experiments. The two

**Table 1** Leaf carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) of ten genotypes grown at three different sites (Lacombe, Castor and Vegreville) in 2008 and 2009

Group	Genotype	Lacombe-2008 <sup>1</sup> $\Delta^{13}\text{C}$ (‰, mean±S.E.)	Castor-2009	Vegreville-2009
High- $\Delta^{13}\text{C}$	18	21.43	18.39±0.74	20.45±0.49
	127	21.09	18.96±0.15	20.35±0.52
	176	22.67	19.33±0.18	20.32±0.96
	196	22.19	18.51±0.21	20.17±0.64
	I60049	22.40±0.24	18.99±0.15	19.97±0.55
	Group mean	21.96	18.84	20.25
Low- $\Delta^{13}\text{C}$	85	20.61	18.17±0.40	18.77±0.15
	116	21.25	18.37±0.39	19.14±0.12
	144	19.84	18.21±0.05	19.24±0.30
	147	20.47	18.81±0.10	19.30±0.44
	W89 <sup>2</sup>	20.71±0.09	17.69±0.56	18.72±0.33
	Group mean	20.58	18.25	19.03
Significance <sup>3</sup>				
Group			***	
Location-year			***	
Group×Location-year			ns	

<sup>1</sup>Data was only from one replicate (except parental lines)

<sup>2</sup>W89: W89001002003

<sup>3</sup>ns, not significant; \*\*\* indicates significance at the 0.1% level

parental lines (W89001002003 and I60049, hereafter referred to as W89 and I60) were previously surveyed for leaf  $\Delta^{13}\text{C}$  at three field locations from 2005 to 2009. Data from 2005 to 2006 were obtained from Anyia et al. (2007) and Chen et al. (2011b). Based on the consistent differences in the leaf  $\Delta^{13}\text{C}$  of W89 and I60, they were used to produce a RIL mapping population using the single seed descent (SSD) approach in 2006. Eight progeny lines were selected from the 200 F<sub>5</sub> RILs for a detailed greenhouse study described below. Selection of the eight progeny lines was based on their observed contrasting levels of leaf  $\Delta^{13}\text{C}$  in previous field evaluations at 3 locations in 2008 and 2009.

### Field experiments

Field experiments were conducted at Lacombe (52°28' N, 113°45' W, 847.3 m altitude), Vegreville (53°31' N, 112°6' W, 639.3 m altitude) and Castor (52°8' N, 111°54' W, 807.7 m altitude) in Alberta, Canada, under rain-fed conditions. The detailed description of site characteristics of each site and experimental design were reported previously by the authors in Chen et al.

(2012). The three sites represent different agroclimatic zones with diverse precipitation and soil moisture profiles. Castor is typically the driest and Lacombe the wettest among the three sites. The average annual precipitation and within season rainfall (June to August) from 1977 to 2007 was 340±89 mm and 172±67 mm at Castor compared with Vegreville which had 382±62 mm and 193±52 mm, and Lacombe which had 440±84 mm and 230±63 mm, respectively (Chen et al. 2012).

In 2008, all genotypes (RILs and parental lines) were evaluated in Lacombe using a completely randomized design with one replicate of each RIL and four replicates of each parental line, while in 2009 they were grown in Vegreville and Castor using a randomized complete block design with three replicates per genotype (Chen et al. 2012).

*Carbon isotope discrimination analysis* Fully expanded penultimate leaves were sampled for measurement of carbon isotope composition ( $\delta^{13}\text{C}$ ) at the jointing stage (BBCH 36 to 39) according to the Zadoks et al. (1974) scale. Samples were randomly collected from 5 plants per plot and bulked. All

samples were oven dried at 70 °C for 48 h and then ground to fine powder with a ball mill (Spex SamplePrep 8000D Mixer, Metuchen, NJ, USA). Samples were analyzed for  $\delta^{13}\text{C}$  using a continuous-flow stable isotope ratio mass spectrometer (Thermo Finnigan Mat GmbH, Bremen, Germany).  $\delta^{13}\text{C}$  was calculated as  $\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{reference}} - 1) \times 1000$ , where R is the ratio of  $^{13}\text{C}/^{12}\text{C}$ , and the reference material is the belemnite carbonate standard (PDB) from the Pee Dee Formation. The  $\Delta^{13}\text{C}$  was calculated as  $\Delta^{13}\text{C} (\text{‰}) = (\delta_{\text{a}} - \delta_{\text{p}})/(1 + \delta_{\text{p}}) \times 1000$  (Farquhar et al. 1989), where  $\delta_{\text{a}}$  and  $\delta_{\text{p}}$  refer to the C isotope ratios of atmospheric  $\text{CO}_2$  (-8.0‰) and plant, respectively.

*Plant height, aerial biomass and grain yield* Plant height was measured on 5 plants per plot at physiological maturity (BBCH 89) for field experiments in 2009. Biomass and grain yield were determined based on a single 1 m<sup>2</sup> sub-plot within each plot for field experiment in 2009, and for experiment in 2008 all plants within each plot were harvested. The harvested plant materials were air dried to a constant weight. Harvest index (HI) was calculated as the ratio of grain yield to total aboveground biomass.

*Leaf area index (LAI)* A LAI-2000 Plant Canopy Analyzer (Li-Cor, Lincoln, NE, USA) was used to measure LAI for the field experiment in 2009 as previously described in Chen et al. (2012).

#### Greenhouse experiment

The experiment was conducted in a greenhouse at Alberta Innovates - Technology Futures in Vegreville, Alberta, Canada, with a 16-h photoperiod of natural illumination and supplemented lighting using sodium halide light bulbs, with 25 °C and 15 °C day and night temperatures, respectively. Seeds of each genotype were sown on December 9<sup>th</sup>, 2009 and plants were harvested in mid-March 2010.

Eight seeds were sown at 3 cm depth in each pot (21 cm diameter × 21 cm height) filled with 3.5 kg of soil mix containing field topsoil and peat moss (Promix 'BX') in a 1:3 ratio (v:v). All pots were flushed with 2 L tap water and drained overnight before seeding. Four seedlings per pot were kept 2 weeks after emergence. Each genotype was subjected to well-watered (ww) and water-deficit (wd) treatments with four replicates using a randomized complete block design. The well-watered

pots were maintained at field capacity (ca. 28.8 ± 1.3% volumetric soil moisture content) during the experiment. The water-deficit treatment was imposed for 7 days from the stem elongation stage (BBCH 31) for all genotypes. Average soil moisture content at the end of the water-deficit treatment on day 7 was 10.9 ± 2.7% vol. At the end of the water-deficit treatment, all pots were re-watered to pre-deficit levels and were then maintained under well-watered conditions until grain maturity. During the water-deficit treatment, pots were weighed every day to monitor water use (Chen et al. 2011b).

*Plant measurements* At the beginning and end of the water-deficit treatment, one plant per pot was cut at ground level and oven-dried at 70 °C to a constant weight, to determine dry matter production. For WUE calculation, initial plant weights were subtracted from final plant weights during the water-deficit treatment period. The WUE was determined by dividing aerial biomass production by the cumulative water transpired during the 7 days. On the last day of the water-deficit treatment, one penultimate leaf per pot was sampled for  $\delta^{13}\text{C}$  analysis as described above for the field experiment. Leaf nitrogen and carbon contents were measured on separate subsamples of the same ground leaf material used for  $\delta^{13}\text{C}$  analysis. A 5–7 mg sample was analyzed using an elemental analyser (NA 2500, CE Instruments, Milan, Italy) coupled by a Conflo II interface to a stable isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Those leaves sampled for  $\delta^{13}\text{C}$  measurement were also used to measure leaf relative water content (RWC), and specific leaf area (SLA). The RWC was determined according to Barrs and Weatherley (1962) as:  $\text{RWC} (\%) = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100\%$ , where FW is fresh weight, DW is dry weight and TW is turgid weight. After measuring the FW, leaves were placed in distilled water for 24 h at room temperature in darkness to achieve complete rehydration. TW was measured by taking the leaves out of water, wiping any surface moisture quickly and lightly with a paper towel, and weighing immediately. The SLA was calculated as the ratio of leaf area to leaf dry weight. Leaf area was calculated as leaf length × leaf width × 0.8, where 0.8 is an empirical coefficient (Rebetzke and Richards 1999). At maturity, plant height was measured and the remaining 2 plants per pot were harvested and oven dried at 70 °C for 2 days to determine aerial biomass and grain yield.

**Gas exchange measurements** The topmost fully expanded and sun-exposed leaf (one leaf per pot) on the main stem was chosen for gas exchange measurements on the last day of the water-deficit treatment, using a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc, Lincoln, NE, USA). Gas exchange measurements were conducted between 9 am and noon under good light conditions with leaf chamber temperature set at ambient, PPFD at  $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ , reference  $\text{CO}_2$  at 400 ppm and flow rate kept constant at  $500 \mu\text{mol s}^{-1}$ . Measurements were made after the reading of parameters became relatively stable (usually about 1 min), and included: net  $\text{CO}_2$  assimilation ( $A$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), and intercellular  $\text{CO}_2$  concentration ( $C_i$ ).  $\text{WUE}_{\text{intrinsic}}$  or  $\text{WUE}_{\text{ic}}$  (the ratio between  $A$  and  $g_s$ ) was calculated from the above measurements.

#### Statistical analysis

Data were analyzed using SAS, version 9.1 (SAS Institute, Inc., Cary, NC). Each year-location combination of the field trials (Lacombe-2008, Castor-2009 and Vegreville-2009) was treated as an environment. Homogeneity of variance and normality of distribution were tested before analysis of variance (ANOVA). Differences among genotypes and between water treatments were examined using the General Linear Model (GLM) procedure. When ANOVA showed significant effects, means were separated by Bonferroni post hoc tests. Correlation analysis was performed to evaluate the relationship between traits using the CORR procedure in SAS. An  $\alpha$  value of 0.05 was chosen to indicate statistical significance. In order to ascertain the most discriminant traits between groups, stepwise discriminant analysis was performed. Initially, all traits were reviewed and evaluated to determine which one contributed significantly to the discrimination between groups, and the trait(s) that contributed most significantly to the discrimination were retained. The process was repeated until no more significant contribution from the remaining traits was found. In the end, all the statistically significant traits from stepwise discriminant analysis were used in multivariate CDA to determine which series of correlated traits best discriminate the genotypes. The squared Mahalanobis distance ( $D^2$ ) was used to express the resemblance or separation between target groups (Loos 1993).

## Results

### Field performance of parental lines across locations and years

The difference of leaf  $\Delta^{13}\text{C}$  between parental lines (W89 and I60) was consistently significant across years and locations (eight different environments), and the significance level was 0.01 for four environments and 0.001 for three environments (data from Chen et al. 2012). The average biomass and grain yield for W89 and I60 at Lacombe-2008 ( $1370$  and  $607 \text{ gm}^{-2}$ ) and Vegreville-2009 ( $1012$  and  $525 \text{ gm}^{-2}$ ) were significantly higher than those at Vegreville-2006 ( $499$  and  $246 \text{ gm}^{-2}$ , data from Chen et al. 2011b) and Castor-2009 ( $251$  and  $121 \text{ gm}^{-2}$ ). The absolute biomass and grain yields of W89 were higher than those of I60 at Lacombe-2008 (Table 2) and Vegreville-2009, but less than those of I60 at Vegreville-2006 and Castor-2009. The HI of W89 ranged from 0.41 to 0.51, and that of I60 ranged from 0.42 to 0.55 (Table 2 and data from Chen et al. 2011b) across four location-year combinations. In 2009, plant height and LAI were significantly reduced at Castor as compared with Vegreville. Of the two parental lines, I60 was taller than W89 at Castor-2009 ( $p=0.011$ ).

### Performance of RILs under field conditions

There were significant differences in leaf  $\Delta^{13}\text{C}$  between the high- $\Delta^{13}\text{C}$  and low- $\Delta^{13}\text{C}$  groups (Table 1). All genotypes had the highest values of leaf  $\Delta^{13}\text{C}$  at Lacombe-2008, followed by Vegreville-2009 and Castor-2009. Considering location means,  $\Delta^{13}\text{C}$  was 1.10‰ lower in Castor-2009 than in Vegreville-2009, 2.72‰ lower in Castor-2009 than in Lacombe-2008. The difference in the field leaf  $\Delta^{13}\text{C}$  between the highest and lowest RILs was 2.83, 1.64 and 1.73‰ at Lacombe-2008, Castor-2009, and Vegreville-2009, respectively.

The ten barley genotypes (RILs and their parents) did not exhibit significant differences in any of the traits measured at Vegreville-2009, but they differed significantly in plant height, biomass and grain yield at Castor-2009 (Table 2). RIL '147' had higher biomass and grain yield than any other genotype at Castor-2009. In contrast, RIL '196' had the lowest biomass, grain yield and plant height at Castor-2009. Amongst all genotypes, RILs '144' and '116' had above average

**Table 2** Mean biomass, grain yield, harvest index (HI), leaf area index during stem elongation stage (L-AI-S), leaf area index during grain filling stage (L-AI-G) and plant height (PH) of ten barley lines under field conditions at three different sites (Lacombe, Castor and Vegreville)

Group	Genotype	Biomass (g m <sup>-2</sup> )		Grain yield (g m <sup>-2</sup> )		HI		LAI-S (m <sup>2</sup> m <sup>-2</sup> )		LAI-G (m <sup>2</sup> m <sup>-2</sup> )		PH (cm)	
		L-08 <sup>1,2</sup>	V-09 <sup>1</sup>	L-08	V-09	L-08	C-09	V-09	C-09	V-09	C-09	V-09	C-09
High- $\Delta^{13}\text{C}$	18	1564	958	244 <sup>b</sup>	495	124 <sup>bc</sup>	0.43	0.51	3.98	1.34	5.62	1.64	86
	127	1274	807	244 <sup>b</sup>	410	124 <sup>bc</sup>	0.41	0.50	3.54	1.47	4.27	1.57	82
	176	1540	723	299 <sup>ab</sup>	364	153 <sup>ab</sup>	0.43	0.49	3.28	1.83	4.00	1.69	71
	196	2403	800	174 <sup>c</sup>	427	75 <sup>d</sup>	0.41	0.53	3.43	1.02	4.23	1.29	73
	I60049	1229	987	265 <sup>b</sup>	521	128 <sup>bc</sup>	0.42	0.53	4.60	1.37	4.89	1.71	73
Group mean	1602	855	245	444	121	0.42	0.51	3.77	1.41	4.60	1.58	77	
Low- $\Delta^{13}\text{C}$	85	1811	596	238 <sup>bc</sup>	271	107 <sup>cd</sup>	0.36	0.45	3.33	1.09	3.83	1.62	72
	116	1790	919	289 <sup>b</sup>	487	131 <sup>bc</sup>	0.48	0.53	4.61	1.48	4.68	1.65	77
	144	2606	946	292 <sup>ab</sup>	487	139 <sup>bc</sup>	0.38	0.51	4.19	1.51	4.64	1.97	88
	147	1549	837	356 <sup>a</sup>	421	194 <sup>a</sup>	0.44	0.50	4.53	1.63	5.11	1.58	74
	W89 <sup>3</sup>	1512	1038	236 <sup>bc</sup>	529	113 <sup>bcd</sup>	0.46	0.51	4.52	1.39	4.54	1.34	67
Group mean	1854	867	282	439	137	0.42	0.50	4.23	1.42	4.56	1.63	76	
Significance													
Group	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Location-year	***	***	***	***	***	***	***	***	***	***	***	***	***
Group × Location-year	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

<sup>1</sup> L-08, V-09, and C-09 stand for location by year (environment), Lacombe-2008, Vegreville-2009, and Castor-2009<sup>2</sup> Data for Lacombe-2008 were only from one replicate (except parental lines), and therefore it was not subjected to ANOVAGenotypic differences across the groups in biomass, grain yield and plant height were only found at Castor-2009, means followed by a different letter were significantly different across the groups and genotypes at  $P < 0.05$ <sup>3</sup> W89: W89001002003<sup>4</sup> ns, not significant; \*\*\* indicates significance level at 0.1%

**Table 3** Correlations between biomass, grain yield, harvest index (HI), leaf area index during stem elongation stage (LAI-S), leaf area index during grain filling stage (LAI-G), plant height (PH) and leaf carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) of 10 barley genotypes at Castor and Vegreville in 2009

	Biomass	Grain yield	HI	LAI-S	LAI-G	PH
<i>Castor-2009</i>						
Grain yield	0.94***					
HI	0.41*	0.69***				
LAI-S	0.54**	0.60***	0.45*			
LAI-G	0.46*	0.38*	ns	0.55**		
PH	0.61***	0.56**	ns	0.44*	0.51**	
$\Delta^{13}\text{C}$	0.38*	0.41*	ns	ns	ns	
<i>Vegreville-2009</i>						
Grain yield	0.99***					
HI	0.69***	0.80***				
LAI-J	0.72***	0.68***	ns			
LAI-G	0.64***	0.59***	ns	0.78***		
PH	0.64***	0.63***	0.41*	0.54**	0.67***	
$\Delta^{13}\text{C}$	0.48**	0.51**	0.44*	ns	0.53**	0.66***

\*, \*\* and \*\*\* indicate 5, 1 and 0.1% significance levels, respectively; ns, not significant

performance in biomass, grain yield and LAI at both locations in 2009. RIL '85' was consistently low in biomass, grain yield, HI and LAI-S in 2009. Leaf  $\Delta^{13}\text{C}$  showed significant positive correlations with biomass and grain yield under field conditions (Table 3). At Vegreville-2009, leaf  $\Delta^{13}\text{C}$  was also positively correlated with HI, LAI-G and plant height (Table 3).

#### Performance of RILs under greenhouse conditions

The RILs from the high- $\Delta^{13}\text{C}$  group exhibited significantly higher leaf area, leaf  $N_m$  (nitrogen content per unit dry mass) and plant height than those of the low- $\Delta^{13}\text{C}$  group under both well-watered and water-deficit conditions (Table 4). Under water-deficit conditions, the high- $\Delta^{13}\text{C}$  group had significantly higher biomass and leaf  $C_m$  (Leaf carbon content per dry mass) but significantly lower WUE compared with the low- $\Delta^{13}\text{C}$  group.

Substantial genotypic diversity was observed under both water availability conditions for biomass, grain yield, HI, WUE, LA, leaf  $N_m$ , plant height and leaf  $\Delta^{13}\text{C}$  (Table 4). The drought treatment significantly reduced biomass, SLA, RWC, plant height,  $A$ ,  $g_s$  and leaf  $\Delta^{13}\text{C}$  (Tables 4 and 5). Among all ten genotypes, RIL '147' showed the lowest decline in  $A$ ,  $g_s$ , internal  $\text{CO}_2$  concentration ( $C_i$ ), and transpiration rate ( $E$ ) on day 7 of withholding irrigation, while RIL '116' exhibited the

largest decline in the four gas exchange parameters (Table 5). However, the genotypic ranking for biomass, grain yield, HI, LA, leaf  $N_m$ , plant height and leaf  $\Delta^{13}\text{C}$  remained stable between well-watered and water-deficit conditions (Table 4).

Leaf  $\Delta^{13}\text{C}$  was negatively correlated with WUE ( $p < 0.01$ , Table 6), and positively correlated with SLA and leaf  $N_m$  under both well-watered and water-deficit conditions. Significant positive relationships among leaf  $\Delta^{13}\text{C}$ , biomass and grain yield under well-watered conditions were observed in the greenhouse experiment (Table 6). A strong positive relationship was found between  $A$  and  $g_s$  under water-deficit conditions ( $r=0.89$ ,  $n=32$ ,  $p < 0.01$ ). There was no significant relationship between WUE and  $\text{WUE}_{ic}$  (Table 6).

#### Performance patterns of barley lines under both field and greenhouse conditions

Stepwise discriminant analysis showed that the most discriminant traits were plant height, leaf  $\Delta^{13}\text{C}$ , HI, grain yield and biomass (Table 7). The multivariate test for differences among the genotypes was significant ( $p < 0.01$ ), whereas the univariate analyses for the combined field and greenhouse data failed to achieve significance ( $p=0.16$ , 0.99, 0.99 and 0.18 for plant height, biomass, grain yield and leaf  $\Delta^{13}\text{C}$ , respectively) except for HI

**Table 4** Abbreviations, descriptions and mean values for plant productivity, leaf structure and leaf function traits measured under well-watered (ww) and water-deficit (wd) conditions in

the greenhouse study. All the traits were recorded on the full set of 10 barley lines. For each trait, general means and significance level were indicated for group and treatment effects.

Variable	Description	Group difference						Genotypic diversity <sup>2</sup>				Drought effect <sup>3</sup>	
		ww			wd			ww		wd		ww vs wd	
		H <sup>1</sup>	L <sup>1</sup>	H vs L	H	L	H vs L	Mean	p	Mean	p	p	r
Biomass	g plant <sup>-1</sup>	20.3	19.2	ns <sup>4</sup>	19.2	17.5	*	19.8	**	18.4	**	**	0.41*
Grain yield	g plant <sup>-1</sup>	9.2	8.3	ns	8.8	7.9	ns	8.7	***	8.3	***	ns	0.68***
HI	Harvest index	0.45	0.43	ns	0.46	0.45	ns	0.44	***	0.45	**	ns	0.72***
PH	Plant height (cm)	90	83.9	*	88	80	**	86.8	***	84	***	*	0.79***
WUE	Water-use efficiency (g kg <sup>-1</sup> )	3.9	4.0	ns	3.6	4.2	*	4.1	**	3.9	*	ns	ns
LA	Penultimate leaf area (cm <sup>2</sup> )	67.1	57.2	***	63.4	56.3	**	62.1	**	59.9	***	ns	0.50**
N <sub>m</sub>	Leaf nitrogen content per dry mass (mg g <sup>-1</sup> DW) <sup>5</sup>	53.8	48.2	*	53.6	47.6	**	50.8	**	50.6	**	ns	0.43*
C <sub>m</sub>	Leaf carbon content per dry mass (mg g <sup>-1</sup> DW)	420	416	ns	419	411	**	417.6	ns	415	ns	ns	ns
SLA	Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )	433	442	ns	416	404	ns	438	ns	410	ns	**	ns
RWC	Relative water content (%)	95.7	95.3	ns	88.6	89.9	ns	95.5	ns	89.3	ns	***	ns
A	Assimilation rate (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	15.07	13.62	*	7.03	8.71	ns	14.4	ns	7.4	ns	***	ns
g <sub>s</sub>	Stomata conductance (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	644.7	569.9	ns	75.7	118.0	ns	608.9	ns	83.2	ns	***	ns
WUE <sub>ic</sub>	Intrinsic water-use efficiency (μmol mol <sup>-1</sup> )	0.02	0.03	ns	0.12	0.10	ns	0.02	ns	0.11	ns	***	ns
Δ <sup>13</sup> C	Leaf carbon isotope discrimination (‰)	22.1	21.4	**	21.6	21.0	**	21.7	***	21.3	***	***	0.48**

<sup>1</sup> H and L stand for high-Δ<sup>13</sup>C group and low-Δ<sup>13</sup>C group<sup>2</sup> Genotypic ranking for each trait were estimated by ANOVA under well-watered and water-deficit conditions, respectively<sup>3</sup> The effects of drought on each trait were estimated by ANOVA and correlations (r) were calculated between well-watered and water-deficit conditions for each trait<sup>4</sup> ns, non-significant, and \*, \*\* and \*\*\* indicate 5, 1 and 0.1% significance levels, respectively<sup>5</sup> DW, dry weight

( $p=0.01$ ). The first two canonical discriminant functions (CAN) accounted for 90% of the genotypic variance. The HI and leaf  $\Delta^{13}\text{C}$  had large loadings on CAN1 (0.45 and 0.37, respectively). The CAN2 was dominated by large loading from plant height and HI (0.38 and 0.48, respectively).

Three clusters of genotypes were identified from the CAN1×CAN2 plane (Fig. 1). Cluster I included genotypes '18', '116', '176', '196' and I60; and cluster II contained RILs '85', and '144'; and cluster III had RILs '127' and '147'. Cluster III was the overlapped part between cluster I and cluster II as suggested by the probability of Mahalanobis distance. The pair-wise distances between RIL '127' and all the genotypes in cluster

I were not significant except with I60 ( $p<0.01$ ), and RIL '127' clustered with the RIL '85' instead of '144' ( $p=0.02$ ) in cluster II. RIL '147' fitted into cluster II and also was not different from '18' and '196' in cluster I. The scatter plot showed that W89 was separated from the other genotypes, except with I60 ( $p=0.054$ ).

## Discussion

### Stability of leaf $\Delta^{13}\text{C}$ across environments

Across eight different environments (location-year combinations) we studied in the field, the leaf  $\Delta^{13}\text{C}$



**Table 5** Leaf carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) and leaf gas exchange parameters (assimilation rate ( $A$ ), stomatal conductance ( $g_s$ ), internal  $\text{CO}_2$  concentration ( $C_i$ ), transpiration rate ( $E$ )), and intrinsic water-use efficiency ( $\text{WUE}_{\text{ic}}$ ) of ten barley genotypes on the 7<sup>th</sup> day of water-deficit (WD) and well-watered (WW) conditions in a greenhouse study. Values presented in the Table are mean $\pm$ SE

Treatment	Group	Genotype	WUE (g kg <sup>-1</sup> )	$\Delta^{13}\text{C}$ (‰)	A ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$g_s$ (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	$C_i$ (ppm)	E (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	$\text{WUE}_{\text{ic}}$ ( $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ )	
WD	High- $\Delta^{13}\text{C}$	18	4.33 $\pm$ 0.39	21.22 $\pm$ 0.10	6.98 $\pm$ 0.91	56.6 $\pm$ 7.9	176 $\pm$ 10	1.52 $\pm$ 0.19	0.124 $\pm$ 0.006	
		127	3.80 $\pm$ 0.09	20.90 $\pm$ 0.19	6.15 $\pm$ 0.27	48.0 $\pm$ 6.4	165 $\pm$ 16	1.35 $\pm$ 0.15	0.132 $\pm$ 0.011	
		176	3.20 $\pm$ 0.25	21.91 $\pm$ 0.05	8.38 $\pm$ 2.23	94.2 $\pm$ 40.6	185 $\pm$ 32	2.17 $\pm$ 0.80	0.117 $\pm$ 0.022	
		196	3.76 $\pm$ 0.27	21.49 $\pm$ 0.11	7.85 $\pm$ 1.76	84.9 $\pm$ 33.6	194 $\pm$ 25	2.04 $\pm$ 0.65	0.112 $\pm$ 0.017	
		I60049	2.75 $\pm$ 0.08	22.76 $\pm$ 0.11	5.79 $\pm$ 1.65	94.4 $\pm$ 51.2	213 $\pm$ 58	2.10 $\pm$ 1.05	0.103 $\pm$ 0.038	
		Group mean	3.56	21.66	7.03	75.7	187	1.84	0.118	
		Low- $\Delta^{13}\text{C}$	85	4.14 $\pm$ 0.37	21.32 $\pm$ 0.19	10.22 $\pm$ 0.15	149.9 $\pm$ 29.4	252 $\pm$ 21	3.41 $\pm$ 0.50	0.074 $\pm$ 0.013
	116		4.00 $\pm$ 0.34	21.52 $\pm$ 0.22	5.05 $\pm$ 1.16	38.4 $\pm$ 10.6	158 $\pm$ 11	1.10 $\pm$ 0.28	0.139 $\pm$ 0.009	
	144		4.58 $\pm$ 0.40	20.70 $\pm$ 0.36	9.87 $\pm$ 2.11	135.3 $\pm$ 35.9	238 $\pm$ 18	3.02 $\pm$ 0.72	0.083 $\pm$ 0.013	
	147		3.82 $\pm$ 0.26	20.84 $\pm$ 0.53	10.95 $\pm$ 1.93	204.5 $\pm$ 74.9	264 $\pm$ 24	3.99 $\pm$ 1.06	0.067 $\pm$ 0.016	
W89 <sup>1</sup>	4.29 $\pm$ 0.54		20.26 $\pm$ 0.15	7.49 $\pm$ 1.94	62.1 $\pm$ 18.9	173 $\pm$ 8	1.66 $\pm$ 0.47	0.126 $\pm$ 0.007		
	Group mean	4.16	20.93	8.71	118.0	217	2.64	0.098		
WW	High- $\Delta^{13}\text{C}$	18	3.31 $\pm$ 0.17	22.61 $\pm$ 0.25	15.33 $\pm$ 0.63	669.4 $\pm$ 25.3	330 $\pm$ 5	8.68 $\pm$ 0.40	0.021 $\pm$ 0.003	
		127	3.35 $\pm$ 0.16	21.97 $\pm$ 0.33	16.72 $\pm$ 0.56	691.6 $\pm$ 101.7	319 $\pm$ 4	8.25 $\pm$ 0.43	0.025 $\pm$ 0.003	
		176	4.28 $\pm$ 0.54	21.91 $\pm$ 0.17	13.69 $\pm$ 1.83	535.2 $\pm$ 49.8	323 $\pm$ 7	7.45 $\pm$ 0.28	0.026 $\pm$ 0.003	
		196	4.72 $\pm$ 0.68	21.11 $\pm$ 0.24	15.86 $\pm$ 1.62	637.9 $\pm$ 68.5	320 $\pm$ 9	7.91 $\pm$ 0.38	0.026 $\pm$ 0.005	
		I60049	3.75 $\pm$ 0.35	22.64 $\pm$ 0.09	13.73 $\pm$ 0.50	689.3 $\pm$ 44.0	336 $\pm$ 5	8.78 $\pm$ 0.43	0.018 $\pm$ 0.003	
		Group mean	3.88	22.05	15.07	644.7	325	8.21	0.023	
		Low- $\Delta^{13}\text{C}$	85	5.02 $\pm$ 0.24	21.14 $\pm$ 0.17	14.36 $\pm$ 0.46	474.5 $\pm$ 70.4	317 $\pm$ 13	7.48 $\pm$ 0.75	0.029 $\pm$ 0.007
	116		2.78 $\pm$ 0.22	22.26 $\pm$ 0.13	14.09 $\pm$ 0.33	629.1 $\pm$ 114.2	326 $\pm$ 5	7.87 $\pm$ 0.51	0.024 $\pm$ 0.003	
	144		4.41 $\pm$ 0.16	21.16 $\pm$ 0.44	12.69 $\pm$ 0.26	675.4 $\pm$ 33.1	337 $\pm$ 2	8.18 $\pm$ 0.12	0.019 $\pm$ 0.001	
	147		3.76 $\pm$ 0.26	21.08 $\pm$ 0.18	13.03 $\pm$ 0.66	543.5 $\pm$ 109.0	324 $\pm$ 8	7.32 $\pm$ 0.68	0.027 $\pm$ 0.005	
W89 <sup>1</sup>	4.10 $\pm$ 0.16		21.31 $\pm$ 0.10	13.95 $\pm$ 0.54	526.9 $\pm$ 76.3	321 $\pm$ 7	7.50 $\pm$ 0.48	0.028 $\pm$ 0.004		
	Group mean	4.01	21.39	13.62	569.9	325	7.67	0.025		

<sup>1</sup> W89: W89001002003

**Table 6** Linear correlations (Pearson's coefficients) among biomass, grain yield, harvest index (HI), water-use efficiency (WUE), penultimate leaf area (LA), leaf nitrogen content ( $N_m$ ), leaf carbon content ( $C_m$ ), specific leaf area (SLA), relative water content (RWC), plant height (PH), assimilation rate ( $A$ ),

stomatal conductance ( $g_s$ ), intrinsic water-use efficiency ( $WUE_{ic}$ ) and leaf carbon isotope discrimination ( $\Delta^{13}C$ ) of ten barley genotypes under well-watered and water-deficit conditions in the greenhouse study

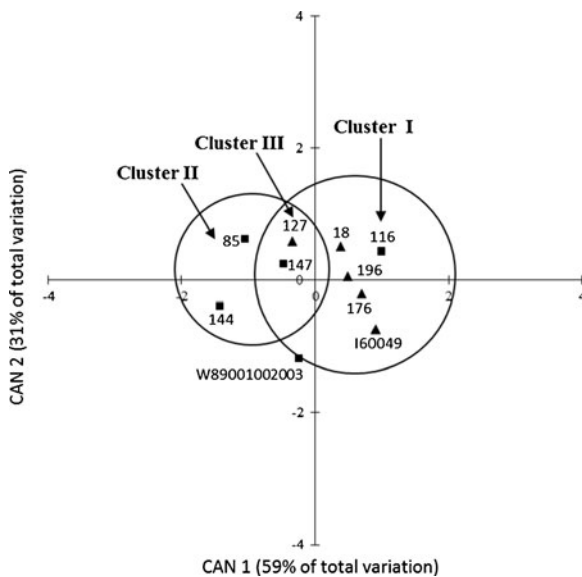
	Biomass	Yield	HI	WUE	LA	$N_m$	$C_m$	SLA	RWC	PH	$A$	$g_s$
<i>Water-deficit</i>												
Yield	0.81***											
HI		0.79***										
LA		0.36*	0.39*									
$N_m$				-0.45**	0.46**							
SLA				-0.41*		0.39*	-0.57***					
PH	0.39*						0.36*					
$A$									0.43*			
$g_s$									0.38*		0.89***	
$WUE_{ic}$											-0.83***	-0.90***
$\Delta^{13}C$				-0.58***	0.47**	0.62***		0.64***				
<i>Well-watered</i>												
Yield	0.88***											
HI	0.44**	0.81***										
WUE	-0.62***	-0.59***	-0.32*									
$N_m$		0.32*										
SLA				-0.35*			-0.60***					
PH					0.45**							
$A$			0.32*		0.39*					0.34*		
$WUE_{ic}$											0.33*	-0.83***
$\Delta^{13}C$	0.39*	0.39*		-0.51***		0.56**		0.51**				

\*, \*\* and \*\*\* indicate 5, 1 and 0.1 % significant level, respectively.

**Table 7** Canonical loadings of the independent variables on the first two canonical discriminant variates of the ten genotypes. The study was conducted under field (Vegreville and Castor) and greenhouse conditions in 2009

Trait	Canonical discriminant function	
	CAN1	CAN2
Plant height	-0.178	0.379
Biomass	0.018	-0.095
Yield	0.036	-0.099
HI	0.452	0.476
Leaf $\Delta^{13}C$	0.366	0.141
Canonical correlation	0.636	0.515
Level of significance (P value)	<0.001	0.020
Variance account for	0.590	0.310

of W89 was consistently lower than that of I60, suggesting that the trait could be intrinsic and under strong genetic control (Anyia et al. 2007; Chen et al. 2011b). This finding is consistent with several other reports on different crops (Hubick et al. 1988; Condon and Richards 1992; Rebetzke et al. 2008; Stiller et al. 2005). For example, Hubick et al. (1988) studied field-grown peanut cultivars and observed that the broad-sense heritability ( $H^2$ ) of  $\Delta^{13}C$  for the whole plant (without the pods and roots) was 0.81, and no significant interaction was found between genotype and environment for  $\Delta^{13}C$ . Condon and Richards (1992) observed that the  $H^2$  of  $\Delta^{13}C$  in wheat was greatest for plant material sampled before or during early jointing stage (0.95 on genotype basis or 0.88 on a single-plot basis). In another study on wheat mapping populations, the narrow-sense heritability ( $h^2$ ) of leaf  $\Delta^{13}C$  varied from 0.37 to 0.91 on a single environment basis



**Fig. 1** Canonical discriminant analysis scatter plot separates the ten barley genotypes into three clusters based on plant height, harvest index and leaf carbon isotope discrimination under field and greenhouse conditions

and from 0.76 to 0.86 on genotype-mean basis across three years (Rebetzke et al. 2008). In a study on cotton, the  $H^2$  for leaf  $\Delta^{13}\text{C}$  (0.68) was higher than that of net photosynthesis (0.65) and lint yield (0.56) (Stiller et al. 2005). These results suggest that leaf  $\Delta^{13}\text{C}$  is under strong genetic control.

#### Leaf $\Delta^{13}\text{C}$ and temporal rainfall distribution across environments

On the Prairies in western Canada, barley relies on stored-moisture from snow melt to deal with the low rainfall situation within the growing season (Anyia et al. 2008). Overall, the difference of the leaf  $\Delta^{13}\text{C}$  between the extreme genotypes was smallest at Castor-2009, largest at Lacombe-2008 and intermediate at Vegreville-2009, and such differences could be ascribed to precipitation (Table 8), especially temporal rainfall distribution, which is critical for crop growth (Bonsal et al. 1999; Chakravartia 1972). The rainfall during June and July (Table 8) was higher at Lacombe-2008, followed by Vegreville-2009 and Castor-2009; this also was the order of mean site leaf  $\Delta^{13}\text{C}$  values of the genotypes. The correlation analysis showed that leaf  $\Delta^{13}\text{C}$  was significantly related to June rainfall ( $r=0.46$ ,  $n=120$ ,  $p<0.01$ ) and total

precipitation ( $r=0.45$ ,  $n=120$ ,  $p<0.01$ ). The low rainfall during June and July may explain the low leaf  $\Delta^{13}\text{C}$  value and narrow leaf  $\Delta^{13}\text{C}$  differences for the extreme genotypes grown at Castor-2009. Teulat et al. (2002) reported similar findings when they observed that both total rainfall and the ratio of rainfall to evapo-transpiration had a significant impact on  $\Delta^{13}\text{C}$ .

#### Relationships between leaf $\Delta^{13}\text{C}$ and agronomic performance

Positive or neutral relationships between  $\Delta^{13}\text{C}$  and grain yield or biomass are often reported in environments with large within-season rainfall or supplemental irrigation, such as wheat and barley grown in the Mediterranean (Araus et al. 2003; Condon et al. 1993; Jiang et al. 2006; Merah et al. 1999; Teulat et al. 2002; Teulat et al. 2001; Voltas et al. 1999). For stored-moisture environments such as eastern Australia and the Canadian Prairies, negative association between  $\Delta^{13}\text{C}$  and grain yield has been reported (Anyia et al. 2007; Condon et al. 1993; Rebetzke et al. 2002). In our study, leaf  $\Delta^{13}\text{C}$  was found to be positively correlated with biomass and grain yield under field conditions (Table 3). A possible explanation of such relationship is suggested by the positive association between leaf  $\Delta^{13}\text{C}$  and  $g_s$ , which has previously been reported in barley (Chen et al. 2011b), common bean (*Phaseolus vulgaris* L.) (Ehleringer 1990) and rice (Kondo et al. 2004; Takai et al. 2009). Plants with a higher  $g_s$  are associated with a higher photosynthetic capacity (Wong et al. 1979), which could in turn increase biomass production and thereby final yield. Low  $g_s$  thus, low  $\Delta^{13}\text{C}$  or high WUE might constrain plant performance under dry environments due to reductions in carbon fixation per unit leaf area as stomata close. Low  $\Delta^{13}\text{C}$  or high WUE under dry conditions can occur at the expense of absolute yield performance (Bloch et al. 2006; Clover et al. 2001). A significantly lower  $g_s$  in W89 than I60 grown in field environments may account for differences in performance between W89 and I60 (Chen et al. 2011b). However, there was no significant correlation between leaf  $\Delta^{13}\text{C}$  and biomass or grain yield under water deficit conditions in the glasshouse. This was likely due to the severity of the imposed water deficit on all genotypes. The leaf  $\Delta^{13}\text{C}$  reflects the time integrated

**Table 8** Monthly precipitation (mm) over the growing season for the three field locations during 2008 and 2009; and the mean, minimum, and maximum air temperature (T<sub>m</sub>) for ten-day periods after sowing are also provided.

Location-year	Sowing date	Precipitation (mm)				Mean T <sub>m</sub> (°C)	Mean min. T <sub>m</sub> (°C)	Mean max. T <sub>m</sub> (°C)
		June	July	August	Total			
Lacombe-2008	May 15 <sup>th</sup>	45.8	48.8	55.5	150.1	14.2	6.7	20.7
Vegreville-2009	May 22 <sup>nd</sup>	32.2	44.6	25.2	102.0	13.1	3.4	20.6
Castor-2009	May 13 <sup>th</sup>	21.5	33.8	51.9	107.2	5.8	-1.3	13.1

measure of the transpiration efficiency (aerial biomass/water transpired) over the period when leaf tissue was formed (Rebetzke et al. 2002). The leaf  $\Delta^{13}\text{C}$  in the present study reflected the integrated WUE from emergence to stem elongation, a critical stage for yield formation in barley (Anyia et al. 2008). Of the three field locations tested, leaf  $\Delta^{13}\text{C}$  values of all genotypes were lowest at Castor-2009, highest at Lacombe-2008, and intermediate at Vegreville-2009 (Table 1); so was the order of the mean performance in biomass and grain yield of all genotypes (Table 2), which also reflected the average soil available moisture for these locations. Bloch et al. (2006) also suggested that  $\Delta^{13}\text{C}$  can be used as a sensitive indicator for water availability during the growing period. The poor performance of all genotypes at Castor-2009 can be ascribed to lower soil moisture resulting in the lower leaf  $\Delta^{13}\text{C}$  values at this location, compared to leaf  $\Delta^{13}\text{C}$  at other locations (Table 1).

In a previous study, Chen et al. (2011b) reported that low leaf  $\Delta^{13}\text{C}$  genotype ‘CDC Cowboy’ maintained its biomass and grain yield at a low  $g_s$ , which were comparable to genotypes with high  $g_s$  under field conditions. In this study, the performance of RIL ‘147’ with low- $\Delta^{13}\text{C}$  was similar to that of ‘CDC Cowboy’. RIL ‘147’ was the most productive one among all the ten genotypes tested under drier conditions at Castor (Table 8), and it also showed the least decline in biomass and grain yield between Vegreville and Castor during the 2009 growing season. Genotypes such as RIL ‘147’ may be suited for the Canadian Prairies, where crops rely heavily on stored soil moisture (with limited rainfall) within the growing season. In low soil moisture environments such as Castor-2009, genotypes with low leaf  $\Delta^{13}\text{C}$  such as ‘147’ that can maintain relatively higher biomass and grain yield at low  $g_s$  than other genotypes should be targeted in breeding programs to achieve a higher stability of yield across locations.

#### Physiological differences under controlled drought conditions

In the greenhouse study, all genotypes showed reduced  $A$  and  $g_s$  when subjected to the water deficit treatment. The positive relationship between  $A$  and  $g_s$  under water-deficit conditions suggested that the decline in  $A$  was driven by stomatal limitations. Similar results have been reported for other crops (Monneveux et al. 2006; Scartazza et al. 1995; Xu et al. 2009). One of the most basic questions regarding  $\Delta^{13}\text{C}$  is whether a low value of leaf  $\Delta^{13}\text{C}$  may arise from reduced  $g_s$  or increased photosynthetic capacity or both (Araus et al. 1997; Condon et al. 1990; Condon et al. 2004; Morgan and LeCain 1991). A proportional variation in both  $A$  and  $g_s$  might have no effect on  $\text{WUE}_{\text{ic}}$ , while a comparable change in  $A$  with  $g_s$  that remains constant would cause a substantial variation in  $\text{WUE}_{\text{ic}}$ , and vice versa (Chen et al. 2011a). In this study, both  $A$  and  $g_s$  decreased under water-deficit conditions with a strong positive correlation, but the variation in  $g_s$  was proportionally greater than  $A$ , which suggested that  $g_s$  caused the variation in  $\Delta^{13}\text{C}$ . A similar pattern has been found by Xu et al. (2009). Roussel et al. (2009) concluded that leaf  $\Delta^{13}\text{C}$  is under strong genetic control, and genetic differences in leaf  $\Delta^{13}\text{C}$  and  $\text{WUE}_{\text{ic}}$  can be ascribed to differences in  $g_s$  and stomatal density instead of  $A$ . RIL ‘147’ maintained the highest  $g_s$  among the ten genotypes on the last day of the water-deficit treatment, which may be responsible for the low leaf  $\Delta^{13}\text{C}$  value of this line.

Genotypic ranking for leaf area, leaf  $N_m$ , plant height and leaf  $\Delta^{13}\text{C}$  was consistent between the well-watered and water-deficit treatments (Table 4,  $r=0.5, 0.43, 0.79$  and  $0.48$ , respectively). The leaf  $\Delta^{13}\text{C}$  was positively correlated with leaf  $N_m$  (Table 6) in this study, and similar results have also been reported in rice (This et al. 2010; Xu et al. 2009). As suggested by This et al.

(2010), the positive relationship between leaf  $\Delta^{13}\text{C}$  and  $N_m$  suggests a tradeoff between WUE and nitrogen use efficiency through regulation between  $A$  and  $g_s$ . The positive relationship between leaf  $\Delta^{13}\text{C}$  and leaf  $N_m$  also implies that diversity in photosynthetic capacity may contribute to the variability of WUE among genotypes. Leaf  $\Delta^{13}\text{C}$  and SLA were also positively correlated under both water conditions, but the genotypic ranking for SLA was not stable between well-watered and water-deficit conditions (Table 4), which may limit its application as a less expensive alternative to leaf  $\Delta^{13}\text{C}$ .

#### Similarities and differences between genotypes

Three distinct clusters of genotypes were identified from CDA primarily based on plant height, HI and leaf  $\Delta^{13}\text{C}$  (Fig. 1). Four out of five genotypes in the high- $\Delta^{13}\text{C}$  group (Table 1) were assigned to cluster I except RIL '127', while the five genotypes in the low- $\Delta^{13}\text{C}$  group were separated into three clusters by CDA (Fig. 1), with W89 and RIL '116' in cluster I, RIL '85' and '144' in cluster II, and RIL '147' in cluster III. This diversity of the low- $\Delta^{13}\text{C}$  group suggests that genotypes with low leaf  $\Delta^{13}\text{C}$  may differ in their level of WUE and drought tolerance. The ability to increase WUE or decrease leaf  $\Delta^{13}\text{C}$  can contribute to drought tolerance, but leaf  $\Delta^{13}\text{C}$  alone is not sufficient to explain the genotypic diversity in drought tolerance as indicated by the multiple patterns through the CDA analysis. The CDA discriminated genotypes largely based on leaf  $\Delta^{13}\text{C}$ , HI and plant height, suggesting HI and plant height also contributed to the discrimination of overall performance of the genotypes.

When assessing the performance of a crop, the trait of ultimate importance is grain yield under the target environment. In this study, different drought tolerance levels were observed as suggested by the cluster patterns from CDA. Cluster I produced more grain yield than the other two clusters at Vegreville-2009, but less grain yield than any other cluster at Castor-2009, suggesting cluster I was drought sensitive. Cluster III showed intermediate performance in grain yield at Vegreville-2009 compared with clusters I and II. Even RILs within the same cluster responded to the low moisture conditions differently. For example, RIL '176' showed less decline in biomass (57%) and grain yield (54%) between the two field locations than the

other five genotypes in cluster I, which may be explained by its early maturity (data not shown). In contrast, the three most productive genotypes (RIL '18', I60 and W89) at Vegreville-2009 were very sensitive to drier conditions experienced at Castor-2009, with the biomass and grain yield declining by 73 and 72%, (RIL '18'), 72 and 75%, (I60), and 77 and 78%, (W89), respectively, at Castor compared with Vegreville. RIL '127' was assigned to cluster III due to its average performance under field and greenhouse conditions. RIL '147' from cluster III was relatively more productive than other genotypes under drier conditions at Castor. It showed the least decline in biomass (56%) and grain yield (52%) among all ten genotypes between Vegreville and Castor, by maintaining a higher stomatal opening and a higher  $A$  than other genotypes evaluated under water-deficit conditions (Table 6).

#### Conclusions

This study demonstrated the stability of leaf  $\Delta^{13}\text{C}$  of RILs derived from parental lines with contrasting levels of leaf  $\Delta^{13}\text{C}$ . The overall performance of RILs was consistent with their leaf  $\Delta^{13}\text{C}$  grouping. In this study, a low leaf  $\Delta^{13}\text{C}$  genotype (RIL '147') was identified as a high yielding line under dry conditions that showed the least decline in biomass and grain yield between Vegreville and Castor sites during the 2009 growing season. Genotypes such as RIL '147' may be of interest for achieving yield stability on the Canadian Prairies, where crops rely on stored soil moisture due to limited rainfall within the growing season. Given the predominant positive correlation between leaf  $\Delta^{13}\text{C}$  and yield in this study, care must be taken when selecting low leaf  $\Delta^{13}\text{C}$  lines such as RIL '147' to avoid yield penalty under favorable conditions. To achieve yield stability, co-selection for low leaf  $\Delta^{13}\text{C}$  and other yield traits (such as HI, plant height and early seedling vigour) is recommended. Additional field testing is needed to further evaluate the yield stability of the parental lines and their RILs used in this study.

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