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# Leaf Carbon Isotope Discrimination as an Accurate Indicator of Water-Use Efficiency in Sunflower Genotypes Subjected to Five Stable Soil Water Contents

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## Keywords

carbon isotope discrimination; soil water content; sunflower; water-use efficiency

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## Abstract

Leaf carbon isotope discrimination (CID) has been suggested as an indirect tool for breeding for water-use efficiency (WUE) in various crops. This work focused on assessing phenotypic correlations between WUE and leaf CID and analysing genotypic variability in four sunflower genotypes grown in a greenhouse in pots with five different stable levels of soil water content (SWC). We measured WUE at whole plant and leaf (intrinsic) level. At whole plant level, WUE was derived from the ratio of total dry aerial biomass (BM) to cumulative water transpired (CWT). At leaf level, intrinsic WUE was calculated as the ratio of light-saturated CO<sub>2</sub> assimilation to stomatal conductance ( $A/g_s$ ) in younger expanded leaves. Significant differences among the four genotypes and the five SWCs were observed for whole plant and leaf WUE and CID. Strong negative correlations were observed between whole plant WUE and CID as well as between intrinsic WUE and CID with decreasing water availability. No relationships appeared between BM production and WUE or CID. Our results can help agronomists and breeders to evaluate sunflower lines with high WUE for adaptation to drought conditions and for reducing water consumption and crop water needs. Leaf CID appears to be a pertinent and valuable trait to select sunflower genotypes with high WUE.

## Introduction

Sunflower (*Helianthus annuus* L.), the fourth important sources of vegetable oil in the world (List 2014), is mainly produced in Ukraine, Russia, European countries and Argentina (USDA 2014). In recent years, sunflower planted area has increased (Labalette et al. 2012) and expanded in the arid region of the Mediterranean and North Africa (Blamey et al. 1997, Kane et al. 2013). However, in southern Europe, it suffers from intense period of water deficit because it is mostly planted in low rainfall areas (Dufresne et al. 2006, Casadebaig et al. 2008). According to Food and Agriculture Organization of the United Nations (FAO) publication reported by Garcia-Via et al. (2012), sunflower yields vary between <0.5 ton ha<sup>-1</sup> in low rainfall areas and >5 ton ha<sup>-1</sup> under ample water supply. In addition, sunflower is considered well adapted to drought, but genotypes

are not homogeneously efficient in the use of water. Systematic analyses of the physiological basis of drought tolerance in sunflower and purposeful attempts to breed for greater drought resistance are still limited (Grieu et al. 2008).

Water availability is considered to be the main factor limiting ecosystem and agrosystem biomass (BM) production. This is because plant growth depends on two closely linked leaf processes, photosynthesis and transpiration. Water-use efficiency (WUE) is the ratio between two physiological (transpiration and photosynthesis) and agronomic (yield and crop water use) entities, and WUE is mostly discussed in terms of plant production rather than gas exchange (Ehleringer et al. 1993, Ebdon and Kopp 2004). On the one hand, improving WUE would reduce the water requirement for a given yield and thus could help save a considerable amount of irrigation water. On the other

hand, an improvement in WUE can significantly increase total BM production as well as yield at a limited and known soil moisture reserve (Impa et al. 2005). Blum (2009) recently proposed that selection for high WUE in breeding for water limited conditions could lead to reduce yield and drought resistance. However, most of authors argued that the prospect of improving agronomic WUE by breeding for greater WUE has been and remain an attractive challenge (Fischer 1981, Ehleringer et al. 1993, Condon et al. 2004).

Direct measurement of WUE relies either on extensive leaf gas-exchange data or long-term measurements of plant water consumption and BM production. This is because WUE can be defined either as the ratio of total plant dry matter produced to total water used over the same period or, at leaf level, as the ratio of photosynthetic carbon gain to transpiration water loss (Condon and Richards 1993, Ehleringer et al. 1993, Donovan et al. 2007). These approaches to WUE are logistically difficult in large-scale individual plant screening efforts. It has been demonstrated, however, that leaf carbon isotope discrimination (CID) can be an excellent surrogate for direct measurement of WUE, and several authors have proposed to use this trait as indirect criterion for yield under drought (Farquhar and Richards 1984, Ehleringer et al. 1993, Condon et al. 2002, Xu et al. 2009).

CID is a measure of the ratio of the stable isotopes of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) in plant material relative to the value of the same ratio in the atmosphere (Farquhar et al. 1989, Condon 2004). The dominant processes leading to CID are fractionations associated with  $\text{CO}_2$  diffusion into leaf intracellular airspaces and with  $\text{CO}_2$  carboxylation by the enzyme Rubisco (that catalyses  $\text{CO}_2$  fixation in the Calvin cycle). Discrimination against  $^{13}\text{C}$  in leaves during photosynthesis decreases with water stress, mainly because of the lowered stomatal conductance (Farquhar and Lloyd 1993, Ebdon and Kopp 2004). Therefore, CID in plant tissues shows subtle but systematic variations among different plant genotypes and/or species grown under different water conditions (Farquhar and Richards 1984).

The relationships between CID and WUE have been widely explored in several species, especially wheat (including durum wheat) and rice. A negative correlation between CID and WUE in some wheat genotypes was reported by Farquhar and Richards (1984), Misra et al. (2010) and Rizza et al. (2012). Other authors such as Dingkuhn et al. (1991), Scartazza et al. (1998) and Centritto et al. (2009) have also reported a negative correlation between CID and WUE in rice genotypes. However, in sunflower, the relationship between CID and WUE has rarely been explored. Lauteri et al. (1993) described a negative correlation between CID and WUE in four sunflower genotypes grown in a greenhouse. In addition, Virgona and Farquhar (1996)

and Lambrides et al. (2004) reported the occurrence of correlations between CID and WUE for a range of sunflower genotypes.

In this study, exploring the possibility of using CID as an indicator to select sunflower genotypes with high WUE, we studied the relationship between CID and WUE in four recombinant inbred lines (RILs) of juvenile sunflowers. We were particularly interested in evaluating the CID and WUE at five levels of soil water content (SWC) which were maintained stable during the experiments.

## Materials and Methods

Two experiments were carried out to measure WUE and CID on sunflower plants grown in a greenhouse at the INRA Auzeville station, Toulouse, France ( $43^{\circ}31'46,94''\text{N}$ ;  $1^{\circ}29'59,71''\text{E}$ ). The first experiment (Exp. 1) was carried out in spring 2012, from 19 March to 1 May 2012 (sowing to harvest). The second experiment (Exp. 2) was carried out in autumn, from 17 September to 30 October 2012 (sowing to harvest).

### Plant sources

Four RILs of sunflower (*Helianthus annuus* L.) from the collection of the Laboratory of Plant–Microbe Interactions (LIPM), INRA Toulouse, France, were used in the two experiments, namely RIL 043, RIL 127, RIL 149 and RIL 200. These four RILs are lines from the INEDI population (Vincourt et al. 2012), which were chosen because of their differing WUE response, determined in a previous experiment (data not shown).

### Experimental design and growth conditions

From sowing to harvest, experiments lasted 40 days. Three seeds were sown in each two-litre pot. Ten days after sowing (DAS), the most vigorous plant (based on morphological criteria) in each pot was selected by cutting down the two others. Each pot was put on a scale (maximum capacity 30 kg, precision 2 g, model SXS, GRAM, Spain) connected by interface wireless communication to a computer with installed software (ENSAT 1.07T, developed by Pesage du Sud Ouest, Launaguet, France).

Starting at 21 DAS, the plants were subjected to different water treatments. Soil water conditions were maintained by daily weighing of the pots and watering on the basis of weight loss (the increase in plant weight was considered negligible).

The experiments were arranged in a randomized complete block design with four RILs, five water treatments and five replicates.

### Water treatments and greenhouse conditions of Experiment 1 (19 March–1 May 2012)

In Exp. 1, water treatments were applied consisting in five levels of SWC: 35 %, 23 %, 21 %, 18 % and 16 %. Pots contained 2 kg of a mixture of soil collected from the field (50 %), organic matter (30 %) and sand (20 %). SWC was determined by the gravimetric method described by Lambe and Whitman (1969).

The trials were carried out under well-controlled conditions. Air temperature ( $T$ ) and relative humidity (RH) were automatically recorded every 30 min. Air vapour pressure deficits (VPD) were calculated as described by Allen et al. (1998):  $VPD = es - ea$ ;  $es = 0.6108 \times \exp[17.27T/(T + 237.3)]$ ;  $ea = es \times (RH/100)$ , where  $es$  is the saturation vapour pressure (kPa);  $T$ , the mean air temperature ( $^{\circ}\text{C}$ ); RH, the relative humidity of the air (%).

During the photoperiod (from 05:30 to 18:30 CET), the air temperatures were minimum ( $T_{\min}$ ) 16.7  $^{\circ}\text{C}$ ; maximum ( $T_{\max}$ ) 23.6  $^{\circ}\text{C}$  and mean ( $T_{\text{me}}$ ) 20.8  $^{\circ}\text{C}$ . The relative humidity was minimum ( $RH_{\min}$ ) 29.4 %; maximum ( $RH_{\max}$ ) 52.3 % and mean ( $RH_{\text{me}}$ ) 36.6 %. The vapour pressure deficits (Fig. 1a) were minimum ( $VPD_{\min}$ ) 1.80 kPa, maximum ( $VPD_{\max}$ ) 4.40 kPa and mean ( $VPD_{\text{me}}$ ) 2.81 kPa.

### Water treatments and greenhouse conditions of Experiment 2 (17 September–30 October 2012)

In Exp. 2, water treatments consisted of five levels of SWC: 25 %, 20 %, 16 %, 13 % and 10 %. Pots were filled with soil extracted from the field and sand in equal proportions.

During the photoperiod (from 05:30 to 17:30 CET), the following parameters were measured:  $T_{\min} = 17.8$   $^{\circ}\text{C}$ ,  $T_{\max} = 26.2$   $^{\circ}\text{C}$  and  $T_{\text{me}} = 23$   $^{\circ}\text{C}$ ;  $RH_{\min} = 31.3$  %,  $RH_{\max} = 61.7$  % and  $RH_{\text{me}} = 48.8$  %;  $VPD_{\min} = 1.14$  kPa,  $VPD_{\max} = 2.26$  kPa and  $VPD_{\text{me}} = 1.61$  kPa (Fig. 1a).

### Trait measurements

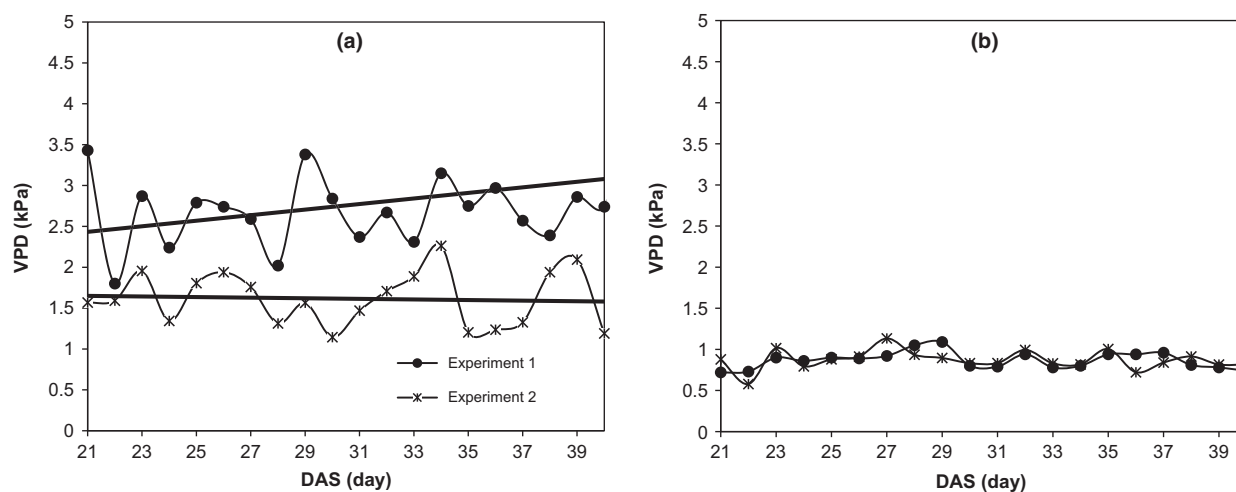
#### *Agronomic traits and water-use efficiency*

At the end of the experiments (23 DAE), the above-ground parts of the plants were harvested. Stems and leaves were oven-dried at 80  $^{\circ}\text{C}$  for 48 h until they reached constant mass to determine total dry aerial BM.

Transpiration water loss (WT) for each plant was estimated every day from the difference in the pot weight. Total transpiration (cumulative water transpired, CWT) for each plant was determined at the end of the experiment by accumulating daily WT. WUE (on a whole plant basis) was determined at the end of the experiments as the ratio of BM to CWT.

#### *Leaf gas-exchange measurements and intrinsic water-use efficiency ( $A/g_s$ )*

Measurements of  $\text{CO}_2$  assimilation rates under saturating light ( $A$ ) and stomatal conductance ( $g_s$ ) were made with a portable Li-6400 (Li-Cor, Lincoln, NE, USA) between 09:00 and 12:00 (Central European Time) in Exp. 2 (from 19 to 21 DAE). All the measurements were taken on a fully expanded leaf (one per plant) under 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD) and 40 Pa  $\text{CO}_2$  partial pressure. Leaf temperature was maintained at  $25 \pm 2$   $^{\circ}\text{C}$  and RH was 50 %.



**Fig. 1** Vapour pressure deficit (VPD) in the day (a) and in the night (b) in greenhouse during the Exp. 1 and Exp. 2. The data represent the mean of VPD in the day (during photoperiod) and in the night. The linear lines in figure 'a' represent the average of VPD in the Exp. 1 and Exp. 2. DAS, days after sowing.

### Carbon isotope discrimination

Oven-dried leaves (including petioles) of each plant were ground into a homogeneous fine powder, and 2–3 mg subsamples was weighed and placed in capsules (Elemental Microanalysis, Okehampton, UK) to be analysed using a continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC Davis Stable Isotope Facility (Davis, CA, USA). Carbon isotope composition ( $\delta$ ) was calculated relative to the international Pee Dee Belemnite (PDB) standard (Farquhar et al. 1989):  $\delta_{\text{plant}} = (R_{\text{sa}} - R_{\text{sd}})/R_{\text{sd}} \times 1000$  [‰] where  $R_{\text{sa}}$  and  $R_{\text{sd}}$  are the  $^{13}\text{C}:^{12}\text{C}$  ratios of the sample and the standard, respectively (Craig 1957). Carbon isotope discrimination (CID) was estimated as:  $\text{CID} = (\delta_{\text{air}} - \delta_{\text{plant}})/(1 + \delta_{\text{plant}}/1000)$  where  $\delta_{\text{air}}$  is the  $^{13}\text{C}$  composition of atmospheric  $\text{CO}_2$ , which is assumed to be  $-8.0$  ‰ (Farquhar et al. 1989).

### Statistical analysis

Data were tested for normal distribution with the Kolmogorov–Smirnov test. All statistical analysis was performed using the statistical package PASW statistics 18 (IBM, New York, NY, USA). Analysis of variance (ANOVA) was used to calculate the effects of genotypes and SWC. For each ANOVA, a trait was considered as a dependent variable. Genotype, SWC and replicate were considered as the fixed factors. Means were compared using a Student–Newman–Keuls (SNK) test ( $P < 0.05$ ). Pearson's correlation coefficients were calculated to determine the phenotypic relationships between WUE, CID and related traits (BM, CWT). Coefficient of determination ( $R^2$ ) was calculated by determining the regressions of main traits, CID and WUE.

## Results

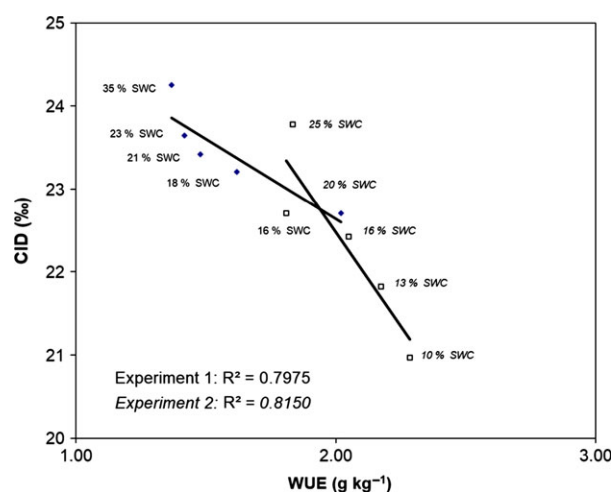
### Relationships between WUE, CID, BM and CWT

In the two experiments, a highly significant negative correlation was observed between WUE and CID (Table 1,  $r_p = -0.66$ ,  $P < 0.001$ ,  $n = 20$  in Exp. 1, and  $r_p = -0.67$ ,  $P < 0.001$ ,  $n = 20$  in Exp. 2), while there were no significant correlations between BM and CID or WUE. In contrast, there was a significant negative correlation between WUE and CWT but only in Exp. 2 ( $r_p = -0.55$ ,  $P < 0.01$ ,  $n = 20$ ). In the two experiments, the coefficient of determination between WUE and CID was high (0.79 in Exp. 1 and 0.81 in Exp. 2; Fig. 2). In the two experiments, there was a concomitant increase in WUE and a decrease in CID from the high to the low SWC for all genotypes. Thus, the highest values of WUE and the smallest values of CID were observed at the smallest SWC, whereas the smallest values of WUE and the highest values of CID were observed at the highest SWC (Fig. 3).

**Table 1** Phenotypic correlations ( $r_p$ ) between water-use efficiency (WUE), carbon isotope discrimination (CID), biomass (BM) and cumulative water transpired (CWT) for four RILs and five soil water contents ( $n = 20$ , average of five replicates)

Traits	WUE ( $\text{g kg}^{-1}$ )	CID (‰)	BM (g)
Experiment 1			
CID (‰)	$-0.66^{***}$		
BM (g)	$-0.09^{\text{ns}}$	$0.44^{\text{ns}}$	
CWT (ml)	$-0.37^{\text{ns}}$	$0.62^{***}$	$0.92^{***}$
Experiment 2			
CID (‰)	$-0.67^{***}$		
BM (g)	$0.39^{\text{ns}}$	$0.18^{\text{ns}}$	
CWT (ml)	$-0.55^{**}$	$0.81^{***}$	$0.50^{**}$

Significance at  $**P < 0.01$ ,  $***P < 0.001$ ; ns, not significant.



**Fig. 2** Relationships between water-use efficiency (WUE) and carbon isotope discrimination (CID) in five soil water contents (SWC) for the Exp. 1 and Exp. 2. For each experiment, values represent mean of four recombinant inbred lines and five replicates ( $n = 5$ ).

### Genotypic variability in WUE and CID in plants growing on five stable SWC

Mean values of WUE were lower in Exp. 1 than in Exp. 2 ( $1.58$  and  $2.03$   $\text{g kg}^{-1}$ , respectively), whereas mean values of CID were higher in Exp. 1 than in Exp. 2 ( $23.45$  ‰ and  $22.37$  ‰, respectively). During Exp. 1, WUE values ranged from  $0.55$  to  $3.13$   $\text{g kg}^{-1}$  and CID values ranged from  $21.50$  to  $24.88$  ‰ (Table 2). The variances of WUE and CID were  $0.34$  and  $0.71$ , respectively. During Exp. 2, WUE values ranged from  $0.79$  to  $4.32$   $\text{g kg}^{-1}$  and CID values ranged from  $21.50$  to  $24.88$  ‰. The variances of WUE and CID were  $0.54$  and  $2.27$ , respectively. These results showed a narrower genotypic variability for WUE and CID in Exp. 1 than in Exp. 2. ANOVA results showed that there were significant effects of genotype and SWC for WUE and CID in

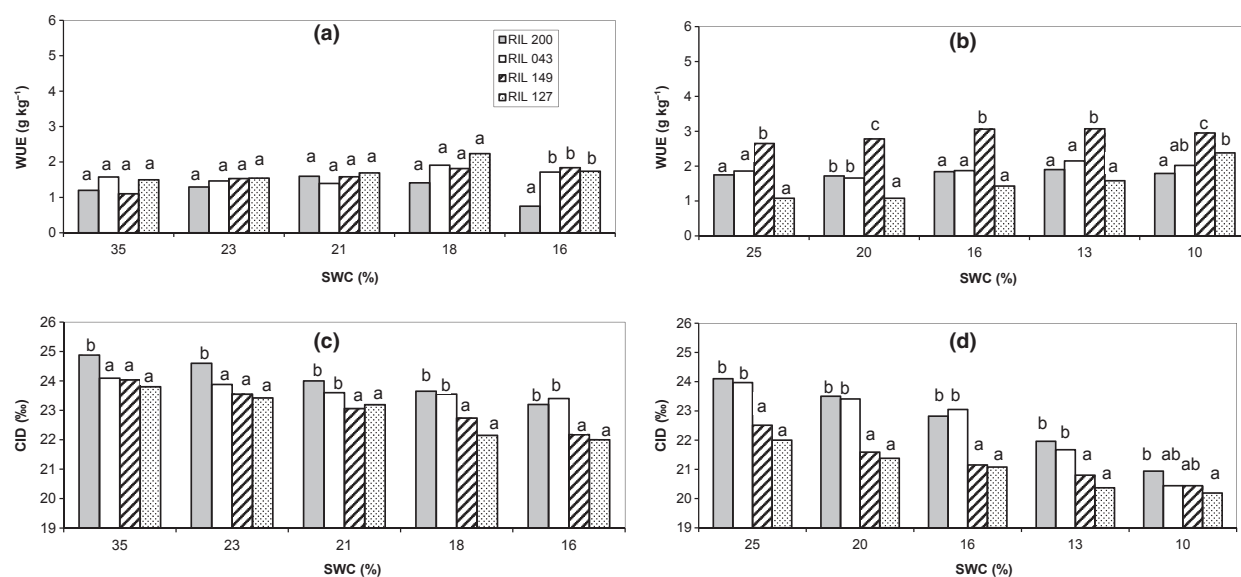
the two experiments. Moreover, there was no significant effect of the genotype and SWC interaction for these two traits in the two experiments.

In Exp. 1, there were no significant differences between genotypes for WUE except for RIL 200 at 16 % SWC (Fig. 3a). WUE values were very low (the power of ANOVA was 0.77; data not shown). In contrast, for CID, significant differences between genotypes appeared at all five SWCs (Fig. 3c). This is consistent with the results for CID of Exp. 2 where genotypes showed differences, with the same ranking as in Exp. 1, under all five SWCs (Fig. 3d). In Exp. 2,

unlike in Exp. 1, significant differences were obtained in WUE between genotypes for all SWCs (Fig. 3b).

### Leaf gas-exchange, intrinsic water-use efficiency ( $A/g_s$ ) and CID in experiment 2

Measurements of gas exchange for the five stable SWCs in Exp. 2 showed a decrease in  $A$  (light-saturated  $\text{CO}_2$  assimilation) and  $g_s$  (stomatal conductance) as water availability decreased. Therefore, high values for  $A$  and  $g_s$  ( $27 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  and  $0.68 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , respectively)



**Fig. 3** Water-use efficiency in Exp. 1 and Exp. 2 (a, b), and carbon isotope discrimination in Exp. 1 and Exp. 2 (c, d), subjected to five soil water contents (SWC) of four genotypes (recombinant inbred lines – RIL 200, RIL 043, RIL 149, RIL 127). Different letters in each SWC level represent significant differences among genotypes (SNK's test,  $P < 0.05$ ).

**Table 2** Genotypic variation, the mean squares of analysis of variance ( $MS_{ANOVA}$ ) for water-use efficiency (WUE), carbon isotope discrimination (CID), biomass (BM) and cumulative water transpired (CWT) among four RILs, five soil water contents (SWC) and five replicates in Exp. 1 and Exp. 2 ( $n = 100$  for each experiment)

Trait	Minimum	Maximum	Mean	S.D.	Variance	MS ANOVA		
						Genotype	SWC	Genotype $\times$ SWC <sup>1</sup>
Experiment 1								
WUE ( $\text{g kg}^{-1}$ )	0.55	3.13	1.58	0.86	0.34	0.94*	1.35***	0.35 <sup>ns</sup>
CID (‰)	21.50	24.88	23.45	0.84	0.71	4.31***	6.19***	0.27 <sup>ns</sup>
BM (g)	0.07	1.87	0.58	0.36	0.13	0.27 <sup>ns</sup>	0.59***	0.06 <sup>ns</sup>
CWT (ml)	121.00	991.00	387.39	180.42	32 552.38	27 573.21 <sup>ns</sup>	438 588.76***	13 247.16 <sup>ns</sup>
Experiment 2								
WUE ( $\text{g kg}^{-1}$ )	0.79	4.32	2.03	0.72	0.51	9.14***	0.85**	0.30 <sup>ns</sup>
CID (‰)	19.68	25.47	22.37	1.51	2.27	12.54***	22.43***	1.61 <sup>ns</sup>
BM (g)	0.21	1.06	0.50	0.19	0.03	0.33***	0.34***	0.02 <sup>ns</sup>
CWT (ml)	105.00	515.00	264.00	95.80	9178.21	26 889.10***	156 976.81***	3264.78 <sup>ns</sup>

Significance at \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; ns, not significant.

<sup>1</sup>Genotype and SWC interaction.

**Table 3** Genotypic variation of net CO<sub>2</sub> assimilation rates (*A*), stomatal conductance (*g<sub>s</sub>*), intrinsic water-use efficiency (*A/g<sub>s</sub>*) among four RILs, five soil water contents (SWC) and five replicates in Exp. 2 (n = 100)

Trait	Minimum	Maximum	Mean	S.D.	Variance	Mean square	
						Genotype	Soil water content
<i>A</i> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	1.70	27	16.68	6.59	43.37	13.53 <sup>ns</sup>	698.12 <sup>***</sup>
<i>g<sub>s</sub></i> (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.02	0.68	0.33	0.18	0.03	0.04 <sup>*</sup>	0.58 <sup>***</sup>
<i>A/g<sub>s</sub></i> (μmol CO <sub>2</sub> mol <sup>-1</sup> H <sub>2</sub> O)	24.29	136.36	59.57	23.04	530.88	1304.38 <sup>**</sup>	5915.00 <sup>***</sup>

Significance at \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; ns, not significant.

**Table 4** Phenotypic correlations (*r<sub>p</sub>*) between carbon isotope discrimination (CID), net CO<sub>2</sub> assimilation rates (*A*), stomatal conductance (*g<sub>s</sub>*), intrinsic water-use efficiency (*A/g<sub>s</sub>*) among four RILs, five soil water contents (SWC) and five replicates in Exp. 2 (n = 100)

Trait	CID (‰)	<i>A</i> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	<i>g<sub>s</sub></i> (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )
<i>A</i> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	0.47 <sup>***</sup>		
<i>g<sub>s</sub></i> (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.45 <sup>***</sup>	0.90 <sup>***</sup>	
<i>A/g<sub>s</sub></i> (μmol CO <sub>2</sub> mol <sup>-1</sup> H <sub>2</sub> O)	-0.30 <sup>***</sup>	-0.47 <sup>***</sup>	-0.72 <sup>***</sup>

Significant at \*\*\*P < 0.001.

were observed at the highest SWC (25 %), whereas low values of *A* and *g<sub>s</sub>* were reached (1.70 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and 0.02 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, respectively) at the smallest SWC level (10 %, Table 3). The values of intrinsic water-use efficiency (*A/g<sub>s</sub>*) ranged from 24.29 to 136.36 μmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O.

ANOVA showed that *A* was not significantly different between genotypes but that significant differences appeared for CO<sub>2</sub> assimilation between SWC levels. By contrast, *g<sub>s</sub>* and *A/g<sub>s</sub>* were significantly different both between genotypes and SWC (Table 3).

Positive correlations were observed between CID and all leaf gas-exchange traits (Table 4). A small but very significant phenotypic correlation was obtained between CID and *A* (*r<sub>p</sub>* = 0.47, P < 0.001, n = 100) as well as between CID and *g<sub>s</sub>* (*r<sub>p</sub>* = 0.45, P < 0.001, n = 100). CID and *A/g<sub>s</sub>* were negatively correlated (*r<sub>p</sub>* = -0.30, P < 0.001, n = 100), and *A/g<sub>s</sub>* was negatively correlated with *A* and *g<sub>s</sub>* (*r<sub>p</sub>* = -0.47, P < 0.001, n = 100 for *A/g<sub>s</sub>* and *A*; *r<sub>p</sub>* = -0.72, P < 0.001, n = 100 for *A/g<sub>s</sub>* and *g<sub>s</sub>*).

## Discussion

In this study, we used five levels of soil moisture, which were maintained rigorously constant throughout the duration of the experiments. This is the first report to our knowledge of such stabilized treatments being used to

study the effect of water limitation on sunflower grown for several weeks in a greenhouse. The levels of SWC defined here covered a large gradient of water availability, leading to differing plant physiological behaviour. This is demonstrated by the marked differences observed in the rates of CO<sub>2</sub> assimilation and values of stomatal conductance between plants grown at the highest or the lowest soil moisture. Changes in SWC led also to changes in whole plant WUE (BM/CWT) and intrinsic leaf WUE (*A/g<sub>s</sub>*), and in leaf CID. WUE and *A/g<sub>s</sub>* were strongly and negatively correlated with CID. This is in accordance with previous work (Lauteri et al. 1993, Lambrides et al. 2004) and agrees with the model of Farquhar and Richards (1984) developed for wheat. WUE in Exp. 1 was lower than in Exp. 2. This can be explained by the differences in average VPD values in the greenhouse during the two experiments (Fig. 1a) as VPD was higher in Exp. 1 than in Exp. 2.

WUE has often been shown to be related to biomass production in plants. The relationship can be positive or negative (Li 1999). In sunflower, a positive correlation between WUE and BM was found by Virgona and Farquhar (1996) and Lambrides et al. (2004). If WUE and BM are positively correlated, plants that use water more efficiently by producing greater biomass for a given quantity of water transpired would also grow more rapidly and produce higher BM (Wright et al. 1993). In the present study with sunflower, no correlation (positive or negative) was found between the two parameters (Table 1). This agrees with the observation of Misra et al. (2010) on 20 durum wheat genotypes. Thus, a plant which displayed high WUE may not produce higher BM. This may be because higher WUE is generally achieved by plant traits than lower transpiration (such as reduced leaf area, moderate growth and low stomatal conductance) reducing photosynthesis and therefore yield.

Variations in WUE are mainly due to leaf diffusive characteristics (such as stomatal conductance, *g<sub>s</sub>*) and intrinsic photosynthetic capacity (such as Rubisco capacity). As BM production is closely associated with transpiration, in plants where WUE is principally determined by intrinsic leaf photosynthetic capacity (capacity type plant), WUE is weakly dependent on transpiration, and high WUE may be



associated with high biomass production. Plants that maximize WUE through a reduction in transpiration ( $g_s$ ) are called 'conductance type' (Farquhar and Lloyd 1993, Impa et al. 2005). Reduction in transpiration to increase WUE often results in reduced crop yield potential under most dryland situations, where crops depend on unpredictable seasonal rainfall rather than a limited and known soil moisture reserve. However, in 'conductance type' plants, higher WUE must lead to higher BM production for a given and limited amount of transpirable soil water. In the present study, WUE was negatively correlated with CWT (Exp. 2). In addition, the absence of relationships between WUE and BM production may be because stable soil moisture (i.e. constant water amount) was maintained in the pot that did not correspond to a limited transpirable soil water reserve.

Leaf CID decreased in sunflower plants grown with decreasing SWC ( $R^2 = 0.79$  for Exp. 1;  $R^2 = 0.81$  for Exp. 2, Fig. 2). This trend of decreasing CID with decreasing water availability has been reported in the previous studies, for example in wheat (Farquhar and Richards 1984), barley (Hubick and Farquhar 1989), Russian wild rye (Frank and Berdahl 2001) and rice (Zhao et al. 2004). However, most of these authors used only two water regimes for the plants (well-watered and markedly water-stressed conditions). Indeed, studies that report CID values for more than two levels of water are scarce: Zhao et al. (2004) found a negative relationship between CID and WUE of two upland rice cultivars under three water regimes, and Erice et al. (2011) found a negative correlation in four alfalfa genotypes subjected to progressive drought. In the present approach, graduated water limitation was imposed on plants through five levels of soil moisture. Often in the literature, soil moisture is not well controlled and it can vary, especially at the beginning of the experiments. In our study, SWC was precisely monitored daily so that leaf CID and WUE values of sunflower plants presented in the present work are related to accurate levels of SWC.

The strong correlation between CID and WUE among sunflower RILs indicates that the relationships may be used to select sunflower varieties with high WUE through leaf CID. Significant differences among four RILs and five SWC were observed for CID and WUE. Such differences in CID and WUE among genotypes have been reported by many authors, for example in rice (Zhao et al. 2004), alfalfa (Erice et al. 2011) and *Eucalyptus microtheca* F. Muell (Li 1999). In our study, two contrasting RILs were observed: RIL 149, identified as having low CID and high WUE, and RIL 200, identified as having high CID and low WUE. The consistency of CID ranking and its strong negative relationship with WUE in two experiments carried out in different seasons, VPD, SWC and on different types of soil reinforce the possibility of using this trait as a pertinent tool for agronomists and breeders in order to select sunflower

genotypes with high WUE. Irrigated agriculture represents up to 85 % of total human water consumption. Thus, considering world population expansion, it is imperative to improve WUE of irrigated but also of rain-fed crops (This et al. 2010).

The wide range observed in this study for CID in Exp. 1 (absolute value of 3.38 ‰, from 21.50 ‰ to 24.88 ‰) and in Exp. 2 (5.79 ‰, from 19.68 ‰ to 25.47 ‰) exceeds the range of 2.8 ‰ reported by Lauteri et al. (1993) on sunflowers grown in a greenhouse. Lambrides et al. (2004) found variations of 4.4 ‰ (absolute units) for 161 sunflower genotypes grown in field conditions. The CID ranges found in the present study are in agreement with these authors. In addition, in previous experiments on a larger number of sunflower genotypes (150 RILs), we observed ranges of 8.95 ‰, 5.82 ‰ and 6.91 ‰ in 2010, 2011 and 2012, respectively (unpublished data). Such wide ranges of CID suggest that it could possibly be used as a selection criterion in sunflower breeding programmes. Due to the wide range of CID, using this trait rather than WUE might be more suitable for comparing genotypes subjected to drought.

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