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Research Article
Carbon Isotope Discrimination as Physiological Marker to Select Tolerant Wheat Genotypes (*Triticum aestivum* L.) Under Water Limited Conditions

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Abstract

Background and Objective: Wheat crop always considered as strategic crop in Algeria and most Algerian land used for wheat production is subjected to water stress. Improvement of drought tolerance in wheat is among objectives for breeder in this region. This experiment was aimed to use carbon isotope discrimination (CID or Δ) as physiological marker to compare the response of ten wheat genotypes (*Triticum aestivum* L.) under different water regimes and to evaluate the relationship between Δ and other traits. **Materials and Methods:** Ten bread wheat genotypes were grown in pots in the absence of stress until 3 weeks of germination, 3 treatments of water regimes were imposed progressively. Treatments resulted from the combination of three irrigation levels (well-watered at 100%, medium-watered at 50% and low-watered at 25% of container capacity). Water stress was imposed after three weeks of germination, the experiment was continued up to 8th week of germination. The data were recorded in terms of dry matter (DM), relative water content (RWC) and carbon isotope ratio (δ) analyzed from shoot dry matter. **Results:** Carbon isotope discrimination varied significantly ($p<0.01$) among genotypes under well-watered and water-stressed conditions (medium-watered and low-watered treatments). Water regimes produced a linear and significant ($r = 0.99$, $p<0.001$) decrease in Δ with DM of all genotypes, the correlation between Δ and dry matter (DM) was positive ($r = 0.71$, $p<0.05$) in case of medium-watered treatment, while Δ wasn't correlated with DM for well-watered and low-watered treatments. The genotypes V9, Hidhab and V6 exhibited the best performance under water stress (medium-watered treatment), with minimum decrease in DM and high carbon isotope discrimination (CID) values were observed in these genotypes as well as close relationship between DM and Δ. **Conclusion:** Data indicated that measurement of carbon isotope discrimination maybe useful tool for selection of drought tolerant wheat genotypes to enhance wheat productivity in drought prone areas.

Key words: Carbon isotope discrimination, water regimes, wheat genotype, drought tolerance, *Triticum aestivum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wheat production in Algeria is mainly practiced in semi-arid areas, as a consequence, grain yield remains very low with annual production of bread wheat 9.52 million quintals and average yield 16.3 q ha^{-1} according to Bachiri *et al.*[1]. Algeria has a Mediterranean type climate and receives an average around 250-500 mm rainfall with about 70% occurring during the cold season from October to February. However, cereal crops suffer additional abiotic stresses such as winter-spring cold (due to altitude) and terminal drought (because of close proximity to the Sahara desert). The low rainfall and high temperatures are the serious threat to its low yield in arid and semi-arid areas. It is reported that the productivity of wheat genotypes under terminal drought is related to their capacity to maintain their photosynthetic activity[2].

The use of carbon isotopes discrimination is an important nuclear technique to screen suitable genotypes for drought prone areas and is less time consuming. Stable isotope ratios have emerged as an approach that integrates physiological processes overtime. Carbon isotope discrimination (Δ) of C_3 plant leaves is related to photosynthetic gas exchange, because Δ is in part determined by C_i/C_a, the ratio of CO_2 concentration in the leaf intercellular spaces (C_i) to that in the atmosphere (C_a)[3]. The ratio C_i/C_a differ among plants because of variation in stomatal opening (affecting the supply rate of CO_2) and because of variation in the chloroplast demand for CO_2 of the model linking C_3 photosynthesis and $^{13}C/^{12}C$ composition, the one developed by Farquhar *et al.*[4] has been most extensively used. In its simplest form, their expression for discrimination in leaves of C_3 plants is:

$$\Delta = a+(b-a)\ C_i/C_a$$

where, a is the fractionation occurring due to diffusion of air through stomata (4.4%) and b is the net fractionation caused by carboxylation [mainly by RuBP carboxylase, approximately (27%)]. Foliar Δ values have been used as an integrated measure of the response of photosynthetic gas exchange to environmental variables such as water availability[5-7], light[8], humidity[9] and salinity[10-13]. Low Δ has been proposed as an indicator of high water use efficiency (WUE) in C_3 plants, negative association between Δ and WUE has been reported by numerous researchers, Gonzalez *et al.*[14], Khazaei *et al.*[15] and Xue *et al.*[16].

The objective of this study was (1) To compare the response of some wheat genotypes (*Triticum aestivum* L.) under different water regimes using carbon isotope discrimination technique and (2) To evaluate the relationship between Δ, dry matter (DM) and relative water content (RWC).

MATERIALS AND METHODS

Plant material and growth conditions: Pot experiment was conducted at Plant Breeding and Genetic Laboratory of the International Atomic Energy Agency (IAEA) Laboratories in Seibersdorf, Austria, using ten wheat genotypes (*Triticum aestivum* L.), eight of them were obtained from International Maize and Wheat Improvement Center (CIMMYT) and two others were from National Institute of Agronomic Research of Algeria (Table 1). The experiment was done during March-May, 2016, wheat seed were hand sown on pots on 21th March and collected samples to record different measurements was carried out after 8 weeks on 17th May, 2016.

The pots containing 4 kg of Seibersdorf soil Typic Eutrocrepts[17], with a clay loam texture plus sand mixed in 1:1 ratio. Five seeds were sown in each pot and three seedlings were maintained one week after germination till sampling. Each pot received an application of 0.22 g kg^{-1} N as urea and 0.25 g kg^{-1} P as super phosphate, the fertilizer were thoroughly mixed with soil using electric mixture.

Table 1: Description of ten wheat genotypes used in this study

Code	Origin	Varieties/pedigree
V1	Algeria	Hidhab
V2	Algeria	Ain Abid
V3	CIMMYT	KINGBIRD#1//INQALAB91*2/TUKURU
V4	CIMMYT	ROLFO*2/KACHU#1
V5	CIMMYT	HEILO//SUNCO/2*PASTOR
V6	CIMMYT	ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOVE/BUC
V7	CIMMYT	VORB/SOKOLL
V8	CIMMYT	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN
V9	CIMMYT	PRL/2*PASTOR*2//FH6-1-7
V10	CIMMYT	BAV92//IRENA/KAUS/3/HUITES/4/GONDO/TNMU/5/BAV92//IRENA

The experiment was performed in split-plot design with 3 replications, treatments have been as whole-plot and genotypes were sub-plots. Three water regimes were imposed (1) Well-watered (WW), water applied at 100% of field capacity throughout the experiment, (2) Medium-watered (MW), water applied at 50% of field capacity and (3) Low watered (LW), water applied at 25% of field capacity, soil moisture in each pot was monitored by weight basis, all pots were watered every third day to maintain, control (well-watered), 50 and 25% plant available water. The three treatments were imposed after 3 weeks of germination and plants were subjected to different water regimes up to eighth week of germination according to the field capacity for each treatment.

Measurements

Carbon isotope discrimination (CID or Δ): The shoot dry parts were ground to a fine powder and about 100 mg was prepared for $\delta^{13}C$ analysis. Carbon isotope composition was determined on 5-10 mg sub-samples with an isotope ratio mass spectrometer (Optima, VG Instruments, UK) at the FAO/IAEA soil and water management and Crop Nutrition Laboratory, Seibersdorf, Austria. Results were expressed as:

$$\delta^{13}C(‰) = \left(\frac{R_{samples}}{R_{reference}}\right)-1\times 100$$

where, R is the isotope ratio of $^{13}C/^{12}C$.

A secondary standard, calibrated against the primary standard, Pee Dee Belemnite (PDB) fossil carbonate, was used as the reference.

The $\Delta^{13}C$ in the plant samples was calculated using the following equation according to Farquhar *et al.*[18]:

$$\delta^{13}C\ (‰) = \left(\frac{\delta a-\delta p}{(1+\delta p)}\right)\times 100$$

Where:

δp = $\delta^{13}C$ of the plant sample

δa = $\delta^{13}C$ of atmospheric (8.0‰)

Dry matter (DM): Shoot parts of the plants for each pot were sampling, then dried in hot air oven for 48 h, dry weight was recorded for each sample.

Relative water content (RWC): Three leaves at the same phenology stage were detached randomly and placed in a sealed container and the fresh weight (FW) was determined. The full turgid weight (TW) was obtained after the re-hydration of the leaves by placing them in a test tube containing distilled water for 24 h at ambient temperature then leaves were placed in hot air oven for 24 h to get the dry weight (DW). The RWC was calculated according to Clarke and McCaig[19]:

$$\text{RWC}\ (\%) = \frac{\text{FW-DW}}{\text{TW-DW}}\times 100$$

Statistical analysis: Data were subjected to factorial analyses of variance (ANOVA) with two factors (genotypes and water regimes) using the GenStat Discovery software package. Then, the differences between the means were compared by Fisher' s Least-significant Difference Test (LSD) at a probability level of 95%. Significance levels were expressed as p = 0.05 and data were significant when p = 0.05.

RESULTS

Genotype differences: Shoot samples analyzed for carbon isotope discrimination (CID) showed that the wheat genotypes discriminate effectively between $^{13}C/^{12}C$ under water limited condition. However, the Δ values decreased under deficit water compared to well-watered treatment (Table 2).

Variance analysis (ANOVA) describe a significant ($p<0.05$) difference between genotypes on carbon isotope discrimination values (Table 2). Under well-watered conditions, the Δ had a range of 1.63% (22.3-20.67%) whereas, underwater-stressed conditions (MW and LW), the span of variation for Δ increased to 1.93% (19.72-17.79). Hidhab cultivar had the maximum Δ value (22.3%) followed by V6 (21.84%) under WW, the CID value was also observed maximum (19.72%) in Hidhab under (MW) but in case of the LW, the cultivar Ain abid record the maximum Δ value (19%). In addition, CID was the only trait which shows a significant genotype treatments interaction (Table 3).

The relative decease in Δ value (Fig. 1) showed that genotypes V8 and V5 had more reduction (15.55 and 14.81%) under MW. On the other hand, it was observed that through V9 had less Δ value in well watered conditions but had maintained quite satisfactorily under LW treatment with only 13.93% of reduction. The higher relative decease in CID value in case of Hidhab (19.06%) and V5 (16.75%) were recorded under LW treatment, but in contrast, these same genotypes showed acceptable dry matter compared to other genotypes, this indicate the performance of these genotypes were affected by degree of water stress but theirs response

Table 2: Mean values of carbon isotope discrimination (Δ), dry matter (DM) and relative water content (RWC) under three water regimes

	$\Delta^{13}C$ (%)			DM (g)			RWC (%)		
Genotypes	WW	MW	LW	WW	MW	LW	WW	MW	LW
Hidhab	22.30^{ab}	19.72^{a}	18.05^{b}	2.50	1.30	0.73	90.69	63.07	36.06
V10	21.74^{ab}	19.27^{ab}	18.19^{ab}	2.95	1.25	0.56	89.94	60.12	32.47
Ain abid	21.31^{bc}	18.68^{bc}	19.00^{a}	2.69	0.89	0.74	90.15	53.76	38.87
V3	21.42^{abc}	18.45^{bc}	17.88^{b}	2.85	0.92	0.64	86.96	66.38	48.20
V4	21.36^{bc}	19.22^{ab}	18.12^{ab}	2.60	1,00	0.66	90.53	64.68	40.07
V5	21.67^{ab}	18.30^{c}	18.04^{b}	3.21	0.87	0.73	93.86	67.51	45.26
V6	21.84^{ab}	19.12^{abc}	18.25^{ab}	3.04	1.29	0.80	93.96	61.01	40.37
V7	21.10^{bc}	18.65^{bc}	18.49^{ab}	3.26	1.11	0.67	88.90	60.29	29.52
V8	21.67^{ab}	18.46^{bc}	18.55^{ab}	2.79	0.94	0.58	91.83	56.99	27.81
V9	20.67^{c}	18.72^{bc}	17.79b	2.94	1.28	0.62	89.29	66.27	41.20
$LSD_{(GxT)}$	0.924*			0.772^{ns}			16.258^{ns}		

*Significant at $p<0.05$, ns: Non-significant. For each genotype values shown are the means of three replications. The means followed by different letters were significantly different at $p<0.05$ by Fisher's test (LSD). WW: Well watered, plants irrigated at 100% of field capacity, MW: Medium watered, water applied at 50% of field capacity and LW: Low watered, water applied at 25% of field capacity

Table 3: Effect of different levels of water regimes on carbon isotope discrimination (Δ), dry matter (DM) and relative water content (RWC) in wheat genotypes *(Triticum aestivum* L.)

	Δ (%)	Dry matter (g)	RWC (%)
Genotype (G)			
Hidhab	20.03^{a}	1.512	63.27
V10	19.73^{ab}	1.592	60.84
Ain abid	19.66^{ab}	1.447	60.93
V3	19.25^{bc}	1.472	67.18
V4	19.57^{ab}	1.424	65.09
V5	19.34^{bc}	1.608	68.88
V6	19.74^{ab}	1.714	65.11
V7	19.41^{bc}	1.686	59.57
V8	19.56^{ab}	1.438	58.88
V9	19.06^{c}	1.613	65.59
Water regime (T)			
WW	21.51^{a}	2.887^{a}	90.61^{a}
MW	18.86^{b}	1.087^{b}	62.01^{b}
LW	18.24^{c}	0.678^{c}	37.98^{c}
ANOVA			
G	0.7009**	0.1011ns	102.47ns
T	90.4185***	41.4389***	20826.18***
G×T	0.4476*	0.0998ns	49.78ns
CV (%)	1.3	14.6	6.6

*Significant at $p<0.05$, **Significant at $p<0.01$, ***Significant at $p<0.001$, ns: Not significant. Mean within a column followed by the same letter do not significantly different to LSD at $p<0.05$

to water stress change according to mechanism developed by each of them. For that, CID wasn't related with dry matter in this case. Moreover, present study results showed no significant differences between genotypes was found regarding to DM and RWC (Table 3).

Water regime effects: Significant variation in mean Δ values ($p<0.001$) due to the treatments were observed for all genotypes ranging from 21.51% for well watered to 18.24% for the LW treatment (Table 3). Water stress during LW treatment had a more severe penalty on DM and RWC than MW treatment. The highest values in DM (1.30 and 0.80 g) were found in Hidhab cultivar and V6 genotype, respectively at MW and LW treatments, which are 48 and 68% less than the lowest DM (2.50 g) produced by Hidhab under well-watered conditions (Table 2).

Variation in RWC were highly significant ($p<0.001$***) through all treatment levels (WW, MW and LW). At MW treatment, the maximum decrease in RWC was recorded in Ain abid and V8 genotype with a rate of reduction 36.39 and 34.84%, whereas, V3 genotype exhibited the lowest reduction in RWC only 20.58% at MW and 44.57% at the LW treatment, it means V3 genotype kept slightly better water status than other genotypes under harsh conditions. But as showed in data (Table 2), there was no significant difference between genotypes in RWC regarding to all treatment levels.

Relationship between Δ and adaptive traits: The results showed that there was more variation on CID in shoots of plants grown under limited water conditions (MW and LW treatments) in comparison to control plants grown under well-watered environment, where Δ had a range of 1.63% for plants under control treatment whereas, under water stress the range of Δ increased to 1.93%. However, Δ exhibited a significant and positive linear correlation ($r = 0.99$) with dry matter averaged across water regime (Fig. 2).

This experiment indicated no relationship between Δ and DM under well watered conditions (Fig. 2) whereas, a significant and positive association ($r = 0.711$, $p<0.05$) was observed between Δ and DM under MW treatment, but there was no correlation ($r = 0.184$) under LW treatment for these two variables, indicating that the level of severity of water stress has varying effects on the performance of genotypes.

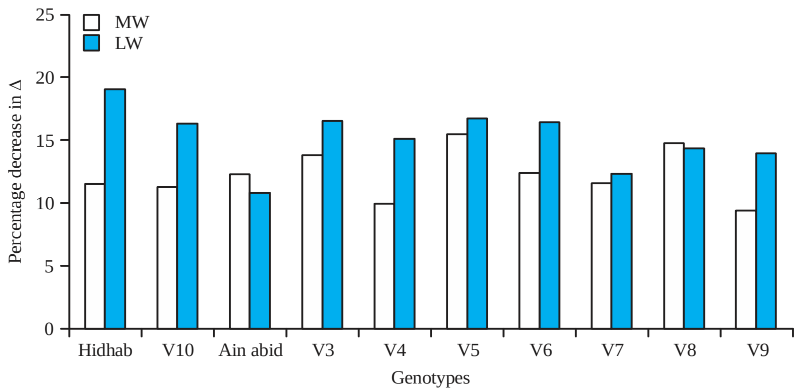

Fig. 1: Relative decrease in Δ values as affected by medium watered (MW) and low watered (LW)

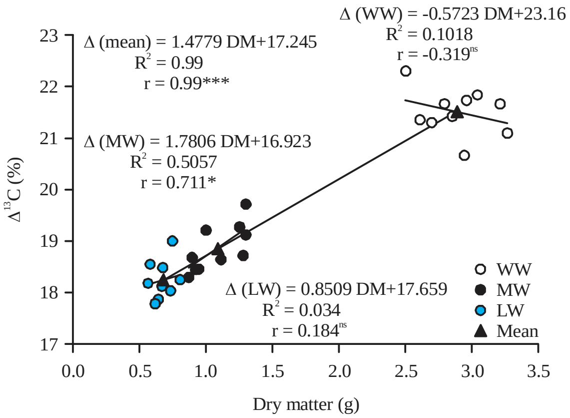

Fig. 2: Relationship between CID (Δ) and dry matter of ten wheat genotypes (*Triticum aestivum* L.) under each of three and mean of three water regimes

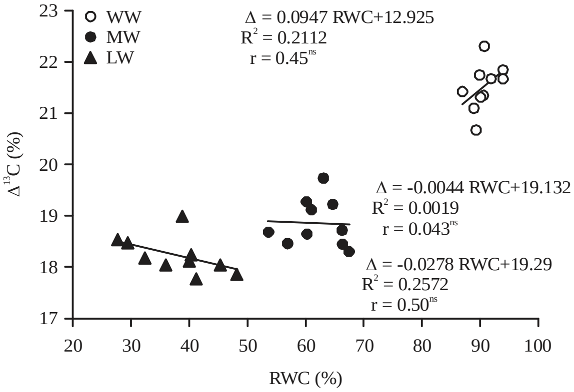

Fig. 3: Relationship between CID (Δ) and RWC of ten wheat genotypes (*Triticum aestivum* L.) under each of three water regimes

The RWC values were significantly lower in water stress than in control conditions, data showed no association between Δ and RWC across all treatments (Fig. 3).

DISCUSSION

Drought stress caused an obvious decrease in CID across the 10 genotypes. The means Δ value under medium watered MW and low watered LW regimes were decreased by 12.31 and 15.20%, respectively as compared to the control (WW) treatment (Table 3). Misra *et al.*[20] have reported a decrease in Δ under limited irrigation(3.44 and 6.25% in two consecutive seasons, respectively). Under MW regime, the higher reduction in dry matter of these genotypes (V5, V8 and V3) was probably due to more decrease in photosynthetic activity. On the other hand, less reduction in dry matter in this group of genotypes (V9, Hidhab and V6) proves well stability in photosynthetic activity under water stress, which might have resulted in maintaining dry matter of these genotypes. In addition, less decrease in dry matter in V9 and Hidhab is also supported by less reduction in CID by only 9.43 and 11.57%, respectively. In contrast, the dry matter in V5 and V3 could not be maintained successfully under water deficit (MW treatment) and showed 72.83 and 67.75% decrease, respectively, indicating their more sensitivity to drought. The sensitivity of these genotypes is well supported by high reduction in CID values, 15.55 and 13.87%, respectively. Similarly, low value of dry matter and high reduction in CID value in V8 (14.81%) also indicate sensitivity to drought by this genotype.

These findings demonstrate significant close relationship (r = 0.711) between shoot dry matter (DM) and CID was observed at moderate water stress (MW treatment), but were non-significant under (WW) optimal conditions and (LW treatment) severe stress (Fig. 2). The decrease in Δ values under drought environment showed a general trend of less discrimination under stress conditions compared to well-watered conditions.

According to Farquhar *et al.*[21], a shift in the carbon isotope discrimination of the leaf tissues due to stress gives, information about the plant succeeded in maintaining water use efficiency (WUE) and confirms the stress induced changes in the C_i/C_a ratio. The variation between the values under well watered and drought condition indicates that the wheat genotypes significantly discriminate between heavier and lighter carbon during photosynthesis. It is also reported that the variation in CID in cereals is known to arise from variation in photosynthetic capacity as well as stomatal conductance[22-24]. Greater photosynthetic capacity, lower stomatal conductance or both, may result in lower values of C_i/C_a. This means that greater photosynthetic capacity should be reflected in lower values of Δ unless stomatal conductance also increases to balance the change in photosynthetic capacity and maintain C_i constant.

Many authors have suggested that Δ could also be related to grain yield (GY) and water use efficiency (WUE)[25], the dry matter production to water consumption ratio[26]. Under Mediterranean conditions, a significant positive correlation was repeatedly found between grain yield and flag leaf Δ under severe stress[27,28].

CONCLUSION

Genetic variation in the response of carbon isotope discrimination to different water regimes was found through this study. A positive correlation between dry matter and Δ was observed during this study. High dry matter (DM) was associated with high Δ under water stress (MW treatment), suggesting that carbon isotope discrimination technique provides an integrated measure as an indirect selection criterion for drought tolerant wheat genotypes. Therefore, carbon isotope discrimination technique can be useful tool integrated in wheat breeding programs.

SIGNIFICANCE STATEMENT

This study discovers possibility to enhance genetic variability of wheat to drought tolerance by using related nuclear technique such as carbon isotope discrimination that can be beneficial for wheat breeding programs. Further, this study will help researchers to uncover critical area of physiological breeding in wheat. However, the tested genotypes have shown possibilities to explore genetic pool of wheat to select adapted genotypes under harsh conditions. Thus, its varieties or advanced lines assessed in green house which have better ability to grown under water stress had lowest reduction values in carbon isotope discrimination and highest in dry matter.

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