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ORIGINAL ARTICLE

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Evaluation of Water Stress Tolerance in Advanced Breeding Lines of Durum and Bread Wheat Using ^{13}C

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Dry matter (DM), nitrogen uptake (TN) along with water (WUE) and nitrogen (15NUE) use efficiencies in twelve advanced breeding lines (ACSAD) and two varieties (Cham1&6) of durum (DW) and bread (BW) wheat grown under well water (I1) and water stress conditions (I2) were evaluated using ^{15}N and $\Delta^{13}C$. Water stress decreased Δ13C in all studied genotypes. The extent of the decrease in Δ by stress was relatively higher in BW (-1.08‰) than DW (-0.8‰). Cham1 (DW) exhibited the highest DM, TN, NUE, WUE and $\Delta^{13}C$ values under I1, indicating its suitability to be grown under irrigated conditions. However, ACSAD1261 (DW) seemed to be a promising line to be grown in semi arid areas due to higher values in the aforementioned criteria under I2. For BW, the highest DM of ACSAD59 under I1 may suggest its suitability to be grown under well irrigated conditions. However, DM of ACSAD883 and 1115 were not affected by watering regime. Additionally, due to the high DM of Cham6 and ACSAD1135 in both watering regimes, and because of the decrease in $\Delta^{13}C$ values under stress, it can be suggested that they could be suitable for both irrigated and water stress conditions. Since $\Delta^{13}C$ values were affected by wheat genotype and watering regime as a result of the effects on the balance between stomatal conductance and carboxilation, it cannot be relied, completely, upon this technique to select drought tolerant genotypes. Therefore, we suggest that using $\Delta^{13}C$ along with agro-physiological parameters are better selection criteria for water stress tolerance in breeding programs than when used separately.

Key words: water stress, Triticum durum, Triticum aestivum, 15N, Δ13C

It is well known that genetic diversity of wheat is very important from agro-ecological, agronomic, economic and socio-cultural standpoints because it offers variation for selection in crop improvement by plant breeders and provides farmers with selected varieties that are adapted to their specific environments, particularly, to the water stress conditions (Bishaw *et al*. 2015). Drought is the most important abiotic factor limiting the productivity of wheat and other crops world-wide. Drought stress influences several physiological, biochemical, and molecular processes in plants, which may facilitate their adaptations to limiting environmental conditions (Fahad *et al.* 2017). As irrigation water sources have become less sufficient, development of crop cultivars with improved adaptation to drought is a major goal in many crop breeding programs. Considerable research and substantial breeding efforts have been devoted to identifying and selecting for morpho-physiological traits that increase WUE and yield under water-limiting conditions (Chen *et al.* 2013; Lonbani & Arzani 2011; Richards *et al.* 2002). Carbon isotope discrimination ($\Delta^{13}C$), in addition to classical breeding methodologies, has been extensively used and demonstrated as an indicator of WUE for many C3 crops; and their negative correlation has been used for indirect selection of genotypes with improved WUE under selected environments (Farquhar *et al.* 1989; Cattivelli *et al.* 2008; Munjonji *et al.* 2017). Substantial genetic variation for Δ was noted in wheat and barley (Craufurd *et al.* 1991; Acevedo 1993; Sayre *et al.* 1995; Voltas *et al.* 1998; Merah *et al.* 2001; Wahbi & Shaaban 2011). During photosynthesis, C3 plants discriminate against the heavy isotope of carbon (^{13}C) leading to a depletion of the plant dry matter in ^{13}C. $\Delta^{13}C$ positively correlates with C_i / C_a (i.e. the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration) and thus provides an integrated measurement of the photosynthesis efficiency in response to environmental conditions prevailing during the plant growth cycle (Farquhar *et al.* 1982 & 1989; Acevedo 1993; Merah *et al*., 2001).

In Syria and elsewhere, wheat is a strategic and important crop for food security (Bishaw *et al*. 2011). It contains important beneficial components for human nutrition (Leváková and Lacko-Bartošová 2017). In the semi-arid areas of the Mediterranean region, wheat is grown on a large scale, where water stress is considered the major limiting abiotic stress. Since water is scarcely available for irrigation in semi-arid areas, selection of drought tolerant genotypes is one of the main objectives of wheat breeding programs (Bishaw *et al.* 2011; Wahbi & Shaaban 2011). There is a need for breeding programs to focus on developing higher yielding genotypes with higher water and nitrogen use efficiencies, particularly, under water stress conditions (Baligar *et al.* 2001; Tambussi *et al.* 2007).Since the Arab Center for the Study of Arid Zones and Dry Lands (ACSAD) was established, researchers have been developing lines of the durum (*Triticum durum*) and bread (*Triticum aestivum*) wheat that are highly adapted for growing in regions of the local environment, with an attention to keep high productivity (Haidar *et al.* 2012). In this sense, the present study was conducted to determine the effects of induced water stress on biomass, nitrogen uptake, nitrogen use efficiency of added fertilizer (NUE), water use efficiency (WUE) and carbon isotope discrimination ($\Delta^{13}C$) in advanced breeding lines of bread (BW) and durum wheat (DW) plants which have been developed by ACSAD. The knowledge developed through this research will be useful in breeding programs to identify suitable water stress tolerant plant lines using agro-physiological and isotopic (^{15}N and ^{13}C) approaches.

MATERIALS AND METHODS

Plant material

Six advanced breeding lines from each of bread wheat, *ie.*, BW (*Triticum aestivum* L.) and durum wheat, *i.e.* DW (*Triticum durum* Desf.) were developed and obtained from the Arab Center for the Study of Arid Zones and Dry Lands (ACSAD), through a previous collaborative research (Haider *et al.* 2012). The denomination for the bread wheat lines were: ACSAD 59, 67, 883, 1059, 1115 and 1135, and those for durum wheat were: ACSAD 65, 357, 1261, 1265, 1277 and 1287. Two released varieties; one bread wheat (Cham 6) and one durum (Cham 1) were also included in the current study.

Soil properties

The soil was classified as clay loam. Soil bulk density was 1.20 g cm^{-3} with an electrical conductivity (EC) of 0.83 dS m^{-1}. Other main chemical soil properties were: pH 7.7; organic matter 0.91%; total nitrogen 0.07%; available phosphorus 6.8 μgg^{-1}; ionic content (meq L^{-1}) chloride (Cl^-) 0.74, bicarbonate (HCO_3^-) 0.97, sulfate (SO_4^{--}) 1.27, calcium (Ca^{++}) 1.1, potassium (K^+) 0.14, sodium (Na^+) 1.27, and magnesium (Mg^{++}) 0.47; cation exchange capacity (CEC) 29.08 meq 100 g^{-1}; nitrate (NO_3^-) 42 μgg^{-1} and ammonium (NH_4^+) 26.1 μgg^{-1}.

Experiment design and treatments

The experiment was conducted in pots, each containing 5 kg of thoroughly mixed soil collected from Deir AL-Hajar agricultural experiment station, located south east of Damascus, Syria (36° 28'E, 33° 21' N; altitude 617 m). Seeds of all tested genotypes were sown in pots, and set outdoors under natural climatic conditions. After germination, seedlings were thinned to three plants per pot. The experiment was laid out in a randomized complete block design with four replicates. For each species, two watering treatments, expressed as percentage of field capacity(I1, well-watering 75–80% and I2 water stress 45–50%) were imposed when the plants were at third leaf formation stage up to physiological maturity. At the end of the experimental period, total amounts of applied water for each irrigation regime were determined. All pots were protected from rainfall by manually operated shelter equipped with movable sheet of transparent flexible plastic.

An equivalent fertilizer rate of 100 kg N ha^{-1} in the form of urea enriched with 2% ^{15}N atom excess was applied to all plants. The N-fertilizer was split in two applications. One third was applied at complete seedling emergence and two third at Zadouks growth-stage 30 (Arslan*et al*. 2000).

Plant harvest and analysis

At physiological maturity (165 DAS) when the spikes had turned yellowish green, all plants were cut at soil level and partitioned into roots, leaves and spikes. Samples were dried to a constant weight at 70 °C and milled to a fine powder. Kjeldahle procedure was used to determine total nitrogen in the samples, and the $^{15}N/^{14}N$ isotopic ratio was determined by the emission spectrometry (Jasco-150, Japan). The N fractions derived from the available sources (*i.e.,* soil Ndfs and fertilizer Ndff) were calculated using the isotopic dilution method by applying equations previously described (Zapata 1990). Nitrogen fertilizer use efficiency (%NUE) was calculated as fertilizer N recovery in the whole plant. Water use efficiency (WUE) was determined as ratio of dry matter to total water applied.

$^{13}C/^{12}C$ ratio was determined on leaf sub-samples (2 mg dry weight) using the continuous-flow isotope ratio mass spectrometry (Integra-CN, PDZ Europea Scientific Instrument, UK). Carbon isotope discrimination ($\Delta^{13}C$‰) values were estimated using the equation of Farquhar *et al.*, (1982):

$$\Delta^{13}C = (\delta^{13}C_{air} - \delta^{13}C_{sample}) / (1 - \delta^{13}C_{sample}/1000)$$

Where $\delta^{13}C_{air}$ is the $\delta^{13}C$ value in air (-8‰) and $\delta^{13}C_{sample}$ is the measured value in the plant.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) test using Stat view 4.5 statistical package, and means were compared using the Least Significant Difference (Fisher's PLSD) test at the 0.05 level of probability ($P<0.05$).

RESULTS

Dry matter production

Dry matter production (DM) in different plant parts (i.e. roots, leaves and spikes) of durum (DW) and bread wheat (BW) lines grown under well watering regime (I1) and water stress conditions (I2) is shown in Table 1. For durum wheat grown under well watering regime (I1), the highest DM yield values were observed in Cham 1 genotype, while, the lowest values were recorded in ACSAD 1261 (Table 1 and Fig. 1). The opposite was true under water stress conditions (I2) where the highest DM values were observed in different plant parts of ACSAD 1261 line which showed a marked increase in total DM (*i.e.,* 24%) as compared with the other studied lines (Table 1 and Fig. 1). In contrast, water stress resulted in a marked decrease in DM of Cham 1 (*i.e.,* decreased by 40%) showing the lowest values. A relatively similar trend to Cham 1 was observed for ACSAD 1287 line which showed a high DM yield under I1 and decreased by 24% under I2. However, water stress did not significantly decrease the whole plant DM of the other DW lines (Fig. 1).

For BW grown under well watering regime (I1), ACSAD 59 produced a significant higher spike DM over the other tested lines with no significant differences being obtained among them (Table 1). Although DM yield of spikes decreased by water stress, no significant differences were observed among the tested lines in I2. With regard to total DM yield, ACSAD 59, 1059, 1135, Cham 6 showed higher values over the other tested lines in I1 (Fig. 1). Waters stress significantly decreased total DM of ACSAD 59 by 38%, whereas, no significant decreases were obtained in other tested lines as compared with values in well watering regime. On the other hand, both DW (except Cham1) and BW genotypes generally showed higher root DM under water stress conditions.

Nitrogen yield

The pattern of total nitrogen yield (TN) was relatively similar to that of DM (Table 2 and Fig. 1). Under well watering regime (I1), the highest TN yield in durum wheat was observed in Cham 1 genotype, and the lowest was in ACSAD 1261 line (Table 2 and Fig. 1). Water stress significantly reduced the TN by 46% in Cham 1 and by 22% in ACSAD 1287. However, TN increased by 22% in water-stressed ACSAD 1261 which showed the highest value among the tested lines. The superiority of this line was also shown in its spikes, leaves and root N yield (Table 2). TN was not significantly decreased by water stress in the other lines (Fig. 1).

With regard to BW lines, the well watered ACSAD 59 had a higher spikes' N yield over ACSAD 883, 1059 and Cham 6. Whereas, values in ACSAD67, 1115 and 1135 did not significantly differ in the aforementioned lines. However, the highest values of total N yield were observed in Cham 6 and ACSAD 1135 either grown under water stress or well watering conditions. Water stress significantly decreased TN by 30, 16, 18 and 18% in ACSAD 59,1115, 1059 and Cham 6, respectively**.,** whereas, no significant decrease of water stress was obtained in other tested lines (i.e., ACSAD 67, 883 and 1135).

Soil (Ndfs) and Fertilizer (Ndff) Nitrogen Uptake

Effect of water stress on nitrogen derived from fertilizer (Ndff) and soil (Ndfs) in the whole plants of durum and bread wheat lines is given in Fig. 2. In well-watered DW plants, the highest amounts of Ndff or Ndfs were noted in Cham 1 genotype, and the lowest values were in ACSAD 1261 line (Fig. 2). Water stress significantly reduced the Ndff by 46% and 24% in Cham1 and ACSAD 1287, respectively. However, it increased by 18% in ACSAD 1261. Also, amounts of Ndfs followed a trend similar to Ndff. For bread wheat, the highest amounts of Ndff or Ndfs were noticed in Cham 6 and ACSAD 1135 under both watering regimes. The highest decrease in the amounts of Ndff (29%) and Ndfs (30%) as a result of water stress was obtained in ACSAD 59.

Nitrogen Use Efficiency

Nitrogen fertilizer use efficiency (%NUE) was calculated as fertilizer N recovery in the whole plant (Fig. 3). In durum wheat, the highest NUE was in well-watered Cham1 (49.5%), followed by ACSAD 1287 (%). Whereas, ACSAD 1261 showed the lowest value (35%), while it showed the highest value under stress (42.6%). Moreover, it can be noticed that the NUE values in DW ranged between 28.3 and 49.5%. These two values are belonging to Cham1 genotype grown under I1 and I2, respectively. In bread wheat, the highest NUE was observed in non-watered stressed Cham 6 (49.5), followed by ACSAD 1135 (47%). Although NUE values declined by water stress, the aforementioned genotypes had also higher values (about 40%).

Water Use Efficiency (WUE) for Biomass

Water uses efficiency in both wheat species and genotypes were determined as ratio of dry matter production to the amount of water applied (Fig. 4). WUE values were generally higher under water stress conditions (I2) as compared to (I1), excepting the Cham 1 genotype which showed a significant decrease in WUE by stress. While some other line (e.g., DW: ACSAD 1287 and BW: ACSAD 59, 67 and 1059) did not show significant differences. In well-watered DW, the highest WUE value was occurred in Cham 1**,** while the lowest was in ACSAD 1261. The opposite was true for these two genotypes under water stress conditions.

Carbon isotope discrimination (Δ^{13}C)

Data presented in Figure 3 show that carbon isotope, expressed as carbon discrimination (Δ^{13}C), was affected by the plant genotype and watering regime. Higher soil moisture level significantly increased Δ^{13}C**,** while lower values of Δ^{13}C were observed under water stressed conditions. Under well watering regime, Δ^{13}C values in DW lines ranged between 18.23‰ (ACSAD 1265) and 19.42‰ (Cham1). For BW, Δ^{13}C values ranged between 18.71‰ (Cham 6) and 19.36‰ (ACSAD 59). Water stress decreased Δ values in all studied lines of both DW and BW as compared to well-watered plants. In DW, the Δ^{13}C values ranged between 17.41‰ and 18.64‰ for ACSAD 1277 and 1261, respectively. While in BW, values ranged between 17.56‰ and 18.66‰ for ACSAD 1115 and 1135, respectively. The extent of the decrease in Δ mean values by stress was relatively higher in BW (-1.08‰) than DW (-0.8‰) lines. On the other hand, based on Δ^{13}C values in each watering regime, the studied lines might be generally classified into two groups, i.e. high >‰19 and low <19‰ for well watered plants; high >18‰ and low <18‰ for water stressed plants. This grouping could have implications for genetic variations among tested line in relation to carbon isotope signature (*i.e.,* genetic variation for Δ). With the exception of ACSAD 65, durum wheat lines having higher Δ values under non-stress conditions (>19‰) had also higher values under stress conditions (>18‰) and *vice versa*. However, Δ values in bread wheat lines did not maintain the previous order.

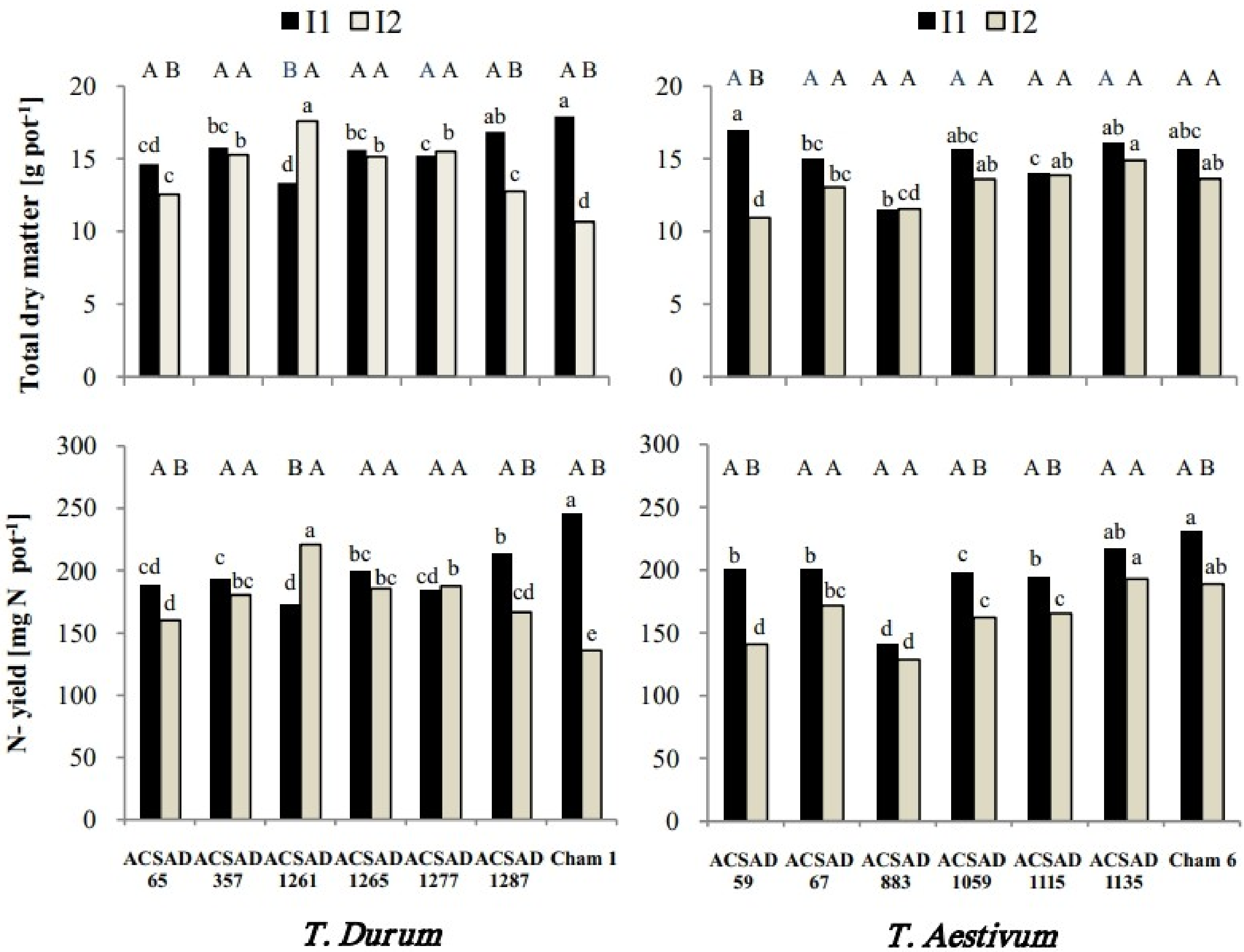

Figure 1. Dry matter production [g pot^{-1}] and total N yield [mg N pot^{-1}] in the whole plants of durum and bread wheat lines grown under well watering regime (I1) and water stress conditions (I2)

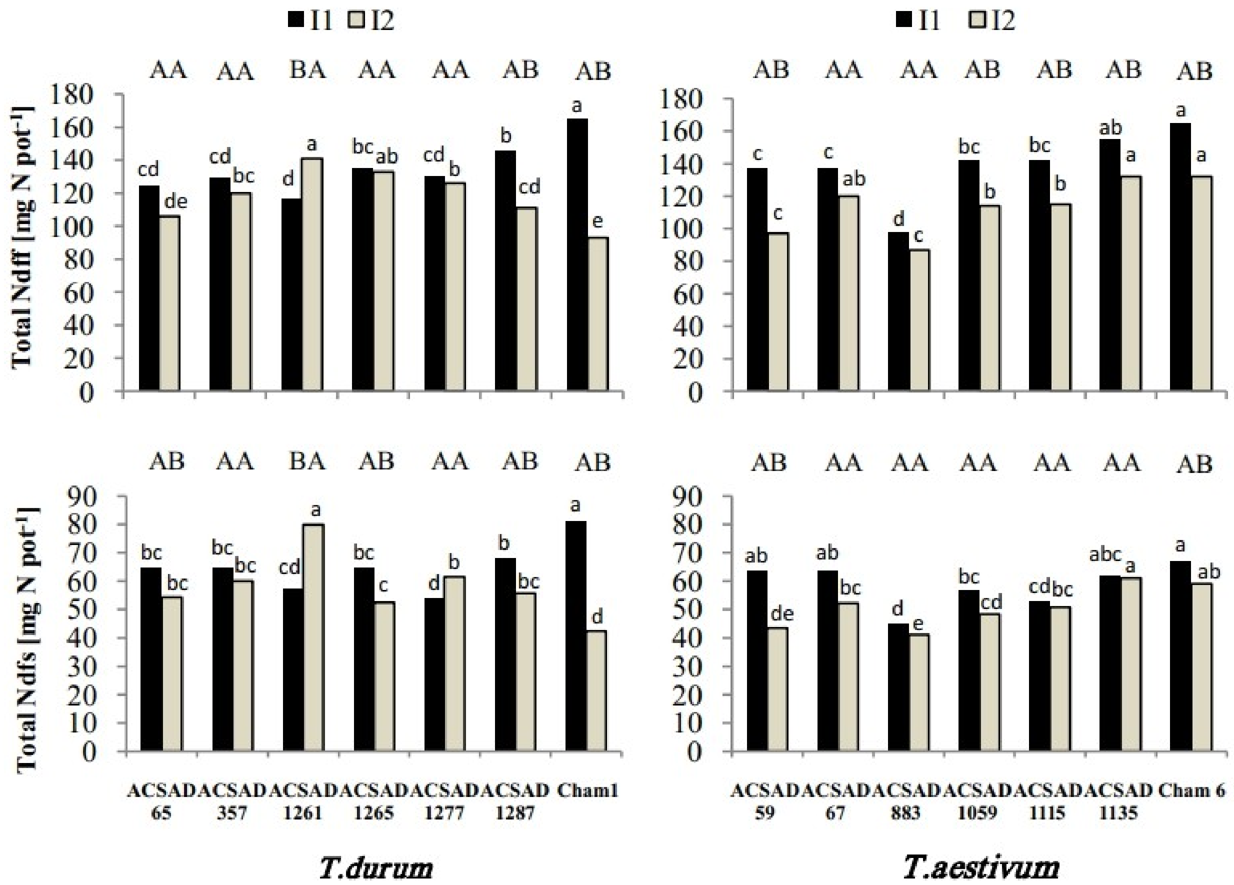

Figure 2. Nitrogen derived from soil (Ndfs) and from fertilizer (Ndff), [mg N pot^{-1}] in the whole plants of durum and bread wheat lines grown under well watering regime (I1) and water stress conditions (I2)

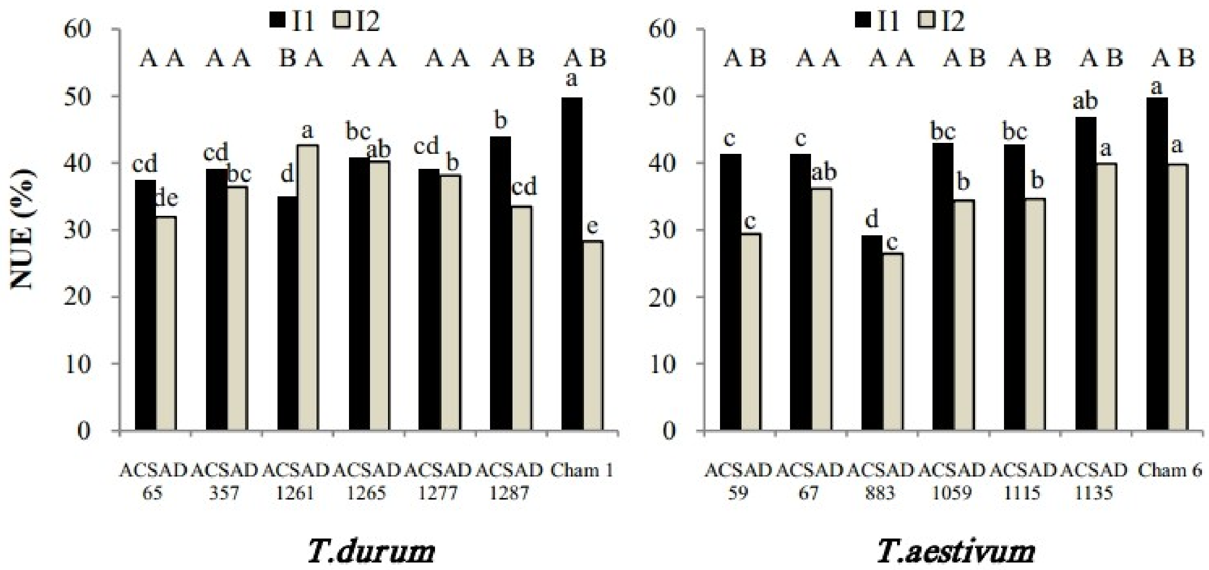

Figure 3. Nitrogen use efficiency of added fertilizer (%NUE), in the whole plants of durum and bread wheat lines grown under well watering regime (I1) and water stress conditions (I2)

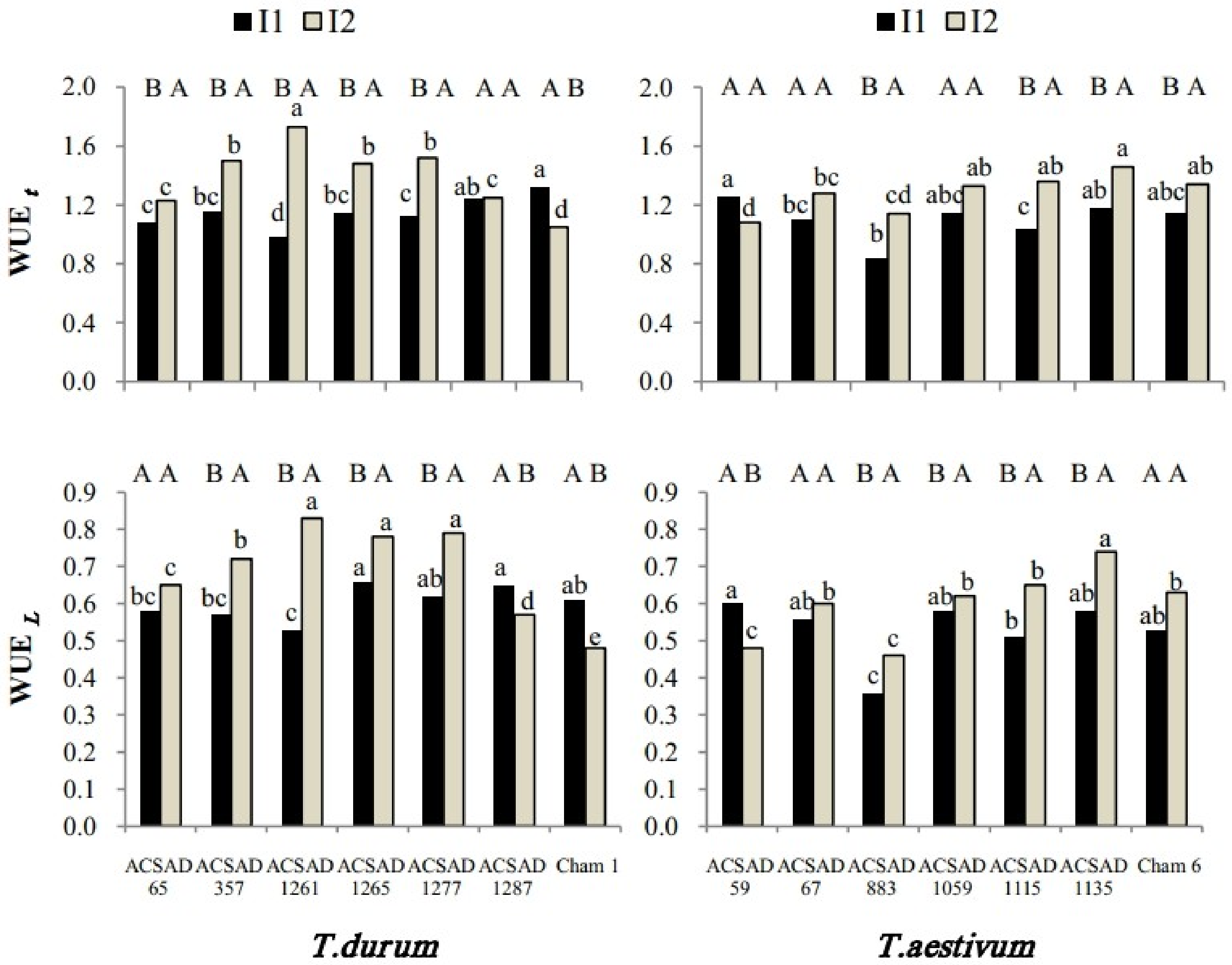

Figure 4. Water use efficiency (WUE) for leaves (WUE_L) and whole plants (WUE_t) of durum and bread wheat lines grown under well watering regime (I1) and water stress conditions (I2)

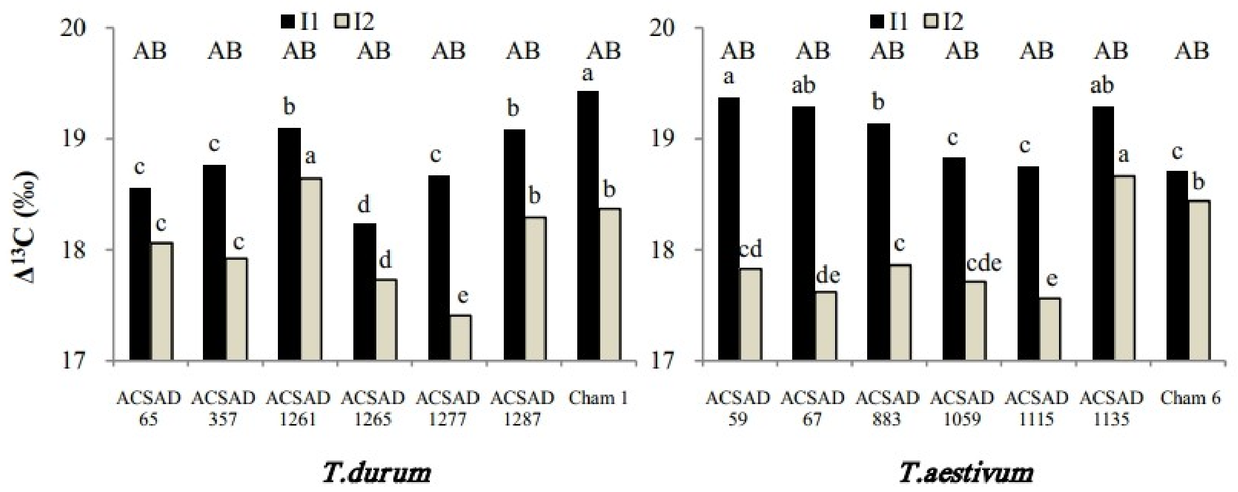

Figure 5. Leaf carbon isotope discrimination (‰$\Delta^{13}C$) of durum and bread wheat lines grown under well watering regime (I1) and water stress conditions (I2)

Table 1. Dry matter production (g pot^{-1}) in different plant parts (i.e. roots, leaves and spikes) durum (*Triticum durum*) and bread (*Triticum aestivum*) wheat genotypes grown under well watering regime (I1) and water stress conditions (I2).

Genotypes	Roots			Leaves			Spikes		
	I1	I2	$LSD_{0.05}$	I1	I2	$LSD_{0.05}$	I1	I2	$LSD_{0.05}$
	Triticum durum								
ACSAD 65	3.30 $^{cd A}$	3.45 $^{bc A}$	*ns*	7.93 $^{bc A}$	6.63 $^{c B}$	*0.89*	3.40 $^{c A}$	2.45 $^{d B}$	*0.50*
ACSAD 357	3.63 $^{bc A}$	4.15 $^{b A}$	*ns*	7.75 $^{bc A}$	7.33 $^{b A}$	*ns*	4.40 $^{b A}$	3.80 $^{a B}$	*0.49*
ACSAD 1261	2.80 $^{e B}$	5.28 $^{a A}$	*0.39*	7.28 $^{c B}$	8.48 $^{a A}$	*0.93*	3.25 $^{c B}$	3.85 $^{a A}$	*0.41*
ACSAD 1265	3.18 $^{d B}$	4.13 $^{b A}$	*0.58*	8.98 $^{a A}$	8.00 $^{a A}$	*ns*	3.45 $^{c A}$	3.0 $^{bcd A}$	*ns*
ACSAD 1277	3.23 $^{d A}$	4.08 $^{b A}$	*ns*	8.40 $^{ab A}$	8.10 $^{a A}$	*ns*	3.60 $^{c A}$	3.33 $^{ab A}$	*ns*
ACSAD 1287	3.68 $^{b A}$	3.80 $^{bc A}$	*ns*	8.85 $^{a A}$	5.83 $^{d B}$	*0.88*	4.33 $^{b A}$	3.13 $^{bc B}$	*0.96*
Cham 1	4.33 $^{a A}$	3.20 $^{c B}$	*0.48*	8.35 $^{ab A}$	4.88 $^{e B}$	*0.75*	5.23 $^{a A}$	2.58 $^{cd B}$	*1.04*
$LSD_{0.05}$	*0.35*	*0.77*		*0.83*	*0.59*		*0.55*	*0.66*	
Triticum aestivum									
ACSAD 59	3.05 $^{bc A}$	2.75 $^{c A}$	*ns*	8.20 $^{a A}$	4.90 $^{c B}$	*1.09*	5.75 $^{a A}$	3.30 $^{a B}$	*0.85*
ACSAD 67	3.08 $^{bc A}$	3.33 $^{abc A}$	*ns*	7.58 $^{ab A}$	6.08 $^{b B}$	*1.14*	4.30 $^{b A}$	3.63 $^{a A}$	*ns*
ACSAD 883	2.33 $^{d B}$	3.10 $^{bc A}$	*0.54*	4.90 $^{c A}$	4.65 $^{c A}$	*ns*	4.20 $^{b A}$	3.80 $^{a A}$	*ns*
ACSAD 1059	3.33 $^{ab A}$	3.73 $^{ab A}$	*ns*	7.85 $^{ab A}$	6.30 $^{b B}$	*1.20*	4.43 $^{b A}$	3.58 $^{a B}$	*0.77*
ACSAD 1115	2.65 $^{cd B}$	3.33 $^{abc A}$	*0.53*	6.95 $^{b A}$	6.60 $^{b A}$	*ns*	4.45 $^{b A}$	3.95 $^{a B}$	*0.33*
ACSAD 1135	3.30 $^{ab A}$	3.60 $^{ab A}$	*ns*	7.83 $^{ab A}$	7.53 $^{a A}$	*ns*	4.93 $^{b A}$	3.75 $^{a B}$	*0.68*
Cham 6	3.80 $^{a A}$	3.95 $^{a A}$	*ns*	7.18 $^{b A}$	6.38 $^{b A}$	*ns*	4.65 $^{b A}$	3.30 $^{a B}$	*0.89*
$LSD_{0.05}$	*0.61*	*0.66*		0.98	*0.88*		*0.76*	*ns*	

Means within a column (small letter) and within a row (capital letter) followed by the same letter are not significantly different (*P*<0.05).

Table 2. Nitrogen yield (mg N pot^{-1}) in different plant parts (i.e. roots, leaves and spikes) of durum (*Triticum durum*) and bread (*Triticum aestivum*) wheat genotypes grown under well watering regime (I1) and water stress conditions (I2)

Genotypes	Roots			Leaves			Spikes		
	I1	I2	$LSD_{0.05}$	I1	I2	$LSD_{0.05}$	I1	I2	$LSD_{0.05}$
	Triticum durum								
ACSAD 65	32.96 ^{dA}	36.67 ^{bcA}	*ns*	92.74 ^{abA}	83.76 ^{bcA}	*ns*	63.11 ^{cdeA}	39.54 ^{cB}	*8.89*
ACSAD 357	39.19 ^{bcA}	41.92 ^{bcA}	*ns*	78.04 ^{cA}	80.62 ^{cA}	*ns*	76.60 ^{bA}	57.93 ^{abB}	*8.16*
ACSAD 1261	36.21 ^{cdB}	56.35 ^{aA}	*4.3*	82.52 ^{bcB}	98.51 ^{aA}	*10.2*	54.41 ^{eB}	66.05 ^{aA}	*10.38*
ACSAD 1265	39.20 ^{bcA}	43.86 ^{bA}	*ns*	100.01 ^{aA}	94.22 ^{aA}	*ns*	60.26 ^{deA}	47.31 ^{cB}	*10.43*
ACSAD 1277	36.14 ^{cdA}	36.52 ^{bcA}	*ns*	81.24 ^{cA}	91.63 ^{abA}	*ns*	66.46 ^{cdA}	59.48 ^{abA}	*ns*
ACSAD 1287	40.87 ^{bA}	41.43 ^{bcA}	*ns*	101.39 ^{aA}	75.47 ^{cB}	*11.9*	71.33 ^{bcA}	49.46 ^{bcB}	*15.03*
Cham 1	47.32 aA	35.59 ^{cB}	*5.3*	100.69 ^{aA}	59.69 ^{dB}	*11.0*	97.71 aA	40.50 ^{cB}	*18.16*
$LSD_{0.05}$	*4.11*	*7.76*		*10.3*	*8.56*		*10.11*	*10.14*	
Triticum aestivum									
ACSAD 59	33.65 ^{cA}	32.26 ^{cdA}	*ns*	69.82 ^{cA}	56.03 ^{dB}	*10.9*	96.72 ^{aA}	52.49 ^{bB}	*14.16*
ACSAD 67	39.60 ^{bcA}	37.66 ^{cA}	*ns*	76.69 ^{bcA}	71.47 ^{bcA}	*ns*	84.24 ^{abA}	62.82 ^{abA}	*ns*
ACSAD 883	25.76 ^{dA}	25.51 ^{dA}	*ns*	40.12 ^{dA}	42.27 ^{eA}	*ns*	75.40 ^{bA}	60.78 ^{abA}	*ns*
ACSAD 1059	40.55 ^{bcA}	39.37 ^{bcA}	*ns*	85.80 ^{abA}	66.93 ^{bcB}	*11.0*	72.07 ^{bA}	55.79 ^{abB}	*11.42*
ACSAD 1115	37.69 ^{bcA}	33.40 ^{cA}	*ns*	71.79 ^{cA}	61.55 ^{cdB}	*8.0*	85.29 ^{abA}	70.36 ^{aB}	*6.2*
ACSAD 1135	42.63 ^{bA}	46.13 ^{bA}	*ns*	90.19 ^{aA}	82.32 ^{aA}	*ns*	84.22 ^{abA}	64.50 ^{abB}	*11.25*
Cham 6	54.76 ^{aA}	58.22 ^{aA}	*ns*	94.95 ^{aA}	76.67 ^{abB}	*17.0*	80.91 $^{b A}$	53.66 ^{bB}	*15.05*
$LSD_{0.05}$	*7.03*	*7.82*		*10.22*	*10.41*		*13.48*	*14.76*	

Means within a column (small letter) and within a row (capital letter) followed by the same letter are not significantly different (*P*<0.05).

DISCUSSION

Water stress decreased $\Delta^{13}C$ values in all studied genotypes of both durum and bread wheat compared to well-watered plants. Previous studies have shown a decrease in Δ accompanied with a decrease in soil water content (Morgan *et al*. 1993; Araus *et al.* 1997; Xu *et al.* 2007; Zhu *et al*. 2008; Kale *et al.* 2017; Munjonji *et al.* 2017. According to Farquhar *et al.* (1989), carbon isotope discrimination (Δ) represents an alternative to gas exchange measurements and is a good long-term indicator of stomatal conductance and transpiration efficiency. The lower Δ value under stress conditions is indicative of lower stomatal conductance which decreases C_i/C_a. (Farquhar *et al.* 1989). In this study, the decrease in Δ was affected by wheat genotypes and was generally more pronounced in bread (-1.08‰) than durum wheat (-0.8‰) lines indicating that the latter might be relatively more tolerant to water stress than the former. Although the reduction in Δ values by water stress occurred in all genotypes, durum wheat genotypes having higher Δ values under non stress conditions had also higher values under stress conditions and *vis versa*. However, Δ values in bread wheat lines did not maintain the previous order. Such an observation may indicate that carbon isotopic signature in studied durum wheat lines was more uniform than that of bread wheat implying a more genotypic diversity in the latter than the former wheat species (*i.e.*, genetic variation for Δ). Likewise, based on data of the biochemical and molecular methods (ISSRs) for the advanced breeding lines used, Haidar *et al.* (2012) showed that the genetic variations of bread wheat lines were higher than those of durum wheat lines. Genetic variation for Δ was noted in wheat and barley (Craufurd *et al*. 1991; Acevedo 1993; Sayre *et al.* 1995; Voltas *et al.* 1998; Merah *et al.* 2001; Wahbi & Shaaban 2011).

Many studies reported that carbon isotope discrimination ($\Delta^{13}C$) can reflect the integrated response of physiological processes to environment. The environmental stress can alter $\Delta^{13}C$ as a result of effects on the balance between stomatal conductance and carboxilation (Farquhar *et al.* 1989). The lower $\Delta^{13}C$ value in the stressed plants compared to the non-stressed plants implies that C_i/C_a ratios were lower under stress. Variation in photosynthetic capacity may also lead to cultivar variation in Δ (Condon *et al.* 1993). 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 constant C_i (Condon *et al*. 1993 & 2002).

The undertaken study showed that dry matter yield and nitrogen uptake in different plant parts of durum and bread wheat plants were affected by the plant genotype and watering regime. Regardless of watering regimes, correlations (r) between $\Delta^{13}C$ and dry matter yield of DW were significantly positive in ACSAD 65 (0.73*), ACSAD 1287 (0.79*) and Cham 1 (0.95***), significantly negative in ACSAD 1261 (-0.95***), weak (not significant) in ACSAD 357 (0.35) and ACSAD 1256 (0.22), and no correlation in ACSAD 1277 (-0.15). For BW, however, the positive correlation was only significant in ACSAD 59 (0.94***), while the other genotypes did not show any correlation. The positive correlation between DM and $\Delta^{13}C$ might indicate that the biological yield was more strongly influenced by stomatal conductance than the variations in photosynthetic capacity (Kirda *et al*. 1992; Condon *et al*. 1987). However, the negative correlations might be explained as caused by variations in photosynthetic capacity (Wright *et al*. 1988). The weak or null relationships between DM and $\Delta^{13}C$ might be due to the effects on the balance between stomatal conductance and photosynthetic capacity.

Since water consumption and its efficient use by crops are correlated with yield (Tambussi et al. 2007), carbon isotope discrimination has been demonstrated to be an indicator of WUE for many C3 crops, and their negative correlation has been used for indirect selection of plants with improved WUE under selected environments (Cattivelli *et al.* 2008). The undertaken study showed negative relationships between $\Delta^{13}C$ and WUE which were obtained for most of DW and BW genotypes with the exception of Cham 1 (r=0.79*), ACSAD 1287 (no correlation), and ACSAD 59 (r=0.71*). It has been reported that genotypic variation in Δ and its relationship with water-use efficiency (WUE) can be exploited for increasing crop productivity by selecting water-use efficient genotypes for target environments (Condon et al. 2004). Selection of plant genotypes for high yield or WUE (i.e. low $\Delta^{13}C$) is desirable to improve crop production in water limited environments (Farquhar *et al.* 1989; Akhter *et al.* 2008; Chen *et al*. 2013). Our study showed that the selection of low $\Delta^{13}C$ as an indirect indicator for higher WUE and greater biomass under water stress is affected by plant genotype. The lowest Δ values obtained in water stressed ACSAD 1277 (DW) and ACSAD 1115 (BW) were associated with high WUE and dry matter yield (low Δ with high WUE & biomass), illustrating the feasibility of using $\Delta^{13}C$ (i.e., low discrimination) as a selection criteria for higher WUE and biomass under water stress. However, for the other tested lines, association between $\Delta^{13}C$ and WUE or biomass was not always negative. Some examples from the studied lines grown under water stress condition could be drawn as follow: a) the water stressed ACSAD 59 (BW) showed low values of the aforementioned criteria compared with the other lines (i.e., low Δ was associated with low WUE & biomass). In contrast, b) the highest WUE and greatest biomass values obtained in ACSAD 1261 (DW) and ACSAD 1135 (BW) under stress were associated with the highest $\Delta^{13}C$ (i.e., high Δ with high WUE & biomass). This result demonstrates the importance of selecting water tolerant plant genotypes having greater $\Delta^{13}C$ and higher biomass under waters stress conditions (Krishnamurthy *et al.* 2013).

The decline in photosynthetic activity in plants upon exposure to water stress predominantly occurred due to low CO_2 availability in response to stomatal closure (stomatal limitation) or/and the modifications of carbon assimilation (metabolic limitation) metabolism (Farquhar *et al.* 1989). Stomatal closure in response to water stress restricts CO_2 entry into leaves thereby decreasing CO_2 assimilation as well as decreasing water loss from leaves leading to higher WUE. In addition, stomatal closure also restricts the diffusion of CO_2 into the leaves leading to decreased net photosynthetic rate (Cornic 2000). Also, Kumar and Singh (2009) reported that the supply of CO_2 to Rubisco may be limited under water stress either by stomatal closure or by stomatal tissue shrinkage, diminishing the intercellular air space. In the present study, the decline in $\Delta^{13}C$ values in Cham 1 and ACSAD 1287 under stress were associated with decline in their WUE, particularly Cham 1, with significant decreases in dry matter yield (i.e. decline in photosynthesis). These results may indicate that the reduction of their growth (e.g., DM, TN.) by water stress was most probably resulted from both stomatal limitation (i.e., closure) and metabolic limitations (carbon assimilation mediated by rubisco). In this context, (Saud *et al.* 2016) reported that stomatal closure along with mesophyll conductance performed a vital responsibility in limiting photosynthesis under drought stress, and diffusional limitations of a leaf were attributed to both of these factors. Likewise, Flexas *et al*. (2006) reported that decreased Rubisco activity during water stress is related to conditions of low stomatal conductance and chloroplast CO_2 concentrations. Also, the decline in $\Delta^{13}C$ and the reduction in DM of ACSAD 65 by stress may be attributed to both stomatal closure and photosynthesis limitation under water stress conditions. On the other hand, the higher $\Delta^{13}C$ in Cham 1 and ACSAD 1287 under well watering regime implying that a maximization of their yield may occur via an enhancement of CO_2 uptake due to stomatal opening (higher C_i/C_a ratio) and carboxylation activity. Therefore, it can be suggested that Cham 1 and ACSAD 1287 might be suitable to be grown in wet conditions. Support for this comes from field results over the years and from recommendation of General Commission for Scientific Agricultural Research (GCSAR) in Syria to grow Cham 1 only under irrigation or in high rainfall areas (Bishaw *et al.* 2011).

The decline in $\Delta^{13}C$ values (e.g., ACSAD 1277, 357, 1265) with water stress was associated with enhancements of WUE, however, DM values were not affected. Thus, it can be suggested that the aforementioned lines might be useful for water limited environments as well as wet environment. Chen *et al.* (2013) reported that improved WUE under stress without a yield reduction offers a promising approach for sustainable agricultural production in semi-arid regions. The increase in WUE with water stress may be because of the higher stomatal conductance reduction (i.e., sharp decrease of $\Delta^{13}C$) than assimilation reduction (Akhter *et al.* 2008). Accordingly, this result may indicate the important role of Rubisco activity in regulating foliar carbon isotope discrimination. Water stress reduces the initial and total Rubisco activity per unit area**,** but does not reduce the amount of Rubisco protein per unit leaf area (Kumar and Singh 2009).

Although $\Delta^{13}C$ values declined under water stress for all durum wheat genotypes, the extent of the decrease was dependent on lines studied. ACSAD 1261 showed a lower decease in $\Delta^{13}C$ compared to other tested lines. This may be attributed to a relatively low partial stomatal closure under water stress conditions. Hence, the higher shoot biomass of ACSAD 1261 with higher $\Delta^{13}C$ under stress as compared with other lines could be resulted from increasing supply of CO_2 to Rubisco (i.e., photosynthetic activity), rather than simple effect on stomatal level. Therefore, this line, may be able to increase its photosynthesis as it tries to recover from the dehydration stress by increasing the root biomass (Table 1). This recovery may allow gradual recovery in stomatal tissue and hence, increased supply of CO_2 to Rubisco, thus, increasing the Rubisco activity (Kumar & Singh 2009). Consequently, this result may illustrate the importance of selecting drought tolerant plant genotypes having greater $\Delta^{13}C$ (as compared with other lines) and higher biomass under waters stress (e.g., ACSAD 1261). It has been reported that under water-limited Mediterranean type environment, the correlation between $\Delta^{13}C$ and yield has been mostly positive and selection for high $\Delta^{13}C$ was thought to be most appropriate in that region (Condon *et al.* 2004; Merah *et al.* 2001; Voltas *et al.* 1998). Also, Krishnamurthy *et al.* (2013), showed that selection for greater $\Delta^{13}C$ (lesser $\delta^{13}C$) or transpiration efficiency balanced the shoot biomass production and harvest index to produce the best seed yield of chickpea grown under water stress.

With regard to bread wheat, water stress did not negatively reduce DM yield of studied lines with the exception of ACSAD 59 which declined by 38% (Fig. 1). However, water stress decreased Δ values in all studied lines as compared to well-watered plants (Fig. 5). The extent of the decrease in Δ mean values was less pronounced in ACSAD 1135 and Cham 6 (-0.45‰) than the other tested lines (-1.35‰). Because of lower dry matter yield in the water stressed ACSAD 59 compared with non stressed plants, it was unlikely that a higher photosynthetic capacity occurred in I2. Therefore, the $\Delta^{13}C$ value decline in this line resulted mainly from stomatal closure induced by stress. The highest DM of ACSAD 59 under well watering regime may suggest the suitability of this line to be grown under well irrigated conditions.

The decline in $\Delta^{13}C$ values in the other tested lines was associated with enhancements of WUE and with unchanged DM. The increase in WUE with water stress might result from lower stomatal conductance rather than assimilation reduction (Akhter *et al.* 2008). With the exception of ACSAD 59, the tested bread wheat lines could be suitable to be grown in wet conditions. Among the tested lines, Bishaw *et al*. (2011) reported that Cham 6 was recommended to be grown under rain-fed and irrigated conditions.

The nitrogen use efficiency (NUE) is defined as the capacity of a given genotype in taking an advantage of the applied nitrogen and transform it in biomass. The evaluation of NUE is useful to differentiate plant species, genotypes and cultivars for their ability to absorb and utilize nutrients for maximum yields (Baligar *et al.* 2001). Genotype that use N more efficiently is one of the main objectives of wheat breeding programs (Sadras & Lemaire 2014). There is a need for breeding programs to focus on developing genotypes with high NUE, particularly, under water stress conditions (Baligar *et al.* 2001). This study showed that the highest (49.5%) and the lowest (28.3%) values of NUE in DW belonged to Cham 1 genotype grown under I1 and I2**,** respectively. This result may indicate the suitability of Cham 1 to be grown in wet environment. However, NUE in ACSAD 1261 under stress (42.6%) was much higher than that under non stress conditions (35%). Also, this line showed higher water use efficiency (WUE) under stress. Quemada & Gabriel (2016) reported that enhancement of WUE and NUE simultaneously may provide advantages over optimization of water and nitrogen inputs separately. Therefore, it can be suggested that the advanced breeding line ACSAD 1261 which is characterized by higher WUE and NUE along with DM and TN might be a promising line for semi-arid areas. In BW, although NUE values declined by water stress, the higher values of Cham 6 and ACSAD 1135 (about 40%) may indicate their suitability for both wet and dry environments.

CONCLUSIONS

The simultaneous use of carbon isotope discrimination ($\Delta^{13}C$) together with agro-physiological traits (DM, TN, N-uptake, NUE and WUE..) provided advantages in selecting water stress tolerance of the advanced breeding lines and genotypes of bread (BW) and durum wheat (DW) over using them separately. Based on the aforementioned criteria, it could be recommended that the tested lines are suitable in water stress environments (e.g., DW: ACSAD 1261) and under well watering conditions (e.g., DW: Cham 1, ACSAD 1287 and ACSAD 65; BW: ACSAD 59.) or under both stressed and non stressed conditions (e.g., most of BW lines except for ACSAD 59; DW: ACSAD 1277, 357, 1265).

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