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ORIGINAL ARTICLE

Growth, Nitrogen Uptake and Carbon Isotope Discrimination in Barley Genotypes Grown under Saline Conditions

Kurdali Fawaz, Farid Al-Ain and Mohammad Al-Chammaa

Atomic Energy Commission, Agriculture Department P.O. Box 6091, Damascus, Syria

[*] *E-mail: ascientific@aec.org.sy*

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The effect of different salinity levels of irrigation water (EC_w range 1-12 dS/m) on dry matter yield, nitrogen uptake, fertilizer nitrogen use efficiency (%NUE), stomatal conductance and carbon isotope discrimination ($\Delta^{13}C$‰) in three barley genotypes originating from different geographic areas (Arabi.Abiad, Syria; Pk-30-136, Pakistan and WI-2291, Australia) was investigated in a pot experiment. An increase in salinity resulted in a decrease in $\Delta^{13}C$ in all the genotypes. Increasing salinity reduced leaf stomatal conductance which was less pronounced in WI-2291 comparing to other genotypes. At high salinity level, the reduction in $\Delta^{13}C$ corresponded to a considerable decrease in the ratio (C_i/C_a) of intercellular (C_i) and atmospheric (C_a) partial pressures of CO_2 in all the genotypes indicating that such a decrease was mainly due to the stomatal closure. Moreover, since the reduction in dry matter yield in all the genotypes grown at 12 dS/m did not exceed 50% in comparison with their controls, the photosynthetic apparatus of all studied genotypes seemed to be quit tolerant to salinity. At the moderate salinity level (8dS/m), the enhancement of leaf dry matter yield in the WI2291 genotype might have been due to positive nutritional effects of the salt as indicated by a significant increase in nitrogen uptake and NUE. Thus, the lower C_i/C_a ratio could result mainly from higher rates of photosynthetic capacity rather than stomatal closure. On the other hand, relationships between dry matter yield or NUE and $\Delta^{13}C$ seemed to be depending on plant genotype, plant organ and salinity level. Based on growth, nutritional and $\Delta^{13}C$ data, selection of barley genotypes for saline environments was affected by salinity level. Therefore, such a selection must be achieved for each salinity level under which the plants have been grown.

Key words: Barley / Salinity / $\Delta^{13}C$‰ / ^{15}N / stomatal conductance

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Key words: Barley / Salinity / $\Delta^{13}C$‰ / ^{15}N / stomatal conductance

Salinity is one of the most important abiotic stresses limiting crop production in arid and semiarid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching. Salt affected lands are increasingly needed for production in order to meet food demands of the ever increasing human population and urbanization on earth. Saline agriculture could be a promising approach since it meets the above-mentioned demands (Qureshi and Barrett-Lennard, 1998). It is profitable since it makes use of genetic resources integration and improves agricultural practices to make better use of saline land and saline irrigation water on a sustainable basis (Qureshi and Barrett-Lennard, 1998).

Barley (*Hordeum vulgare* L.) is a salt-tolerant crop (Maas and Hoffman, 1977) with considerable economic importance in salinity-affected arid and semiarid regions of the world. Salt tolerance of barley has been of interest for a long time and has resulted in a considerable body of data from studies using physiological (Cramer *et al.,* 1990; Munns and Rawson, 1999), genetic (Mano and Takeda, 1997; Ellis *et al.,* 2002), and cytogenetic approaches (Forster *et al.,* 1997). Salinity affects many morphological, physiological, and biochemical processes, including seed germination, plant growth, water and nutrient uptake (Zhao *et al.,* 2007; Marschner, 1995).

During photosynthesis, C3 plants discriminate against the heavy isotope of carbon (^{13}C) leading to less ^{13}C in plant dry matter. Many previous studies demonstrate that carbon stable isotope composition 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 (Bellaloui, 2011). Since water consumption and its efficient use by crops are related with yield (Tambussi *et al.,* 2007), carbon isotope discrimination ($\Delta^{13}C$) could facilitate genotypic selection for yield owing to its negative relationship with water use efficiency in C3 species (Farquhar *et al.,* 1989). Carbon isotope discrimination positively correlates with C_i/C_a, i.e. the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (Farquhar *et al.,* 1982). Measurement of $\Delta^{13}C$ gives an estimation of the assimilation-rate-weighted value of C_i/C_a. A lower C_i/C_a ratio may result either from higher rate of photosynthetic activity or from stomata closure induced by stress. Determination of $\Delta^{13}C$ was widely used as an indirect selection criterion for higher yield in cereals such as wheat (Rebetzke *et al.,* 2002), barley (Craufurd *et al.,* 1991; Teulat *et al.,* 2001) and rice (Impa *et al.,* 2005). The work of Condon *et al.* (1987) suggests that screening for high yielding wheat varieties, based on ^{13}C discrimination, leads to similar results irrespective of whether the plants are grown in pots or in the field. Low $\Delta^{13}C$ values under salinity have been reported for wheat (Ansari *et al.,* 1998, Shaheen and Hood-Nowotny, 2005a, Arslan *et al.,* 1999), barley (Isla *et al.,* 1998) rice (Shaheen and Hood-Nowotny, 2005b) and Kentuky bluegrass (Qian *et al.,* 2004). Isla *et al.* (1998) reported that the behavior of barley plants grown under salinity stress is different from that under drought stress. Carbon isotope discrimination is a useful indicator for yield potential in barley grown under non-saline conditions; whereas, grain yield remain only reliable means for identifying salt tolerance in barley. The objectives of this study were to (i) compare plant growth (e.g. dry matter production, nitrogen uptake and fertilizer nitrogen use efficiency as determined by the ^{15}N isotope technique) and salinity tolerance of three barley genotypes originated from various countries and (ii) examine variations of carbon isotope discrimination ($\Delta^{13}C$‰) and stomatal conductance (gs) values among three barley genotypes over a range of salinity levels of irrigation water (e.g. EC_w range 1-12 dS/m).

MATERIALS AND METHODS

Soil properties

The used soil for this experiment was collected from Der-Alhajar Research Station located in the south-east of Damascus, Syria (33˚ 21′ N, 36˚ 28′ E) at 617 m above sea level. The experiment was conducted in pots, each one containing 5 kg of thoroughly mixed soil. The soil is sandy clay loam in texture, water content at field capacity varied from 30.7 to 36.1% by volume, and wilting point from 11.5 to 17.1%. Soil bulk densities ranged from 1.11 to 1.21 g cm^{-3} throughout the 0.6 m soil profile (Hussein *et al.,* 2011). The main physical and chemical soil properties are: pH 7.7; Ec_e 0.83 dS /m; organic matter 0.91%; cations mmol (e) /L (Ca^{2+} 1.10, Mg^{2+} 0.47, K^+ 0.14, Na^+ 1.27); anions mmol (e) /L (SO_4^{2-} 1.27, HCO_3^- 0.97, Cl^- 0.74); available P (Olsen) 6.8 µg/g; total N 0.07%; NO_3^- 42.0µg/g; NH_4^+ 26.1µg/g; Cation exchange capacity (CEC) 29.08 meq/100g soil; Exchangeable cations: Na^+ 2.99, K^+ 1.81 Ca^{2+} 41.51 and Mg^{2+} 6.76 meq/100g soil.

Planting procedure

Seeds of three barley genotypes originated from various countries (Arabi Abiad, Syria; Pk-30-136, Pakistan and WI-2291, Australia) were used in the experiment. After germination, plants were thinned to 3 plants per pot. The pots were placed outdoors under natural climatic conditions.

An equivalent fertilizer rate of 50 kg N/ha urea enriched with 2 atom %^{15}N was applied to the soil. The N fertilizer was used in two split applications at one month interval after planting. Soil moisture level in pots was maintained around 70% of field capacity throughout the experimental period.

Salinity treatments

During the first two weeks, pots were irrigated with non-saline water. Thereafter, five saline irrigation treatments (EC1, EC2, EC3, EC4, and EC5) were applied. EC1 is the control which is ordinary non-saline water (ECw 1.1 dS/m). EC2, EC3, and EC4 were obtained by mixing EC1 with a saline ground water (ECw 19 dS/m) obtained from the Euphrates valley until the solutions EC levels were 4, 8, and 12 dS/m, respectively. As for the last treatment (EC5), plants were irrigated with gradually increased levels of water salinity (1, 4, 8 and 12 dS/m) at 15-day intervals throughout the whole experimental period.

Stomatal conductance (gs)

Midday abaxial stomatal conductance measurements were taken with a porometer (Type AP4, England) on the flag leaves of three barley genotypes. The measurements were made in triplicates for each pot in the control and salinity treatments.

Plant harvest and analysis

Plants were harvested 18 weeks after planting. Leaf, spike and root samples were oven dried at 70 °C, weighed and ground to a fine powder. Concentration of total N was measured by ***Kjeldahl procedure***. $^{15}N/^{14}N$-isotope ratio was measured using an emission spectrometry (Jasco-150, Japan) to calculate nitrogen fertilizer use efficiency. Concentration of C, and $\delta^{13}C$ were determined on sub-samples (2 mg dry weight) of different plant parts using the continuous-flow isotope ratio mass spectrometry (Integra-CN, PDZ Europea Scientific Instrument, UK). Isotopic compositions are expressed using delta notation (δ) in parts per thousand (‰): δ (‰) = [($R_{sample}/R_{standard}$) -1] 1000

where R is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The nitrogen isotope ratios are expressed relative to atmospheric air as the standard. The carbon isotope ratio in the plant sample is expressed relative to Pee

Dee belemnite (PDB) standard which is a fossil of *Belemnita americana* from the Pee Dee geologic formation in South Carolina. Carbon isotope discrimination ($\Delta^{13}C$) was calculated according to 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.

Whole plant $\Delta^{13}C$ (‰) was calculated as an average of leaves (L), spikes (S) and roots (R) $\Delta^{13}C$ weighted by the total C content (mg) of leaves, spikes and roots:

$$\text{Whole-plant } \Delta^{13}C = [(L\ \Delta^{13}C \times L_C) + (S\ \Delta^{13}C \times S_C) + (R\ \Delta^{13}C \times R_C)] / (L_C + S_C + R_C)$$

Statistical analysis

The data were subjected to analysis of variance (ANOVA) test, and means were compared using the Least Significant Difference (Fisher's PLSD) test at the 0.05 level confidence.

RESULTS

Dry matter and nitrogen yield

Dry matter yield (DM) in different plant parts was affected by barley genotype and salinity levels (Table 1 and Fig. 1). The highest salinity level (12 dS/m) reduced leaf DM by 18, 32 and 18% for A. Abiad, Pk-30-136 and WI-2291, respectively. Irrigation with saline water produced a significant decrease in leaf dry matter yield of PK-30-136 genotype. No significant decrease was observed in A. Abiad at EC_w of 4 and 8 dS/m. However, DM in WI-2291 genotype increased as salt level increased to 8 dS/m and decreased with increases salinity to 12 dS/m. Leaf DM in plants irrigated with gradually increased levels of water salinity (EC5) increased by 12% in A. Abiad and decreased by 13% in Pk-30-136, while it was relatively similar to that in the control of WI2291 genotype. For roots, significant decreases in DM were observed in all the genotypes. From a spike production standpoint, although A. Abiad surpassed the other genotypes in maintaining higher spike yields under different salinity treatments, no significant differences were observed between EC1, EC2 and EC4 treatments. However, the Pk-30-136 showed greater spike yield under saline conditions in comparison with its control. The WI-2291 showed a relatively constant spike yield under different salinity treatments (1-12dS/m). In EC5 treatment, significant increases in spike DM were observed in all the genotypes. In the whole plant, only the highest salinity level (12 dS/m) significantly reduced DM. The observed values were 21, 17 and 16% lower than those of the control for A. Abiad, PK-30-136 and WI-2291, respectively.

Total N (TN) yield in different plant parts is shown in Table 2. No significant decrease was observed in A. Abiad at various salinity treatments. Irrigation with saline water produced a significant decrease in leaf nitrogen matter yield of Pk-30-136 genotype. However, TN in WI-2291 genotype increased as salt level increased to 8 dS/m and decreased with increases salinity to 12 dS/m. For roots, significant decreases in TN were observed only in A. Abiad and Pk-30-136 genotypes at the highest salinity level (12 dS/m). In spikes, A. Abiad surpassed the other genotypes in maintaining higher spike N yields under different salinity treatments with no significant differences being obtained between the control and 1, 4, 8 and 12dS/m treatments. However, the Pk-30-136 showed greater spike N yield under saline treatments in comparison with its control. The WI-2291 showed a greater N yield in the EC3 treatment (8dS/m) in comparison with EC1, EC2 and EC4 treatments. Moreover, the highest TN in the whole plant was observed in WI-2291 genotype at 8dS/m salinity level where the percent increment was 51% over its control (Fig.2). In EC5 treatment, whole plant nitrogen increased by 24% over the control in the Pk-30-136 genotype; whereas, no significant increases were observed for the other genotypes.

Nitrogen uptake from soil and fertilizer

Data presented in Table 3 shows that nitrogen uptake from soil (Ndfs) and fertilizer (Ndff) were affected by the genotype and salinity levels. The highest Ndfs in the whole plant was observed in WI-2291 genotype at 8dS/m salinity level where the percent increment was 68% over its control. An increase in salinity (1-12dS/m) resulted in a decrease in Ndff in A. Abiad and Pk-30-136 genotypes. However Ndff in WI-2291 genotype increased as salt level increased to 8 dS/m and decreased with increases salinity to 12 dS/m. The highest salinity level reduced Ndff of all genotypes by 19, 22 and 23% for A. Abiad, PK-30-136 and WI-2291, respectively.

Nitrogen fertilizer use efficiency (%NUE) was calculated as fertilizer N recovery in the whole plant using the ^{15}N isotopic data. An increase in salinity resulted in a decrease in %NUE values of A. Abiad and Pk-30-136 genotypes. In contrast, NUE in WI-2291 genotype increased as salt level increased to 8 dS/m (61% over the control) and decreased with increasing salinity to 12 dS/m (Table 3).

Stomatal conductance

Significant differences in stomatal conductance (gs) were observed in leaves of the genotypes grown under non-stress conditions (control). A. Abiad showed a higher leaf stomatal conductance comparing with Pk-30-136 and WI-2291 genotypes. An increase in salinity resulted in a decrease in stomatal conductance in leaves of all the genotypes (Fig.3). In comparison with the control, the reduction in stomatal conductance of plants grown under salinity was less pronounced in the WI-2291 genotype than those of A. Abiad and Pk-30-136 as compared with their controls. Irrigation of A. Abiad, Pk-30-136 and WI-2291 genotypes with mild saline water showed a decline in stomatal conductance values by 24, 29 and 16% in EC2 and by 50, 54 and 14% in EC3 as compared to their controls. At the highest salinity treatment (EC4), stomatal conductance values of the above mentioned genotypes were 74, 66 and 43% lower than those of the control. Correspondingly, the average stomatal conductance values of the three barley genotypes measured in the high salinity treatment was 62% lower that measured in the control.

Variations in ^{13}C natural abundance

Data presented in Table 4 shows that carbon isotope data, expressed as carbon discrimination (‰$\Delta^{13}C$), were different in the various plant parts and were affected by the genotype and salinity levels. Generally, $\Delta^{13}C$ values in leaves were higher than those in roots or spike; whereas, they were somewhat parallel to those of the whole plants. Carbon isotope discrimination values (‰$\Delta^{13}C$) ranged from 16.21‰ to 20.33‰ in shoots, from 16.15‰ to 19.25‰ in roots, and from 14.56‰ to 17.27‰ in spikes. Whole plant ‰$\Delta^{13}C$ ranged from 15.93‰ to 18.85.4‰ (Fig.4). Negative correlations were observed between $\Delta^{13}C$ and salinity. An increase in salinity (1-12dS/m) resulted in a decrease in $\Delta^{13}C$ discrimination in different plant parts of the three barley genotypes. The lowest $\Delta^{13}C$ values were often obtained in the highest salinity treatment (EC4). The difference in $\Delta^{13}C$ between each two salinity levels was approximately 1‰.

Moreover, A. Abiad grown under different salinity treatments generally showed higher Δ^{13}C values than the other genotypes. On the other hand, Δ^{13}C values of the whole plants in the EC5 treatments were approximately similar to those in EC2.

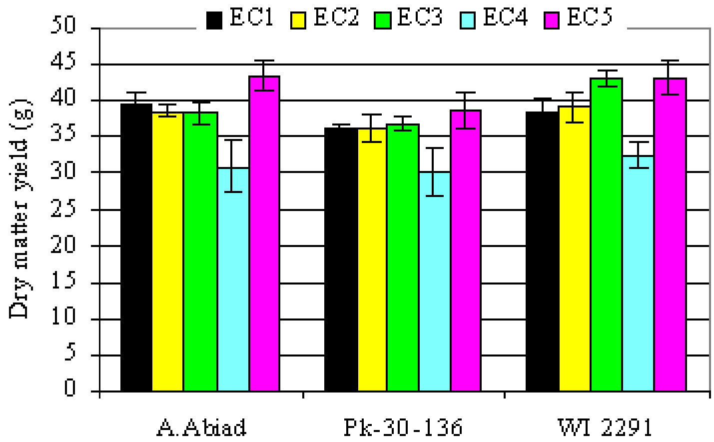

Figure 1: Total dry matter yields (g/pot) of three barley genotypes as affected by increasing levels of salinity in the irrigation water.

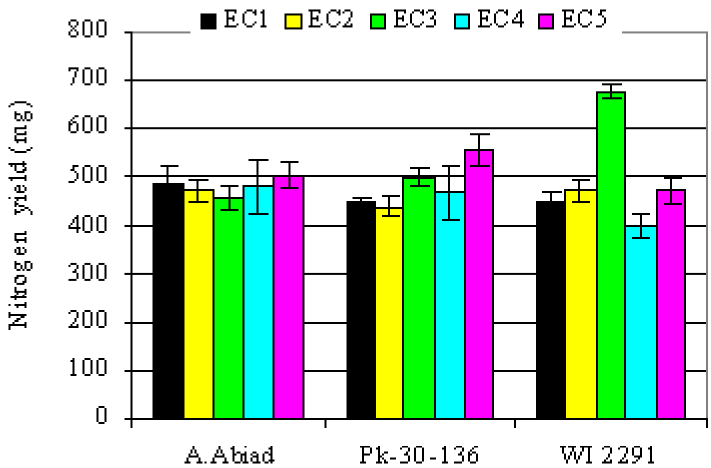

Figure 2: Total nitrogen yields (mg N/pot) of three barley genotypes as affected by increasing levels of salinity in the irrigation water.

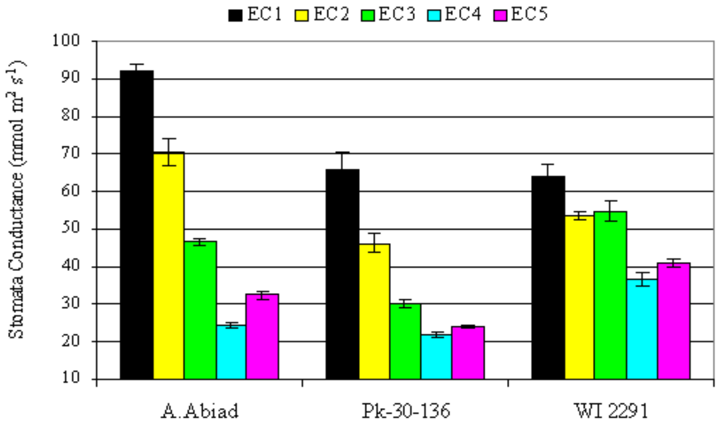

Figure 3: Stomata conductance (mmol m^2s^{-1}) in leaves of three barley genotypes as affected by increasing levels of salinity in the irrigation water.

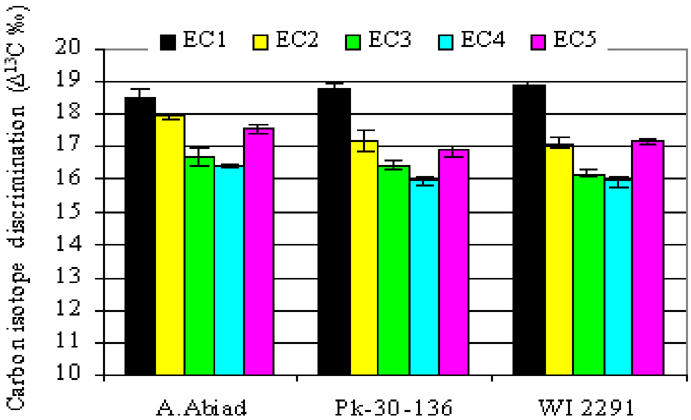

Figure 4: Carbon isotope discrimination ($\Delta^{13}C$ ‰) in the whole plant (leaves, spikes and roots) of three barley genotypes as affected by increasing levels of salinity in the irrigation water.

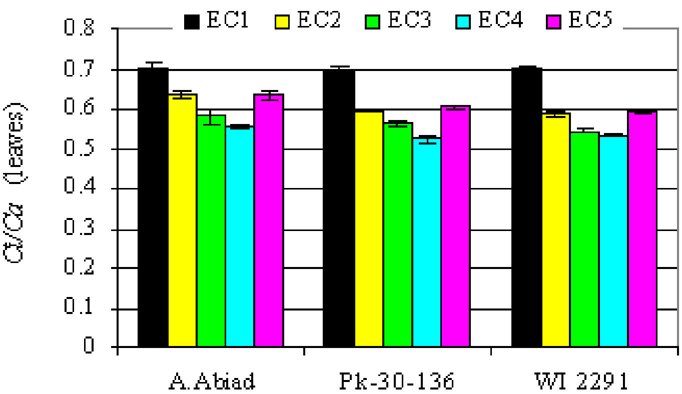

Figure 5: Intercellular CO_2 partial pressure to ambient CO_2 partial pressure (C_i/C_a) in leaves of three barley cultivars as affected by increasing levels of salinity in the irrigation water.

Table 1: Dry matter yields (g/pot) in different plant parts of three barley cultivars as affected by increasing levels of salinity in the irrigation water

Cultivars	Salinity levels dS/m					
	EC1	EC2	EC3	EC4	EC5	LSD $_{0.05}$
	Leaves					
Arabi Abiad	16.58B,b	16.40C,b	15.10C,bc	13.63B,c	18.60B,a	***1.57***
Pk-30-136	20.93A,a	18.93B,b	19.68Bb	14.30B,c	18.25Bb	***1.76***
WI- 2291	21.40A,b	22.93A,a	24.18Aa	17.63A,c	22.85A,ab	***1.49***
LSD $_{0.05}$	***1.51***	***1.77***	***1.61***	***1.98***	***1.64***	
	Roots					
Arabi Abiad	7.88B,b	7.35B,b	8.00A,a	4.98A,c	6.65A,b	***1.47***
Pk-30-136	9.58A,a	9.08A,a	6.23B,bc	5.90A,c	7.40A,b	***1.45***
WI- 2291	7.40B,a	6.50B,a	7.80A,a	5.53A,b	6.70A,ab	***1.19***
LSD $_{0.05}$	***1.40***	***1.33***	***1.24***	***N.S***	***N.S***	
	Spikes					
Arabi Abiad	14.88A,b	14.70A,b	15.18A,b	12.33A,c	18.03A,a	***2.00***
Pk-30-136	5.58C,d	7.95B,c	10.80B,b	9.85B,b	12.93B,a	***1.22***
WI- 2291	9.65B,b	9.70B,b	11.08B,b	9.30B,b	13.38B,a	***1.89***
LSD $_{0.05}$	***1.70***	***2.04***	***1.18***	***2.12***	***2.09***	

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

Table 2: Nitrogen yields (mg N/pot) in different plant parts of three barley cultivars as affected by increasing levels of salinity in the irrigation water

Cultivars	Salinity levels dS/m					
	EC1	EC2	EC3	EC4	EC5	LSD $_{0.05}$
	Leaves					
Arabi Abiad	143.13B,a	149.53C,a	139.31C,a	154.52B,a	144.42C,a	***N.S***
Pk-30-136	253.07A,a	198.11B,b	192.59B,b	187.83A,b	193.85B,b	***24.77***
WI- 2291	228.73A,bc	242.31A,b	353.52A,a	175.66AB,d	214.28A,c	***21.34***
LSD $_{0.05}$	***27.76***	***19.40***	***17.66***	***28.89***	***18.70***	
	Roots					
Arabi Abiad	96.42A,a	85.04A,ab	81.86A,ab	72.63A,b	71.30B,b	***19.28***
Pk-30-136	101.09A,a	82.07AB,ab	89.64A,ab	80.13A,b	97.43A,ab	***19.37***
WI- 2291	73.16B,b	69.61B,b	92.56A,a	63.55A,b	67.10B,b	***13.37***
LSD $_{0.05}$	***18.32***	***13.97***	***N.S***	***N.S***	***21.7***	
	Spikes					
Arabi Abiad	249.35A,b	235.16A,b	235.74A,b	252.90A,ab	287.49A,a	***37.60***
Pk-30-136	93.86C,d	159.24B,c	216.36A,b	200.09B,b	263.68A,a	***28.77***
WI- 2291	145.37B,c	158.18B,c	231.10A,a	158.67B,c	189.69B,b	***29.43***
LSD $_{0.05}$	***36.22***	***33.74***	***N.S***	***42.66***	***31.96***	

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

Table 3: Amounts of nitrogen derived from soil (Ndfs) and from fertilizer (Ndff), (mg N/pot) and % nitrogen fertilizer use efficiency (NUE) in the whole plant of three barley cultivars as affected by increasing levels of salinity in the irrigation water

Cultivars	Salinity levels dS/m					
	EC1	EC2	EC3	EC4	EC5	$LSD_{0.05}$
	Ndfs					
Arabi Abiad	297.94A,a	306.66A,a	299.10C,a	325.10AB,a	321.57B,a	*N.S*
Pk-30-136	273.62B,c	278.79B,c	338.26B,ab	331.83A,b	371.40A,a	***36.86***
WI- 2291	282.25AB,bc	300.14AB,b	474.64A,a	270.06B,c	291.46B,bc	***25.90***
$LSD_{0.05}$	***23.46***	***22.62***	***30.83***	***56.64***	***37.30***	
	Ndff					
Arabi Abiad	190.97A,a	163.08A,b	157.81B,c	154.95A,c	181.65A,ab	***21.65***
Pk-30-136	174.41AB,ab	160.63A,b	160.31B,b	136.22AB,c	183.56A,a	***17.65***
WI- 2291	165.01B,c	169.96A,bc	202.54A,a	127.81B,d	179.60A,b	***14.44***
$LSD_{0.05}$	***18.29***	***N.S***	***11.28***	***26.21***	***N.S***	
	%NUE					
Arabi Abiad	57.69A,a	49.27A,bc	47.68B,c	46.81A,c	54.88A,b	***6.54***
Pk-30-136	52.69AB,ab	48.53A,b	48.43B,b	41.16AB,c	55.46A,a	***5.38***
WI- 2291	49.85B,c	51.35A,bc	61.19A,a	38.61B,d	54.26A,b	***4.36***
$LSD_{0.05}$	***5.53***	***N.S***	***3.41***	***7.92***	***N.S***	

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

Table 4: Carbon isotope discrimination ($\Delta^{13}C$‰) in different plant parts of three barley cultivars as affected by increasing levels of salinity in the irrigation water

Cultivars	Salinity levels dS/m					
	EC1	EC2	EC3	EC4	EC5	$LSD_{0.05}$
	Leaves					
Arabi Abiad	20.33A,a	18.78A,b	17.53A,c	16.96A,d	18.76A,b	***0.40***
Pk-30-136	20.14A,a	17.90B,b	17.13AB, c	16.21B,d	18.03B,b	***0.35***
WI- 2291	20.29A,a	17.65B,b	16.71B,c	16.46B,d	17.78C,b	***0.24***
$LSD_{0.05}$	***N.S***	***0.49***	***0.46***	***0.25***	***0.19***	
	Roots					
Arabi Abiad	17.82A,b	17.17A,c	16.36A,d	19.25A,a	17.67A,b	***0.20***
Pk-30-136	16.70B,b	15.87C,d	16.15A,cd	18.04B,a	16.43B,bc	***0.30***
WI- 2291	16.39C,c	16.47B,bc	16.33A,c	16.66C,b	17.41A,a	***0.21***
$LSD_{0.05}$	***0.23***	***0.18***	***N.S***	***0.23***	***0.38***	
	Spikes					
Arabi Abiad	16.81A,b	17.27A,a	16.08A,d	14.91A,e	16.36A,c	***0.29***
Pk-30-136	16.27B,a	16.41B,a	15.32B,b	14.56B,c	15.45C,b	***0.34***
WI- 2291	16.91A,a	16.12B,b	15.03C,c	14.73AB,d	15.98B,b	***0.21***
$LSD_{0.05}$	***0.19***	***0.43***	***0.29***	***0.20***	***0.34***	

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

DISCUSSION

The effect of salinity on dry matter production seems to vary from negative to positive effects depending on plant genotype and plant organ (Table 2 and Fig.1). However, an increase in salinity (1-12dS/m) resulted in a decrease in $\Delta^{13}C$ discrimination in the different plant part of three barley genotype. Such a decrease indicates that imposition of salt stress leads to less discrimination against the heavier isotope. Low $\Delta^{13}C$ values under salinity have been reported for wheat (Ansari *et al.,* 1998, Shaheen and Hood-Nowotny, 2005a, Arslan *et al.,* 1999), Barley (Isla *et al.,* 1998), rice (Shaheen and Hood-Nowotny, 2005 b) and Kentuky bluegrass (Qian *et al.,* 2004).

During photosynthesis, C3 plants discriminate against the heavy isotope of carbon (^{13}C) leading to less ^{13}C in plant dry matter. Carbon isotope discrimination positively correlates with C_i/C_a, i.e. the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (Farquhar *et al.,* 1982). Higher $\Delta^{13}C$ is caused by a higher C_i/C_a ratio mainly due to a large stomata conductance, which leads to higher photosynthetic rate and hence higher yield, i.e. positive relationship between $\Delta^{13}C$ and yield but if stomatal conductance remains constant and photosynthetic capacity varies, the increasing photosynthetic capacity should lower C_i/C_a and $\Delta^{13}C$ but elevate assimilation rate per unit leaf area. This condition will lead to negative relationship between $\Delta^{13}C$ and yield (Condon *et al.,* 1987).

In C3 plants, several researchers have examined the association between $\Delta^{13}C$ and yield. Reported correlations vary from positive (Condon *et al.,* 1987; Kirda *et al.,* 1992) to negative (Condon *et al.,* 2002; Arslan *et al.,* 1999). This contrast could be explained by differences related to growth stage sampling and environmental conditions under which the plants have been grown (Monneveux *et al.,* 2005). The relationship between $\Delta^{13}C$ and yield in barley, and hence its utility as an indicator of yield, has often been found to be positive (Febrero *et al.,* 1994; Romagosa and Araus, 1991), indicating that higher yield is associated with low WUE. In this study, relationships between $\Delta^{13}C$ and dry matter yield seemed to be depending on plant genotype and plant organ (data not shown). In A. Abiad genotype, relationship between $\Delta^{13}C$ and dry matter yield was positive in leaves and spikes and negative in roots. On the other hand, only leaves of the Pk-30-136 genotype showed positive relationship between between $\Delta^{13}C$ and dry matter yield. However, no such relations were found in WI-2291. For the whole plant, weak positive correlations were found in A. Abiad and Pk-30-136.

The higher spike yields (Table 1) observed in A. Abiad as compared with the other two genotypes was associated with a high $\Delta^{13}C$ (Table 4) and a high carbon yield (data not shown). This observation may indicate that A. Abiad could be a drought tolerant genotype. In this context, Craufurd *et al.* (1991) reported that earlier barley cultivars may have fixed a large proportion of their grain carbon early in the season and therefore giving a high C_i/C_a and $\Delta^{13}C$. These authors suggested that cultivars with high $\Delta^{13}C$ and high grain yield (earlier cultivars) have an advantage under terminal water stress relative to those having low $\Delta^{13}C$ (later cultivars). On the other hand, significant differences in stomatal conductance were observed in leaves of the genotypes grown under non-stress conditions (control). A. Abiad showed a higher leaf stomatal conductance (i.e favoring stomatal opening) and greater spike yield comparing with Pk-30-136 and WI-2291 genotypes implying that a maximization of yield may occurs via a maximization of stomatal opening (Isla *et al.,* 1998).

Using the equation of Farquhar *et al.* (1982), we calculated the average C_i/C_a in the leaves of the studied genotypes among different salinity treatment (Fig.5). The reduction in $\Delta^{13}C$ as salinity increased from 1 to 12 dS/m corresponded to a decrease in C_i/C_a from 0.7 (control) to 0.56, 0.53

and 0.52 in A. Abiad, WI-2291 and Pk-30-136, respectively. A lower C_i/C_a ratio could result either from stomatal closure induced by salt stress or from higher rates of photosynthetic capacity or a combination of both (Condon *et al.,* 2002). However, considering the lower leaf dry matter yield in the EC4 treatment, it was unlikely that a higher photosynthetic capacity occurred at this salinity treatment. Thus, the decrease in C_i/C_a ratio because of salinity (12 dS/m) was mainly due to the stomatal closure, i. e. decline in stomatal conductance. The average stomatal conductance values of the three barley genotypes measured in the high salinity treatment was 62% lower that measured in the control. Similar results were obtained with barley by Isla *et al.* (1998) and Shen *et al.* (1994). Moreover, since the reduction in dry matter yield in all the genotypes grown at 12 dS/m salinity level did not exceed 50% in comparison with the control, the photosynthetic apparatus of all studied genotypes seemed to be quit tolerant to salinity. Thus, the observed decline in C_i/C_a ratio could mainly be attributed to the decreases in stomatal conductance.

At moderate salinity levels, particularly at 8dS/m, the enhancement of leaf dry matter yield in the WI-2291 genotype reflects a higher photosynthetic capacity. Consequently, the lower C_i/C_a ratio in this genotype could result mainly from higher rates of photosynthetic capacity rather than stomatal closure. Likewise, the rate of stomatal conductance reduction because of salinity was less pronounced in WI2291 comparing to other genotypes (Fig.3). The average stomatal conductance value of this barley genotype measured at 8dS/m salinity level was only 13% lower that measured in the control. Whereas, the corresponding value observed in A. Abiad and Pk-30-136 was about 50%.

Carbon isotope discrimination ($\Delta^{13}C$) in C3 plants has been shown to be negatively correlated with water use efficiency (WUE). Low $\Delta^{13}C$ has been proposed as an indicator of high WUE (Farquhar and Richards, 1984). In this study, irrigation of WI-2291 genotype with saline water having EC_w of 8dS/m showed a decline in $\Delta^{13}C$ (high WUE) with a corresponding increase in dry matter yield. Shaheen and Hood-Nowotny (2005b) reported that the increase in dry mater yield and WUE in salt stressed wheat might have been due to positive nutritional effects of the salt. Yeo (1983) reported that a factor that may increase WUE as a result of salinity is that stomatal conductance may decline without a corresponding fall in assimilation capacity. In this study, WI-2291 genotype grown at mild salinity level (8dS/m) showed such benefits as indicated by a significant increase in the amount of total nitrogen uptake, i.e. from soil and fertilizer (Table 3).

Generally, $\Delta^{13}C$ values in leaves were higher than those in roots or spikes; whereas, they were somewhat parallel to those of the whole plants. This indicates differences in chemical composition among plant parts along with different patterns of carbon allocation between leaves and seeds (Hubick *et al.,* 1986; Knight *et al.,* 1995). Similarly, Shaheen and Hood-Nowotny (2005 a,b) reported higher $\Delta^{13}C$ in leaves of wheat and rice plants. Numerous studies have reported different $\Delta^{13}C$ values depending on the tissues used for analysis (Knight *et al.,* 1995). Declining contents of non-structural carbohydrates which have a less negative $\delta^{13}C$ than the remaining biomass (Brugnoli *et al.,* 1988) or raising levels of lipids with a more negative $\delta^{13}C$

(Robinson *et al.,* 1983) may account for higher $\Delta^{13}C$ (Shaheen and Hood-Nowotny, 2005b).

Carbon isotope discrimination is suggested to be a potential tool to identify genotypes for high water uses efficiency (WUE) and high nitrogen fertilizer use efficiency (%NUE) in salt affected soil (Arslan *et al.,* 1999). In the present study, an increase in salinity (1-12dS/m) resulted in a decrease in %NUE as well as in $\Delta^{13}C$ values of A. Abiad and Pk-30-136 genotypes. In contrast, an increase in salinity (1-8dS/m) resulted in an increase in NUE in the WI-2291 genotype. However, irrigation of this genotype with saline water having EC_w of 12 dS/m (EC4 treatment) drastically decreased NUE (Table 3). The highest NUE (61%) observed in WI-2291 at 8dS/m was associated with the lowest $\Delta^{13}C$ (16.19‰).

Arslan *et al.* (1999) reported a high negative correlation between $\Delta^{13}C$ and NUE of wheat genotypes grown in salt affected soil. In this study, however, NUE was positively correlated with $\Delta^{13}C$ in A. Abiad (r^2=0.76) and Pk-30-136 (r^2= 0.76) genotypes with no correlation being obtained in WI-2291. But, if EC4 data of the WI-2291 genotype were excluded, a negative correlation (r^2= -0.70) could be obtained between NUE and $\Delta^{13}C$. Therefore, it can be concluded that, associations between $\Delta^{13}C$ and NUE seemed to be depending on plant genotype and salinity level.

Isla *et al.* (1998) reported that carbon isotope discrimination is a useful indicator for yield potential in barley grown under non-saline conditions; whereas, grain yield remains only reliable means for identifying salt tolerance in barley. Overall, the present study revealed that, from a productivity standpoint, A. Abiad surpassed the other genotypes in maintaining higher spike yields under different treatments (1-8dS/m). The Pk-30-136, which is known as a salt tolerant genotype (Charbaji *et al.,* 2003) showed greater spike yield in saline treatments comparing with its control. The WI-2291 showed a relatively constant spike yield under different salinity levels. However, based on growth, nutritional parameters and ^{13}C discrimination, selection of barley genotypes for saline environments was affected by salinity level under which the plants have been grown. Under non-saline conditions, the performance of A. Abiad was higher than the other genotypes owing to its lower $\Delta^{13}C$, greater DM, NY, spike yield and NUE. At low (4ds/m) and high salinity (12ds/m) levels, this genotype also showed greater spike yield and NUE with a more $\Delta^{13}C$. However, under mild salt stress (8dS/m) WI-2291 seemed to be more salt tolerant than A. Abiad and Pk-30-136 evidenced as it exhibits by a smaller $\Delta^{13}C$ (i.e. higher WUE) with higher leaf gs, DM, N-uptake from soil and from fertilizer and greater NUE. In conclusion, the results of this study emphasize the importance of selecting barley genotypes for saline environments using growth, nutritional and ^{13}C discrimination criterions in a particular salinity level under which the plants have been grown.

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