RESEARCH PAPER

Effect of salinity and water stress during the reproductive stage on growth, ion concentrations, δ{}^{13}C, and δ{}^{15}N of durum wheat and related amphiploids

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Abstract

The physiological performance of durum wheat and two related amphiploids was studied during the reproductive stage under different combinations of salinity and irrigation. One triticale, one tritordeum, and four durum wheat genotypes were grown in pots in the absence of stress until heading, when six different treatments were imposed progressively. Treatments resulted from the combination of two irrigation regimes (100% and 35% of container water capacity) with three levels of water salinity (1.8, 12, and 17 dS m\(^{-1}\)), and were maintained for nearly 3 weeks. Gas exchange and chlorophyll fluorescence and content were measured prior to harvest; afterwards shoot biomass and height were recorded, and \(\Delta{}^{13}C\), \(\delta{}^{15}N\), and the concentration of nitrogen (N), phosphorus, and several ions (K\(^+\), Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\)) were analysed in shoot material. Compared with control conditions (full irrigation with Hoagland normal) all other treatments inhibited photosynthesis through stomatal closure, accelerated senescence, and decreased biomass. Full irrigation with 12 dS m\(^{-1}\) outperformed other stress treatments in terms of biomass production and physiological performance. Biomass correlated positively with N and \(\delta{}^{15}N\), and negatively with Na\(^+\) across genotypes and fully irrigated treatments, while relationships across deficit irrigation conditions were weaker or absent. \(\Delta{}^{13}C\) did not correlate with biomass across treatments, but it was the best trait correlating with phenotypic differences in biomass within treatments. Tritordeum produced more biomass than durum wheat in all treatments. Its low \(\Delta{}^{13}C\) and high K\(^+\)/Na\(^+\) ratio, together with a high potential growth, may underlie this finding. Mechanisms relating \(\delta{}^{15}N\) and \(\Delta{}^{13}C\) to biomass are discussed.

Key words: \(\Delta{}^{13}C\), \(\delta{}^{15}N\), durum wheat, leaf photosynthesis, potassium, salinity, sodium, triticale, tritordeum, water limitation.

Introduction

Water scarcity is the main factor limiting agricultural productivity in the Mediterranean region (Araus, 2004). This limitation is likely to increase in the future as climatic change is expected to decrease precipitation and increase evapotranspiration (World Bank, 2007; Lobell et al., 2008), and at the same time competition for water resources due to population growth and the development of economical sectors other than agriculture (e.g. industry or tertiary activities such as tourism) will also grow (Araus, 2004). Under such circumstances agriculture will be limited by reduced water supply and water of lower quality, particularly for crops with a water productivity (i.e. cash per unit water consumed) lower than that of horticultural or other intensive crops (Hsiao et al., 2007). Deficit irrigation,
defined as the application of water below full crop water requirements, is one of the alternatives to sustain productivity (Fereres and Soriano, 2007). For durum wheat under Mediterranean conditions, deficit irrigation may improve water use efficiency (biomass produced per unit water applied) as well as water productivity compared with purely rainfed or fully irrigated crops (Oweis et al., 1998; Hsiao et al., 2007).

Durum wheat is one of the most cultivated herbaceous crops in the south and east Mediterranean basin (www.fao.org/statistics/yearbook). These environments, where durum and other cereals are cultivated, are characterized by ‘terminal stress’ in the sense that drought develops during the last part of the crop cycle. As this stress occurs during the reproductive period, it may affect yield, either impeding grain set or further affecting grain filling (García del Moral et al., 2003). Under such circumstances, one possible way of increasing (or at least, stabilizing) productivity in semi-arid environments is to apply supplemental irrigation during the reproductive part of the crop cycle. As a drawback, the available water could be of low quality, which may compromise yield and critically expose soils to progressive salinization (World Bank, 2007). Therefore it would be advisable to use genotypes with improved salt tolerance (Munns et al., 2002; Munns, 2008). This is particularly important in durum wheat, since it is much more salt sensitive than other cereals such as barley or even bread wheat (Munns et al., 2002).

Morphophysiological traits for breeding of salt tolerance are extensively reviewed elsewhere (Munns and Tester, 2008, and references therein). They are based on the understanding that the mechanisms of salinity tolerance fall into any of the following categories: tolerance to osmotic stress; Na+ exclusion from leaves; or tolerance of tissue to accumulated Na+ (Munns and Tester, 2008). Moreover, salinity also affects N metabolism, reducing, for example, the levels and activity of nitrate reductase (Rao and Gnanam, 1990; Foyer et al., 1998; Carillo et al., 2005). In that regard, the stable nitrogen isotope signature (δ¹⁵N) of dry matter might be useful as a screening tool (Yousfi et al., 2009), even though the mechanisms underlying the genotypic and environmental relationships between δ¹⁵N and biomass are not fully elucidated.

Nevertheless, salt tolerance is a complex phenomenon where plant response depends on the phenological stage at which stress is experienced (Munns et al., 2006). In this context, the suitability of screening techniques may depend not only on the severity of the saline conditions, but also on the plant stage at which salinity is imposed (Leland et al., 1989). Moreover, the interaction between deficit irrigation and salinity may exacerbate the effect of salinity. Thus, while a toxicity-mediated effect may take time to develop (Munns, 2002), a premature senescence may also be produced if the drought effect is severe enough.

In a previous study (Yousfi et al., 2009), the genotypic performance was evaluated during the first part of the plant cycle under full irrigation with different saline conditions. It was shown that nitrogen isotope composition (δ¹⁵N) was better for tracking genotypic differences in salinity tolerance than carbon isotope discrimination (Δ¹³C) and other widely accepted traits such as the accumulation and ratios of ions such as Na+, K+, and Ca²⁺. However, for growth conditions such as those resembling supplemental (either fully or deficit) irrigation with brackish water during the later stages (i.e. anthesis and grain filling) of the crop cycle, the plant’s response to salinity and, therefore, the traits that best reflect its performance, may be different. In fact, there are differences in the specific stage at which the plant first encounters salinity, and the duration of the stress may also be variable.

In this work the response of durum wheat and related amphiploids to either deficit irrigation or salinity, or both in combination, that was imposed at anthesis was evaluated. The main objective was to determine the most informative physiological traits on genotype performance within, as well as across, growing conditions. To this end, two outstanding recombinant inbred lines (RILs), along with their common parents, of a population tested in an earlier study under continuous salinity during the vegetative stage were evaluated (Yousfi et al., 2009). These two RILs were among the most salt tolerant in the entire population, while they exhibited a high growth in the absence of stress. One genotype each of the amphiploids triticale and tritordeum was also included. These two cereals were obtained after interspecific hybridization, having durum wheat as one of the parents, and have been reported to be comparatively better adapted to drought and salinity conditions than durum wheat (Gallardo and Fereres, 1989; Giunta et al., 1993; Martín et al., 1999, 2000; Villegas et al., 2010), and therefore may represent a genetic bridge for the introgression of useful stress adaptation traits into wheat. Salinity tolerance was defined as genotypic differences in ‘absolute’ shoot biomass after growing plants under several saline conditions. Traits evaluated were the same as in Yousfi et al. (2009). It is postulated that natural abundance signatures of ¹³C and ¹⁵N measured in plant dry matter may be better at tracking genotypic performance within and across treatments than more conventional parameters such as ion concentration (Yousfi et al., 2009).

Materials and methods

Plant material and growth conditions

The different species used in this study were durum wheat, triticale, and tritordeum. Four durum wheat [Triticum turgidum L. ssp. durum (Desf.) Husn.] genotypes were tested: two RILs (here termed as RIL47 and RIL85), obtained by single seed descent from the cross between Jennah Khetifa (also termed ‘Lahn’) and Cham 1 (hereafter Cham), and the two parents. This cross was performed in 1991 at the Tel Hadya research station (Aleppo province, Syria) by the CIMMYT/ICARDA durum breeding programme for Mediterranean dryland (Nachit et al., 2001). These two RILs are among the best lines selected from a set of 112 belonging to the Jennah Khetifa×Cham population evaluated in a previous study (Yousfi et al., 2009) for tolerance to different levels of continuous salinity during the vegetative stage (comprising from shortly after planting to booting). Jennah
Khetifa is a landrace that shows specific adaptation to the North African continental drylands, being tall and moderately resistant to drought and cold. Cham is a variety that has been released for commercial production in several countries of the Mediterranean basin. It exhibits broad adaptation and has both high yield potential and yield stability. A hexaploid tritordeum (× Tritordeum Asch & Graeb) was also included, which is a fertile amphiploid derived from crosses between Hordeum chilense Roem. et Schult. and durum wheat. The genome of H. chilense confers tritordeum with a certain degree of drought and salt tolerance (Martín et al., 1999, 2000). The line tested was HT621 (reg. no. GP-7, PI 636334) developed and released in 2001 by the Institute for Sustainable Agriculture (CSIC), Córdoba, Spain. Although HT621 is not suitable for commercial cultivation due to its brittle rachis, hard glumes, excessive height, and therefore a low harvest index, it is very adapted to Mediterranean environments (Ballesteros et al., 2005). Triticale (× Triticosecale Wittm.) is an allopolyploid obtained from combining the chromosomes of wheat (Triticum spp.) and rye (Secale cereale L.). The variety tested was Imperioso, a hexaploid triticate having durum wheat as a parental line, registered in 2006 by Agrovetejal, S.A., Seville, Spain, and characterized by high and stable productivity and good grain quality.

Plants were grown in a greenhouse at the Experimental Fields of the University of Barcelona, Spain. Plants were planted in a mixture of peat, perlite, and vermiculite (2:1:1). The average temperature during the experiment was 26/18 °C day/night. Relative humidity ranged from 50% to 68% and the maximum photosynthetic photon flux density (PPFD) was ~1000 μmol m⁻² s⁻¹. Two seeds were planted in 3 dm² pots and watered to field capacity to facilitate germination. After a week, only one plant was left per pot.

The combinations of two water regimes and three levels of salinity were tested, accounting for a total of six different treatments. Water regimes corresponded to full irrigation (FI) (100% of container capacity) and deficit irrigation (DI) (35% of container capacity), respectively. The three salinity levels were 1.8 dS m⁻¹ (which corresponds to half-strength normal Hoagland solution; Hoagland and Arnon, 1950), 12 dS m⁻¹, and 17 dS m⁻¹. A completely randomized design was used to accommodate the three-way factorial experiment, with genotype, water level, and salinity stress as factors. Three single-pot replicates per factorial combination were used, totalling 108 pots. All plants were grown in the absence of water stress and supplied with a half-concentrated Hoagland solution until heading. The DI regime was imposed progressively over 1 week by decreasing irrigation, and then the two salinity treatments were imposed by adding NaCl progressively to the nutrient solution, starting with a salt concentration of 4 dS m⁻¹. This concentration was increased progressively during 1 week to reach the final salt levels of 12 dS m⁻¹ (~120 mM NaCl) or 17 dS m⁻¹ (~170 mM NaCl). A total of six treatments were studied: (i) FI, full irrigation (i.e. control) with normal Hoagland solution; (ii) FI-12 dS m⁻¹, full irrigation with Hoagland solution at 12 dS m⁻¹; (iii) FI-17 dS m⁻¹, full irrigation with Hoagland solution at 17 dS m⁻¹; (iv) DI, deficit irrigation with normal Hoagland solution; (v) DI-12 dS m⁻¹, deficit irrigation with Hoagland solution at 12 dS m⁻¹; and (vi) DI-17 dS m⁻¹, deficit irrigation with Hoagland solution at 17 dS m⁻¹. The different treatments were fully established at anthesis and then the plants were grown for ~3 weeks when they were harvested. Plants were grown for a total of 4 months. Except for triticale, which was 2–3 d earlier, all other genotypes reached anthesis simultaneously.

Gas exchange measurements

Leaf gas exchange was measured at the end of treatments. Measurements were made with an open IRGA LI-COR 6400 system (LICOR Inc., Lincoln, NE, USA). For each treatment and genotype, measurements were carried out in three randomly chosen, fully expanded flag leaf blades, each one from a different pot, at 10–15 h (solar time) under saturated PPFD conditions (>1200 μmol m⁻² s⁻¹), at a temperature of 25 °C, and chamber CO₂ concentration of 400 μmol mol⁻¹. The gas exchange parameters were light-saturated net CO₂ assimilation (A_sat), transpiration rate (T), and stomatal conductance (g_s). Subsequently, the ratio of intercellular to ambient CO₂ concentration (C_i/C_a) was calculated according to Sharkey and Raschke (1981). The efficiency of excitation energy captured by open PSII reaction centres (F_v/F_m) was also estimated in the same leaves.

Plant growth and leaf chlorophyll content

Chlorophyll content was measured in the same flag leaves monitored for gas exchange. Four measurements were performed from the middle of the leaf blade just before harvesting using a portable meter (Minolta SPAD 502 Meter). The height of the main shoot of each plant was measured with a ruler prior to harvest, with a precision of ~1 mm. After harvesting, shoots were oven dried at 70 °C for 48 h, weighed, and finely ground for subsequent analyses.

Ion analysis

For each shoot sample analysed, 100 mg of dry material was digested with 3 ml of concentrated HNO₃ and 2 ml of H₂O₂. The samples were placed overnight in a microwave at 90 °C. After digestion, each sample was then brought up to 30 ml final volume with pure water. The amount of Na⁺, Ca²⁺, K⁺, P, and Mg²⁺ in the sample was then determined with an Inductively Coupled Plasma Emission Spectrometer (L3200RL, Perkin Elmer, Germany) at the Scientific Facilities of the University of Barcelona. Ion concentrations were expressed as mmol per g of dry weight.

Total nitrogen concentration and stable carbon and nitrogen isotope signatures

Total nitrogen concentration and the stable carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N) isotope ratios in the shoots were measured using an elemental analyser (Flash 1112 EA; ThermoFinnigan, Germany) coupled with an isotope ratio mass spectrometer (Delta C IRMS, ThermoFinnigan, Germany), operating in continuous flow mode. Samples of ~1 mg and reference materials were weighed into tin capsules, sealed, and then loaded into an automatic sampler (ThermoFinnigan, Germany) prior to EA-IRMS analysis. Measurements were carried out at the Scientific Facilities of the University of Barcelona.

Nitrogen was expressed as either concentration (mmol per g of dry weight) or total content (g) of the aerial part (shoot nitrogen). The ¹³C/¹²C ratios were expressed in δ notation (Coplen, 2008):

\[ \delta^{13}C = \frac{^{13}C_{\text{sample}} / ^{12}C_{\text{standard}} - 1}{^{13}C_{\text{standard}}} \times 1000 \]  

where ‘sample’ refers to plant material and ‘standard’ to Pee Dee Belemnite (PDB) calcium carbonate. The same δ notation was used for the ¹⁵N/¹⁴N ratio (δ¹⁵N), but in this case the standard referred to N₂ in air. Atropine was used as a system check in the elemental analyses of nitrogen. International isotope secondary standards of known ¹³C/¹²C ratios (IAEA CH7 polyethylene foil, IAEA CH6 sucrose, and USGS 40 l-glutamic acid) were used for calibration to a precision of 0.1%. For nitrogen, isotope secondary standards of known ¹⁵N/¹⁴N ratios (IAEA N₁ and IAEA N₂ ammonium sulphate and IAEA NO₃ potassium nitrate) were used. The mean δ¹⁵N of the fertilizer provided by the Hoagland solution was 0.6 %o.

The carbon isotope discrimination (Δ¹³C) of shoots was calculated as:
where the subscripts a and p refer to air and the plant, respectively (Farquhar et al., 1989). Air samples were taken inside the greenhouse and analysed by the GC-C-IRMS technique, as previously described in Nogués et al. (2004). Air analyses were carried out at the Scientific Facilities of the University of Barcelona. The δ13C_p was −11.3‰.

Statistical analysis

Data for the set of morphophysiological traits were subjected to factorial analyses of variance (ANO瓦s) to test for the effects of treatment (irrigation, salinity), genotype, and their first- and second-order interactions. Means were compared by Duncan’s test (P < 0.05). The morphophysiological data set was then subjected to stepwise discriminant analyses to ascertain which traits best discriminated between genotypic or treatment groups. The two procedures made use of neither the ‘biomass’ variable (since it was used as a dependent variable in subsequent stepwise regression analyses) nor the ‘total shoot N’ variable (whose values were tightly related to those of ‘biomass’). The ‘control’ (i.e. full irrigation or salinity effects. The significance level corresponding in the discriminate analysis of treatment groups so as to highlight tightly related to those of ‘biomass’). The ‘control’ (i.e. full irrigation or salinity effects. The significance level corresponding in the discriminate analysis of treatment groups so as to highlight tightly related to those of ‘biomass’).

Data were analysed using SPSS (SPSS Inc., Chicago, IL, USA) and SAS (SAS Institute Inc., Cary, NC, USA) statistical packages.

Results

The effect of treatments and genotypes on growth parameters

Growing conditions other than control (FI) significantly decreased aerial biomass, plant height, leaf chlorophyll, nitrogen concentration, and total shoot nitrogen content (Table 1). The combination of deficit irrigation and salinity most affected all growth traits under study. Thus FI-12 dS m⁻¹ limited growth less than DI, while no significant differences existed between the latter treatment and FI-17 dS m⁻¹. Genotypes significantly differed for all traits, whereas significant interaction between genotype and growing conditions (G×T) only existed for chlorophyll and N concentration (Table 1). Overall, Cham was the genotype with the lowest biomass and shoot N concentration across the four most stressful treatments (FI-17 dS m⁻¹, DI, DI-12 dS m⁻¹, and DI-17 dS m⁻¹), while tritordeum and triticale showed the highest values at DI and DI-12 dS m⁻¹(Supplementary Table S1 available at JXB online). Tritordeum and triticale also showed the highest biomass in the absence of stress (FI). The relative decrease (i.e. compared with FI) in biomass for each of the six genotypes at any of the five stress treatments was also studied. Overall, RIL47 was the genotype least affected (i.e. showed the least reduction in biomass) by the various treatments, followed by RIL85. In contrast, Cham and triticale were usually the most affected genotypes (except for DI-12 dS m⁻¹), while tritordeum and Lahn showed intermediate responses (Fig. 1).

The effect of irrigation, salinity, and genotype on photosynthesis and stable isotope signatures

Compared with control, all the other treatments strongly decreased A_sat, g_s, C_i/C_a, T, F_v'/F_m’, Δ13C, and δ15N. FI-17 dS m⁻¹ and DI-17 dS m⁻¹ were the treatments that most affected these traits, and DI was the treatment with the least effect (Table 2). There were no differences for A_sat among treatments other than control, even though g_s and T were slightly, but significantly, higher at FI-12 dS m⁻¹ as well as for DI. Treatment effect was higher than genotypic effect for δ15N, while the opposite occurred for Δ13C. Cham showed the highest Δ13C value and the lowest records for other traits, apart from g_s. In turn, titordeum, followed by triticale, showed the lowest Δ13C values. Transpiration, g_s, δ15N, and Δ13C were the only traits not showing a significant G×T interaction.

The effect of irrigation, salinity, and genotype on ion concentration

Treatments significantly affected the concentration of ions in the shoots. Thus, all treatments with saline water strongly increased Na⁺, and slightly decreased K⁺, Ca²⁺, Mg²⁺, and P, as compared with control conditions. Therefore, the ratios K⁺/Na⁺ and Ca²⁺/Na⁺ decreased markedly. DI was the treatment that affected ion concentration the least. There were also genotypic differences for all traits, with triticale exhibiting the highest K⁺/Na⁺ and Ca²⁺/Na⁺ ratios (Table 3). Except for Ca²⁺, all ions and their selected ratios showed significant genotype by treatment interactions. Thus, for example, tritordeum and triticale showed the highest K⁺/Na⁺ ratios under FI, FI-12 dS m⁻¹, and DI, but this was not the case for the other treatments (Supplementary Table S1 at JXB online).

Overall differences across treatments

The stepwise discriminant analysis indicated that 10 traits contributed the most to the differentiation among treatments. These were (ranked by order of inclusion in the model): Na⁺, K⁺/Na⁺, leaf chlorophyll (SPAD), K⁺, δ15N, F_v'/F_m’, Ca²⁺, T, N concentration, and plant height. Hotelling’s T²-statistic testing for between-treatment differences was significant for all pairwise comparisons. This was also suggested by the outcome of a canonical discriminant
analysis (Fig. 2), with most of the between-treatments to
within-treatments variability explained by the first two
canonical axes (CAN1 and CAN2). The discriminant
loadings for each variable (or simple correlations of the
variable and the discriminant scores for each axis), imposed
on the plot representation as attribute points, provided
a more complete interpretation of the analysis. Following
the direction denoted by these points, CAN1 was mainly
concerned with Na+, hence separating FI-17 dS m
\(-1\)/C0
1 from the other, lower Na+, treatments, which
tended to exhibit higher Ca2+,K+/Na+, T,
Fv/
Fm
, and
d
15N values and correspond to the three deficit irrigation
treatments. In turn, CAN2 corresponded closely to Ca2+, K+, N concentration, and leaf chlorophyll, with FI-
12 dS m
\(-1\)/C0
1 as the only group having higher values of these
attributes.

Relationships between biomass and physiological traits
across treatments

Relationships between biomass and physiological variables
were assessed across full irrigation (i.e. combining FI, FI-12
dS m
\(-1\), and FI-17 dS m
\(-1\)) and deficit irrigation (i.e. DI,
DI-12 dS m
\(-1\), and DI-17 dS m
\(-1\)) treatments, indepen-
dently. Overall, biomass was negatively related to Na+
concentration and positively related to the ratio K+/Na+.
However, relationships were not linear and they followed

Table 1. Effect of different levels of salinity, water stress, and the combination of the two stresses on the shoot biomass and nitrogen (N) concentration, the total shoot nitrogen content, plant height, and chlorophyll content of the flag leaf of durum wheat (Cham, Lahn, RIL47, and RIL85), triticale (Imperioso), and tritordeum (HT621)

| Genotype       | Biomass (g) | Plant height (cm) | Leaf chlorophyll (SPAD units) | Shoot N concentration (mmol g
\(-1\) DW) | Total shoot N (g) |
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<tbody>
<tr>
<td>Cham</td>
<td>51.08 a</td>
<td>66.36 ab</td>
<td>20.74 b</td>
<td>3.22 a</td>
<td>1.42 a</td>
</tr>
<tr>
<td>Lahn</td>
<td>64.88 b</td>
<td>66.65 bc</td>
<td>23.37 bc</td>
<td>2.57 b</td>
<td>1.88 abc</td>
</tr>
<tr>
<td>RIL47</td>
<td>63.39 b</td>
<td>74.94 c</td>
<td>28.07 d</td>
<td>2.72 b</td>
<td>1.84 abc</td>
</tr>
<tr>
<td>RIL85</td>
<td>62.27 ab</td>
<td>70.05 b</td>
<td>25.44 cd</td>
<td>2.20 a</td>
<td>1.55 ab</td>
</tr>
<tr>
<td>Triticale</td>
<td>68.56 b</td>
<td>81.50 d</td>
<td>21.49 b</td>
<td>2.65 b</td>
<td>2.07 c</td>
</tr>
<tr>
<td>Tritordeum</td>
<td>67.50 b</td>
<td>64.52 a</td>
<td>15.86 a</td>
<td>1.86 a</td>
<td>2.00 bc</td>
</tr>
</tbody>
</table>

| Treatment      | Biomass (g) | Plant height (cm) | Leaf chlorophyll (SPAD units) | Shoot N concentration (mmol g
\(-1\) DW) | Total shoot N (g) |
<table>
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<tbody>
<tr>
<td>FI</td>
<td>121.81 d</td>
<td>78.65 c</td>
<td>48.90 d</td>
<td>3.94 e</td>
<td>4.75 d</td>
</tr>
</tbody>
</table>
| FI-12 dS m
\(-1\) | 73.00 c     | 73.89 bc          | 31.12 c                      | 2.76 d                           | 2.00 c          |
| FI-17 dS m
\(-1\) | 60.06 b     | 70.07 ab          | 15.67 b                      | 2.11 b                           | 1.29 b          |
| DI             | 39.17 a     | 68.76 a           | 14.45 b                      | 2.41 c                           | 0.96 a b        |
| DI-12 dS m
\(-1\) | 29.18 a     | 65.06 a           | 10.12 a                      | 1.79 a                           | 0.55 a          |

ANOVA

<table>
<thead>
<tr>
<th>G</th>
<th>3693.27**</th>
<th>3506.95***</th>
<th>924.81***</th>
<th>6.40***</th>
<th>6.93***</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>73367.21***</td>
<td>1916.70***</td>
<td>15364.23***</td>
<td>42.60***</td>
<td>161.98***</td>
</tr>
<tr>
<td>G×T</td>
<td>10805.36 ns</td>
<td>1335.46 ns</td>
<td>3036.73***</td>
<td>6.54***</td>
<td>12.33 ns</td>
</tr>
</tbody>
</table>

FI, full irrigation (i.e. control) with normal Hoagland solution; FI-12 dS m
\(-1\), full irrigation with Hoagland solution at 12 dSm
\(-1\); FI-17 dS m
\(-1\), full irrigation with Hoagland solution at 17 dS m
\(-1\); DI, deficit irrigation (35% pot capacity) with normal Hoagland solution; DI-12 dS m
\(-1\), deficit irrigation with Hoagland solution at 12 dS m
\(-1\); DI-17 dS m
\(-1\), deficit irrigation with Hoagland solution at 17 dS m
\(-1\); G, genotype; T, treatment; G×T: genotype by treatment interaction.

Fig. 1. Relative decrease in shoot biomass of the six different
genotypes under the different growing conditions compared with
control conditions. For each genotype and growing condition
values are expressed as a percentage of the average of the same
 genotype under full irrigation with Hoagland solution (FI). FI-S1, full
irrigation with Hoagland solution at 12 dS m
\(-1\); FI-S2, full irrigation
with Hoagland solution at 17 dS m
\(-1\); DI, deficit irrigation (35% pot capacity) with normal Hoagland solution; DI-S1, deficit irrigation
with Hoagland solution at 12 dS m
\(-1\); DI-S2, deficit irrigation
with Hoagland solution at 17 dS m
\(-1\).
Table 2. Effect of different levels of salinity, water stress, and the combination of the two stresses on leaf net CO2 assimilation (A\text{sat}), stomatal conductance (gs), the ratio of intercellular to ambient CO2 concentration (C1/Ca), the transpiration rate (T), efficiency of excitation energy capture by open PSII reaction centres (Fv/Fm\text{r}), stable carbon isotope discrimination (\Delta^{13}\text{C}), and stable nitrogen isotope composition (\delta^{15}\text{N}) of durum wheat (Cham, Lahn, RIL47, and RIL85), triticale (Imperioso), and tritordeum (HT621).

Gas exchange measurements were performed in flag leaf blades and stable isotopes analysed in shoots sampled 3 weeks after anthesis. Abbreviations for treatments and ANOVA analysis are as defined in the footnotes of Table 1. Means followed by different letters were significantly different (P < 0.05) by Duncan’s test. The associated sum of squares and probabilities (ns, not significant; **P < 0.01; ***P < 0.001) are shown.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A\text{sat} (\mu\text{mol CO2 m}^{-2} \text{s}^{-1})</th>
<th>gs (\text{mol CO2 m}^{-2} \text{s}^{-1})</th>
<th>C1/Ca</th>
<th>Fv/Fm\text{r}</th>
<th>T (\text{mmol H2O m}^{-2} \text{s}^{-1})</th>
<th>\Delta^{13}\text{C} (%)</th>
<th>\delta^{15}\text{N} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cham</td>
<td>4.62 a</td>
<td>0.07 ab</td>
<td>0.29 a</td>
<td>0.18 a</td>
<td>0.98 a</td>
<td>21.12 c</td>
<td>3.41 a</td>
</tr>
<tr>
<td>Lahn</td>
<td>5.70 bc</td>
<td>0.09 ab</td>
<td>0.32 a</td>
<td>0.30 bc</td>
<td>1.66 bc</td>
<td>20.93 bc</td>
<td>5.03 cd</td>
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<tr>
<td>RIL47</td>
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<td>0.06 ab</td>
<td>0.37 ab</td>
<td>0.25 ab</td>
<td>1.47 ab</td>
<td>21.47 d</td>
<td>5.96 e</td>
</tr>
<tr>
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<td>5.11 ab</td>
<td>0.05 a</td>
<td>0.34 a</td>
<td>0.22 ab</td>
<td>1.10 ab</td>
<td>20.90 bc</td>
<td>5.37 de</td>
</tr>
<tr>
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<td>0.10 b</td>
<td>0.51 c</td>
<td>0.39 d</td>
<td>2.19 c</td>
<td>20.84 b</td>
<td>4.44 bc</td>
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<tr>
<td>Triticum</td>
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<td>0.06 ab</td>
<td>0.46 bc</td>
<td>0.36 cd</td>
<td>1.20 ab</td>
<td>20.34 a</td>
<td>4.13 ab</td>
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<table>
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<th>Treatment</th>
<th>A\text{sat} (\mu\text{mol CO2 m}^{-2} \text{s}^{-1})</th>
<th>gs (\text{mol CO2 m}^{-2} \text{s}^{-1})</th>
<th>C1/Ca</th>
<th>Fv/Fm\text{r}</th>
<th>T (\text{mmol H2O m}^{-2} \text{s}^{-1})</th>
<th>\Delta^{13}\text{C} (%)</th>
<th>\delta^{15}\text{N} (%)</th>
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<td>0.80 d</td>
<td>0.66 d</td>
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<td>21.30 c</td>
<td>7.27 c</td>
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<td>0.02 a</td>
<td>0.33 b</td>
<td>0.22 b</td>
<td>0.75 bc</td>
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<td>FI-17 dS m^{-1}</td>
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<td>0.02 a</td>
<td>0.08 a</td>
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<td>0.04 a</td>
<td>0.61 c</td>
<td>0.38 c</td>
<td>1.38 c</td>
<td>21.12 bc</td>
<td>5.61 b</td>
</tr>
<tr>
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<td>2.72 a</td>
<td>0.02 a</td>
<td>0.20 a</td>
<td>0.36 bc</td>
<td>0.51 ab</td>
<td>21.06 bc</td>
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<tr>
<td>DI-17 dS m^{-1}</td>
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<td>0.02 a</td>
<td>0.20 a</td>
<td>0.09 a</td>
<td>0.38 ab</td>
<td>20.75 a</td>
<td>3.59 a</td>
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| ANOVA | G 53.43** | 0.02 ns | 0.68*** | 0.66*** | 18.95*** | 9.61*** | 70.70*** |
|       | T 4441.68*** | 1.46*** | 5.42*** | 4.45*** | 368.02*** | 3.4*** | 125.51*** |
| G x T | 145.82** | 0.07 ns | 1.18*** | 1.33*** | 21.45 ns | 5.38 ns | 42.94 ns |

Overall genotypic differences

The stepwise discriminant analysis indicated that nine attributes contributed the most to the differentiation among genotypes. These were (ranked by order of inclusion in the model): \Delta^{13}\text{C}, plant height, P, K\text{}/Na\text{+}, \delta^{15}\text{N}, C1/Ca, Ca\text{2+}, Mg\text{2+}, and N concentration. However, Hotelling’s T$^2$-statistic testing for between-genotypes differences was in some cases not significant. In particular, Lahn was not statistically distinguishable from RIL85 (P=0.25), nor was RIL47 from RIL85 (P=0.12). This was also suggested by the outcome of a canonical discriminant analysis (Fig. 6), with most of the between-genotypes to within-genotypes variability explained by the first two canonical axes (CAN1 and CAN2). The discriminant loadings for each variable, imposed on the plot as attribute points, provided a better interpretation of the analysis. The first dimension correlated closely (and negatively) with \Delta^{13}\text{C} and P, and separated the two amphiploids (tritordeum and triticale) from the durum wheat genotypes satisfactorily. Differences between durum wheat genotypes were smaller. Even so, Cham was placed away from the other three durum wheat genotypes. On the other hand, Cham and also triticale displayed a negative value for this dimension, while Lahn occupied an intermediate position in the figure.

Both tritordeum and triticale exhibited the highest biomass and the lowest \Delta^{13}\text{C}, \delta^{15}\text{N}, and Na\text{+} content, and the highest K\text{+}/Na\text{+} ratio under FI and DI (Supplementary Table S1 at JXB online). Triticum also exhibited the highest biomass at FI-12 dS m^{-1} and FI-17 dS m^{-1}, followed by Lahn and the two RILs, respectively. Under FI-12 dS m^{-1} a high biomass was again accompanied by low \Delta^{13}\text{C} and Na\text{+}, together with high K\text{+}/Na\text{+} and C1/Ca ratios. Triticale and tritordeum also showed the highest biomass at DI-12 dS m^{-1} and DI-17 dS m^{-1}, respectively, while Lahn ranked second in both cases. Triticale also showed the highest C1/Ca and A\text{sat} at DI-12 dS m^{-1}, together with the lowest Na\text{+} and the highest K\text{+}/Na\text{+} ratio. In turn, tritordeum showed the highest N concentration and A\text{sat} at DI-17 dS m^{-1}, together with the lowest \Delta^{13}\text{C}. Cham was the genotype with the least growth in most treatments (Supplementary Table S1 at JXB online). On the other hand, Cham and triticale were the genotypes exhibiting the
The highest relative decrease in biomass at both fully and deficit irrigation conditions as a response to Na+ accumulated in the plant (Supplementary Fig. S2 at JXB online).

Relationships between biomass and physiological traits across genotypes

Stepwise regressions were performed for each growing condition using biomass as the dependent variable and combining all individual measurements for the six genotypes (Table 4). Except for the most severe treatment (DI-17 dS m⁻¹/C₀), the first trait selected was related to plant photosynthetic performance; either $g_s$ (FI), $D_{13C}$ (FI-12 dS m⁻¹/C₀ and FI-17 dS m⁻¹/C₀), or $C_i/C_a$ (DI and DI-12 dS m⁻¹/C₀). Moreover, $D_{13C}$ ranked second in the selection process under DI and DI-12 dS m⁻¹/C₀, and $C_i/C_a$ also ranked second at DI-17 dS m⁻¹/C₀ (Table 4). $D_{13C}$ and biomass were negatively correlated within each treatment except for FI and DI-17 dS m⁻¹/C₀ (Fig. 7A, B). $C_i/C_a$ had a positive influence on biomass under DI and DI-12 dS m⁻¹/C₀, while it had a negative influence under DI-17 dS m⁻¹/C₀, the most severe treatment. A positive influence of $K^+/Na^+$ on biomass was only detected under FI-17 dS m⁻¹/C₀. A high N content was chosen as the first trait at DI-17 dS m⁻¹/C₀.

Discussion

Growing conditions and genotypes both differed significantly for biomass and plant height; traits that can be considered useful for screening durum wheat germplasm...
under salinity and water stress (Islam and Sedgley, 1981; Nicolas et al., 1993; Munns and James, 2003). Compared with control conditions (FI), all treatments decreased plant height and shoot biomass, but plant height was far less affected than biomass because salinity and water stress were applied during the reproductive stage (i.e. late in the crop cycle). Thus, while biomass at DI-17 dS m\(^{-1}\) was reduced by ~75% relative to the control, plant height was reduced by just 17%. Even shoot biomass decreased by 40% under FI-12 dS m\(^{-1}\), which is in line with the ~50% decrease reported in previous studies using similar levels of salinity (Ayers and Westcot, 1989; Yousfi et al., 2009), but represented a reduction in height of only 6%.

FI-12 dS m\(^{-1}\), after the control (FI), was the second best treatment in terms of biomass, while the most stressful treatments resulted from a combination of deficit irrigation with saline water (DI-12 dS m\(^{-1}\) and DI-17 dS m\(^{-1}\)). A major difference between the full irrigation treatments with saline solution versus deficit irrigation (even if with no saline solution) is the total amount of water available. Under fully irrigated treatments, a large (essentially unlimited) amount of water at a constant, low water potential is available. Durum wheat and other cereals may adjust osmotically under saline conditions through the incorporation of available ions such as Na\(^+\), which allows plants to access water in the substrate for growth (Munns, 2002; Cuin et al., 2009). Thus in the present study, Na\(^+\) concentration in shoots increased as the amount of salt provided in the growing medium increased, reaching the highest value at FI-17 dS m\(^{-1}\). These Na\(^+\) concentrations at least doubled those reported in previous studies with durum and bread wheat exposed to saline conditions during the vegetative stage (Husain et al., 2004; Yousfi et al., 2009). In the current work, salinity treatments were imposed on plants with fully developed leaves, so no dilution effect was produced as new leaves appeared.

On the other hand, the two most stressful treatments in terms of biomass (DI-12 dS m\(^{-1}\) and DI-17 dS m\(^{-1}\)) induced very low Na\(^+\) concentration (0.34–0.45 mmol g\(^{-1}\) DW) and K\(^+\)/Na\(^+\) ratios far higher (between 3 and 4) than those reported by Yousfi et al. (2009) under FI-12 dS m\(^{-1}\) and FI-17 dS m\(^{-1}\). Only FI-17 dS m\(^{-1}\) produced a K\(^+\)/Na\(^+\) ratio <0.5, comparable with those reported previously for vegetative durum wheat at FI-12 dS m\(^{-1}\) (Yousfi et al., 2009).

Plant growth responds to salinity in two phases: a rapid, osmotic phase that parallels that of drought stress; and a slower, ionic phase that accelerates the senescence of mature leaves (Munns and Tester, 2008). In spite of higher Na\(^+\) accumulation and lower K\(^+\)/Na\(^+\) ratios, full irrigation with saline solutions delayed senescence compared with
deficit irrigation with similar saline solutions; this is supported by a higher N concentration and SPAD values in the former treatments. Moreover, salinity is reported to have a strong effect of accumulating amino acids (mostly proline and glycine betaine) in durum wheat leaves, which may act as protective compounds (Carillo et al., 2008).

Effect of treatments on gas exchange and $\Delta^{13}C$

Compared with control, all the other treatments induced a decrease in photosynthetic rates. Besides an accelerated senescence, the drop in photosynthesis also seems to be due to stomatal limitation, as concluded from the drop in the $C_i/C_a$ ratio. This is in agreement with previous reports on durum and bread wheat (Ouerghi et al., 2000; Zheng et al., 2008; Yousfi et al., 2009), triticale (Morant-Manceau et al., 2004), and other species (Isla et al., 1998; Rasmuson and Anderson, 2002; Shaheen and Hood-Nowotny, 2005). Flexas et al. (2004) conclude that salt and drought stress predominantly affect diffusion of CO$_2$ in the leaves through a decrease of stomatal and mesophyll conductances, but not the biochemical capacity to assimilate CO$_2$.

The $C_i/C_a$ measured at the end of the treatment showed a positive relationship with biomass (Supplementary Fig. S1B at JXB online). However, a time-integrative estimate of $C_i/C_a$ such as $\Delta^{13}C$ did not correlate with biomass across treatments (Supplementary Fig. S1A at JXB online). In the present study, salinity decreased $\Delta^{13}C$ by just 0.03$\%$ and 0.02$\%$ per unit increase in electrical conductivity (EC) under full irrigation and deficit irrigation conditions, respectively. These values are about one order of magnitude lower than those reported for durum wheat leaves (Yousfi et al., 2009) and barley kernels (Isla et al., 1998) under long-term salinity conditions, and even 3–5 times smaller than those reported for wheat leaves grown in pots (Shaheen and Hood-Nowotny, 2005) and barley shoots under hydroponics (Handley et al., 1997) exposed to salinity for short periods. It is likely that in the present study, shoot $\Delta^{13}C$ reflects the plant carbon assimilation history as weighed by the impact of the pre- and reproductive phases (i.e. before and after imposing the treatment) on plant functioning.

Effect of treatments on $\delta^{15}N$

In the present study $\delta^{15}N$ decreased about 0.2$\%$ and 0.12$\%$ per unit increase in ECs under full irrigation and deficit
irrigation conditions, respectively. Yousfi et al. (2009) reported a nearly 0.2% decrease in shoot $^{15}$N per unit increase in ECs. Several studies on barley under hydroponics have reported rates of shoot $^{15}$N that decreased by nearly half (Ellis et al., 1997; Handley et al., 1997), probably due to the fact that barley is more tolerant to salinity than durum wheat, and salinity conditions were mild (Munns et al., 2002). Deficit irrigation also reduced $^{15}$N as compared with control conditions (Robinson et al., 2000; Raimanová and Haberle, 2010). However, in other studies with different species including cereals (Handley et al., 1999; Lopes et al., 2004; Lopes and Araus, 2006) water stress caused an increase in $^{15}$N, which suggests that drought affects plant $^{15}$N in a different way from salinity.

Isotope fractionation of nitrogen may occur during uptake from the medium into root cells, or during subsequent enzymatic assimilation into other $N$ forms. Further fractionation may also occur if biochemical components of varying isotopic composition are lost through translocation, exudation, or volatilization (Evans, 2001; Pritchard and Guy, 2005). Reduced stomatal conductance, due to either salinity or water stress, or a combination of both factors, should lead to a reduction in the loss of ammonia and nitrous oxide, decreasing $^{15}$N (Farquhar et al., 1980; Smart and Bloom, 2001). In fact, $^{13}$C and $^{15}$N were positively related across treatments, and the same pattern was observed considering either full or deficit irrigation (Supplementary Fig. S3A at JXB online). A positive relationship between $^{13}$C and $^{15}$N has also been reported for durum wheat seedlings across full irrigation treatments (Yousfi et al., 2009). Moreover, $^{15}$N correlated positively with $C/C_i$ (Supplementary Fig. S3B at JXB online), as well as with $g_s$ and transpiration ($r=0.61$ and $0.69$, $P<0.01$, respectively) across full irrigation treatments. $C/C_i$ may integrate leaf permeability together with the functional status of a major N pool (carboxylation enzyme), which would explain why $^{15}$N correlates with $C/C_i$ better than with $g_s$ or with transpiration.

Mechanisms other than N loss may also lead to salinity increasing the discrimination against $^{15}$N, such as a high external N concentration relative to a modest demand (Mariotti et al., 1982). Consequently, the suboptimal growing conditions associated with any stress may produce a decrease in demand relative to a constant external N concentration. This may have the same effect as increasing the external concentration (Mariotti et al., 1982), leading to greater isotopic discrimination (Vitousek et al., 1989; Handley et al., 1997). Thus the positive relationship found between $^{15}$N and biomass might be due to differences across treatments in $^{15}$N being associated with assimilation capacity and N demand (Robinson et al., 2000; Pritchard and Guy, 2005; Coque et al., 2006). In this regard, a positive relationship across growing conditions between $^{15}$N and biomass has already been reported in durum wheat (Yousfi et al., 2009). Alternatively, Handley et al. (1997) suggest that salt stress would make $^{15}$N less positive than in controls due to down-regulation of assimilating enzymes. Thus the N concentration decreases with increased salinity under full irrigation, and this would agree with a decrease in plant enzyme activity. In fact $^{15}$N/14N fractionation may occur during either nitrate assimilation by nitrate reductase or ammonium assimilation by glutamine synthetase (Evans, 2001). These two enzymes have apparently similar in vitro discrimination factors (Ledgard et al., 1985; Yoneyama et al., 1993), and their activity is reduced by salinity in wheat (Carillo et al., 2005). However, other works indicate that moderate salinity levels (100–150 mM NaCl) in wheat increase activities of glutamine synthetase while decreasing

### Table 4

Multiple linear regressions (stepwise) explaining biomass variation across genotypes in each growing condition as a dependent variable, and all the physiological parameters (plant height, ion concentrations and ratios, gas exchange traits, nitrogen concentration, and chlorophyll content and stable isotope signatures) measured (excluding total shoot N) in the same particular growing condition as independent variables.

Abbreviations for treatments and ANOVA analysis are as defined in the footnotes of Table 1. For the calculations all the individual measurements of the six different genotypes were used ($n=8$). *$P<0.05$; **$P<0.01$; ***$P<0.001$.

<table>
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<tr>
<th>Model treatments</th>
<th>Variable chosen</th>
<th>$R^2$</th>
<th>Final stepwise model</th>
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<td>Biomass FL</td>
<td>$g_s$</td>
<td>0.44***</td>
<td>199.7 $g_s$+53.7</td>
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<td>Biomass FL-12 dS m$^{-1}$</td>
<td>$^{13}$C</td>
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<td>$^{15}$C-2.6 $A_{sat}$+434.8</td>
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<td>$A_{sat}$</td>
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<td>Biomass FL-17 dS m$^{-1}$</td>
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<td>0.61***</td>
<td>$^{15}$C+77.5 $K^+/Na^+$+858.4</td>
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<td>Biomass DI</td>
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<td>27.6 $C/C_i$-10.1 $^{13}$C+261.8</td>
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<td>$^{13}$C</td>
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<td>Biomass DI-12 dS m$^{-1}$</td>
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<td>Biomass DI-17 dS m$^{-1}$</td>
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<td>11.45 N-16.4 $C/C_i$+11.96</td>
</tr>
<tr>
<td></td>
<td>$C/C_i$</td>
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</table>

The table show only the parameters significantly entering in the models. $g_s$, stomatal conductance; $^{13}$C, shoot carbon isotope discrimination; $K^+/Na^+$, ratio of potassium to sodium; $C/C_i$, the ratio of intercellular to ambient CO$_2$ concentration; N, leaf nitrogen concentration.
Comparative responses of different cereals to water stress and salinity

Abbreviations of treatments are as defined in the legend of Fig. 1.

levels of nitrate reductase (Wang et al., 2007; Carrillo et al., 2008), whereas at 300 mM the activity of glutamine synthetase decreases (Wang et al., 2007). In agreement with δ15N changes mediated by a decrease in enzyme activity due to salinity, when genotypes were combined across growing conditions under full irrigation, δ15N positively correlated not just with biomass but also with N concentration (Fig. 5B) and total shoot nitrogen content (Fig. 5C). Similar results were reported by Yousfi et al. (2009), but Handley et al. (1997) did not find such relationships; in this latter case, the salinity treatment imposed was probably too short to modify nitrogen content and shoot biomass.

It may be concluded that regardless of the mechanism(s) affecting plant δ15N, this trait seems to relate to the plant’s ability to use available nitrogen. Moreover, under the present experimental conditions, N concentration and δ15N are better at reflecting differences in biomass caused by short-term treatments applied at the end of the crop cycle than Δ13C. This reflects the far less mobile nature of carbon, mostly accumulated in support structures (i.e. cell walls) that were already developed before imposition of treatment, compared with the N invested in metabolism, which is more affected by differences in the current growing conditions, including accelerated senescence and remobilization to growing grains.

Fig. 7. Relationship between stable carbon isotope discrimination (Δ13C) and shoot biomass across the six genotypes within each of the six growing conditions assayed. Each point represents the individual value for a given replication and genotype within a growing condition. ns, not significant; *P < 0.05; **P < 0.01. Abbreviations of treatments are as defined in the legend of Fig. 1.

Relationships of biomass to physiological traits across treatments and genotypes

Relationships of biomass to the physiological traits included in this study (Na+, Na+/K+, C/Ca, N, and δ15N) were different across full irrigation from deficit irrigation treatments, and usually stronger in the first case. This may reflect the different nature of drought and salinity (Chaves et al., 2009), and consequently the potential interactions between both stresses.

In a previous study δ15N, rather than Δ13C, was the best informative trait on genotypic differences in tolerance to salinity (Yousfi et al., 2009). In the present study, the genotypic effect for Δ13C was much more relevant than the treatment factor, while the reverse was true for δ15N. The phenotypic negative association between Δ13C and biomass for each stress treatment suggests that genotypes with lower gs (and, thus, less transpiration) were most tolerant. A negative relationship between biomass and Δ13C has also been reported by Yousfi et al. (2009) at 12 dS m⁻¹. For barley, Isla et al. (1998) reported a positive correlation between Δ13C and grain yield in the absence of stress, whereas no association was observed under highly saline conditions. Munns and James (2003) suggest that screening for high gs may be the most effective way of selecting genotypes that will grow fast in saline soil. In the present study, however, gs was not chosen as a variable related to biomass in the stepwise analysis, which may be due to the fact that all treatments other than control induced very low gs values. It was only under control conditions that gs entered the model, with genotypes having higher gs yielding more. Instead, C/Ca, probably associated with gs, was included as the first trait in the stepwise models for DI and DI-12 dS m⁻¹.

Physiological mechanisms of genotypic performance

The results suggest that a superior growth potential (i.e. in the absence of stress) may confer a better performance in terms of total biomass under the different salinity and drought combinations. Thus, tritordeum and Cham were genotypes with the most and least biomass, respectively, under control conditions, but also under the different stress treatments. Previous studies also support a positive role for constitutive high growth, rather than high physiological tolerance (i.e. small phenotypic plasticity) (Rawson et al., 1988; Isla et al., 1998; Yousfi et al., 2009), conferring adaptation to salinity.

Besides its high potential growth under control conditions, tritordeum also exhibited the lowest Δ13C across treatments, while durum wheat genotypes, particularly Cham, showed the highest values (Supplementary Table S1 at JXB online). Tritordeum has higher water use efficiency at Centro Internacional de Mejoramiento de Maiz y Trigo on September 28, 2010
tritordeum has been reported to maintain greater $g_s$ than wheat and triticale under water deficit conditions (Gallardo and Fereres, 1989), which agrees with the present results under DI. Triticale was, together with tritordeum, the best genotype in terms of biomass at DI and DI-12 dS m$^{-1}$ (Supplementary Table S1 at JXB online). Giunta et al. (1993) conclude that triticale is more drought resistant than durum wheat due (at least in part) to its greater ability to extract water from the soil. Aranjuelo et al. (2009) also reported higher $g_s$ values in triticale compared with wheat and tritordeum. In the present study, however, it did not show a consistently higher $g_s$ than durum wheat genotypes at the end of treatments DI and DI-12 dS m$^{-1}$. Rather the opposite was seen, as triticale (as tritordeum) showed lower $\Delta^{13}$C than durum wheat, which suggests lower $g_s$ during the period of treatment.

Differences in Na$^+$ accumulation may also be involved in genotypic performance under saline conditions (Zheng et al., 2008), which suggests that toxic ions may accumulate more in the susceptible genotypes due to higher transpiration. Thus, and except for the two treatments with the highest salinity level (17 dS m$^{-1}$), tritordeum and triticale exhibited a lower accumulation of Na$^+$ and higher K$^+$/Na$^+$ ratio than the four durum wheat genotypes. Na$^+$ exclusion and a subsequent high K$^+$/Na$^+$, usually from leaves and shoots, have been proposed as screening traits for tolerance to moderate salinity (e.g. Dvorak et al., 1994; Chhipa and Lal, 1995; Colmer et al., 2005; Yousfi et al., 2009). Moreover, both triticale and tritordeum showed the highest N concentration in the most stressful treatments (17 dS m$^{-1}$) while, compared with control, $\delta^{15}$N decreased the least across saline treatments. In fact, triticale is reported as tolerant to salinity (Morant-Manceau et al., 1993), with thylakoid functions being preserved and senescence delayed in salt stress conditions.

Among durum wheat genotypes the two RILS performed slightly better in terms of biomass and senescence than Lahn, and consistently better than Cham, the other parent. In fact, Cham showed higher $\Delta^{13}$C together with lower K$^+$/Na$^+$ ratios and concentrations of ions other than Na$^+$. This genotypic ranking agrees with Yousfi et al. (2009), and suggests that genetic variability for salinity tolerance in durum wheat is maintained through the entire crop cycle. Therefore, genotypic evaluation during the first part of the crop cycle (perhaps even at the seedling stage) may be a valid option to select for salt tolerance in durum wheat. This study also points to tritordeum and, to a lesser extent, triticale as potential sources to introgress genes for tolerance to salinity, drought, or, what is most common under field conditions, both stresses in combination.

**Supplementary data**

Supplementary data are available at JXB online.

**Figure S1.** Relationship between biomass and (A) the carbon isotope discrimination ($\Delta^{13}$C) of shoots and (B) the intercellular to ambient CO$_2$ concentration ($C_i$/C$_a$) of flag leaves across the six genotypes assayed under full irrigation (filled circles: FI, FI-S1, and FI-S2) and deficit irrigation (open circles: DI, DI-S1, and DI-S2) conditions. Each point represents the individual value for a given replicate and genotype within a growing condition. Gas exchange measurements and shoot sampling were performed ~3 weeks after anthesis. Abbreviations of treatments are as defined in the legend of Fig. 1.

**Figure S2.** Relationship between biomass and Na$^+$ values across treatments. Biomass is expressed as a percentage of the value reached by each genotype grown in the absence of salinity, and Na$^+$ is expressed as the absolute difference between Na$^+$ concentration in shoot dry matter in the presence or absence of salinity for a given genotype. Left: full irrigation treatments (normal Hoagland, 12 dS m$^{-1}$, and 17 dS m$^{-1}$). Right: deficit irrigation treatments (normal Hoagland, 12 dS m$^{-1}$, and 17 dS m$^{-1}$). Circles represent the average genotypic value for each treatment.

**Figure S3.** Relationship between the nitrogen isotope composition ($\delta^{15}$N) of shoots and (A) the carbon isotope discrimination (\(\Delta^{13}\)C) of shoots and (B) the ratio of intercellular to ambient CO$_2$ concentration ($C_i$/C$_a$) of flag leaves at the end of the treatment across the six genotypes assayed under full irrigation (filled circles: FI, FI-S1, and FI-S2) and deficit irrigation (open circles: DI, DI-S1, and DI-S2) conditions. Each point represents the individual value for a given replication and genotype within a growing condition. Abbreviations of treatments are as defined in the legend of Fig. 1. ns, not significant; **$P<0.01$

**Table S1.** Effect of different levels of salinity, water stress, and the combination of the two stresses on the shoot biomass, concentration of Na$^+$, the ratio K$^+$/Na$^+$, stable carbon isotope discrimination (\(\Delta^{13}\)C), stable nitrogen isotope composition ($\delta^{15}$N), and nitrogen concentration in shoots, leaf net CO$_2$ assimilation ($A_{sat}$) and the ratio of intercellular to ambient CO$_2$ concentration ($C_i$/C$_a$) of durum wheat (Cham, Lahn, RIL47, and RIL85), triticale (Imperioso), and tritordeum (HT621). The data shown are the mean of the three replicates for each genotype in each treatment. Abbreviations for treatments and ANOVA are as defined in Table 1. Means followed by different letters were significantly different ($P<0.05$) by Duncan’s test.

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