Journal of Experimental Botany, Vol. 61, No. 13, pp. 3529–3542, 2010
doi:10.1093/jxb/erq184 Advance Access publication 21 July, 2010

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

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

Salima Yousfi[1], Maria Dolores Serret[1], Jordi Voltas[2] and José Luis Araus[1,3,*]

[1] Unitat de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain
[2] Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida, Lleida, Spain
[3] International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico

* To whom correspondence should be addressed. E-mail: j.araus@cgiar.org

Received 5 March 2010; Revised 30 April 2010; Accepted 3 June 2010

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,

Abbreviations: A_{sat}, light-saturated net CO_2 assimilation rate; C_i/C_a, ratio of intercellular to ambient CO_2 concentration; DI, DI-12 dS m^{-1} and DI-17 dS m^{-1}, deficit irrigation with normal nutrient solution, and with 12 dS m^{-1} and 17 dS m^{-1} conductivity nutrient solutions, respectively; FI, FI-12 dS m^{-1} and FI-17 dS m^{-1}, full irrigation with normal nutrient solution, and with 12 dS m^{-1} and 17 dS m^{-1} conductivity nutrient solutions, respectively; g_s, stomatal conductance; F_v'/F_m', efficiency of excitation energy captured by open PSII reaction centres; T, transpiration rate; $\Delta^{13}C$, carbon isotope discrimination; $\delta^{15}N$, nitrogen isotope composition.

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 herein). 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 ($\delta^{15}N$) 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 $\delta^{15}N$ 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 ($\delta^{15}N$) was better for tracking genotypic differences in salinity tolerance than carbon isotope discrimination ($\Delta^{13}C$) and other widely accepted traits such as the accumulation and ratios of ions such as Na^+, K^+, and Ca^{2+}. 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 genotypic 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 ^{13}C and ^{15}N 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 triticale having durum wheat as a parental line, registered in 2006 by Agrovegetal, 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 dm^3 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 CO_2 concentration of 400 μmol mol^{-1}. The gas exchange parameters were light-saturated net CO_2 assimilation (A_{sat}), transpiration rate (T), and stomatal conductance (g_s). Subsequently, the ratio of intercellular to ambient CO_2 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 HNO_3 and 2 ml of H_2O_2. 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^+, Ca^{2+}, K^+, P, and Mg^{2+} 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 ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) 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 $^{13}C/^{12}C$ ratios were expressed in δ notation (Coplen, 2008):

$$\delta^{13}C = (^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1 \text{ (Farquhar et al., 1989)}$$

where 'sample' refers to plant material and 'standard' to Pee Dee Belemnite (PDB) calcium carbonate. The same δ notation was used for the $^{15}N/^{14}N$ ratio ($\delta^{15}N$), but in this case the standard referred to N_2 in air. Atropine was used as a system check in the elemental analyses of nitrogen. International isotope secondary standards of known $^{13}C/^{12}C$ 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 $^{15}N/^{14}N$ ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3 potassium nitrate) were used. The mean $\delta^{15}N$ of the fertilizer provided by the Hoagland solution was 0.6 ‰.

The carbon isotope discrimination ($\Delta^{13}C$) of shoots was calculated as:

$$\Delta^{13}C(‰) = (\delta^{13}C_a - \delta^{13}C_p)/[1+(\delta^{13}C_p)/1000],$$

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 $\delta^{13}C_a$ was –11.3‰.

Statistical analysis

Data for the set of morphophysiological traits were subjected to factorial analyses of variance (ANOVAs) 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 with normal nutrient solution) records were not included in the discriminate analysis of treatment groups so as to highlight differences in the data set that had only arisen from deficit irrigation or salinity effects. The significance level corresponding to the F-value for developing or retaining a specific trait was set at P=0.15. All traits that remained in the models once the stepwise regression processes stopped were considered to discriminate significantly between groups (either genotype or treatment). For the selected traits, Hotelling's T^2 statistics were calculated to test for significance of between-group differences. Canonical discriminant analysis was then used to perform graphical representations of the classifications. Either treatment means (for distinguishing between genotype groups) or genotype means (for distinguishing between treatment groups) across replicates were used as input for the canonical analyses. In order to test the association between biomass and the set of morphophysiological traits measured, further linear stepwise models across genotypes were constructed that were independent for each growing condition, with P=0.05 as the criterion for variables to be either included or removed from the model. 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 in A_{sat}, g_s, C_i/C_a, T, F_v'/F_m', $\Delta^{13}C$, and $\delta^{15}N$. 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 $\delta^{15}N$, while the opposite occurred for $\Delta^{13}C$. Cham showed the highest $\Delta^{13}C$ value and the lowest records for other traits, apart from g_s. In turn, titordeum, followed by triticale, showed the lowest $\Delta^{13}C$ values. Transpiration, g_s, $\delta^{15}N$, and $\Delta^{13}C$ were the only traits not showing a significant G×T intercation.

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^+, Ca^{2+}, Mg^{2+}, and P, as compared with control conditions. Therefore, the ratios K^+/Na^+ and Ca^{2+}/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 Ca^{2+}/Na^+ ratios (Table 3). Except for Ca^{2+}, 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^+, $\delta^{15}N$, F_v'/F_m', Ca^{2+}, T, N concentration, and plant height. Hotelling's T^2-statistic testing for between-treatment differences was significant for all pairwise comparisons. This was also suggested by the outcome of a canonical discriminant

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)

For each genotype values shown are the means of three repetitions. The 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.

	Biomass (g)	Plant height (cm)	Leaf chlorophyll (SPAD units)	Shoot N concentration (mmol g^{-1} DW)	Total shoot N (g)
Genotype					
Cham	51.08 a	66.36 ab	20.74 b	2.32 a	1.42 a
Lahn	64.88 b	66.65 ab	23.37 bc	2.57 b	1.88 abc
RIL47	63.39 b	74.94 c	28.07 d	2.72 b	1.84 abc
RIL85	62.27 ab	70.05 b	25.44 cd	2.20 a	1.55 ab
Triticale	68.56 b	81.50 d	21.49 b	2.65 b	2.07 c
Tritordeum	67.50 b	64.52 a	15.86 a	2.82 b	2.00 bc
Treatment					
FI	121.81 d	78.65 c	48.90 d	3.94 e	4.75 d
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}	58.33 b	68.33 a	17.81 b	2.30 bc	1.35 b
DI	60.06 b	70.07 ab	15.67 b	2.11 b	1.29 b
DI-12 dS m^{-1}	39.17 a	68.76 a	14.45 b	2.41 c	0.95 a b
DI-17 dS m^{-1}	29.18 a	65.06 a	10.12 a	1.79 a	0.55 a
ANOVA					
G	3693.27**	3506.95***	924.81***	6.40***	6.93***
T	73367.21***	1916.70***	15364.23***	42.60***	161.98***
G×T	10805.36 ns	1335.46 ns	3036.73***	6.54***	12.33 ns

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 dSm^{-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.

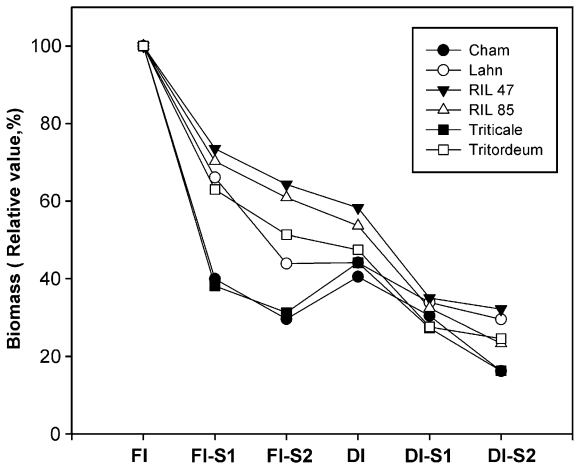

Fig. 1. Relative decrease in shoot biomass of the six different genotypes under the different growing conditions compared with control conditions. For each genotype and growing condition values are expressed as a percentage of the average of the same genotype under full irrigation with Hoagland solution (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 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}.

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} (with high Na^+) from the other, lower Na^+, treatments, which tended to exhibit higher Ca^{2+}, K^+/Na^+, T, F_v'/F_m', and $\delta^{15}N$ values and correspond to the three deficit irrigation treatments. In turn, CAN2 corresponded closely to Ca^{2+}, K^+, N concentration, and leaf chlorophyll, with FI-12 dS m^{-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, independently. 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 2. Effect of different levels of salinity, water stress, and the combination of the two stresses on leaf net CO_2 assimilation (A_{sat}), stomatal conductance (g_s), the ratio of intercellular to ambient CO_2 concentration (C_i/C_a), the transpiration rate (T), efficiency of excitation energy capture by open PSII reaction centres (F_v'/F_m'), stable carbon isotope discrimination ($\Delta^{13}C$), and stable nitrogen isotope composition ($\delta^{15}N$) 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.

	A_{sat} (μmol CO_2 m^{-2} s^{-1})	g_s (mol CO_2 m^{-2} s^{-1})	C_i/C_a	F_v'/F_m'	T (mmol H_2O m^{-2} s^{-1})	$\Delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Genotype							
Cham	4.62 a	0.07 ab	0.29 a	0.18 a	0.98 a	21.12 c	3.41 a
Lahn	5.70 bc	0.09 ab	0.32 a	0.30 bc	1.66 bc	20.93 bc	5.03 cd
RIL47	5.74 bc	0.06 ab	0.37 ab	0.25 ab	1.47 ab	21.47 d	5.96 e
RIL85	5.11 ab	0.05 a	0.34 a	0.22 ab	1.10 ab	20.90 bc	5.37 de
Triticale	6.89 c	0.10 b	0.51 c	0.39 d	2.19 c	20.84 b	4.44 bc
Tritordeum	5.55 b	0.06 ab	0.46 bc	0.36 cd	1.20 ab	20.34 a	4.13 ab
Treatment							
FI	22.14 b	0.35 b	0.80 d	0.66 d	6.03 d	21.30 c	7.27 c
FI-12 dS m^{-1}	2.76 a	0.02 a	0.33 b	0.22 b	0.75 bc	20.89 ab	4.31a
FI-17 dS m^{-1}	1.99 a	3×10–3 a	0.16 a	0.02 a	0.08 a	20.72 a	3.60 a
DI	2.68 a	0.04 a	0.61 c	0.38 c	1.38 c	21.12 bc	5.61 b
DI-12 dS m^{-1}	2.72 a	0.02 a	0.20 a	0.36c	0.51ab	21.06 bc	4.46 a
DI-17 dS m^{-1}	1.80 a	0.02 a	0.20 a	0.09 a	0.38 ab	20.75 a	3.59 a
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×T	145.82**	0.07 ns	1.18***	1.33***	21.45 ns	5.38 ns	42.94 ns

a different pattern under full irrigation compared with deficit irrigation (Fig. 3A, B). Nitrogen concentration was related positively to biomass, but the linear relationship was different across the two irrigation regimes (Fig. 4). $\delta^{15}N$ also correlated positively with total biomass, but only when full irrigation treatments were combined, while no correlation existed across deficit irrigation treatments (Fig. 5A). $\Delta^{13}C$ did not relate to biomass across full irrigation or deficit irrigation treatments (Supplementary Fig. S1A at *JXB* online). However the ratio C_i/C_a was positively related to biomass across treatments, although again full and deficit irrigation followed different patterns (Supplementary Fig. S1B at *JXB* online).

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): $\Delta^{13}C$, plant height, P, K^+/Na^+, $\delta^{15}N$, C_i/C_a, Ca^{2+}, Mg^{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 $\Delta^{13}C$ 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 one hand, RIL47 and RIL85 (as well as tritordeum) had centroids with positive values of CAN2, which was basically related to higher plant height, $\delta^{15}N$, and Mg^{2+} values. 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 $\Delta^{13}C$, $\delta^{15}N$, and Na^+ content, and the highest K^+/Na^+ ratio under FI and DI (Supplementary Table S1 at *JXB* online). Tritordeum 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 $\Delta^{13}C$ and Na^+, together with high K^+/Na^+ and C_i/C_a 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 C_i/C_a and A_{sat} at DI-12 dS m^{-1}, together with the lowest Na^+ and the highest K^+/Na^+ ratio. In turn, tritordeum showed the highest N concentration and A_{sat} at DI-17 dS m^{-1}, together with the lowest $\Delta^{13}C$. 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

Table 3. Effect of different levels of salinity, water stress, and the combination of the two stresses during growth on the ion concentration of shoots of durum wheat (Cham, Lahn, RIL47, RIL85), tritordeum (Imperioso), and triticale (HT621)

Measurements were performed ~3 weeks after anthesis. Abbreviations for treatments and ANOVA analysis are as defined in the footnotes of Table 1. The values shown are the means of three replicates of each genotype. Concentrations are expressed as mmol per g of dry weight. Means followed by different letters are different by Duncan's test (P <0.05). The associated sum of squares and probabilities (ns, not significant; ** P <0.01; *** P <0.001) are shown.

	Na^+	K^+	Ca^{2+}	Mg^{2+}	P	K^+/Na^+	Ca^{2+}/Na^+
Genotype							
Cham	0.87 a	1.14 ab	0.20 d	0.14 a	0.24 d	5.11 a	0.94 a
Lahn	1.07 b	1.22 bc	0.16 b	0.15 a	0.22 c	6.90 a	1.04 a
RIL47	0.73 a	1.19 bc	0.18 c	0.14 a	0.19 b	6.14 a	1.03 a
RIL85	0.85 a	1.18 ab	0.19 c	0.15 a	0.20 c	5.24 a	0.91 a
Triticale	0.82 a	1.26 c	0.14 a	0.15 a	0.18 b	23.20 b	3.17 b
Tritordeum	1.05 b	1.13 a	0.19 c	0.18 b	0.16 a	6.90 a	1.20 a
Treatment							
FI	0.07 a	1.32 d	0.27 d	0.18 d	0.22 c	31.50 d	5.59 c
FI-12 dS m^{-1}	0.92 c	1.23 c	0.20 c	0.17 d	0.18 ab	1.94 ab	0.30 a
FI-17 dS m^{-1}	3.31 d	1.10 b	0.11 a	0.01 a	0.17 a	0.34 a	0.03 a
DI	0.09 a	1.27 cd	0.17 b	0.15 c	0.21 c	17.40 c	2.25 b
DI-12 dS m^{-1}	0.34 b	1.00 a	0.15 b	0.14 bc	0.20 b	3.30 b	0.52 a
DI-17 dS m^{-1}	0.45 b	1.20 c	0.14 b	0.13 b	0.21 c	3.88 b	0.49 a
ANOVA							
G	1.16***	0.18**	0.04***	0.02***	0.05***	7548.82***	142.24***
T	133.61***	1.07***	0.20***	0.07***	0.03***	16304.85***	473.80***
G×T	7.78***	1.47***	0.01 ns	0.01**	0.03***	20877.91***	472.97***

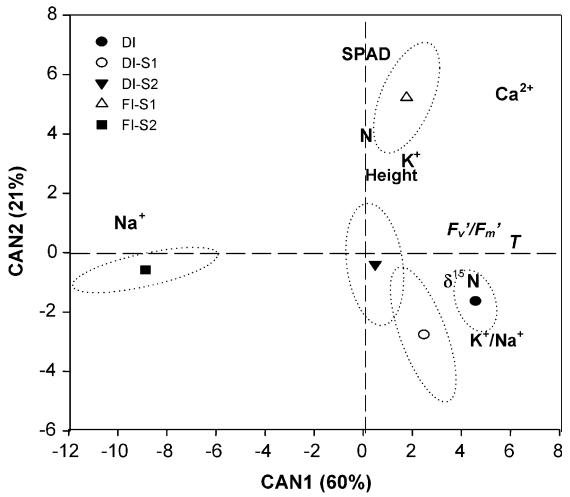

Fig. 2. Plot of the centroids (mean values) and their 95% confidence ellipses for the first two canonical variables of full and deficit irrigation treatments. Rescaled discriminant loadings of the explanatory variables are included in the plots. Abbreviations for treatments are as defined in the legend of Fig. 1. Full irrigation with normal Hoagland solution was not included in the analysis. Abbreviations of variables are as follows: $\delta^{15}N$, stable nitrogen isotope composition; F_v'/F_m', efficiency of excitation energy capture by open PSII reaction centres; height, plant height; K^+, Na^+, Ca^{2+}, dry matter concentrations of K^+, Na^+, and Ca^{2+}, respectively; K^+/Na^+, ratio of K^+ to Na^+ concentrations in dry matter; N, nitrogen concentration; SPAD, leaf chlorophyll content; T, transpiration rate. Except for ion concentrations and ratios, which were analysed in the whole shoot, and plant height, all other parameters were measured in the flag leaf blade.

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

Discussion

Growing conditions and genotypes both differed significantly for biomass and plant height; traits that can be considered useful for screening durum wheat germplasm

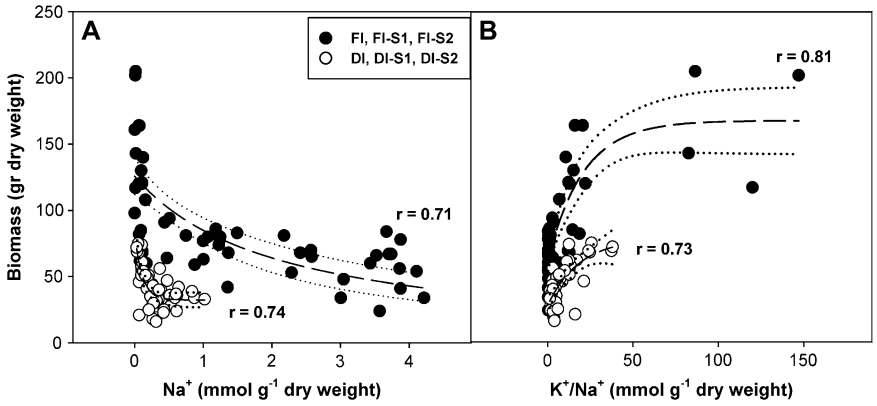

Fig. 3. Exponential relationship between biomass and (A) the Na^+ concentration and (B) the K^+/Na^+ ratio in shoots across the six genotypes (four of durum wheat, one of triticale, and one of tritordeum) assayed under full irrigation (filled circles: FI, FI-S1, and FI-S2) and deficit irrigation (open circles: DI, DI-S1, and DI-S2) conditions. Each point represents the individual value for a given replication and genotype within a growing condition. Plants were sampled ~3 weeks after anthesis after 3 weeks of treatments. Abbreviations for treatments are as defined in the legend of Fig. 1.

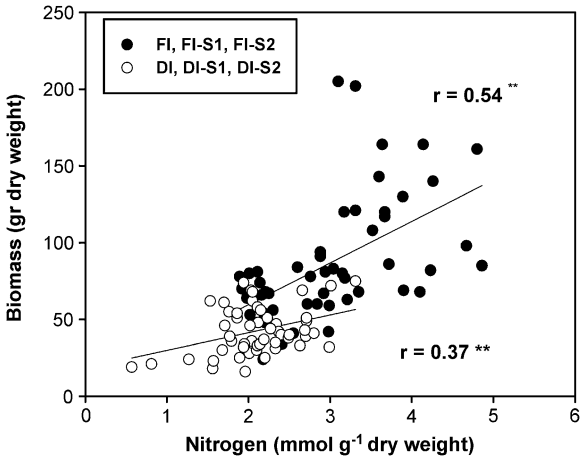

Fig. 4. Relationship between biomass and the nitrogen concentration in the shoot across the six genotypes (four of durum wheat, one of triticale, and one of tritordeum) assayed under full irrigation (filled circles: FI, FI-S1, and FI-S2) and deficit irrigation (open circles: DI, DI-S1, and DI-S2) conditions. Each point represents the individual value for a given replication and genotype within a growing condition. The coefficient of regression for the linear relationship combining all data of the six treatments together was 0.66. Abbreviations of treatments are as defined in the legend of Fig. 1. $^{**}P$ <0.01

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

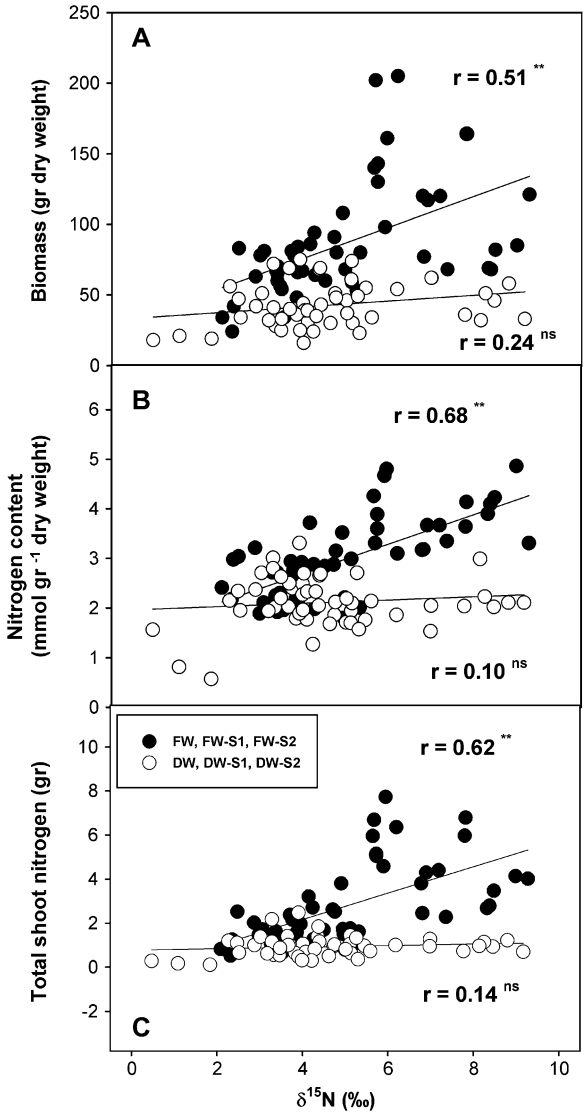

Fig. 5. Relationship between the nitrogen isotope composition ($\delta^{15}N$) of shoots and (A) the shoot biomass, (B) the nitrogen concentration, and (C) the total nitrogen content of shoots across the six genotypes assayed under full irrigation (filled circles: FI, FI-S1, and FI-S2) and deficit irrigation (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. Abbreviations of treatments are as defined in the legend of Fig. 1. ns, not significant; $^{**}P$ <0.01

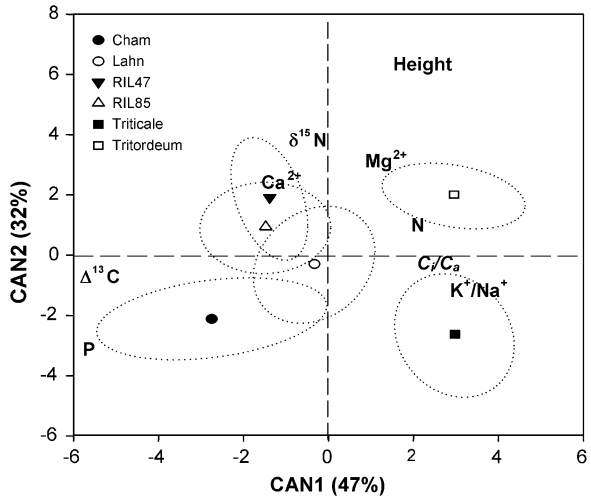

Fig. 6. Plot of the centroids (mean values) and their 95% confidence ellipses for the first two canonical variables of durum wheat, triticale, and tritordeum genotypes. Rescaled discriminant loadings of the explanatory variables are included in the plots. Abbreviations of variables are as in Fig. 2 and Tables 2 and 3.

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

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 <0.01; ***P <0.001.

Model treatments	Variable chosen	R^2	Final stepwise model
Biomass FI	g_s	0.44***	199.7 g_s+53.7
Biomass FI-12 dS m^{-1}	$\Delta^{13}C$	0.52***	–16.6 $\Delta^{13}C$–2.6 A_{sat}+434.8
	$\Delta^{13}C$, A_{sat}	0.64 ***	
Biomass FI-17 dS m^{-1}	$\Delta^{13}C$	0.61***	–38.8 $\Delta^{13}C$+75.7 K^+/Na^++858.4
	$\Delta^{13}C$, K^+/Na^+	0.74***	
Biomass DI	C_i/C_a	0.23**	27.6 C_i/C_a–10.1 $\Delta^{13}C$+261.8
	C_i/C_a, $\Delta^{13}C$	0.50**	
Biomass DI-12 dS m^{-1}	C_i/C_a	0.51***	43.5 C_i/C_a–7.9 $\Delta^{13}C$+202.5
	C_i/C_a, $\Delta^{13}C$	0.73***	
Biomass DI-17 dS m^{-1}	N	0.42***	11.45 N–16.4 C_i/C_a+11.96
	N, C_i/C_a	0.57***	

The table show only the parameters significantly entering in the models. g_s, stomatal conductance; $\Delta^{13}C$, shoot carbon isotope discrimination; K^+/Na^+, ratio of potassium to sodium, C_i/C_a, the ratio of intercellular to ambient CO_2 concentration; N, leaf nitrogen concentration.

irrigation conditions, respectively. Yousfi *et al.* (2009) reported a nearly 0.2‰ decrease in shoot $\delta^{15}N$ per unit increase in ECs. Several studies on barley under hydroponics have reported rates of shoot $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$, which suggests that drought affects plant $\delta^{15}N$ 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 $\delta^{15}N$ (Farquhar *et al.*, 1980; Smart and Bloom, 2001). In fact, $\Delta^{13}C$ and $\delta^{15}N$ 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 $\Delta^{13}C$ and $\delta^{15}N$ has also been reported for durum wheat seedlings across full irrigation treatments (Yousfi *et al.*, 2009). Moreover, $\delta^{15}N$ correlated positively with C_i/C_a (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_i/C_a may integrate leaf permeability together with the functional status of a major N pool (carboxylation enzyme), which would explain why $\delta^{15}N$ correlates with C_i/C_a 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 $\delta^{15}N$ and biomass might be due to differences across treatments in $\delta^{15}N$ 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 $\delta^{15}N$ and biomass has already been reported in durum wheat (Yousfi *et al.*, 2009). Alternatively, Handley *et al.* (1997) suggest that salt stress would make $\delta^{15}N$ 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 $^{15}N/^{14}N$ 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

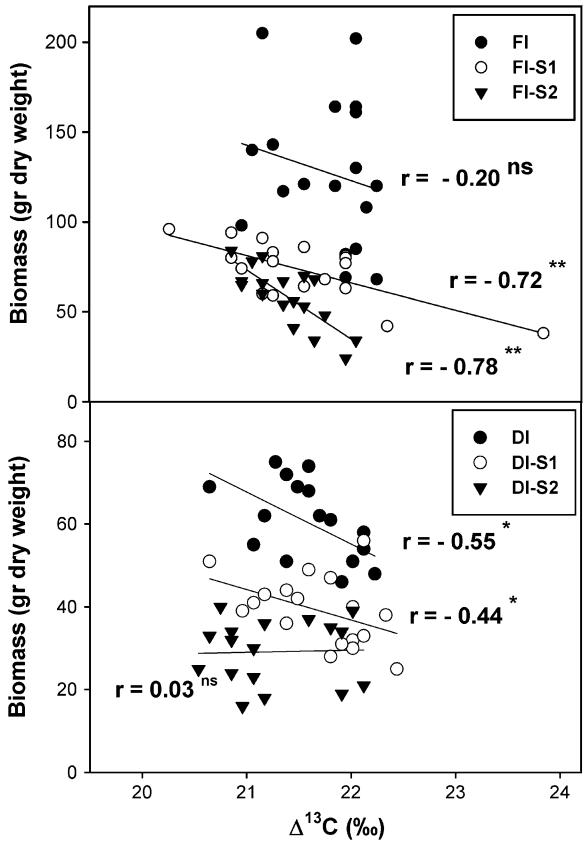

Fig. 7. Relationship between stable carbon isotope discrimination ($\Delta^{13}C$) and shoot biomass across the six genotypes within each of the six growing conditions assayed. Each point represents the individual value for a given replication and genotype within a growing condition. ns, not significant; *P <0.05; **P <0.01. Abbreviations of treatments are as defined in the legend of Fig. 1.

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 $\delta^{15}N$ changes mediated by a decrease in enzyme activity due to salinity, when genotypes were combined across growing conditions under full irrigation, $\delta^{15}N$ 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 $\delta^{15}N$, this trait seems to relate to the plant's ability to use available nitrogen. Moreover, under the present experimental conditions, N concentration and $\delta^{15}N$ are better at reflecting differences in biomass caused by short-term treatments applied at the end of the crop cycle than $\Delta^{13}C$. 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.

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_i/C_a, N, and $\delta^{15}N$) 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 $\delta^{15}N$, rather than $\Delta^{13}C$, was the best informative trait on genotypic differences in tolerance to salinity (Yousfi *et al.*, 2009). In the present study, the genotypic effect for $\Delta^{13}C$ was much more relevant than the treatment factor, while the reverse was true for $\delta^{15}N$. The phenotypic negative association between $\Delta^{13}C$ and biomass for each stress treatment suggests that genotypes with lower g_s (and, thus, less transpiration) were most tolerant. A negative relationship between biomass and $\Delta^{13}C$ 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 $\Delta^{13}C$ 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 g_s may be the most effective way of selecting genotypes that will grow fast in saline soil. In the present study, however, g_s 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 g_s values. It was only under control conditions that g_s entered the model, with genotypes having higher g_s yielding more. Instead, C_i/C_a, probably associated with g_s, 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 $\Delta^{13}C$ across treatments, while durum wheat genotypes, particularly Cham, showed the highest values (Supplementary Table S1 at *JXB* online). Tritordeum has higher water use efficiency than wheat (Martin *et al.*, 1999) probably associated with a constitutively (i.e. in the absence of stress) low g_s (Aranjuelo *et al.*, 2009). On the other hand, the genome of *H. chilense* seems to confer a certain degree of tolerance to drought and salt on this cereal (Martín *et al.*, 2000). Thus,

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.*, 2004), 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 o 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.

Acknowledgements

This study was supported in part by the European research projects TRITIMED (INCO-CT-2004-509136) and OPTIWHEAT (INCO STREP no. 015460) and by the Spanish Ministry of Science and Technology project, AGL2009-13539-C02-01 (subprograma AGR). We thank Llorenç Cabrera-Bosquet and Florence Baptist for their comments.

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