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Durum wheat seedlings in saline conditions: Salt spray versus root-zone salinity

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ABSTRACT

Salinity is an increasingly serious problem with a strong negative impact on plant productivity. Though many studies have been made on salt stress induced by high NaCl concentrations in the root-zone, few data concern the response of plants to saline aerosol, one of the main constraints in coastal areas. In order to study more in depth wheat salinity tolerance and to evaluate damage and antioxidant response induced by various modes of salt application, seedlings of *Triticum turgidum* ssp. *durum*, cv. Cappelli were treated for 2 and 7 days with salt in the root-zone (0, 50 and 200 mM NaCl) or with salt spray (400 mM NaCl + 0 or 200 mM NaCl in the root-zone). Seedlings accumulated Na^+ in their leaves and therefore part of their ability to tolerate high salinity seems to be due to Na^+ leaf tissue tolerance. Durum wheat, confirmed as a partially tolerant plant, shows a higher damage under airborne salinity, when both an increase in TBA-reactive material (indicative of lipid peroxidation) and a decrease in root growth were recorded. A different antioxidant response was activated, depending on the type of salt supply. Salt treatment induced a depletion of the reducing power of both ascorbate and glutathione while the highest contents of proline were detected under salt spray conditions. In the short term catalase and ascorbate peroxidase co-operated with glutathione peroxidase in the scavenging of hydrogen peroxide, in particular in salt spray-treated plants. From our data, the durum wheat cultivar Cappelli seems to be sensitive to airborne salinity.

1. Introduction

Salinity, that is one of the main environmental stressors, can have a negative impact on crop productivity. At least 20% of cultivated land is affected by salinity, predicted to be in the future an increasingly serious problem, exacerbated by the concomitant increase in the need for food due to the continuous increase in world population. As a consequence, some researchers screen plant species to assess their salt tolerance while others try to understand the mechanisms of tolerance to develop salt-tolerant plants able to grow on marginal areas affected by salinity. In this context, the use of coastal areas for agricultural purposes could be of great interest; in this habitat salinity is often mainly in the form of seawater aerosol (Rozema et al., 1985). Air-borne salt, just as salt applied to the root-zone, can cause Na^+ and Cl^- accumulation as polar solutes are able to penetrate through leaf cuticles (Kekere, 2014). Though many studies have been made on plants treated with salt in the root-zone (Amor et al., 2007; Kong et al., 2011; Canalejo et al., 2014; Gengmao et al., 2014) few reports concern the response of plants to airborne salt (Griffiths, 2006; Scheiber et al., 2008; De Vos et al., 2010). Injury from salt spray to plants living near coastal areas is well documented mainly in terms of growth inhibition (Scheiber et al., 2008 and literature therein) but few studies (De Vos et al., 2010) exist on physiological parameters and in particular on oxidative stress and antioxidant response of plants subjected to airborne salinity.

Wheat is one of the most important crops and salinity in the root-zone is known to have a negative impact on its growth and yield, with different cultivars often differing in their tolerance to salinity (Ashraf and McNeilly, 1988; Płażek et al., 2013). It has been reported that wheat salinity tolerance can be due to its ability to exclude Na^+ from the shoot (Munns and James, 2003); however the capacity to accumulate and compartimentalize Na^+ minimalizing metabolic damages could contribute to salt tolerance (Rajendran et al., 2009). Durum wheat is traditionally the main crop in southern peninsular Italy, Sicily and Sardinia. It is well adapted to

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the constraints of the Mediterranean habitat, in which not only arid conditions but also soil salinization by seawater intrusion can be experienced (Borrelli et al., 2011). Less tolerant than bread wheat (Munns and James, 2003), durum wheat is regarded as a moderately tolerant species, with significant yield decrease only at high salinity (Borrelli et al., 2011).

Based on the lack of studies on the response of durum wheat to salt supplied as a spray, in the present study plants of *Triticum turgidum* L. ssp. *durum* (Desf.) were subjected for 2 and 7 days to saline stress by the application of salt in the root-zone and/or as a spray to leaves. For salt spray a NaCl concentration similar to seawater was used to create a situation comparable to the coastal environment. The ancient cultivar Cappelli, recently rediscovered and revalued (Dinelli et al., 2013), due to its superior organoleptic properties, has been used.

Our aims were:

to assess if the two different modes of salt application can induce comparable damage to wheat
to study more in depth if wheat salinity tolerance can be associated only with its ability to exclude Na^+ from the shoot or if also a tissue tolerance may be involved
to ascertain if root-zone and airborne salinity can both induce an active antioxidant response and if this response is differentially modulated in the two different types of salt supply

Besides the physiological aspect, the evaluation of oxidative stress and antioxidant response of seedlings could give a preliminary indication to assess if coastal areas subjected to airborne salinity may be suitable for the cultivation of durum wheat.

2. Materials and methods

2.1. Experimental setup and leaf sample collection

Caryopses (referred to in this paper as grains) of *T. turgidum* L. ssp. *durum* (Desf.) cv. Cappelli were obtained from plants cultivated in fields specifically used for experimental purposes near Pisa, Italy. Fully viable grains (11% moisture content, 100% germination after 48 h of imbibition) were surface sterilised for 3 min in NaOCl (1%, v/v, available chlorine) and rinsed before use. Wheat grains were germinated as in Spanò et al. (2008) in Petri dishes (10 replicates each of 100 grains) on water-moistened Whatman No. 2 filter paper at 23 ± 1 °C in the dark for 72 h. Plants were randomly divided into six different treatment groups (100 plants each) transplanting them into 4 l polyethylene pots filled with deionised water and submitted to 12/12 h day/night photoperiod with a photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} and a relative humidity of 70%, at 23 °C. After six days deionised water was substituted by ¼ x Hoagland solution (Sigma) and after 4 more days (two weeks after imbibitions) salt treatments were started. For salt treatments at the root level 0 (control), 50, and 200 mM NaCl were added to the Hoagland solution. To avoid an osmotic shock, salt concentration was gradually increased (50 mM NaCl per day, until 200 mM). All solutions were continuously aerated. For salt spray treatments deionised water (control spray, CS) or a solution containing 400 mM NaCl (De Vos et al., 2010), reproducing seawater sodium chloride concentration, (salt spray, SS) were applied using a nebulisation system. They were sprayed two times per day, at 9 am and at 2 pm, on plants grown on Hoagland solution. Salt spray treatment corresponded to about 200 mg NaCl dm^{-2} leaf area d^{-1}. One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS). After 2 and 7 days of treatment, 50 plants were collected, measured and all of the leaves, after washing, were used as fresh material (for pigment determination) or fixed in liquid nitrogen and stored at −80 °C until use (for all the other analyses).

2.2. Leaf chemical characteristics

Na^+, K^+ and Cl^- were determined by atomic adsorption spectrometry (Thomas, 1982). Values were expressed on the dry matter basis (%).

2.3. Growth measurement

After collections, both leaf and root length (limited to this parameter only the longest ones were considered) were recorded. Leaf dry matter was determined as described in the following section and sensitivity rate index (IS) was calculated as in Rejili et al. (2006) with the formula:

$$IS = [(DW_{NaCl} - DW_{control})/DW_{control}] \times 100$$

DW_{NaCl} = leaf dry weight of NaCl-treated plants

$DW_{control}$ = leaf dry weight in control (0) or CS plants

2.4. Determination of water content and of relative water content

Calculations of leaf fresh weight, dry weight and moisture content were based on weights determined before and after oven drying of leaf samples. Water content percentage was estimated on the fresh weight basis. Leaf relative water content, RWC, was determined as in Balestri et al. (2014) and calculated with the formula:

RWC = [(FW-DW)/(TW-DW)] × 100.
FW = Fresh weight.
DW = Dry weight.
TW = Turgid weight.

Fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed in water over night, blotted dry and then weighed to get the turgid weight. The leaves were then dried in an oven at 100 °C to constant weight (48 h) and reweighed to obtain the dry weight.

2.5. Pigment determination

Chlorophylls (*a, b* and total) and carotenoids were extracted and determined according to Hassanzadeh et al. (2009) and to Lichtenthaler (1987) respectively. 100 mg of fresh leaves were homogenised in 80% acetone (6 ml) and the extracts were centrifuged for 10 min at 6000 *g* at 4 °C. The supernatants were collected and the pellets were re-suspended and extracted with 80% acetone until they resulted colourless. The collected supernatants were read using spectrophotometer at 645, 663 and 470 nm. Pigment contents were expressed as mg g^{-1}DW.

2.6. Extraction and determination of hydrogen peroxide

H_2O_2 content of leaves was determined according to Jana and Choudhuri (1982). Leaves (250 mg) were ground in a mortar and homogenised with phosphate buffer 50 mM pH 6.5 (15 ml). The homogenate was centrifuged at 6000 *g* for 25 min. To determine the H_2O_2 content, 3 ml of extracted solution were mixed with 1 ml of 0.1% titanium chloride in 20% (v/v) H_2SO_4, then the mixture was centrifuged at 6000 *g* for 15 min and the supernatant absorbance at 410 nm was read. The amount of H_2O_2 in the

extracts was calculated from a standard curve and expressed as μmol g^{-1}DW.

2.7. Thiobarbituric acid reactive substances (TBARS) determination

Lipid peroxidation in leaves was measured by determining the amount of TBARS determined by the thiobarbituric acid (TBA) reaction, according to Hartley-Whitaker et al. (2001) with minor modifications. Leaves (250 mg) were mixed with 4 ml of TBA reagent (10% w/v trichloroacetic acid + 0.25% w/v thiobarbituric acid), heated (95 °C for 30 min), cooled for 15 min and centrifuged at 2000 *g* for 15 min. The level of TBARS was measured as specific absorbance at 532 nm by subtracting the non-specific absorbance at 600 nm and calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}. TBA-reactive materials was expressed as nmol g^{-1} DW.

2.8. Extraction and determination of proline

Proline concentration was determined according to the method of Bates et al. (1973) with minor modifications, as in Spanò et al. (2013). Leaf tissue (250 mg) was homogenised with 5 ml of 3% sulfosalicylic acid. The supernatant was incubated with glacial acetic acid and ninhydrin reagent (1:1:1) and boiled in a water bath at 100 °C for 60 min. After cooling the reaction mixture, toluene was added, and the absorbance of toluene phase was read at 520 nm. Calculations were made on the base of a standard curve and proline content was expressed as μmol g^{-1}DW.

2.9. Extraction and determination of ascorbate and dehydroascorbate

Ascorbate, reduced form (ASA) and oxidised form (dehydroascorbate, DHA), extraction and determination were performed according to Kampfenkel et al. (1995) with minor modifications.

Briefly, leaves (250 mg) were ground in a chilled mortar and homogenised with 1.875 ml of 5% (w/v) TCA. The homogenate was centrifuged at 12 000 g for 10 min at 4 °C and the supernatant was used for the determination (Kampfenkel et al., 1995). The assay for ASA and total ascorbate (ASA + DHA) measurement is based on the reduction of Fe^{3+} to Fe^{2+} by ASA in an acidic solution and on the subsequent formation of complexes of Fe^{2+} with bipyridyl, giving a pink colour with maximum absorbance at 525 nm. Total ascorbate was determined after reduction of DHA to ASA by dithiothreitol and DHA level was estimated on the basis of the difference between total ascorbate and ASA value. Calculations were made on the base of a standard curve and correction was made for colour development in the blank (absence of sample). Content was expressed as μmol g^{-1}DW.

2.10. Extraction and determination of glutathione

Glutathione was extracted and determined according to Gossett et al. (1994). Leaves (250 mg) were homogenised in (0.75 ml) of ice-cold 6% (w/v) *m*-phosphoric acid (pH 2.8) containing 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 20,000 *g* for 15 min at 4 °C and the supernatant was collected and stored in liquid nitrogen until use. Total glutathione (reduced form, GSH + oxidised form, GSSG) was determined by the 5,5'-dithio-bis-nitrobenzoic acid (DTNB)-glutathione reductase recycling procedure and the reaction was monitored as the rate of change in absorbance at 412 nm. GSSG was determined after removal of GSH from the sample extract by 2-vinylpyridine derivatization. GSH was detected by subtracting the amount of GSSG from total glutathione and calculations were made on the base of a standard curve. A blank was made in the absence of the extract and content was expressed as μmol g^{-1}DW.

2.11. Enzyme extraction and assays

Leaves were ground in liquid nitrogen with a mortar and pestle. Extraction was made as in Spanò et al. (2013). All the extractions were performed at 4 °C. The homogenate was then centrifuged at 15,000 *g* for 20 min. For ascorbate peroxidase, 2 mM ascorbate was added to the extraction medium. For glutathione reductase the supernatant was desalted on a Sephadex G-25 column. Supernatants were collected and stored in liquid nitrogen until their use for enzymatic assays.

Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (1981). Enzyme activity was assayed from the decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) as ascorbate was oxidised and enzyme extract contained 25 μg protein ml^{-1}. Correction was made for the low, non enzymatic oxidation of ascorbate by hydrogen peroxide (blank).

Glutathione reductase (GR) activity was determined as described by Rao et al. (1995) following the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mM^{-1} cm^{-1}). Enzymatic extract contained 25 μg protein ml^{-1}. A correction for the non-enzymatic reduction of GSSG was carried out in the absence of the enzyme sample (blank).

Glutathione peroxidase (GPX) activity was determined according to Navari-Izzo et al. (1997) following the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mm^{-1} cm^{-1}). Enzymatic extract contained 12.5 μg protein ml^{-1}.

Catalase (CAT) activity was determined as described by Aebi (1984). Enzymatic extract contained 12.5 μg protein ml^{-1}. A blank containing only the enzymatic solution was made. Specific activity was calculated from the 39.4 mM^{-1} cm^{-1} extinction coefficient.

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was determined as described by Arezky et al. (2001) using as substrate 1% guaiacol. Enzymatic extract contained 5 μg protein ml^{-1}. Enzymatic activity was determined following guaiacol oxidation by H_2O_2 (extinction coefficient 26.6 mM^{-1} cm^{-1}) at 470 nm, one unit oxidising 1.0 μmole guaiacol per min.

Superoxide dismutase (SOD) activity was determined as in Beyer and Fridovich (1987) with minor modification. The reaction mixture containing potassium phosphate buffer pH 7.5, 0.1 mM EDTA, 13 mM L-methionine, 75 μM nitroblue tetrazolium (NBT), 2 μM riboflavin and 25 μg protein ml^{-1}, was kept under a fluorescent light for 15 min at 25 °C. One SOD unit is defined as the amount required to inhibit the photoreduction of nitroblue tetrazolium by 50% determined spectrophotometrically at 550 nm.

All enzymatic activities were determined at 25 °C and expressed as U mg^{-1} protein. Protein measurement was performed according to Bradford (1976), using BSA as standard.

2.12. Statistical analysis

The data were the mean of at least three replicates from three independent experiments.

Statistical significance was determined by ANOVA tests followed by post hoc Bonferroni multiple comparison test. *Post hoc* statistical significance is indicated in figures and tables by different letters. Correlation analyses were performed using the Pearson's correlation coefficient (r).

3. Results

3.1. Leaf chemical characteristics

The contents of Na^+, K^+ and Cl^- are reported in Table 1. After two days of treatment both Na^+ and Cl^- progressively increased in treated plants and 200 mM NaCl and 200 mM NaCl + SS leaves showed the highest contents of these ions. The same treatments were characterised by low K^+/Na^+ ratios, however not significantly different from SS plants. 50 mM NaCl-treated material had an intermediate value of this ratio among treated and control plants (0, CS), characterised by significantly higher K^+/Na^+ ratio. After 7 days 50 mM NaCl-treated leaves had contents of Na^+ and Cl^- not significantly different from control plants (0, CS) while SS material showed concentrations of these ions similar to 200 mM NaCl leaves. The highest contents were reached in 200 mM NaCl + SS plants. The highest values of K^+/Na^+ were typical of controls (0 and CS), while the lowest one after 7 days was detected in 200 mM NaCl + SS plants.

3.2. Growth, water content, relative water content and pigments

Growth and water status are shown in Fig. 1 and in Table 2. Salt treatment induced a significant decrease in leaf dry matter, reported as IS (sensitivity rate index, Fig. 1), when salt was supplied as a spray. In the short term the decrease was −17 and −12%, in comparison with the control (CS) for SS and 200 mM NaCl + SS plants respectively. In the longer period, there was a further decrease only in SS plants. Salt in the root zone induced after 2 days of treatment a salt concentration-dependent decrease in dry matter that remained unchanged in the longer period. Both after 2 and 7 days, leaf length (Table 2) was significantly higher in controls and 200 mM NaCl + SS plants; the minimum value characterised SS plants showing also the lowest root length. In the longer period while 200 mM NaCl + SS plants reached root lengths not significantly different from control (0), CS and SS roots were still significantly shorter than control (0). Both in the short and in the longer period SS plants showed the minimum root length (about 61% and 60% of the control, respectively). This material was also characterised by the highest shoot/root length ratio. The minimum value of this ratio was recorded in 200 mM NaCl plants.

Water content (Table 2) did not show significant differences among different materials either after 2 or after 7 days of treatments. Relative water content (RWC) (Table 2) only after 7 days significantly decreased in 200 mM and 200 mM NaCl + SS plants.

There were not significant differences in chlorophyll contents (Table 3) among the different materials, while the content of carotenoids generally increased in salt-treated plants both in the short and in the longer period. Neither Chla/Chlb nor Car/Tot Chl significantly differed among different materials.

3.3. Hydrogen peroxide and TBA-reactive material

The lowest hydrogen peroxide content (Fig. 2A) was detected in control plants (0), both after 2 and 7 days of treatment. Control spray plants had always higher contents of this ROS than control ones (0) at both investigated times. The highest contents of H_2O_2 were recorded in 200 mM NaCl + SS plants. The content of TBA-reactive material (Fig. 2B), indicative of lipid peroxidation and of membrane damage, was generally in accordance with hydrogen peroxide content. The highest values were detected in 200 mM NaCl + SS plants, followed by salt spray plants characterised, after 7 days of treatment, by values significantly higher than plants suffering from root-zone salinity.

3.4. Ascorbate, glutathione and proline

Total ascorbate content (Table 3) was never significantly different among plants with the exception of 200 mM NaCl + SS material after 2 days and, in the longer period, of 200 mM NaCl leaves, characterised by the highest concentration of this low molecular weight antioxidant. After 2 days of treatment reducing power of the ASA/DHA couple (Table 3) was significantly higher in 200 mM NaCl reaching the maximum value in 200 mM NaCl + SS

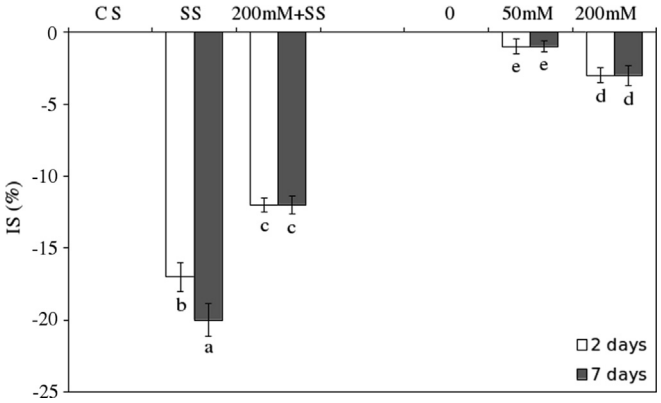

Fig. 1. Sensitivity rate index (IS) in leaves from seedlings of *Triticum turgidum* spp. *durum* cv. Cappelli subjected for 2 and 7 days to salt treatments at the root level: 0 (control, C), 50, and 200 mM NaCl or to salt spray treatments: deionised water (control spray, CS) or a solution containing 400 mM NaCl (salt spray, SS). One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS). Data are the mean of at least three replicates from three independent experiments ± SE. Means followed by the same letters are not significantly different at 1%.

Table 1
Chemical characteristics of leaves of seedlings of *Triticum turgidum* spp. *durum* cv. Cappelli subjected for 2 and 7 days to salt treatments at the root level: 0 (control, C), 50, and 200 mM NaCl or to salt spray treatments: deionised water (control spray, CS) or a solution containing 400 mM NaCl (salt spray, SS). One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS).

Treatments	CS		SS		200 mM NaCl + SS		0		50 mM NaCl		200 mM NaCl	
	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days
Na^+ (%)	0.43 ± 0.06 f	1.01 ± 0.14 ef	2.23 ± 0.45 de	4.16 ± 0.36 bc	3.19 ± 0.32 cd	6.26 ± 0.68 a	0.58 ± 0.06 f	0.60 ± 0.09 f	1.10 ± 0.11 ef	1.73 ± 0.21 ef	4.02 ± 0.48 bc	4.64 ± 0.42 b
K^+ (%)	6.37 ± 0.96 a	5.90 ± 0.78 a	4.65 ± 0.93 a	5.18 ± 0.65 a	5.03 ± 0.50 a	4.31 ± 0.53 a	6.24 ± 0.83 a	5.16 ± 0.77 a	5.45 ± 1.04 a	5.51 ± 0.50 a	5.47 ± 0.39 a	4.90 ± 0.94 a
Cl^- (%)	0.41 ± 0.06 e	1.69 ± 0.14 de	3.63 ± 0.72 d	7.14 ± 0.64 b	5.32 ± 0.48 c	11.61 ± 0.89 a	0.41 ± 0.05 e	1.11 ± 0.17 e	1.08 ± 0.31 e	1.90 ± 0.19 de	7.82 ± 0.94 b	8.10 ± 0.99 b
K^+/Na^+	14.94 ± 2.57 a	5.83 ± 0.89 c	2.08 ± 0.48 e	1.25 ± 0.15 e	1.58 ± 0.18 e	0.69 ± 0.09 e	10.82 ± 1.47 b	8.62 ± 1.51 b	4.94 ± 0.88 d	3.18 ± 0.40 de	1.36 ± 0.16 e	1.05 ± 0.18 e

Data are the mean of at least three replicates ± SE. Means followed by the same letters within the same row are not significantly different at 1%.

Table 2
Growth, water status (H_2O) and relative water content (RWC) of leaves of seedlings of *Triticum turgidum* spp. *durum* cv. Cappelli subjected for 2 and 7 days to salt treatments at the root level: 0 (control, C), 50, and 200 mM NaCl or to salt spray treatments: deionised water (control spray, CS) or a solution containing 400 mM NaCl (salt spray, SS). One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS).

Treatments	CS		SS		200 mM NaCl + SS		0		50 mM NaCl		200 mM NaCl	
	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days
Leaf length (cm)	30.26 ± 0.61 bc	31.43 ± 0.73 ab	27.02 ± 0.29 e	27.93 ± 0.50 de	30.11 ± 0.56 bc	31.61 ± 0.14 ab	31.30 ± 0.40 ab	33.01 ± 0.58 a	28.91 ± 0.46 cd	30.87 ± 0.46 bc	28.90 ± 0.49 cd	29.34 ± 0.36 cd
Root length (cm)	22.98 ± 0.50 c	22.91 ± 0.39 c	15.93 ± 0.54 d	16.08 ± 0.33 d	24.28 ± 0.57 bc	24.56 ± 0.46 bc	26.14 ± 0.45 a	26.49 ± 0.40 ab	24.17 ± 0.51 bc	25.35 ± 0.72 ab	26.62 ± 0.50 a	25.84 ± 0.52 ab
Leaf/root	1.32 ± 0.03 bc	1.37 ± 0.03 b	1.70 ± 0.03 a	1.74 ± 0.03 a	1.24 ± 0.03 c	1.29 ± 0.02 c	1.20 ± 0.02 cd	1.25 ± 0.02 c	1.20 ± 0.03 cd	1.22 ± 0.03 c	1.08 ± 0.02 e	1.13 ± 0.02 de
H_2O (%)	90.20 ± 0.30 a	90.30 ± 0.26 a	91.30 ± 0.42 a	90.70 ± 1.22 a	91.27 ± 0.69 a	90.20 ± 0.10 a	90.13 ± 0.20 a	89.51 ± 0.51 a	90.43 ± 0.58 a	90.30 ± 0.38 a	89.67 ± 0.32 a	90.07 ± 0.10 a
RWC (%)	96.47 ± 0.67 a	96.07 ± 1.85 a	94.00 ± 1.33 a	86.00 ± 5.14 ab	94.40 ± 3.63 a	79.63 ± 5.98 b	91.77 ± 1.26 a	97.76 ± 0.47 a	95.95 ± 1.90 a	97.53 ± 0.76 a	93.00 ± 0.32 a	80.17 ± 1.29 b

Data are the mean of at least three ± SE. Means followed by the same letters within the same row are not significantly different at 1%.

Table 3
Contents of chlorophylls (Chl), carotenoids (Car), total ascorbate (reduced ascorbate, ASA + dehydroascorbate, DHA), total glutathione (reduced form, GSH + oxidised form, GSSG), and ASA/DHA and GSH/GSSG ratios in leaves of seedlings of *Triticum turgidum* spp. *durum* cv. Cappelli subjected for 2 and 7 days to salt treatments at the root level: 0 (control, C), 50, and 200 mM NaCl or to salt spray treatments: deionised water (control spray, CS) or a solution containing 400 mM NaCl (salt spray, SS). One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS).

Treatments	CS		SS		200 mM NaCl + SS		0		50 mM NaCl		200 mM NaCl	
	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days	2 days	7 days
Total Chl ($mg\ g^{-1}DW$)	15.45 ± 0.53 a	17.58 ± 0.81 a	17.00 ± 1.46 a	16.92 ± 1.25 a	17.89 ± 1.44 a	13.52 ± 1.90 a	16.46 ± 1.00 a	15.24 ± 0.95 a	20.34 ± 2.73 a	16.09 ± 0.97 a	14.52 ± 1.30 a	13.62 ± 0.93 a
Chla/Chlb	1.46 ± 0.07 a	1.65 ± 0.13 a	1.76 ± 0.04 a	1.54 ± 0.17 a	1.62 ± 0.13 a	1.60 ± 0.10 a	1.44 ± 0.12 a	1.48 ± 0.06 a	1.32 ± 0.05 a	1.61 ± 0.11 a	1.73 ± 0.19 a	1.63 ± 0.12 a
Car ($mg\ g^{-1}DW$)	1.33 ± 0.04 c	1.80 ± 0.17 ab	1.91 ± 0.10 ab	1.79 ± 0.22 ab	1.92 ± 0.08 ab	1.93 ± 0.12 ab	1.27 ± 0.17 c	1.55 ± 0.02 bc	1.40 ± 0.03 bc	1.78 ± 0.05 b	1.72 ± 0.07 bc	2.01 ± 0.11 a
Car/Total Chl	0.09 ± 0.00 a	0.10 ± 0.01 a	0.11 ± 0.00 a	0.11 ± 0.02 a	0.11 ± 0.01 a	0.15 ± 0.10 a	0.08 ± 0.01 a	0.10 ± 0.01 a	0.07 ± 0.01 a	0.11 ± 0.01 a	0.12 ± 0.01 a	0.15 ± 0.02 a
Total ascorbate ($\mu mol\ g^{-1}DW$)	6.99 ± 0.58 d	8.71 ± 0.84 bcd	7.62 ± 0.85 cd	8.31 ± 0.29 bcd	10.66 ± 1.05 b	8.83 ± 0.20 bcd	6.93 ± 0.25 d	6.79 ± 0.30 d	7.77 ± 0.30 cd	6.88 ± 0.38 d	10.44 ± 1.14 bc	15.13 ± 0.58 a
ASA/DHA	1.37 ± 0.14 d	2.37 ± 0.31 d	4.63 ± 0.48 d	1.94 ± 0.09 d	23.01 ± 1.71 a	1.97 ± 0.12 d	1.92 ± 0.13 d	1.53 ± 0.28 d	1.95 ± 0.16 d	13.58 ± 1.74 c	17.86 ± 1.62 b	2.40 ± 0.11 d
Total glutathione ($\mu mol\ g^{-1}DW$)	1.22 ± 0.07 b	0.78 ± 0.04 de	1.08 ± 0.03 c	0.58 ± 0.01 f	1.48 ± 0.03 a	0.32 ± 0.02 g	0.76 ± 0.02 de	0.66 ± 0.02 ef	0.99 ± 0.04 c	0.81 ± 0.06 d	0.83 ± 0.02 d	0.37 ± 0.03 g
GSH/GSSG	3.57 ± 0.09 b	2.12 ± 0.07 d	1.47 ± 0.04 f	1.86 ± 0.05 e	2.37 ± 0.04 d	2.32 ± 0.09 d	5.34 ± 0.10 a	3.06 ± 0.06 c	1.03 ± 0.09 g	1.63 ± 0.11 ef	1.01 ± 0.07 g	2.29 ± 0.14 d

Data are the mean of at least three replicates ± SE. Means followed by the same letters within the same row are not significantly different at 1%.

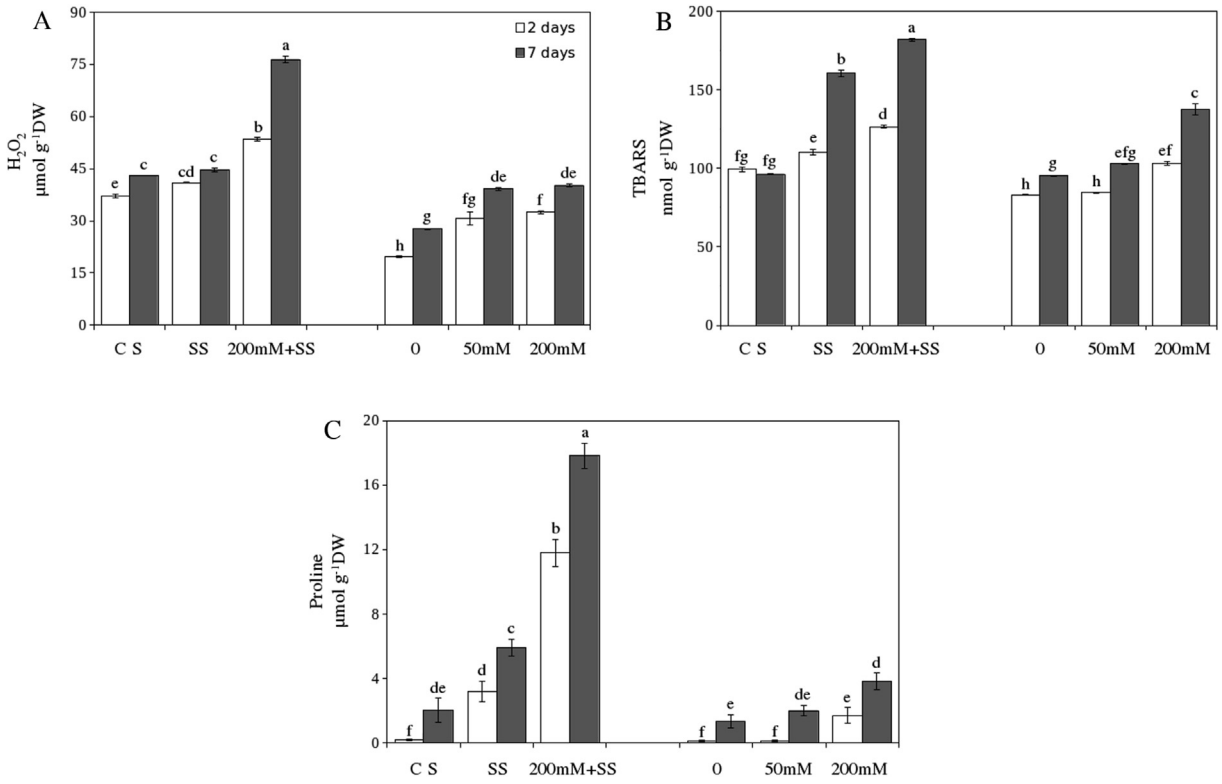

Fig. 2. Content of hydrogen peroxide (A), TBA-reactive material (TBARS, B), and proline (C) in leaves from seedlings of *Triticum turgidum* spp. *durum* cv. Cappelli subjected for 2 and 7 days to salt treatments at the root level: 0 (control, C), 50, and 200 mM NaCl or to salt spray treatments: deionised water (control spray, CS) or a solution containing 400 mM NaCl (salt spray, SS). One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS). Data are the mean of at least three replicates from three independent experiments ± SE. Means followed by the same letters are not significantly different at 1%.

plants. After 7 days only 50 mM NaCl leaves showed a reducing power significantly higher than other materials.

After 2 days, the content of total glutathione (Table 3) was relatively low in control (0) plants, significantly higher in CS and afterwards it progressively decreased in salt spray, 50 mM NaCl and 200 mM NaCl plants to rise again in 200 mM NaCl + SS material, where the highest value of this molecule was recorded. After 7 days glutathione content was significantly lower in comparison with 2 days and the lowest values were detected in 200 mM NaCl and 200 mM NaCl + SS plants. The reducing power of this antioxidant molecule, expressed as GSH/GSSG ratio, was always higher in control (0) and CS than in treated plants after 2 days of treatment, while in the longer period control plants had the maximum value of GSH/GSSG ratio. The lowest values were detected in SS and in 50 mM NaCl plants.

The highest contents of proline (Fig. 2C), barely detectable after 2 days in controls (0 and CS) and in 50 mM NaCl plants, were always recorded in 200 mM NaCl + SS samples. SS leaves had contents of this protective molecule significantly higher than plants subjected to salinity in the root-zone both in the short and in the longer period.

3.5. Antioxidant enzymes

After 2 days the highest activities of APX (Fig. 3A) were detected in control plants (0), while the lowest values of activity were recorded in plants treated with salt in the root zone and in 200 mM NaCl + SS leaves, not significantly different from CS plants. In the longer period there was a significant increase of activity in CS, SS, and 200 mM NaCl leaves, while the remaining materials maintained the values of activity measured after 2 days. Among salt treatments, the highest activity was recorded in SS plants. While after 2 days there was a gradual increase in GPX activity (Fig. 3B), starting from controls (0 and CS), with 200 mM NaCl and 200 mM NaCl + SS plants characterised by the highest activity of this H_2O_2 scavenging enzyme, after 7 days there were not significant differences among the different treatments. After 2 days salt treatments generally induced a decrease in POD activity (Fig. 3C), with the exception of SS plants that showed an activity of this scavenger not significantly different from control (0 and CS) plants. After 7 days POD activity increased in particular in treated materials, reaching the highest value in 200 mM NaCl + SS plants. Both after 2 and 7 days GR activity (Fig. 3D) was significantly higher only in CS plants, while all the other materials have lower and not significantly different activity of this enzyme. After 2 days of treatment, CAT activity (Fig. 3E) was significantly higher in control (0) and SS plants, that however were both characterised in the longer period by values of activity similar to other materials. In the short period, control plants (0) showed the maximum of SOD activity (Fig. 3F). Afterwards the activity of this enzyme decreased until values were not significantly different from the other materials.

4. Discussion

High salinity causes both ionic and osmotic stresses leading to reduced growth rates and eventually to plant death. Many of the studies done on salt stress, consider the effects of salt applied at the root-zone (Amor et al., 2007; Kong et al., 2011; Canalejo et al., 2014; Gengmao et al., 2014) and few data, and never on wheat, concern response of plants to airborne salt (Griffiths, 2006; Scheiber et al., 2008; De Vos et al., 2010).

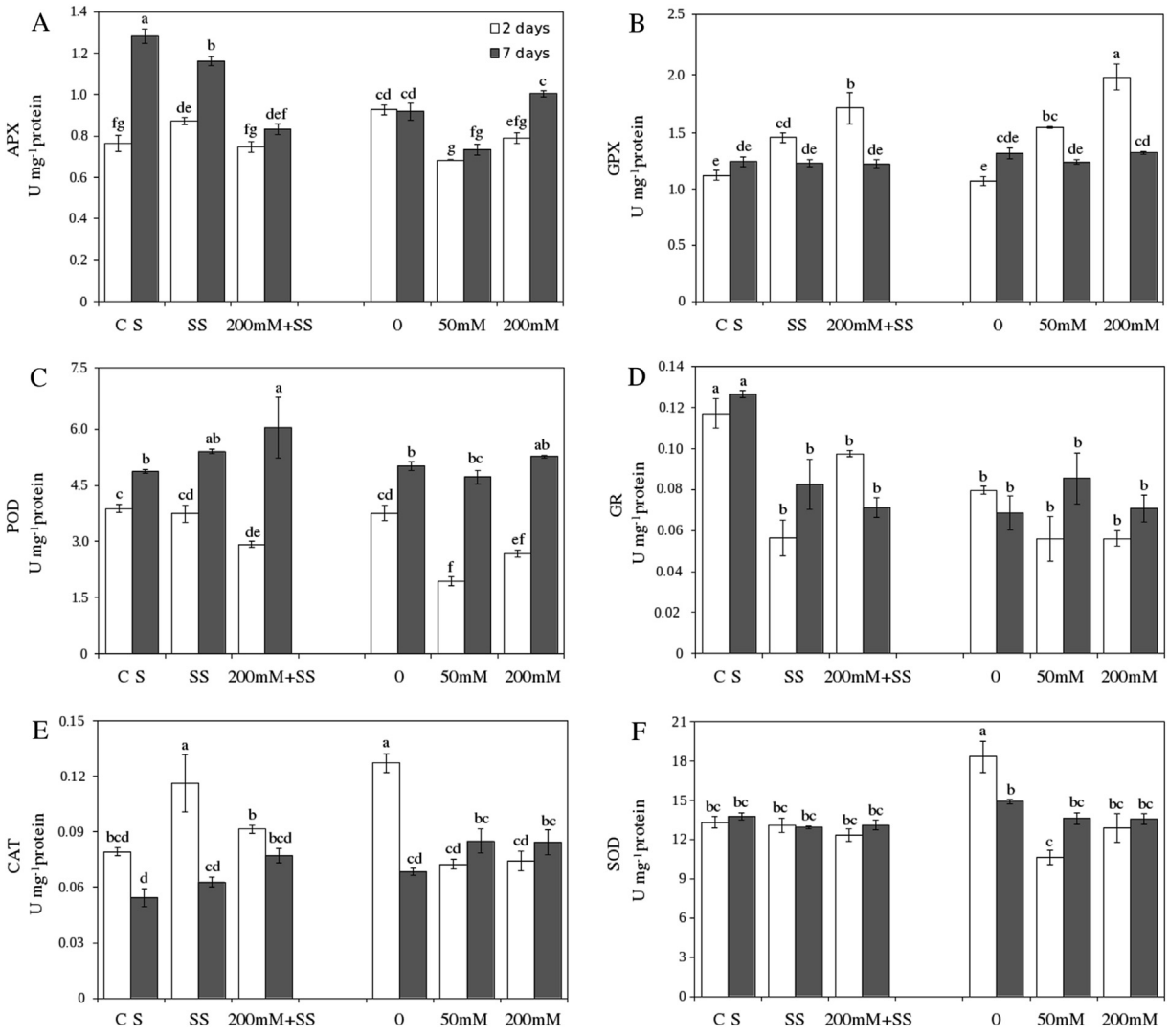

Fig. 3. Activities of ascorbate peroxidase (APX, A), glutathione peroxidase (GPX, B), guaiacol peroxidase (POD, C), glutathione reductase (GR, D), catalase (CAT, E), and superoxide dismutase (SOD, F) in leaves from seedlings of *Triticum turgidum* spp. *durum* cv. Cappelli subjected for 2 and 7 days to salt treatments at the root level: 0 (control, C), 50, and 200 mM NaCl or to salt spray treatments: deionised water (control spray, CS) or a solution containing 400 mM NaCl (salt spray, SS). One lot of plants experienced both salt at the root level and salt spray (200 mM NaCl + SS). Data are the mean of at least three replicates from three independent experiments ± SE. Means followed by the same letters are not significantly different at 1%.

To have an insight into the effects of different ways of supplying salt, plants of *T. turgidum* spp. *durum* were subjected to saline irrigation and/or to salt spray. The content of both Na^+ and Cl^- progressively increased with the increasing of salt treatment and the maximum contents of these ions were recorded in 200 mM NaCl + SS plants after 7 days. Due to these increases, though K^+ content did not differ significantly among the different materials, K^+/Na^+ ratio was significantly higher in control plants than in treated ones, regardless of the type of treatment. Both treatments, salt spray and root-zone salinity, were therefore able to induce disturbance in ionic balance, with a low K^+/Na^+ ratio, a typical trait of plants subjected to salt stress (Spanò et al., 2013). However, though high K^+/Na^+ ratio is considered essential for an appropriate leaf water potential (Devitt et al., 1981), in spite of the ionic disturbance, water balance, as indicated by the value of RWC, was significantly lower than controls only in the longer period in 200 mM NaCl and 200 mM NaCl + SS plants. Interestingly SS plants had at this time an RWC value that was intermediate between controls and 200 mM NaCl and 200 mM NaCl + SS plants. The ability to accumulate Na^+ ions in leaves in the absence of a negative effect on water content stability (H_2O percentage never differed significantly among the different plants), could indicate at least a partial tolerance of our cultivar to salt stress. Although it has been suggested that wheat salinity tolerance can be associated with its ability to exclude Na^+ from the shoot (Munns and James, 2003) our data seem to sustain an "inclusive" behaviour (Rejili et al., 2006) in which salt tolerance is the result also of a Na^+ tissue tolerance (Rajendran et al., 2009).

Growth responses reflect the tolerance of plants to salinity with a significant reduction of growth in salt-sensitive species under saline conditions (Munns and Tester, 2008). In addition, it is reported that plants are often more sensitive to salt spray than to NaCl applied at the root-zone (Benes et al., 1996). In accordance, in our experimental conditions, the reduction in leaf dry matter accumulation, evaluated as a sensitivity rate index, was significantly higher in conditions of airborne salinity than under root-zone salinity. Although both salt treatments had a slight inhibiting effect on leaf and root length, of particular interest is

the significant reduction in root length of SS plants, which seems to be partially recovered by salt treatment in the root-zone, as shown in 200 mM NaCl + SS plants. The reduction in root length is reflected in SS plants on a higher ratio shoot/root. Salt spray seems therefore to modify the growth habit with a preferential development of the shoot than the root. The lack of a significant difference in chlorophyll content among plants receiving different treatments seems to confirm the partial tolerance of our cultivar to salt treatment, as a reduction of pigment content is often reported as indicative for plants suffering abiotic stress (Jaleel et al., 2008). On the other hand, the increase in carotenoid content detected in treated plants is a response to salt stress also found in other plants (Borghesi et al., 2011). However as changes in Chla/Chlb and Car/Total Chl ratios are stress indicators (Rout and Shaw, 2001), the lack of significant differences in these values among treatments showed only a slight effect of salt on pigments.

Saline conditions are known to induce oxidative stress through over-production of ROS that can cause damage to cellular macromolecules resulting in oxidative stress.

As reported in literature about salt in the root-zone (Chaparzadeh et al., 2004; Kong-ngern et al., 2012), also salt spray exposure was able to induce an increase in hydrogen peroxide content. In conditions of airborne salinity the contents of this signalling molecule were generally higher than under saline irrigation. However, as in the longer period there were not significant differences between CS and SS plants, periodic spraying in itself seems to be detrimental for our cultivar. The contents of H_2O_2 were well correlated with the level of lipid peroxidation both after 2 and 7 days of treatment ($r = 0.94$ and 0.91 respectively) suggesting that salt can induce an oxidative stress ROS-mediated (Hu et al., 2012). In the longer period both hydrogen peroxide content and lipid peroxidation strongly correlated with the leaf Na^+ content ($r = 0.79$ and 0.96 for root-zone salinity and salt spray respectively). The lack of correlation in the shorter period could further indicate a partial tolerance of our cultivar to salt.

To counteract oxidative stress, plants have evolved protective antioxidant systems, including both enzymatic and non-enzymatic molecules. Among the non-enzymatic molecules, ascorbate and glutathione play an important role. They may directly scavenge ROS or they may enter in the ascorbate–glutathione cycle interacting with antioxidant enzymes. In accordance with Chaparzadeh et al. (2004) salt induced an increase in total ascorbate content in the shorter period associated with the even more important increase in ASA/DHA ratio. In the longer period although total ascorbate reached the maximum value in 200 mM NaCl-treated plants, there was a strong decrease in the antioxidant power of this molecule. This was parallel with the increase in oxidative damage as indicated by TBARS content indicative of lipid peroxidation. Interestingly only 50 mM NaCl-treated plants in the longer period were characterised by a high value of ASA/DHA ratio and this saline concentration seems to represent our salt concentration beyond which leaves are no longer able to maintain a strong prevalence of ascorbate in its reduced form. *Triticum* plants contained high levels of glutathione, generally higher in salt-treated leaves, only in the short term, with a significant decrease of this protective molecule after seven days of treatment. The reducing power of glutathione was lower in treated plants than in control ones in particular under saline irrigation. On the whole, under salt treatment our cultivar showed a depletion of the reducing power of both ascorbate and glutathione, underlining the only partial salt tolerance of our cultivar. In salt-treated plants there is a good correlation ($r = 0.94$) between the reducing power of glutathione and the activity of GR, highlighting the importance of this enzyme in maintaining the redox status of this antioxidant molecule in stress conditions.

Proline is a compatible solute that can accumulate in several stress conditions. It contributes not only to osmotic adjustment, but also to protein and membrane protection and quenching of reactive oxygen species (Mudgal et al., 2010). In salt stress conditions, both increase and decrease in proline content are reported (Kong-ngern et al., 2012). In our study, salinity in root-zone and salt spray were able to enhance the level of this protective molecule, airborne salinity inducing the highest contents of this aminoacid. This is in partial contrast with previous results on the coastal plant *Crambe maritima* (De Vos et al., 2010) where salt spray leaves were characterised by a proline content not significantly different from plants subjected to 50 and 100 mM NaCl treatment in the root-zone but significantly lower than in 200 mM NaCl-treated plants. This is a further confirmation of the particular sensitivity of our wheat cultivar to airborne salt.

Low molecular weight antioxidants are complemented in their protective action by antioxidant enzymes. In accordance with literature (Chaparzadeh et al., 2004), in our cultivar there was not a similar trend for all the enzymes and both increases and decreases in activity have been recorded under saline conditions. SOD is able to directly modulate the levels of H_2O_2 and in our durum wheat, airborne salinity had little effect on the activity of this enzyme, while salinity in the root zone lead to a reduction in SOD activity. This is in accordance with data in literature where a decrease in this activity was recorded and could indicate a salt sensitivity of our cultivar (Chaparzadeh et al., 2004). APX, GPX, CAT, POD are all able to scavenge hydrogen peroxide. The activity of APX was generally higher after 7 days than after 2 days of treatment, in accordance with higher contents of H_2O_2 detected in the longer period. At this time, among treatments, the highest activity was recorded in salt spray plants. In the short term airborne salinity induced a significant increase in CAT activity, while salt in the root-zone seemed to have a negative impact on the activity of this enzyme as well evidenced by the activity in 200 mM NaCl + SS leaves, significantly lower than in SS ones. On a time basis, POD and GPX had an opposite trend: while in the shorter term salt induced a decrease in POD and an increase in GPX activity, in the longer term there was an increase in POD and a decrease in GPX activity in comparison with controls. Therefore, the different antioxidant enzymes seem to cooperate in the regulation of hydrogen peroxide content playing roles of varying relevance in different treatments. In the short term airborne and root-zone salinity induced different responses: GPX seemed to play an important role in both treatments, but only in plants subjected to salt spray was there a significant involvement of the scavenging action of APX and in particular CAT. In the long term POD seems to play a crucial role in the antioxidant enzymatic machinery, with significant increase in comparison with the short term.

In conclusion, the durum wheat *T. turgidum* cv. Cappelli is able to activate in saline conditions an antioxidant response, differentially modulated depending on time of treatment and on the type of salt supply. Seedlings show a partial tolerance to salinity as most of the stress indicators reach higher values only in our longer period and in the presence of the highest salt concentrations. In addition, at least part of their tolerance seems to depend also on Na^+ tissue tolerance. When comparing the two types of salt supply, salt spray seems to be more detrimental than root-zone salinity as at the same Na^+ leaf content oxidative damage is significantly higher in plants subjected to airborne salinity. From our preliminary results, the durum wheat cultivar Cappelli, being already particularly sensitive to salt spray from the seedling stage, seems less able to face airborne salinity conditions. It could be interesting to analyze salt spray tolerance also in other cultivars in order to assess if the particular sensitivity to airborne salinity is a common trait in durum wheat.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzym. 105, 121–125.
Amor, N.B., Jimenez, A., Megdiche, W., Lundqvist, M., Sevilla, F., Abdelly, C., 2007. Kinetics of the antioxidant response to salinity in the halophyte Cakile maritima. J. Int. Plant Biol. 49, 1–11.
Arezky, O., Boxus, P., Kevers, C., Gaspar, T., 2001. Changes in peroxidase activity, and level of phenolic compounds during light-induced plantlet regeneration from *Eucalyptus camaldulensis* Dhen. nodes in vitro. Plant Growth Regul. 33, 215–219.
Ashraf, M., McNeilly, T., 1988. Variability in salt tolerance of nine spring wheat cultivars. J. Agron. Crop Sci. 160, 14–21.
Balestri, M., Bottega, S., Spanò, C., 2014. Response of *Pteris vittata* to different cadmium treatments. Acta Physiol. Plant. 36, 767–775.
Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of proline for water stress studies. Plant Soil 39, 205–207.
Benes, S.E., Aragues, R., Grattan, S.R., Austin, R.B., 1996. Foliar and root absorption of Na and Cl in maize and barley: implications for salt tolerance and screening and the use of saline sprinkler irrigation. Plant Soil 180, 75–86.
Beyer, W.F., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161, 559–566.
Borghesi, E., Gonzáles-Miret, M.L., Escudero-Gilete, M.L., Malorgio, F., Heredia, F.J., Meléndez-Martínez, A.J., 2011. Effects of salinity stress on carotenoids, anthocyanins, and color of diverse tomato genotypes. J. Agric. Food Chem. 59, 11676–11682.
Borrelli, G.M., Ficco, D.B.M., Giuzio, L., Pompa, M., Cattivelli, L., Flagella, Z., 2011. Durum wheat salt tolerance in relation to physiological, yield and quality characters. Cereal Res. Commun. 39, 525–534.
Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Canalejo, A., Martinez-Domínquez, D., Córdoba, F., Torronteras, R., 2014. Salt tolerance is related to a specific antioxidant in the halophyte cordgrass, *Spartina densiflora*. Estuar. Coast. Shelf Sci. 146, 68–75.
Chaparzadeh, N., D'Amico, M.L., Khavari-Nejad, R.A., Izzo, R., Navari-Izzo, F., 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol. Biochem. 42, 695–701.
Devitt, D., Jarrell, W.M., Stevens, K.L., 1981. Sodium-potassium ratios in soil solution and plant response under saline conditions. Soil Sci. Soc. Am. J. 45, 80–86.
De Vos, A.C., Broekman, R., Groot, M.P., Rozema, J., 2010. Ecophysiological response of *Crambe maritima* to airborne and soil-borne salinity. Ann. Bot. 105, 925–937.
Dinelli, G., Marotti, I., Di Silvestro, R., Bosi, S., Bregola, V., Accorsi, M., Di Loreto, A., Benedettelli, S., Ghiselli, L., Cationе, P., 2013. Agronomic, nutritional and nutraceutical aspects of durum wheat (*Triticum durum* Desf.) cultivars under low input agricultural management. Italian J. Agron. 8, 85–93.
Gengmao, Z., Quanmei, S., Yu, H., Shihui, L., Changhai, W., 2014. The physiological and biochemical responses of a medicinal plant (*Salvia miltiorrhiza* L.) to stress caused by various concentrations of NaCl. Plos One 9, 1–6.
Gossett, D.R., Millhollon, E.P., Lucas, M.C., 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. 34, 706–714.
Griffiths, M.E., 2006. Salt spray and edaphic factors maintain dwarf stature and community composition in coastal sandplain heathlands. Plant Ecol. 186, 69–86.
Hartley-Whitaker, J., Ainsworth, G., Meharg, A.A., 2001. Copper- and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant, Cell Environ. 24, 713–722.
Hassanzadeh, M., Ebadi, A., Panahyan-e-Kivi, M., Eshghi, A.G., Sh, Jamaati-e-Somarin, Saeidi, M., Zabihi-e-Mahmoodabad, R., 2009. Evaluation of drought stress on relative water content and chlorophyll content of Sesame (*Sesamum indicum* L.) genotypes at early flowering stage. Res. J. Environ. Sci. 3, 345–360.
Hu, W., Yuan, Q., Wang, Y., Cai, R., Deng, X., Wang, J., Zhou, S., Chen, M., Chen, L., Huang, C., Ma, Z., Yang, G., He, G., 2012. Overexpression of a wheat aquaporin gene, TaAQP8, enhances salt stress tolerance in transgenic tobacco. Plant Cell Physiol. 53, 2127–2141.
Jaleel, C.A., Lakshmanan, G.M.A., Gomathinayagam, M., Panneerselvam, R., 2008. Triadimefon induced salt stress tolerance in Withania somnifera and its relationship to antioxidant defense system. South Afr. J. Bot. 74, 126–132.
Jana, S., Choudhuri, M.A., 1982. Glycolate metabolism of three submerged aquatic angiosperm during aging. Aquat. Bot. 12, 345–354.
Kampfenkel, K., Montagu, M.V., Inzé, D., 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Anal. Biochem. 225, 165–167.
Kekere, O., 2014. Responses of *Kyllinga peruviana* Lam. to sea water spray. J. Plant Stud. 3, 30–38.
Kong, X., Luo, Z., Dong, H., Eneji, A.E., Li, W., 2011. Effects of non-uniform root zone salinity on water use, Na^+ recirculation, and Na^+ and H^+ flux in cotton. J. Exp. Bot. 63, 2105–2116.
Kong-ngern, K., Bunnag, S., Theerakulpisut, P., 2012. Proline, hydrogen peroxide, membrane stability and antioxidant enzyme activity as potential indicators for salt tolerance in rice (*Oryza sativa* L.). Int. J. Bot. 8, 54–65.
Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzym. 148, 350–382.
Mudgal, V., Madaan, N., Mudgal, A., 2010. Biochemical mechanisms of salt tolerance in plants: a review. Int. J. Bot. 6, 136–143.
Munns, R., James, R.A., 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant Soil 253, 201–218.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Navari-Izzo, F., Meneguzzo, S., Loggini, B., Vazzana, C., Sgherri, C.L.M., 1997. The role of the glutathione system during dehydration of Boea hygroscopica. Physiol. Plant. 99, 23–30.
Płażek, A., Tatrzańska, M., Maciejewski, M., Kościelniak, J., Gondek, K., Bojarczuk, J., Dubert, F., 2013. Investigation of the salt tolerance of new Polish bread and durum wheat cultivars. Acta Physiol. Plant. 35, 2513–2523.
Rao, M.V., Beverley, A.H., Ormrod, D.P., 1995. Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide. Role of antioxidant enzymes. Plant Physiol. 109, 421–432.
Rajendran, K., Tester, M., Roy, S.J., 2009. Quantifying the three main components of salinity tolerance in cereals. Plant Cell Environ. 32, 237–249.
Rejili, M., Vadel, A.M., Guetat, A., Neffati, M., 2006. Effect of NaCl on the growth and the ionic balance K^+/Na^+ of two populations of *Lotus creticus* (L.) (Papillionaceae). Lotus News Lett. 36, 34–53.
Rout, N.P., Shaw, B.P., 2001. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci. 160, 415–423.
Rozema, J., Bijwaard, P., Prast, G., Broekman, R., 1985. Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. Vegetatio 62, 499–521.
Scheiber, S.M., Sandrock, D., Alvarez, E., Brennan, M.M., 2008. Effect of salt spray concentration on growth and appearance of 'Gracillimus' maiden grass and 'Hamelin' fountain grass. Hort. Tecnol. 18, 34–38.
Spanò, C., Bruno, M., Bottega, S., 2013. *Calystegia soldanella*: dune versus laboratory plants to highlight key adaptive physiological traits. Acta Physiol. Plant. 35, 1329–1336.
Spanò, C., Buselli, R., Grilli, I., 2008. Dormancy and germination in wheat embryos: ribonucleases and hormonal control. Biol. Plant. 52, 660–667.
Thomas, G.V., 1982. Exchangeable cations. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2 Chemical and Microbiological Methods. ASA Monograph, Wisconsin, USA, pp. 159–165. Madison.