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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