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Research article

# Salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance: *In planta* evidence for cross-tolerance

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ABSTRACT

Salinity tolerance in plants is dependent on their abilities to adjust osmotically to reduced soil water potential and to keep intracellular ROS levels under control. Both these processes are believed to rely on de novo synthesis of organic osmolytes (traditionally defined as *compatible solutes*). However direct *in planta* evidence for anti-oxidant roles of compatible solutes are scarce. In this work, we induced changes in the level of endogenous organic osmolytes by exposing plants to various levels of NaCl (salinity stress; 50–300 mM range) and then studying sensitivity of leaves to oxidative (UV–B) stress. Increase in the external NaCl concentrations was accompanied by the progressive accumulation in leaf $Na^+$. This accumulation was much higher in old leaves compared with young ones. In old leaves, three major inorganic ions ($Na^+$, $Cl^-$ and $K^+$) have made 67.7% and 70.4% of leaf osmotic potential (in wheat and barley, respectively) when exposed to 200 mM NaCl treatment, while in young leaves their contribution was only 43.9% and 46.8%, respectively. Here, organic osmolytes played a substantial role in leaf osmotic adjustment. Increased accumulation of organic osmolytes correlated strongly with activity of PSII in leaves exposed to oxidation inducing UV-B treatment in both species ($R^2 = 0.50$ for wheat and 0.71 for barley). We conclude that salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance and provides the evidence for a mechanism of cross-tolerance between these two stresses.



## 1. Introduction

Compatible solutes (CS) are organic compounds with low molecular weight and good solubility in water, which cells accumulate in response to osmotic stress and which are defined by having a supposedly minimal disturbance to metabolic processes (hence the adjective 'compatible') (Brown and Simpson, 1972). As a result, CS can make a significant contribution to plant osmotic potential and are often referred to in the literature as organic osmolytes. CS are chemically diverse and include four major groups: sugars, polyols, amino acids, and quaternary ammonium compounds (Delauney and Verma, 1993). The levels of compatible solutes are highly variable and are significantly increased in response to environmental stresses, including salinity (Bohnert et al. 1995; Hasegawa et al. 2000; Chen and Murata, 2008).

While it is obvious from four decades of research that CS can play an important role in resistance to a wide range of abiotic stresses (Hasegawa et al. 2000; Yancey, 2005), specific mechanisms beyond this phenomenon are still not fully understood. Putative mechanisms include contribution to osmotic adjustment (Colmer et al. 1995), stabilising proteins during destabilising stresses (Papageorgiou and Murata, 1995; Yancey, 2005), stabilising lipid membranes subjected to dehydration (Hincha et al. 2003), suppressing photorespiration (Sivakumar et al. 2002), maintaining redox balance (Yancey, 2005), scavenging free radicals (Smirnoff and Cumbes, 1989), binding toxic metals (Sharma and Dietz, 2006), maintaining intracellular potassium homeostasis (Cuin and Shabala, 2007a,b), acting as antifreeze (Yancey, 2005), and activating transcription factors responsible for stress responses (Gupta et al. 2012).

Plants in hostile environments experience a rapid and significant increase in reactive oxygen species (ROS) production (Miller et al. 2010). This increase is observed in response to literally every known abiotic and biotic stress, including salinity (Munns,

*Abbreviations:* ROS, reactive oxygen species; LSD, Least Significance Difference; CS, compatible solutes; MDA, Malondialdehyde.

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2002; Gagneul et al. 2007; Daneshmand et al. 2010). At the same time, salinity-induced increase in the level of CS is also a widely reported phenomenon, and it was frequently suggested that the latter is essential for alleviation or prevention of oxidative stress (Akashi et al. 2001; Liu et al. 2011; Duan et al. 2012). However, direct *in planta* evidence are scarce. Most reported evidence comes from either *in vitro* studies (Smirnoff and Cumbes, 1989; Akashi et al. 2001), or from experiments with transgenic plants in which plant ability to produce CS was increased many folds via genetic engineering (Kishor et al. 1995; Sheveleva et al. 1997). In the former case, the levels of CS used are often non-physiologically high, while in the case of transgenic plants numerous pleiotropic effects (Romero et al. 1997; Knipp and Honermeier, 2006; Dörffling et al. 2009) as well as unrealistically high CS levels (Nishizawa et al., 2008; He et al., 2008) leave some doubt regarding a direct causal relationship between increased production of CS and oxidative stress tolerance. Thus, the role of CS in oxidative stress tolerance under physiologically relevant conditions is yet to be proven.

A recent publication from our laboratory (Shabala et al. 2012) showed that the extent of the UV-B damage to the photosynthetic machinery in a halophyte species *Chenopodium quinoa* was much less in young leaves compared with old ones. The observed protective effect in the young leaves was attributed to the much larger pool of organic osmolytes. As halophytes are rather special cases, in the present work we wanted to investigate whether similar mechanisms are also at work in two glycophyte crop species: wheat and barley. Our overall mechanistic hypothesis was that in glycophytes endogenous organic osmolytes would protect photosynthetic machinery from oxidative stress damage (caused by UV-B in this study), and that this effect would be more pronounced in young leaves, (which accumulate more CS), than in old leaves. By exposing plants to various levels of salinity, we have modulated the level of endogenous organic osmolytes and, thus, were also able to investigate if the protective effects of endogenous CS were dose-dependent. The main objective of this work was two-fold: (1) investigate the role of compatible solutes in oxidative stress tolerance in glycophytes, and (2) provide *in planta* evidence for a possibility of cross-tolerance between salinity and oxidative stresses.

We now show that in both species, increased accumulation of organic osmolytes correlated strongly with activity of PSII in young leaves exposed to oxidative (UV–B) treatment ($R^2$ 0.5 for wheat and 0.71 for barley), but not in old leaves. We conclude that salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance and thus provides evidence for a mechanism of cross-tolerance between these two stresses.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Wheat (*Triticum* spp. cv. Brennan) and barley (*Hordeum vulgare* cv. Gairdner) were used in experiments. The cultivar Brennan was bred by CSIRO from a cross between Mercia and Hartog that was back crossed to Mercia. The barley variety Gairdner is a two-row malting barley bred by Agriculture Western Australia. Plants were grown from seeds in 1.5 L pots using the standard potting mix (Hariadi et al. 2011) under natural light in a temperature-controlled glasshouse (maximum day/night temperatures during growth season 28/18 °C; average humidity 65%) at the University of Tasmania (Hobart, Australia). The experiment was repeated twice, in July–Sept 2012 and 2013, with consistent results. The natural day length varied between ~9.5 h (July) to 11.5 h (September) and was extended to 14 h by using incandesced lamps in the glasshouse. Control plants were irrigated with tap water twice per day. Salinity treatment was applied to plants by hand-watering with appropriate NaCl solution (50, 100, 150, 200 and 300 mM) on a daily basis. Salt accumulation in the soil was avoided by allowing excess irrigation water to drain out of the pots (monitored by periodic measurements of soil EC). Salinity stress commenced when plants were at the second leaf stage and lasted for 35 days.

### 2.2. UV-B treatment

At the end of the experiment, young and old leaves were excised, and 30–40 mm long segments were placed in closed Petri dishes on top of wetted filter paper. Young leaves were taken from the growing tip of the plant and were the smallest leaves that could be measured with the fluorometer. Old leaves were chosen for being the lowest non-senesced leaves. Leaves were brought into a temperature-controlled room (24 °C) and exposed to two levels of light treatment for 20 h. Control leaves were exposed to white light of 150 μmol $m^{-2}$ $s^{-1}$ provided by fluorescent tubes (Sylvania GRO-LUX F36W; Sylvania, Danvers, MA) while other leaves were subjected to oxidative stress by exposing them to UV-B light (312 nm) in addition to the white light. UV-B light came from two Vilber-Lourmat T-20M UV-B tubes (Vilber-Lourmat GmbH, Eberhardzell, Germany) set 20 cm away from the leaves. In a separate set of experiments, the above UV-B treatment was also applied to intact leaves (i.e. attached to plants growing in pots) in the same way as described above. The length of treatment (20 h) was chosen for being long enough to cause oxidative stress to leaves, without being protracted enough to cause any leaf browning or severe desiccation of excised leaves. Methodological experiments have suggested that the impact of UV treatment on chlorophyll fluorescent characteristics was not related to circadian clock, as it was the overall UV dose but not duration of exposure that mattered (data not shown). The differing light times during growth phases and treatment phases are therefore of no consequence.

### 2.3. Chlorophyll fluorescence measurements

The relative effects of the UV-B were quantified by comparing maximum photochemical efficiency of PSII, measured as chlorophyll fluorescence Fv/Fm ratio using an Optiscan OS-3OP fluorometer (Optiscience, Hudson, NH) at the end of the 20 h of light treatment. Leaves were adapted to darkness for 15 min prior to measuring. At least five leaves were measured for each treatment, and each leaf was measured at two sites, giving a minimum of 10 readings for each treatment.

### 2.4. Measurement of leaf osmolality, and potassium, sodium and chloride contents

A minimum sample of 5 old leaves and 5 young leaves were taken from each combination of salt and light treatment. Representative leaf samples were taken from the petri dishes promptly at the end of the 20 h light treatment period and frozen at −20 °C in centrifuge tubes. Close to the time of measurement, frozen samples were thawed for 10 min and squeezed to extract sap as described in Cuin et al., 2009. An amount of 10 μL sap was taken from each sample for measuring leaf osmolality using a vapour pressure osmometer (Vapro model 5520, Wescor Inc., Logan, Utah). A further 20 μL of sap was taken from each sample and appropriately diluted with distilled water to measure $Na^+$ and $K^+$ content using a flame photometer (Bibby Scientific Limited, Staffordshire, UK). Chloride concentrations were estimated as 1.3-fold of the measured sodium concentration, as described by James et al. (2006). As wheat plants were dead by the end of experiment when exposed to the highest (300 mM NaCl) treatment, sap extraction from these leaves was not