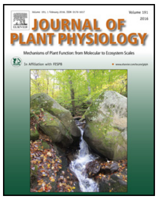
Journal of Plant Physiology 201 (2016) 1–8

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph



Physiology

Acclimation improves salt stress tolerance in *Zea mays* plants

Camilla Pandolfi[a,b,*], Elisa Azzarello[b], Stefano Mancuso[b], Sergey Shabala[a]

[a] *School of Land and Food, University of Tasmania, Private Bag 54, Hobart, Tas 7001, Australia*
[b] *Department of Agrifood and Environmental Science, University of Florence, Viale delle Idee 30, 50019 Sesto Fiorentino, FI, Italy*

ARTICLE INFO

Article history:
Received 6 March 2016
Received in revised form 15 June 2016
Accepted 16 June 2016
Available online 23 June 2016

Keywords:
Acclimation
Ion channels
Priming salinity
Zea mays
Vacuolar sequestration

ABSTRACT

Plants exposure to low level salinity activates an array of processes leading to an improvement of plant stress tolerance. Although the beneficial effect of acclimation was demonstrated in many herbaceous species, underlying mechanisms behind this phenomenon remain poorly understood. In the present study we have addressed this issue by investigating ionic mechanisms underlying the process of plant acclimation to salinity stress in *Zea mays*. Effect of acclimation were examined in two parallel sets of experiments: a growth experiment for agronomic assessments, sap analysis, stomatal conductance, chlorophyll content, and confocal laser scanning imaging; and a lab experiment for *in vivo* ion flux measurements from root tissues. Being exposed to salinity, acclimated plants (1) retain more K$^+$ but accumulate less Na$^+$ in roots; (2) have better vacuolar Na$^+$ sequestration ability in leaves and thus are capable of accumulating larger amounts of Na$^+$ in the shoot without having any detrimental effect on leaf photochemistry; and (3) rely more on Na$^+$ for osmotic adjustment in the shoot. At the same time, acclimation affect was not related in increased root Na$^+$ exclusion ability. It appears that even in a such salt-sensitive species as maize, Na$^+$ exclusion from uptake is of a much less importance compared with the efficient vacuolar Na$^+$ sequestration in the shoot.

1. Introduction

Salt stress in plants is one of the main causes limiting agricultural productivity in the world's irrigated land. A way to tackle the problem is to try to enhance plant tolerance to salt stress by understanding basic natural mechanisms that naturally occur in plants under changing environmental conditions. As such, acclimation to external environmental changes occurs in plants thanks to internal adjustments within tissues and cells, enabling cell metabolism to proceed under these somewhat altered conditions (Demmig-Adams et al., 2008). It was reported that salt tolerance of many plant species can be increased by previous exposure to a low level of stress for a certain period of time (Amzallag et al., 1990; Bethke and Drew, 1992; Umezawa et al., 2000; Silveira et al., 2001; Djanaguiraman et al., 2006). Reported beneficial effects included improved survival, growth rate and yield (Amzallag et al., 1990; Djanaguiraman et al., 2006). However, the physiological mechanisms beyond this acquired resistance have not been clearly elucidated. Umezawa et al. (2000) related the better performance of acclimated soybean to a reduced accumulation of Na$^+$ in plant *leaves*, whereas Saha et al. (2010) and Ottow et al. (2005) related it to an improvement in the ability to withstand osmotic stress. At a cellular level, salinity stress can be distinguished between its ionic and osmotic component thanks to the work of Munns (1993) who developed a model for the whole-plant level. In our recent work on peas (Pandolfi et al., 2012) we have shown that acclimation in non-ionic (ie polyethylene glycol) isotonic media was not as efficient as in NaCl, suggesting that acclimation to salinity is related to the ion-specific rather than the osmotic component. Furthermore, metabolic acclimation *via* previous exposure to a low level of salinity was induced primarily in roots and was related to a better regulation of xylem ion loading (Pandolfi et al., 2012).

One of the hallmarks of detrimental effects of salinity at the tissue level is K$^+$ efflux from plant roots (Shabala and Cuin, 2008; Cuin et al., 2012; Wu et al., 2013) *via* both depolarization-activated outward-rectifying K$^+$ (KOR; Chen et al., 2007) and ROS-activated non-selective cation (NSCC; Bose et al., 2014) channels. This efflux disturbs cytosolic K$^+$ homeostasis (Cuin et al., 2003), with major implications to cell metabolism and its fate (*e.g.* transition to programmed cell death; Shabala et al., 2007; Shabala and Pottosin, 2014). For this reason, a strong correlation between plant's K$^+$

Abbreviations: DW, dry weight; E_K, equilibrium potential; FW, fresh weight; Gs, stomatal conductance; KOR, outwardly rectifying potassium channel; MIFE, microelectrode ion flux estimation technique; NHX, vacuolar Na+/H+ antiporters; NSCC, non-selective cation; SOS1, salt overlay sensitive antiporters 1; PM, plasma membrane; PCD, programmed cell death.

* Corresponding author at: Department of Agrifood and Environmental Science, University of Florence, Viale delle Idee 30, Sesto Fiorentino, FI, 50019, Italy
E-mail address: camilla.pandolfi@unifi.it (C. Pandolfi).

http://dx.doi.org/10.1016/j.jplph.2016.06.010
0176-1617/

retention ability and salt tolerance has been observed in both root (Chen et al., 2007; Cuin et al., 2008; Smethurst et al., 2008) and leaf (Wu et al., 2013) tissues in several species. Another key determinant of salinity tolerance is Na$^+$ exclusion from the cytosol. Na$^+$/H$^+$ antiporters are thought to drive the active transport of Na$^+$ out of plant cells (Apse and Blumwald, 2007), either back to external media, or into vacuole. Overexpression of the plasma membrane Na$^+$/H$^+$ antiporter SOS1 has been found to reduce Na$^+$ accumulation and improve salinity tolerance in transgenic *Arabidopsis* (Shi et al., 2003), while efficient sequestration of Na$^+$ in the vacuoles by means of Na$^+$/H$^+$ antiporters from the NHX family was also essential to confer salinity tolerance in a range of plant species (Apse et al., 1999). In the latter case, in addition to avoiding accumulation of toxic Na$^+$ in the cytosol, vacuolar Na$^+$ sequestration also contributes to the turgor maintenance (Zhang et al., 2001; Yokoi et al., 2002). Compartmentalization of sodium in the vacuole has been reported as one of the clue to salt adaptation (Munns and Tester, 2008). Vacuolar NHX proteins (NHX1 and NHX2; NHX = Na$^+$/H$^+$ exchanger) are considered the main players in sodium compartmentalization in the vacuoles (Apse et al., 1999, 2003; Blumwald, 2000). More recently, vesicle trafficking has been described as a contributor for sodium compartmentation (Liu et al., 2007; Hamaji et al., 2009; Qiu, 2012). Control of Na$^+$-permeable slow (SV) and fast (FV) vacuolar channels is also essential for effective Na$^+$ retention in vacuole (Bonales-Alatorre et al., 2013).

The aim of this study was to reveal the role and relative contribution of the ionic mechanisms that play a role in plant acclimation to salinity. This was achieved by a whole-plant physiological assessment of plants pre-treated with NaCl and by studying patterns of ion flux across cellular membranes in salt-exposed acclimated and non-acclimated roots. In addition, we aimed to see if acclimation effect reported earlier for C3 *Pisum sativum* species (Pandolfi et al., 2012) could be also observed in more tolerant C4 *Zea mays* plants, where Na$^+$ is considered to be a beneficial nutrient (Subbarao et al., 2003). Our results suggest that exposing *Zea mays* to moderate salinity activates a set of physiological adjustments enabling plants to withstand severe saline conditions, and that it is the acclimation to the ion toxicity component of salt stress that play a major role in plant acclimation. This acclimation takes place in both root and shoot tissues. At a root level, it involves better potassium retention and, as a result, a better control of intracellular K/Na ratio. In leaves, acclimation results in a better sequestration of sodium in the vacuoles. The implications for this results will be discussed the in the following paragraphs.

2. Materials and methods

2.1. Growth experiment

Maize plants (*Zea mais L* cv B73; a kind gift of Dr Trevor Garnett, Univ Adelaide) were grown from seeds between November and December 2010. Seeds were placed in 4 l plastic pots, 4 seeds for each pot, in a standard potting mixture (70% composted pine bark; 20% coarse sand; 10% sphagnum peat; Limil at 18 kg m^{-3}; and dolomite at 18 kg m^{-3}). Plant nutrient balance was maintained by adding the slow release Osmocote Plus^{TM} fertilizer (at 6 kg m^{-3}) plus ferrous sulphate (at 500 g m^{-3}). Plants were grown under ambient light in a temperature-controlled glasshouse (day/night temperature 26 °C/19 °C; average humidity at 65%) at the University of Tasmania (Hobart, Australia). Plants were hand watered on a daily basis to achieve full water-holding capacity and leach out any possible salt accumulating in root rhizosphere to ensure uniform and constant EC values in soil solution (tested by periodic measurements of soil electric conductivity; data not shown). Seedlings were established under control conditions (no salt) until 10 days old; then 4 of the 12 pots were irrigated with a 25 mM NaCl solution for one week (Fig. 1). These plants are referred as "acclimated" in this study. After one week of acclimation period, these pots were irrigated with a solution containing 100 mM NaCl, for two weeks, alongside with 4 non- acclimated pots. The plants were irrigated with the final concentration of 100 mM NaCl without any progressive increments, to mimic conditions observed in the field brought by raising saline water tables. The remaining pots were irrigated daily with water and used as control. At the beginning of acclimation plants' height was 12–15 cm, and they had three fully developed leaves. The three different sets of plants were termed as follow: Control (non-acclimated, non-stressed), NA (non-acclimated, stressed), A(25) (acclimated, stressed). The salinity levels were chosen on the base of the following consideration. Pre-treatment with 25 mM was selected to exclude the possibility of (i) a strong reduction of growth during the acclimation period, in order to have acclimated and not-acclimated plants of comparable sizes at the start of the salinity treatment; and (ii) on the basis of our previous experiment of the salt-sensitive *Pisum sativum* (Pandolfi et al., 2012), in which two pre-treatments were tested (10 mM and 25 mM) and only the lower one triggered a beneficial reaction in the acclimated plants. The final salinity treatment was set at 100 mM to ensure a significant reduction of the growth as reported in previous experiments (*e.g.* Rodríguez et al., 1997).

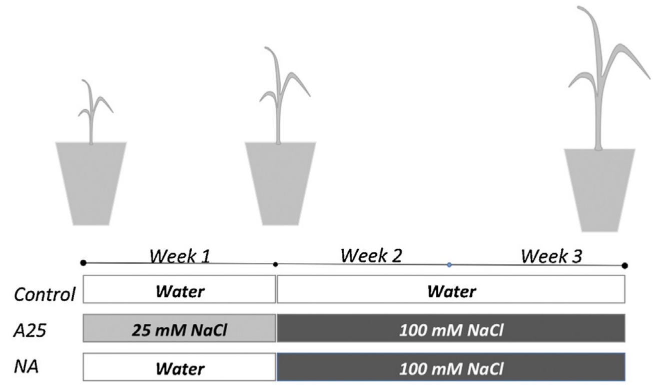


Fig. 1. Experimental procedure for the acclimation experiment. Seedlings were established under control conditions (no salt) until 10 days old; then a four pots were irrigated with a 25 mM NaCl solution for one week (A25) and after one week, were irrigated with a solution containing 100 mM NaCl, for two weeks, alongside with non-acclimated pots (NA). The remaining pots were irrigated daily with water and used as control.

2.2. Agronomical assessment

Eight plants were harvested for each treatment at the end of acclimation (day 17) and at the end of NaCl stress period (day 31). Plants were divided into leaves and roots, and their fresh weight (FW) was measured. Samples were then dried at 70 °C for 72 h, and their dry weight (DW) then determined.

2.3. Sap analysis for K$^+$, Na$^+$ and osmolarity

For each plant, the third from the bottom (fully expanded but not senescing) leaf was collected at the end of acclimation and treatment periods. Root samples were also collected by rinsing them thoroughly in 10 mM CaCl$_2$ for 2 min to remove apoplastic NaCl and then blotting them dry with paper towels. Samples were collected in Falcon tubes and stored at −20 °C. Leaf and root sap was extracted using the freeze-thaw method as described in (Cuin et al., 2010) and its osmolarity was determined using a vapour pressure osmometer (Vapro, Wescor Inc Logan, Utah, USA). For the determination of Na$^+$ and K$^+$ contents, samples were diluted 1:50 and