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Enhanced oxidative stress in the ethylene-insensitive (*ein3-1*) mutant of *Arabidopsis thaliana* exposed to salt stress

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ABSTRACT

To better understand the role of ethylene signaling in plant stress tolerance, salt-induced changes in gene expression levels of ethylene biosynthesis, perception and signaling genes were measured in *Arabidopsis thaliana* plants exposed to 15 days of salinity. Among the genes analyzed, *EIN3* showed the highest expression level increase under salt stress, suggesting a key role for this ethylene-signaling component in response to salt stress. Therefore, we analyzed the salt stress response over 15 days (by adding 100 mM NaCl to the nutrient solution) in the *ein3-1* mutant compared to the wild-type (Col-0) in terms of growth, oxidative stress markers (lipid peroxidation, foliar pigments and low-molecular-weight antioxidants) and levels of growth- and stress-related phytohormones (including cytokinins, auxins, gibberellins, abscisic acid, jasmonic acid and salicylic acid). The *ein3-1* mutant grew similarly to wild-type plants both under control and salt stress conditions, which was associated with a differential time course evolution in the levels of the cytokinins zeatin and zeatin riboside, and the auxin indole-3-acetic acid between the *ein3-1* mutant and the wild-type. Despite showing no signs of physiological deterioration under salt stress (in terms of rosette biomass, leaf water and pigment contents, and PSII efficiency) the *ein3-1* mutant showed enhanced lipid peroxidation under salt stress, as indicated by 2.4-fold increase in both malondialdehyde and jasmonic acid contents compared to the wild-type. We conclude that, at moderate doses of salinity, partial insensitivity to ethylene might be compensated by changes in endogenous levels of other phytohormones and lipid peroxidation-derived signals in the *ein3-1* mutant exposed to salt stress, but at the same time, this mutant shows higher oxidative stress under salinity than the wild-type.

Introduction

Acclimation of plants to changes in their environment requires a new state of cellular homeostasis achieved by a delicate balance between multiple pathways that reside in different cellular compartments. This coordination may, however, be disrupted during salt stress, especially when the cell or the entire plant is exposed to a rapid decrease in water potential, or when additional environmental parameters are involved (Miller et al., 2010). Damage caused by high salinity is often associated with three different mechanisms (Abdelly et al., 2008). First, ion toxicity is caused by excessive accumulation of Na^+ and Cl^- in the cytoplasm, leading to an ionic imbalance. This can be counteracted by an increased transport intensity of the ions to the vacuole. Second, even if massive ion compartmentalization occurred in the vacuole, the cytosol water potential must be lowered to balance a low external water potential, allowing water intake in plant cell and preventing macromolecule damage. Third, a high cellular NaCl concentration causes increased formation of reactive oxygen species (ROS), which is considered to be the primary event under a variety of stress conditions. It has been generally accepted that enhanced production of ROS during stress can disturb cellular redox homeostasis, by enhancing oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damage (Bartels and Sunkar, 2005; Miller et al., 2010).

Ethylene, the simplest unsaturated hydrocarbon, regulates many diverse metabolic and developmental processes in plants, ranging from seed germination to senescence, and is considered to play a major role as a signal molecule in the tolerance of several species to environmental constraints, including salt stress (for review, see Bleecker and Kende, 2000; Xu et al., 2008). The use of different experimental approaches, such as (i) exogenous

Abbreviations: ABA, abscisic acid; DHA, dehydroascorbate; IAA, indole-3-acetic acid; JA, jasmonic acid; MDA, malondialdehyde; RWC, relative leaf water content; SA, salicylic acid; Z, zeatin; ZR, zeatin riboside.

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application of ethylene precursors or ethylene-enriched atmospheres (Munné-Bosch et al., 2004; Wang et al., 2009), (ii) determination of endogenous concentrations of ethylene prior to and during a salt stress period (Kukreja et al., 2005; Zapata et al., 2007), and (iii) the use of transgenic plants and mutants (He et al., 2005; Cao et al., 2006, 2007), have provided evidence that ethylene is involved in the regulation of plant tolerance to salt stress. However, the role of ethylene signal transduction in response to abiotic stress, and particularly salt stress, remains to be completely understood.

Based on the mutant analysis the triple response of etiolated seedlings treated with ethylene, an ethylene signal transduction pathway has been proposed in *Arabidopsis thaliana* that involves five ethylene receptors, including ETR1, ERS1, ETR2, EIN4 and ERS2, as well as other important signaling components, such as CTR1, EIN2, and EIN3 (Bleecker and Kende, 2000; Guo and Ecker, 2004; Chen et al., 2005; Kendrick and Chang, 2008). The *EIN3* gene is an important component of ethylene signaling that encodes a transcription factor that is required, together with EIL1, for full activation of ethylene responses (Chao et al., 1997; Solano et al., 1998; An et al., 2010). The *ein3-1* loss-of-function mutant of *A. thaliana* displays partial insensitivity to ethylene, and *EIN3* has been shown to participate in plant responses to salt stress (Cao et al., 2007). Cao et al. (2007) showed that an alteration in receptor function or the loss of *EIN2* function may lead to salt-sensitive responses. However, a mutation in *EIN3* did not lead to the same phenotype, despite the *ein3-1* mutant showing increased electrolyte leakage compared to the wild-type under salt stress (Cao et al., 2007). Unfortunately, it is still unknown whether such changes in electrolyte leakage are associated with increased oxidative stress, nor what mechanisms are involved in conferring salt stress tolerance in the *ein3-1* mutant.

To better understand the role of ethylene signaling in the tolerance to salt stress, our aim was to evaluate the effects of the loss of *EIN3* function on salt stress tolerance in *A. thaliana*. In particular, we examined the possible compensatory roles of other phytohormones and oxidative stress-derived signals in the *ein3-1* mutant.

Materials and methods

Plant material and treatments

Seedlings of the *Arabidopsis thaliana* Columbia ecotype (Col-0) wild-type plants and the *ein3-1* mutant, which shows reduced responsiveness to ethylene (AT3G20770, N8052, Chao et al., 1997) were used in the present study. Plants were grown in separate pots containing a mixture of peat/perlite/vermiculite (1:1:1 by volume) in a constant-environment chamber (8-h photoperiod, 90–110 μmol quanta m^{-2} s^{-1}, air temperature 21–23 °C) and were irrigated with Hoagland solution. Wild-type and *ein3-1* plants were placed on different trays and mixed following a randomized distribution. After 45 days from seed germination, plants were assigned to control (irrigation with Hoagland solution) or salt stress (irrigation with Hoagland solution with an addition of 100 mM NaCl) treatments. Irrigation was applied every 3 days; one day after irrigation, the remaining solution was removed from each tray to prevent salt precipitation. Control plants were irrigated following the same system as salt-stressed plants. Plants remained in the vegetative (pre-reproductive) state during the whole experiment. With these plants, two different experiments (samplings) were performed including:

(i) *Experiment 1*. To evaluate expression levels of ethylene biosynthesis, perception and signaling genes in wild-type plants under salt stress, a sampling was performed at day 15 after the start of treatments (control and salt stress).

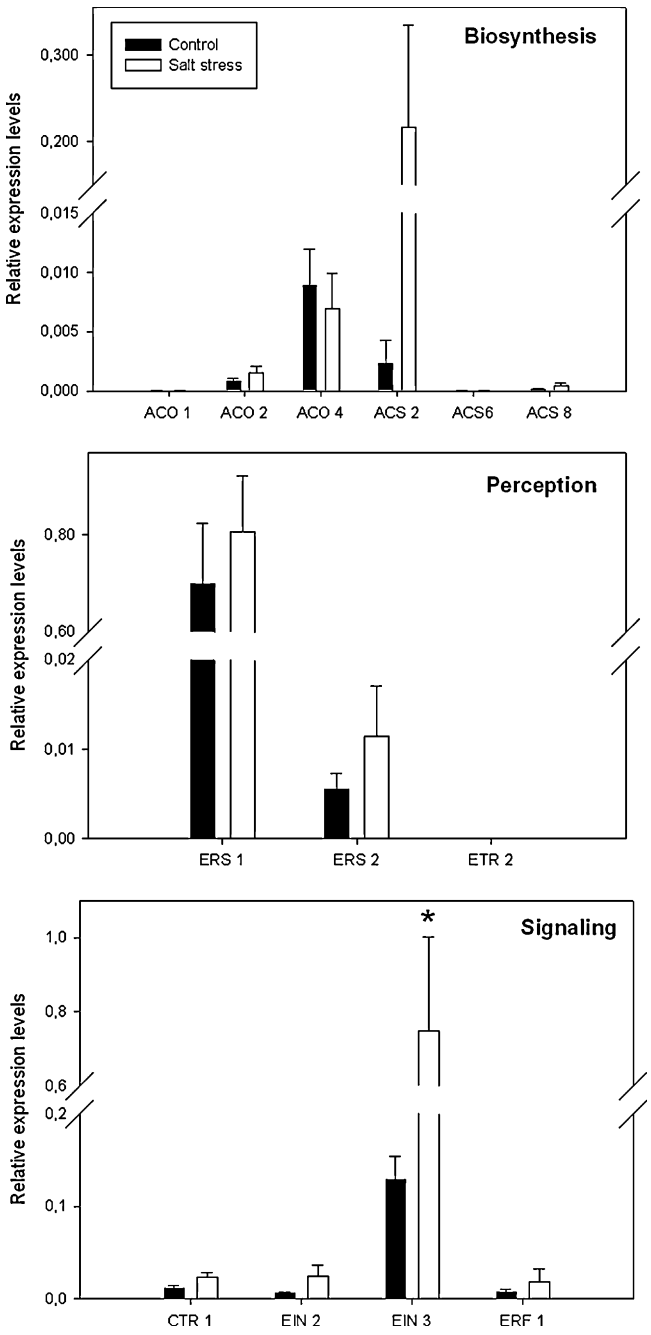

Fig. 1. Changes in the relative gene expression of ethylene biosynthesis, perception and signaling genes in wild-type plants exposed to 15 days of salt stress. Data represent the mean ± S.E. of 4 individuals. An asterisk indicates significant differences in gene expression between control and salt stress (Student's *t*-test, $P \leq 0.05$). Values are given relative to the reference gene *GAPDH* (AT1G13440.1), which encodes for glyceraldehyde 3P dehydrogenase.

(ii) *Experiment 2*. To evaluate the salt stress response of *ein3-1* mutants in terms of growth and phytohormones, samplings were performed at day 0 (immediately before the first treatment irrigation), and 6, 11 and 15 days after the start of treatments. Moreover, we evaluated the salt stress response of *ein3-1* mutants in terms of oxidative stress and *ERF1* gene expression with a sampling performed at day 15 after the start of treatments (control and salt stress).

In all cases, measurements were made in the middle of the photoperiod, except for F_v/F_m, which was measured at pre-dawn