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Short Communication

The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance

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ABSTRACT

The plant hormones abscisic acid (ABA) and auxin (IAA, IBA) play important roles in plant responses to environmental stresses such as salinity. Recent breeding improvements in terms of salt resistance of maize have lead to a genotype with improved growth under saline conditions. By comparing this salt-resistant hybrid with a sensitive hybrid, it was possible to show differences in hormone concentrations in expanding leaves and roots. In response to salinity, the salt-resistant maize significantly increased IBA concentrations in growing leaves and maintained IAA concentration in roots. These hormonal adaptations may help to establish favorable conditions for growth-promoting agents such as β-expansins and maintain growth of resistant maize hybrids under salt stress. Moreover, ABA concentrations significantly increased in resistant maize leaves under salt stress, which may contribute to acidifying the apoplast, which in turn is a prerequisite for growth.

Introduction

Salinity is one of the most common environmental stress factors in modern agriculture, having detrimental effects on plant growth and development (Munns and Tester, 2008). Phytohormones are of great significance for the plant's stress responses and adaptation. Maize as one of the most important crop plants is considered as a salt sensitive species compared to barley or cotton (Maas et al., 1983). Recently, a more salt-resistant hybrid (SR03) was developed by combining a sodium-excluding inbred line with an osmotic-resistant inbred line (Schubert et al., 2009). The newly bred salt-resistant SR hybrid is able to maintain growth and reproduction under moderate or high salinity treatments. Moreover, the salt-resistant hybrid maintained the expression of growth-promoting agents such as β-expansins, whereas the abundance of β-expansins was decreased in the salt-sensitive maize hybrid under salt stress (Geilfus et al., 2010, 2011). By comparing this salt-resistant hybrid with a salt-sensitive hybrid (Lector, Limagrain, Edemissen, Germany), it was possible to investigate differences in hormonal responses and to correlate these differences to salinity resistance.

To date, the role of auxin in mediating cell wall expansion and growth under conditions of NaCl stress is not clear. Following the acid growth theory, an auxin-mediated acidification of the leaf apoplast is the major requirement for an increase in wall extensibility (Hager et al., 1971). Auxin is assumed to activate the proton pump of the plasma membrane pumping protons from the cytosol into the apoplast, resulting in wall loosening and cell expansion (Moriau et al., 1999).

The physiological mechanisms underlying the leaf-growth inhibition that occurs immediately in response to the salt outside the roots are not fully understood (Munns and Tester, 2008). Although the ability of the plant to take up water is reduced (Munns et al., 2006), the cell turgor is not the growth-limiting factor (DeCosta et al., 2007). Moreover, it has been demonstrated that shoot turgor and growth are maintained in salt-resistant hybrids under salt stress through significant increases in osmolality of the shoot sap (DeCosta et al., 2007). In this context, DeCosta et al. (2007) discussed a possible growth promoting effect under the control of different auxin concentrations as responsible for enhanced salt resistance (osmotic part) for the salt-resistant SR3 hybrid. Indole-3-butyric acid (IBA) is an endogenous auxin that can be found in many plant species such as *Zea mays* (Epstein and Ludwig-Müller, 1993). Several physiological effects of IBA may be mediated by the hormone itself (Ludwig-Müller et al., 1997), but there is also evidence that IBA acts via the conversion to IAA by β-oxidation (Zolman and Bartel, 2004). Indole-3-acetic acid (IAA) is an auxin that also may

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contribute to maintenance of growth in resistant maize genotypes under salinity by the mechanism described above.

The phytohormone abscisic acid (ABA) plays a major role in signaling and adaptation to abiotic stresses such as water, drought, and salt stress (Leung and Giraudat, 1998). Salt stress is reported to cause apoplastic accumulation of ABA during growth of tobacco cells (Singh et al., 1987). Root growth under stress involves ABA (Sharp and LeNoble, 2002). Endogenous ABA strongly accumulates in maize roots two hours after addition of 200 mM NaCl, but rapidly declines in plants, indicating an early recovery from water deficit (Zhu et al., 2005). Wang et al. (2001) found that ABA is increased in response to salinity. The involvement of ABA in alleviating salt stress resistance in maize has also been addressed (DeCosta et al., 2007). However, there is a remaining information gap to properly understand the role of ABA in sensitive and resistant maize genotypes under salinity.

The precise roles of ABA, IBA and IAA under conditions of salt stress, while unclear, are of current interest.

Materials and methods

Plant cultivation

The salt-sensitive maize hybrid Lector (Limagrain, Edemissen, Germany) and the highly salt-resistant maize hybrid SR03 (Schubert et al., 2009) were grown in a greenhouse in hydroponic conditions. The experimental set-up consisted of three independent replicates of the salt-treated plants (100 mM NaCl) and the corresponding control plants (without salt stress). Each biological replicate was run in triplicate in a completely randomized design. The seedlings were imbibed in 1 mM $CaSO_4$ in an aerated solution at 25 °C for one day and placed between filter papers moistened with 1 mM $CaSO_4$ for a period of three days. Subsequently, the seedlings were transferred to 4.5 L plastic pots (three plants per pot) containing one-quarter-strength nutrient solution. After two days of cultivation, the concentration of nutrients was increased to half-strength and, after four days of cultivation, to full-strength. The nutrient solution had the following composition (Fortmeier and Schubert, 1995): 2.5 mM $Ca(NO_3)_2$, 1.0 mM K_2SO_4, 0.2 mM KH_2PO_4, 0.6 mM $MgSO_4$, 5.0 mM $CaCl_2$, 1 mM NaCl, 1.0 μM H_3BO_4, 2.0 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.005 μM $(NH_4)_6Mo_7O_{24}$, 200 μM Fe–EDTA. The solution was changed every second day to avoid nutrient depletion. To prevent osmotic shock, NaCl treatment was started two days after the full nutrient concentration had been reached and was increased stepwise in moderate amounts by 25 mM increments every second day. Temperature was kept constant at 26 °C for the light period and at 18 °C for the dark period; relative humidity was set to about 70%. Salt treatment was started after four weeks of growth, when plants developed the fifth leaf. In this study, only young expanding shoot material that had developed entirely under the full influence of the 8-day 100-mM NaCl treatment was harvested. After the measure of fresh weight, leaf and root material was immediately ground in liquid nitrogen and was lyophilized for hormone analysis.

Analysis of free abscisic acid (ABA), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) in expanding leaves and roots of maize

At least 10 mg dry powder was used for one sample to determine the plant hormones ABA, IAA and IBA. Three technical replicates were performed for each biological replicate. The samples were extracted with a mixture of iso-propanol and acetic acid (95:5, v/v) for 2 h under continuous shaking at 4 °C. Before the start of the extraction procedure 100 ng of heavy labeled ABA, IAA and IBA, respectively, were added to each sample. $[^2H_6]$-ABA was from the Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Canada; $[^{13}C_6]$-IAA from Cambridge Isotope Laboratories, Andover, MA, USA; and $[^{13}C_8,^{15}N_1]$-IBA (Strader et al., 2010) a generous gift from Dr. Jerry D. Cohen, University of Minnesota, USA. Further sample preparation was performed according to Meixner et al. (2005). Briefly, the samples were centrifuged for 10 min at 10,000 × *g*, the supernatant removed and evaporated to dryness under a stream of N_2. The residue was resuspended in methanol, centrifuged again for 10 min at 10,000 × *g*, and the supernatant was removed and placed in a glass vial. The methanol was evaporated under a stream of N_2 and the sample was resuspended in 100 μl ethyl acetate and methylated according to Cohen (1984) with freshly prepared diazomethane.

GC–MS analysis was carried out on a Varian Saturn 2100 ion-trap mass spectrometer using electron impact ionization at 70 eV, connected to a Varian CP-3900 gas chromatograph equipped with a CP-8400 autosampler (Varian, Walnut Creek, CA, USA). For the analysis, 1 μl of the methylated sample dissolved in 30 μl ethyl acetate was injected in the splitless mode (splitter opening 1:100 after 1 min) onto a Phenomenex (Aschaffenburg, Germany) ZB-5 column (30 m × 0.25 mm × 0.25 μm) using He carrier gas at 1 ml min^{-1}. The injector temperature was 250 °C and the temperature program was 60 °C for 1 min, followed by an increase of 25 °C min^{-1} to 180 °C, 5 °C min^{-1} to 250 °C, 25 °C min^{-1} to 280 °C, then 5 min isothermically at 280 °C. The μSIS mode (Varian Manual; Wells and Huston, 1995) was used for greater sensitivity. The endogenous hormone concentrations were calculated by the principles of isotope dilution (Cohen et al., 1986), using the ions at m/z 190/194 (endogenous and labeled standard; while the molecular ion of ABA would have six deuterium incorporated, during fragmentation of ABA two deuteriums are lost and the fragmentation ion at m/z 194 has only four deuterium retained) for methylated ABA (Walker-Simmons et al., 2000), the ions at m/z 130/136 for methylated IAA (Cohen et al., 1986), and at m/z 130/139 for methylated IBA (Strader et al., 2010).

Results and discussion

Shoot growth under salt stress

All measurements were based on expanding leaf material derived from plants entirely grown under the influence of 100 mM NaCl for 8 days. Thus, only leaves that exclusively grew under stress were taken into account. Under these conditions, biomass decreased differently in both genotypes. In response to the salt treatment, a significant decrease in shoot fresh weight of 64% was measured for the salt-sensitive hybrid Lector when compared with control plants. In contrast, only a slight decrease of 9% in expanding shoot fresh weight was measured for the salt-resistant SR03. These data are in line with earlier findings with the use of similar genotypes (Geilfus et al., 2010). The maize plants of this survey with only 8 days of treatment were in the first phase of the stress reaction. Plant ion concentrations demonstrating that plants were still in the first phase have been provided elsewhere (Schubert et al., 2009). The growth reduction of maize plants in the first phase of salt stress is characterized by osmotic effects and, to a lesser extent, by sodium toxicity (Sümer et al., 2004).

IAA, and IBA concentrations in leaves grown under salinity

Fig. 1 shows the free IAA (a) and the free IBA (b) concentrations measured in leaves and roots of the salt-resistant SR03 and the sensitive hybrid Lector under control and salt stress conditions. Generally, the free IAA and IBA concentration was higher in leaves compared with roots. The free IAA concentration was significantly reduced in the roots of the salt-sensitive hybrid Lector but remained