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Salinity stress effects on direct and indirect defence metabolites in maize

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ABSTRACT

In nature, plants are often exposed to multiple stress factors at the same time. The effects of single biotic or abiotic stresses on plant metabolism are well documented but how plants respond to a combination of these is little researched. Here we studied the effects of high salinity and herbivory on levels of secondary compounds and gene expression associated with defences against insects. Hydroponically grown maize plants were subjected to sodium chloride (1, 50, 100 mM NaCl) and/or damage by caterpillars of *Spodoptera exigua*. Salt-stressed plants showed stunted growth, reduced chlorophyll fluorescence and enhanced levels of reactive oxygen species and 1,4-benzoxazin-3-one aglycones (aBX). Herbivory induced higher transcript levels of the *Zm-Bx1* gene involved in aBX biosynthesis and of the *Zm-SerPIN* gene coding for a serine proteinase inhibitor which might affect plant feeding insects. Herbivory also triggered the emission of volatile organic compounds (VOCs) that are attractive signals for parasitoids and predators and thus regarded as an indirect defence. Herbivore-induced metabolites were differentially affected in salt-stressed plants. High salinity resulted in transient priming of jasmonic acid while aBX levels were reduced in double-stressed plants. Salt stress led to lower herbivore-induced VOC emission per plant but not per unit biomass. However, quantitative shifts in individual compounds were found in both cases. Our study confirms the notion that combined stresses produce a unique phenotype that cannot be derived from single-stress effects. The ecological implications of these changes for organisms from different trophic levels and for plant fitness remain to be tested.

1. Introduction

Increased soil salinity constrains the cultivation of agricultural crops in many arid and semi-arid regions of the world and is often caused by non-adapted irrigation practices (Plaut et al., 2013). High concentrations of salts, in particular sodium chloride (NaCl), generally have profound detrimental impacts on major plant physiological processes, although different species vary greatly in their ability to tolerate salt stress (Munns and Tester, 2008). To some extent, salinity effects resemble drought and cold stresses as the uptake of water by the root is restricted. In addition, salt-stressed plant cells need to cope with disturbances in ion homeostasis and the toxic effects of excess Na^+ in the cytoplasm. Affected plants show reduced growth due to the inhibition of cell division and cell elongation and are compromised in their photosynthetic capacity as a result of oxidative stress (Tester and Davenport, 2003). The impact of soil salinity on plant growth, photosynthesis, ion regulation, tolerance mechanisms and primary metabolism have been well studied over past decades (Munns and Tester, 2008; Parida and Das, 2005). However, little information is available on how salinity affects secondary plant defence compounds (Ballhorn and Elias, 2014; Ballhorn et al., 2011).

In nature, plants are frequently exposed to multiple abiotic and biotic stress factors at the same time. For instance, they may need to grow and reproduce in soil affected by drought, heavy metals or salinity while coping with an attack by pathogens and herbivores. Both abiotic and biotic stresses interact at the cellular level, inducing the same signalling pathways that involve reactive oxygen species (ROS), abscicic acid (ABA), salicylic acid (SA) and jasmonic acid (JA)/ethylene. The responses to a combination of stress factors, however, is often unique and cannot be extrapolated from studying these stresses individually (Atkinson and Urwin, 2012; Polle and Luo, 2014; Prasch and Sonnewald, 2015; Suzuki et al., 2014). A comprehensive microarray study on Arabidopsis

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ecotypes, for example, showed that approximately 60% of transcriptome changes were not predictable when the plants were exposed to double stresses (Rasmussen et al., 2013).

Plants cope with biotic stress factors such as insect herbivory by maintaining large amounts of secondary metabolites in their cells (constitutive defence) or by rapidly synthesizing defence compounds in response to an attack (induced defence). These plant secondary metabolites can have deterrent, repellent or toxic effects on insects (direct defence) or may act as volatile signals that attract carnivorous arthropods (indirect defence) (Karban and Baldwin, 1997; Schoonhoven et al., 2005). In maize and other Poaceae the most common secondary metabolites are 1,4-benzoxazin-3-one derivatives (BX), which are stored as inactive glycosides in the vacuole. Upon tissue disruption by insect feeding, the glycosides are hydrolysed by plastidic β-glucosidases and yield toxic 1,4-benzoxazin-3-one aglycones (aBX) (Morant et al., 2008; Niemeyer, 2009). Maize tissue contains high constitutive concentrations of 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc) and rather low levels of 2-β-D-glucopyranosyloxy-4,7-dimethoxy-1,4-benzoxazin-3-one (HDMBOA-Glc) and other 1,4-benzoxazin-3-ones. However, in response to herbivory by *Spodoptera* spp., HDMBOA-Glc is rapidly induced (Glauser et al., 2011). The breakdown products of both aBX (DIMBOA and MBOA) have shown strong activity against insects (Glauser et al., 2011; Niemeyer, 2009; Rostás, 2007).

Apart from secondary metabolites, plants also produce an array of highly inducible defensive proteins such as proteinase inhibitors (PIs) to counteract insect damage. PIs inactivate cysteine and serine proteinases in the midgut of insects, which restricts the uptake of essential amino acids in non-adapted herbivores. Induction of PIs is regulated by the JA signalling pathway and occurs locally at the damage site but also systemically in distant plant parts (Zhu-Salzman et al., 2008).

Throughout their life cycle or during specific developmental stages, most plants continuously emit a blend of volatile organic compounds (VOCs) from their below- and aboveground parts. The release of VOCs such as isoprene or derived terpenes is thought to protect leaf metabolic processes from thermal and oxidative stress (Loreto and Schnitzler, 2010) but constitutive VOCs can also be exploited by foraging herbivorous insects as cues to locate their host plant (Kalberer et al., 2001; Rostás et al., 2015). In return, plants respond to herbivore attack by an upregulation of the JA cascade and the synthesis of large amounts of VOCs that differ in their composition from the constitutive blend. Induced compounds comprise fatty acid derivatives, terpenoids, phenyl propanoids and benzenoids. Herbivore-induced VOCs can have multiple ecological functions such as regulating herbivore competition (De Moraes et al., 2001) or acting as signals that prime adjacent plants for future attack (Kim and Felton, 2012; Ruther and Kleier, 2005). Primarily though they are known as synomones (signals that benefit both emitter and recipient) that can restrict further insect damage by attracting parasitic wasps, predators or entomopathogenic nematodes that kill damaging herbivores (Mumm and Dicke, 2010; Turlings et al., 2012). Abiotic factors may cause considerable variation in the quality of the herbivore-induced VOC blend by changing the release rates of individual compounds or the total bouquet (Winter et al., 2012). A modified blend can thus constitute a signal with altered information content for natural enemies and the potential to disrupt plant-insect interactions (D'Alessandro et al., 2006; Wäschke et al., 2013; Winter and Rostás, 2010).

In this study we investigated the combined effects of salinity stress and insect herbivory on direct and indirect defence responses in *Zea mays*. Young maize plants and caterpillars of the genus *Spodoptera* are an agronomically important crop–pest association. Herbivore defence responses of maize and their impact on the multitrophic network are well characterised (Turlings and Tumlinson, 1992) and therefore this system has been widely used as a model for studying the effects of abiotic and biotic stresses (Gouinguene and Turlings, 2002; Schmelz et al., 2003; Winter et al., 2012; Winter and Rostás, 2010). Furthermore, maize is classified as a salt-sensitive plant that is particularly vulnerable during the early vegetative stages of its growth (Fortmeier and Schubert, 1995). Our results suggest that *Z. mays* exposed to salinity stress are compromised in their ability to mount direct and indirect defence responses to their full potential.

2. Material and methods

2.1. Plant, insects and salt-stress treatments

Seeds of *Zea mays* L. var. Hibiscus (Advanta Seeds, France) were soaked in water for one day and left to germinate on expanded clay (3–5 mm grain, pH 9.1, Lamstedt Ton, Germany) in a climate chamber (Percival E-36L) with a L16:D8 photoperiod (PAR: 140 μmol photons m^{-2} s^{-1} at 50 cm distance from lamps), a 29/20 °C (light/dark) temperature regime and 75% relative humidity. Six days after sowing, seedlings (6–8 cm) were transferred to black plastic containers (Rotilabo-Drehstapelwannen, Carl Roth GmbH, Germany) and grown in modified Hoagland solution as described in Winter and Rostás (2010). After 2 days of acclimation, sodium chloride (NaCl) was added to the hydroponic solution resulting in three treatments defined as control (1 mM NaCl), medium (50 mM NaCl) and high (100 mM NaCl). Electric conductance and pH of the hydroponic cultures were checked daily and were adjusted if necessary to maintain identical growth conditions. Plants were 11 days old when the experiment started unless stated otherwise.

Eggs of *Spodoptera exigua* (Lepidoptera) were kindly provided by Bayer CropScience AG (Monheim, Germany) on a weekly basis and reared to the second larval stage as described in Rostás (2007).

2.2. Growth analysis

The performance of salt-stressed maize was monitored by measuring the fresh weight, shoot height and primary root length of intact plants. Measurements were carried out on 8-day-old plants before salt treatment started (day 1) and on days 3 and 5.

2.3. Chlorophyll fluorescence measurement

Photosynthetic efficiency was assessed on the same plants that were used for growth measurements. Chlorophyll fluorescence was recorded from tip to base of the second leaf at four points using a PAM-2000 fluorometer (Walz Mess-u. Regeltechnik, Effeltrich, Germany). Plants were dark-adapted for 1 h before the experiment was carried out. Maximum photochemical yield of photosystem II (PSII) was measured as the ratio of variable (F_v) to maximal (F_M) chlorophyll fluorescence at room temperature with $F_v/F_M = (F_M - F_0)/F_M$ (Schreiber et al., 1986). Minimum fluorescence (F_0) was excited at 655 nm and 600 Hz modulation frequency, and maximum fluorescence (F_M) was measured with 100 kHz modulation frequency. The F_M was elicited by saturating pulses of 0.8 s duration from a built-in halogen lamp. Measurements were carried out before adding NaCl (day 1) and then every 2 days (day 3 and 5) between 10:00 and 12:00 h.

2.4. Quantification of hydrogen peroxide

Plants (8 days old) were exposed to salt stress for 4 days. Determination of H_2O_2 was then carried out with the Quanti-Chrom™ Peroxide Assay Kit (BioAssay System, CA, USA) according