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Diferulic acids in the cell wall may contribute to the suppression of shoot growth in the first phase of salt stress in maize

Md. Nesar Uddin [1], Stefan Hanstein *, Franziska Faust, Philipp T. Eitenmüller, Britta Pitann, Sven Schubert

Institute of Plant Nutrition, Interdisciplinary Research Center (IFZ), Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

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ABSTRACT

In the first phase of salt stress the elongation growth of maize shoots is severely affected. The fixation of shape at the end of the elongation phase in *Poaceae* leaves has frequently been attributed to the formation of phenolic cross-links in the cell wall. In the present work it was investigated whether this process is accelerated under salt stress in different maize hybrids. Plants were grown in nutrient solution in a growth chamber. Reduction of shoot fresh mass was 50% for two hybrids which have recently been developed for improved salt resistance (SR 03, SR 12) and 60% for their parental genotype (Pioneer 3906). For SR 12 and Pioneer 3906, the upper three leaves were divided into elongated and elongating tissue and cell walls were isolated from which phenolic substances and neutral sugars were determined. Furthermore, for the newly developed hybrids the activity of phenolic peroxidase in the cell wall was analysed in apoplastic washing fluids and after sequential extraction of cell-wall material with $CaCl_2$ and LiCl. The concentration of ferulic acid, the predominant phenolic cross-linker in the grass cell wall, was about 5 mg g^{-1} dry cell wall in elongating and in elongated tissue. The concentration of diferulic acids (DFA) was 2–3 mg g^{-1} dry cell wall in both tissues. Salt stress increased the concentration of ferulic acid (FA) and DFA in the parental genotype Pioneer 3906, but not in SR 12. Both genotypes showed an increase in arabinose, which is the molecule at which FA and DFA are coupled to interlocking arabinoxylan polymers. In SR 12, the activity of phenolic peroxidase was not influenced by salt stress. However, in SR 03 salt stress clearly increased the phenolic peroxidase activity. Results are consistent with the hypothesis that accelerated oxidative fixation of shape contributes to growth suppression in the first phase of salt stress in a genotype-specific manner.

Introduction

The agricultural production of maize (*Zea mays* L.) in a saline environment is threatened by a strong shoot-growth inhibition during the first phase of salt stress (Hatzig et al., 2010; Munns et al., 2000; Pitann et al., 2009; Uddin et al., 2013). Even though a lot of studies have described reasons for shoot growth retardation in response to phytohormones, the physiological and biochemical mechanisms of shoot growth reduction during the first phase of salt stress are still unclear (Munns and Tester, 2008). Several investigations have shown that salt stress imposed by NaCl addition to the nutrient solution causes a persistent decrease of the elongation rate of maize leaves (Chazen et al., 1995; Cramer, 1992; Cramer and Bowman, 1990; Neumann, 1993). The elongation rate of an individual cell depends on two factors: (1) the growth-effective turgor exerted on the surrounding cell wall and (2) the cell-wall extensibility (Kutschera, 1996; Lockhart, 1965). Under saline conditions, maize leaves re-establish shoot turgor pressure, while the leaf elongation rate only partially recovers (Cramer, 1992; De Costa et al., 2007). A similar response pattern occurs when maize shoots are subjected to water stress (Lu and Neumann, 1998; Van Volkenburgh and Boyer, 1985). The recovery of turgor during salt stress can also be inferred from the observation that water uptake from the saline solution (Schubert, 2011) and assimilate supply to the growing shoot (De Costa et al., 2007) are maintained during salt stress. The sustained impairment of elongation rate despite of turgor recovery must be attributed to a loss of cell-wall extensibility (Cramer, 1994; Neumann, 1993).

Abbreviations: CW, cell wall; DM, dry mass; FM, fresh mass; GAX, glucuronoarabinoxylan; PMSF, phenylmethanesulfonyl fluoride; FA, ferulic acid; DFA, diferulic acid; AWF, apoplastic washing fluid.

* Corresponding author. Tel.: +49 (0)641 99 39175; fax: +49 (0)641 99 39169.
E-mail address: stefan.m.hanstein@ernaehrung.uni-giessen.de (S. Hanstein).
[1] Present address: Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

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According to Cosgrove (1997) several factors may reduce the cell-wall extensibility during cell maturation: (1) a decrease in wall-loosening processes, (2) an increase in cross-linking of cell-wall polymers, and (3) a change in cell-wall composition, resulting in a more tightened wall structure or one which is less susceptible to wall loosening. The loosening of the cell wall is induced by a decrease of apoplastic pH (Hager, 2003). Elongation growth and extensibility of maize leaves are highly dependent on apoplastic pH (Van Volkenburgh and Boyer, 1985). The plasmalemma H^+-ATPase plays the key role in controlling apoplastic pH by pumping protons from the cytosol into the apoplast. H^+-ATPase-mediated acidification has recently been investigated for two maize hybrids with improved salt resistance, which were developed as described by Schubert et al. (2009). Only one of them (SR 03) can maintain H^+-ATPase-mediated acidification under salt stress, while the other hybrid (SR 12) and the more salt-sensitive parental hybrid Pioneer 3906 (synonym cv. Ornella) cannot maintain wall acidification (Hatzig et al., 2010; Pitann et al., 2009).

Since under salt stress shoot growth of SR 12 is improved compared to Pioneer 3906, although apoplastic acidification is impaired, other factors which may favorably influence the cell-wall extensibility of SR 12 have to be taken into consideration. A mechanism with a high potential to counteract cell-wall expansion is peroxidase-catalyzed cross-linking of wall polymers. The better growth of SR 12 during salt stress may reflect that in this genotype the cross-linking mechanism is less responsive to salt stress. The suppression of oxidative cross-linking in the cell wall was first suggested as a mechanism of hormonal growth regulation by Fry (1979). For suspension-cultured spinach cells he discovered concentrations of wall-bound ferulic acid (FA), which were sufficiently high to decrease the cell-wall extensibility through oxidative cross-linking. Furthermore, the growth-promoting hormone gibberillic acid proved to suppress the secretion of peroxidase into the spinach cell wall. In the following years, cross-linked aromatic compounds have been recognized as the structural components in *Poaceae* which "lock cells into shape" at the end of cell expansion (Carpita, 1996; Carpita and Gibeaut, 1993; Fry, 2004; Hatfield et al., 1999; Parker et al., 2005). Various diferulic acids (DFA) and even triferulates have been isolated from leaves, grains and bran of maize (Bunzel, 2010). It has been confirmed that in the cell wall these compounds are esterified to the arabinose moieties of glucuronoarabinoxylans which represent the predominant component of the hemicellulose matrix of *Poaceae*. The increase in the wall-bound DFA and FA shows a close correlation to a decrease in cell-wall extensibility in coleoptiles of oat, maize and rice (Kamisaka et al., 1990; Parvez et al., 1997; Tan et al., 1991). Furthermore, factors preventing the accumulation of FA and DFA also prevent a decrease of cell-wall extensibility in rice and wheat coleoptiles (Kawamura et al., 2000; Wakabayashi et al., 1997a, 1997b). Ferulic acid in cell walls isolated from maize suspension cultures is cross-linked upon addition of peroxidase and H_2O_2 (Grabber et al., 1995; Hatfield et al., 1999). In the cell wall of maize cells grown in suspenion culture ferulic acid cross-linking requires apoplastic peroxidases and apoplastic H_2O_2 (Encina and Fry, 2005). There is evidence that these two factors are important for the *in vivo* regulation of FA cross-linking in these cells (Burr and Fry, 2009; Lindsay and Fry, 2008). Accordingly, observations at the leaf tissue level show that a transient increase in apoplastic peroxidase precedes cessation of elongation growth (De Souza and MacAdam, 2001, 1998).

The overall targets of this investigation were to evaluate (1) whether the biochemical system for oxidative cross-linking in the expanding maize cell wall could contribute to salt-induced growth suppression and (2) whether the salt response of this system shows genotypic variation. The investigation was based on the isolation of the involved structural components including the cross-linking products (DFA) and on the isolation of the apoplastic peroxidases. These substances were extracted from the youngest tissue at the leaf base (upper shoot elongating zone) and as a reference for more mature tissue from the distal leaf portion which is addressed in this paper as upper shoot mature zone. Data from three experiments were compiled in this manuscript (Table 1).

Table 1
Overview of experiments.

Experiment number	Maize hybrids	Parameters studied
1	Pioneer 3906, SR 12	Phenolics, neutral sugars
2	SR 03, SR 12	Peroxidase activities
3	SR 03, SR 12	Neutral sugars

The main target was to determine the structural components of the oxidative cross-linking system of Pioneer 3906 and SR 12. The second experiment provided data on apoplastic peroxidase activities in upper shoot elongating zone of SR 12 and SR 03. Peroxidase activities were investigated after various extraction procedures with two different substrates. The third experiment investigated the hemicellulose matrix of SR 12 and SR 03 with a simplified harvesting scheme in which tissue from the elongation zone was not separated from the elongated zone.

The salt treatment was similar to the treatments which were reported to cause a persistent decrease of cell-wall extensibility in expanding maize leaves (Cramer, 1994; Neumann, 1993). The treatment did not cause ion toxicity and is therefore classified as salt stress in the first phase (Munns and Tester, 2008; Schubert, 2011).

Results

Shoot growth during the first phase of salt stress

Salt stress (100 mM NaCl) caused a significant reduction in shoot fresh mass compared to the control treatment (1 mM NaCl) in all three genotypes (Fig. 2a and b). The relative reduction in shoot fresh mass was significantly higher (52%) for the salt-sensitive Pioneer 3906 compared to the salt-resistant SR 12 (42%). In the second experiment, genotype SR 12 was compared with SR 03, and it was found that the relative reduction in shoot fresh-mass was 37% and 41% in SR 03 and SR 12, respectively (Fig. 2b).

Sugar composition of the cell-wall matrix

In the cell-wall matrix of the upper shoot elongating zone, salt stress caused a significant increase of arabinose concentration of Pioneer 3906 and SR 12 in the order of 20% (Table 2a). In addition, the cell-wall matrix of SR 12 showed a significant increase by 40% for galactose. In the upper shoot mature zone, the concentration of arabinose increased by 53% for SR 12, while no significant difference was found for Pioneer 3906. For SR 12, consistent results were obtained in the experiment without separation between elongating zone and mature zone, addressed as third experiment thoughout this paper: Salt stress significantly increased the concentrations of arabinose and of galactose in the cell walls.

Monomeric phenols in the cell-wall matrix

The most abundant monomeric phenols in the cell walls of both genotypes and in both shoot fractions were *trans*-FA and *trans*-*p*-coumaric acid (Fig. 4a), which is typical for primary cell walls of *Poaceae*. Among the monomeric phenols which are bound to the cell wall, ferulic acid is the most important substance for oxidative cross-linking in the expanding primary cell wall (Bunzel, 2010). Salt stress significantly increased the concentrations of wall-bound