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Transcript profiling of the salt-tolerant *Festuca rubra* ssp. *litoralis* reveals a regulatory network controlling salt acclimatization

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Summary

We report an analysis of salt-stress responses in the monocotyledonous halophyte *Festuca rubra* ssp. *litoralis*. Salt-dependent expression of transcripts encoding a *PIP2;1* aquaporin, V-ATPase subunit B, and the Na^+/H^+ antiporter *NHX* was characterized. Transcription of *FrPIP2;1*, *FrVHA-B*, and *FrNHX1* was induced in root tissue of *F. rubra* ssp. *litoralis* by salt treatment, and during salt-stress *F. rubra* ssp. *litoralis* accumulated sodium in leaves and roots. Cell specificity of *FrPIP2;1*, *FrVHA-B*, and *FrNHX1* transcription was analyzed by *in situ* PCR in roots of *F. rubra* ssp. *litoralis*. Expression of the genes was localized to the root epidermis, cortex cells, endodermis, and the vascular tissue. In plants treated with 500 mM NaCl, transcripts were repressed in the epidermis and the outer cortex cells, whereas endodermis and vasculature showed strong signals. These data demonstrate that transcriptional regulation of the aquaporin *PIP2;1*, V-ATPase, and the Na^+/H^+ antiporter *NHX* is correlated with salt tolerance in *F. rubra* ssp. *litoralis* and suggests coordinated control of ion homeostasis and water status at high salinity in plants. Salt-induced transcript accumulation in *F. rubra* ssp. *litoralis* was further monitored by cDNA-arrays with expressed sequence tags derived from a cDNA subtraction library. The salt-regulated transcripts included those involved in the control of gene expression and signal transduction elements such as a serine/threonine protein kinase, an *SNF1*-related protein kinase, and a WRKY-type transcription factor. Other ESTs with salt-dependent regulation included transcripts encoding proteins that function in metabolism, general stress responses, and defense and transport proteins.

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Introduction

Salinity is a major problem for agricultural crop production that particularly affects irrigated soils. Worldwide, more than one-third of irrigated land is salinized, and increasing loss of agricultural yields due to secondary soil salinization has been predicted (Ashraf, 1994; Munns, 2002; Wang H. et al., 2003; Wang W. et al., 2003; Flowers, 2004). High salinity primarily induces cellular imbalances in ion homeostasis and osmotic potential, accompanied by secondary effects as increased generation of active oxygen species that leads to oxidative stress (Wang H. et al., 2003; Wang W. et al., 2003; Tausz et al., 2004). Plant adaptation to salinity requires alterations in various cellular, physiological, and metabolic mechanisms that are controlled at the transcriptional level, and increasing knowledge on salt-induced regulation of gene expression has emerged. Osmotic adjustment is achieved by salt-induced synthesis and cytoplasmic accumulation of osmoprotective solutes such as proline, quaternary ammonium compounds such as glycine betaine, polyols, and sugars (McNeil et al., 1999; Hasegawa et al., 2000). In the halotolerant *Mesembryanthemum crystallinum*, a salt-induced increase of expression and activity of NADP-specific isocitrate dehydrogenase, which is involved in proline biosynthesis, has been reported, and a proline transporter was specifically up-regulated in root vascular cells, suggesting stimulated proline translocation (Popova et al., 2002, 2003). Salt-dependent regulation of aquaporins has been shown, indicating adjustment of water fluxes across the plasma membrane and tonoplast in salinity-exposed plants (Kirch et al., 2000; Katsuhara et al., 2002). Cytoplasmic Na^+/K^+ discrimination involves transcriptional regulation of K^+ uptake systems as AKT1-type K^+ channels and HAK-type K^+ transporters, as well as HKT1-type Na^+ transporters (Uozumi et al., 2000; Horie et al., 2001; Rus et al., 2001; Golldack et al., 2002, 2003). Stimulation of vacuolar H^+-ATPase energizes vacuolar Na^+ detoxification that is mediated by secondary-activated NHX-type Na^+/H^+-antiporters (Apse et al., 1999; Lehr et al., 1999; Chauhan et al., 2000; Ratajczak, 2000; Golldack and Dietz, 2001; Xia et al., 2002).

Although basic salinity-adaptive mechanisms have been identified and investigated, most knowledge on molecular response pathways is derived from studies on *Arabidopsis thaliana*, a typical glycophyte that does not possess particular salt tolerance. A halophyte that has been analyzed as a model plant for molecular mechanisms in salt adaptation is *M. crystallinum*. Knowledge of salinity responses in *M. crystallinum* is, however, not directly transferable to the salt-sensitive crop species. *M. crystallinum* has special morphological and physiological characteristics such as succulence and salt-inducible CAM (Adams et al., 1998). To identify mechanisms with the potential to confer salt tolerance to crop plants, it will be necessary to analyze, both on the molecular and the morphological levels, regulation of molecular salinity responses in halotolerant species that are closely related to crop plants.

In this study, we have chosen the facultative halotolerant grass *Festuca rubra* ssp. *litoralis* (red fescue) as a model for an analysis of salt-stress responses. We characterized salt-dependent expression of transcripts encoding a *PIP2*-homologous aquaporin, an *NHX*-type Na^+/H^+-antiporter, and V-ATPase subunit B. We observed coordinated regulation of these key determinant mechanisms of salt adaptation that indicate correlated control of the transcriptional changes by distinct salt-inducible ion homeostasis. As a next step, transcripts specifically regulated in salt-stressed plants were obtained by differential subtraction and used for assembling cDNA-arrays. Using these cDNA-arrays, the transcript accumulation patterns of *F. rubra* ssp. *litoralis* were monitored in control and stressed plants during the initial phase of salt stress. The analysis allowed us to identify regulatory networks of genes that are expressed in the salt-tolerant grass in response to salinity.

Materials and methods

Plant material

Festuca rubra ssp. *litoralis* was grown under controlled conditions in a growth chamber with 14 h light ($300\,\mu E\,m^{-2}\,s^{-1}$, 25 °C) and 10 h dark (21 °C) with 50% relative humidity. Seeds were germinated in vermiculite soaked with half-strength Hoagland's nutrition solution (Golldack et al., 2002). *F. rubra* ssp. *litoralis* plants were transferred to aerated hydroponic tanks with half-strength Hoagland's nutrition solution 3 weeks after germination. Experiments were performed at the age of 6 weeks. For salt stress, the nutrient solution was supplemented with 125, 250, and 500 mM NaCl, respectively, for 48 h. Unstressed control plants were grown in parallel and harvested at the same time. Plants were harvested 5 h after the onset of illumination.