Article Addendum

Comparison of salt-responsive gene regulation in rice and in the salt-tolerant *Festuca rubra* ssp. *litoralis*

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To identify novel elements of plant salt stress adaptation, salt-induced transcript accumulation was compared in the model crop plant rice and in the halotolerant grass *Festuca rubra* ssp. *litoralis* by cDNA-array hybridizations. Results of the study show major differences in transcript expression profiles between the salt sensitive rice and the naturally halotolerant grass species. The data indicate that the salt tolerance strategy of *F. rubra* ssp. *litoralis* involves activated expression of genes with functions in osmotic and ion homeostasis, in metabolism, and general stress defense that is missing in rice. In addition, transcripts with a function in regulation of transcription, translation, signal transduction and protein turnover showed different transcriptional responses. Among other signaling elements that were regulated by salt in the halotolerant *F. rubra* ssp. *litoralis* but not in rice, the putative serine/threonine protein kinase *SnRK1b* (sucrose non-fermenting-1-related kinase 1) was identified. It is hypothesized that modification of signal transduction pathways and transcriptional control in salt-sensitive species according to regulatory mechanisms identified in related halophytes can activate the complex network of molecular processes that lead to an improved tolerance of salinity.

Transcriptome Analysis of the Salt Stress Response in Rice and *F. rubra* ssp. *litoralis*

Most knowledge on molecular mechanisms involved in plant salt adaptation has been derived from analyses of the glycophytic

models *Arabidopsis thaliana* and rice.¹⁻⁴ In contrast, naturally halotolerant species have been studied less although these species might allow identifying and investigating novel regulatory elements and pathways of salt adaptation with the potential to confer salinity tolerance to salt-sensitive plants.

To identify differently regulated transcripts in the salt sensitive rice and the salt tolerant grass *F. rubra* ssp. *litoralis*, a subtracted cDNA library that was enriched for salt-responsive transcripts was synthesized from *F. rubra* ssp. *litoralis* and used for the generation of cDNA-arrays.⁵ Orthologous genes of rice and *F. rubra* ssp. *litoralis* share in average identities of about 80% on the nucleic acid sequence level.⁵ Accordingly, the cDNA-arrays could be probed with cDNAs from both species for a direct comparison of transcriptional salt stress responses.

In *F. rubra* ssp. *litoralis* the number of genes with salt-induced modification of expression increased with time and with the concentration of salt (Fig. 1A)⁵ whereas in rice the proportion of salt-responsive genes decreased with the duration of stress (Fig. 1B). Metabolic enzymes with different transcription under salt stress included the delta I-pyrroline-5-carboxylate synthetase with a function in the synthesis of the osmoprotectant amino acid proline, aldehyde dehydrogenase and carbonate dehydratase (Fig. 1C). In addition, transporters as the NHX1-type Na⁺/H⁺ antiporter, subunit A of V-ATPase, and an ABC transporter responded differently to salt in both species (not shown).

Multiple Signaling Factors are Salt Responsive in *F. rubra* ssp. *litoralis* but not in Rice

Differently regulated transcripts in rice and *F. rubra* ssp. *litoralis* included those involved in the control of gene expression and signal transduction such as a WRKY-type transcription factor, a cyclic nucleotide-binding transporter, and a wall-associated protein kinase (Fig. 1D).⁵ In addition, a SUI-homologous translation initiation factor eIF-1 and the sucrose nonfermenting 1-related protein kinase2 (*SnRK2*) *SAPK4* were differently regulated in response to salinity in both species. Another transcript that showed transcriptional upregulation in *F. rubra* ssp. *litoralis* in response to salt was a serine/threonine protein kinase *SnRK1b* (sucrose non-fermenting-1-related kinase 1) homologue that was not regulated

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Figure 1. (A and B) Hierarchical clustering of signal ratios [salt-treated plants/control plants] of transcript levels monitored by array hybridization with leaf cDNA obtained from *F. rubra* ssp. *litoralis* (Fr; A) and rice var. IR29 (Os; B) stressed with 125 mM NaCl for 1 h and 6 h (C and D) Expression of genes within different functional categories as monitored by cDNA array hybridization. (C) Metabolism. (D) Transcription, translation, signal transduction, protein turnover. 1—rice stressed with 125 mM NaCl, 6 h, 2–4—*F. rubra* ssp. *litoralis* stressed with 125 mM (2), 250 mM NaCl (3), 500 mM NaCl (4) NaCl for 6 h.

in rice (Fig. 2). Translational fusion of rice *SnRK1b* with the green fluorescent protein (GFP) showed subcellular localization in the nucleus as it has been reported also for other kinases as for example a calcium/calmodulin-dependent protein kinase functioning in Nod-factor signaling from *Medicago truncatula* and the rice SnRK2-type kinase OSRK1 (Fig. 3).^{6,7}

Conclusions

Plants adapt to changes in salinity with the activation of cellular and metabolic mechanisms that are triggered by several signaling and regulatory pathways.^{8–10} Comparative studies of salt adaptation in naturally halotolerant and in stress-sensitive plants will allow identifying novel elements involved in signaling and transcriptional activation in response to salt stress. These elements might effectively activate down-stream regulated target genes of adaptive pathways when overexpressed in salt-sensitive species as it has been shown for rice *SAPK4* and *eIF1*.^{11,12} Thus, comparative analyses of the salt-stress response in related glycophytic and halophytic species provide a novel strategy with which to identify global transcription regulators of major salt stress mechanisms that are suitable for genetically engineering improved salt tolerance in plants.

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Figure 3. Subcellular localization of SnRK1b-protein. (A) Nuclear localization of SnRK1b-GFP fusion proteins in onion epidermal cells. The arrow points to the nucleus. (B) The signal of the SnRK1b-GFP fusion protein was merged with a light microscopic image of the transformed onion epidermal cell. (C) Onion epidermal cells transformed with a translational construct of GFP as a positive control showed localization throughout the cell with strongest signals in cytoplasm and nucleus. (D) Onion epidermal cells transformed with the empty vector as a background control.

Figure 2. Northern hybridization of the expression of *SnRK1b* in leaves of *F. rubra* ssp. *litoralis* and rice. The plants were treated with 0, 125, 250 and 500 mM NaCl for 48 h. Hybridization of actin is shown as a loading control.

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