Cell type-specific transcriptional responses of plants to salinity

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Abstract

Soil salinity reduces the growth of glycophytic plants such as *Arabidopsis thaliana* and rice. In vascular plants, roots are organized into concentric layers of cells and each layer has a specific biological function coordinated with other cell types in the root. Therefore, genes differentially expressed in response to a salt stress are also likely to be changing only in specific cell types, and thus may not be revealed at the organ level. In order to identify novel salt-responsive genes, cell-type specific transcriptomic approaches were undertaken in *Arabidopsis thaliana* and rice, with application of physiologically reasonable salt stress (50 mM) over 48 hours. Two cell-types from the root were chosen in both species for their potential role in salt storage and transport: cortical and pericycle/stelar cells respectively. Cell-types of interest expressing specifically Green Fluorescent Protein (GFP) were isolated from the rest of the root using fluorescence-activated cell sorting (FACS).

The outer layer of the root was found to be responding more than the inner part of the root after 48 hours of salt stress, with an overall down-regulation in both rice and Arabidopsis. Arabidopsis cortical cells responding to salt seem to regulate the cell wall biosynthesis, which may modulate the shape of the cells or alter the apoplastic movements of solutes in response to salt. Genes related to transport were affected by salt in Arabidopsis, with the crucial role of cortical cells in the movement of solutes being evident. Rice cortical cells respond to salt by showing a more extreme defense reaction in changing the protein metabolism and the regulation of transcription. The response of the inner part of the rice root to 48 hours of mild salt stress showed up-regulation of genes implicated in broader functional categories. The biological relevance of genes revealed using cell type-specific transcriptomics was demonstrated in a salt assay using knock-out (KO) lines of candidate genes from both cell-types in *Arabidopsis thaliana*. Three KO mutant lines showed 20% reduction in shoot sodium after 5 weeks of salt stress and were also able to maintain a higher shoot dry weight.

These transcriptomic studies of isolated stelar and cortical cells in response to a mild salt stress have revealed salt responsive genes and pathways, indicating new areas for further study, and contributing to our understanding of the complex responses of plants to their environment at the cellular level.

Résumé

La salinité du sol affecte la croissance des plantes glycophytes telle que *Arabidopsis thaliana* et le riz. Chez les plantes vasculaires, les racines sont composées de divers types de cellules organisées en cercles concentriques. Chaque type de cellules racinaires possède une fonction biologique spécifique et coordonnée avec les autres cellules qui composent cette même racine. Il est probable que la réponse des gènes au stress salin soit spécifique du type cellulaire, ce qui ne peut être révélé par des études à l'échelle de l'organe entier. Afin d'étudier les réponses spécifiques, notre approche a été de générer des profils de transcriptome pour deux types de cellules racinaires chez les plantes modèles, Arabidopsis et riz. Les deux types de cellules étudiées ont été choisis en raison de leur rôle possible soit dans le stockage du sodium dans les cellules corticales, soit dans son transport dans les cellules du péricycle chez Arabidopsis ou du cylindre central chez le riz. Des plantes exprimant la protéine fluorescente verte (GF) spécifiquement dans un type de cellule racinaire furent utilisées pour cette analyse. Les cellules ont donc pu être isolées chez le riz et Arabidopsis grâce à la technique de cytométrie en flux.

L'analyse du transcriptome des cellules du péricycle et du cylindre central montrent que les cellules corticales sont plus réactives au stress salin et qu'une large majorité des gènes est sous–exprimée chez les deux plantes modèles. D'après les analyses d'expression des cellules du cortex d'Arabidopsis, trois voies métaboliques sont significativement sous-exprimées en réponse au stress salin: la voie de biosynthèse des phénylpropanoïdes, le transport de l'eau and le métabolisme secondaire. La régulation de gènes impliqués dans le transport de l'eau et des nutriments démontre l'importance des cellules corticales dans le mouvement des solutés. Chez le riz, les profils des deux types cellulaires étudiés révèlent une forte réaction de défense ; en effet le métabolisme protéique et la régulation de la transcription sont fortement sous-exprimés dans les cellules corticales alors que les cellules du cylindre central modifient et activent les gènes correspondant à divers catégories fonctionnelles telles que la réplication de l'ADN et le transport. Des gènes candidats ont été sélectionnés dans les deux types cellulaires d'Arabidopsis. Des lignées mutantes n'exprimant pas ces gènes ont été testées en stress salin dans des conditions hydroponiques. Les résultats ont révélé

un phénotype accumulant moins de sodium dans les parties aériennes (20% par rapport au génotype sauvage) pour certaines de ces lignées mutantes.

Ce travail est la première étude de transcriptome utilisant des types spécifiques de cellules racinaires chez le riz. L'identification de gènes et voies métaboliques répondant au stress salin dans le cortex et le cylindre central de la racine ouvre de nouveaux axes de recherche et va permettre d'élucider la complexité des processus biologiques d'adaptation au stress salin.

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%, percent
^{o}C, degrees Celsius
ABA, abscisic acid
AKT, Arabidopsis potassium channel
Ath, *Arabidopsis thaliana*
AVP, vacuolar H^+-pyrophosphatase
B, boron
Ca^{2+}, calcium
CBL, calcineurin B-like proteins
cDNA, complementary DNA
cRNA, complementary RNA
CHX, cation/H^+ exchangers
CIPK, CBL-interacting protein kinases
CSLD, cellulose synthase like D subfamily
DEPC, diethylpyrocarbonate
DNA, deoxyribonucleic acid
dNTPs, mixture of equal equivalents of dATP, dTTP, dCTP and dGTP
DRE, dehydration response element
EDTA, ethylenediaminetetraacetic acid
Fe, iron
FACS, fluorescently activated cell sorting
FDR, false rate discovery
GFP, green fluorescent protein
H^+, proton
HKT, high affinity potassium transporter
ICP-OES, inductively coupled plasma optical emission spectrometry
IRGSP, international rice genome sequencing project
IVT, *in vitro* transcription
K, potassium
KAT, potassium Arabidopsis transporter
KO, knock-out
LB, luria and bertani medium
Mb, megabase
MES, 2-(*N*-morpholino)ethanesulfonic acid
Mg, magnesium
mM, millimolar
MM, mismatch
Mn, manganese
MS, Murashige and Skoog medium
Na^+, sodium
NaCl, sodium chloride
NCBI, National Center for Biotechnology Information

NHX, Na^+/H^+ antiporter
Os, *Oryza sativa*
P, phosphate
PCA, principal component analysis
PCR, polymerase chain reaction
PM, perfect match
PVPP, polyvinylpolypyrrolidone
Q-PCR, quantitative real time polymerase chain reaction
RMA, robust multi-array average
RNA, ribonucleic acid
RGAP, rice annotation genome project
RGR, relative growth rate
RO, reverse osmosis
ROS, reactive oxygen species
S, sulfate
SDS, sodium dodecyl sulphate
SEM, standard error of the mean
SKOR, stelar K^+ outward rectifyer
SOS, salt overly sensitive
SUMO, small ubiquitin-like modifier proteins
TAE, Tris base, acetic acid and EDTA buffer
T-DNA, transfer DNA
UV, ultra violet
XTH, xyloglucan endotransglycosylase/hydrolase
Zn, Zinc