Role of AtCIPK16 in Arabidopsis abiotic tolerance

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List of Abbreviations

Abbreviation	Full term
3′	Three prime, of nucleic acid sequence
5′	Five prime, of nucleic acid sequence
#	Number
%	Percent
±	Plus and minus
×	Times
°C	Degree Celsius
aa	Amino acid
ABA	Abscisic acid
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
Agrobacterium	*Agrobacterium tumefaciens*
AKT	Arabidopsis potassium channel
amiRNA	Artificial micro ribonucleic acid
ANOVA	Analysis of variance
Arg	Arginine
Asp	Aspartic acid
At	*Arabidopsis thaliana*
AVP	Vacuolar H^+-pyrophosphatase
BLAST	Basic Local Alignment Search Tool
BNS	Basal nutrient solution
bp	Base pairs, of nucleic acid
BSA	Bovine serum albumin
C-terminal	Carboxyl terminal
C-terminus	Carboxyl terminus
Ca^{2+}	Calcium ion
$Ca(NO_3)_2$	Calcium nitrate
$CaCl_2$	Calcium chloride
cAMP	Adenosine 3', 5'-cyclic monphophate
CaMV	Cauliflower mosic virus
Cat.#	Catalogue number
CBL	Calcineurin B-like proteins
cDNA	Complementary deoxyribonucleic acid
CHX	Cation/H^+ exchangers
CIPK	CBL-interacting protein kinases
Cl^-	Chloride ion
cm	Centimetre(s)
$CoCl_2$	Cobalt chloride
Col-0	Columbia-0

$CuSO_4$	Cupric sulfate
d	Day(s)
Da	Dalton
DEPC	Diethylpyrocarbonate
dH_2O	Deionised water
DNA	Deoxyribonucleic acid
dNTPs	Mixture of equal equivalents of dATP, dTTP, dCTP and dGTP
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agricultural Organization of the United Nations
FW	Fresh weight
g	Gram(s)
g	Gravity
gDNA	Genomic deoxyribonucleic acid
GFP	Green fluorescent protein
H_2O	water
H_3BO_3	Boric acid
HCl	Hydrochloric acid
His	Polyhistidine tag
h	Hour(s)
H^+	Proton
K^+	Potassium ion
KAT	Potassium Arabidopsis transporter
kb	Kilo base pairs, of nucleic acid
KCl	Potassium chloride
KDa	Kilo Dalton
KH_2PO_4	Monopotassium phosphate
KNO_3	Potassium nitrate
LB	Luria and Bertani medium
Leu	Leucine
LR	Ligation reaction
Lys	Lysine
M	Molar
MES	2-(N-Morpholino) ethanesulfonic acid, 4-morpholineethanesulfonic acid
Met	Methionine
mg	Miligram(s)
Mg^{2+}	Magnesium ion
$MgSO_4$	Magnesium sulphate
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
Mn^{2+}	Manganese ion
$MnCl_2$	Manganese choride

mRNA	Messenger RNA
MS media	Murashige and Skoog media
mV	millivolt
n	Sample size
N-terminal	Amine terminal
N-terminus	Amine terminus
N/A	Not applicable
Na+	Sodium ion
Na_2HPO_4	Sodium phosphate dibasic
Na_2MoO_4	Sodium molybdate
NaCl	Sodium chloride
NaFe(III)EDTA	Sodium iron EDTA
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NH_4NO_3	Ammonium nitrate
NHX	Na^+/H^+ antiporter
$NiCl_2$	Nickel chloride
nM	Nanomolar
No.	Number
NO^{3-}	Nitrate ion
nosT	Bacterial nopaline synthase terminator sequence
ng	Nanogram(s)
OD_{600}	Optical density measured at 600 nm
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
pI	Isoelectric point
PI	Proidium iodide
PO_4^{3-}	Phosphate ion
Q-PCR	Quantitative real time polymerase chain reaction
QTL	Quantitative trait loci
RNA	Ribonucleic acid
RO	Reverse osmosis
ROS	Reactive oxygen species
sec	Second(s)
SEM	Standard error of the mean
Ser	Serine
SDS	Sodium dodecyl sulphate
SKOR	Stelar K^+ outward rectifier
SOS	Salt overly sensitive
T-DNA	Transfer deoxyribonucleic acid
T_1	Primary Arabidopsis transformant
T_2	Progeny of T_1 plant

TAE	Tris base, acetic acid and EDTA buffer
TE	Tris-EDTA
Thr	Threonine
Tm	Melting temperature, of primers
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
Trition X-100	Toctylphenoxypolyethoxyethanol
Trp	Tryptophan
Tyr	Tyrosine
U	Units
UTR	Untranslated region
V	voltage
v/v	Volume per volume
w/v	Weight per volume
Xenopus	*Xenopus laevis*
YFP	Yellow fluorescent protein
Zn^{2+}	Zinc ion
$ZnSO_4$	Zinc sulfate

Abstract

Soil salinity is a significant environmental problem affecting agriculture around the world leading to reduced crop yield. High concentrations of Na^+ affect cell metabolism and compete with K^+ for the binding sites of enzymes which play important roles in cellular function. One mechanism for improving salinity tolerance of crop plants is to minimise the accumulation of Na^+ in the shoot. *AtCIPK16* (Calcineurin B-like-interacting protein kinase 16) has been identified as a novel candidate gene important in increasing salinity tolerance (Roy *et al.* 2013). Over-expression of *AtCIPK16* has been shown to reduce the shoot sodium in a number of species. In both hydroponic and soil culture, Arabidopsis with constitutive over-expression of *AtCIPK16* show significant reductions in Na^+ concentration in shoot, compared with wild type and nulls, while Arabidopsis with amiRNA knockdown of *AtCIPK16* exhibit an increase of Na^+ concentration in shoot (Roy *et al.* 2013). While it can be clearly seen that alterations in the expression of *AtCIPK16* result in increased salinity tolerance, little is known, however, about the role the protein plays in tolerance mechanisms. It is therefore important to identify its cellular location, upstream and downstream targets, and which abiotic stresses it is involved in to elucidate its function in plants.

Yeast two hybrid systems were used to identify the potential upstream CBL partners of AtCIPK16. The assay revealed 6 AtCBLs (AtCBL1, AtCBL2, AtCBL4, AtCBL5, AtCBL9 and AtCBL10) could interact with AtCIPK16. Bimolecular Fluorescence Complementation (BiFC) assays were then employed to confirm the result from Y2H and showed one more interacting AtCBL partner, AtCBL3. Additionally, BiFC demonstrated possible plasma membrane localization of the complexes of AtCBL1-AtCIPK16, AtCBL4-AtCIPK16, AtCBL5-AtCIPK16 and AtCBL9-AtCIPK16; and cytoplasm localization of the complexes of AtCBL2-AtCIPK16, AtCBL3-AtCIPK16 and AtCBL10-AtCIPK16 using transient co-expression in *Nicotiana benthamiana* leaves. Moreover, a pull-down assay was planned to identify downstream target proteins of AtCIPK16.

The radioactive tracer $^{22}Na^+$ was used to quantify net Na^+ accumulation in the different part of transgenic Arabidopsis overexpressing *AtCIPK16* and nulls to determine if this gene can alter Na^+ influx or Na^+ translocation in plants. Only one transgenic line showed lower Na^+ accumulation in root compare to nulls under salt stress, while all three transgenic lines demonstrated slightly lower but not significant Na^+ translocation rate and shoot Na^+ accumulation compare to nulls under 50 mM NaCl treatment. Furthermore, to examine the function redundancy of AtCIPK24 and AtCIPK16 in salt stress, complementary lines of constitutively expressing *AtCIPK16* in the *atcipk24/sos2* knockout lines background were

generated and analysed with plate assay and soil assay. The study revealed constitutive expression of *AtCIPK16* could not complement the salt sensitivity phenotype of *atcipk24/sos2* knockout mutants, suggest their different functions which are non-complementary in each other's signalling pathway.

The phenotypes of *35S:AtCIPK16* were characterized under osmotic, drought, cold, low K^+ stresses and ABA treatment to examine the potential function of AtCIPK16 in other stresses. This study revealed that over-expressing *AtCIPK16* plants were more sensitive to ABA and had increased K^+ root accumulation when grown under low K^+ stress, it appears that AtCIPK16 is involved with processes involving the transport of monovalent cations. No significant phenotypic variation was observed in cold, drought, osmotic and high KCl stresses, suggesting AtCIPK16 could be not involved in other stresses which typically require the production of compatible solutes or enzymes which mop up reactive oxygen species. However, the function of AtCIPK16 in salinity tolerance and in the response to other abiotic stresses still requires further characterization.

Declaration

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