

Title:

Athyrium yokoscense, a cadmium-hypertolerant fern, exhibits two cadmium stress mitigation strategies in its roots and aerial parts

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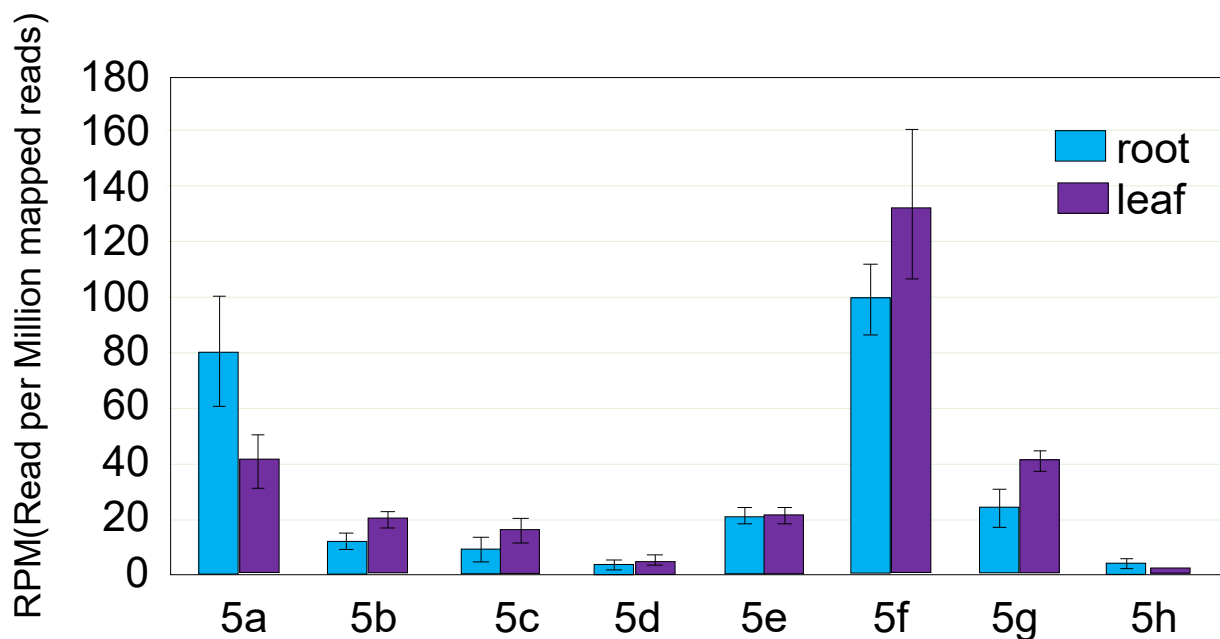
color figures: 4

supplementary files: two figures and one table

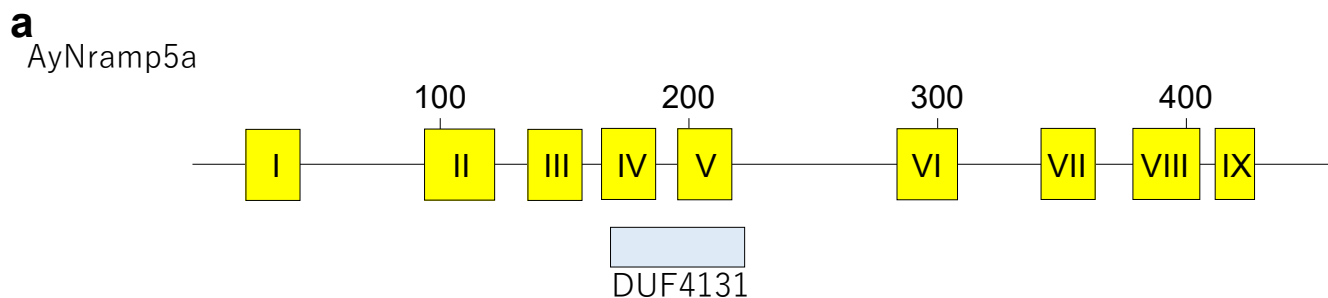
a Homologs of Nramp5 in *A. yokoscense*

Name	Size (a.a.)	Homology to OsNramp5 (%)	ID	Acc. No.
<i>AyNramp5a</i>	397	66.0	Athyo15474	ICTA0100001
<i>AyNramp5b</i>	506	59.7	Athyo2574	ICTA0100002
<i>AyNramp5c</i>	330	57.3	Athyo88288	ICTA0100003
<i>AyNramp5d</i>	329	56.2	Athyo32359	ICTA0100004
<i>AyNramp5e</i>	380	53.9	Athyo45774	ICTA0100005
<i>AyNramp5f</i>	492	42.1	Athyo44278	ICTA0100006
<i>AyNramp5g</i>	448	32.1	Athyo17600	ICTA0100007
<i>AyNramp5h</i>	45	60.0	Athyo5667	ICTA0100008

b

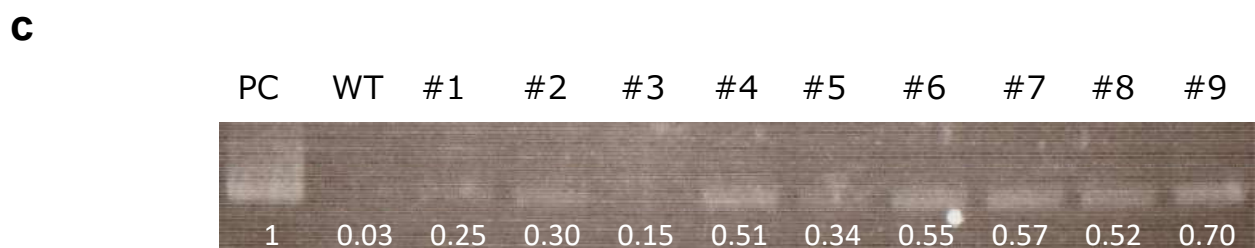


Supplementary Figure S1 (a) List of *A. yokoscense* Nramp5 genes. "Size" indicates the number of amino acid residues in the registered cDNA. The cDNA of *AyNramp5h* contains a partial sequence without translational termination. ID and Acc. No. indicates the corresponding ID names in the dataset registered by DRA008924, and accession numbers of the DDBJ database, respectively. (b) Suggestive expression levels of the predicted *Athyrium yokoscense* Nramp5 genes. Expression levels are represented as the numbers of read per million mapped reads (RPM), whose original data have been reported previously (Ukai et al. 2020). Nramp5 genes are indicated by names without the prefix "AyNramp", such by 5a and 5b.



b

GGATCC**ATG**GGGGAGCTGTTACTATATCAACTCAGTTTGCAGGACTGGCCACCCCATAGC
 ACTCTTCTTCTGTGGTGTTCGGTGCTCTCAAACCCGCTTAGCCTCAGCCTTCACCTGCTT
 TGCACCATAATGGATGCCGGCCAGACCAGATTTTCGTCCCCGAAAACGAGGACTGCGT
 AACTCTTAGCTTACCTGGGACCGGGTTTCTTGCTCGCAATAGCGTACATCGATCCAGGA
 AAC**TTTGAGTCTGACCTGAAATCGGGCGCCAAGTACAAGTACGAGTTGCTATGGATCATC**
GTCATCGCCAGTTGTGCAGCGTTGTTAATTCAGAGTCTCGCCGCCAATCTCGGCGTTGTA
ACAGGCAAGCACTTAGCGGAGCATTGCAGAACGGAGTATCCTCGCAAAGTGAACCTCCTG
CTTTGGATAGCAGCTGAAGTCTCCATTGTGGCCTCTGACATCCCTGAAGTGCTGGGAAC
GCCTTTGCCCTCAACCTTCTCTTCAAGATCCCGTTTGGGCAGGAGTGATCCTGACAGGT
CTGTCCACGTTGATTCTCCTTGCGCTGCAACAGTACGGCGTACGCAAGCTAGAGTTCTTC
ATAGCCCTACTGGTCTTCACCATGGCTGGCTGCTTCTTTGCAGAGCTAGCTTATGCTAGG
CCACCTGTGGTTGAAGTCTTGAAAGGCATGTTCAATTCCTCGGCTTAATGGCGATGGTGCG
ACTAGGCTGGCTATCTCATTGTTGGGTGCCATGGTAATGCCTCACAATCTGTTCTTGCA
TCTGCCCTGGTGCTTTCACGCCGTACGCCGAGAACAGTTCGCGGGATAAAGGATGCGTGC
AAATTCTACTTCATGGAGTCGGCTTTTGCCTGTTTGTGCGGTTTCTCATCAACATCTTT
GTGAACAGCGTCAGTGGTGCCGTCTGTTCCGATCCGACTTTAGCGGCTGGAGACAAGGAG
AAATGCGAGAACCTAGACCTCAATCAAGCGAGCTTTCTCCTGAAGAACGTGCTAGGCTCC
TGGTCGAGTAAGCTCTTCGGCATAGCGTTGTTGGCCTCAGGGCAAAGCTCTACGATTACA
GGCACCTATGCAGGGCAGTATGTGATGCAAGGGTTTCTCAATCTCCGGTTCGTTCCCTGG
GTTTCGGAACCTTCTCACCAGGTCTGTTGCCATTGGACCATCACTCCTCGCAGCGATTTTT
GGCGGGTCCTCAGGTGCAGGGAATCTCATCATCATCTCCAGCATTATCCTCTCTTTCGAA
CTCCCCTTCGCTTTATTGCCCCTGCTCAAGTTCACGTCTCGAAGACCAAGATGGGCCCCG
TACAAGAATCCGCTTACCGGGATTTCGAGCTCATGATTACATGGTCAATCGGATT**CACCAC**
CATCATCACCAT**TG**AGAATTC



Supplementary Figure S2 (a) Schematic representation of the structure of AyNramp5a. Yellow boxes indicate the transmembrane domains. DUF4131 region is indicated by a light blue box. (b) Nucleotide sequence of the chemically synthesized AyNramp5a-6 × His gene, which was applied for expression in rice cells. Red letters indicate the initiation and termination codons, and the sequence of 6 × His is underlined. Blue letters indicate the Nramp domains, and green letters indicate the region corresponding to DUF4131 domains. (c) Gene expression analysis of AyNramp5a in transgenic plants by RT-PCR. PC : pGWB2 – AyNramp5 – 6 × His plasmid, WT: wild type, #1 - #9: independent transformant callus line

Supplementary Table 1 Analytical conditions of μ -XRF imaging

Figure number	X-ray energy /keV	Beam size /μm(V) x μm(H)	Step size /μm(V) x μm(H)	Imaging area /μm(V) x μm(H)	Dwell time /s point ⁻¹
Fig. 4a	30	0.85 x 1.6	2 x 2	122 x 232	0.3
Fig. 4b	30	0.85 x 1.6	2 x 2	252 x 168	0.3

The detailed conditions shown in Fig. 4 are summarized.