Supplemental data

Table I. Analyses of rice root response to barrier with or without jasmonic acid (1µM) using Proscope digital microscope. Seeds from WT and RNAi::*OsHOS1*#1 were surface sterilized and germinated on solid Yoshida's medium as described on the Material and Methods section. After germination, the seeds from both lines were transferred to fresh Yoshida's plates or to medium supplemented with 1µM of jasmonic acid, and transferred to light at 24-30 ºC and with a 12/12 h photoperiod for another 2 days. To facilitate root imaging, the roots were kept between two layers of solid growth medium (with or without jasmonic acid). The roots grew vertically in these conditions overnight prior to imaging. The barrier test was performed using 2 glass-coverslips placed perpendicularly in front (1-2 mm) of the growing root tip. Root curling could be observed after 20-22 h.

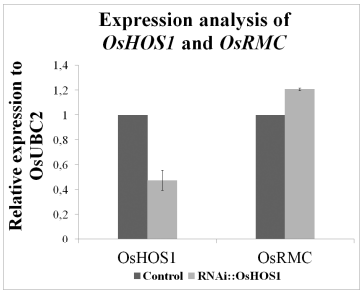
Each rice seedling was analyzed individually, each representing a single experiment. We considered a root curling score when RNAi::*OsHOS1*#1 seedlings showed a morphological alteration when hitting the barrier (similar to what is shown in Figure 3C). None of the RNAi::*OsHOS1*#1 seedlings showed a total reversion of the phenotype to mimic WT seedlings. Total WT seedlings analyzed = 21. Total RNAi::*OsHOS1*#1 seedling analyzed = 18.

	-JA		+JA	
	Curled	Not curled	Curled	Not curled
WT	11	2	8	0
RNAi::*OsHOS1*#1	1	8	9	0

Table II. Oligonucleotide sequences used in the cloning procedures and gene expression studies. The underlined region of the primer corresponds to adapter sequences and the remaining region is specific to the target DNA.

Primer name	**Primer sequence 5´- 3´**
GW-OsEREBP1-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTACAAGCAATCCACCACTGCA
GW-OsEREBP1-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTGCCTCCCAATCTCCAATAG
GW-OsEREBP2-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTATATGACGGTGGCGGGGGCGTCGGAGCT
GW-OsEREBP2-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTGGACAGAATCCGGCGGCTACTGCGTGTGC
GX-OsEREBP1-Fw	ATATGAATTCATGTGCGGCGGCGCCATCATCC
GX-OsEREBP1-Rv	ATATCTCGAGGAATTCCTCAATAGAAATCGCTAACGGGCAT
GX-OsEREBP2-Fw	ATATGAATTCATGACGGTGGCGGGGGCGTCGGAGCT
GX-OsEREBP2-Rv	ATATCTCGAGGGACAGAATCCGGCGGCTACTGCGTGTGC
qPCR-OsEREBP1-Fw	ACGTCGTCGAGATCAAGCC
qPCR-OsEREBP1-Rv	TTTGGCAGACTTTGCAGCAG Tm = 58 ºC RT-qPCR efficiency = 97%
qPCR-OsEREBP2-Fw	TCGGAGTCGAGCTATCACCA

qPCR-OsEREBP2-Rv	AATCTGCGACGTCCATCTCC Tm = 60 ºC RT-qPCR efficiency = 99%
qPCR-OsRMC-Fw	CAGGTGGTGGGACACGTT
qPCR-OsRMC-Rv	CCTACTCACGCAGCACCAC Tm = 59 ºC RT-qPCR efficiency = 88%
sqPCR-OsRMC-Fw	GTTCGACATCACGCTGGA
sqPCR-OsRMC-Rv	ATAATCCGGTTACAGCTTAGATAGAT
qPCR-OsUBC2-Fw	TTGCATTCTCTATTCCTGAGCA
qPCR-OsUBC2-Rv	CAGGCAAATCTCACCTGTCTT Tm = 58 ºC RT-qPCR efficiency = 93%
sqPCR-OsUBC2-Fw	CAAAATTTTCCACCCGAATG
sqPCR-OsUBC2-Rv	ATCACATGAATCAGCCATGC
GW-OsHOS1-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCGTCGACCAATCGGAGATG
GW-OsHOS1-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTCCTCAAACAAATCGCAGTTACA
qPCR-OsHOS1-Fw	TGTTGGCCTTAACAGCAGTG
qPCR-OsHOS1-Rv	TGAACTCAGGGGAACTGGTC Tm = 59 ºC RT-qPCR efficiency = 98%
S1-OsHOS1-Fw	CAGCTGGCATCCATTGATCT
L2-OsHOS1-Rv For sqPCR	GTGTGCCTTGAGGGTATGTCC
qPCR-OsAOS1-Fw	GCCCGGTCATCTTATTTTCC
qPCR-OsAOS1-Rv	ACCAGTGCAACTCCGTATCC Tm = 58 ºC RT-qPCR efficiency = 88%



Supplemental Figure 1. Gene expression analyses for *OsHOS1* (JQ866627) and *OsRMC* (LOC_Os04g25650), was performed by semi-quantitative RT-PCR (RT-sqPCR) in control (protoplast transformation procedure without plasmid) rice protoplasts or transiently down-regulating *OsHOS1* (RNAi::*OsHOS1*) protoplasts. The specific primers are described in Supplemental Table II. The transcript level for *Ubiquitin-Conjugating Enzyme E2* (*OsUBC2*, LOC_Os02g42314) was used to normalize the expression as internal control for the RT-sqPCR. The expression value of the control protoplasts sample was set to 1. Values represent means ± SE (n≥3 independent protoplasts transformations).

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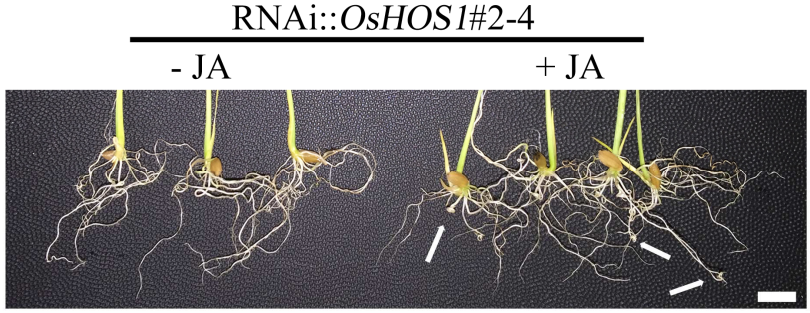

Supplemental Figure 2. Root curling response of 10-DAG RNAi::*OsHOS1*#2-4 seedlings grown in liquid Yoshida's medium on test tubes under control conditions. The seedlings were in (from 2-DAG to 10-DAG) control conditions (1% v/v ethanol) or jasmonic acid treated (2 µM). At least 19 seedlings per treatment were used in two independent experiments. Under control conditions, roots in RNAi::*OsHOS1*#2-4 were similarly curled to the ones shown in Fig. 3B. Approximately 20-30% of the total number of RNAi::*OsHIOS1*#2-4 seedlings in control conditions showed root curling. Under jasmonic acid treatment, the RNAi::*OsHOS1*#2-4 seedlings with root curling were approx. 60-75% (data not shown). Arrows show root curling phenotype in the RNAi::*OsHOS1*#2-4 seedlings under jasmonic acid treatment. Scale bar = 1 cm.

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Supplemental Video 1. Vertical growth of wild-type rice root (cv. Nipponbare) on solid medium against a glass barrier. Photographs were taken every 5min for 22h using the Proscope digital microscope (Bodelin Technologies, USA) with a 50x magnification. Images were compiled into a 17 seconds movie file (16 frames *per* second).

Supplemental Video 2. Vertical growth of RNAi::*OsHOS1* rice root on solid medium against a glass barrier. Photographs were taken every 5min for 19h using the Proscope digital microscope (Bodelin Technologies, USA) with a 50x magnification. Images were compiled into a 14 seconds movie file (16 frames *per* second).