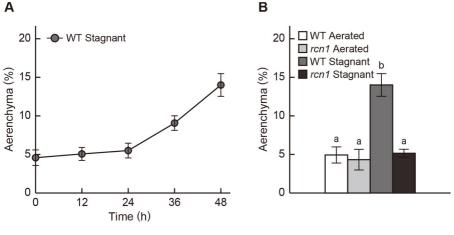


conditions. Emerged adventitious root numbers of the wild type and the rcn1 mutant grown under aerated conditions were counted every day until day 10. Values are means  $(n = 15) \pm SE$ . No Significant differences were observed between the wild type

and the *rcn1* mutant at P < 0.01 (two sample t test).

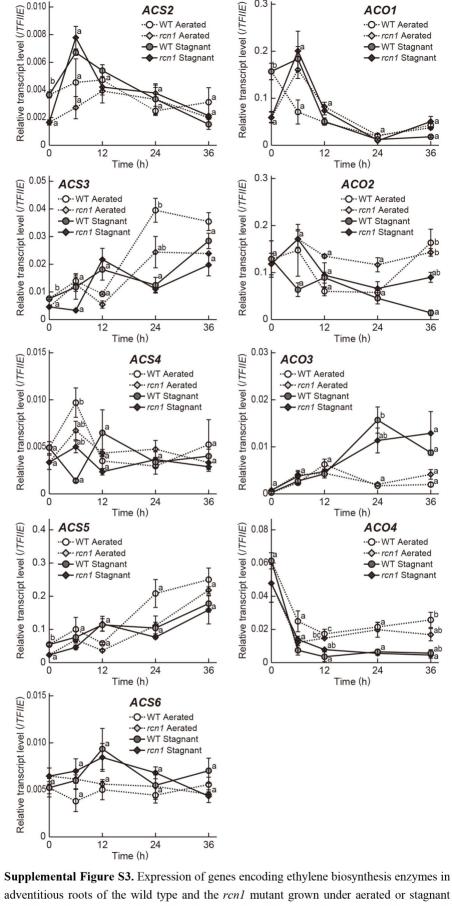
the wild type and the ren1 mutant grown under aerated



roots in the wild type and the rcn1 mutant. Twenty-d-old aerobically grown rice seedlings were transfered to aerated or stagnant conditions. Time-course of aerenchyma formation at 20 mm from the tips of adventitious roots in the wild type under stagnant conditions (A). The percentage of aerenchyma of root-cross-sectional area at 20 mm from the tips grown under aerated or stagnant conditions for 48 h (B). Values are means (n = 6)  $\pm$  SE. Different lower-case letters denote significant differences between the wild type and the rcn1 mutant in each condition (P < 0.01, one-way ANOVA and then Tukey's test for multiple

comparisons).

**Supplemental Figure S2.** Aerenchyma formation at 20 mm from the tips of adventitious



В

ACS2

0.3

ACO1

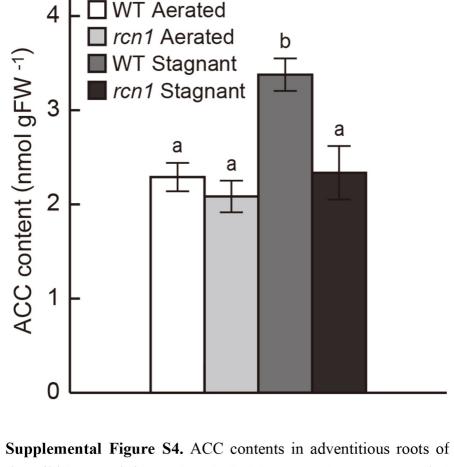
A

0.010

adventitious roots of the wild type and the rcn1 mutant grown under aerated or stagnant conditions. Time-course qRT-PCR analyses of the ACS genes (A) and the ACO genes (B) using RNAs extracted from adventitious roots at 10 mm (±2 mm) from the root tips grown under aerated or stagnant conditions. The transcription initiation factor TFIIE gene was used as a control. Values are means (n = 3)  $\pm$  SE. Different lower-case letters denote

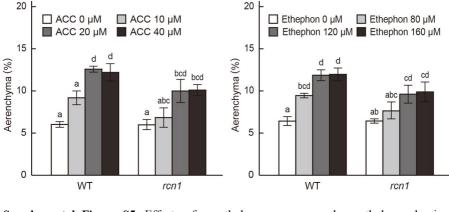
significant differences between the wild type and the rcn1 mutant in each condition (P <

0.01, one-way ANOVA and then Tukey's test for multiple comparisons).



the wild type and the *rcn1* mutant. ACC content was measured at 10 to 30 mm from the root tips grown under aerated or stagnant conditions for 12 h. Values are means  $(n = 3) \pm SE$ . Different lower-case letters denote significant differences between the wild

type and the rcn1 mutant in each condition (P < 0.01, one-way ANOVA and then Tukey's test for multiple comparisons).

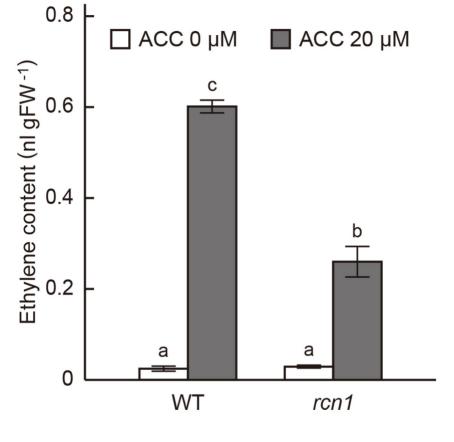


В

**Supplemental Figure S5.** Effects of an ethylene precursor and an ethylene-releasing compound on aerenchyma formation at 20 mm from the tips of adventitious roots of the wild type and the rcn1 mutant. The percentage of aerenchyma of root-cross-sectional area

at 20 mm from the tips of adventitious roots grown under aerated conditions for 48 h with 0, 10, 20 or 40 µM ACC treatment (A), and grown under aerated conditions for 48 h with 0 80, 120 or 160  $\mu$ M ethephon treatment (B). Values are means (n = 6)  $\pm$  SE. Different lower-case letters denote significant differences between the wild type and the rcn1 mutant in each condition (P < 0.01, one-way ANOVA and then Tukey's test for multiple

comparisons).



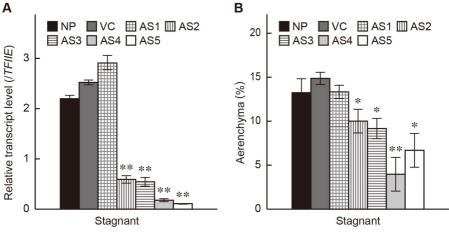
initiation of growth under aerated conditions with or without 20  $\mu$ M ACC treatment. Values are means (n = 3)  $\pm$  SE. Different lower-case letters denote significant differences between the wild type and the *rcn1* mutant in

Supplemental Figure S6. Ethylene contents in roots of the wild type and

the rcn1 mutant grown under aerated conditions with or without 20 µM

ACC treatment. Ethylene content was measured in roots at 24 h after

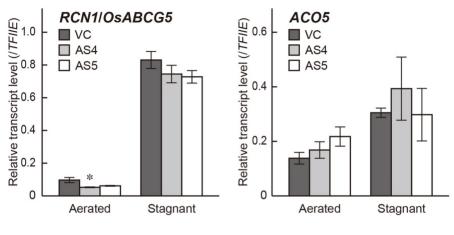
each condition (P < 0.01, one-way ANOVA and then Tukey's test for multiple comparisons).



Supplemental Figure S7. Expression of the CUT1L gene and aerenchyma formation in adventitious roots of the pACT1::CUT1L-antisense T<sub>0</sub> transgenic lines. The percentage of aerenchyma of root-cross-sectional area at 10 mm (A) and at 20 mm (B) from the tips of adventitious roots of the wild type (Nipponbare; NP), the pACT1::CUT1L-antisense T<sub>0</sub>

transgenic lines (AS1 to AS5) and the vector control (VC) grown under stagnant conditions for 48 h. All transgenic lines had a cv. Nipponbare background. Values are means (n = 3)  $\pm$  SE.

Significant differences between each of the transgenic lines and the vector control at P < 0.01or at P < 0.05 (two sample t test) are denoted by \*\* or \*, respectively.



B

**Supplemental Figure S8.** Expression of RCNI/OsABCG5 and ACO5 in adventitious roots of the pACT1::CUTIL-antisense  $T_1$  transgenic lines. qRT-PCR analyses of the RCNI/OsABCG5 gene (A) and the ACO5 gene (B) using RNAs extracted from adventitious roots at 10 mm ( $\pm 2$  mm) from the root tips of the pACT1::CUTIL-antisense  $T_1$  transgenic lines (AS4 and AS5) and the vector control (VC) grown under aerated or stagnant conditions for 36 h. All transgenic lines had a cv. Nipponbare background. The transcription initiation factor TFIIE gene was used as a control. Values are means (n = 3)  $\pm$  SE. Significant differences between each of the transgenic lines and the vector control at P < 0.05 (two sample t test) are denoted by \*.

Supplemental Table S1. List of primers used for qRT-PCR analysis

Gene Name	ID (RAP-DB)	Forward primer sequence	Reverse primer sequence
ACC synthase (A	CS)		
ACS1	Os03g0727600	5'-ACAAAACCACACCATGTCCA-3'	5'-CGAAAGGAATCTGCTACTGCTGC-3'
ACS2	Os04g0578000	5'-CACCACCACCTCAGC-3'	5'-GACGTAGTAAGGCGCAGCAT-3'
ACS3	Os05g0196600	5'-GAGGCGAAGCTGAACATCTC-3'	5'-CATGTTGTTCTTGCTCCCATT-3'
ACS4	Os05g0319200	5'-AGCTGAGGCTGTGGGACA-3'	5'-GTGGCCAGGCTCATGTTC-3'
ACS5	Os01g0192900	5'-GCTTGGACACGCTGGATCTT-3'	5'-TTATTGCTGTTCTTGCTGCTG-3'
ACS6	Os06g0130400	5'-GGATGGTTCAGGTGTTGCTT-3'	5'-CCTGGCAAAGCAGTTATTCC-3'
ACC oxidase (AC	(O)		
ACO1	Os09g0451000	5'-GATAGCGTGTGTACCACAGCGACC-3'	5'-AGGTAGAAAACGCGAGCTGA-3'
ACO2	Os09g0451400	5'-AAGTCCATGGAAACCGAGAC-3'	5'-CCACAGTTCATGCACACACA-3'
ACO3	Os02g0771600	5'-GAGGTTCGTGTTCGAGGACT-3'	5'-CGCAGCCGTAGCTAGTGAAG-3'
ACO4	Os11g0186900	5'-GCATGGCCAACATTGCTC-3'	5'-GTTCGCCAGGGCTGCGAACC-3'
ACO5	Os05g0149400	5'-CGAGTACCCGGAGTACGTGTT-3'	5'-ATTTTGGCGCCTTGACGGCC-3'
ACO7	Os01g0580500	5'-GGACTACTACCAGGGCACCA-3'	5'-GATTAGCGCACGCGATTTTA-3'
G-type ABC trans	sporter		
RCN1/OsABCG5	Os03g0281900	5'-AAGTGGGAGTGCCTCTGGAT-3'	5'-GGTGAGACGAGGTGACGAT-3'
Fatty acid elongas	se (β-ketoacyl-CoA sy	nthase; KCS)	
CUT1L	Os03g0220100	5'-AAGTGCCTCCGCACAGTC-3'	5'-ACCATGGATTCGATCACAGC-3'
CUT1L 5'-UTR p	rimers for the <i>CUT1L</i>	- antisense transgenic lines	
CUT1L5UTR	Os03g0220100	5'-CCATCACCAACACACCACTG-3'	5'-TCCTACGTACGACTTTAGTTTCTTC-3'
Control			
TFIIE	Os10g0397200	5'-GTGCAGCCCAAGGCTAAG-3'	5'-CGTCGAATAAGCGTAGAGCA-3'