

Supplementary Materials for Coordinated Responses to Oxygen and Sugar Deficiency Allow Rice Seedlings to Tolerate Flooding

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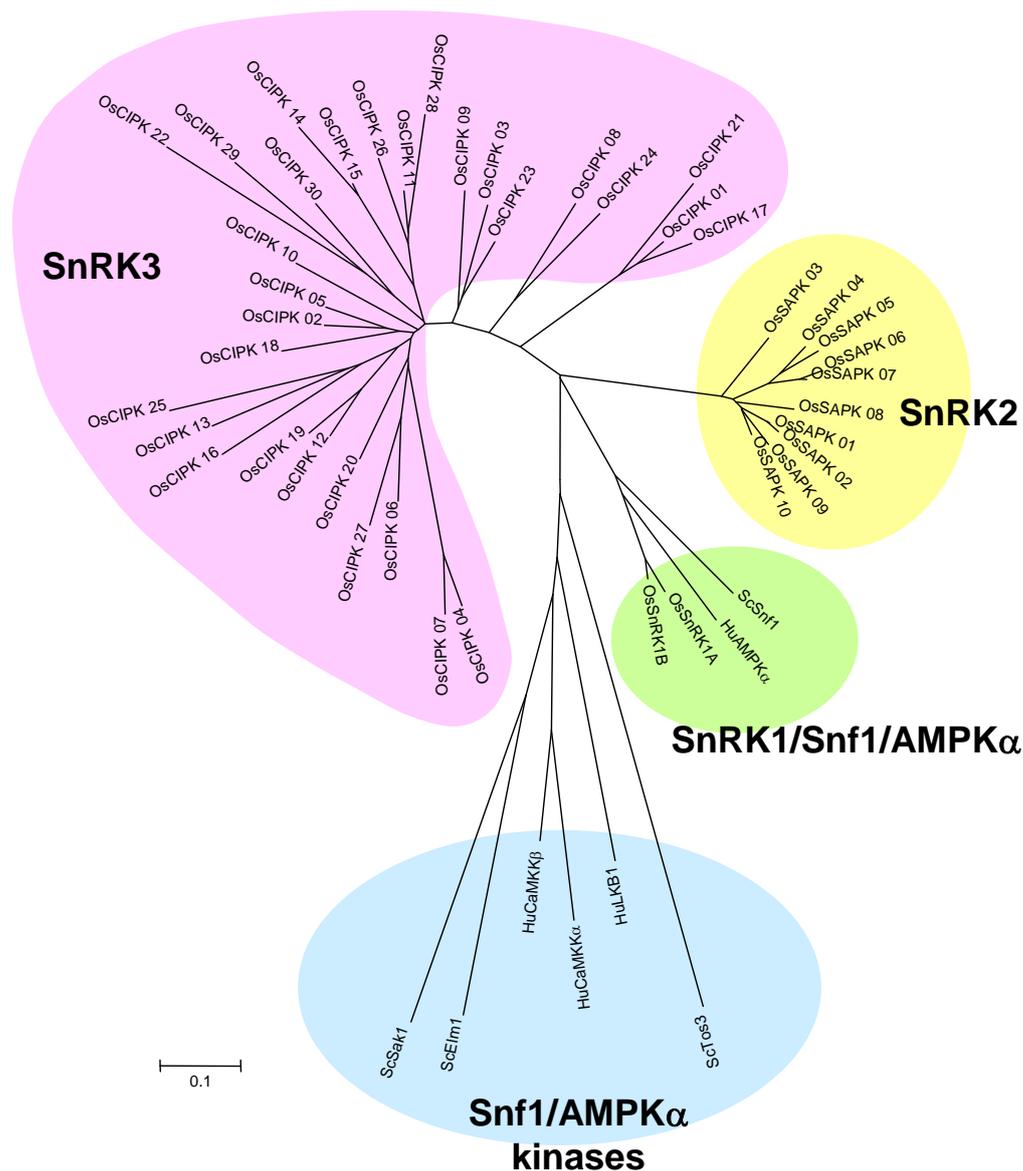


Fig. S1. Dendrogram showing the evolutionary relationship of members of the rice CIPK family and other Snf1-related protein kinases from rice, mammals and yeast. Deduced amino acid sequences of the rice SnRK1, SnRK2 and SnRK3 (CIPK) families were identified by BLAST search of the NCBI and Swiss-Prot/TrEMBL databases using sequences of the human AMPK and yeast Snf1 upstream protein kinases: human LKB1 (Q15831), human CaMKK α (Q8N5S9), human CaMKK β (Q96RR4), yeast Elm1 (P32801), yeast Tos3 (Q5CJ76), and yeast Sak1 (P38990). Sequences of the rice CIPK family have also been reported (1). The amino acid sequence alignment and phylogenetic analysis were carried out using AlignX (Vector NTI, version 9.0.0, Invitrogen), GeneScan (The New GENSCAN Web Server at MIT) and MEGA 3.1 (Center for Evolutionary Functional Genomics) programs. The scale value of 0.1 indicates 0.1 amino acid substitutions per site.

Reference:

1. U. Kolukisaoglu, S. Weinl, D. Blazevic, O. Batistic, J. Kudla, Calcium sensors and their interacting protein kinases: genomics of the Arabidopsis and rice CBL-CIPK signaling networks. *Plant Physiol* **134**, 43-58 (2004).

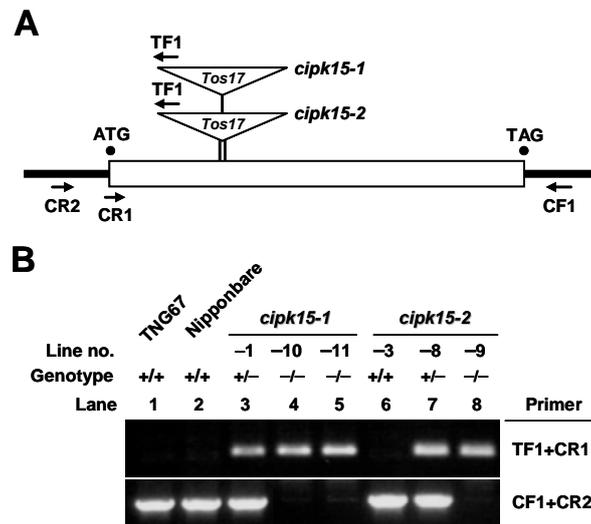


Fig. S2. Genotyping identified two allelic *Tos17*-tagged *CIPK* homozygous ($^{-/-}$) and heterozygous ($^{+/-}$) Nipponbare rice mutants. Only the homozygous mutant displays hypoxia sensitive phenotype while the heterozygous mutant was as normal as the segregated wild type. **(A)** Diagram shows positions of retrotransposon *Tos17* insertions at positions 1059 and 1065 bp downstream of the translation initiation codon ATG in coding regions of *CIPK15* in mutants *cipk15-1* (NE3531) and *cipk15-2* (NE3540), respectively. Box indicates the single exon and lines indicate 5' and 3' untranslated regions of *CIPK15*. Triangles indicate *Tos17*. ATG indicates the translation initiation codon. Arrows indicate positions of DNA primers used for PCR analyses. **(B)** Genotyping of *cipk15-1* and *cipk15-2*. PCR with DNA primers CF1 and CR2 produced a product of 1623 bp from wild-type ($^{+/+}$) rice genomic DNA, and with DNA primers TF1 and CR1 produced a product of 375 bp from the rice genomic DNA-*Tos17* junction region in *cipk15* mutant.

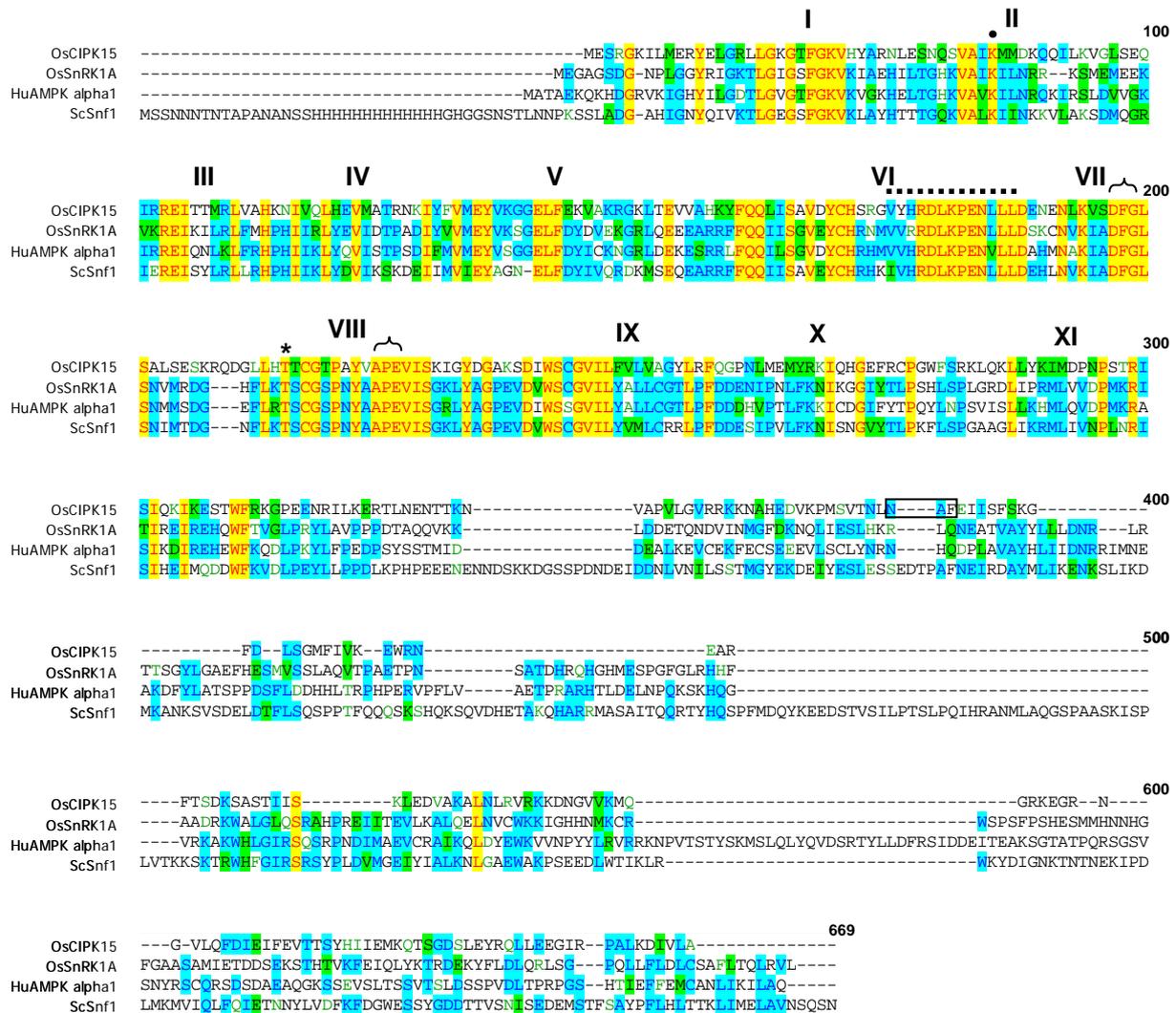


Fig. S3. Amino acid sequence of CIPK15 closely resembles that of the rice SnRK1A, mammalian AMPK α 1 and yeast Snf1 protein kinases. Amino acid sequences of the rice CIPK15 (AK121773), rice SnRK1A (BAA36298), human AMPK α 1 (P54645), and yeast Snf1 (AAA35058) were compared. Gaps have been introduced to maximize the alignment. Roman numerals above sequences indicate the 11 conserved sub-domains of Ser/Thr protein kinases (2). Identical, conserved and similar amino acid residues are highlighted in yellow, blue, and green, respectively. The ATP binding site is marked by a dot, the phosphorylation site (Thr) is marked by an asterisk, the catalytic center is marked by a dashed line, and the conserved Asp-Phe-Gly (DFG) and Ala-Pro-Glu (APE) motifs that define the activation loop are marked by brackets. The NAF domain is boxed.

Reference:

2. S. K. Hanks, A. M. Quinn. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. *Methods Enzymol* **200**, 38-62 (1991).

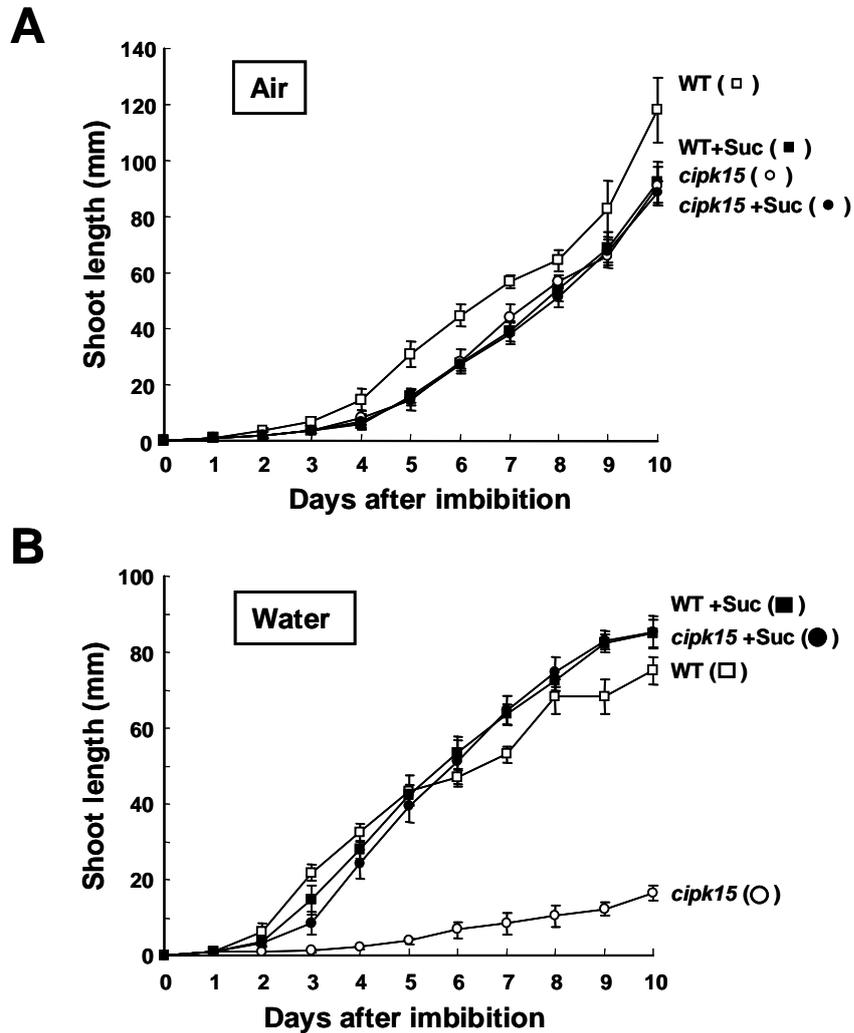


Fig. S5. Sucrose rescues underwater seedling shoot development in the *cipk15* mutant. (A) WT and *cipk15*^(-/-) seeds were germinated in air, by placing on filter papers containing water or 90 mM sucrose (Suc) solution for various lengths of time. Shoot elongation of *cipk15* was slightly retarded compared to WT. (B) WT and *cipk15*^(-/-) seeds were germinated under water or 90 mM sucrose solution for various lengths of time. *cipk15* seeds germinated but both shoot and root growth were severely retarded; however, sucrose recovered shoot growth to the same extent as in WT. Shoot length of seedlings at each time points was measured daily. Error bars indicate the S.E. of shoot length (n = 30). (ANOVA; p < 0.01)

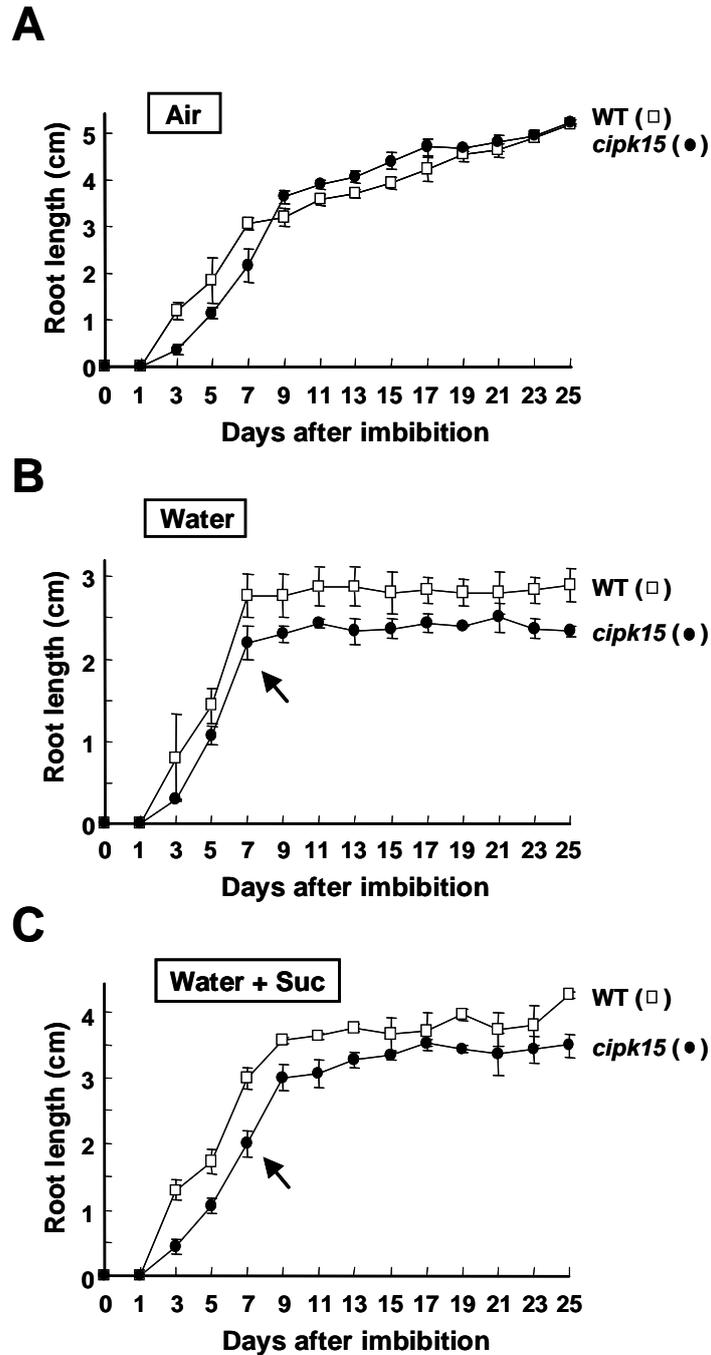


Fig. S6. CIPK15 controls underwater root development. WT and *cipk15*^(-/-) seeds were germinated on the surface of 5-cm high 0.3% agarose gel in a 500 mL glass bottle, and seedlings were grown at 28°C under a 16-hr light / 8-hr dark condition for 7 days. Some seedlings were then submerged by filling up the glass bottle with water or 90 mM sucrose (Suc) solution, and the root length was measured daily up to 25 days after imbibition. (A) Roots growth in air. (B) Roots growth under water. (C) Roots growth under 90 mM sucrose solution. Error bars indicate the S.E. of root lengths (n = 5). Arrow indicates the date starting treatments. (ANOVA; p < 0.01)

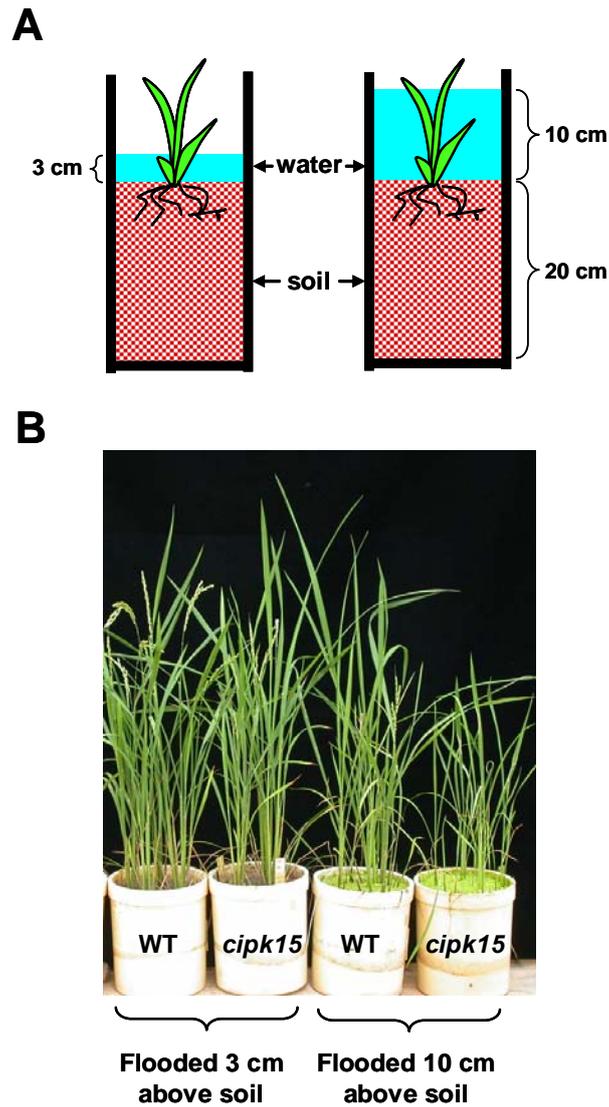


Fig. S7. CIPK15 control mature plant growth under partially-flooded conditions. **(A)** Diagram showing how rice plants were flooded with water. Two-week-old rice seedlings were grown in pot soil (approximately 20-cm deep) and flooded with water, either 3-cm (left) or 10-cm (right) high above soil surface. **(B)** The growth of WT and *cipk15*^(-/-) plants was similar when flooded with 3 cm water but slower in *cipk15*^(-/-) than in the WT when flooded with 10 cm water. Rice plants were grown in the greenhouse and the photo was taken 9 weeks after flooding.

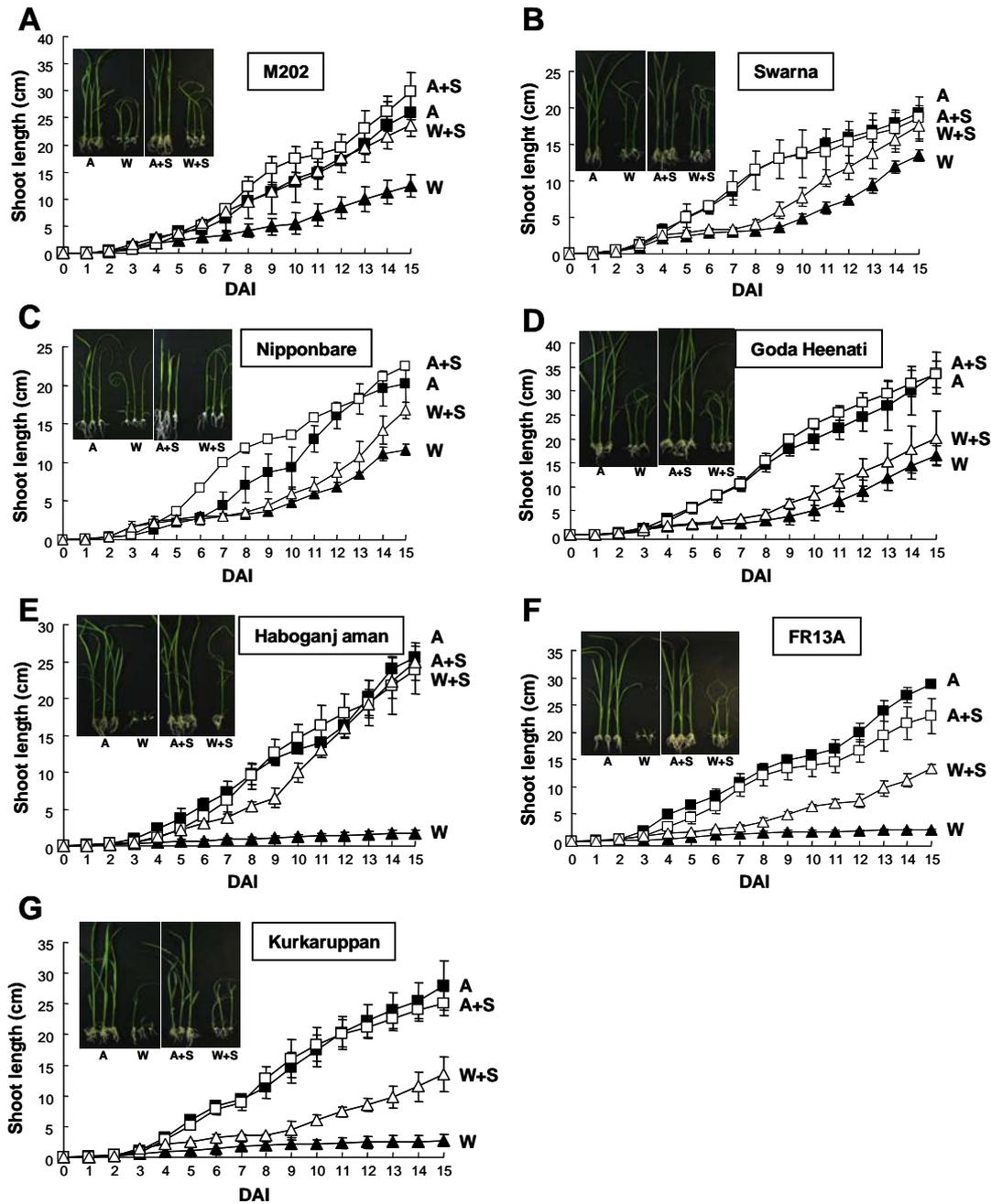


Fig. S8. Seedling hypoxia tolerance varies among rice varieties. Rice seeds were germinated in air or in water with or without 90 mM sucrose for various lengths of time. Shoot length of seedlings were measured daily. Error bars indicate the S.E. of shoot length ($n = 30$). (ANOVA; $p < 0.01$) (A-D) seedlings of varieties tolerant to hypoxia. (E-G) seedlings of varieties intolerant to hypoxia. A, in air; A+S, in air with sucrose; W, under water; W+S, under water with sucrose. Sucrose improved seedling development in all rice varieties. DAI, days after inhibition. These rice varieties correspond to those shown in Table 1.

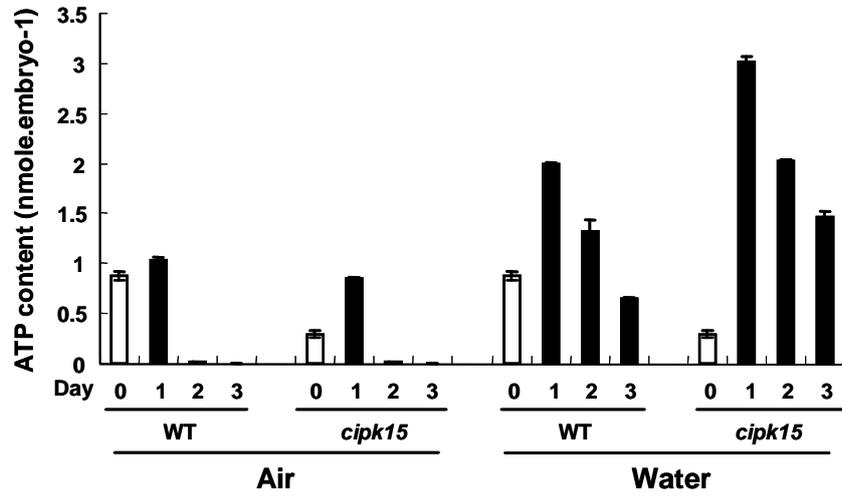


Fig. S9. ATP is consumed more slowly in WT and *cipk15* embryos germinated under water than in air.

Table S1. The IRRI Germplasm Center (IRGC) accession number of rice germplasms used in the present study.

Germplasm name	IRGC accession number
<i>Oryza sativa</i> cv. FR13A	72966
<i>Oryza sativa</i> cv. Kurkaruppan	15449
<i>Oryza sativa</i> cv. Goda Heenati	31393
<i>Oryza sativa</i> cv. Habiganj Aman 7	47641
<i>Oryza sativa</i> cv. Swarna	117278
<i>Oryza sativa</i> cv. M202	117270

Table S2. Primers used in the present study.

Target gene	Primers	Annealing temperature (°C)	Fragment size (bp)
<i>CIPK15</i> (WT genotyping)	15CF1: 5'-CGGGATCCCCTGTCTCAACATGAACTCTCC-3' 15CR2: 5'-CGGGATCCCCTCCATCCACGATCATGACC-3'	60	1623
<i>CIPK15</i> (Mutant genotyping)	15TF1: 5'-ATTGTTAGGTTGCAAGTTAGTTAAGA-3' 15CR1: 5'-GCGTGGAAAGCTTACAGAGG-3'	55	375
<i>CIPK15</i> (RT-PCR)	15F2: 5'-TAAGCCTTCAAATTCTTCG-3' 15R3: 5'-TATAAACAAAACCAGGCATC-3'	60	133
<i>CIPK15</i> (ORF)	15OF1: 5'-ATCCACGATCATGACCTTGG-3' 15OR1: 5'-TGTCTCAACATGAACTCTCC-3'	50	1302
<i>CIPK11</i> (ORF)	11OF1: 5'-GCG GATATCGGCCGTTATGAGGTTGGGA-3' 11OR1: 5'-GCG GATATCCTAATTCTTTGTTTGTCTGC-3'	58	1506
<i>CIPK21</i> (ORF)	21OF1: 5'-GCG GATATCCGGATGGGGAAGTACGAG-3' 21OR1: 5'-GCG GATATC TCAAAAGCTACTGTTATATCG-3'	58	1491
<i>αAmy3</i> (RT-PCR)	3RTF1: 5'-CAGGCTCTCTAGCCTTAGG-3' 3RTR1: 5'-ACCTGACATATATATGCACC-3'	58	103
<i>αAmy3</i> (Promoter)	3PF1: 5'-CTACTCCACTGCTCCATAGG-3' 3PR1: 5'-GCGTCCAGGTCGTAGAGACG-3'	50	1440
<i>αAmy3</i> (ORF)	3OF1: 5'-AGGCCAAGTCGACGACATCG-3' 3OR1: 5'-CACCAACGGTTACAAACTGC-3'	50	1.5
<i>αAmy7</i>	7RT1: 5'-GACGAGACTCTCAGTTTAGC-3' 7RT2: 5'-TGCATCCGTAATTCGGATCG-3'	58	120
<i>αAmy8</i>	8RT1: 5'-GGTTCCTGCCGGTAGAAAGC-3' 8RTB: 5'-GGATCGAAACGAACAGTAGC-3'	58	266
<i>SnRK1A</i>	SnRK1aF1: 5'-AACCAGAGGTAACAGGCAGG-3' SnRK1aR1: 5'-CATCTGTCAAGGAATGCAGG-3'	58	218
<i>MYB S1</i>	S1RTF1: 5'-ATGGACGGACATGAGCAGC-3' S1RTR1: 5'-GCTTTCACCGGGTGTACG-3'	58	234
<i>Adh1</i>	Adh1F1: 5'-CCAGTTCAGCAGGTACTTGC -3' Adh1R1: 5'-CAGGATACACAGAAGAACCG -3'	54	484
<i>Adh2</i>	Adh2F1: 5'-CACGAGGTGCTGATTGAG -3' Adh2R1: 5'-CAAGATCAAGCCATGAAAGG -3'	54	583
<i>Sub1C</i>	Sub1CF1: 5'-CGTGGTAGTGACAAGTGCCTC -3' Sub1CR1: 5'-CGTTTATGTTGCTCCTGAATCTGC -3'	60	535
<i>EXPA7</i>	Exp7F1: 5'-TCTCCTACCGCCGGGTGC -3' Exp7R1: 5'-CCAACCAACATGATGCAGTATCC -3'	60	623
<i>α-Tubulin</i>	TubF1: 5'-GAGGAGTCGTCGTCGCTGG-3' TubR1: 5'-GCACACAGGTTACAACAACG-3'	60	182
<i>18S rRNA</i>	18SF1: 5'-CCTATCAACTTTCGATGGTAGGATA-3' 18SR1: 5'-CGTTAAGGGATTAGATTGTACTCATT-3'	58	229

Gene accession number: *CIPK15* (LOC_Os11g02240), *CIPK11* (LOC_Os01g60910), *CIPK21* (LOC_Os07g44290), *αAmy3* (LOC_Os08g36910), *αAmy7* (LOC_Os02g52710), *αAmy8* (LOC_Os08g36900), *SnRK1A* (AF062479.1), *MYBS1* (AY151042), *Adh1* (LOC_Os11g10480), *Adh2* (LOC_Os11g10510), *Sub1C* (DQ453965), *ExpA7* (LOC_Os03g60720), *α-Tubulin* (LOC_Os07g38730), *18S rRNA* (LOC_Os03g0684600).