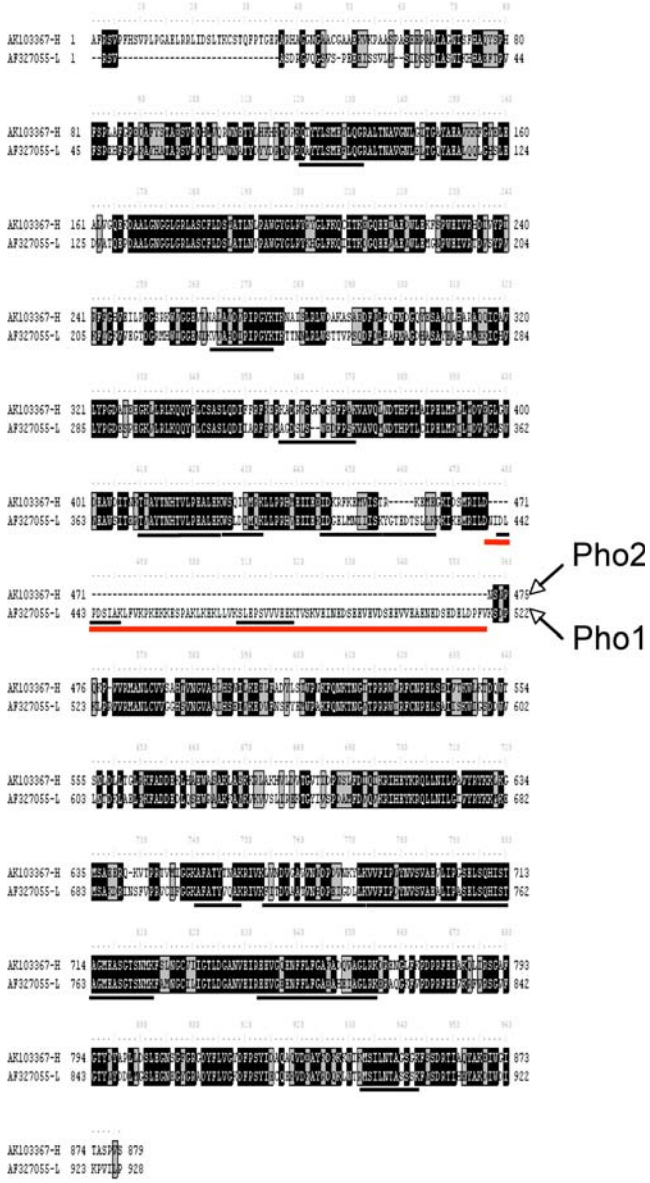
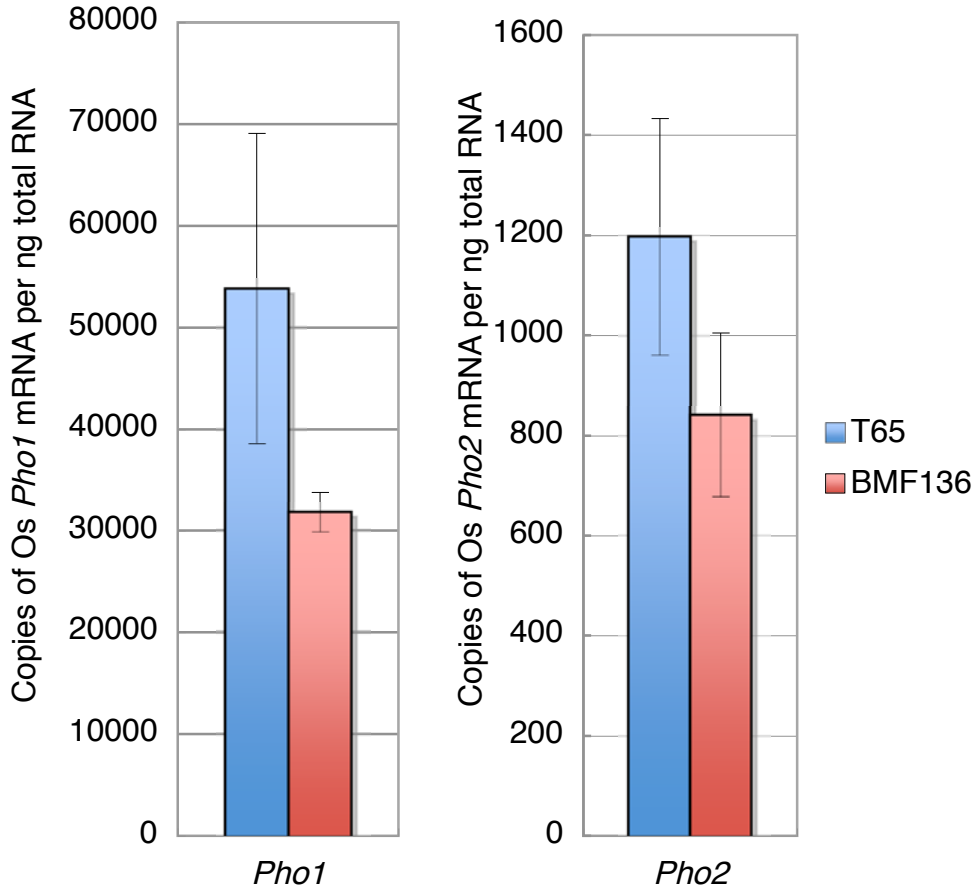
Supplemental Data. Satoh et al. (2008). Mutation of the plastidial α-glucan phosphorylase gene in rice affects the synthesis and structure of starch in the endosperm.



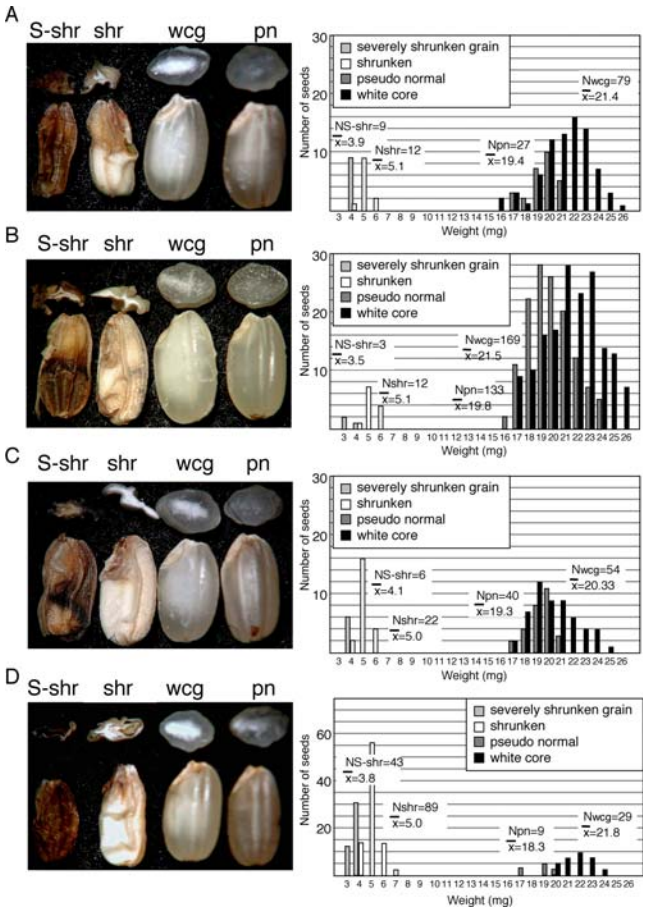
Supplemental Figure 1. Amino acid sequences of the 106 kDa protein fragments analyzed by LC-MS/MS.

The Pho1 and Pho2 primary sequences are aligned showing their sequence similarity and the presence of the extra 80 amino acid insert (red underline) unique in Pho1. The blackly underlined sequences represent tryptic fragments that were identified by mass spectrometry, which align with the Pho1 primary sequence.



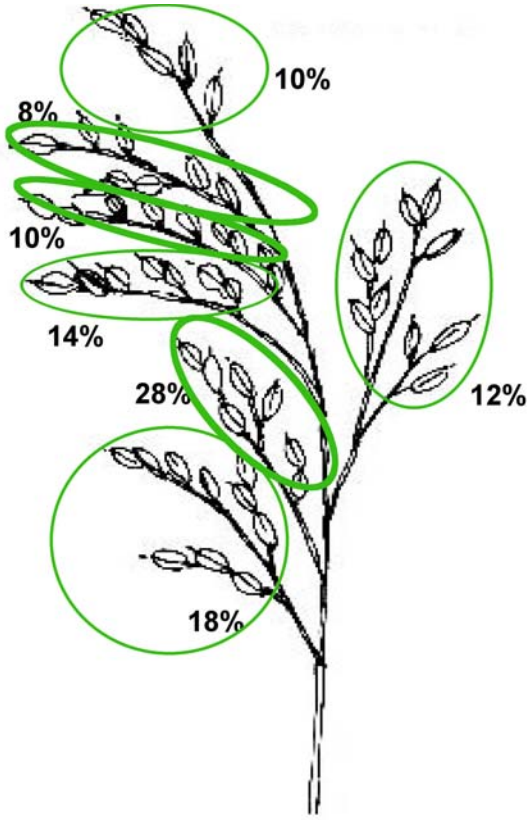
Supplemental Figure 2. Transcript levels of *Pho1* and *Pho2* genes determined by real-time RT-PCR.

The transcript levels were expressed as copy numbers of *Pho1* and *Pho2* mRNA in endosperm. Spikelets were harvested at about 10 days after flowering. An aliquot of the first strand cDNA mixture corresponding to 5 ng of the total RNA was used as a template for quantitative real-time RT-PCR. The data represent the average ± SD of at least four independent measurements. (A) *Pho1* mRNA. (B) *Pho2* mRNA.



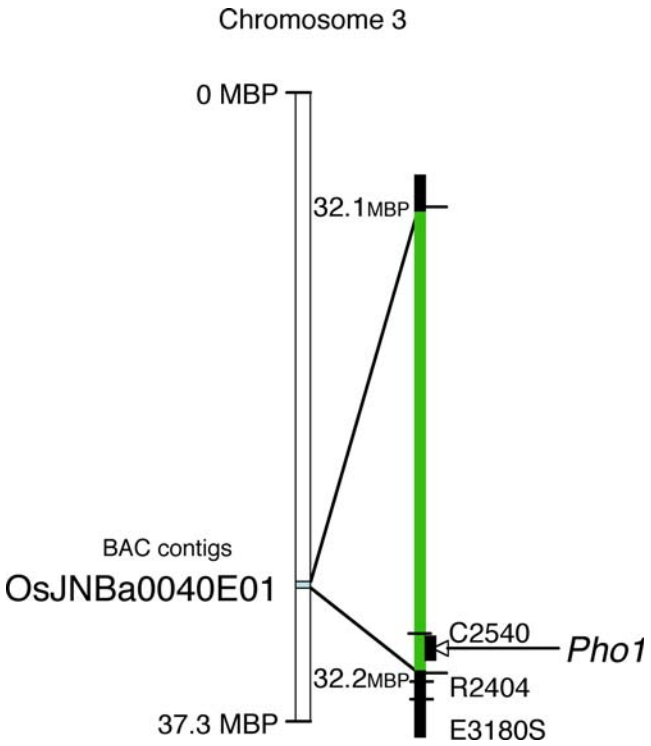
Supplemental Figure 3. Effects of *pho1* mutations on the morphology of rice kernels grown under field condition.

The left panel shows views of representative samples of sectioned (upper row) and intact (lower row) seeds for (A), EM640; (B), BMF134 (EM719); (C), BMF136 (EM755); and (D), EM876. The right panel depicts the distribution of seed weights for the various seed phenotypes. S-shr, severely shrunken seeds; shr, shrunken seeds; wcg, white-core grains; pn, pseudo-normal grains; NS-shr, number of severely shrunken seeds; Nshr, number of shr seeds; Npn, number of pn seeds; and Nwcg, number of white-core grains.

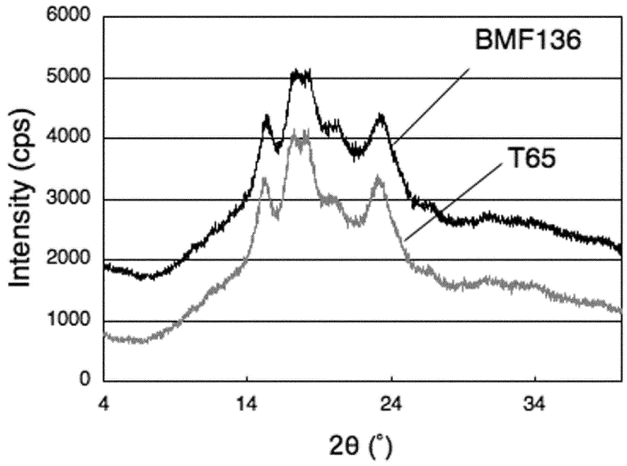


Supplemental Figure 4. Distribution of shrunken seeds on the panicles of a *pho1* mutant plant.

The position of 157 shrunken seeds was determined in a *pho1* mutant (BMF136). Figure shows percentage of shrunken seed set on respective rachis-branches of a single mutant plant.

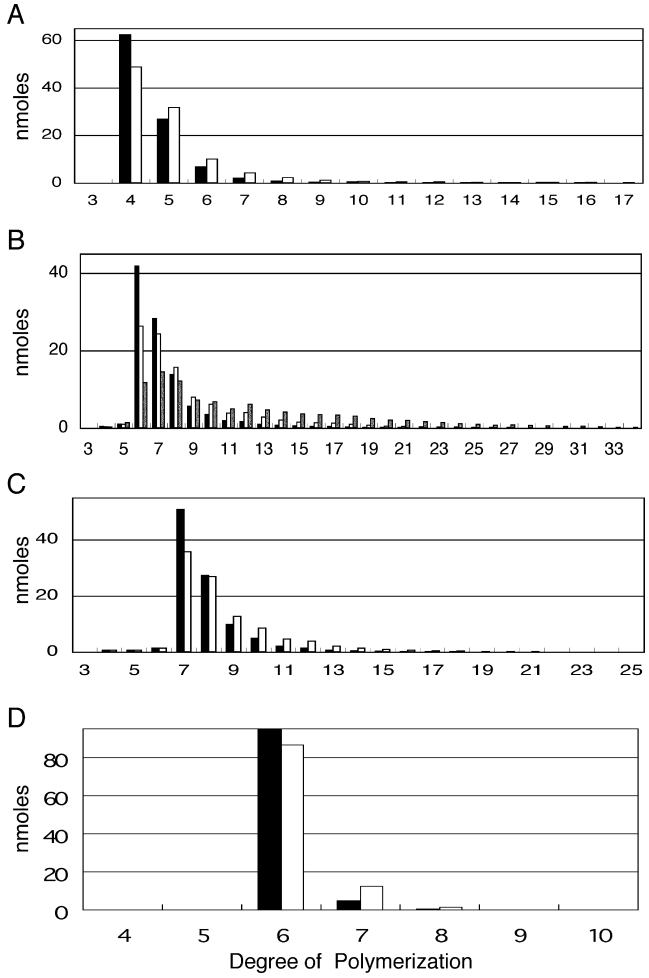


Supplemental Figure 5. Location of the *Pho1* gene on chromosome 3 in rice. Mapping of *Pho1* gene (OsJNBa0040E01) on chromosome 3 was based on the database of Oryzabase (http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp).



Supplemental Figure 6. X-ray diffraction pattern of starch in the endosperm of T65 and a *pho1* mutant line BMF136.

The figure shows a typical A pattern in the diffractograms of both starches.



Supplemental Figure 7. Chain-elongation Reaction of rPho1 and rSSIIa using Malto-oligosaccharides as Primers.

(A) to **(C)** Products of rPho1 reactions. **(D)** Products of rSSIIa reactions. Malto-oligosaccharides used as primers were maltotetraose (A), malto-hexaose (B and D) or maltoheptaose (C), respectively. The rPho1 content used in the 0.2 ml of the reaction mixture was 0.35 μg protein (closed bar and open bar) or 0.70 μg protein (gray bar), and the reaction time was 5 min (closed bar) or 10 min (open bar and gray bar). The rSSIIa content used in the 0.2 ml of the reaction mixture was 5.4 μg protein (closed bar) or 10.8 μg protein (open bar), and the reaction time was 60 min.

Supplemental Table 1. *Pho* genes of primers for real-time RT-PCR

Enzyme	Gene name	EC No.	Acc. No.	Chr.	Primer sequence
Starch phosphorylase 1	OsPho1	2.4.1.1	AK063766	3	[F] ttggcaggaaggtttcgct
					[R] cgaagcctgaagtgaacttgct
Starch phosphorylase 2	OsPho2	2.4.1.1	AK103367	1	[F] caccaagacgaagctcatcaag
					[R] ttcactcgttgctgggttctc

Supplemental Table 1. The effect of phosphate on the biosynthetic activity of rice starch phosphorylase1 (Pho1).

G1P	Activity (units/mg)			% activity remaining	
	no Pi	1mM Pi	10mM Pi	1mM Pi	10mM Pi
0.2 mM	0.57 ± 0.03	0.49 ± 0.01	0.03 ± 0.01	86	6.1
1 mM	3.0 ± 0.03	2.4 ± 0.08	0.2 ± 0.04	81	8.2
5 mM	16 ± 0.2	15 ± 0.4	5.3 ± 0.06	91	36
10 mM	25 ± 0.4	24 ± 0.2	12 ± 0.3	93	51

1.0 μg of the purified Pho1 was used for each reaction. Reaction was carried out at 37°C in 0.1 ml of 100 mM MES-NaOH, 10 mM $MgCl_2$, 0.1% amylopectin, and various concentrations of [^{14}C] G1P and potassium phosphate (pH 6.5). After 20 min, 4 volumes of methanol were added to stop the reaction and samples were left at room temperature for 10 min. Starch pellets were obtained by centrifugation for 2 min and washed once with 0.5 ml of 80 % of methanol. The pellets were dissolved in 1N HCl and then placed in boiling water for 2 min. The amount of [^{14}C] G1P incorporated into the amylopectin was measured using a liquid scintillation counter. One unit of starch phosphorylase activity is defined as the amount of enzyme which incorporate one μmole of G1P into amylopectin per minute under the defined assay conditions.

Supplemental Table 2. Primer list for sequencing analysis of *Pho1* gene

Primer name		Sequence
SPH1L	5'	ggaggagcaaaagggctagg
SPH1R	5'	tttttgcggggaacctattt
SPH2L	5'	cacccctattcttaaggtacccctat
SPH2R	5'	gcttgcctgacgtgtagtga
SPH3L	5'	tgcggttcactacacgtcag
SPH3R	5'	caggaagggcaagggtaaag
SPH4L	5'	ttgcgcaaattcctcatactc
SPH4R	5'	cgacggctgggaattaaaac
SPH5L	5'	aaaacagaaggcccaagagc
SPH5R	5'	cagccgcgtaccttcctc
SPH6L	5'	ctcgctctgacacacttgaca
SPH6R	5'	atctccccgcgtaaactcag
SPH7L	5'	ggggagatgaactgggtgtt
SPH7R	5'	aagaacgatgcagcttttgc
SPH8L	5'	gcaaaagctgcatcgttctta
SPH8R	5'	aggcttgaggaagcagcata
SPH9L	5'	ctgcttcctcaagcctcatc
SPH9R	5'	acaggaagctaaccggccta
SPH10L	5'	tcatttccttccctttgttctg
SPH10R	5'	tctcccaaggatttcccatc
SPH11L	5'	gatgggaaatccttgggaga
SPH11R	5'	aaagccatttcaccatgtgc
SPH12L	5'	tatggtcgcatccactaggc
SPH12R	5'	ccaagcctcattccagctta
SPH13L	5'	gctggaatgaggcttggagt
SPH13R	5'	ggctccagagatttgacaagc
SPH14L	5'	tggagcctagtgttgtggttg
SPH14R	5'	tgttcaaaacccaatcatcaga
SPH15L	5'	ggatctgatgattgggttttga
SPH15R	5'	tgctttcccaccaaaatatgc

(Continued)

SPH16L	5'	gcatatttggtgggaaagca
SPH16R	5'	tggcagtctggcaaaaactc
SPH17L	5'	tgccagactgccaactgtaa
SPH17R	5'	cagtgcgcaaaaagaagagg
SPH18L	5'	tgaatgccaggagaaggttg
SPH18R	5'	tgcaatcggaatagaagatgc
SPH19L	5'	gcatcttctattccgattgcag
SPH19R	5'	aggatgccactaaccgatcc
SPH20L	5'	agggatcggttagtggcatc
SPH20R	5'	tcaaacgctcgagttcccta
SPH21L	5'	agccatggaccacagttgac
SPH21R	5'	ggcaactaacacccccatct