

**Figure S1.** Transmission electron microscopy analysis of anthers in wild type and *osgpat*3-2-2 mutant at stage 8. The transverse sections of the wild-type (**A**,**C**,**E**,**G**,**I**,**K**,**M**,**O**) and *gpat*3-2 (**B**,**D**,**F**,**H**,**J**,**L**,**N**,**P**) anthers at stage 8a (**A**–**H**) and stage 8b (**I**–**P**) are compared. (**A**,**B**,**I**,**J**) Anthers of the wild type (**A**,**I**) and *osgpat*3-2 (**B**,**J**), showing the four layers of the anther wall with pollen mother cell at stage 8a and tetrads at stage 8b. (**C**,**D**,**K**,**L**) The four layers of the anther wall of the wild type (**C**,**K**) and *osgpat*3-2 (**D**,**L**). (**E**,**F**,**M**,**N**) Higher magnification of the tapetum cells showing Ubisch body of the wild type (**E**,**M**) and *osgpat*3-2 (**F**,**N**). (**G**,**H**) Pollen mother cell of the wild type (**G**) and *osgpat*3-2 (**H**) at stage 8a. (**O**,**P**) Tetrads of the wild type (**O**) and *osgpat*3-2 (**P**) at stage 8b. The arrows in (**E**,**F**,**M**,**N**) indicate an Ubisch body (Ub). E, Epidermis; En, Endothecium; ML, Middle Layer; Nu, Nucleus; PE, Priexine; PMC, Pollen Mother Cell; T, Tapetum; Tds, Tetrads. Bars = 10 µm in (**A**,**B**,**I**,**J**); 5 µm in (**C**,**D**,**J**,**K**); 0.5 µm in (**E**,**F**,**M**,**N**), and 2 µm in (**G**,**H**,**O**,**P**).



**Figure S2.** Alignments of OsGPAT3 sequence between the wild type (Zh8015, WT), three CRISPR/Cas9-induced mutants, and two allelic mutants. Analysis performed with DNASTAR-Lasergene v6/Megalign, and GENEDOC software. The signal peptide (black box), the conserved phospholipid/glycerol acyltransferase domain (GPAT, red line), transmembrane region (black dotted box), and four conserved acyltransferase motifs are indicated above the alignment (AT-I, AT-II, AT-II, and AT-IV, marked in red dotted box) were indicated by the BLAST search program (http://www.ncbi.nlm.nih.gov/blast/). Identical sequences, mutation sites, and different sequences after mutation of amino acid residues are shaded black, red, and green, respectively.

Combination	Seed-Setting - Rate of F1	F2			
		No. of Wild Type Plants	No. of Mutant Plants	χ²(3:1)	$\chi^{2_{0.05}}$
gpat3-2/Zh8015	76.75	289	91	0.17	2.94
gpat3-2/02428	82.31	472	138	1.71	5.84

Table S1. Segregation	analysis of the	e gpat3-2 allele.
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**Table S2.** Inferred function of candidate genes in the *gpat3-2* locus on rice Chr. 11.

Gene Number	Putative Function	Biological Process Involved	Specific Expression Tissue of Homologs in <i>Arabidopsis</i> .t	
LOC_Os11g45390	von Willebrand Factor Type A	Poot Dovelonment	Root Meristem, Shoot Stem	
	Domain Containing Protein	Root Development	Cells, Root Quiescent Center	
LOC_Os11g45400	Glycerol-3-Phosphate	Anther Development	Seedlings, Leaves, Shoots,	
	Acyltransferase	and Pollen Formation	Panicles and Anthers, Siliques	
LOC_Os11g45410	Tetratricopeptide Repeat		Guard Cell, Shoot Apex	
	(TPR)-like Superfamily Protein			

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Primer	Forward Primer (5-3')	Reverse Primer (5-3)	Purpose	
RM27172	GAAAGAAGGGATGTCTTGCATGAGG	GAACATCCTAACCACGTCGGAAGC		
RD1110	GTCAGAGGAGTCGAATACG	ACCAACCAAACACCTAAAA	Linkaga analysis	
RM27273	ATGCGTTTGCCGTGAAGAAAGG	GTCTGGGCCCACATGTCAATAGC	Linkage analysis	
RM27326	ATCAACGAGTACGCAACAGTCC	TCCTGTCTCTTCACATCCTAATCG		
ZH-3	TGTCGAGGTTCTTGCAGACG	GACGTCGACCACTCCGAA		
ZH4	GTTCTCCGAGGGGGTTGTTC	ATACGCAAAAACTCCTGCCC	Fine mapping	
ZH5	CTAAACTCGTCTATCACCATCGTTC	CCATCGTATATTACCCATGAACAT		
ZH-6	GCGGATTCGACCAACAGTA	ATCAAGTAAACATCTTAACACAACT		
ZH-7	GATCGGCCGAATTCCACAAT	ACACCAACCGGAAACTAAAGA		
ZH-8	GGCGACAATGTAACCGTCAG	ATCAATGATTGCACGATCAGC	Sequencing	
ZH-9	ATTTTATTTCCCCAACCACTTTT	CAATGACTTAACATCAATTCTTAGCA		
CAPS-1	TTGAAGGTCATGGCAATGGT	CAAGCAACACCTCCTCTTGA	Enzyme Degistion	
CAPS-2	TACCCATTGCTATGTGTCATGG	AAGAACCCCCAAATCACCTT		
GP-COM-BamH I	CGGTACCCGGGGATCCCGGGACAAGACGGTCCCAATC	CGACTCTAGAGGATCC <u>TAGTGGCATGTGGATTGCATTG</u>	Complementation vector construction	
GP-Cas-Aar I	AGATGATCCGTGGCA <u>CTAGTACTCGACGTCGAAGGCG</u>	GCATAGCTCTAAAACCCGCCTTCGACGTCGAGTACTAG	CRISPR/Cas9 vector construction	
	<u>G</u> GTTTTAGAGCTATGC	TGCCACGGATCATCT		
Cas-Seq	GGCTGTTCTCTGCCTTGGTAT	TCTTTGTCACACACCCCTCC	Sequencing	
OsGPAT3	GCAGAGGAGGATGGGCG	TGGTTGTCTTGGGTGGTGTT		
LOC_Os05g38350	GCGATCCTTCTTGCCCTTC	GGAACGGAGGGCATTTGTG	qPCR	
LOC_Os10g42720	GCCATAATGTTGTTCTGATGTC	CTTCAGACTACGAGTATTTGC		

 Table S3. Primer sequences used in this study.