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***OsMATL* mutation induces haploid seed formation in *indica* rice**

Li Yao^{1,4}, Ya Zhang^{1,4}, Chunxia Liu¹, Yubo Liu¹, Yanli Wang¹, Dawei Liang¹, Juntao Liu¹, Gayatri Sahoo² and Timothy Kelliher^{3*} 

¹Syngenta Beijing Innovation Center, ZhongGuanCun Life Science Park, Beijing, China. ²Syngenta India Limited, Technology Centre, Medchal Mandal, India. ³Seeds Research, Syngenta Crop Protection, LLC, Research Triangle Park, NC, USA. ⁴These authors contributed equally: Li Yao, Ya Zhang.

*e-mail: tim.kelliher@syngenta.com

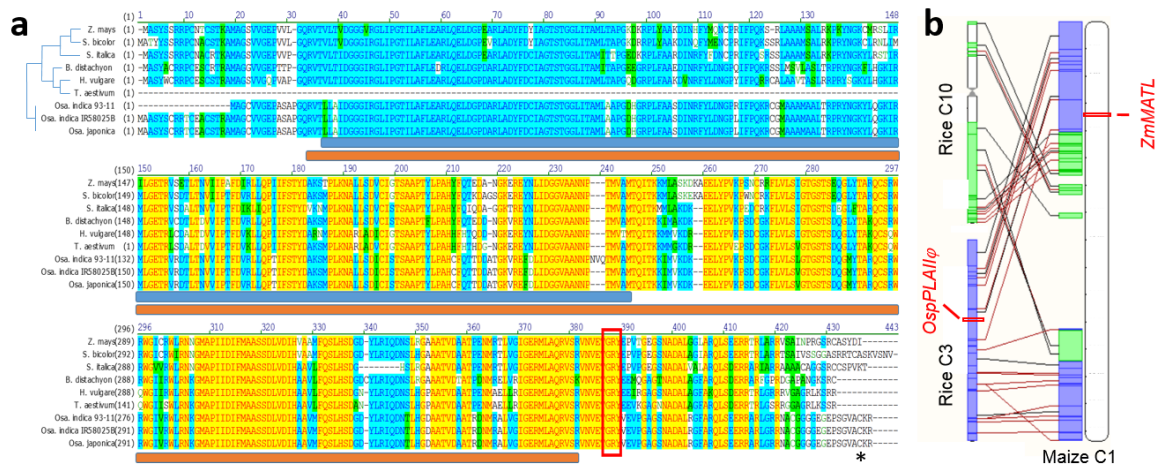


Figure S1. The rice ortholog *OsMATL*. **a**, Amino acid alignment of MATL orthologs in seven grass species, including maize (*Zea mays*), sorghum (*Sorghum bicolor*), foxtail millet (*Setaria italica*), barley (*Hordeum vulgare*), *Brachypodium*, rice (*Oryza sativa* v. *indica* and *japonica*), and bread wheat (*Triticum aestivum*). The blue bar indicates a patatin /phospholipase A2-like domain (IPR002641); brown bar indicates an acyl transferase/acyl hydrolase/lysophospholipase domain (IPR016035); red box highlights the mutation site in the native maize *matl* allele. The black star marks the predicted S-palmitoylation or S-farnesylation site at C430 in the rice ortholog. **b**, Synteny alignment of *ZmMATL* and *OsMATL*, (produced at www.gramene.com)

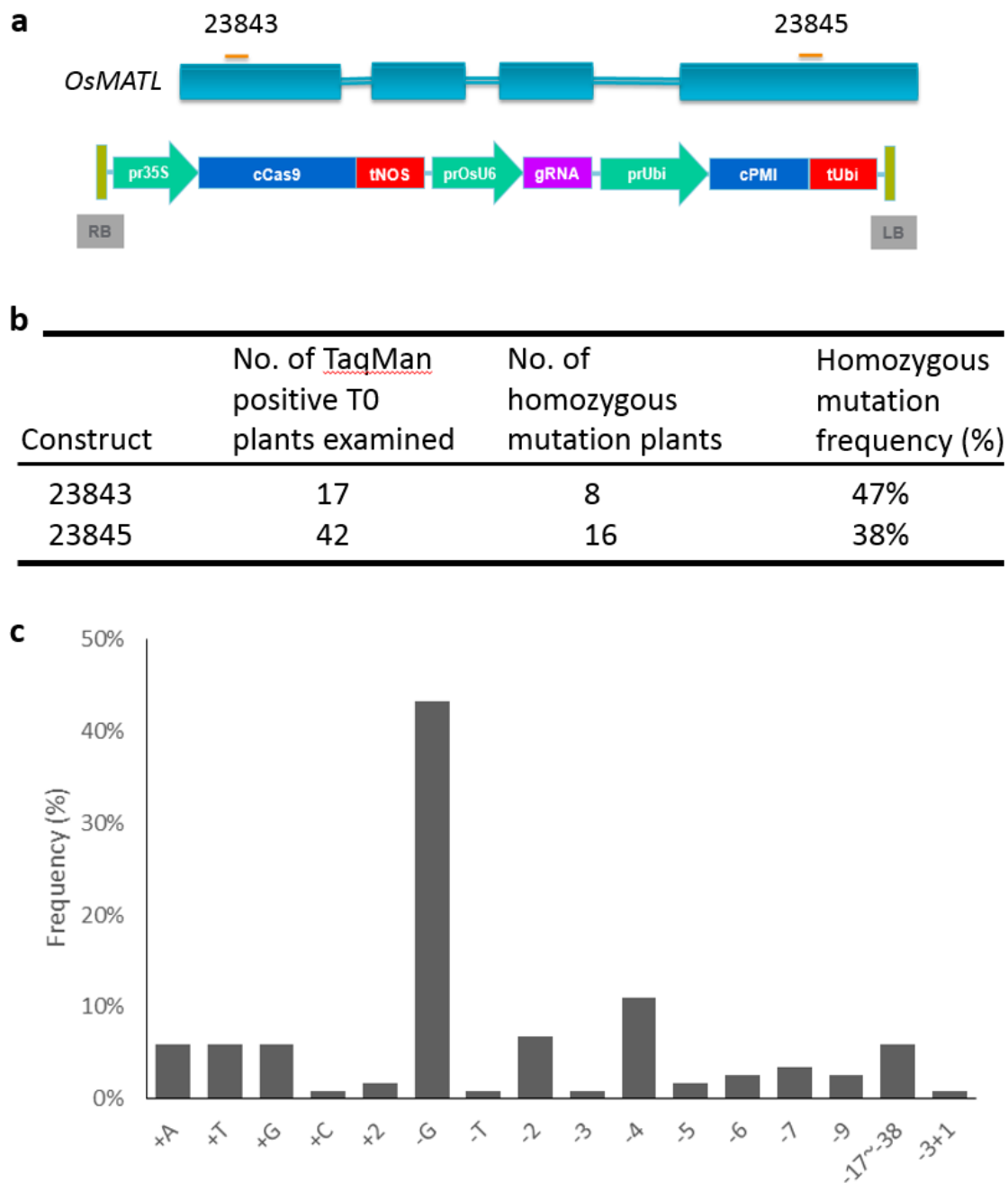


Figure S2. Editing construct design, transformation and editing results. **a**, Map of construct design. Guide RNA targeting sites in exon 1 and exon 4 of the *OspPLAII* gene, and vector components map. **b**, Editing efficiency of the two constructs. **c**, summary of different editing types found in the transformed T0 plants based on PCR colony sequencing.

Supplementary Tables

Table S1. Maize TILLinG mutants and crossing results.

Line Id	Genomic Position	Amino Acid change	Mutation Impact	Crosses Done	# of Seed Germinated	Diploid progeny	Haploid progeny	HIR
2414	2650	R406K	Tolerated	4	400	372	0	0
2658	2142	A237T	Tolerated	4	400	367	0	0
2732	2587	T385I	Tolerated	4	400	369	0	0
3594	1167	P26L	Tolerated	4	400	359	0	0
1139	1128	C13Y	Tolerated	4	400	360	0	0
0505	1431	P114L	Not Tolerated	3	238	228	0	0
1900	1433	R115C	Not Tolerated	0	M4: 50	50	0	0
1983	2501	M356I	Not Tolerated	3	300	251	3	1.2%

Table S2. TILLinG alleles in rice Os03g27610 (*OspPLAII*φ). BLOSUM matrix was used to predict the significance of each nucleotide change.

GSOR Line	Genomic change	Amino Acid change	Mutation Impact	Seed Set (% of normal)	Haploid Progeny	Diploid Progeny	HIR
403878	G366A	splice	Not tolerated	36 %	0	121	0
404675	G558A	V156M	Tolerated	14 %	0	22	0
405205	C738T	P187L	Not tolerated	57 %	0	250	0
405490	G761A	D195N	Tolerated	18 %	0	68	0
404794	C792T	T205I	Tolerated	40 %	0	170	0
406317	G803A	A209T	Tolerated	30 %	0	142	0
404534	A1021G	T246A	Tolerated	39 %	0	74	0
403403	G1163A	G293E	Not tolerated	50 %	0	59	0
406250	G1189A	G302R	Not tolerated	54 %	0	66	0

Table S3. Outcross haploid induction rate (HIR) of 049A line with 2 tester lines.

Tester line	# of progeny tested	# of diploids	# of haploids	HIR
TB12	195	186	9	4.6%
MN024529	113	111	2	1.8%