

Table S1. Altered fatty acid profile of 48 mutant lines and wild type Pungsannamul.

Lines		Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
PE529	Pool PCR amplicons #1	8.5	2.7	49.1	30.2	9.4
PE908		8.6	3.3	44.6	35.2	8.3
PE1326		9.9	2.8	32.0	45.2	10.2
PE1470		10.0	2.8	32.4	46.3	8.5
PE1759		9.4	3.3	41.2	38.7	7.4
PE1889		9.6	3.2	29.1	48.6	9.4
PE1962		9.2	3.0	39.8	39.1	8.9
PE2166		8.8	3.2	37.5	40.7	9.8
PE640	Pool PCR amplicons #2	9.1	2.9	35.3	44.4	8.3
PE743		9.5	3.3	33.7	45.4	8.2
PE867		10.0	2.7	35.1	44.7	7.5
PE1117		10.0	3.0	34.5	44.2	8.3
PE1669		10.0	3.1	32.6	45.9	8.4
PE1975		9.1	3.0	36.0	43.2	8.7
PE2009		10.4	3.2	29.5	46.5	10.5
PE2020		9.6	3.7	32.9	44.8	9.0
PE2972	Pool PCR amplicons #3	9.5	3.0	33.6	44.6	9.2
PE3177		10.0	3.3	33.0	44.9	8.7
PE204		9.6	3.2	35.2	42.8	9.2
PE219		9.5	3.1	36.5	43.0	7.8
PE1018		10.2	2.7	33.6	46.2	7.3
PE1101		10.2	2.7	31.2	47.2	8.7
PE1664		9.7	2.9	34.4	45.2	7.8
PE1804		10.2	3.2	31.8	46.5	8.5
PE2062	Pool PCR amplicons #4	9.8	2.3	33.9	44.5	9.4
PE2719		10.1	2.9	34.3	42.9	9.8
PE3097		9.6	3.2	33.9	44.3	9.0
PE3167		9.8	3.1	31.8	46.8	8.4
PE458		8.5	2.8	33.9	41.1	13.7
PE576		9.4	2.5	33.4	45.4	9.3
PE884		10.1	2.7	31.7	47.6	8.0
PE1260		9.8	3.2	32.4	45.9	8.7
PE1281	Pool PCR amplicons #4	9.2	3.2	33.8	45.2	8.6
PE1996		10.0	3.2	31.5	46.1	9.1
PE2184		10.7	2.2	33.6	43.1	10.5
PE2243		9.9	3.4	33.4	44.4	8.8
PE2707		10.0	3.4	33.1	44.5	9.1
PE2971		9.2	2.9	34.6	45.0	8.2
PE75		9.7	3.0	31.2	46.7	9.4
PE478		9.0	3.6	32.0	46.6	8.8

PE757	Pool PCR amplicons #5	9.7	3.1	35.4	44.2	7.5
PE882		10.0	2.7	33.4	45.3	8.5
PE907		10.0	2.8	32.8	46.0	8.4
PE1012		9.3	2.8	35.3	42.8	9.7
PE1482		10.1	2.9	31.9	46.9	8.1
PE1696		9.7	3.4	32.3	46.4	8.2
PE1787		9.6	3.0	32.7	45.9	8.8
PE1972		9.6	3.8	32.4	45.0	9.3
Pungsannamul		10.7	3.0	28.1	50.4	7.7
Williams 82		10.1	3.5	26.4	52.9	7.0

Pungsannamul and Williams 82 are soybean cultivars with normal oleic acid concentrations and contain functional

FAD2-1A gene.

Table S2. Primers and the size of amplicons for the *FAD2-1A* and *FAD2-1B* genes

Genes	Forward primer	Reverse primer	Amplicon size (bp)
<i>FAD2-1A</i>	5'-ACTGCATCGAATAATACAAGCC-3'	5'-TCACGGTCAAGGGAACCTGT-3'	922
	5'-CTGGTGTGTGGGTGATTGCT-3'	5'-TGATATTGTCCCGTGCAGC-3'	1094
<i>FAD2-1B</i>	5'-CCCGCTGTCCCTTTTAAACT-3'	5'-AAGGGAACCCGTGTTGGAGT-3'	978
	5'-TACTGGCGTGTGGGTGATTG-3'	5'-AGCCATGGATCGCTACAAGC-3'	1000

The *FAD2-1A* and *FAD2-1B* indicates functional microsomal delta-12 fatty acid desaturase 2 gene which is similar to the Williams 82 reference sequence for *Glyma.10G278000* and *Glyma.20G111000*, respectively.

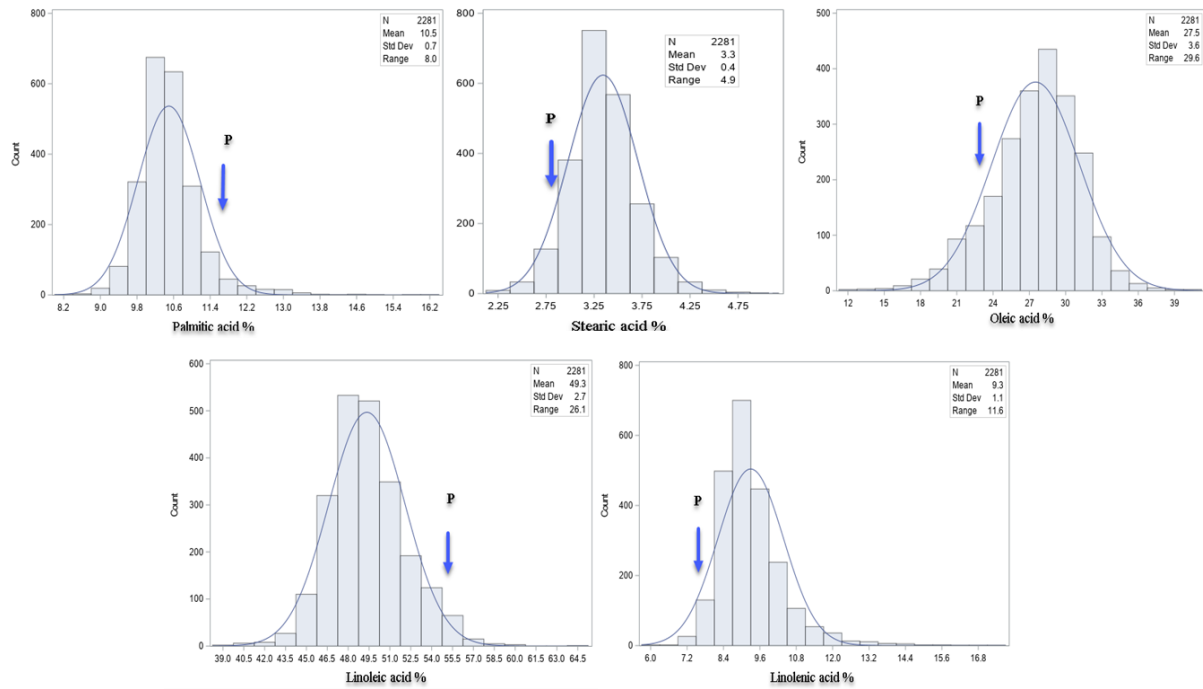


Figure S1. Distribution of fatty acid concentration in the 2,281 EMS mutant lines. It represents percentage of each of the five major fatty acid of soybeans seeds as measured from the 2,281 mutant lines by gas chromatography. The blue arrow labelled with letter “P” denotes the mean for each fatty acid content for Pungsannamul.

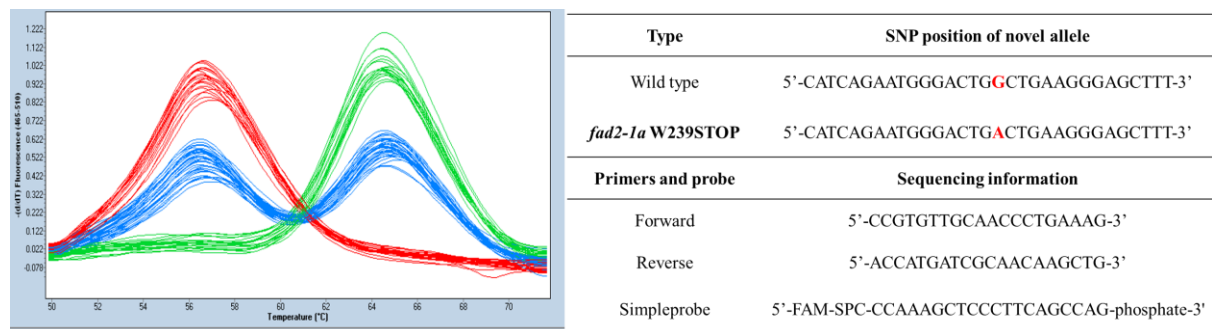


Figure S2. Genotyping of *FAD2-1A* W293STOP allele, sequencing information, primers and simpleprobe. Green peaks are homozygous of *FAD2-1A* W293STOP allele. Blue peaks indicate heterozygous. Red peaks are for homozygous of *fad2-1a* W293STOP allele. A Nucleotide with red color indicates the SNP position for *FAD2-1A* W293STOP allele. The *FAD2-1A* indicates functional microsomal delta-12 fatty acid desaturase 2 gene which is similar to the Williams 82 reference sequence for *Glyma.10G278000*. The *fad2-1a* indicates mutant allele of *FAD2-1A* W293STOP in PE529.