Seed-Specific Expression of Apolipoprotein A-I$_{Milano}$ Dimer in Rice Engineered Lines

SUPPLEMENTAL DATA

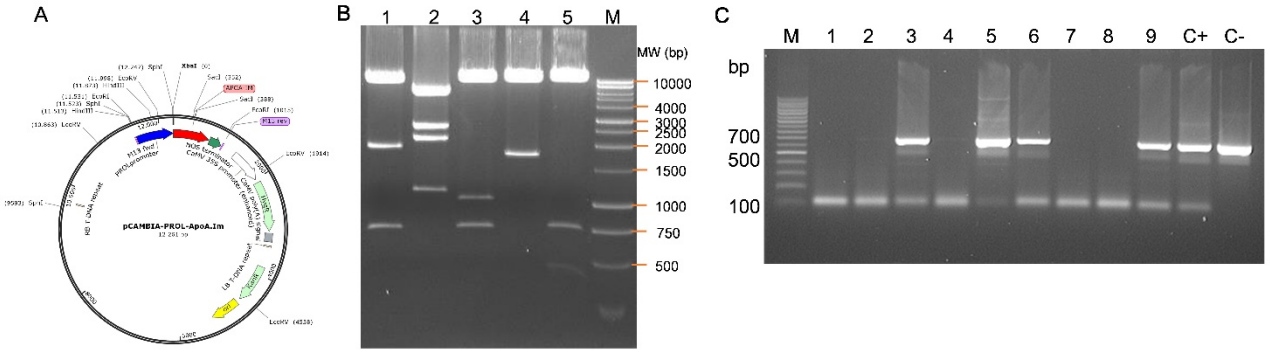

Fig. S1. Expression vector and molecular analysis of putative transformed plants.

A, Schematic diagram of expression vector pCambia-PROL-ApoA-I$_M$ used for rice transformation.

B, Restriction analysis of expression vector to confirm the correct insertion of gene and promoter. M, Molecular weight marker. Lane 1, *Sph*I restriction analysis (linearized plasmid, 724 bp and 1 990 bp fragments); Lane 2, *Eco*RV restriction analysis (linearized plasmid, 2 624 bp, 2 177 bp and 1 135 bp fragments); Lane 3, *Xba*I/*Eco*RI restriction analysis (linearized plasmid, 1 015 bp and 730 bp fragments); Lane 4, *Eco*RI restriction analysis (linearized plasmid and 1 745 bp fragment); Lane 5, *Sac*I/*Hind*III restriction analysis (linearized plasmid, 740 bp and 360 bp fragments). The restriction analysis confirms the correct insertion of ApoA-I$_M$ gene and 13 kDa prolamin promoter.

C, Agarose gel 0.8% of PCR assay of ApoA-I$_M$ gene in different T$_0$ rice plants. M, Molecular weight marker (100 bp ladder); Lanes 1–9, Hygromycin resistant plants; C+, Positive control (pCAMBIA-PROL-ApoA-I$_M$); C-, Untransformed rice. The positive plants show a band the same weight as the positive control (732 bp).

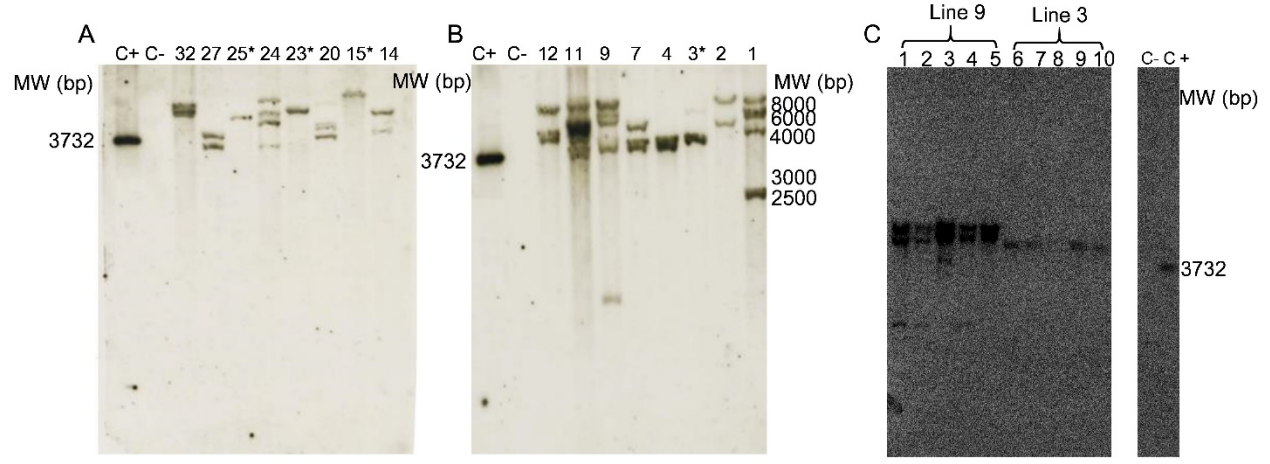

Fig. S2. Southern blot analysis of engineered rice plants.

DNA was digested with *Xba*I, separated on a 0.8% agarose gel, transferred to a nylon membrane, and probed with a DIG-labelled ApoA-I$_M$ gene. In **A** and **B**, 1, 2, 3, 4, 7, 9, 11, 12, 14, 15, 20, 23, 24, 25, 27, and 32 represent the engineered rice plants. The engineered rice plants that present ApoA-I$_M$ in a single copy was marked with *. In **C**, 1, 2, 3, 4, 5 are representative samples of plants from line 9; and 6, 7, 8, 9, 10 are representative samples of plants from line 3. 'C+' indicates positive control (3 732 bp *Xba*I fragment from pGEMT-ApoA-I$_M$) and 'C-' indicates untransformed control plant.

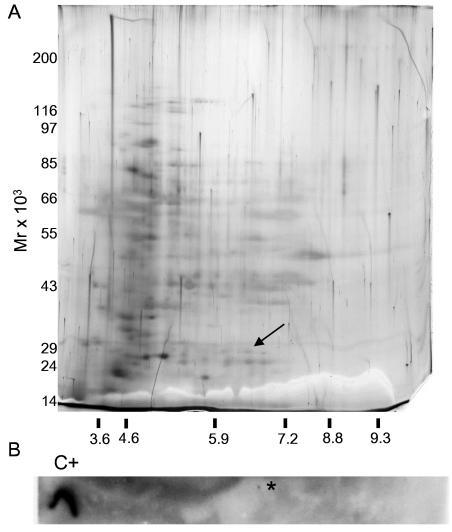

Fig. S3. Two-dimensional gel electrophoresis (A) and western blotting of bidimensional gel (B) of 50 µg of rice polypeptides extracted from ApoA-I transformed seeds.

Polypeptides were silver stained. C+, ApoA-I; * indicates the signal of the hybridized spot.

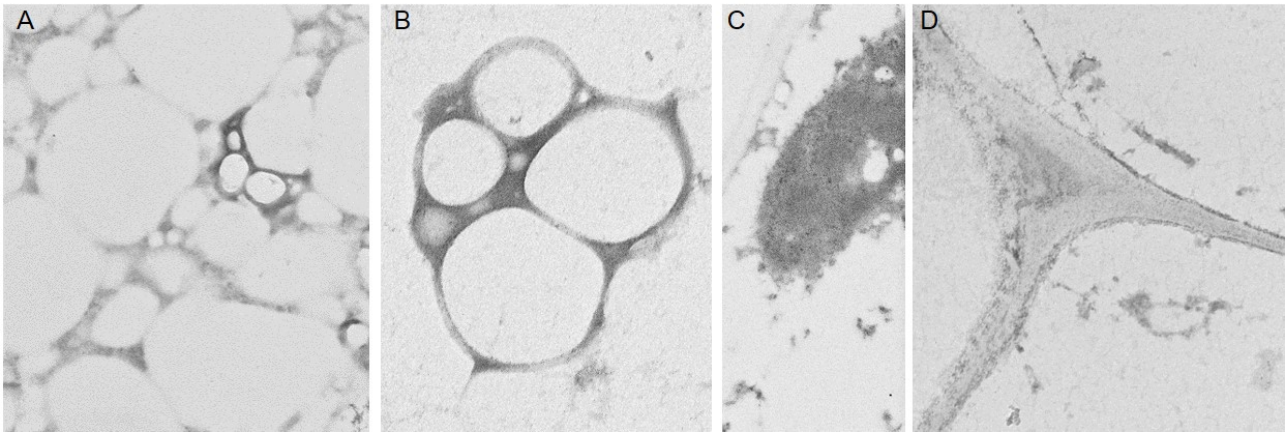

Fig. S4. Negative control of seed tissues did not show antibody staining.

Storage vacuoles (**A**), amyloplast (**B**), chloroplast (**C**) and cell wall (**D**) were not decorated by antibodies.

Table S1. Oligonucleotides used in this study for amplification ApoA-I gene and 13 kDa prolamin promoter.

Primer name	Nucleotide sequence	Ref-seq	Amplicon size (bp)	PCR cycle
Apo-fw	**GGATCC**GATGAACCCCCCCAGAGCC	X00566	732	95 ºC × 3 min; 95 ºC × 45 s, 58 ºC × 1 min, 72 ºC × 1 min, recycle 30×; 72 ºC × 5 min
Apo-rv	**GATATCTCA**CTGGGTGTTGAGCTTCTTAG			
Prol-fw	**CTGCAG**GAATTCCTTCTACATCGGCTTAGG	D63901	729	
Prol-rv	**TCTAGA**CGCAGAGGCATTGCATGCAACAATAGC			

Specific restriction site used for the cloning in the expression vector was underlined.

Table S2. T_0 rice plants obtained from transformation, relative controls (PCR, Western blotting, and Sothern analysis), and ApoA-I_M quantification by indirect competitive ELISA.

ID	PCR analysis	WB reducing	WB no-reducing	Southern (Insertion number)	Expression level (mg/kg)
1	+	+	-	4	8.7
2	+	+	-	2	9.6
3	+	+	-	1	9.0
4	+	+	+	2	82.0
5	-				
6	+	-			
7	+	+	+	3	78.0
8	-				
9	+	+	+	5	38.0
10	-				
11	+	+	+	5	20.0
12	+	+	+	3	75.0
13	+	-			
14	+	+	+	2	31.8
15	+	+	+	1	45.0
16	-				
17	-				
18	+	-			
19	+	-			
20	+	+	+	4	40.0
21	+	-			
22	+	-			
23	+	+	+	1	58.0
24	+	+	+	4	67.5
25	+	+	-	1	15.2
26	+	-			
27	+	+	+	2	82.0
28	-				
29	-				
30	-				
31	-				
32	+	+	+	3	54.0