Supplementary Data

Knockout of *OsGAPDHC7* Gene Encoding Plastidial Glyceraldehyde-3-Phosphate Dehydrogenase Affects Energy Metabolism in Rice Seeds

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Figure S2. Breeding process of *gapdhc7* transgenic plants. (A) Schematic diagram of pBOsC Vector for monocot transformation. sgRNA is integrated into the vector via the *Aar* I restriction enzyme site. (B) Production of transgenic plants through tissue culture. Shoots and roots are generated from rice calli grown in 2N6 medium, which develop into regenerated plants. (C) Selection of transgenic plants through PCR analysis.

T-DNA insertion is confirmed in regenerated plants by amplifying the *bar* gene/nos terminator region in pBOsC vector.

Supplementary Table S1. Mutation frequencies at on-target and potential off-target sites among selected sgRNA target of the *OsGAPDHC7* genes in rice genome using RGEN tools (http://www.rgenome.net/cas-designer/).

	RGEN Target (5' to 3')	Direction	GC Contents(%, w/o PAM)	Out-of-frame Score	Mismatches			
					0	1	2	3
sgRNA1	GCGTATTGCAACCAGTAGAGAGG	-	50	50	1	0	0	0
sgRNA2	CTGTTGTCGACTTGACCTGCCGG	+	55	67.6	1	0	0	0

Supplementary Table S2. GAPDH enzyme activity assay in roots and shoots of WT and *gapdhc7* mutants.

Line	Total GAPDH activity (U/g)		NAD1-dependent GAPDH activity (U/g)	
	roots	shoots	roots	shoots
WT	1.37 ± 0.09	1.27 ± 0.03	1.23 ± 0.23	1.41 ± 0.07
gapdhc 7-2	0.93 ± 0.01	0.74 ± 0.02	1.17 ± 0.18	0.37 ± 0.01
gapdhc 7-13	0.86 ± 0.21	0.79 ± 0.11	1.37 ± 0.14	0.49 ± 0.02

Supplementary Table S3. The primers list used in this study.

Primer name	Sequence (primer direction 5′-3′)	purpose
J67 pBOsC sgSEQ - FW	CAGCTTGGCTCTAGTCGACC	
K20 RGEN scaaffold regoin RV	CGGTGCCACTTTTTCAAGTT	
OsGAPDHC7 NAD binding domain_sg1 up	ggcagCTCGCCCAAGAGCAGAGTCT	Vector construction
OsGAPDHC7 NAD binding domain_sg1 down	aaacAGACTCTGCTCTTGGGCGAGc	
OsGAPDHC7 C-terminal domain_sg2 up	ggcagGGGAATCATAGGATATGTTG	
OsGAPDHC7 C-terminal domain_sg2 down	aaacCAACATATCCTATGATTCCCc	
T-DNA confirm-Nos ter Fw	TTGCGCGCTATATTTTGTTTT	T-DNA confirm
T-DNA confirm-Bar R Rv	CGTCAACCACTACATCGAGA	
OsGAPDHC7 NAD_sg1 1st F1	TGCTCGCTCAAAGAGATGTT	
OsGAPDHC7 NAD_sg1 1st R1	TGCCAATTTGTTGGAATGAA	
OsGAPDHC7 NAD_sg1 2nd F1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTTTGATTTTCGTGTGGT	
OsGAPDHC7 NAD_sg1 2nd R1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACGCTCGCTACCATAAACA	deep-sequencing
OsGAPDHC7 C-terminal_sg2 1st F1	GACGAATTGCTTGCTGTTCA	
OsGAPDHC7 C-terminal_sg2 1st R1	TGCTCGACCTGAGTCAAAGA	
OsGAPDHC7 C-terminal_sg2 2nd F1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGCAGAACTGCACAAGTTA	
OsGAPDHC7 C-terminal_sg2 2nd R1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTAACCACTTAGTTTGGAGCA	
OsACTIN FW	CAACACCCCTGCTATGTACG	
OsACTIN RV	ATCACCAGAGTCCAACACAA	
OsGAPDHC1 qRT-PCR Fw	TTGACCTGCCGGATTGAAAA	qRT-PCR analysis
OsGAPDHC1 qRT-PCR Rv	TGCATCACCAACGAGTCATT	
OsGAPDHC2 qRT-PCR Fw	AGCTGACTGGAATGGCTTTC	
OsGAPDHC2 qRT-PCR Rv	CCCTCAGCCTCCTACTTGAT	

OsGAPDHC5 qRT-PCR Fw	CAGAAGACCGTTGATGGACC
OsGAPDHC5 qRT-PCR Rv	CGGAAAGCCATACCAGTCAA
OsGAPDHC6 qRT-PCR Fw	GTCGACGGTCCTTCAATGAA
OsGAPDHC6 qRT-PCR Rv	CCATACCAGTGAGTTTCCCG
OsGAPDHC7 qRT-PCR Fw	CCCAAAAGACCGTTGATGGA
OsGAPDHC7 qRT-PCR Rv	ATTCCCGTAAGCTTGCCATT
OsSBEI qRT-PCR Fw	GGCATTGCACTCCAAAAGAT
OsSBEI qRT-PCR Rv	GCTCCAGTTGTTGCCTTCTC
OsSS qRT-PCR Fw	TCCGAGAGGTTCAGGTCATC
OsSS qRT-PCR Rv	ATGAGCTCCTCGGCGTAGTA
OsPGK qRT-PCR Fw	TGGGGTGATTGAGTCTCTGT
OsPGK qRT-PCR Rv	AAGAGAGAAACACCCTTCGC

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          WT      1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKTLLLGEKPVTVFGIRNPDEIPWAEAGAEYVVES 100
   gapdhc 7-1     1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKMLLLGEKPVTVFGIRNPDEIPWAEAGAEYVVES 100
   gapdhc 7-2     1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKTWARSRSPFSASGTLTRFRGLRLVLSMSWSPPV 100
gapdhc 7-11       1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKM---GEKPVTVFGIRNPDEIPWAEAGAEYVVES  97

          WT    101 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 200
   gapdhc 7-1    101 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 200
   gapdhc 7-2    101 SSLTRRRLLLT*                                                                                        111
gapdhc 7-11       98 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 197

          WT    201 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGYVEEDLVSTDFVGDSRSSIFDAK 300
   gapdhc 7-1    201 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGYVEEDLVSTDFVGDSRSSIFDAK 300

gapdhc 7-11     198 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGYVEEDLVSTDFVGDSRSSIFDAK 297

          WT    301 AGIALNDNFVKLVAWYDNEWGYSNRVIDLIRHMAKTQ*                                                              337
   gapdhc 7-1    301 AGIALNDNFVKLVAWYDNEWGYSNRVIDLIRHMAKTQ*                                                              337

gapdhc 7-11     298 AGIALNDNFVKLVAWYDNEWGYSNRVIDLIRHMAKTQ*                                                              335
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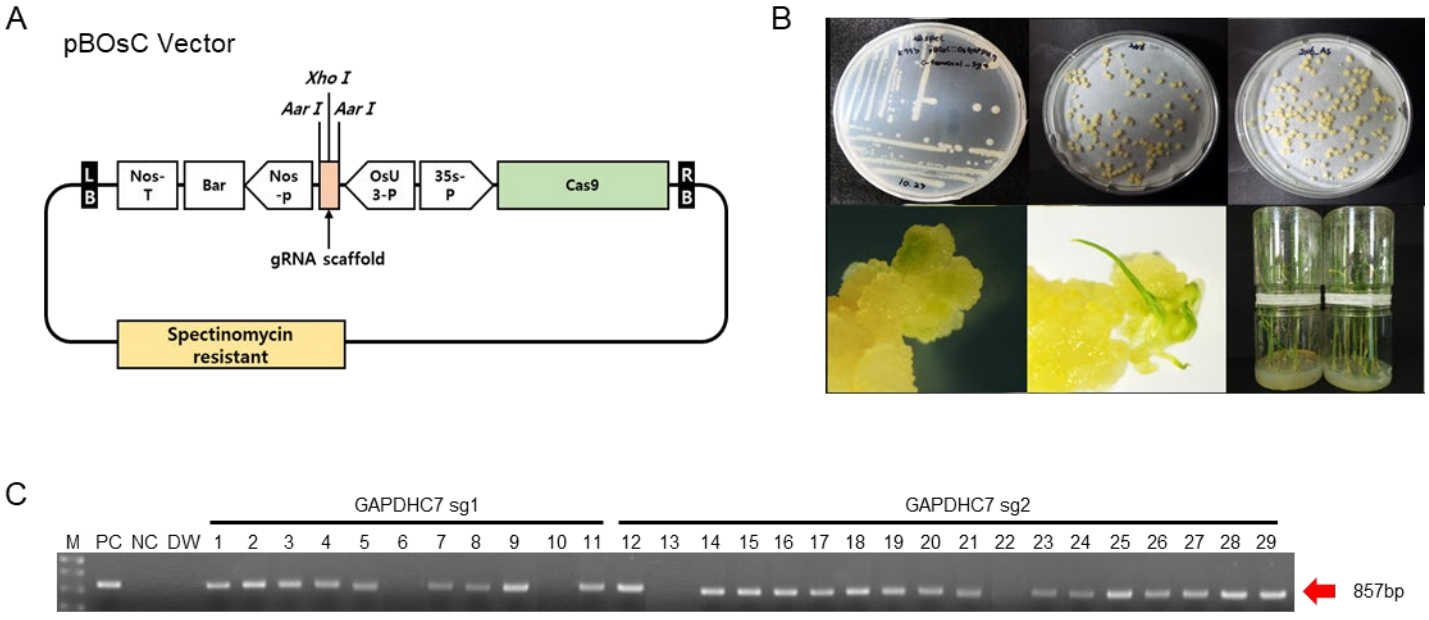
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   gapdhc 7-13    1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKTLLLGEKPVTVFGIRNPDEIPWAEAGAEYVVES 100
   gapdhc 7-19    1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKTLLLGEKPVTVFGIRNPDEIPWAEAGAEYVVES 100
   gapdhc 7-23    1 MGKIKIGINGFGRIGRLVARVALQSEDVELVAVNDPFITTDYMTYMFKYDTVHGQWKHSDIKIKDSKTLLLGEKPVTVFGIRNPDEIPWAEAGAEYVVES 100

          WT    101 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 200
   gapdhc 7-13   101 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 200
   gapdhc 7-19   101 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 200
   gapdhc 7-23   101 TGVFTDKEKAAAHLKGGAKKVVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAKVIHDNFGIIEGLMTTVHAITATQKTVDGPSSKDWRG 200

          WT    201 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGYVEEDLVSTDFVGDSRSSIFDAK 300
   gapdhc 7-13   201 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGC*                      237
   gapdhc 7-19   201 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGGRPGFY*                  242
   gapdhc 7-23   201 GRAASFNIIPSSTGAAKAVGKVLPDLNGKLTGMSFRVPTVDVSVVDLTVRIEKAASYDAIKSAIKSASEGKLKGIIGYV*                     238

          WT    301 AGIALNDNFVKLVAWYDNEWGYSNRVIDLIRHMAKTQ*                                                              337
   gapdhc 7-13
   gapdhc 7-19
   gapdhc 7-23
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Supplementary Figure S1. Amino acid sequences of WT and *gapdhc7* mutants. "-" indicates deletion, "*" indicates stop codon. Blue letters indicate target positions, and bold underlined letters indicate amino acid sequences changed by frame shift through gene editing.



Supplementary Figure S2. Breeding process of *gapdhc7* transgenic plants. (A) Schematic diagram of pBOsC Vector for monocot transformation. sgRNA is integrated into the vector via the *Aar* I restriction enzyme site. (B) Production of transgenic plants through tissue culture. Shoots and roots are generated from rice calli grown in 2N6 medium, which develop into regenerated plants. (C) Selection of transgenic plants through PCR analysis. T-DNA insertion is confirmed in regenerated plants by amplifying the *bar* gene/nos terminator region in pBOsC vector.