Supporting information

CRISPR/Cas9-mediated knockout of *BnaFAH* genes enhanced *Brassica napus* resistance to *Plutella xylostella* under 2-day short-day photoperiod.

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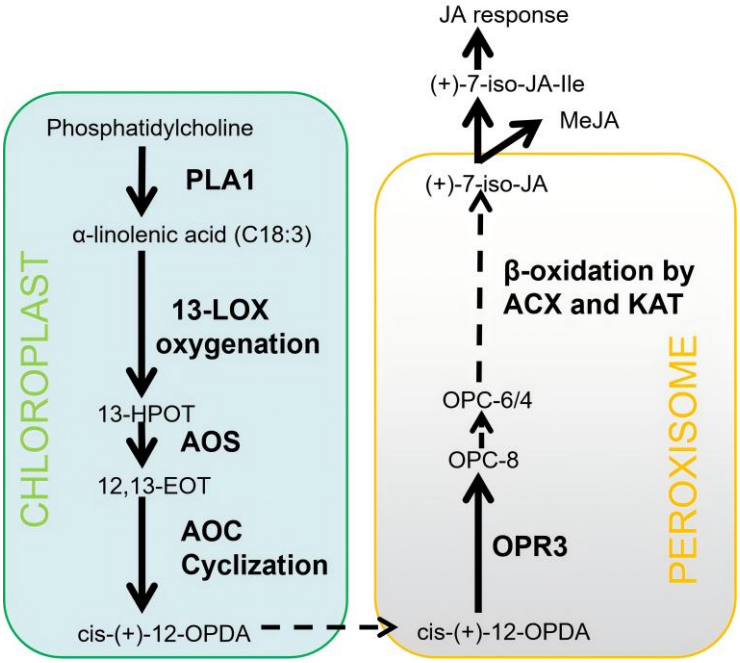

Figure S1. Synthesis of oxylipins and their intracellular transport. Abbreviations: ACX, acyl-CoA oxidase; AOC, allene oxide cyclase; AOS, allene oxide synthase; 12,13-EOT, 12,13(S)-epoxy-9(Z),11,15(Z)-octadecatrienoic acid; 13-HPOT, (13S)-hydroperoxy-octadecatrienoic acid. (+)-7-iso-JA, (+)-7-iso-jasmonic acid; (+)-7-iso-JA-Ile, (+)-7-iso-jasmonoyl-L-isoleucine; KAT, l-3-ketoacyl-CoA thiolase; 13-LOX, 13-lipoxygenase; OPC-8, 3-oxo-2-(2-[Z]-pentenyl)cyclopentane-1-octanoic acid; cis-(+)-12-OPDA, cis-(+)-12-oxo-phytodienoic acid; OPR3,12-oxo-phytodienoic acid reductase; PLA1, phospholipase A1.

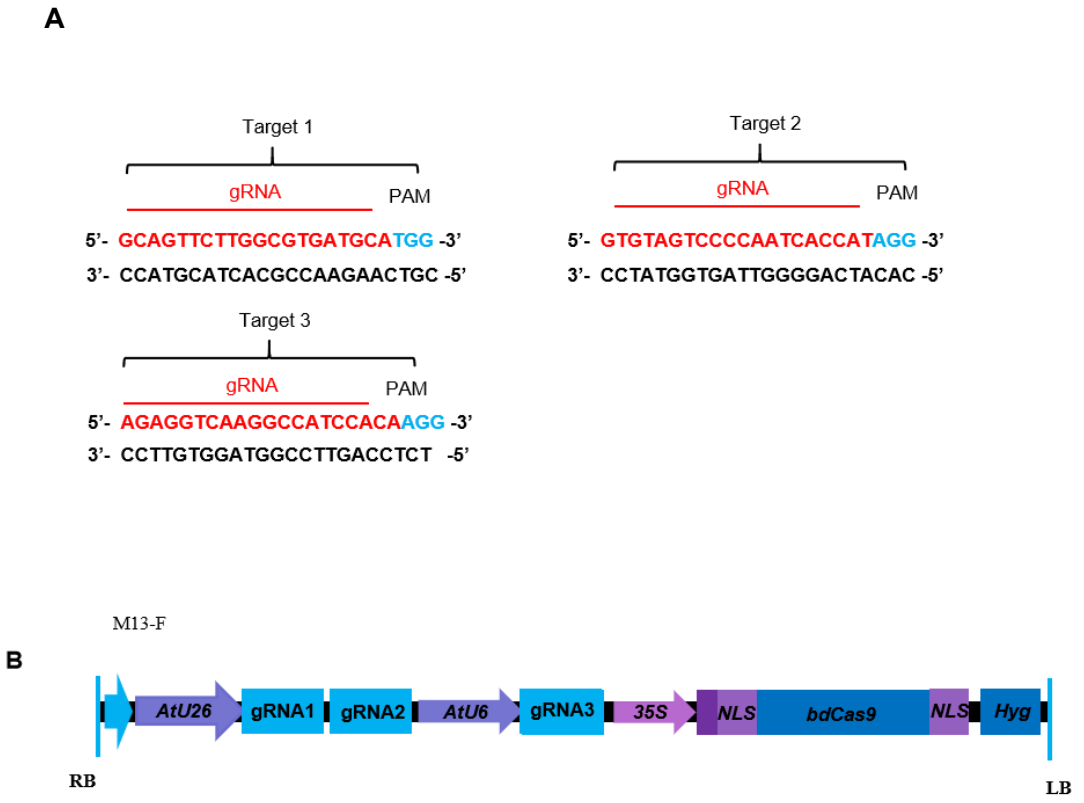

Figure S2. CRISPR/Cas9 induced targeted mutagenesis of *BnaFAH* gene in *Brassica napus*. L. (A) The detail underneath shows partial sequences of *BnaC05FAH* and *BnaA06FAH*. The sequences of target 1 (locating on exon 4 of *BnaFAH*), target 2 (locating on exon 4 of *BnaFAH)* and target 3 (locating on exon 6 of *BnaFAH*) are are highlighted in red, respectively. PAM sites (5′-NGG-3′) are highlighted in blue. (B) Schematic presentation of the recombination CRISPR/Cas9 vectors.

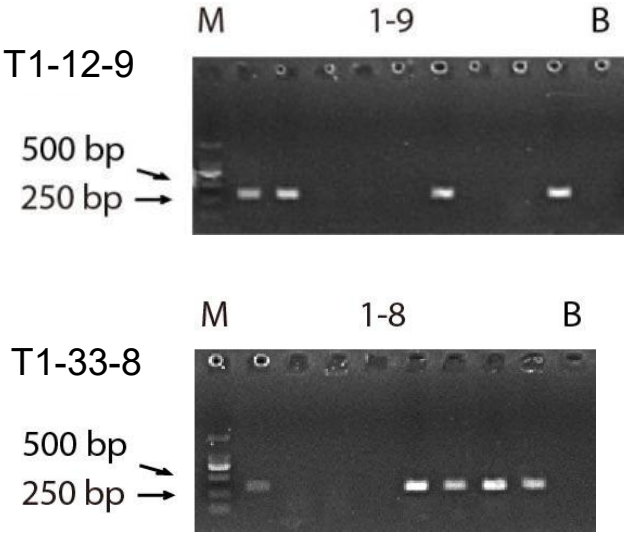

Figure S3. Isolation of transgene-free T_1 plants from T12 and T33 mutant lines. schematic description of the location/direction of the primer sets used for the analysis for the presence of *Cas9* sequences in T_1 plants of T12-9 and T33-8 mutant lines. T_0 lines containing T-DNA in their genome, self-pollination allows the removal of the T-DNA in the T1 generation plants. DNA fragments of *Cas9* were not detected by PCR in genomic DNA isolated from some of the T_1 plants. M, Mark, 2000bp. B, blanket

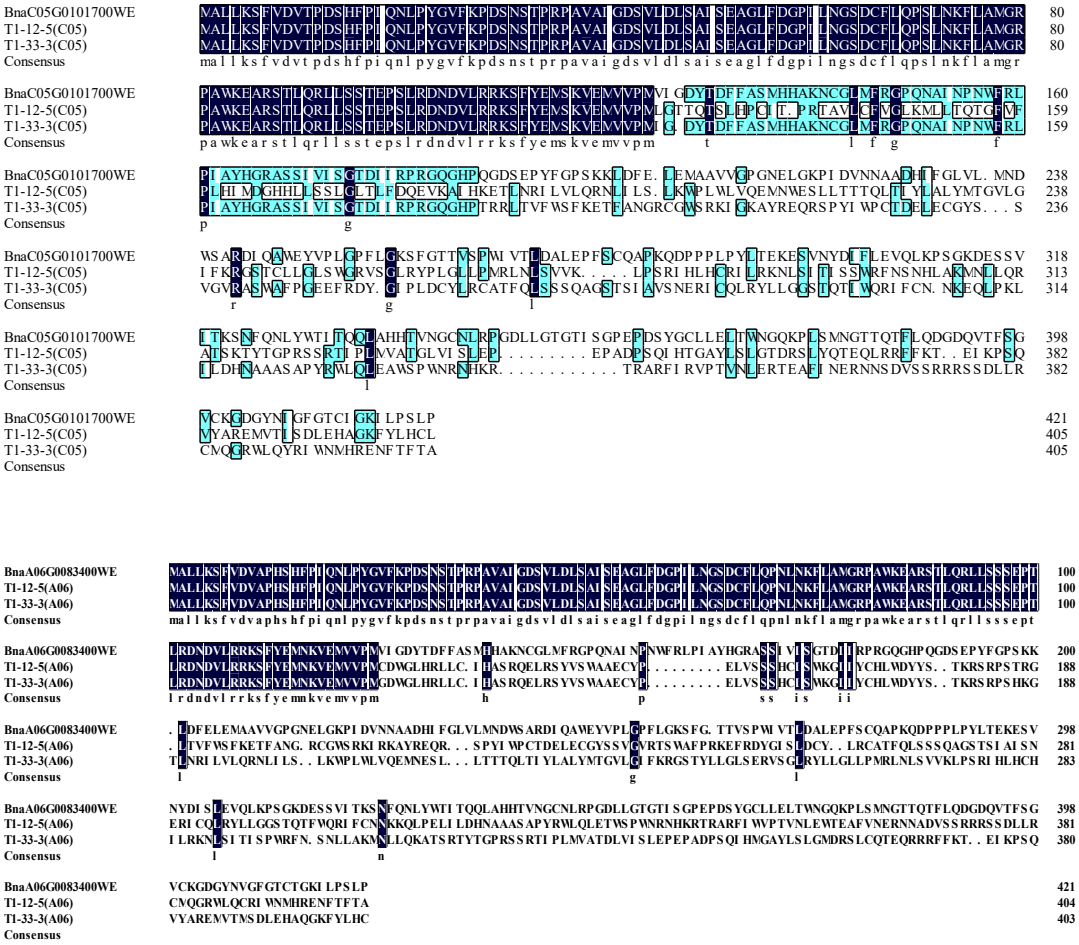

Figure S4. Multiple alignment of the amino acid sequences of the mutant

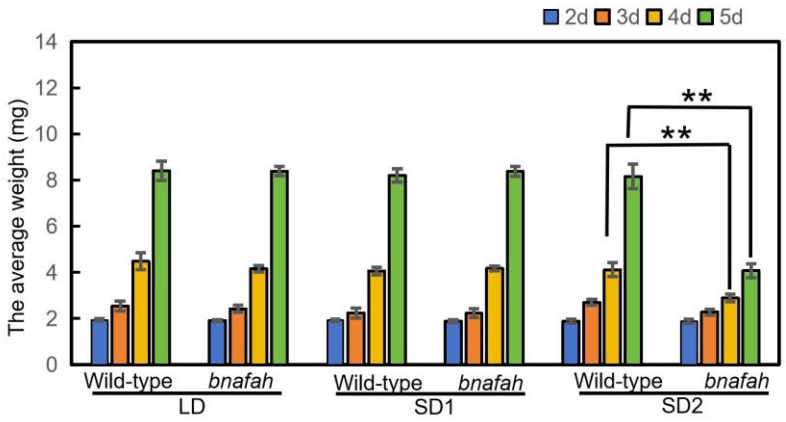

Figure S5. The average weight of *Plutella xylostella* larva exposed to 0-days short-day (LD), 1-days short-day (SD1) and 2-days short-day (SD2) wild-type and *bnafah* under long day for 2-5 days.

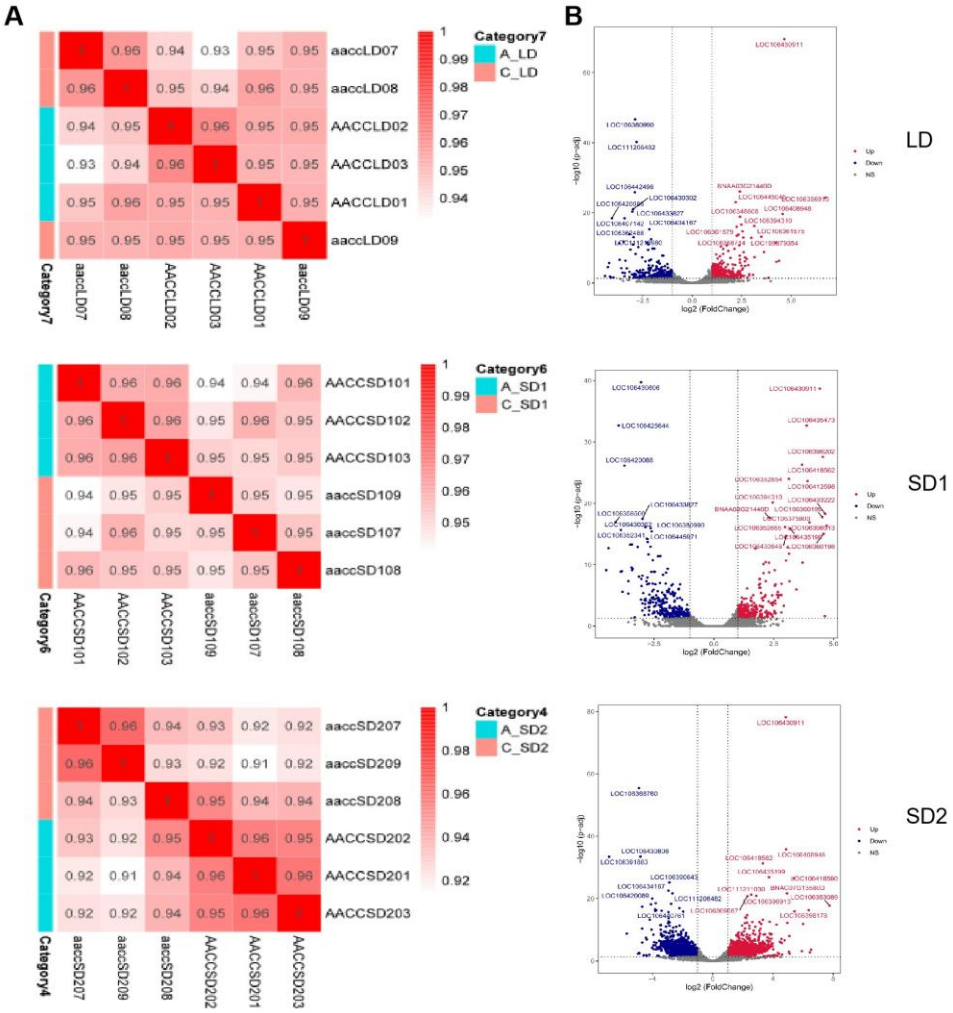

Figure S6. Spearman correlation heat map between TPM expression (**A**) and Difference Analysis Volcano Plot (**B**) in wild-type (AACC) and *bnafah* (aacc) grown under short day for 0 day (LD), 1 day(SD1) and 2 days (SD2).

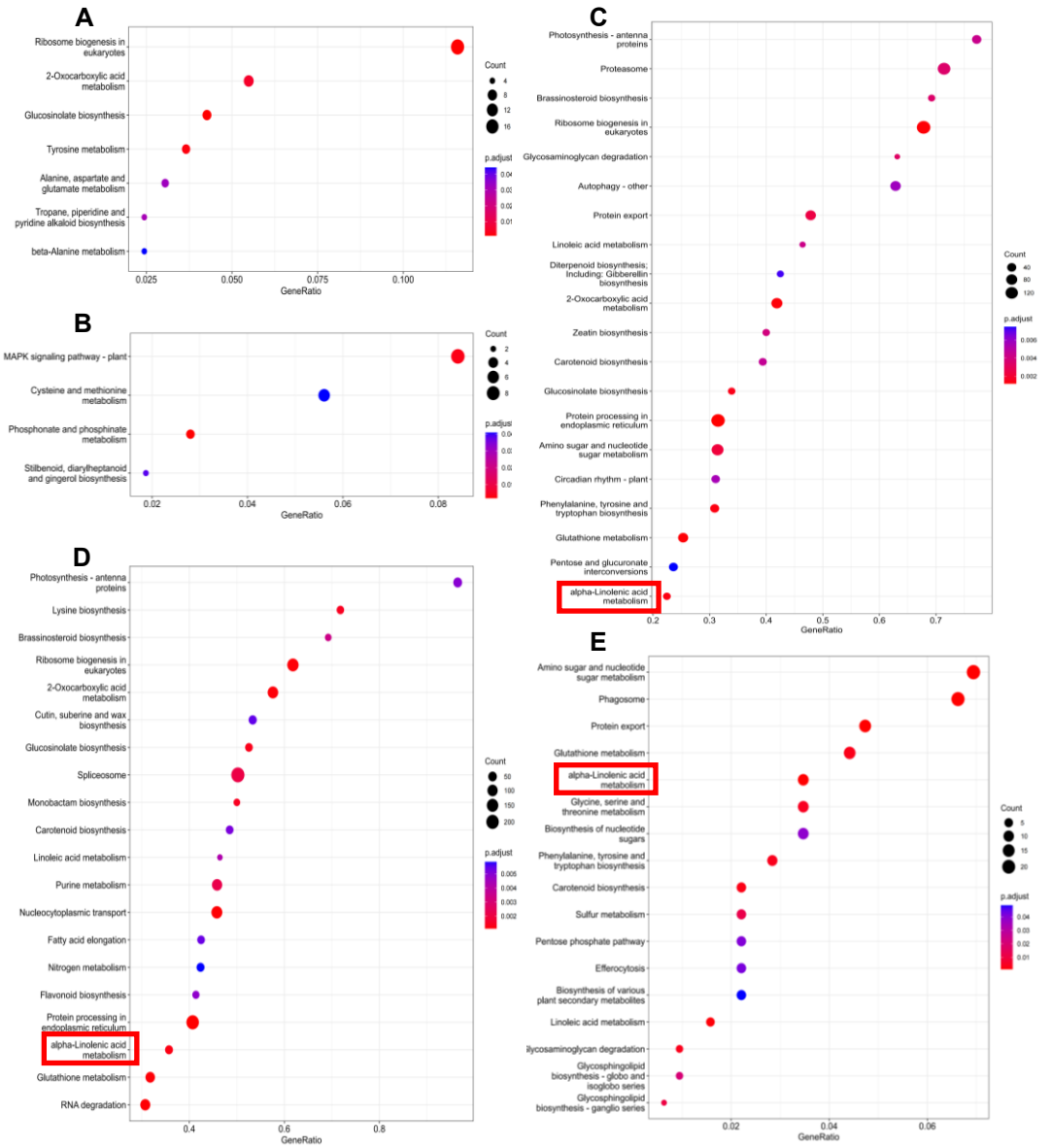

Figure S7. Transcriptome analysis in *bnafah* mutant under different photoperiod compared with different control. (**A**) KEGG enrichment of DEGs in *bnafah* mutant compared with wild type under long day. (**B**) KEGG enrichment of DEGs in *bnafah* mutant compared with wild type under short day for 1 day. (**C**) KEGG enrichment of DEGs between wild-type and *bnafah* under short day for 2 days. (**D**) KEGG enrichment of DEGs in *bnafah* under short day for 2 days compared with *bnafah* under long day. (**E**) KEGG enrichment of DEGs in *bnafah* under short day for 2 days compared with *bnafah* under short day for 1 day.

Table S1. Primer sets used in this study

Primer	Sequence (5′ to 3′)	Use of PCR products
BnFAH-A1-F	TTGAAGTCATGCATCTTGTAAGG	Detect mutations at *BnFAH* target site 1 (A genome)
BnFAH-A1-R	CAATGAAGTTCGTTAATAACTCT	
BnFAH-A2-F	TGATTATTGCTGACACAGGTGA	Detect mutations at *BnFAH* target site 2 (A genome)
BnFAH-A2-R	GCCAAAGTATAAGACCTTACAAGA	
BnFAH-A3-F	CAGCTACTGAACCCACCTTAC	Detect mutations at *BnFAH* target site 3 (A genome)
BnFAH-A3-R	GGTAATATCATGTAGGTTACAG	
BnFAH-C1-F	GAACCAGAACTGGAGGATAGT	Detect mutations at *BnFAH* target site 1 (C genome)
BnFAH-C1-R	AAAGGTTTAAGTTAGTTGCGG	
BnFAH-C2-F	ATTTGAATTGTGGGGATATTGG	Detect mutations at *BnFAH* target site 2 (C genome)
BnFAH-C2-R	CACCAAGAAACACCTCAGC	
BnFAH-C3-F	GAACCAGAACTGGAGGATAGT	Detect mutations at *BnFAH* target site 3 (C genome)
BnFAH-C3-F	AAAGGTTTAAGTTAGTTGCGG	
Cas9-F	GATCACCGACGAGTACAAGG	Detect *Cas9* transgene in T_1 plants
Cas9-R	GGGTACTTCTCGTGGTAGGC	

qBnaA06FAH-F	TACATCATCCGTTTATCGTC	qPCR of *BnaA06FAH*
qBnaA06FAH-R	GAGTTGGAATCGGGCTTG	
qBnaC05FAH-F	CGTCATCTGTTTGTTTATCG	qPCR of *BnaC05FAH*
qBnaC05FAH-R	AGGACGGGGAGTGGAGTT	
qBnaActin7-F	GCTGACCGTATGAGCAAAG	qPCR of *BnaActin7*
qBnaActin7-R	AAGATGGATGGACCCGAC	
qBnaLOX3-F	GGGTGTGGCAGCTCGCTAAA	qPCR of *BnaLOX3*
qBnaLOX3-R	AATCACACCGTCTGCACTGATC	
qBnaAOS-F	GTCAGAGACTCCTCCTGATCTA	qPCR of *BnaAOS*
qBnaAOS-R	AATTGGTAGACCGTAGCTTCC	
qBnaAOC2-F	AACTAGACCGACACAGCCCAAA	qPCR of *BnaAOC2*
qBnaAOC2-R	AGTCGCCGAAGTAGAAGCTG	
qBnaOPR3-F	TCTCTCTCACAGAGTGGTTCT	qPCR of *BnaOPR3*
qBnaOPR3-R	TGGTGCGTTGAGCGTAAT	

Table S2. CRISPR/Cas9-meditated targeted mutagenesis of two *BnaFAH* genes in transgenic rape plants.

Number	Plant ID	*BnaC05FAH*			*BnaA06FAH*		
		Target 1	*Target 2*	*Target 3*	*Target 1*	*Target 2*	*Target 3*
1	$T_{0\text{-}1}$	×	H	×	×	×	×
2	$T_{0\text{-}2}$	H	×	×	×	H	H
3	$T_{0\text{-}3}$	×	×	×	×	×	H
4	$T_{0\text{-}12}$	×	√	×	×	H	H
5	$T_{0\text{-}13}$	×	×	H	×	×	H
6	$T_{0\text{-}14}$	×	H	×	×	×	×
7	$T_{0\text{-}15}$	×	√	×	×	H	×
8	$T_{0\text{-}17}$	×	×	×	×	×	H
9	$T_{0\text{-}18}$	×	×	×	×	×	H
10	$T_{0\text{-}19}$	×	H	×	×	H	H
11	$T_{0\text{-}22}$	×	×	H	×	H	×
12	$T_{0\text{-}23}$	×	√	×	×	×	×
13	$T_{0\text{-}24}$	×	×	×	×	H	×
14	$T_{0\text{-}26}$	×	√	×	×	×	H
15	$T_{0\text{-}27}$	×	×	√	H	×	×
16	$T_{0\text{-}32}$	×	×	H	×	×	×
17	$T_{0\text{-}33}$	×	√	√	×	√	H
18	$T_{0\text{-}34}$	×	×	H	H	×	×
19	$T_{0\text{-}38}$	×	√	×	×	H	H
20	$T_{0\text{-}43}$	H	×	×	H	×	×
21	$T_{0\text{-}45}$	×	×	H	×	×	×
22	$T_{0\text{-}50}$	×	×	H	×	H	×
23	$T_{0\text{-}52}$	H	√	H	×	H	H
24	$T_{0\text{-}57}$	×	×	H	×	√	×
25	$T_{0\text{-}60}$	×	√	×	×	×	H

“H” represent gene was heterozygous. “×” represent gene was no changed. “√” represent gene was homozygous.

Table S3. Detailed genotyping of CRISPR/Cas9-edited *BnaFAH* mutant lines.

Line ID	*Target Gene*	*Target sites*	*Genotype*	*Induced mutations*	*Zygosity*[1]	*Transgene Status*[2]
$T_{1\text{-}12\text{-}5}$	*BnaC05FAH*	Target 2	cc	Deletion 4bp	Hom	Cas9-free
$T_{1\text{-}12\text{-}5}$	*BnaA06FAH*	Target 2	aa	Insertion 1bp	Hom	Cas9-free
$T_{1\text{-}33\text{-}3}$	*BnaC05FAH*	Target 2	cc	Deletion 3bp	Hom	Cas9-free
$T_{1\text{-}33\text{-}3}$	*BnaC05FAH*	Target 3	cc	Insertion 1bp	Hom	Cas9-free
$T_{1\text{-}33\text{-}3}$	*BnaA06FAH*	Target 2	aa	Insertion 1bp	Hom	Cas9-free
$T_{1\text{-}33\text{-}3}$	*BnaA06FAH*	Target 3	aa	Insertion 1bp	Hom	Cas9-free
$T_{1\text{-}52\text{-}4}$	*BnaC05FAH*	Target 1	Cc	Insertion 1bp	Hom	Cas9-free
$T_{1\text{-}52\text{-}4}$	*BnaC05FAH*	Target 2	cc	Deletion 1bp	Hom	Cas9-free
$T_{1\text{-}52\text{-}4}$	*BnaC05FAH*	Target 3	cc	Insertion 1bp	Hom	Cas9-free
$T_{1\text{-}52\text{-}4}$	*BnaA06FAH*	Target 2	aa	Deletion 4bp	Hom	Cas9-free
$T_{1\text{-}52\text{-}4}$	*BnaA06FAH*	Target 3	aa	Deletion 4bp	Hom	Cas9-free

[1]Zygosity：Hom = Homozygous, Het = Heterozygous .

[2]Transgene Status：T1 plants were screened for the Cas9 transgene by PCR with specific primers.