Applying genome editing technology for molecular breeding of rapeseed

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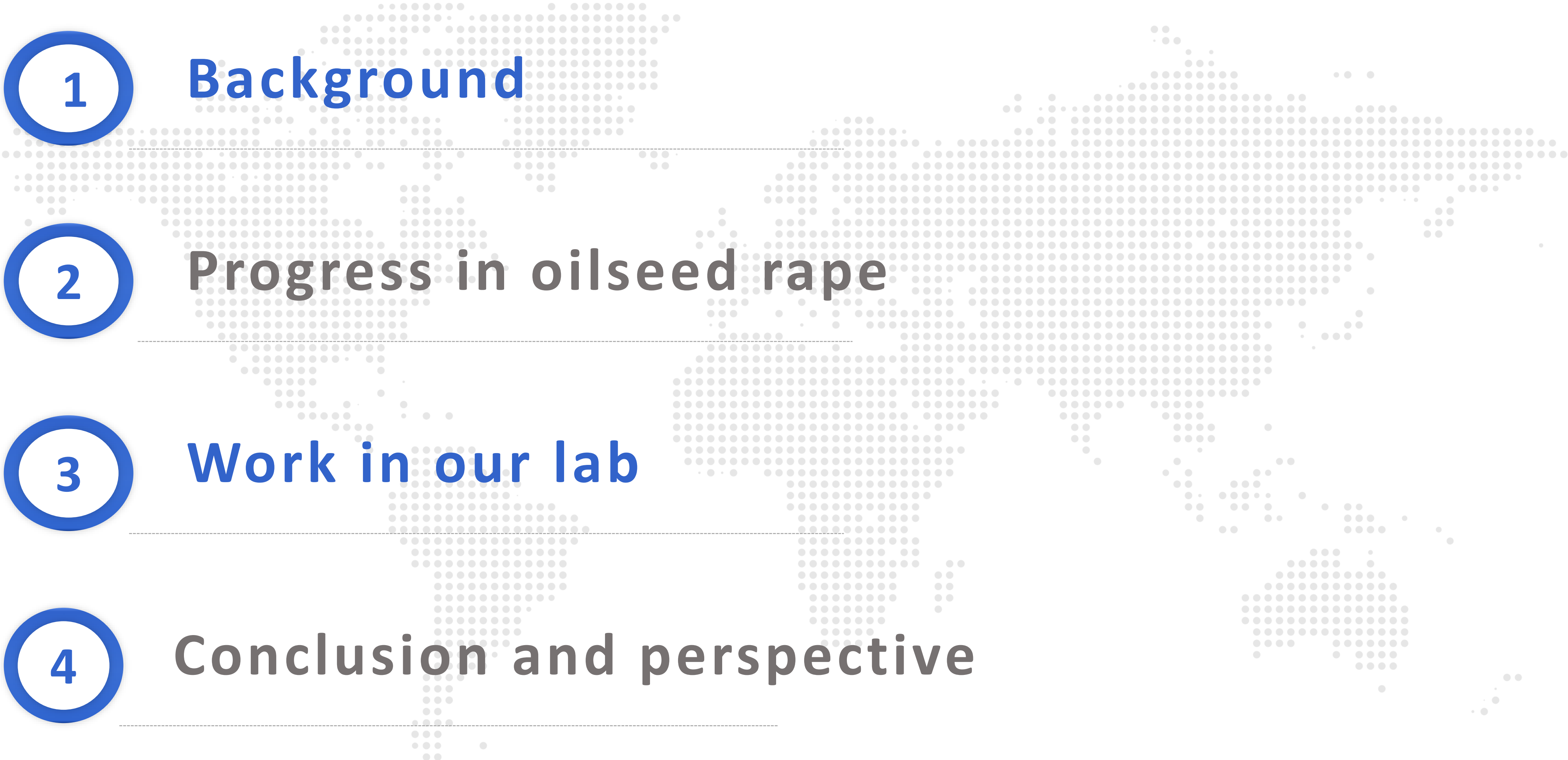
Outlines



1. Background
2. Progress in oilseed rape
3. Work in our lab
4. Conclusion and perspective

1.Background- Rapeseed is a major source of edible oil and feed protein in China

China: Rapeseed Production

USDA Foreign Agricultural Service
U.S. DEPARTMENT OF AGRICULTURE

Source: National Bureau of Statistics of China (data excluding Taiwan)
Average Rapeseed Production 2015-2019

- **Annual average planting area:** 6.99 million hectares in 2021.
- **Annual average production:** >14 million ton rapeseed grain, 6 million ton oil and 8 million ton of high-protein animal feed.
- **Largest oilseed crop and fifth largest crop**

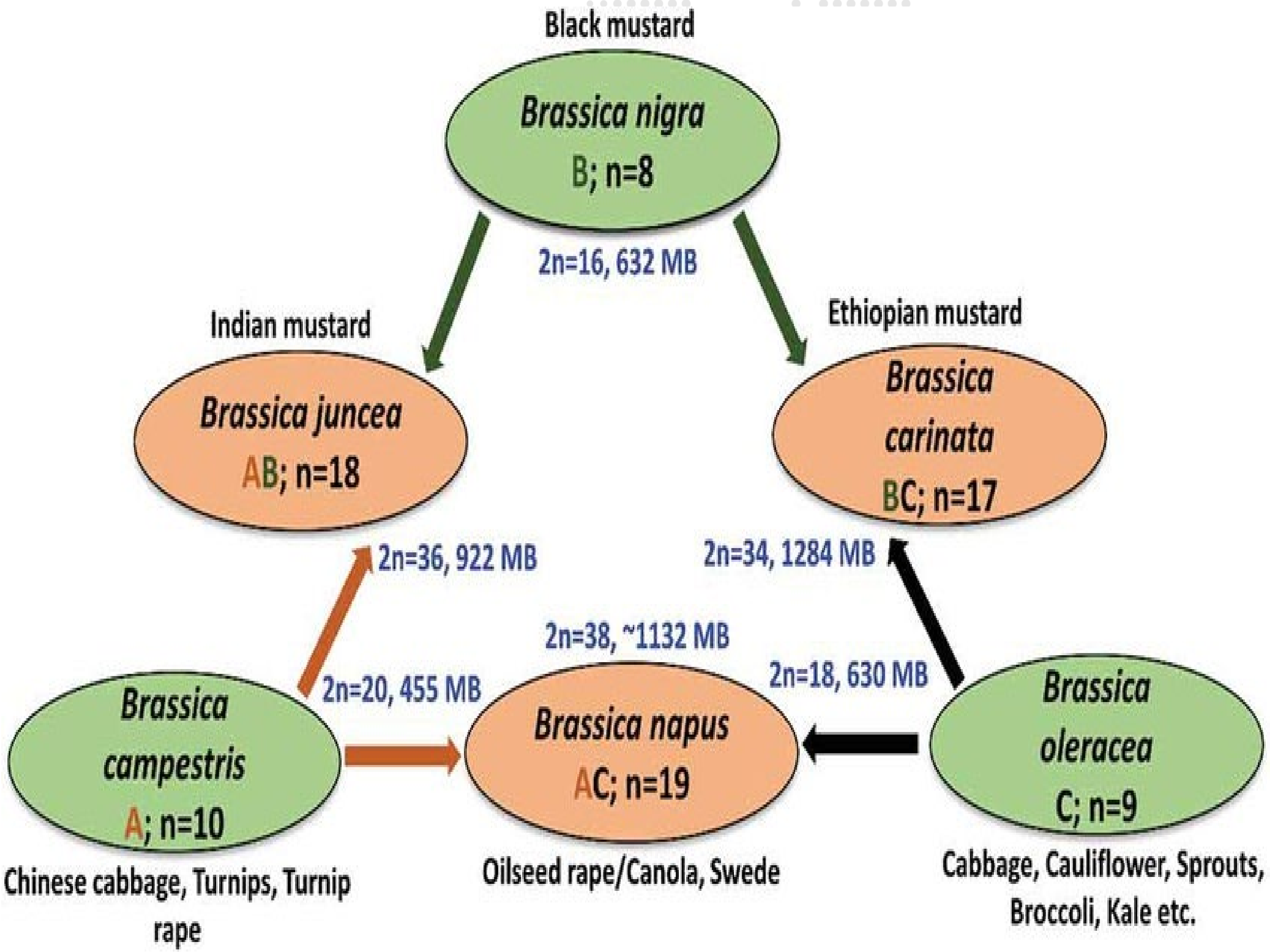
B. napus: Unlocking its Multifaceted Commercial Potential

Oilseed rape stem and leaves, containing multiple vitamins, selenium, calcium and other microelements, have become a very popular vegetable.

B. napus serves as a highly valuable tourism resource

With over 45 vibrant colors, oilseed rape serves as a valuable resource for developing rural tourism industry. Flowers have the potential for multiple by-products, honey and cosmetics.

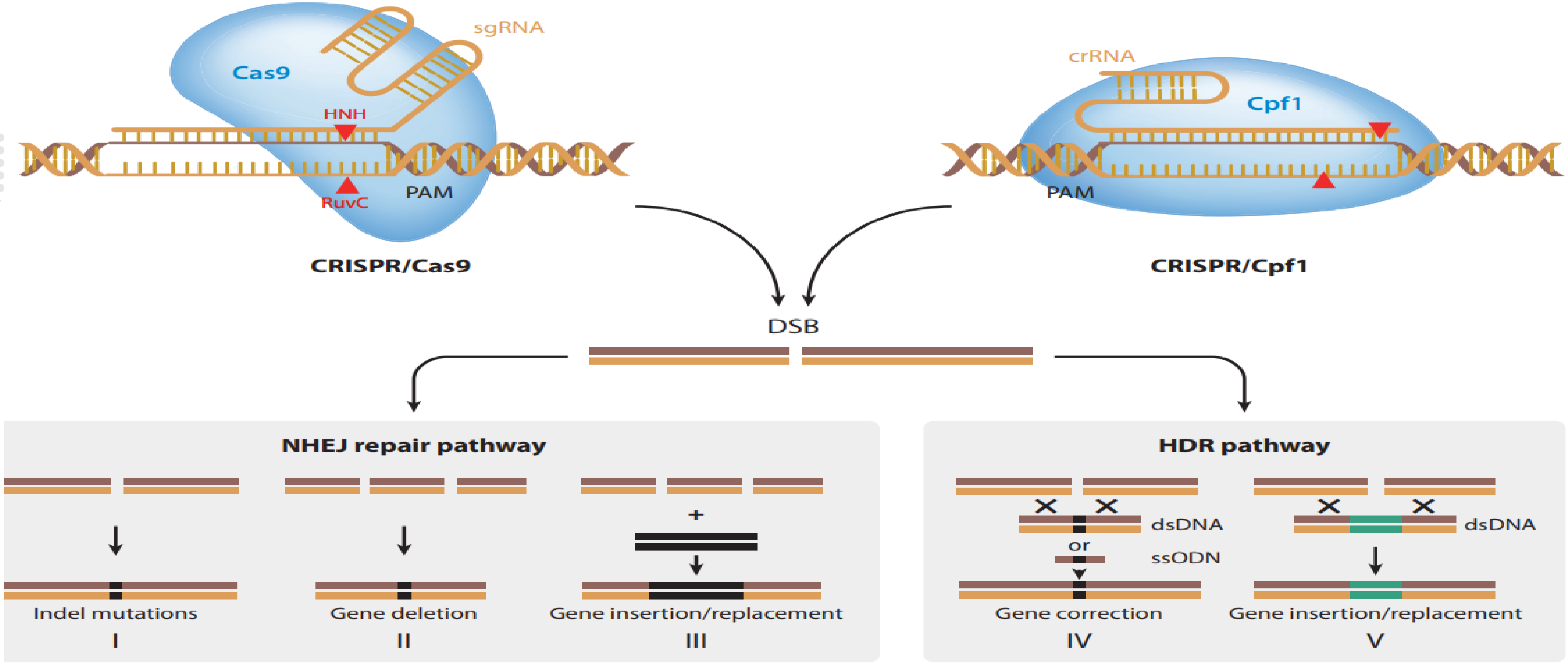
Challenges in Genetic Improvement of *B. napus*



- **Low genetic diversity:** narrow germplasm background hampers breeding applications

- **Complex genome:** Each gene in *B. napus* typically has at least two homoeologs, potentially exhibiting redundant functions.

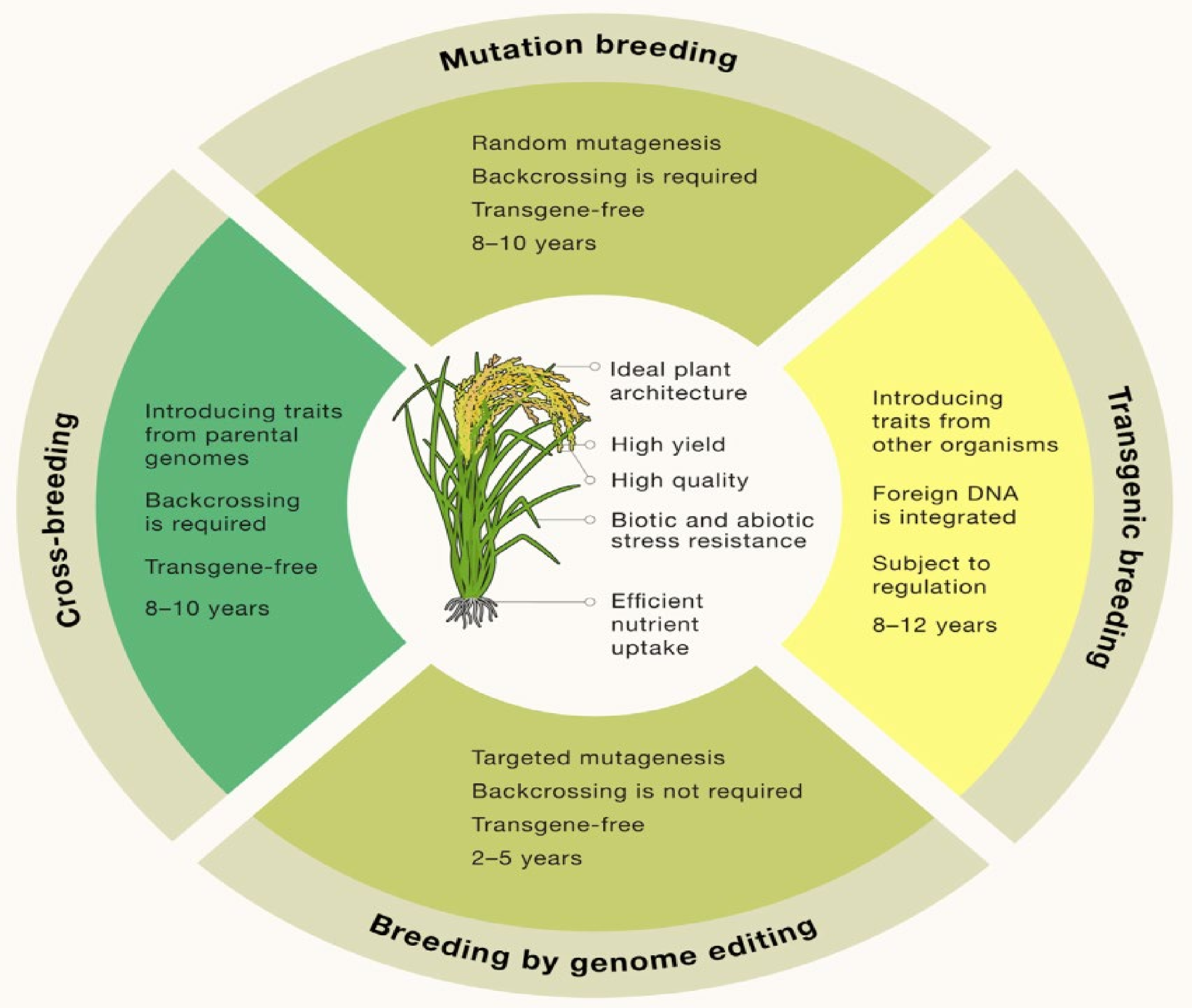
Gene Modification by CRISPR/Cas9 Genome Editing



Under the action of guide RNA, CAS protein binds to target region, causing DSB; through DNA repair processes, enabling precise gene modifications and alterations

Chen et al., 2019， Annual Review in Plant Biology

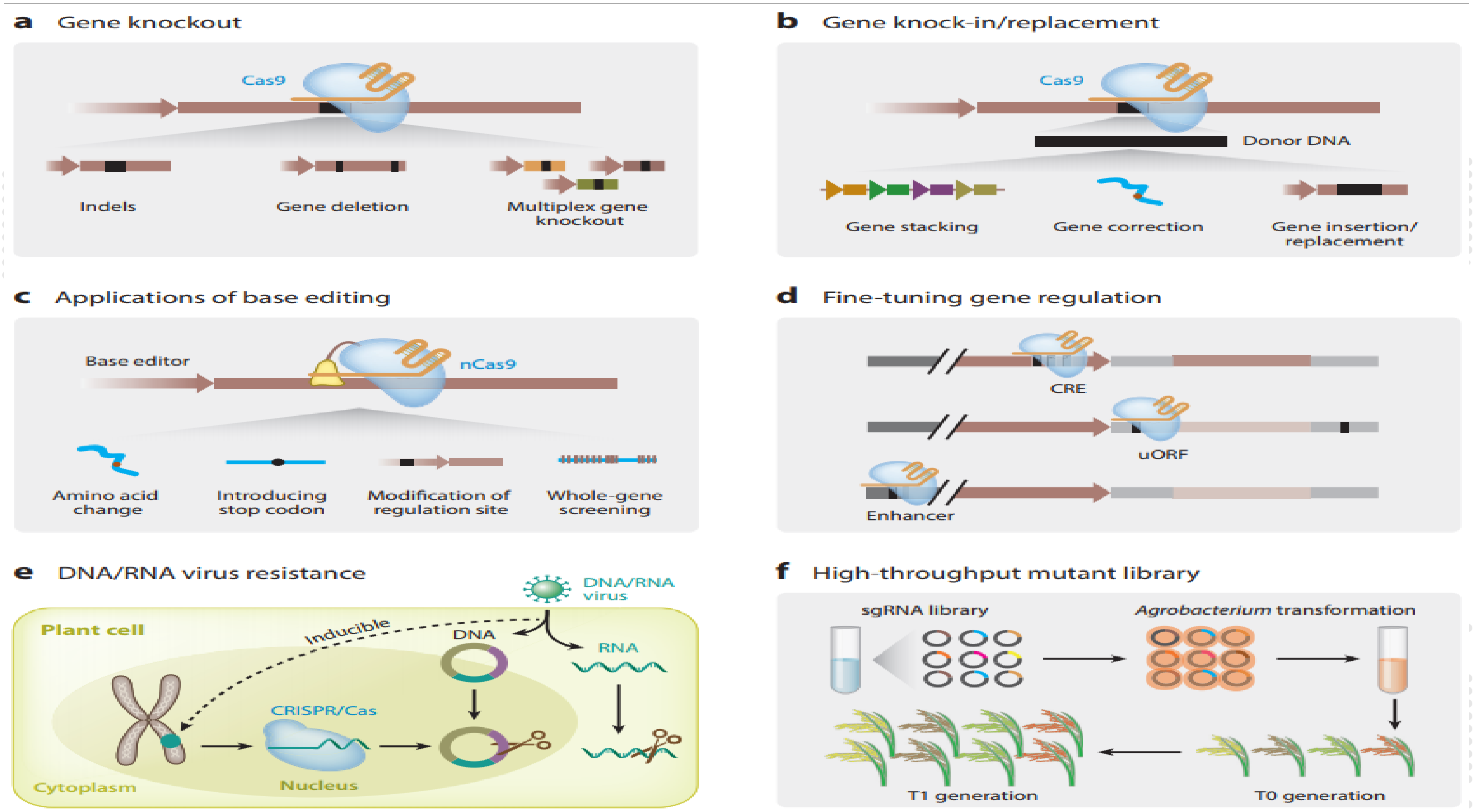
Developing CRISPR/Cas Genome Editing for Precision Plant Breeding



- **Targeted mutation**
- **No requirement of backcrossing**
- **Thansgene-free**
- **Labor- and time-saving**

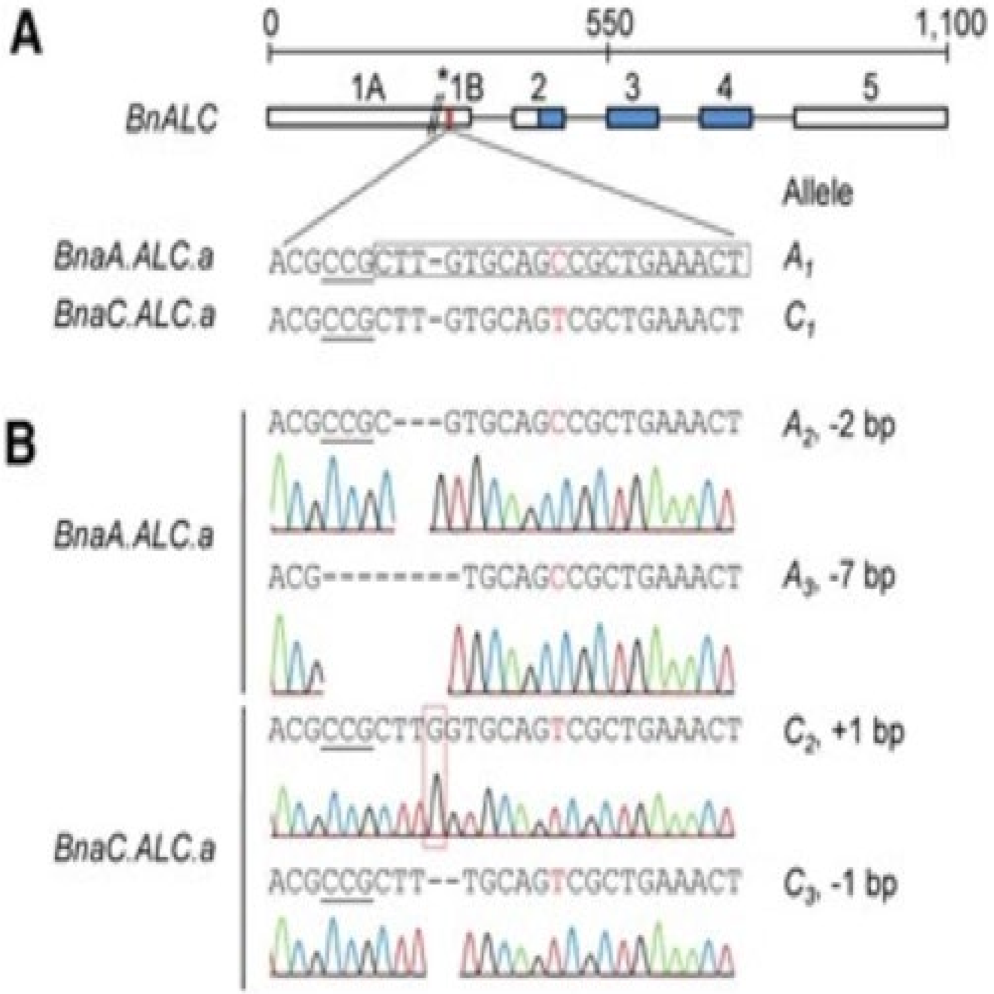
Gao, 2021, Cell

Potential Applications of CRISPR/Cas Genome Editing system

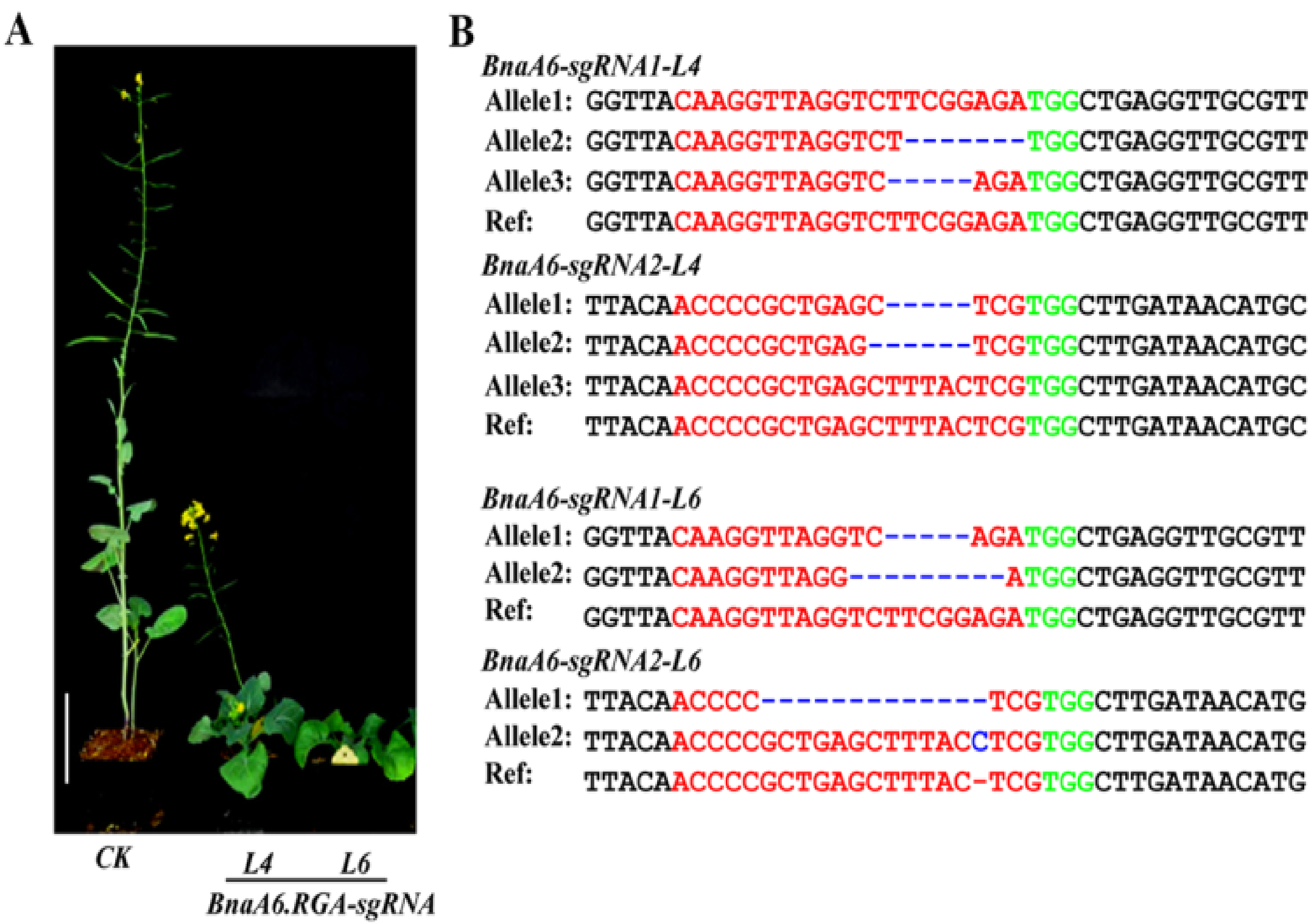


Chen et al., 2019， Annual Review in Plant Biology

2. Progress of CRISPR/Cas9 genome editing in *B. napus*

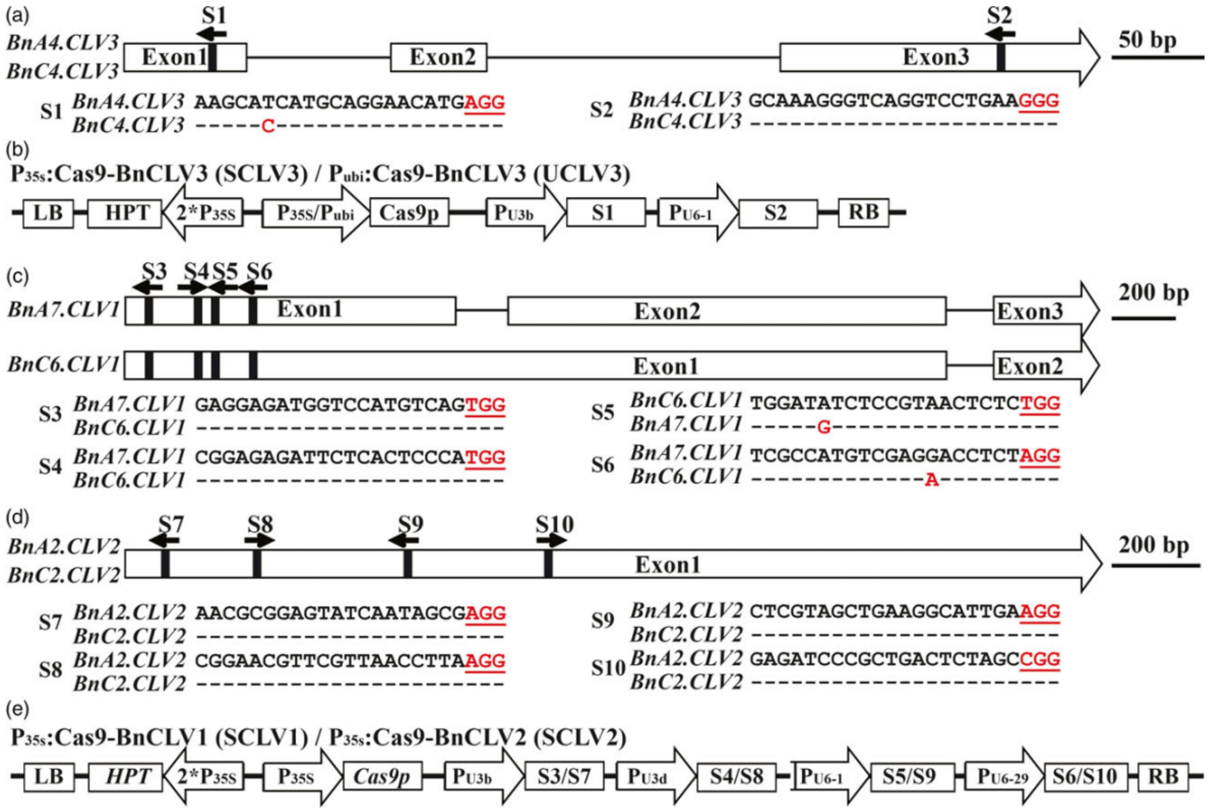


Braatz et al., 2017, Plant physiology



Hong et al., 2017, Scientific reports

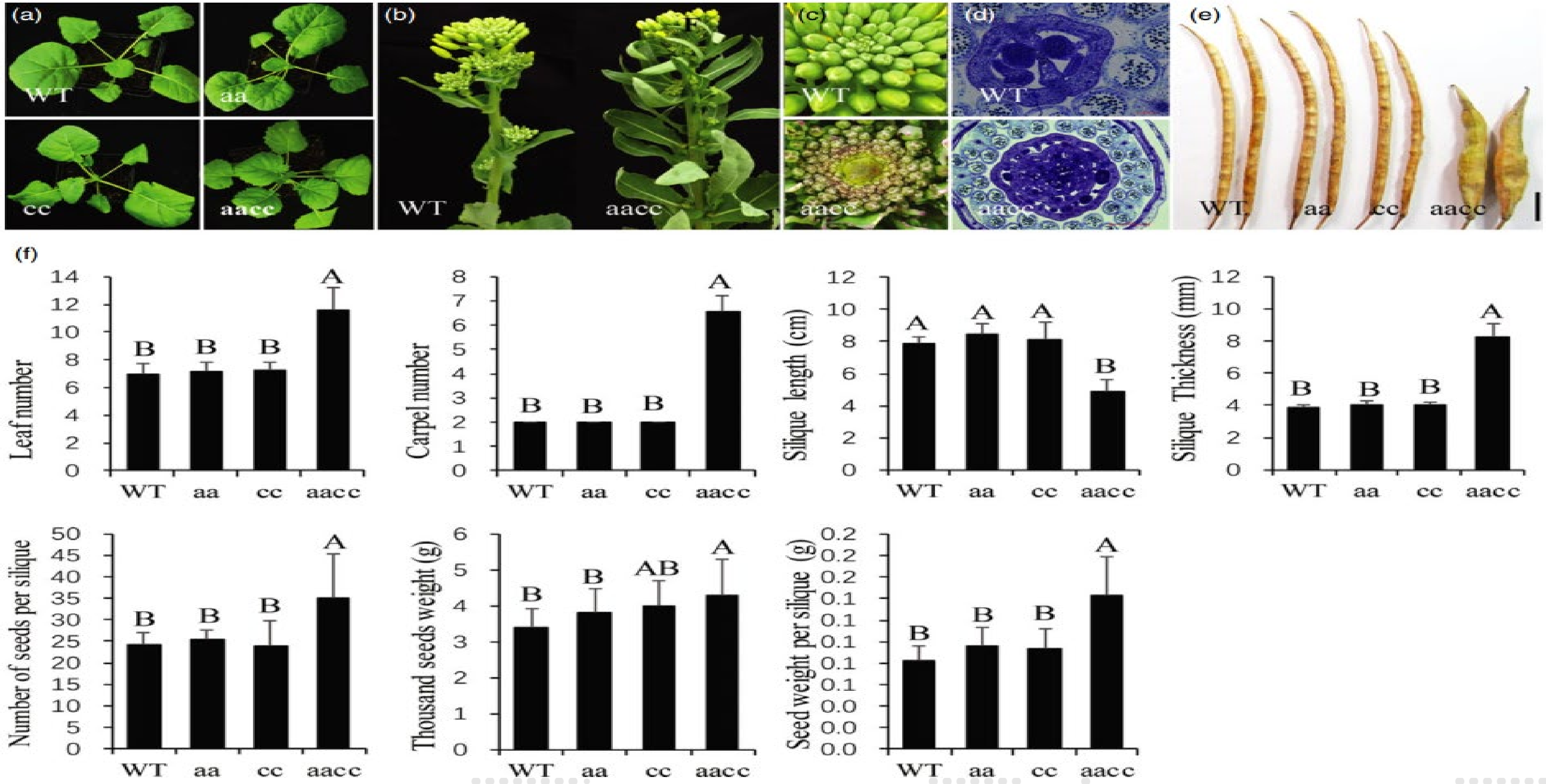
Generating Multilocular Silique by gene editing of CLAVATA



Efficient knockout of rapeseed homologues of CLAVATA3 (CLV3) and its receptors CLV1 and CLV2 in CLV signaling pathway using CRISPR/Cas9 system

Yang et al., 2018. Plant Biotechnology J

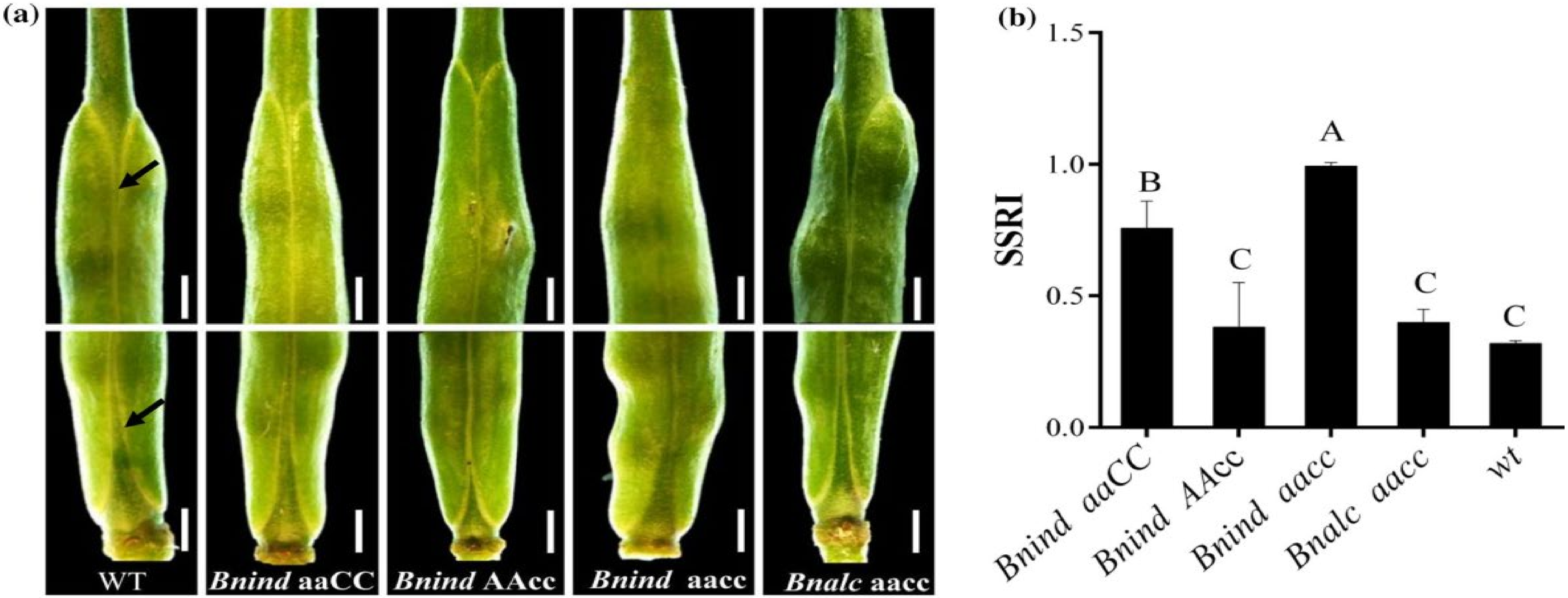
Generating Multilocular Silique by gene editing of CLAVATA



BnCLV3 mutant exhibited increased leaf production and multilocular siliques, with a significantly higher number of seeds per silique and increased seed weight compared to wild-type.

Yang et al., 2018. Plant Biotechnology J

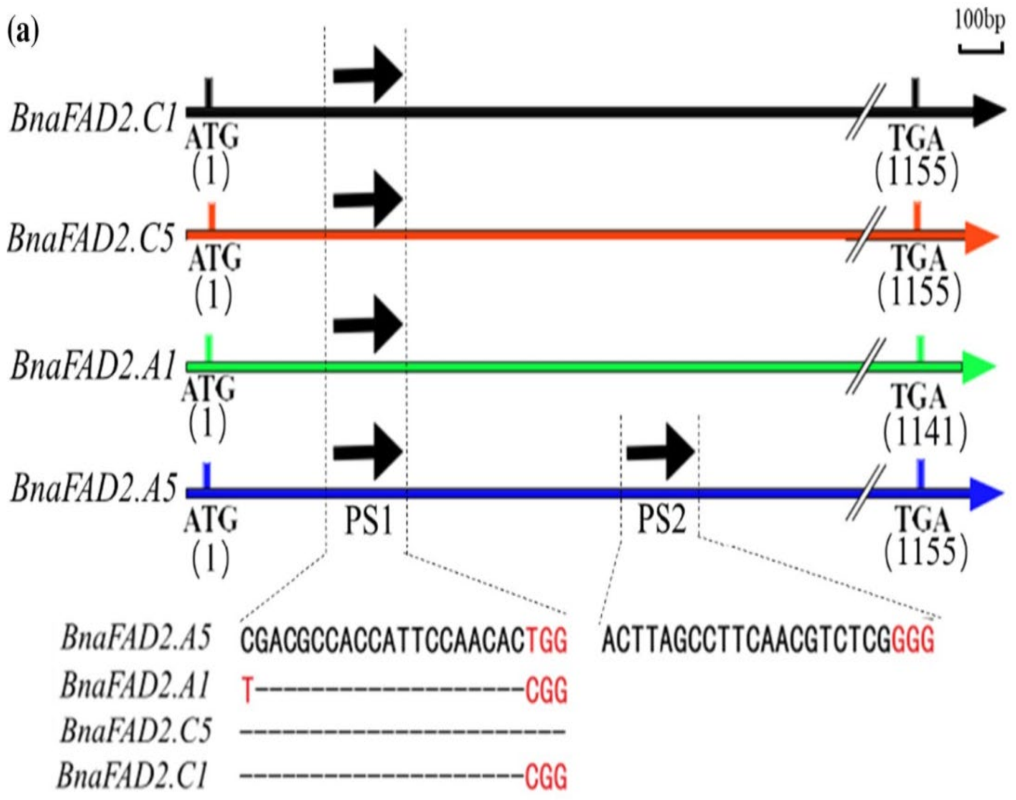
Increasing pod shatter resistance by CRISPR/Cas9



BnIND has two functionally redundant homologous copies, *BnIND-A03* and *BnIND-C03*, and mutations can significantly enhance resistance to pod shattering

Zhai et al., , 2019, Theoretical and Applied Genetics

Increasing Oleic Acid content by CRISPR/Cas9



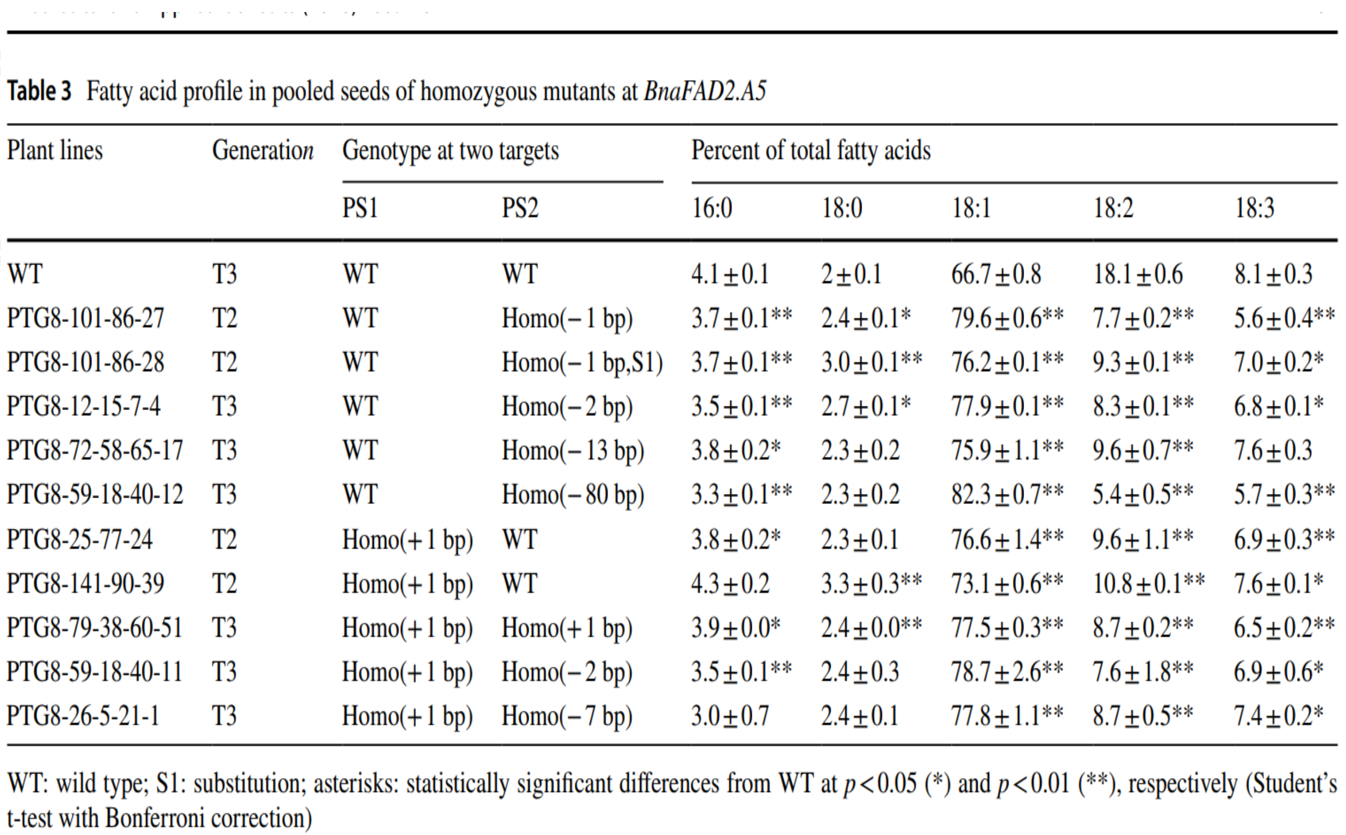


Table 3 Fatty acid profile in pooled seeds of homozygous mutants at *BnaFAD2.A5*

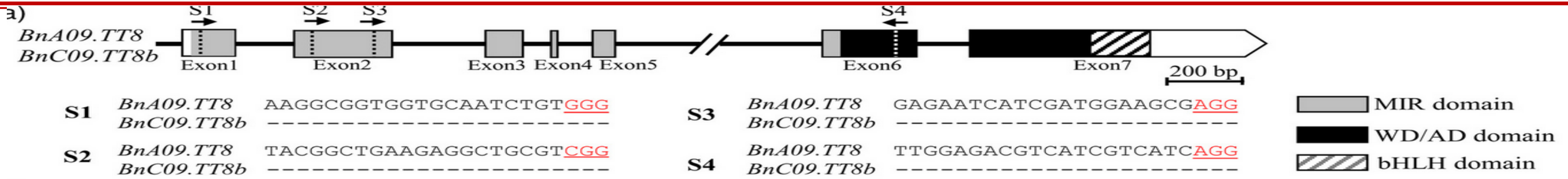
Plant lines	Generation	Genotype at two targets		Percent of total fatty acids				
		PS1	PS2	16:0	18:0	18:1	18:2	18:3
WT	T3	WT	WT	4.1±0.1	2±0.1	66.7±0.8	18.1±0.6	8.1±0.3
PTG8-101-86-27	T2	WT	Homo(−1 bp)	3.7±0.1**	2.4±0.1*	79.6±0.6**	7.7±0.2**	5.6±0.4**
PTG8-101-86-28	T2	WT	Homo(−1 bp,S1)	3.7±0.1**	3.0±0.1**	76.2±0.1**	9.3±0.1**	7.0±0.2*
PTG8-12-15-7-4	T3	WT	Homo(−2 bp)	3.5±0.1**	2.7±0.1*	77.9±0.1**	8.3±0.1**	6.8±0.1*
PTG8-72-58-65-17	T3	WT	Homo(−13 bp)	3.8±0.2*	2.3±0.2	75.9±1.1**	9.6±0.7**	7.6±0.3
PTG8-59-18-40-12	T3	WT	Homo(−80 bp)	3.3±0.1**	2.3±0.2	82.3±0.7**	5.4±0.5**	5.7±0.3**
PTG8-25-77-24	T2	Homo(+1 bp)	WT	3.8±0.2*	2.3±0.1	76.6±1.4**	9.6±1.1**	6.9±0.3**
PTG8-141-90-39	T2	Homo(+1 bp)	WT	4.3±0.2	3.3±0.3**	73.1±0.6**	10.8±0.1**	7.6±0.1*
PTG8-79-38-60-51	T3	Homo(+1 bp)	Homo(+1 bp)	3.9±0.0*	2.4±0.0**	77.5±0.3**	8.7±0.2**	6.5±0.2**
PTG8-59-18-40-11	T3	Homo(+1 bp)	Homo(−2 bp)	3.5±0.1**	2.4±0.3	78.7±2.6**	7.6±1.8**	6.9±0.6*
PTG8-26-5-21-1	T3	Homo(+1 bp)	Homo(−7 bp)	3.0±0.7	2.4±0.1	77.8±1.1**	8.7±0.5**	7.4±0.2*

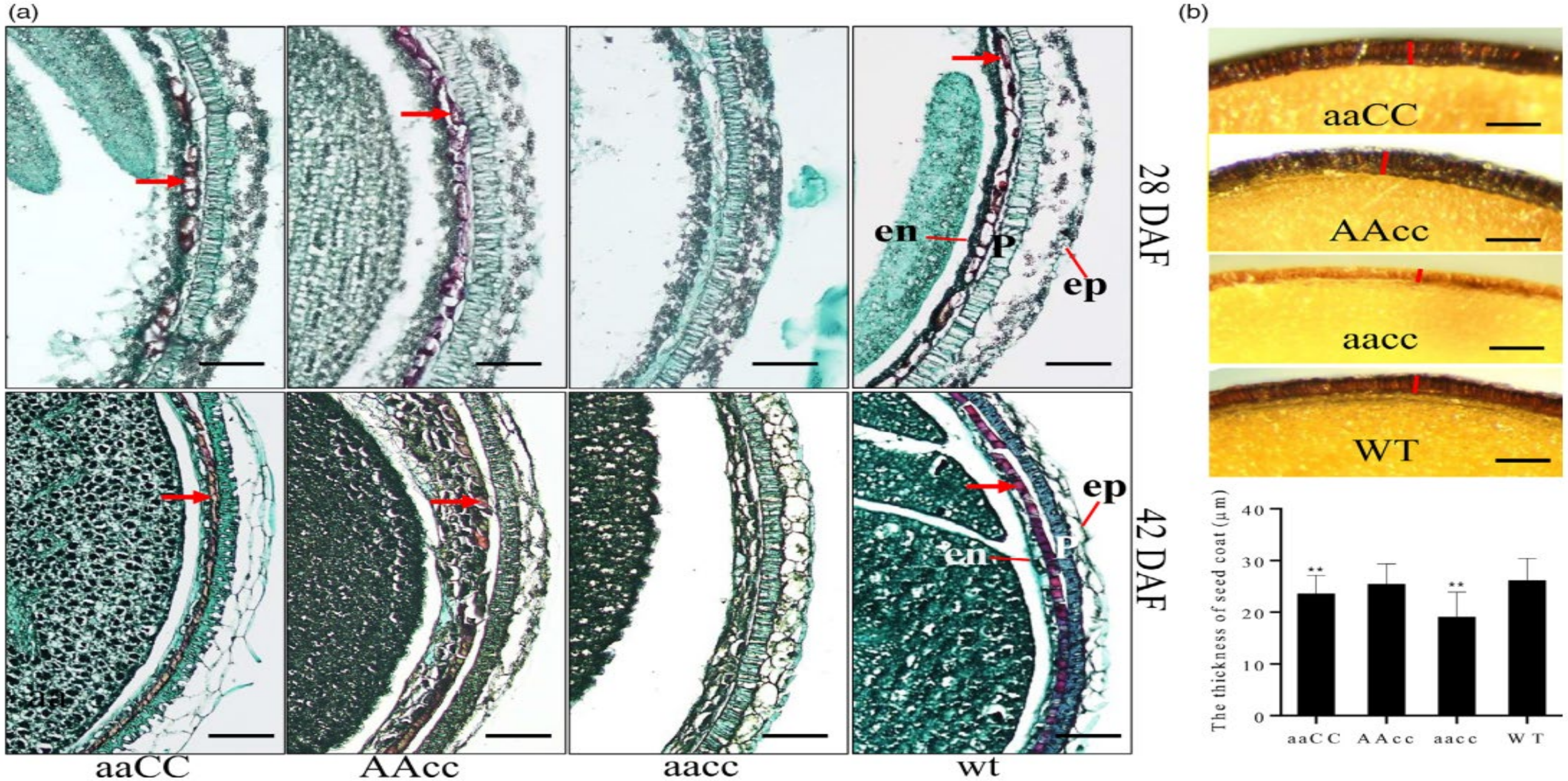
WT: wild type; S1: substitution; asterisks: statistically significant differences from WT at $p < 0.05$ (*) and $p < 0.01$ (**), respectively (Student's t-test with Bonferroni correction)

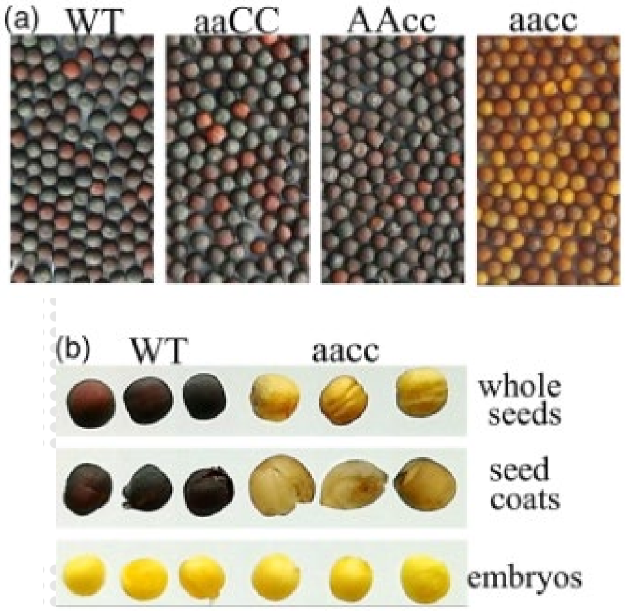
Mutation of *BnFAD2* resulted in a significant increase in oleic acid content in seeds of mutants, with the highest content exceeding 80%, compared to wild type with 66.43%.

Huang , 2020, Theoretical and Applied Genetics

Target mutation of *transparent testa 8 (TT8)* resulted in yellow seed



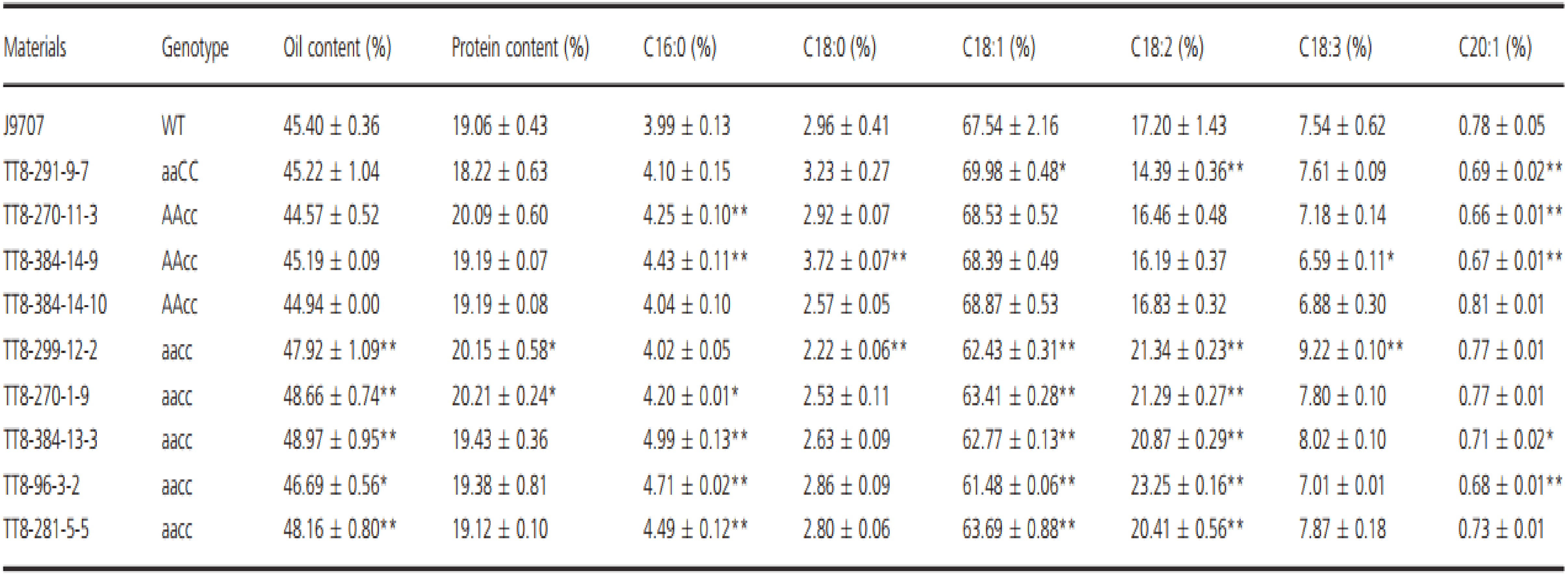




Mutation of *BnTT8* impaired proanthocyanidins (PA) deposition in inner seed coat.

Zhai et al., 2020. Plant Biotechnology J

Increasing seed oil and protein contents（TT8）

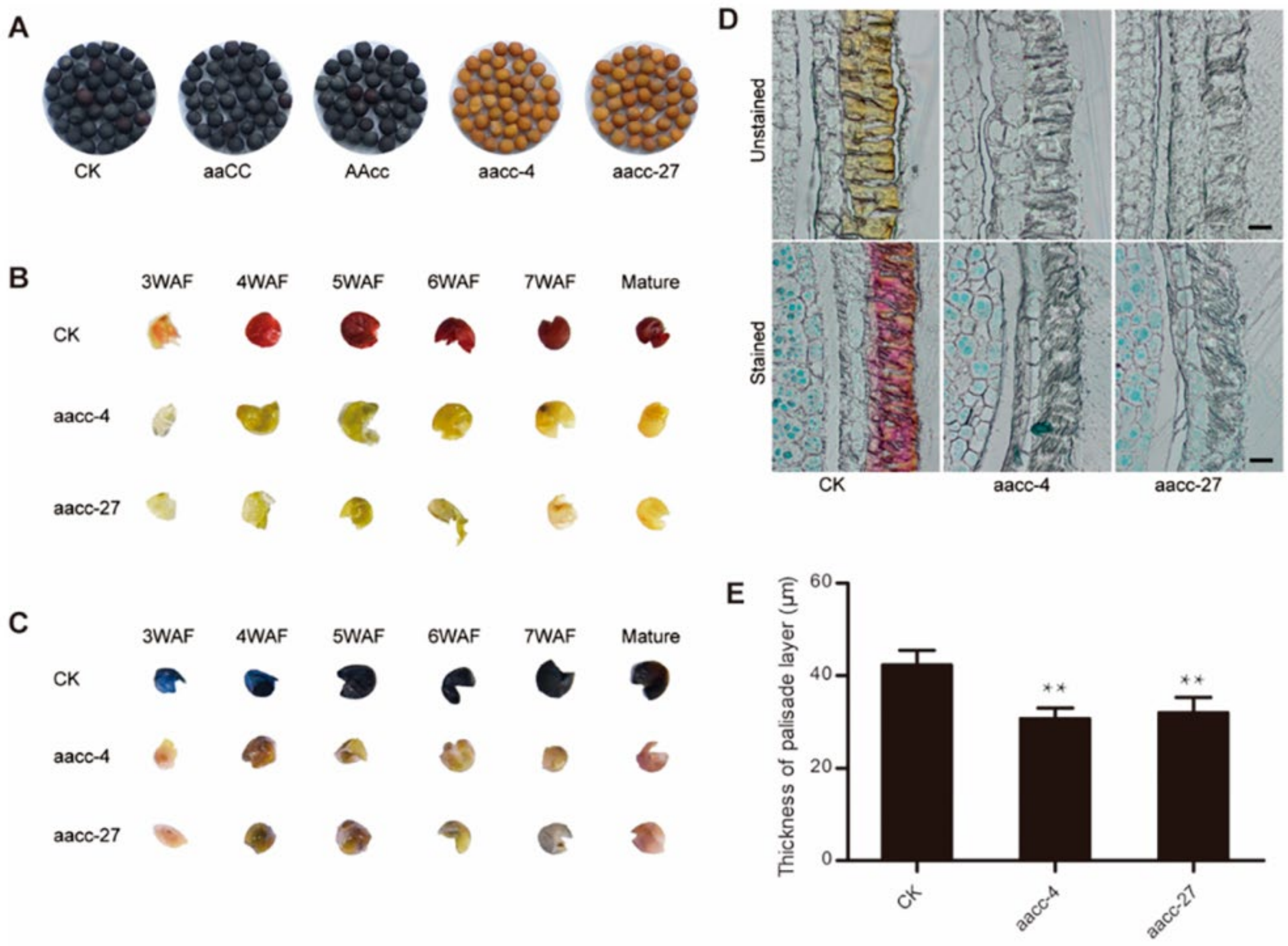


Materials	Genotype	Oil content (%)	Protein content (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	C20:1 (%)
J9707	WT	45.40 ± 0.36	19.06 ± 0.43	3.99 ± 0.13	2.96 ± 0.41	67.54 ± 2.16	17.20 ± 1.43	7.54 ± 0.62	0.78 ± 0.05
TT8-291-9-7	aaCC	45.22 ± 1.04	18.22 ± 0.63	4.10 ± 0.15	3.23 ± 0.27	69.98 ± 0.48*	14.39 ± 0.36**	7.61 ± 0.09	0.69 ± 0.02**
TT8-270-11-3	AAcc	44.57 ± 0.52	20.09 ± 0.60	4.25 ± 0.10**	2.92 ± 0.07	68.53 ± 0.52	16.46 ± 0.48	7.18 ± 0.14	0.66 ± 0.01**
TT8-384-14-9	AAcc	45.19 ± 0.09	19.19 ± 0.07	4.43 ± 0.11**	3.72 ± 0.07**	68.39 ± 0.49	16.19 ± 0.37	6.59 ± 0.11*	0.67 ± 0.01**
TT8-384-14-10	AAcc	44.94 ± 0.00	19.19 ± 0.08	4.04 ± 0.10	2.57 ± 0.05	68.87 ± 0.53	16.83 ± 0.32	6.88 ± 0.30	0.81 ± 0.01
TT8-299-12-2	aacc	47.92 ± 1.09**	20.15 ± 0.58*	4.02 ± 0.05	2.22 ± 0.06**	62.43 ± 0.31**	21.34 ± 0.23**	9.22 ± 0.10**	0.77 ± 0.01
TT8-270-1-9	aacc	48.66 ± 0.74**	20.21 ± 0.24*	4.20 ± 0.01*	2.53 ± 0.11	63.41 ± 0.28**	21.29 ± 0.27**	7.80 ± 0.10	0.77 ± 0.01
TT8-384-13-3	aacc	48.97 ± 0.95**	19.43 ± 0.36	4.99 ± 0.13**	2.63 ± 0.09	62.77 ± 0.13**	20.87 ± 0.29**	8.02 ± 0.10	0.71 ± 0.02*
TT8-96-3-2	aacc	46.69 ± 0.56*	19.38 ± 0.81	4.71 ± 0.02**	2.86 ± 0.09	61.48 ± 0.06**	23.25 ± 0.16**	7.01 ± 0.01	0.68 ± 0.01**
TT8-281-5-5	aacc	48.16 ± 0.80**	19.12 ± 0.10	4.49 ± 0.12**	2.80 ± 0.06	63.69 ± 0.88**	20.41 ± 0.56**	7.87 ± 0.18	0.73 ± 0.01

BnTT8 double mutation enhances seed oil and protein contents, alters fatty acid composition

Zhai et al., 2020. Plant Biotechnology J

Knockout *BnTT2* also resulted in yellow seeds



Targeted knockout of *BnTT2* leads to yellow-seeded *B. napus* with reduced flavonoid content.

Xie et al., 2020. J. Agric. Food Chem

BnTT2 mutants showed increased oil content

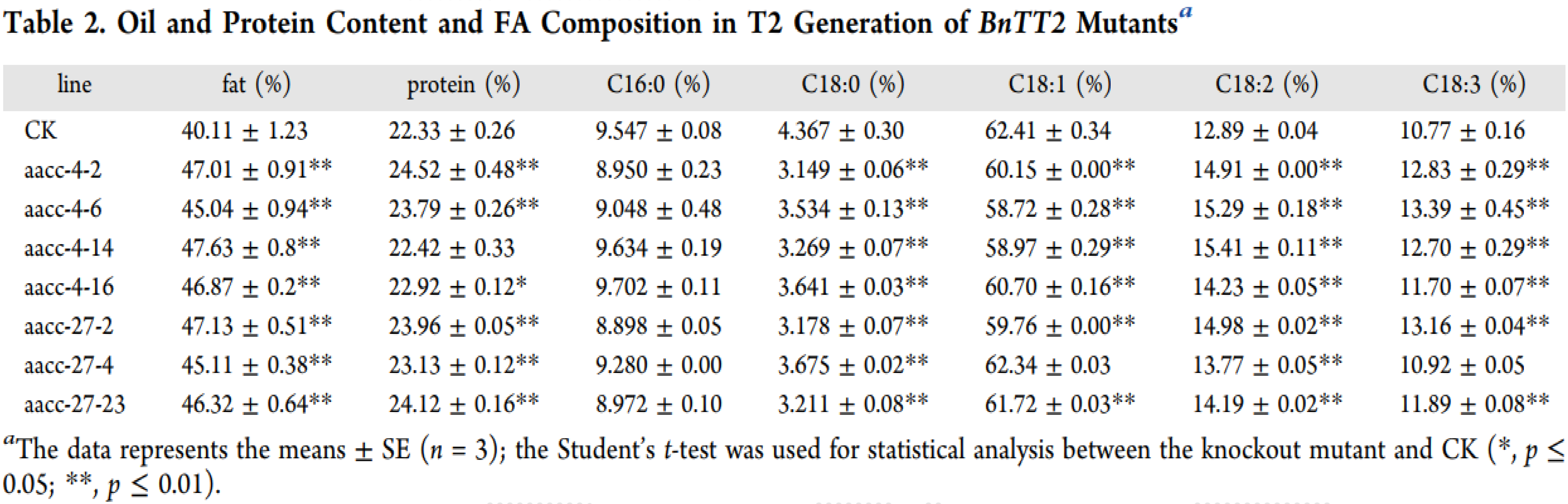


Table 2. Oil and Protein Content and FA Composition in T2 Generation of *BnTT2* Mutants[a]

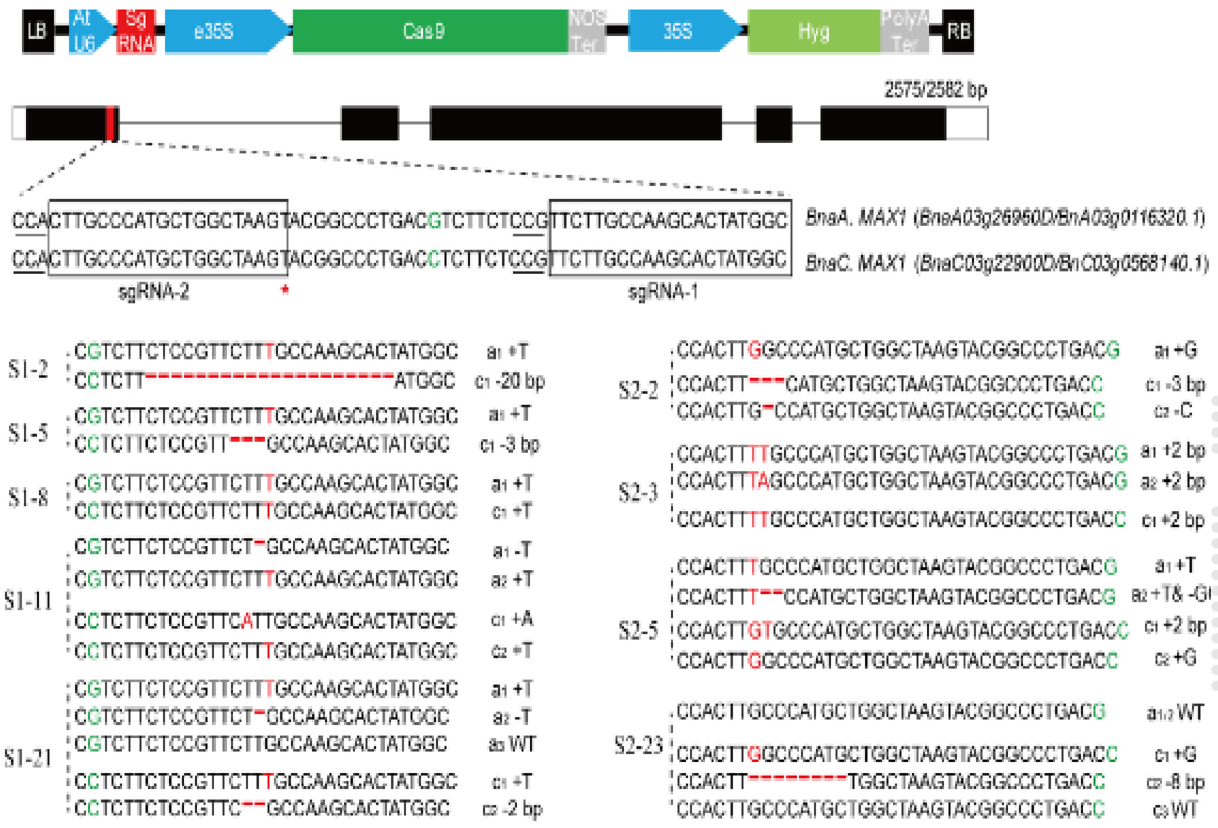
line	fat (%)	protein (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)
CK	40.11 ± 1.23	22.33 ± 0.26	9.547 ± 0.08	4.367 ± 0.30	62.41 ± 0.34	12.89 ± 0.04	10.77 ± 0.16
aacc-4-2	47.01 ± 0.91**	24.52 ± 0.48**	8.950 ± 0.23	3.149 ± 0.06**	60.15 ± 0.00**	14.91 ± 0.00**	12.83 ± 0.29**
aacc-4-6	45.04 ± 0.94**	23.79 ± 0.26**	9.048 ± 0.48	3.534 ± 0.13**	58.72 ± 0.28**	15.29 ± 0.18**	13.39 ± 0.45**
aacc-4-14	47.63 ± 0.8**	22.42 ± 0.33	9.634 ± 0.19	3.269 ± 0.07**	58.97 ± 0.29**	15.41 ± 0.11**	12.70 ± 0.29**
aacc-4-16	46.87 ± 0.2**	22.92 ± 0.12*	9.702 ± 0.11	3.641 ± 0.03**	60.70 ± 0.16**	14.23 ± 0.05**	11.70 ± 0.07**
aacc-27-2	47.13 ± 0.51**	23.96 ± 0.05**	8.898 ± 0.05	3.178 ± 0.07**	59.76 ± 0.00**	14.98 ± 0.02**	13.16 ± 0.04**
aacc-27-4	45.11 ± 0.38**	23.13 ± 0.12**	9.280 ± 0.00	3.675 ± 0.02**	62.34 ± 0.03	13.77 ± 0.05**	10.92 ± 0.05
aacc-27-23	46.32 ± 0.64**	24.12 ± 0.16**	8.972 ± 0.10	3.211 ± 0.08**	61.72 ± 0.03**	14.19 ± 0.02**	11.89 ± 0.08**

[a] The data represents the means ± SE ($n = 3$); the Student's t-test was used for statistical analysis between the knockout mutant and CK (*, $p \leq 0.05$; **, $p \leq 0.01$).

BnTT2 **homozygous mutants showed enhanced oil content and fatty acid composition, with higher linoleic acid and linolenic acid.**

Xie et al., 2020. J. Agric. Food Chem

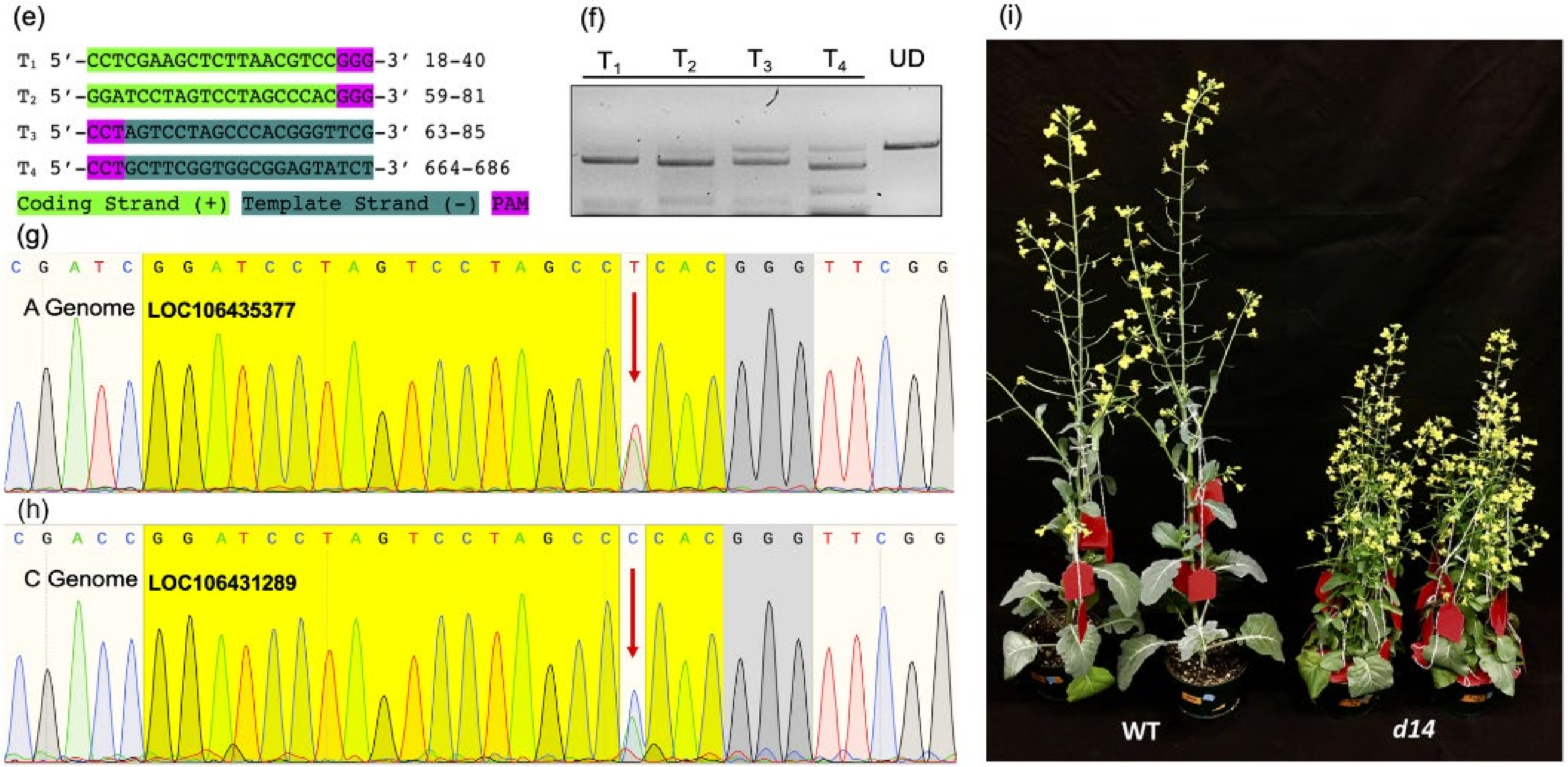
CRISPR/Cas9-Mediated Improvement of Plant Architecture and Yield



Knock out all four *BnaMAX1* alleles simultaneously resulted in semi-dwarf and increased branch traits with a greater number of siliques, leading to an elevation of yield per plant.

Ming Zheng et al., 2019. Plant Biotechnology J

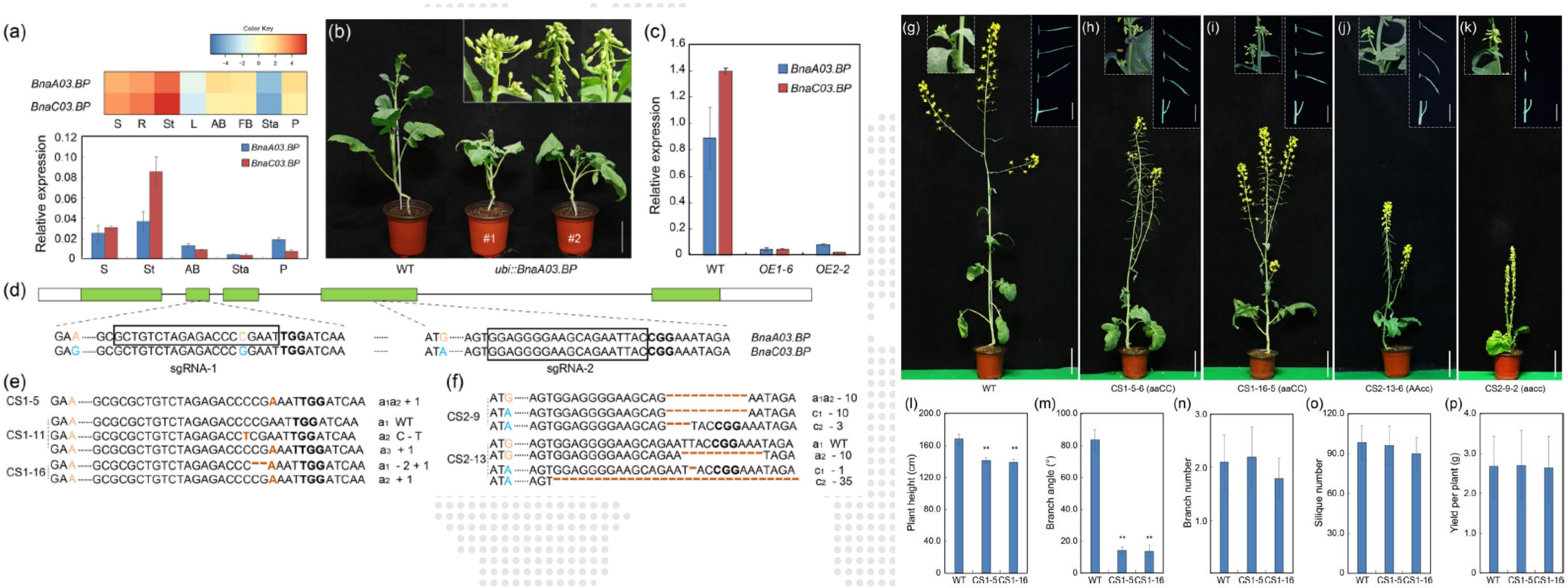
Modifying Plant Architecture using CRISPR/Cas9



Genome-edited D14 gene plants exhibited enhanced branching (200% higher mean total branches) and a dwarfed phenotype (34% reduction in mean plant height and decreased internode length).

Matija et al., 2020. Plant Biotechnology J

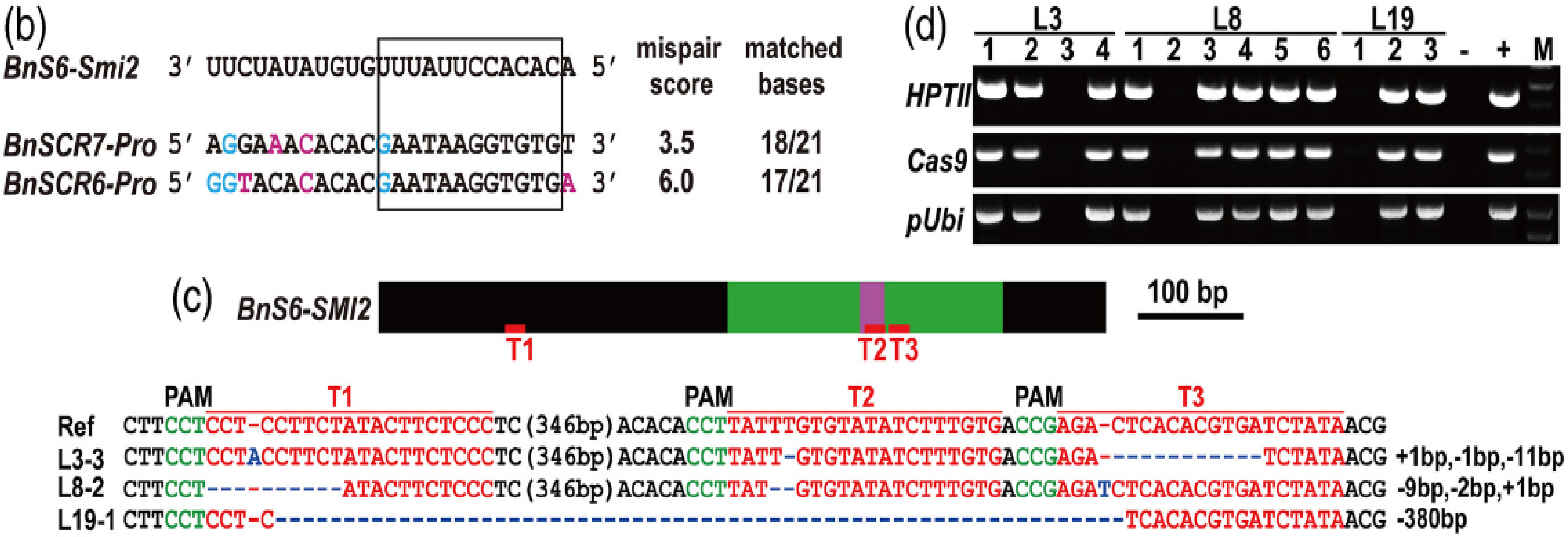
CRISPR/Cas9-Mediated Improvement of Plant Architecture



CRISPR/Cas9-targeted mutagenesis of the *BnaA03.BP* gene confers semi-dwarf and compact architecture in *B. napus*.

Fang et al., 2021. Plant Biotechnology J

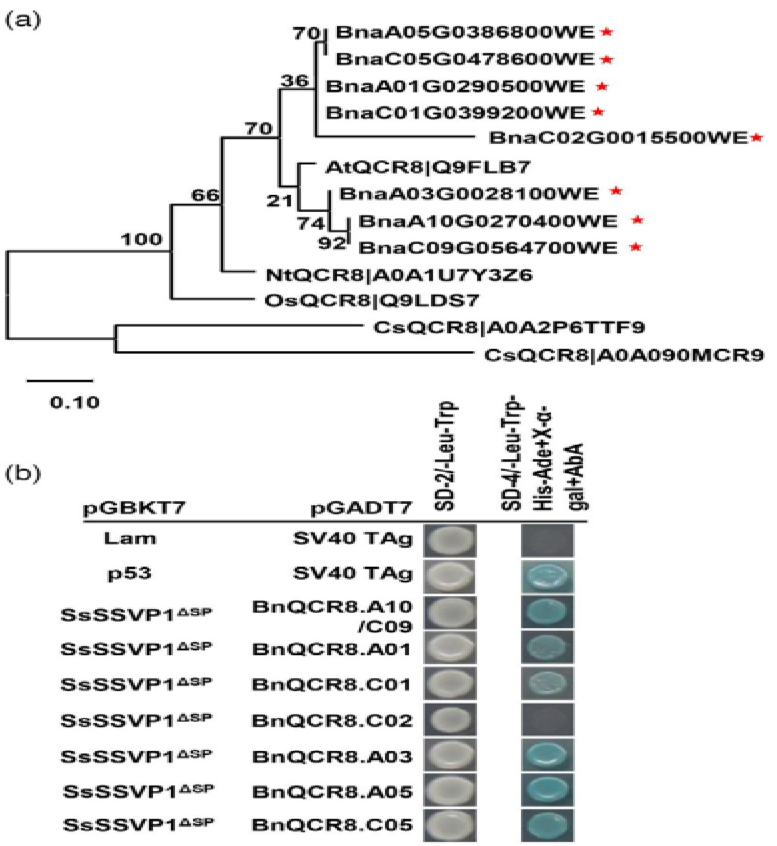
Generation of novel self-incompatibity by CRISPR/Cas9

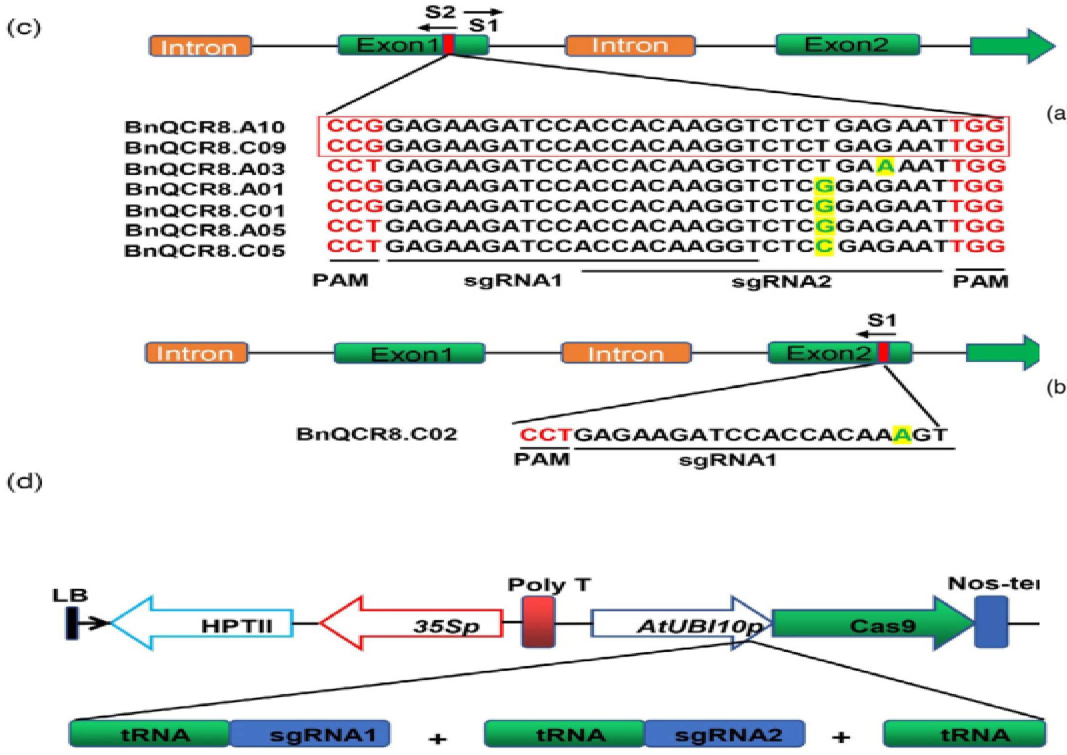


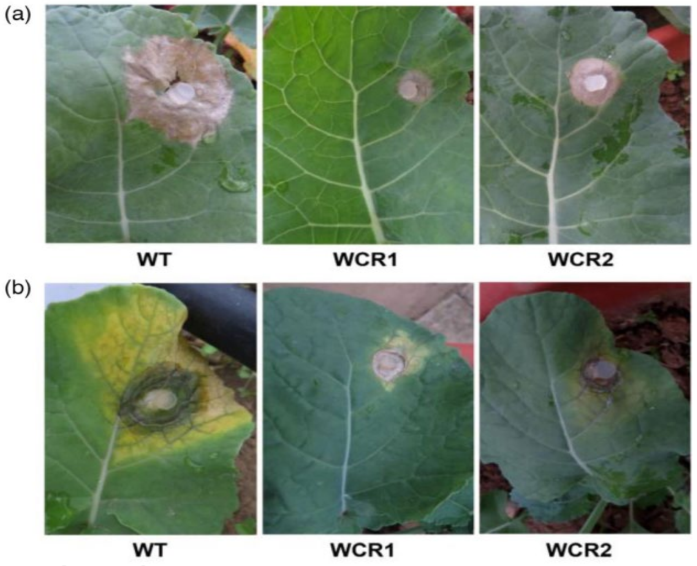
Utilizing CRISPR/Cas9 to generate self-incompatible *B. napus* by specifically targeting BnS6-Smi2 gene.

Dou et al., , 2021, Plant Biotechnology J

Increasing pathogen resistance by genome editing of *BnQCR8*



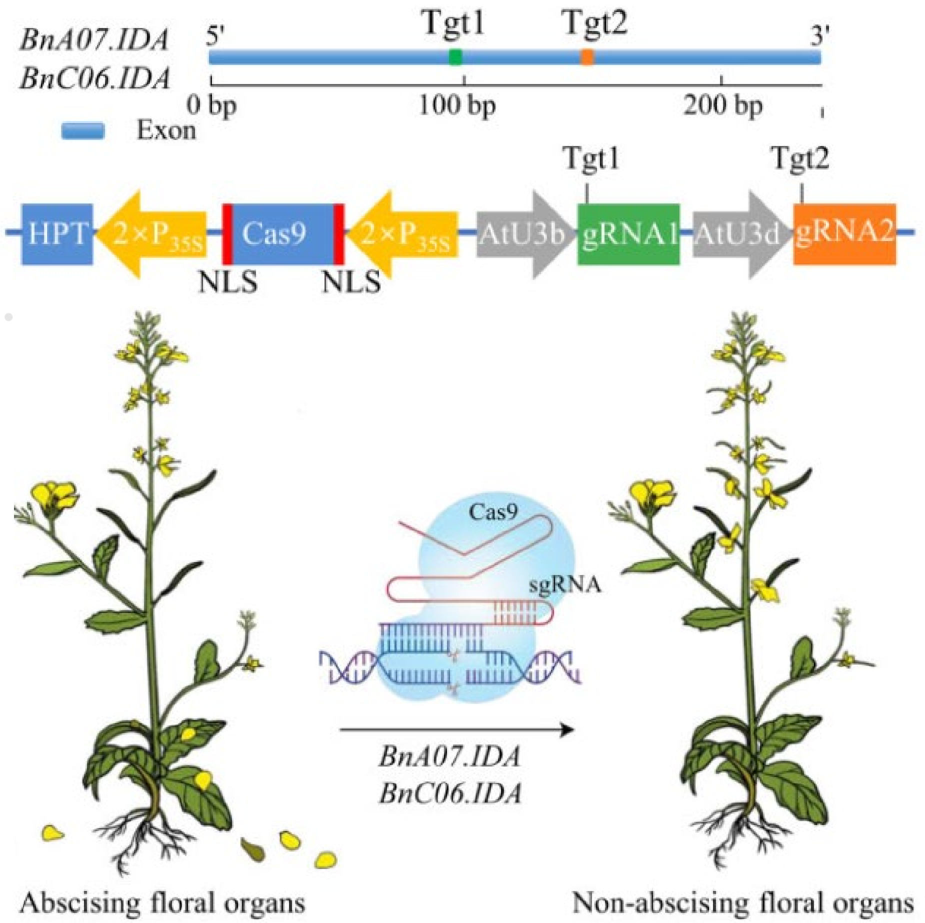


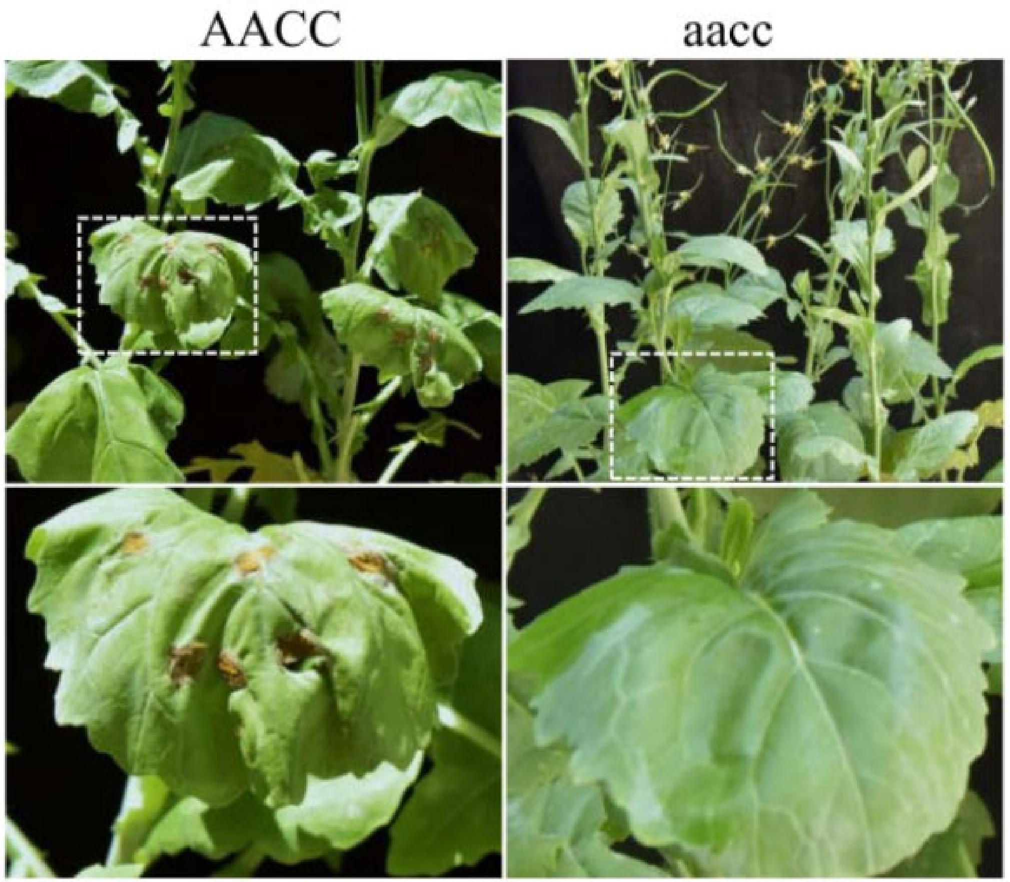


BnQCR8-edited mutants showed strong resistance against *S. sclerotiorum* and *Botrytis cinerea*

Zhang et al., 2021, Plant Biotechnology J

Generating non-abscising floral organs Using CRISPR/Cas9

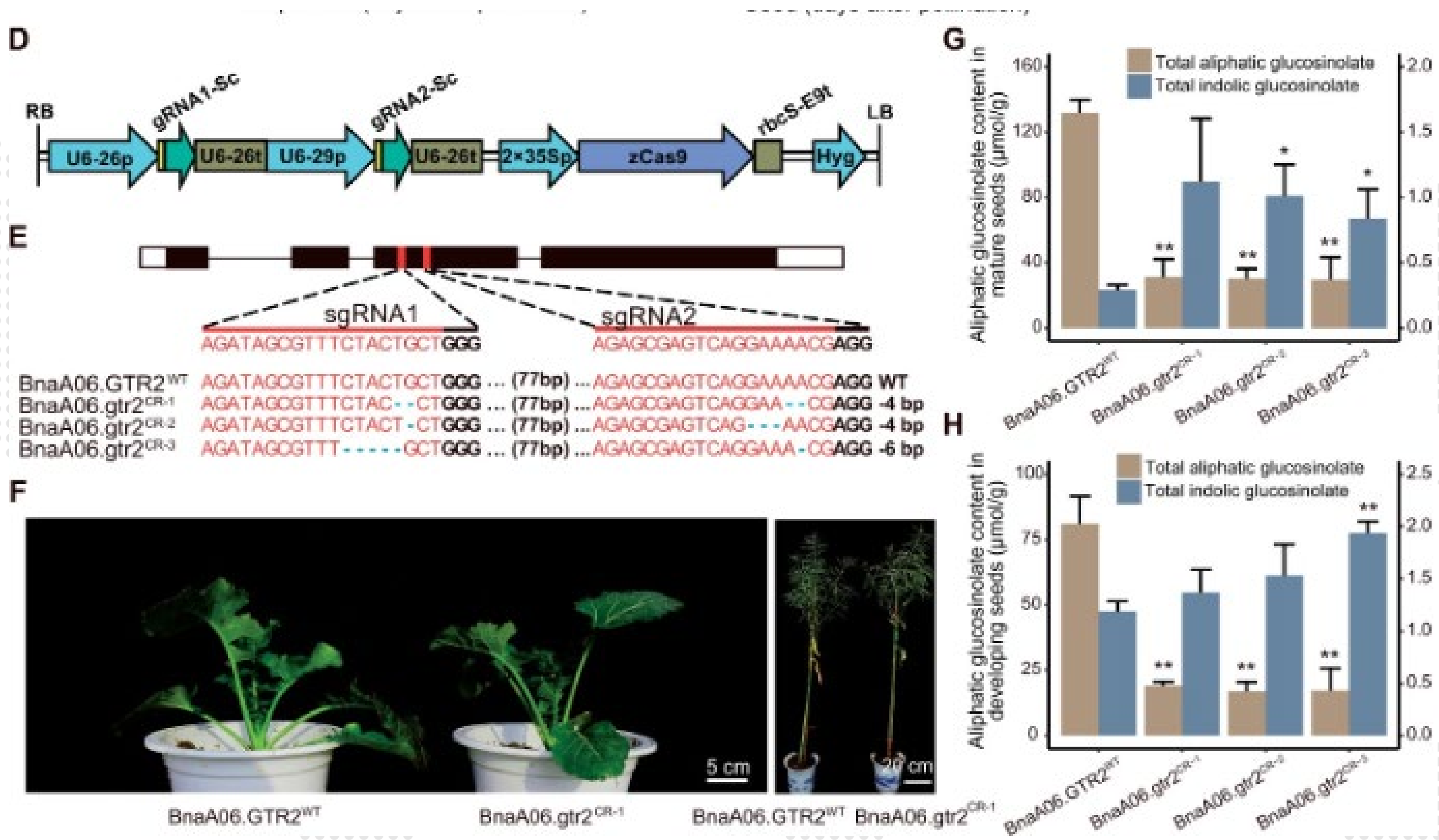




Knock out *BnA07.IDA* and *BnC06.IDA* genes can lead toe generation of mutants with non-abscising floral organs, which have the potential to prevent *Sclerotinia* infection through abscised petals and stamens.

Wu et al., 2022, Plant Physiology
Geng et al., 2022, Plant Communication

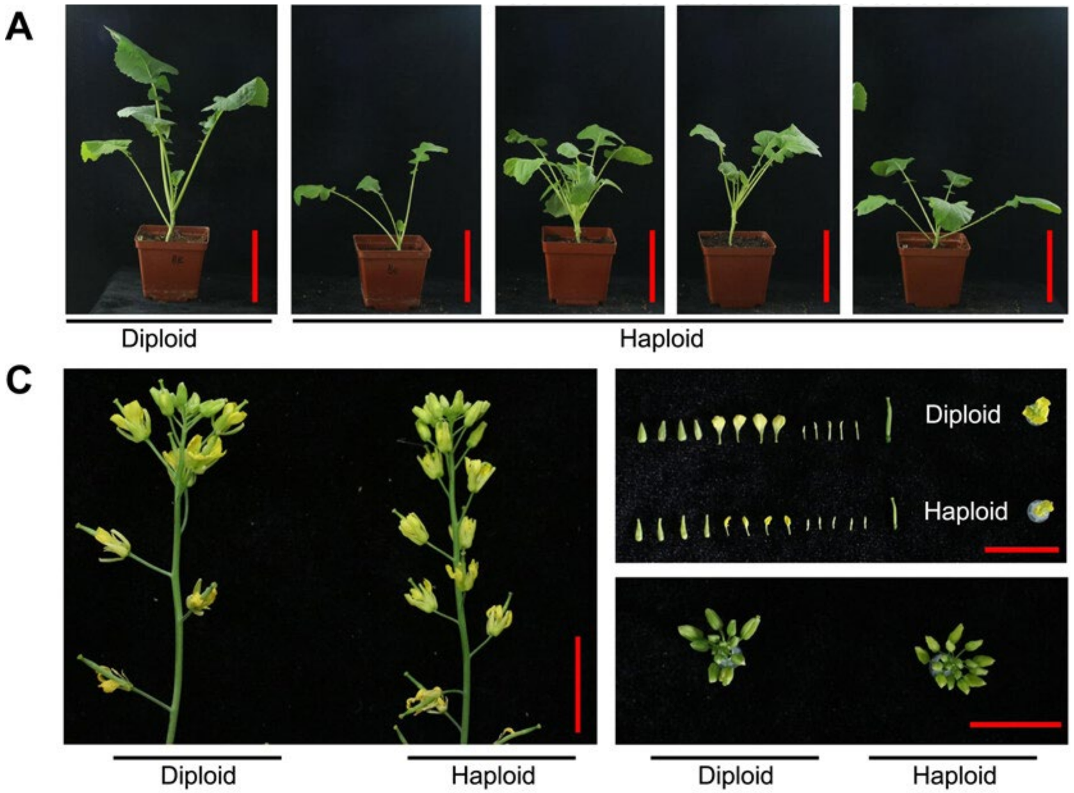
Creating Low Seed Glucosinolate Resource Using CRISPR/Cas9

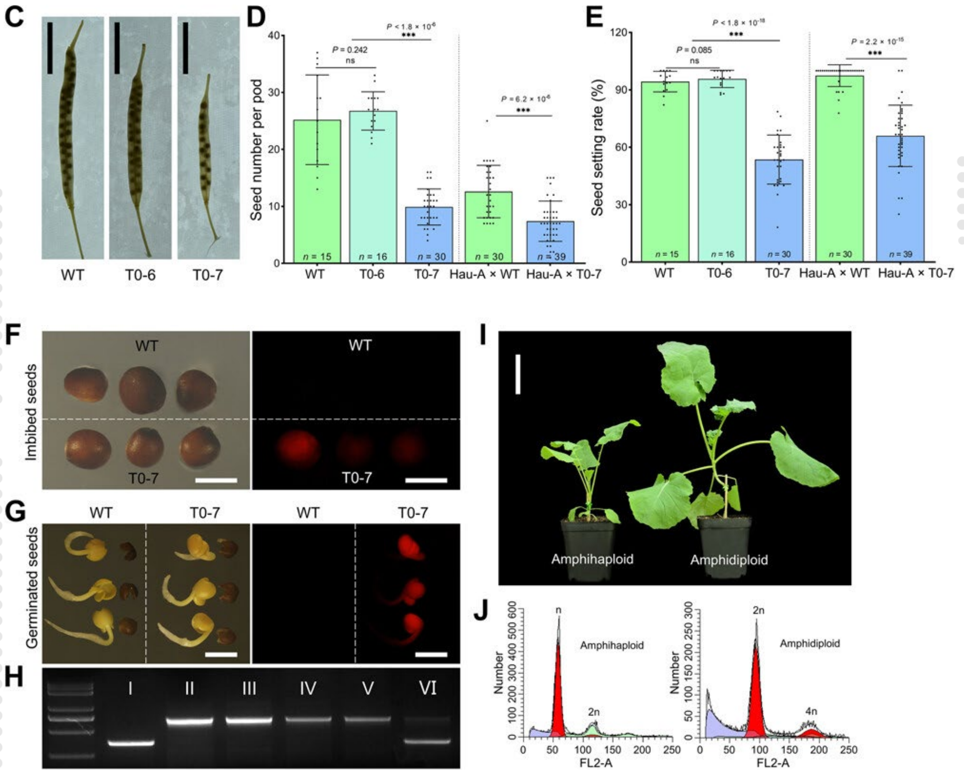


The seed glucosinolate content in the *BnaA06.GTR2* knockout mutant decreased by 76.05% compared to the transgenic negative control.

He et al., 2022, Plant Physiology

Establishment of *dmp* based maternal haploid induction system

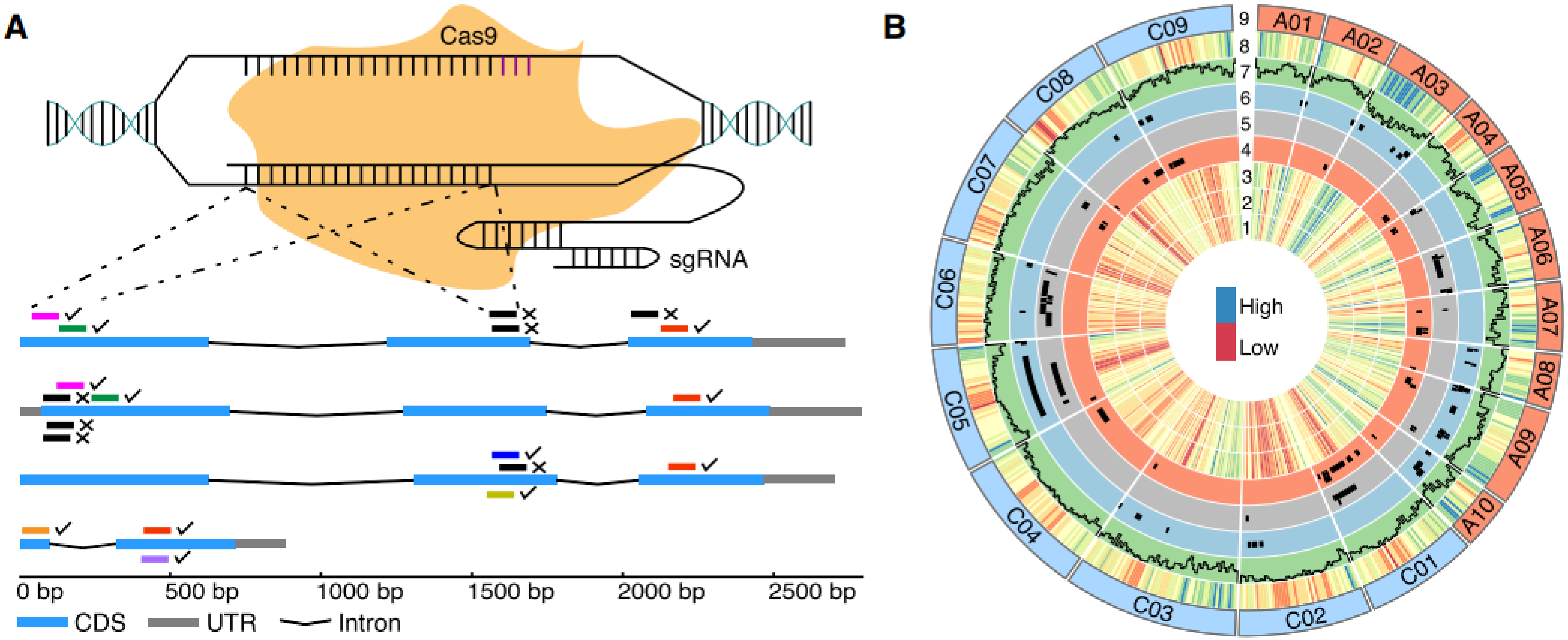




Knocked out *BnaDMP* genes generated an in planta haploid induction system in *B. napus* with low efficiency (2.53%).

Li et al., 2022, JIPB
Zhong et al., 2022, JIPB

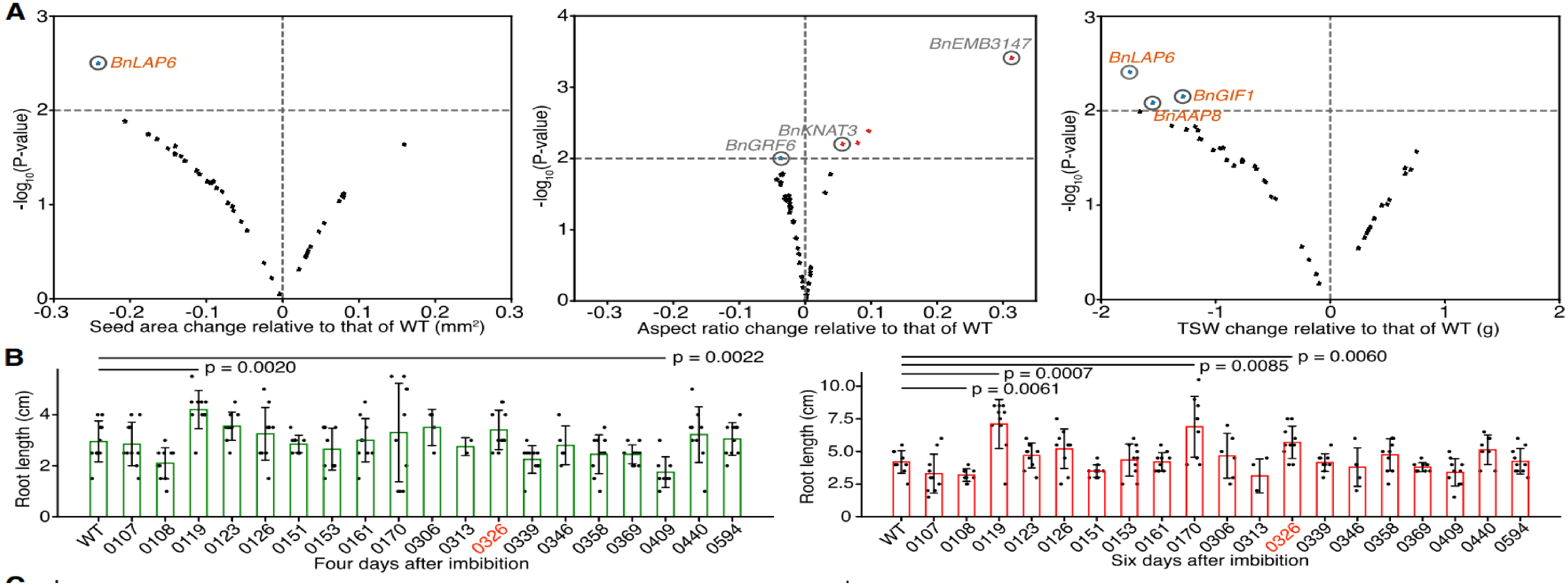
Genome-Scale Targeted Mutagenesis using a Pooled CRISPR Library



A comprehensive set of 18,414 sgRNAs was designed to specifically target 10,480 genes of interest. A total of 1,104 regenerated transgenic plants were successfully obtained, collectively hosting 1,088 unique sgRNAs.

He et al., 2023, Genome Research

Genome-Scale Targeted Mutagenesis using a Pooled CRISPR Library

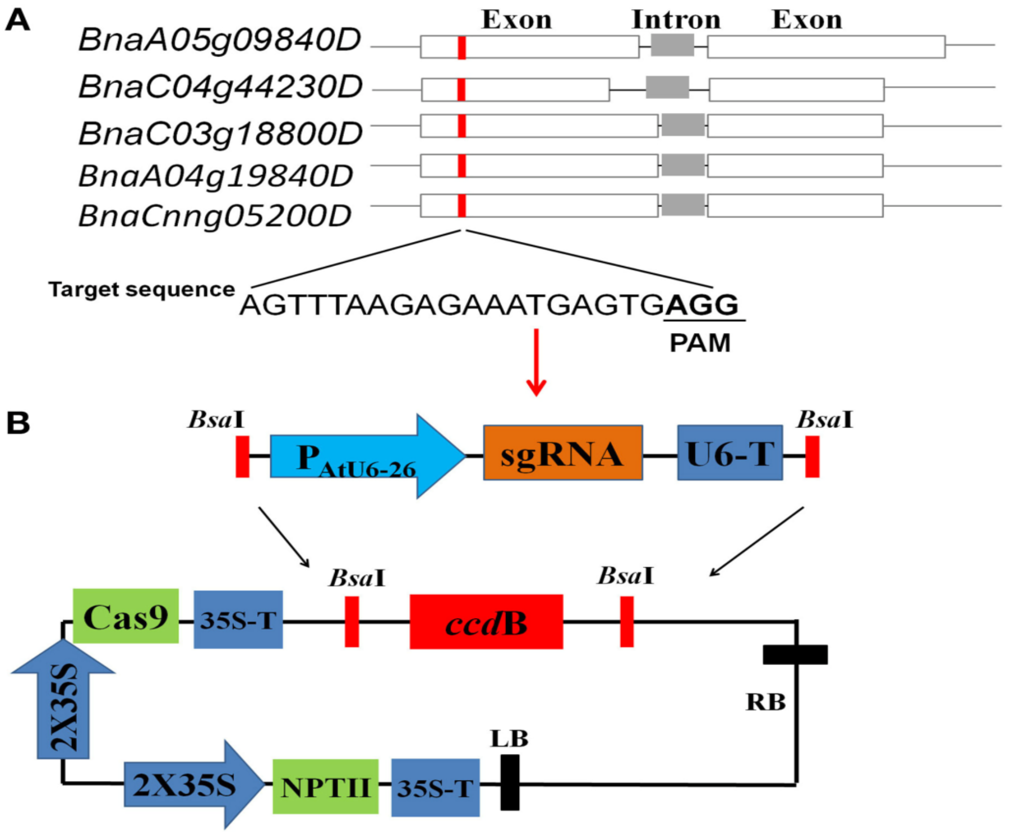


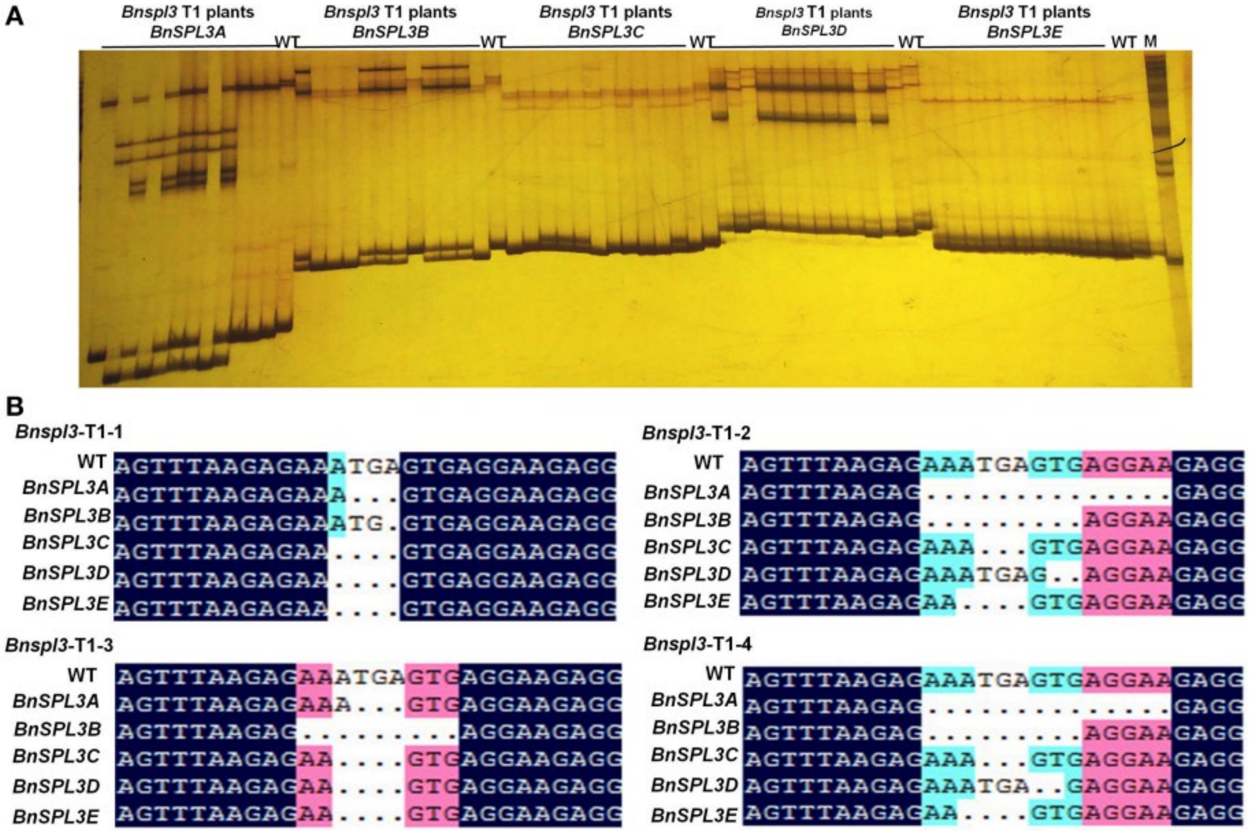
A pooled CRISPR library is valuable for conducting reverse genetic screening to study various traits, such as seed-related traits and root length.

He et al., 2023, Genome Research

3. Work in our lab-Established CRISPR/Cas9 system in *B. napus*

- We established CRISPR/Cas9 system in oilseed rape by optimization of U6 promoter.





Li et al., 2018, Frontiers in Plant Science

Increasing pod shatter resistance Using CRISPR/Cas9

BnJAG-33

JAG-33.A08-Mut:
JAG-33.A08-WT :

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CGCAACAATACATTTCGTTTCCTCATCAAGG
CGCAACAATACATTTC..CTCCTCATCAAGG
cgcaacaatacatttc    tcctcatcaagg
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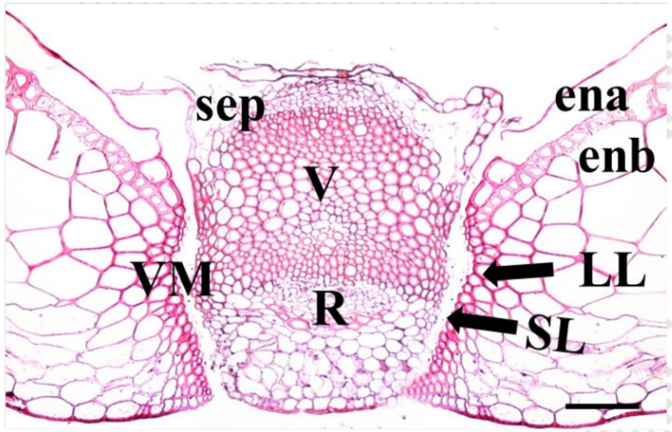
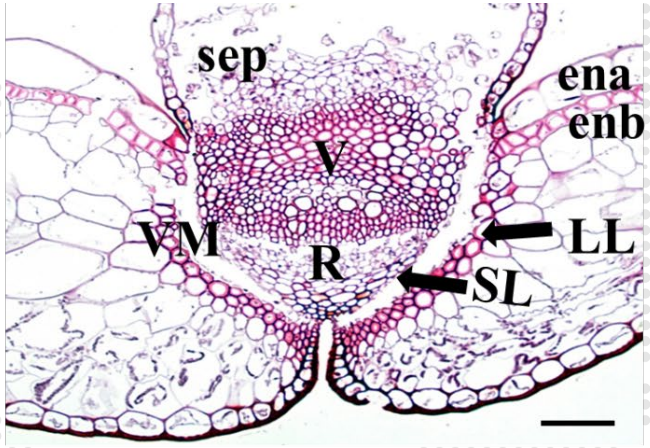
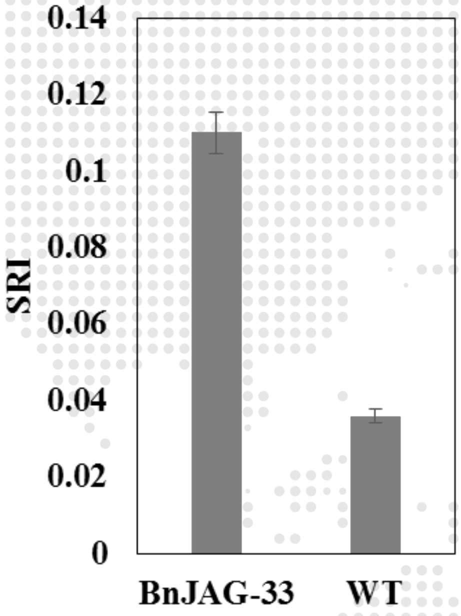
+3



BnJAG-33 Mutant



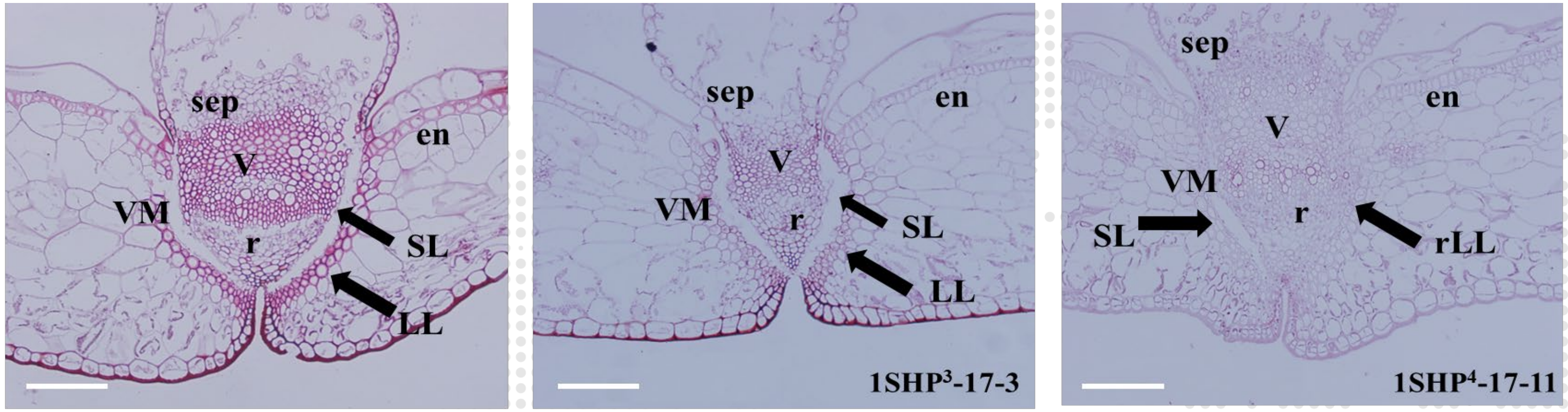
Wild Type



- *BnJAG* has 5 copies in *B. napus* genome.
- A mutation in *BnJAG.A08* leads to approximately 5-fold increase in pod shattering resistance index.

Zaman et al.,2019. *Biomolecules*

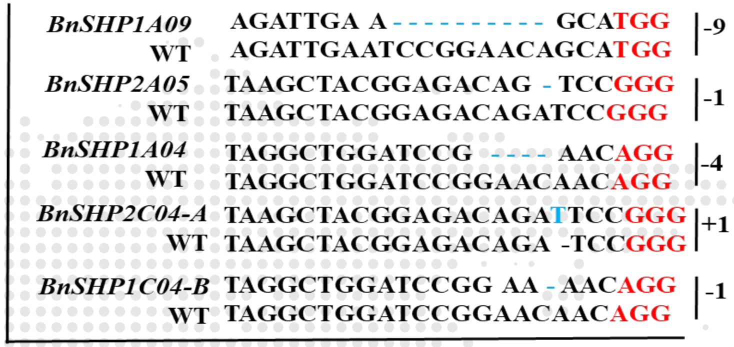
Increasing pod shatter resistance using CRISPR/Cas9



WT

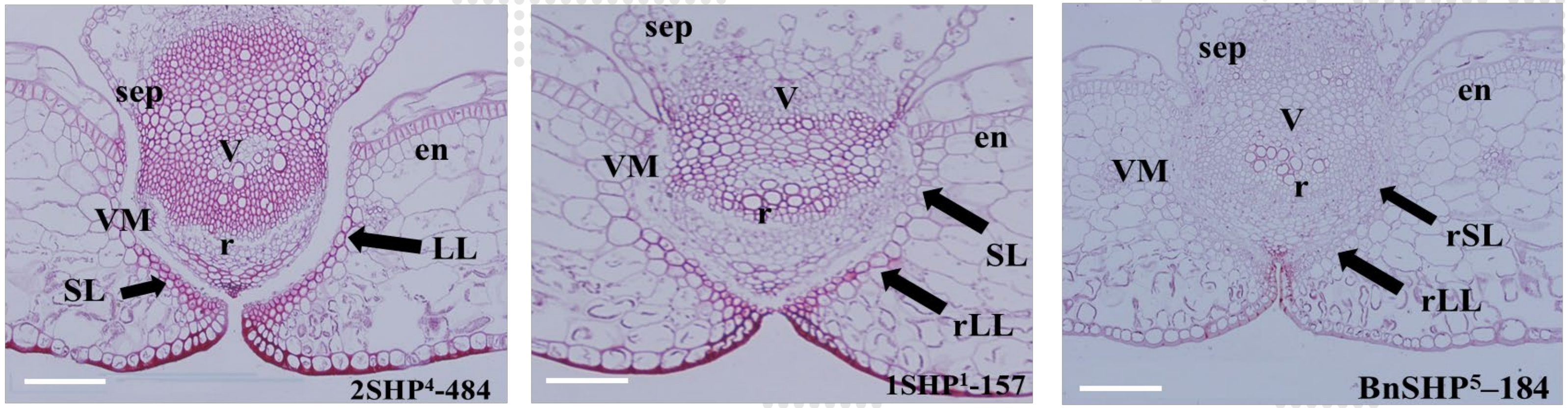
BnSHP1C06/C08/C09

BnSHP1A09/C06/C08/C09



BnSHP1A09	AGATTGA A - - - - - - - - - - - GCATGG	-9
WT	AGATTGAATCCGGAACAGCATGG	
BnSHP2A05	TAAGCTACGGAGACAG - TCCGGG	-1
WT	TAAGCTACGGAGACAGATCCGGG	
BnSHP1A04	TAGGCTGGATCCG - - - - AACAGG	-4
WT	TAGGCTGGATCCGGAACAACAGG	
BnSHP2C04-A	TAAGCTACGGAGACAGATTCCGGG	+1
WT	TAAGCTACGGAGACAGA -TCCGGG	
BnSHP1C04-B	TAGGCTGGATCCGG AA - AACAGG	-1
WT	TAGGCTGGATCCGGAACAACAGG	

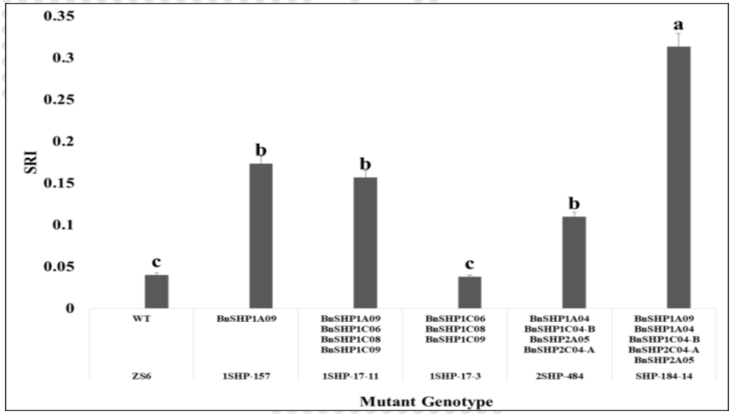
BnSHP^5–184



BnSHP1C04-B/A04, BnSHP2C04-A/A05

BnSHP1A09

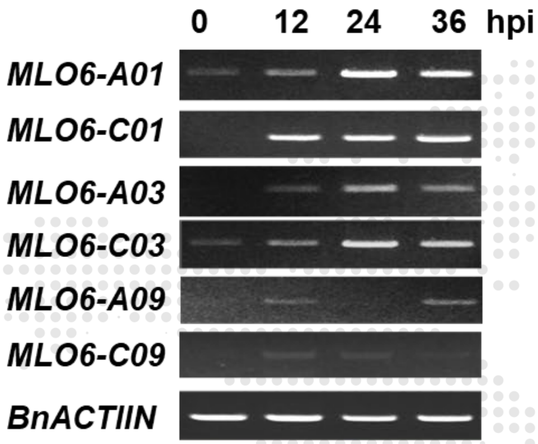
BnSHP1A09/C04-B/A04, BnSHP2C04-A/A05



Among 8 *BnSHP* copies, A09 copy is crucial for lignin accumulation and regulation of silique dehiscence, along with *BnSHP1C04-B/A04* and *BnSHP2C04-A/A05* copies.

Zaman et al., 2021. The CRISPR JOURNAL

Increasing pathogen resistance by CRISPR/Cas9




A

BnMLO6-A03
Allele1 CAGCCG------------------GATCAT -15bp
Allele2 CAGCCG------------------GATCAT -15bp

BnMLO6-C03
Allele1 CAGCCG----------------GTGATCAT -13bp
Allele2 CAGCCGCTT---TCAGCCCGGTGATCAT -2bp

BnMLO6-A01
Allele1 CAGCCGCTTG---CAGCCCGGTGATCAT -2bp
Allele2 CAGCCGCTTGGTTCAGCCCGGTGATCAT +1bp

BnMLO6-C01
Allele1 CAGCCGCTTGTTCAGCCCGGTGATCAT WT
Allele2 CAGCCGC---------------GTGATCAT -12bp

BnMLO6-A09
Allele1 CAGCCGCTTGTTCAGCCCGGTGATCAT WT
Allele2 CAGCCGCTT-----CAGCCCGGTGATCAT -3bp

BnMLO6-C09
Allele1 CAGCCGCTTGTTCAGCCCGGTGATCAT WT
Allele2 CAGCCGCT---TTCAGCCCGGTGATCAT -2bp

B

BnMLO6-A03
WT GEIVKGMPLVQPGDHLFWFGR
-15bp GEIVKGMP---------DHLFWFGR

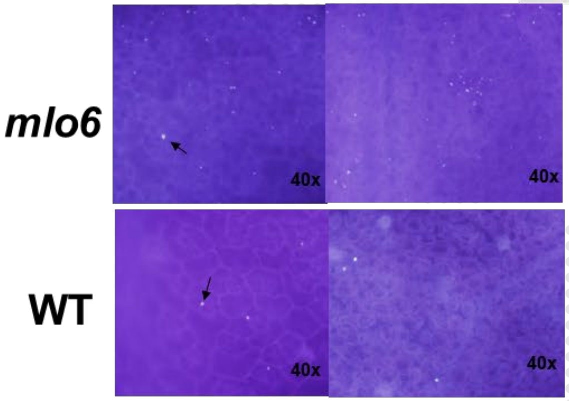
BnMLO6-C03
WT GEIVKGMPLVQPGDHLFWFGR
-13bp GEIVKGMPVIIFSGSVVHVSTF
-2bp GEIVKGMPLSARSSFLVRSFSS

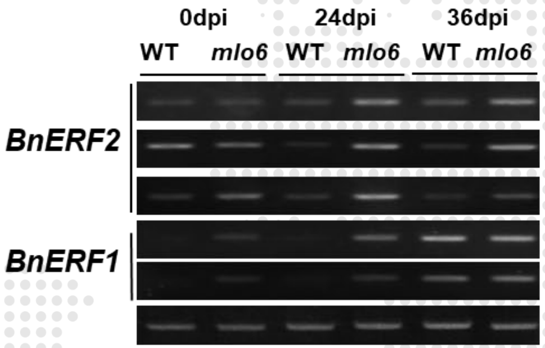
BnMLO6-A01
WT DIVKGMPLVQPGDHLFWFGR
-2bp DIVKGMPLAARSSLLVRSSTF
+1bp DIVKGMPLGSARSSLLVRSSTF

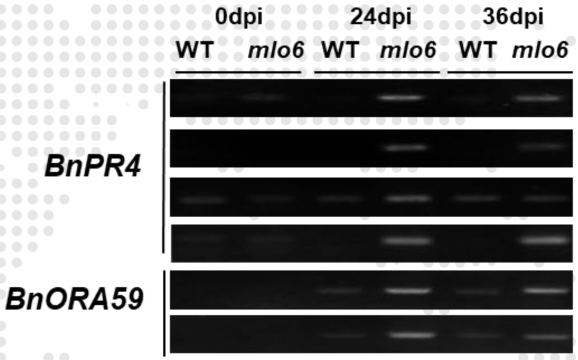
BnMLO6-C01
WT DIVKGMPLVQPGDHLFWFGR
-12bp DIVKGMPR--------DHLFWFGR

BnMLO6-A09
WT DIVKGMPLVQPGDHLFWFGR
-3bp DIVKGMPL--QPGDHLFWFGR

BnMLO6-C09
WT DIVKGMPLVQPGDHLFWFGR
-2bp DIVKGMPLSARSPLLVRSPTF





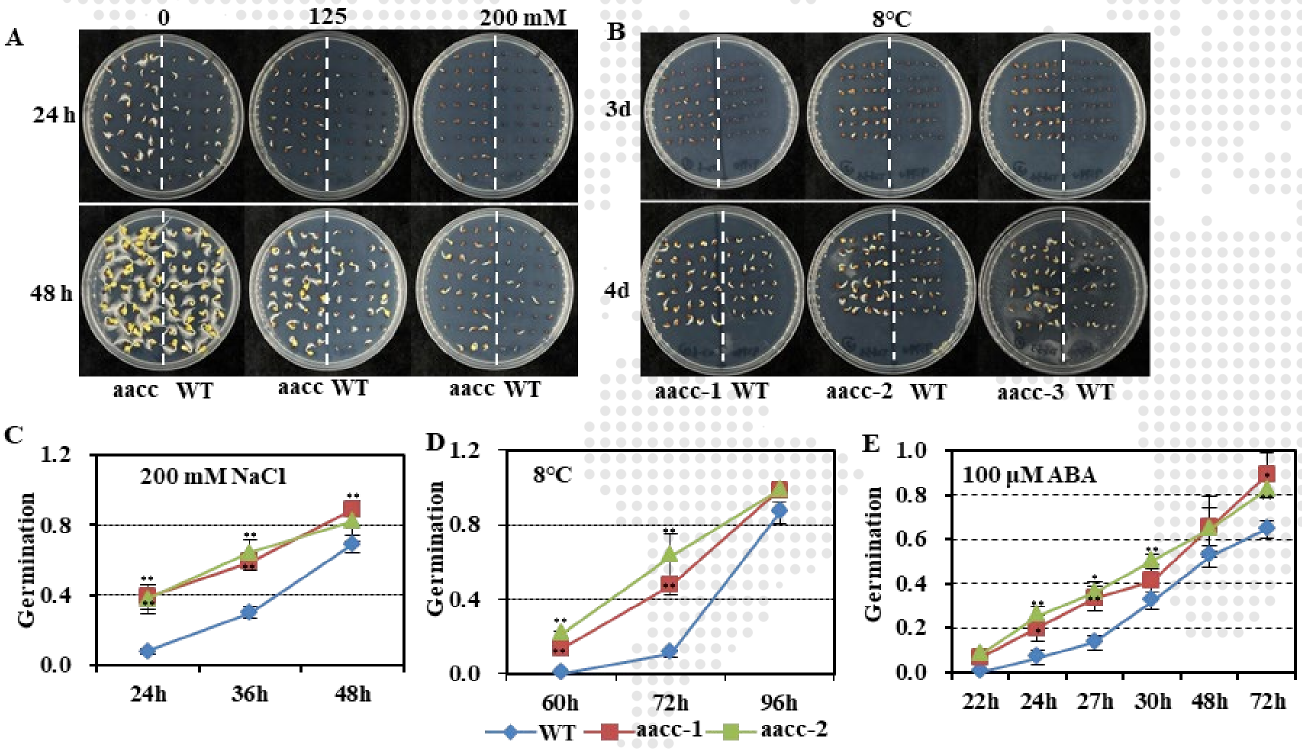


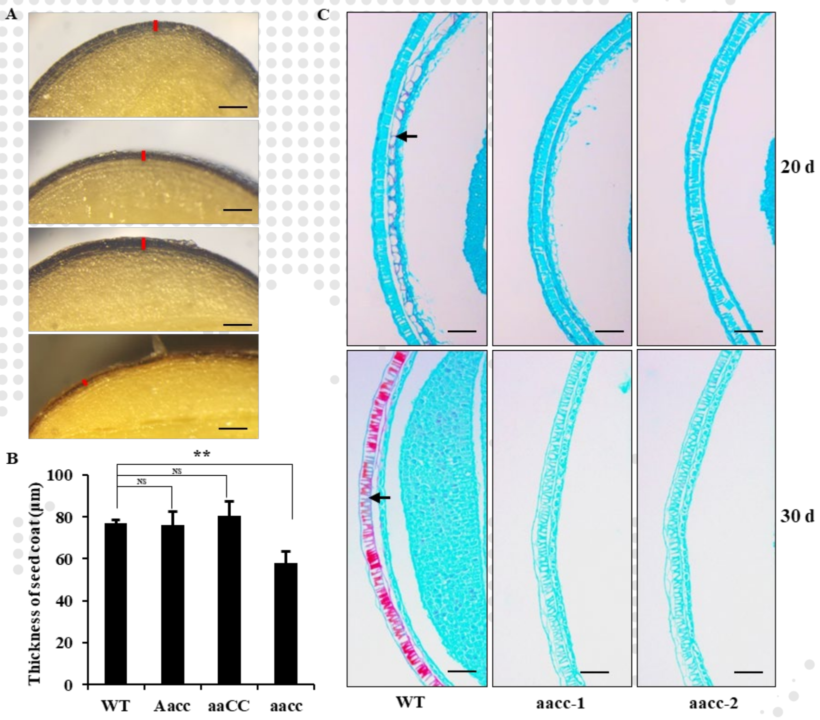
Mutating all six copies of *BnMLO6* gene enhanced resistance of rapeseed to powdery mildew and *S. sclerotiorum*.

Shi et al□ CROP journal□ 2021

Targeted mutagenesis of *BnGT1* increased oil content and seed germination

- Mutation of *BnGT1* generating yellow-seeded oilseed rape genetic resources with increase oil content, modified FA composition and resistance to multiple abiotic stresses (salt/low temp/ABA).

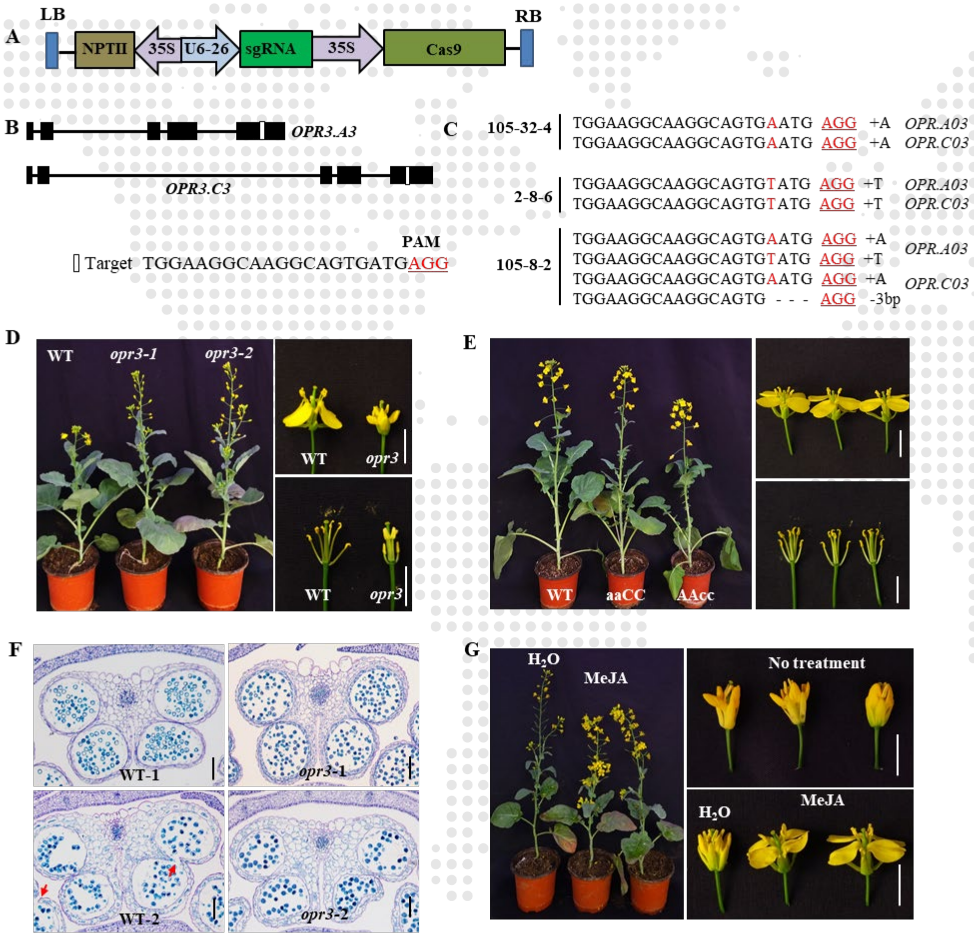


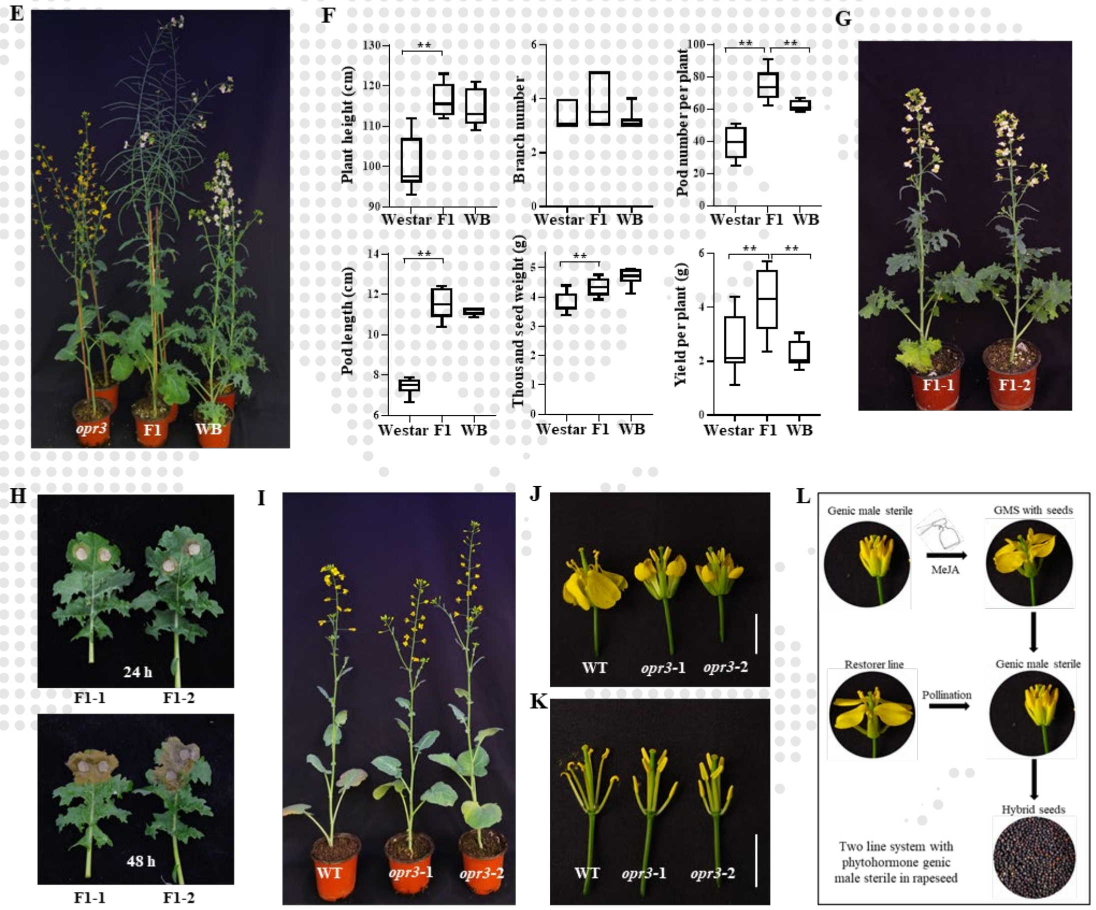


Cheng et al., Under review

Establishment of new two-line system by targeting mutation of *BnOPR3*

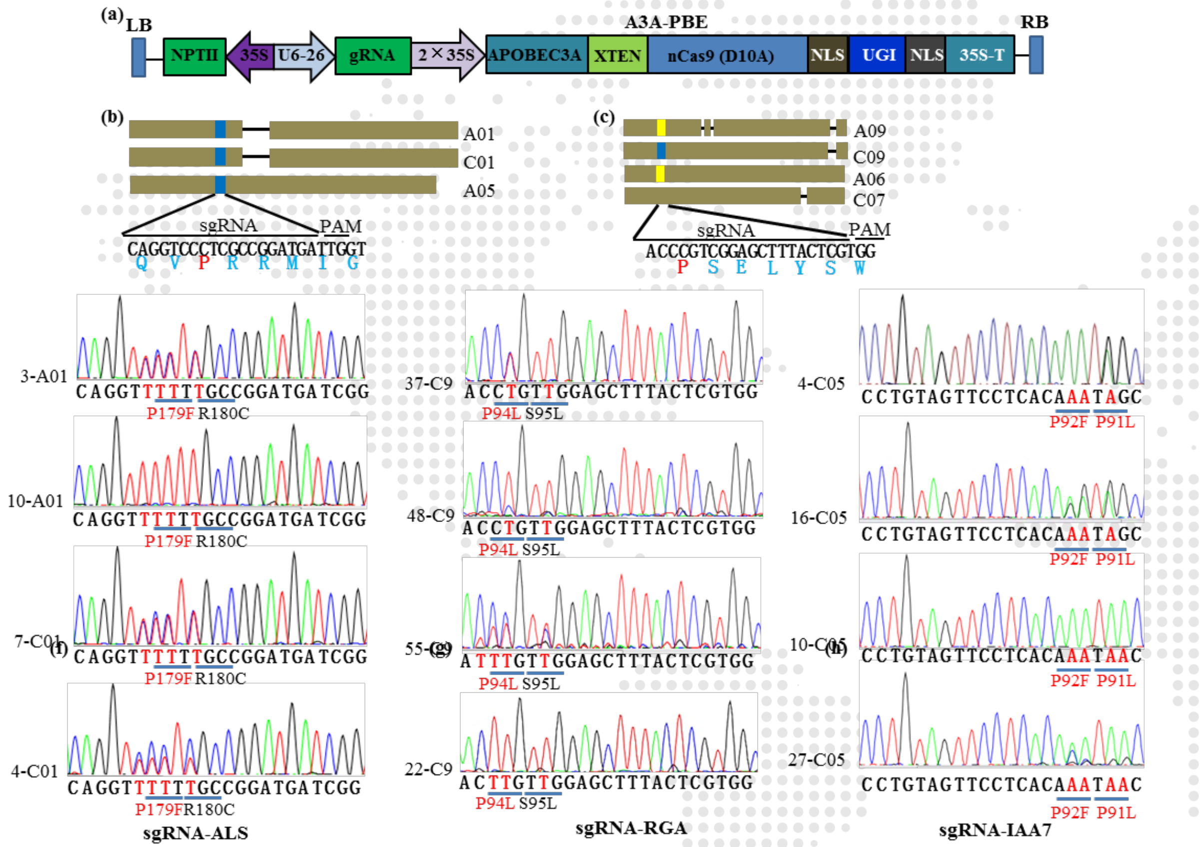
- Mutation of *BnOPR3* generating stable male sterile line, which can be conveniently restored fertility and sets seeds by treated with exogenous MeJA (P216)





Cheng et al., Under review

Developing A3A-PBE base editing system in *B. napus*



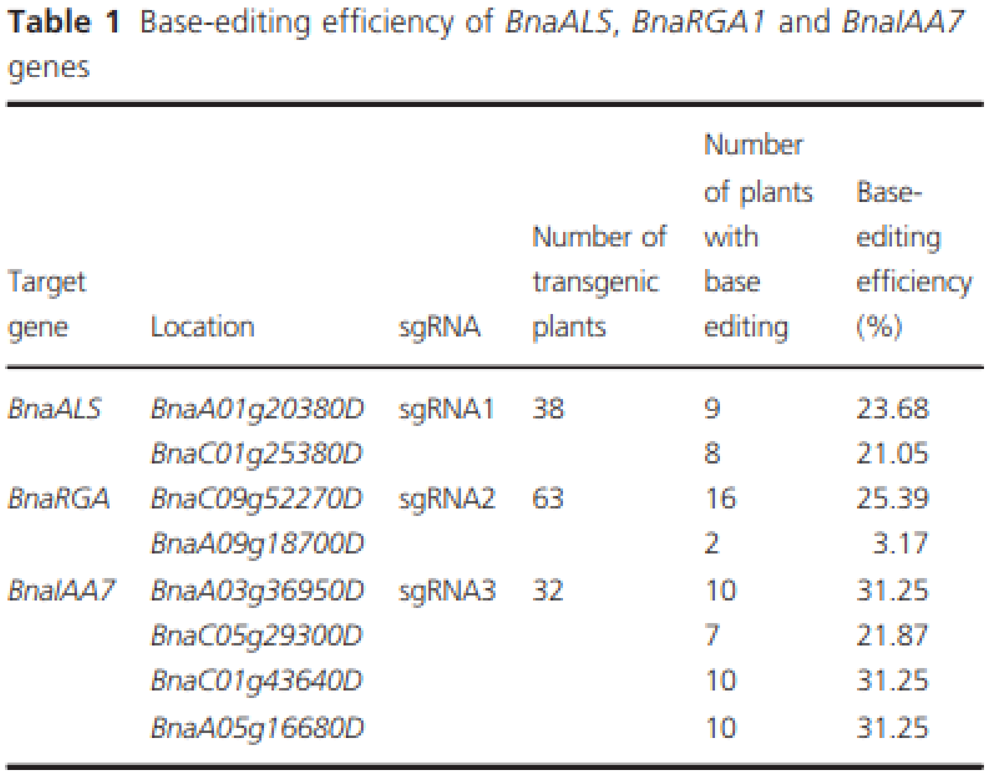


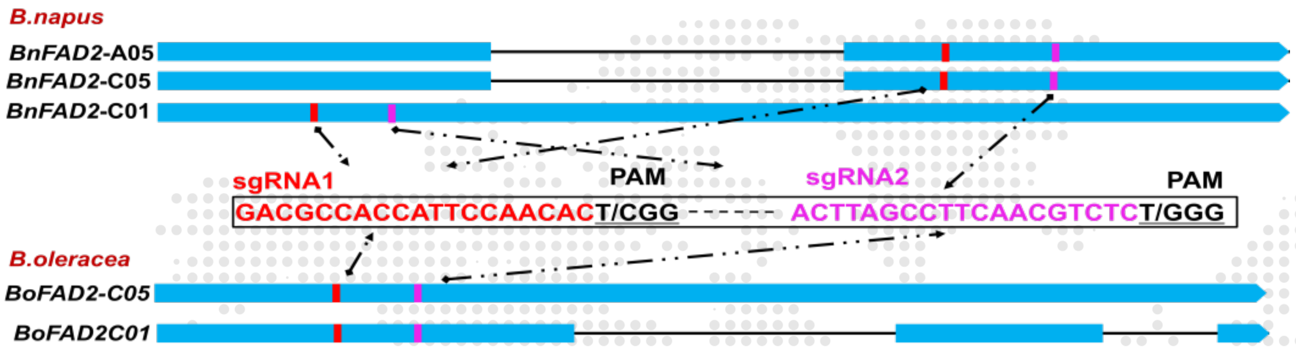
Table 1 Base-editing efficiency of *BnaALS*, *BnaRGA1* and *BnaIAA7* genes

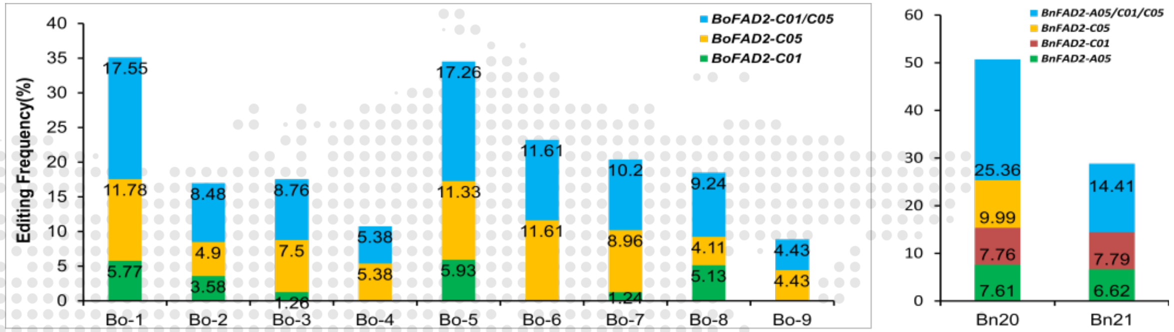
Target gene	Location	sgRNA	Number of transgenic plants	Number of plants with base editing	Base-editing efficiency (%)
BnaALS	*BnaA01g20380D*	sgRNA1	38	9	23.68
	BnaC01g25380D			8	21.05
BnaRGA	*BnaC09g52270D*	sgRNA2	63	16	25.39
	BnaA09g18700D			2	3.17
BnaIAA7	*BnaA03g36950D*	sgRNA3	32	10	31.25
	BnaC05g29300D			7	21.87
	BnaC01g43640D			10	31.25
	BnaA05g16680D			10	31.25

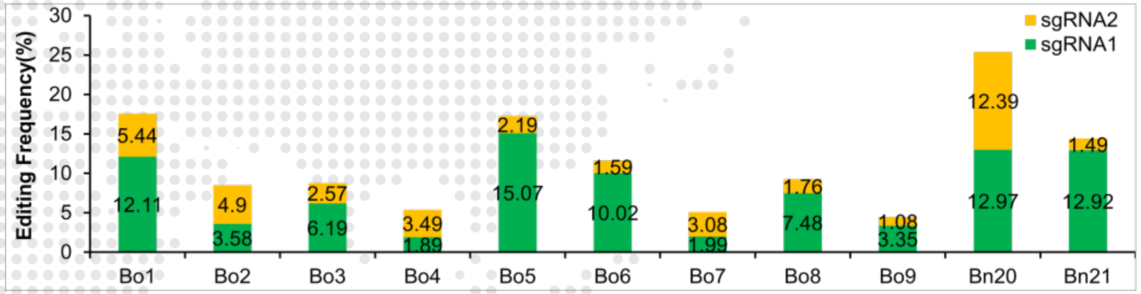
◆A3A-PBE can convert C to T substitutions effectively and enables efficient single-base editing of multiple homologous copies in rapeseed, with a mutation efficiency over 20%.

Cheng et al., 2020, Plant Biotechnology J

Doubled haploid inducer-mediated genome-editing system







Brassica Doubled Haploid Inducer was used as a carrier for CRISPR/Cas9-mediated site-specific mutagenesis in *B. oleracea* and *B. napus* plants. The editing efficiency ranged from 12.92% to 12.97% for *B. napus* and from 1.89% to 12.11% for *B. oleracea*.

Li et al., 2021, Plant Biotechnology J

4. Conclusion and perspective

- CRISPR/Cas9 and A3A-PBE base editing systems are robust tools for gene functional analysis and genetic improvement.
- Important agronomic traits, such as resistance to pod shattering, pathogens, and herbicides, as well as plant architecture could be modified via genome editing in rapeseed.
- The doubled haploid inducer-mediated genome-editing system can enhance breeding applications, but editing efficiency needs optimization.
- More genome editing system should be developed in oilseed rape, such as ABE and Prime editing with high efficiency.

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- Prof. Yaoguang Liu
- Prof. Shaohong Fu

N S F C

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The Agricultural Science and Technology Innovation Program of China

Key R&D Projects in Hubei Province

Recruiting people working on gene editing and abiotic stress resistance

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