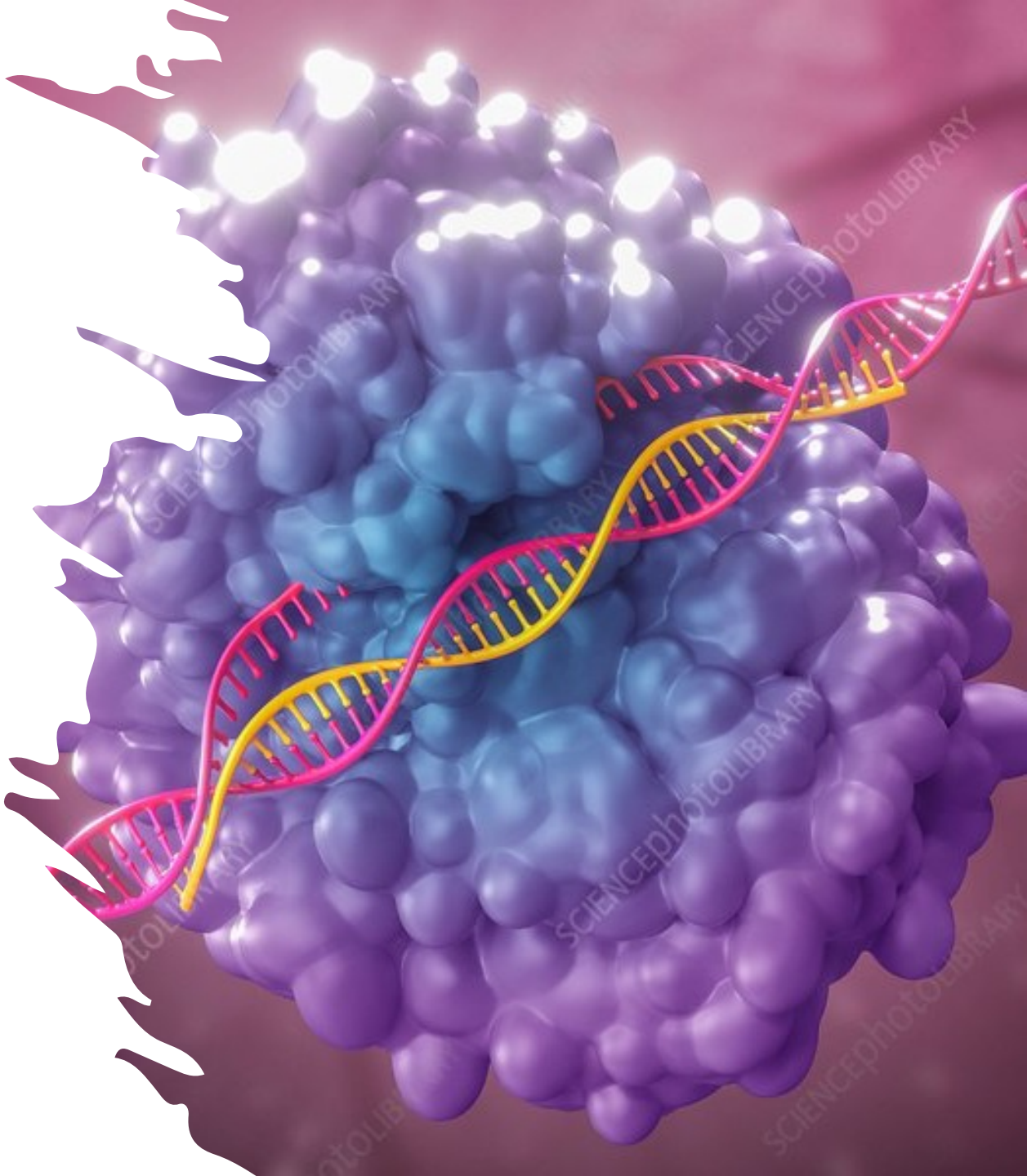
Presentation And Discussion Of The Paper:

Engineering herbicide-resistant watermelon variety through CRISPR/ Cas9-mediated base-editing



By: Kawthar Akbar

29th Jun, 2021

Outline

- The paper
- Aim of the study
- The CRISPR/cas9 system
- CRISPR/cas9 Base Editing
- CRISPR/cas9 delivery into plants
- Methods
- Results
- Discussion
- Conclusions

The paper

Plant Cell Reports
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FOCUS ARTICLE

Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing

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Keywords Base-editing · Herbicide-resistant watermelon · Transgene-free

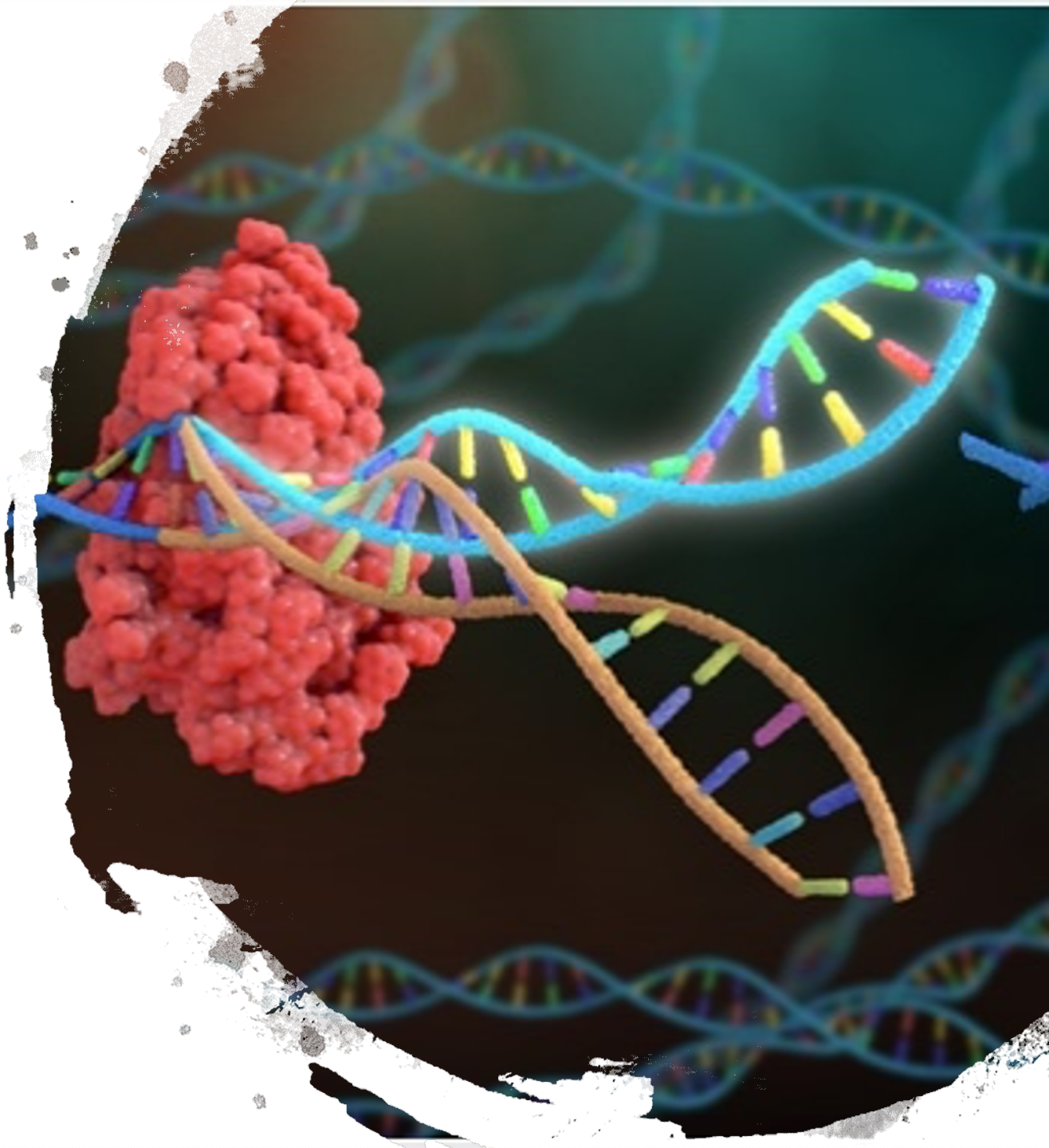
Aim of the study

Utilizing CRISPR/ Cas9 base editing to produce herbicide-resistant-watermelon (broadleaved weed)

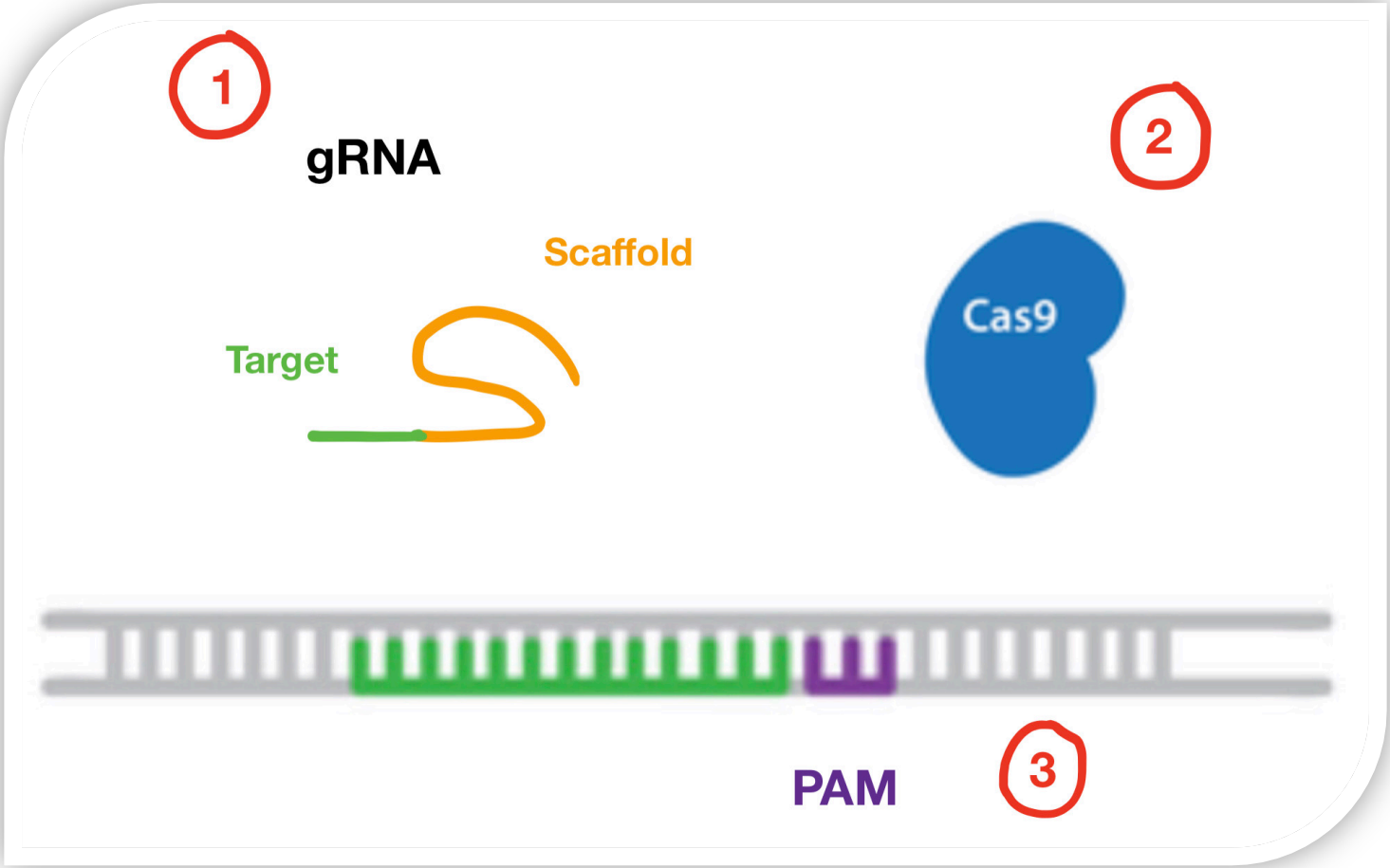
Broadleaved weed

Previous studies

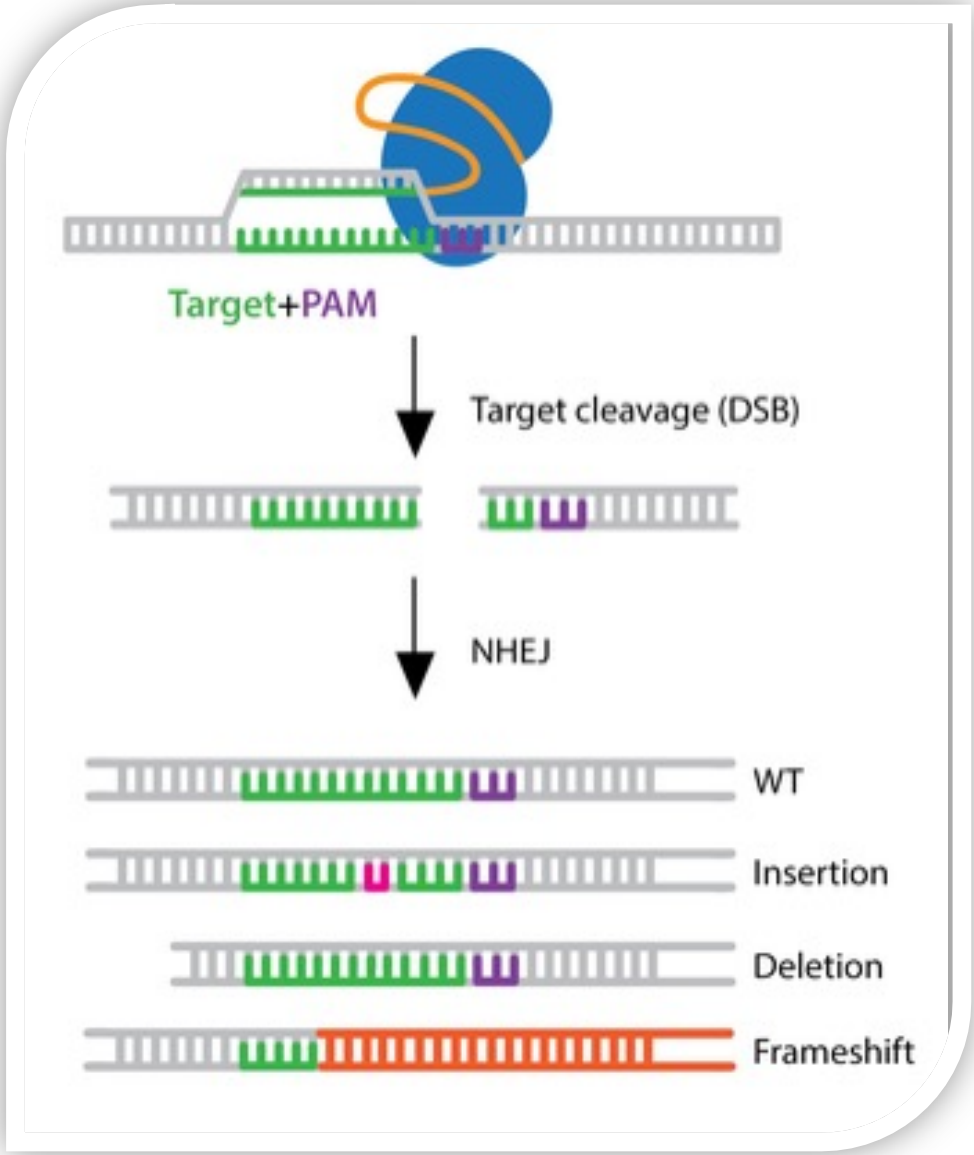
CRISPR/cas9 system



CRISPR/cas9 System Components



Non-Homologous End Joining



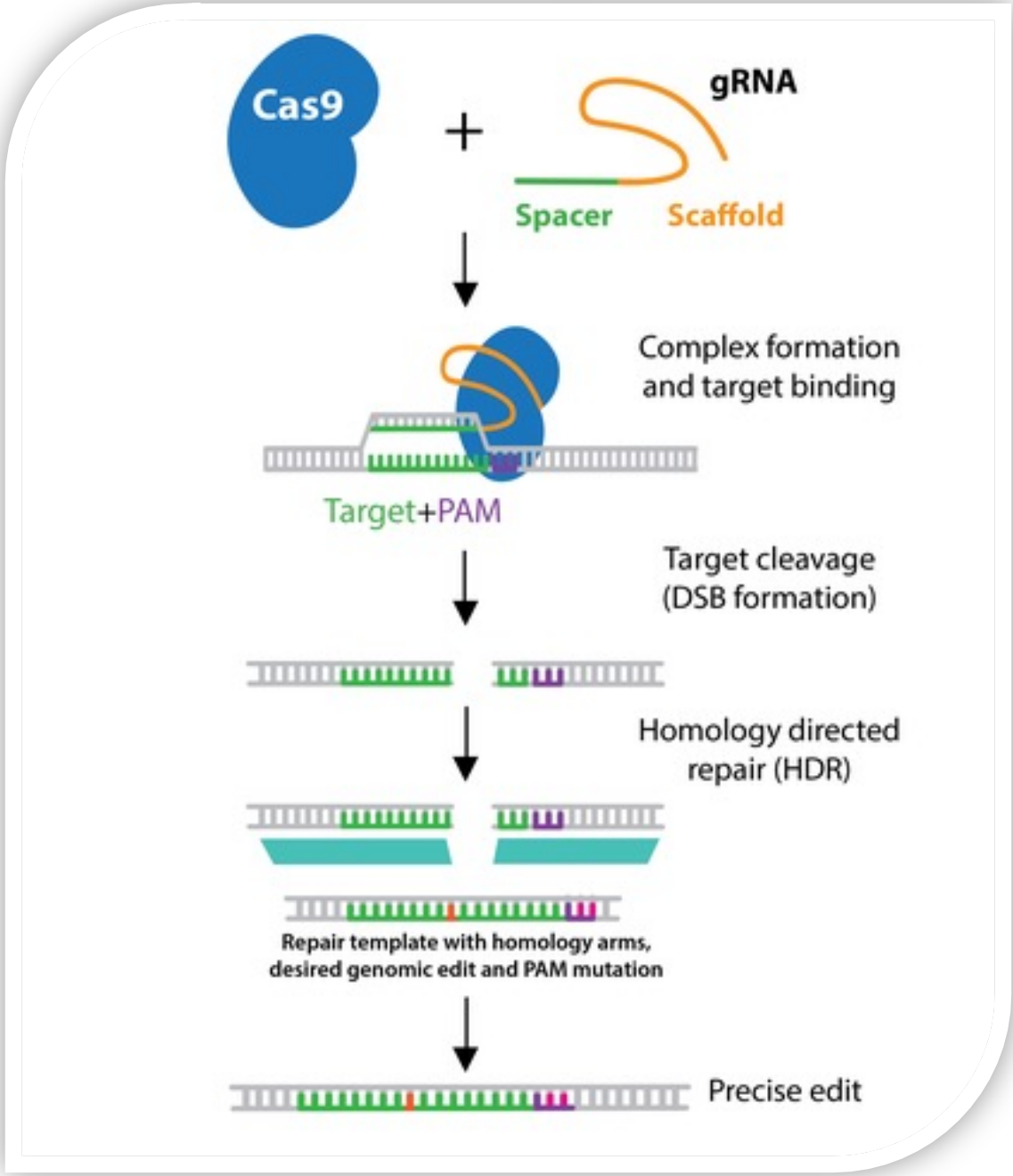
Homology Directed Repair

Limitations

Difficulties delivering

sufficient template DNA

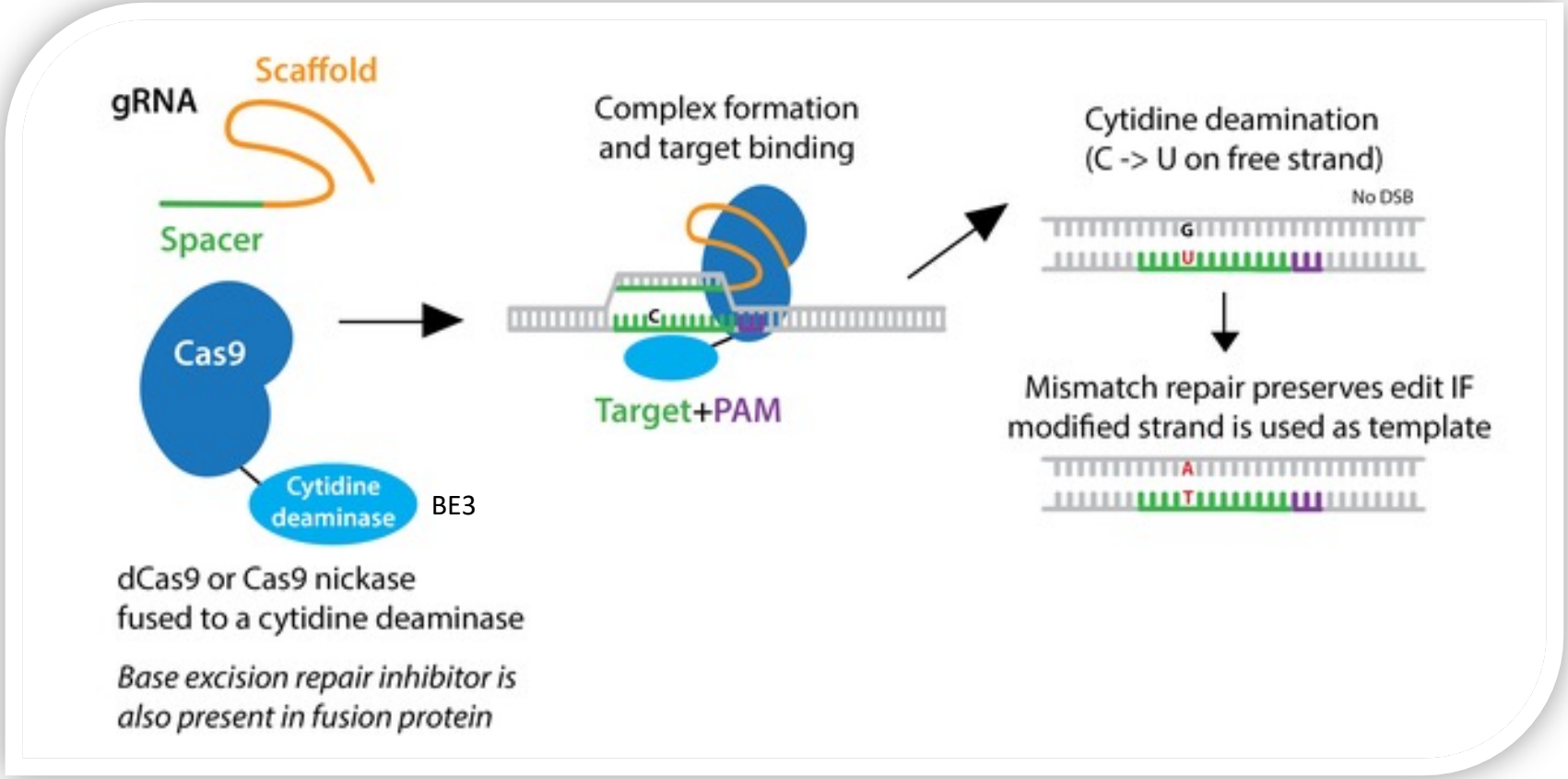
when repairing the double strand break in homology directed repair



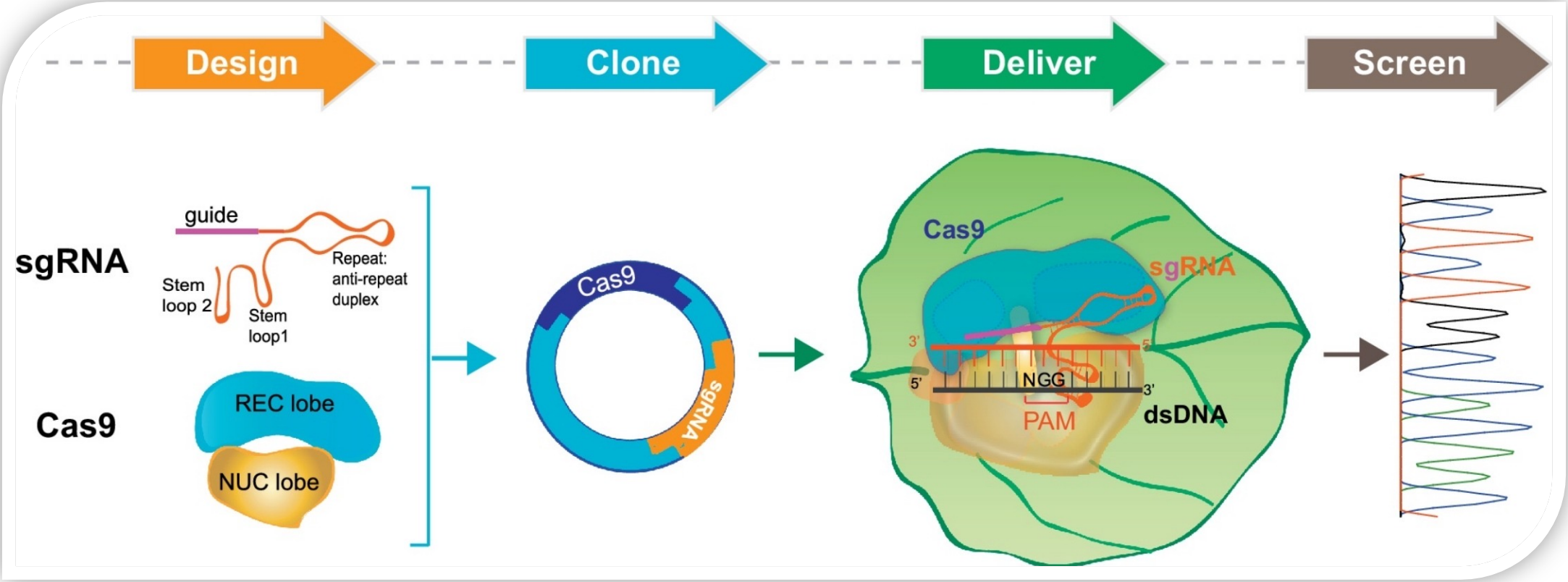
CRISPR/cas9 Base Editing

Cystosine Base Editors (CBEs) C to T

adenine Base Editors (ABEs) A to G



CRISPR/cas9 delivery into plants



Methods

The Target Gene

Watermelon Acetolactate Synthase gene
CIALS

↓

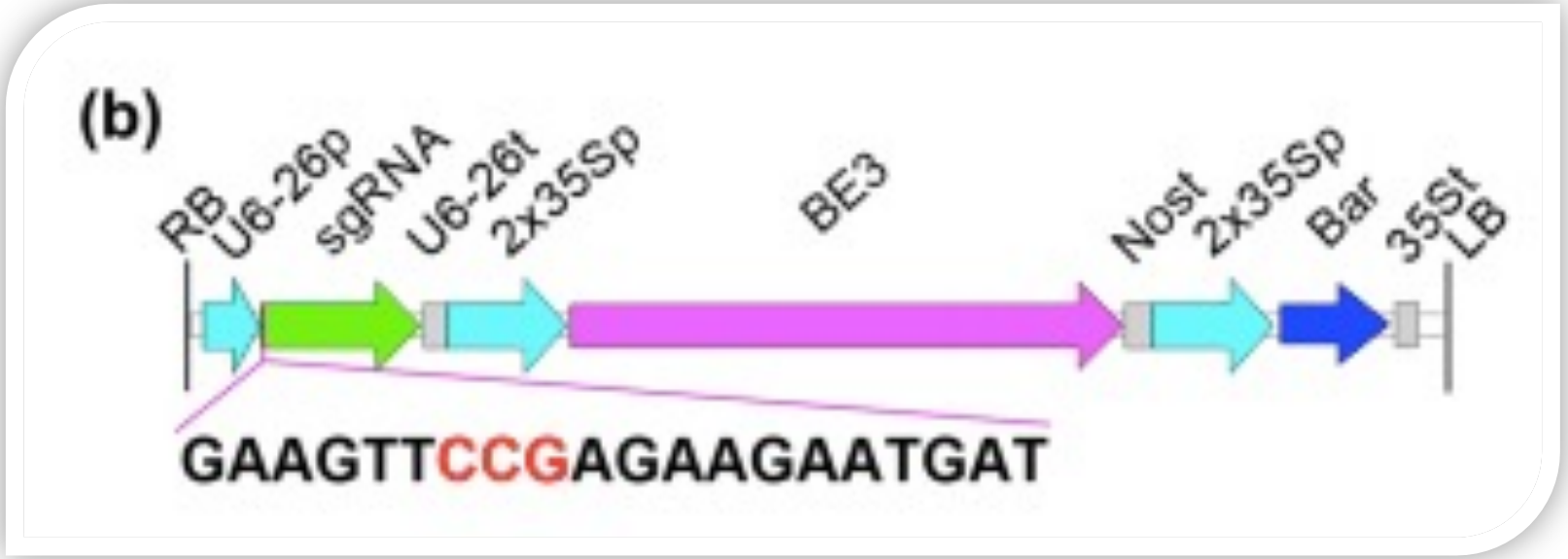
Point mutation
C to T In Pro190 (CCG)

←

Herbicide resistance

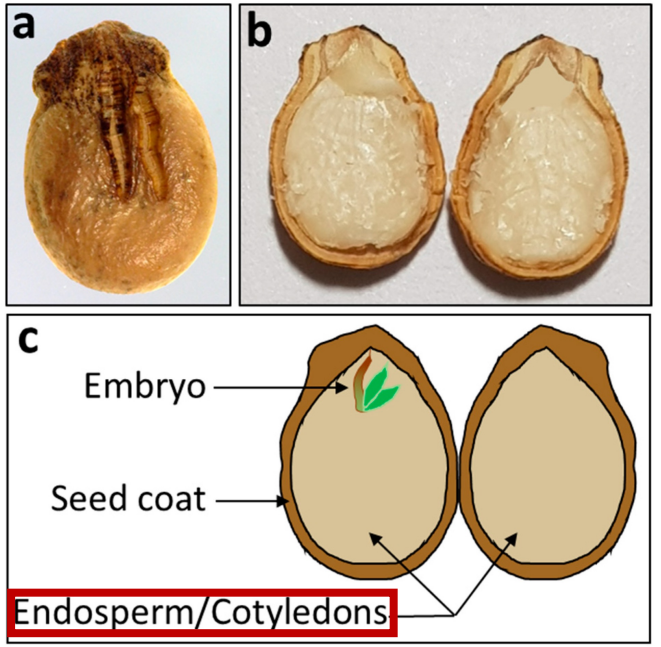
Transformation vector

pBSE901



Agrobacterium transformation

EHA105



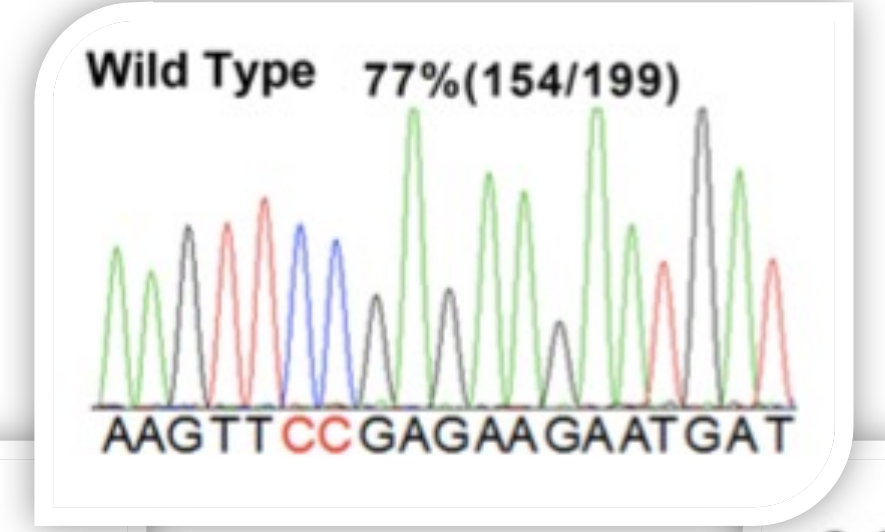
Watermelon
ZG94

Results

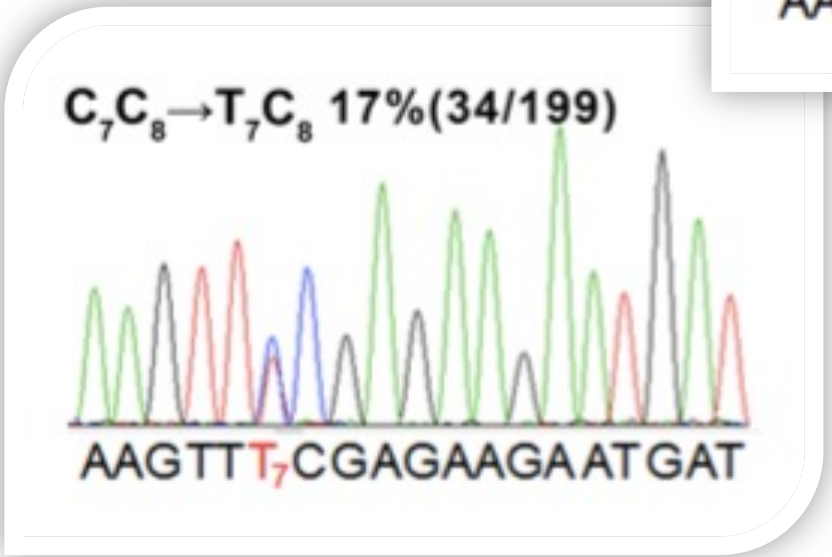
PCR

all candidate plants contained genes of *BE3* and *gRNA*

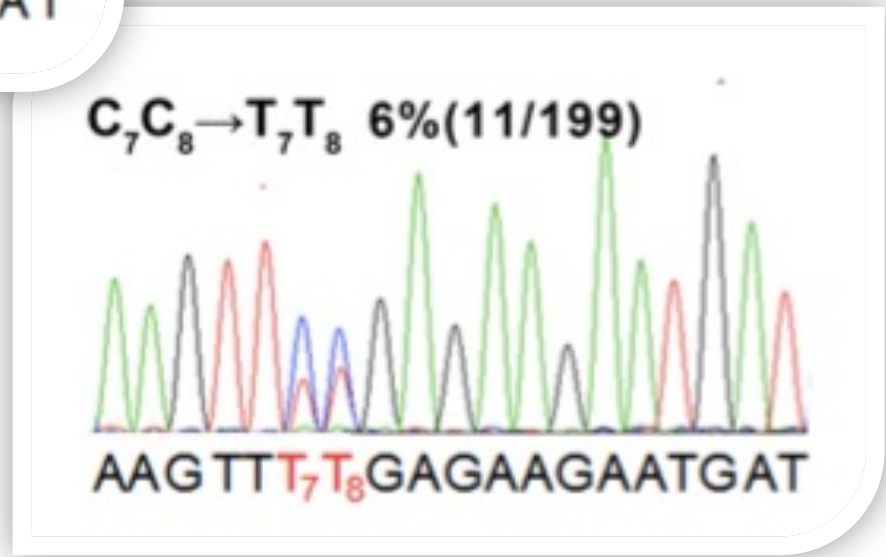
Sanger Sequencing (T0)



P190S



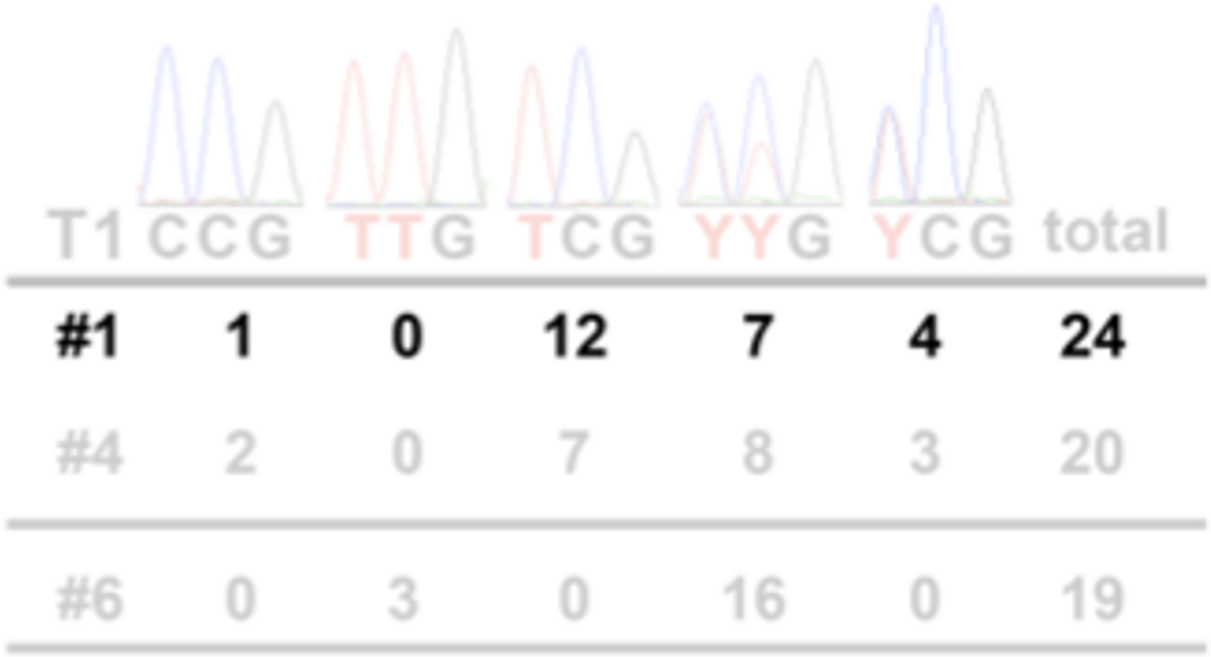
P190L



Inheritance

T0: CCG to TCG

Wild	4% (1/24)
Homozygous	50% (12/24)
Hetrozygous	46% (11/24)

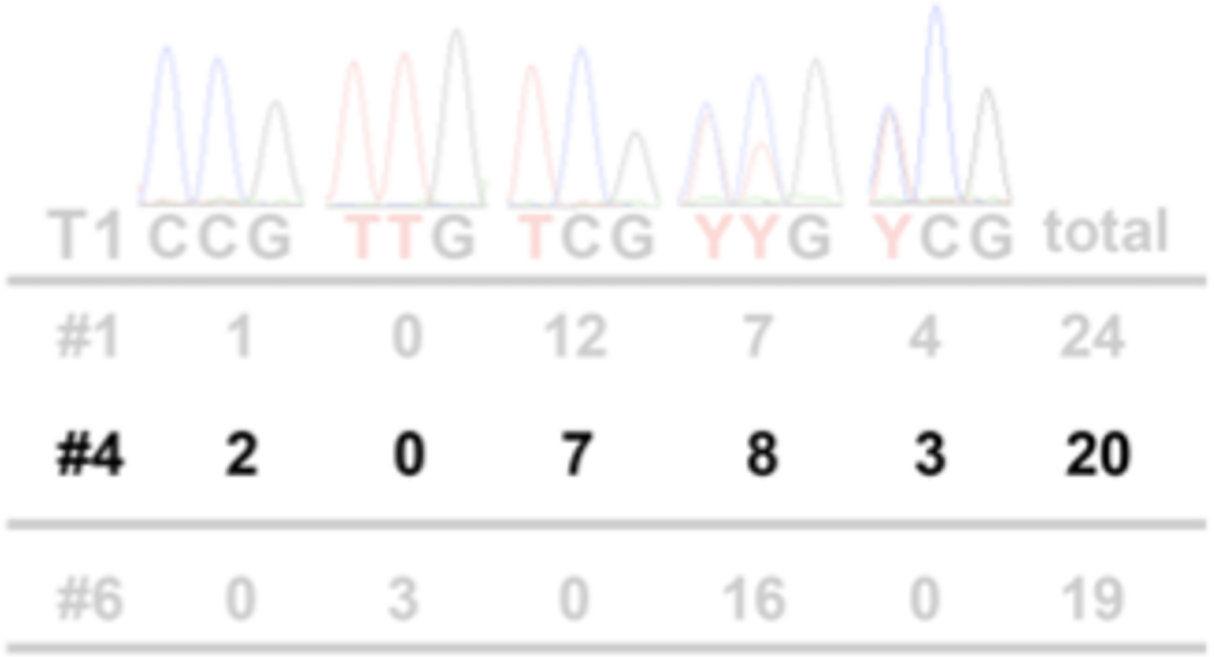


T1	CCG	TTG	TCG	YYG	YCG	total
#1	**1**	**0**	**12**	**7**	**4**	**24**
#4	2	0	7	8	3	20
#6	0	3	0	16	0	19

Inheritance

T0: CCG to TCG

Wild	10% (2/20)
Homozygous	35% (7/20)
Hetrozygous	55% (11/20)



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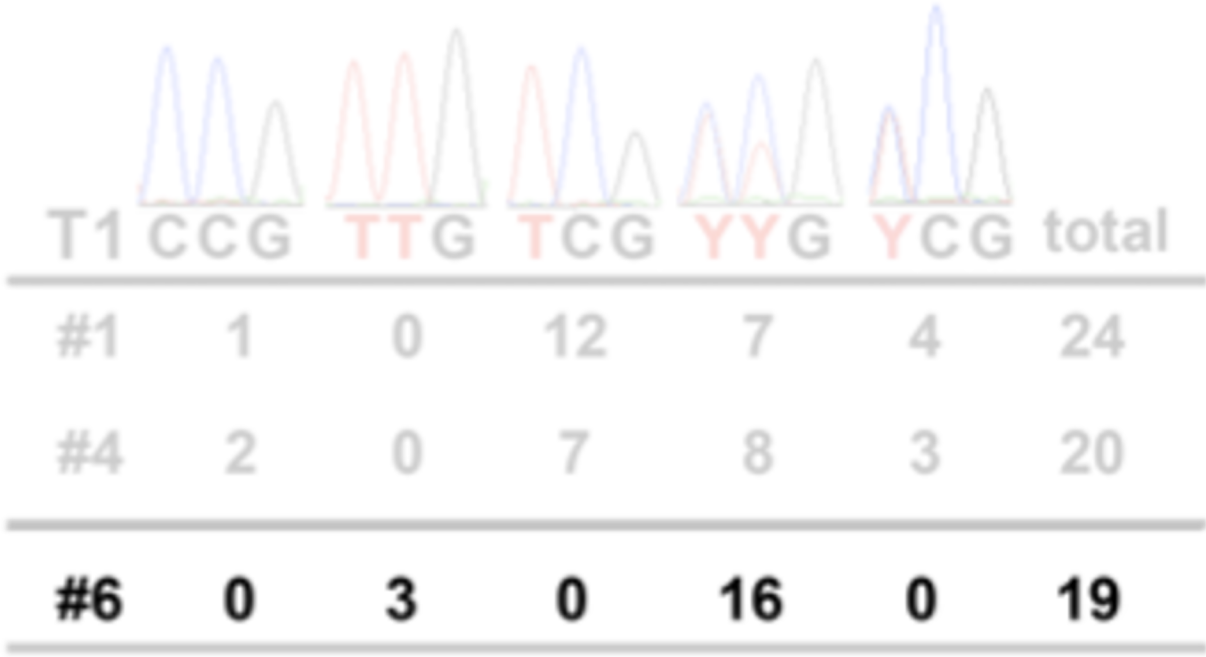
#1	1	0	12	7	4	24
#4	**2**	**0**	**7**	**8**	**3**	**20**
#6	0	3	0	16	0	19

Inheritance

T0: CCG to TTG

Wild	null
Homozygous	16% (3/19)
Hetrozygous	84% (16/19)



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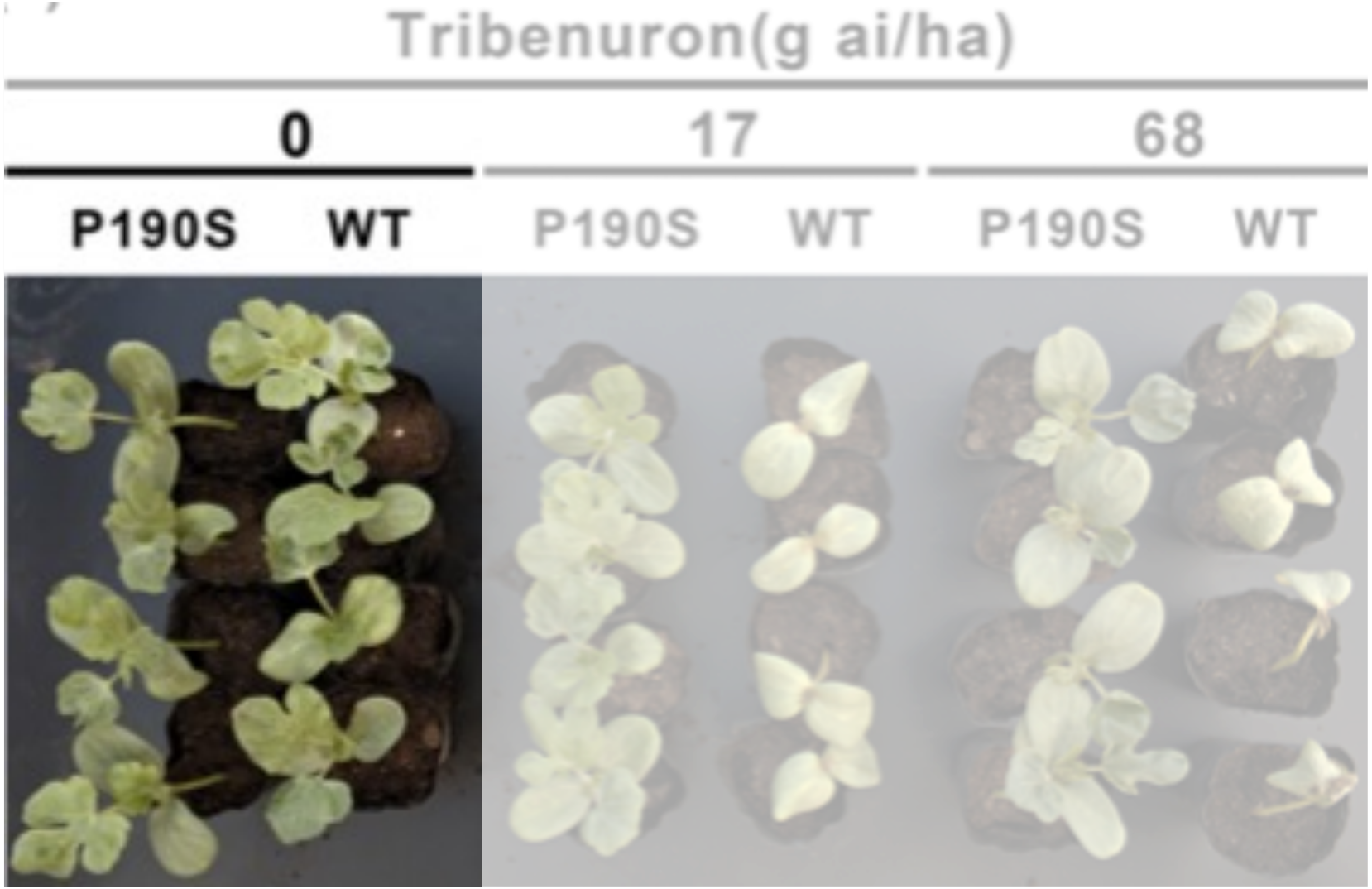
Herbicide Resistance

14 days after herbicide treatment



Herbicide Resistance

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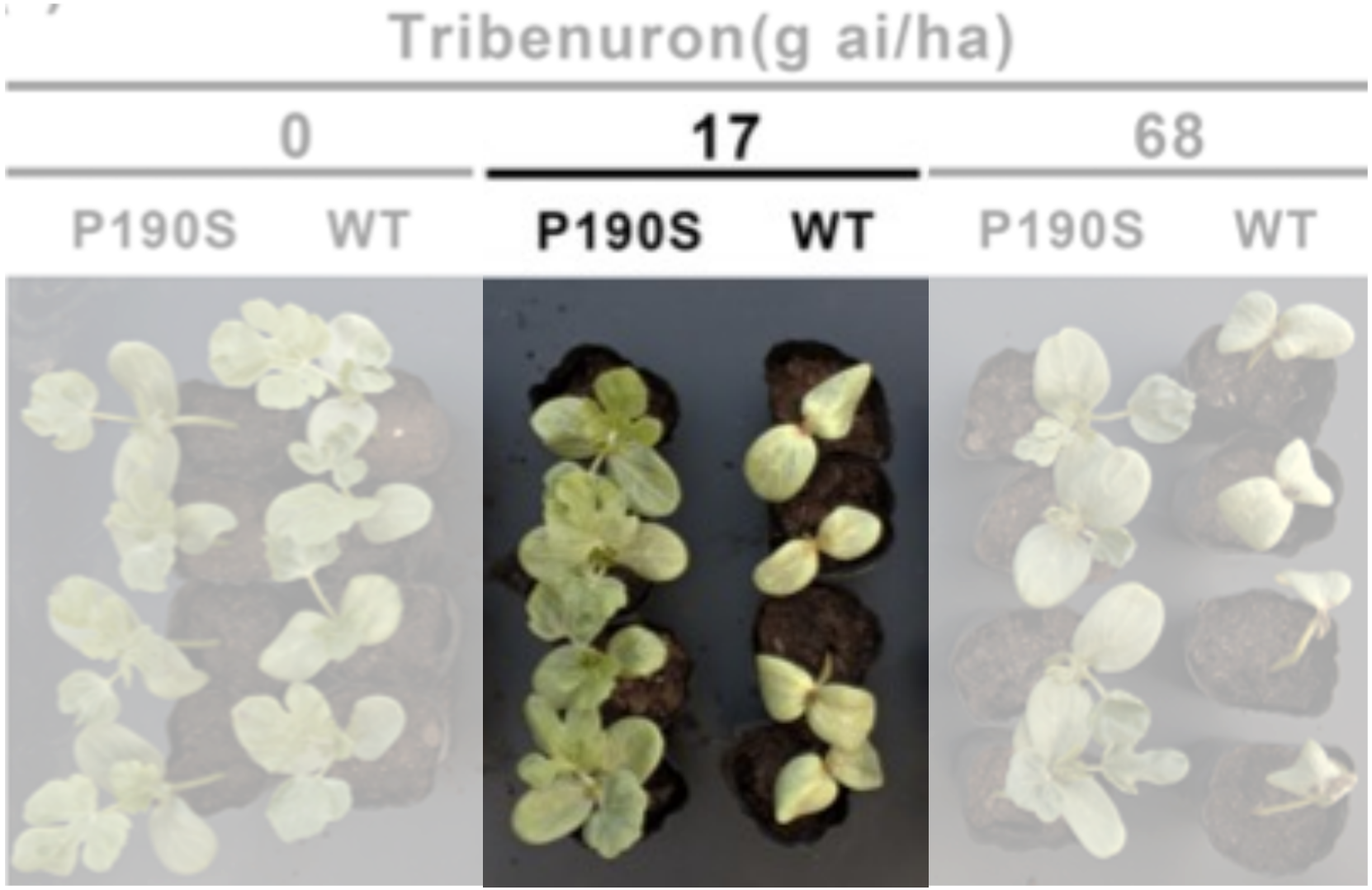

Herbicide Resistance

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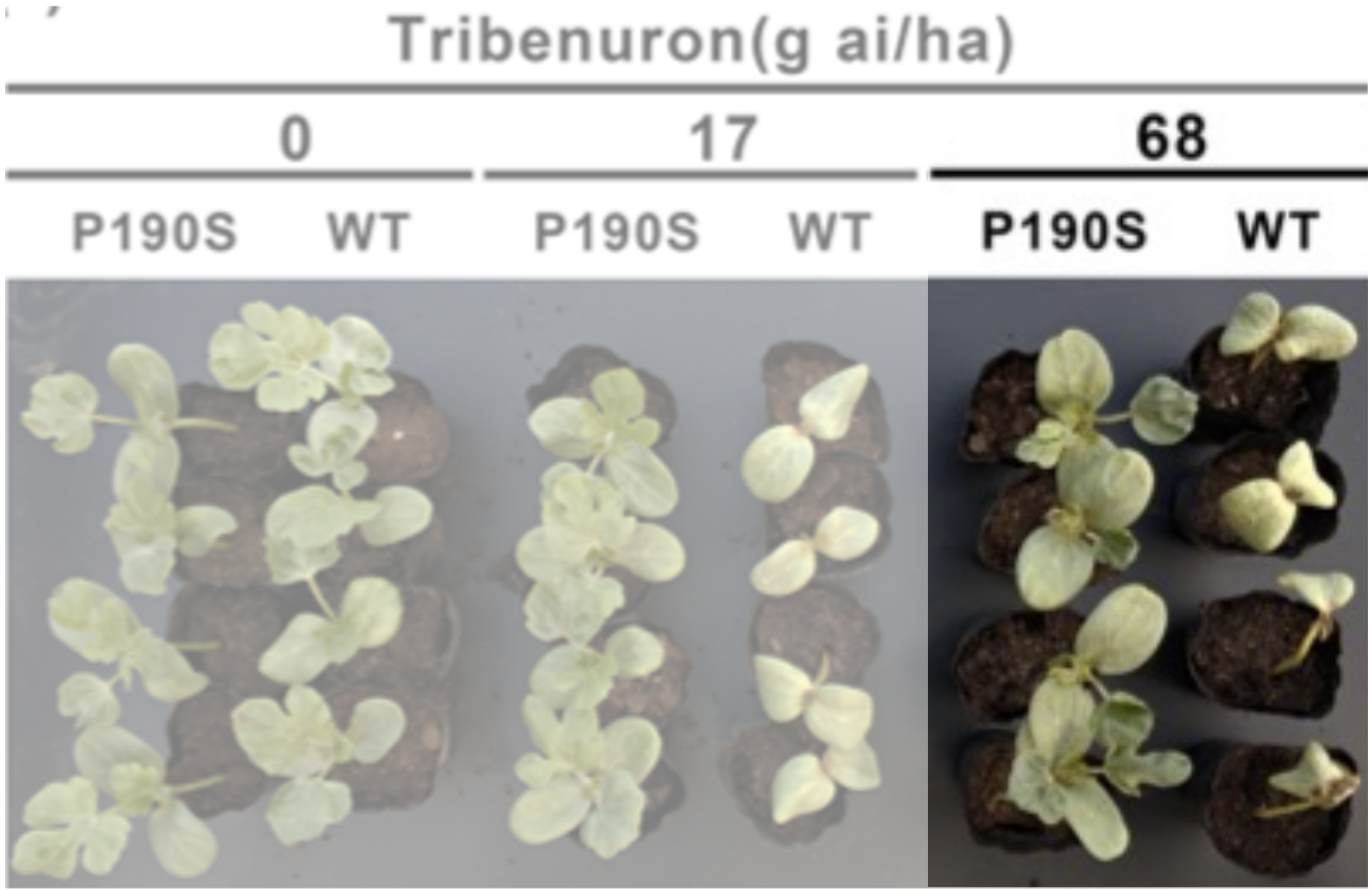
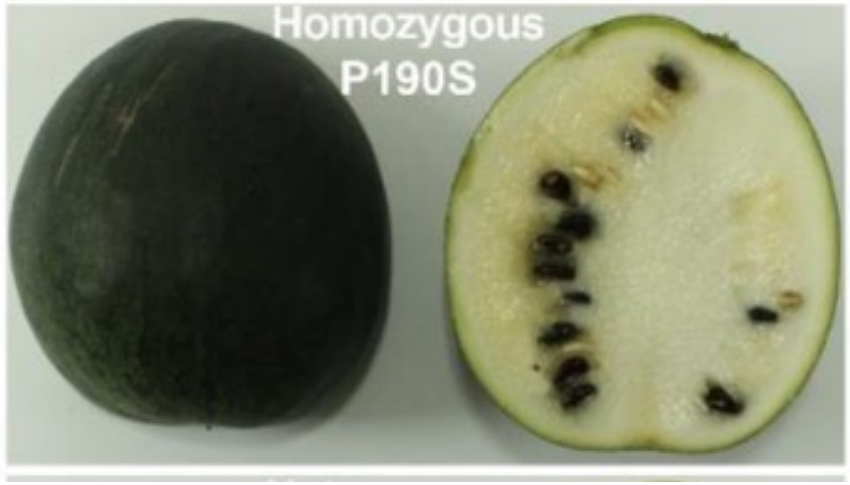


Discussion

What do you think the problems with this technique would be ?

Properties of the base edited watermelon

Fruit size and colour did not change



Properties of the base edited watermelon

	[a]Seeds Number	[b]Seeds Length(cm)	[c]Seeds Weight(g)
Wild type	205±7	10.37±0.12	2.31±0.04
Homozygous	213±4.5	10.16±0.11	2.33±0.03
Heterozygous	209±4.7	10.33±0.15	2.30±0.02

The seed number per fruit, seed length and weight per 10 seeds
Did not change

The seed size did not change

Off-Target potential

Whole genome search

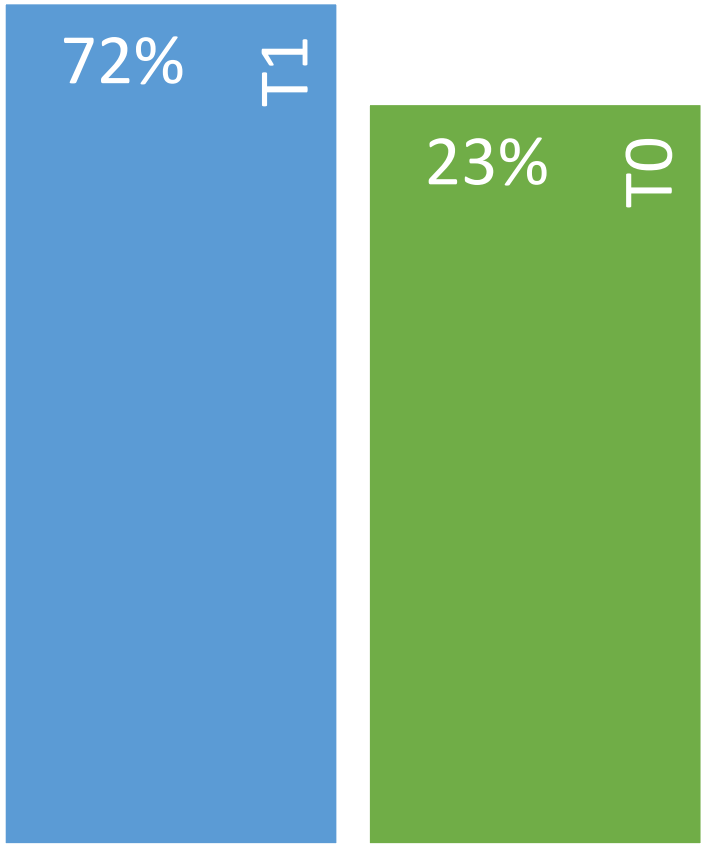
5 locations with mismatches

PCR + Sanger sequencing

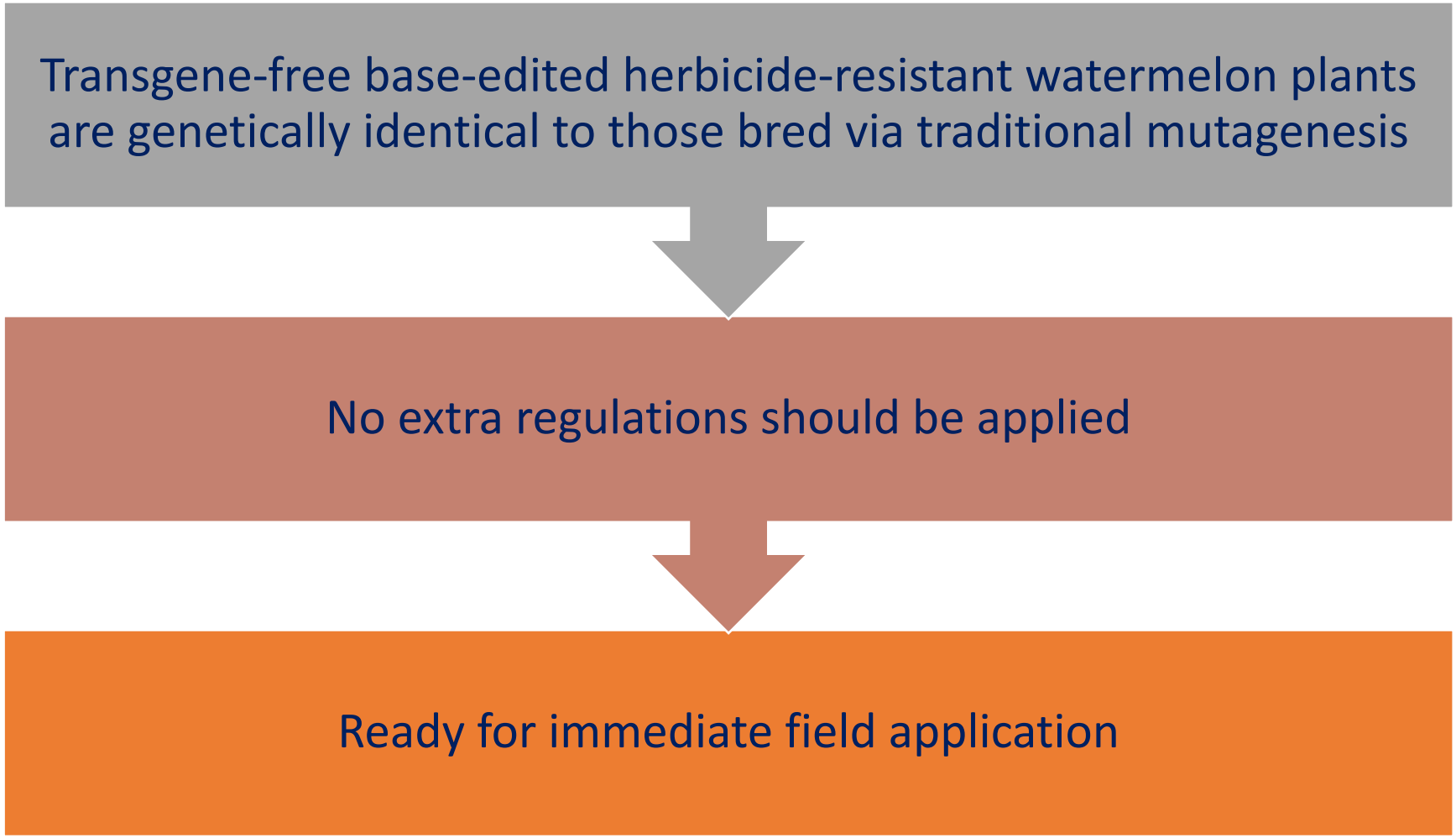
No off-target edits found or indels

Conclusions

System efficiency



Implementation




Thank you
for
listening