5TH INTERNATIONAL
STRAWBERRY CONGRESS

WELCOME

Connecting the dots in a complex world of strawberries

Antwerp – Belgium

17 – 20 September 2025

5TH INTERNATIONAL STRAWBERRY CONGRESS

Wednesday

Parallel Session 1: Strawberry Breeding & Physiology

Genome editing of Fra a 1.01 to reduce allergen in strawberry

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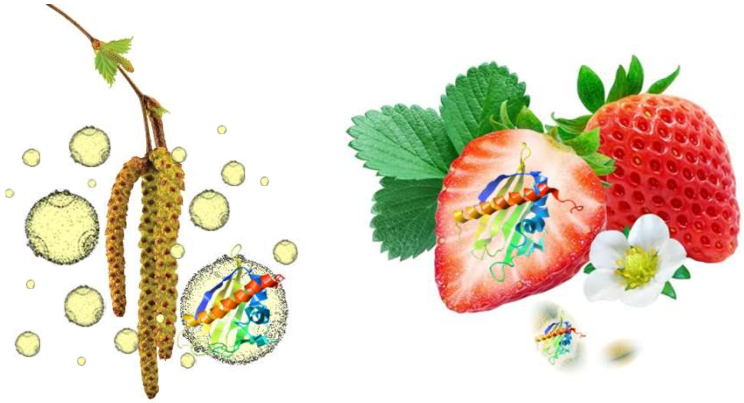
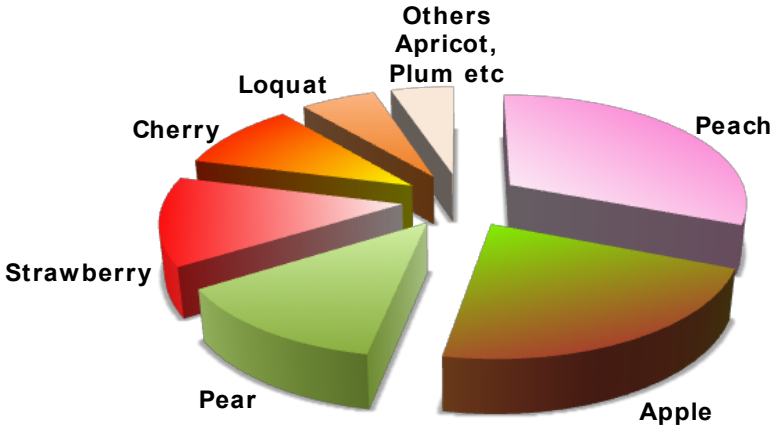
Background



Bet v 1 (birch)　　Fra a 1.01

Strawberry Allergy

- Cross-reactivity with birch pollen allergen Bet v 1
- Oral allergy syndrome: swelling and itching of the oral mucosa
- Strawberry pollinosis in farmers (Kobayashi et al.,1973)



(Ono et al., 2007)

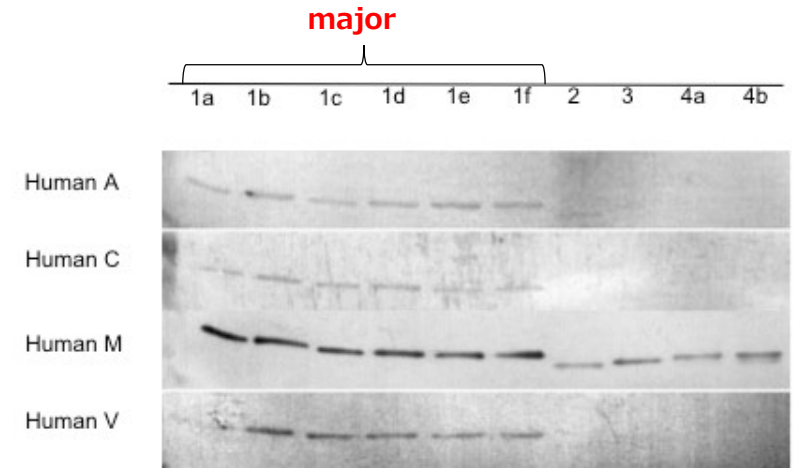
Rosaceae fruits allergy

- Strawberry OAS patients ranks third among Rosaceae fruits
- Estimated strawberry OAS is 1%<
- General pollinosis: 0.42million(1996)→0.63(2014)

Background

class	function	cluster		paralogs
Fra a 1	PR10	Fra a	1.01	9
			1.02	11
			1.03	4
			Others	9
Fra a 3	nsLTP1			
Fra a 4	Profilin			

(Who/IUIS, Allergen Nomenclature; Ishibashi et al., 2018)



Strawberry Allergen

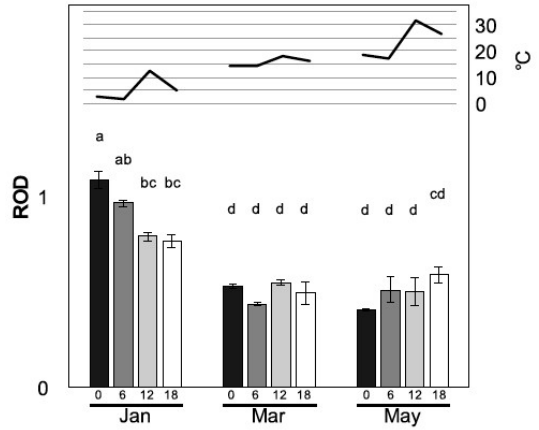
- Fra a 1 is a major allergen of strawberry
- At least 33 paralogs encode the Fra a 1 family protein

Binding to IgE in patient sera

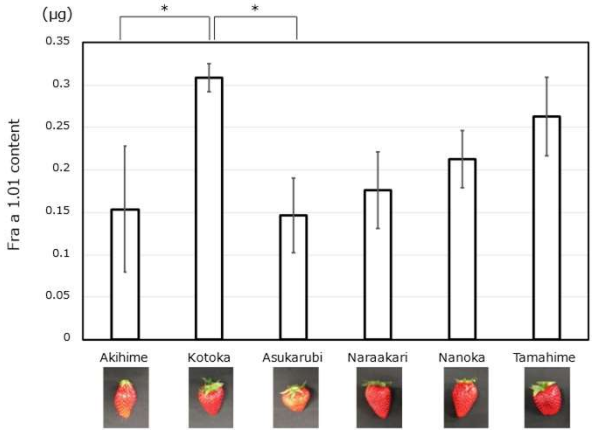
- Fra a 1.01 binds to sera from pollinosis patient, indicating major paralogs

(Ishibashi et al., 2018)

Background



(Ishibashi et al., 2023)



(Tatei et al., 2024)

Environmental response

- Fra a 1.01 contents were increased by cold environments
- Reduced in warm conditions, but still remains

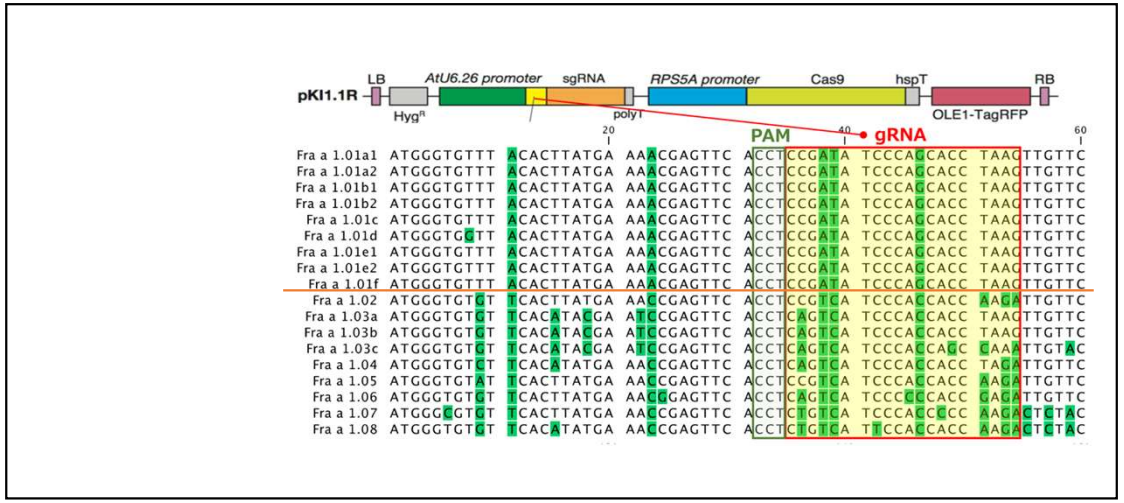
Breeding is better way than cultivate condition to reduce allergen.

Varietal difference in Fra a 1

- There are not Fra a 1-defective breeding materials

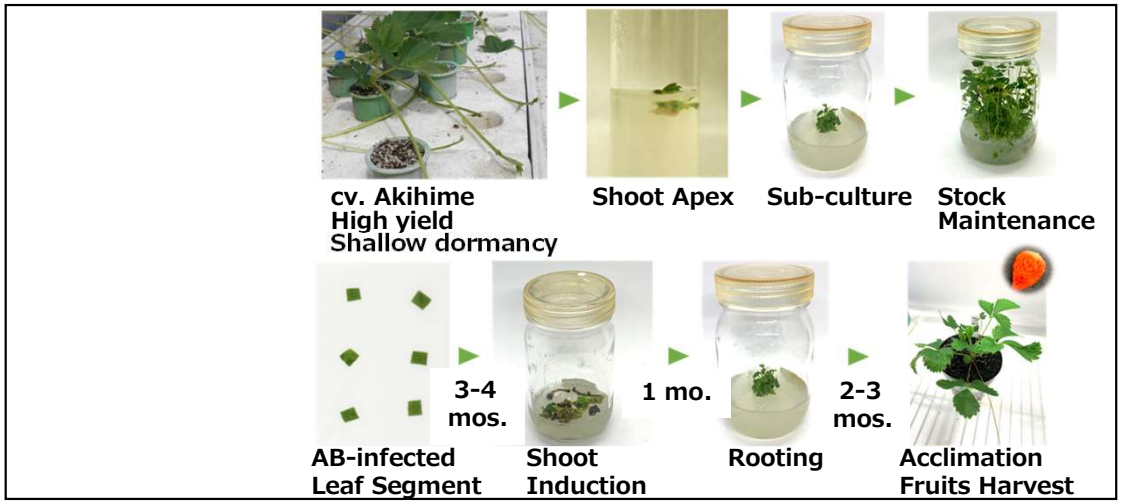
It is hard to reduce allergen by cross breeding.

Materials and Methods



Construction of the vector

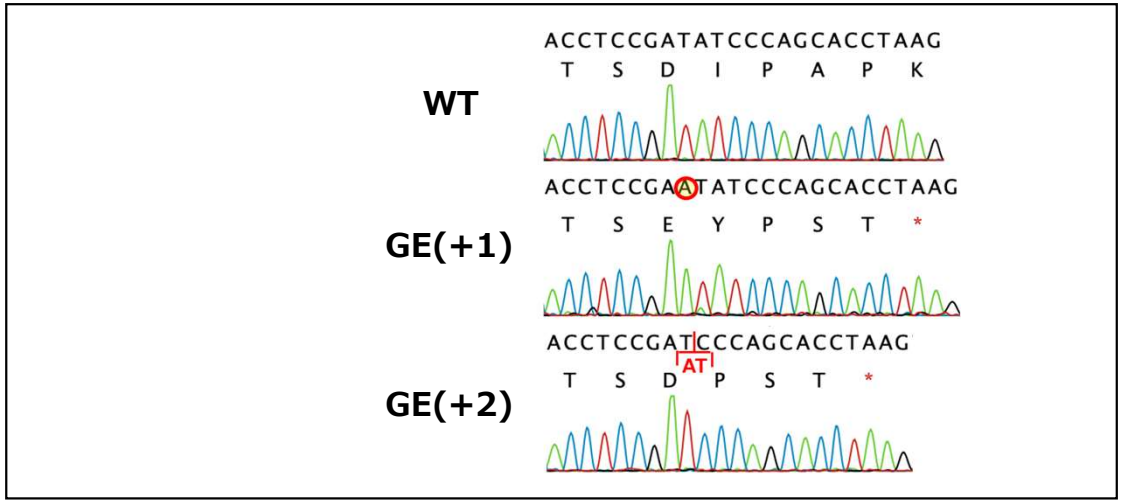
- CRISPR /Cas9 vector, pKI1.1R (Tsutsui and Higashiyama, 2017)
- Targeting the common sequence of Fra a 1.01



Transformation of strawberry

- Six times infection by *Agrobacterium*.
- Screening with antibiotics
- 8 lines were selected by PCR

Editing of Target Sequence



Verification of target mutations

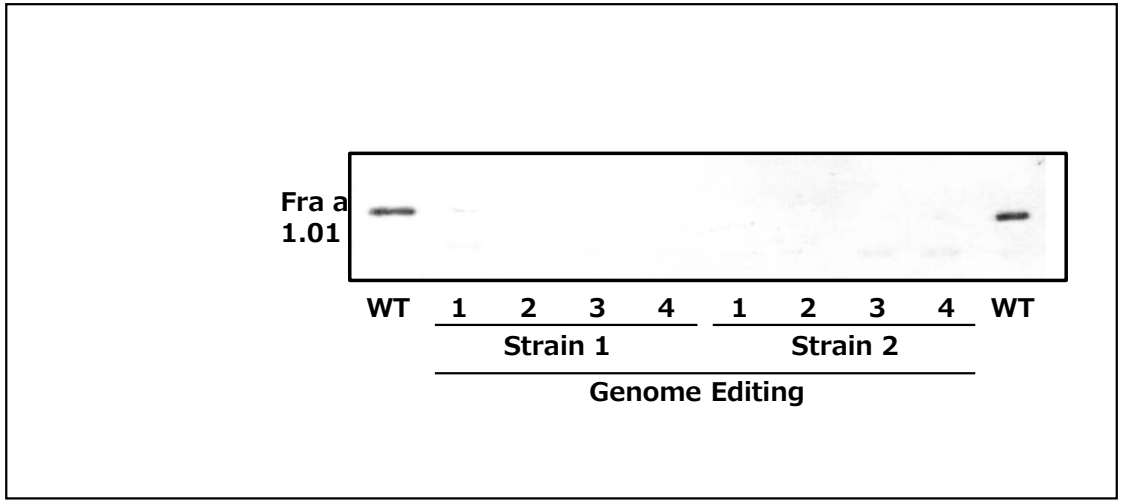
- PCR / Sanger-sequencing from two lines

Line	Paralog	Target (PAM+gRNA)
wt	Fra a 1.01	CCTCCGATATCCCAGCACCTAAG
CR1-20-1	e2	CCTCCGAATATCCCAGCACCTAAG
	b1	CCTCCGAATATCCCAGCACCTAAG
		CCTCCGA(+148)TATCCCAGCACCTAAG
	c1	CCTCCGAATATCCCAGCACCTAAG
		CCTCCGA(–2)ATCCCAGCACCTAAG
		CCTCCGA(+148)TATCCCAGCACCTAAG
	f1	CCTCCGA(–2)ATCCCAGCACCTAAG
	g	CCTCCGAATATCCCAGCACCTAAG
CR1-10-1	b1	CCTCCGAATATCCCAGCACCTAAG
		CCTCCGA(–3)CCCAGCACCTAAG
	c1	CCTCCGAATATCCCAGCACCTAAG
		CCTCCGA(–3)CCCAGCACCTAAG
	e1	CCTCC (–4)TCCCAGCACCTAAG
	e2	CCTCCGAATATCCCAGCACCTAAG
		CCTCCG(–17)
	g	CCTCC (–4)TCCCAGCACCTAAG
		CCTCCGAATATCCCAGCACCTAAG

Variation in target mutations

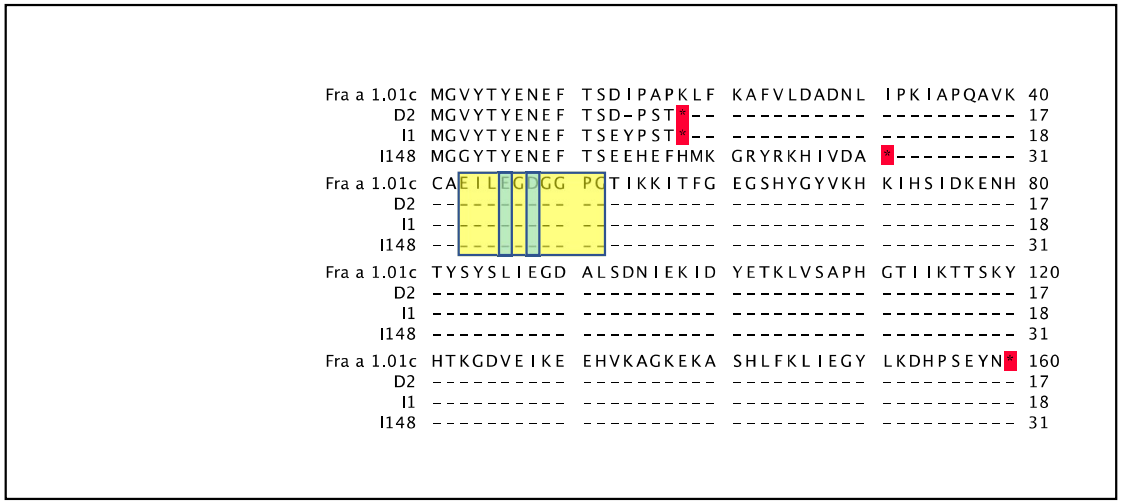
- Insertion / deletion of 1-148 bases were detected
- At least 5-6 paralogs had premature stop codons

Confirmation of Fra a 1.01 suppression



Immunoblot using anti-Fra a 1 antibody

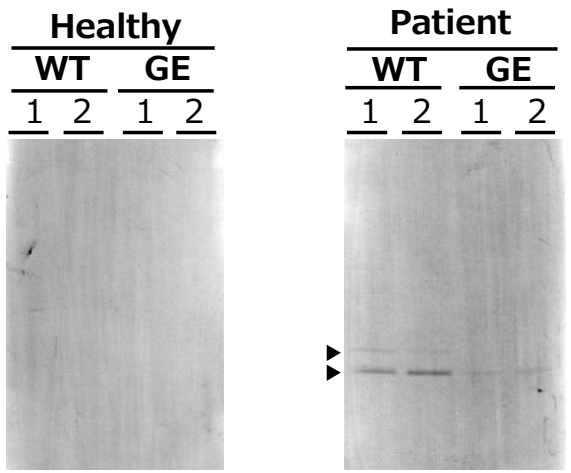
- Fra a 1.01 decreased to undetectable levels by genome-editing



Editing site and epitope position

- The 42-52 amino acid region of birch Bet v 1 is important for antibody binding (Mirza et al., 2000)
- In Fra a 1.02, Glu46 and Asp48 affect antibody binding (Orozco-Navarrete et al., 2020)

Verification of low allergenicity by human sera



	sera samples	Fra a 1.01 Positive	
		Wild type	GE
Healthy	10	0	0
Patients	22	5	0

Immunoblot by human sera

- The patient group with strawberry OAS or birch pollen allergy (self-proclaimed)
- The reactivity of Fra a 1.01 to patient IgE in wild type was reduced in two genome-edited lines

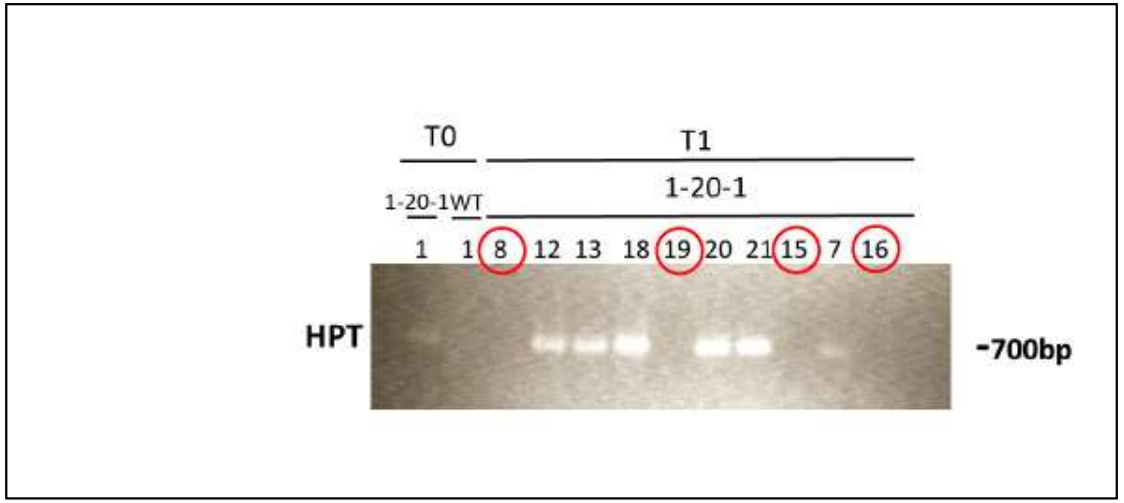
(allowed by Ethics Committee of KOBE-U)

Results summary

- Among 22 patients, 5 had specific IgE react with Fra a 1.01 in WT, but not in GE

Still in the developmental stage, genome editing of Fra a 1.01 has contributed to the breeding of a hypoallergenic strawberry.

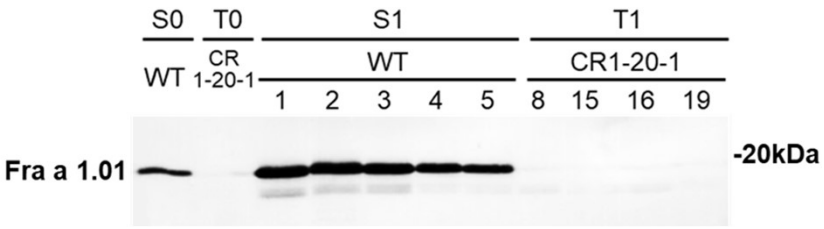
Obtaining next-generation



T_1 generation

- 403 seeds
- 21 stocks were grown
- 4 null segregants were screened by PCR, indicating without foreign gene.

Line	Paralog	Target(PAM+gRNA)
WT		CCTCCGATATCCCAGCACCTAAG
T1CR1-20-1-15	a	CCTCCGAATATCCCAGCACCTAAG
	b	CCTCCGAATATCCCAGCACCTAAG
	c	CCTCCGAATATCCCAGCACCTAAG
	e	CCTCCGAATATCCCAGCACCTAAG
	f	CCTCCGA(-2)TCCCAGCACCTAAG



Null segregant candidates

- Sequencing and immunoblot confirms that edited sequences were inherited by the next generation

Roles of PR-10 type allergens in plants

- pathogenesis-related (PR) proteins
- response to abiotic stress; drought, cold, or UV
- hydrophobic pocket binding to various ligands:

  Quercetin-3-O-glucuronide

  Naringenin

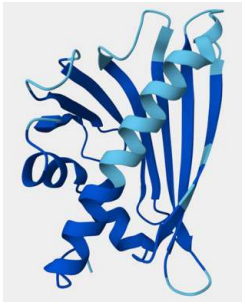
  Cytokinins

  Brassinosteroids

  Tryptamine

  Alkaloids

  Fatty acids

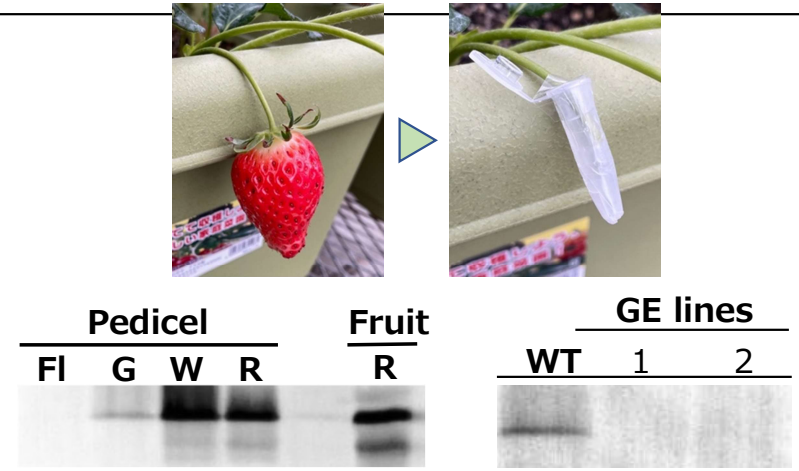


allergen

worthless

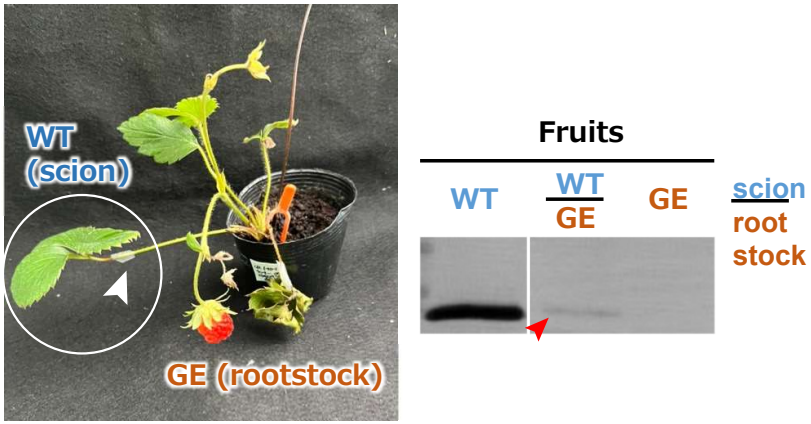
beneficial ?

Roles of allergens in plants



Allergen in pedicel exudate

- Fra a 1.01 were found in pedicel at fruit maturity stages
- Transporting ligand compounds essential for fruit development and ripening



Verification by grafting

- Fra a 1.01 was detected in genome-edited fruits when WT leaf was grafted.

Allergen moves in plants !

It cannot be evaluated by gene expression levels (PCR is not possible; immuno-detection is possible).

Conclusion and Acknowledgement

Hypoallergenic strawberry was produced by genome editing

- At least 5 paralogs have premature stop codons, leading to the non-detection of the proteins by polyclonal antibodies.
- IgE reactivity decreased in patients with strawberry allergy.
- Four null segregants were obtained, and many edited sequences were maintained in them.

We are grateful to (under) graduate students for their technical assistance. This work was supported by JST Adaptable and Seamless Technology transfer Program through Target driven R&D (A-STEP) Grant Number JPMJTM22DJ, and partially supported by JSPS KAKENHI Grant Number 23H02204.

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Wednesday

Networking Break

- Sponsors & Exhibitors Fair
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