Part I. Case examples showing contribution of genome editing

10:50-11:20 Japanese efforts on applying genome editing technologies for agriculture, forestry and fisheries in SIP program

Dr. Hiroshi Ezura, Professor, University of Tsukuba, Director of Tsukuba Plant Innovation Research Center, Representative of SIP, NBT Genome Editing Breeding Consortium, Japan

Japanese efforts on applying genome editing technologies for agriculture, forestry and fisheries in SIP program

Hiroshi Ezura
Tsukuba Plant Innovation Research Center, University of Tsukuba
Tennodai 1-1-1, Tsukuba 305-8572, Ibaraki, Japan.
Email: ezura.hiroshi.fa@u.tsukuba.ac.jp

In the last decade, we have made remarkable progress in genome editing technologies which allow us to induce mutations into target genes of interest. It is an innovation of mutation breeding, and will contribute to establish a global food security. On the basis of such progress, Japanese government has created a novel project named as “Establishment of new breeding techniques”, leaded by this presenter, in which we are applying genome editing techniques for agriculture, forestry and fisheries under the frame of the Cross-ministerial Strategic Innovation Promotion Program (SIP). The project includes four research groups, Group1/Next-Generation Breeding Techniques Consortium, Group2/Omics Breeding Technology Consortium, Group3/Genome Editing Breeding Consortium, and Group4/Social Implementation Consortium. The Group1 aims to develop novel genome editing techniques for plants and animals, the Group2 aims to search target genes for genome editing, the Group3 aims to create genome editing crops and fishes which are capable of implement to our society, and the Group4 aims to develop methodologies for social implementation of the genome editing products. So far, the Group3 are trying to create genome editing products including super-yielding rice, high value-added tomato, natural toxin-free potato with health-promoting function and easy-aquafarming tuna fish, which can contribute to establish the next-generation agriculture, forestry and fisheries systems in Japan. In this presentation, I am going to briefly introduce the research activities and current status of application of genome editing techniques in the Group 3 of SIP program, specially focused on tomato genome editing. I also would like to discuss challenges in the implementation of such genome editing products.

筑波大学
University of Tsukuba

Japanese efforts on applying genome editing technologies for agriculture, forestry and fisheries in SIP program

Hiroshi EZURA

Faculty of Life and Environmental Sciences
The University of Tsukuba, Japan
Tsukuba Plant Innovation Research Center,
The University of Tsukuba

2017.07.10@ILSI-WS

筑波大学 University of Tsukuba

Strategy and goal of "establishment of new breeding techniques" SIP program

Improve international competitiveness of agriculture by dramatically increase of productivity

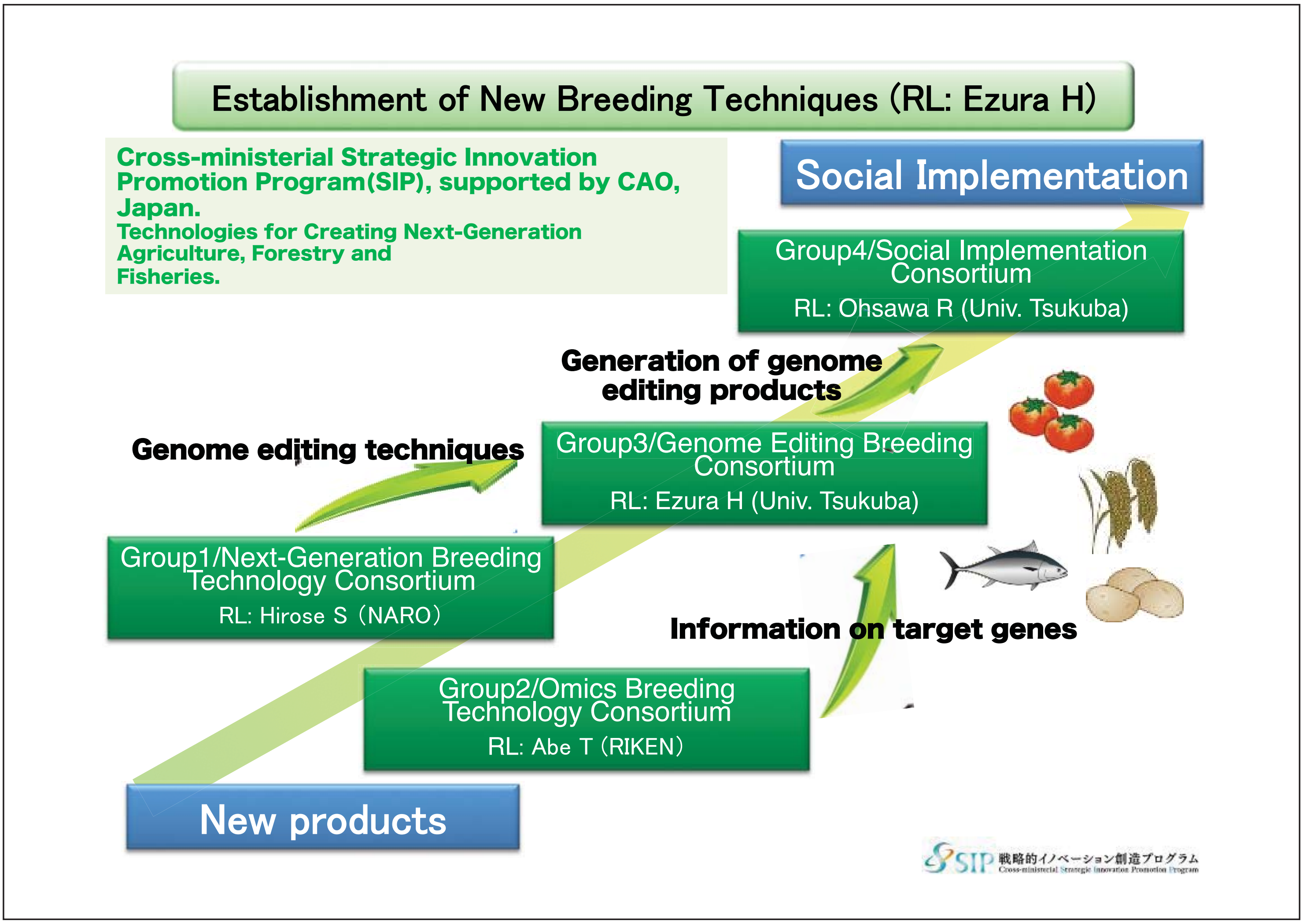
Major policy issue	Action plan of R&D
○Reduce production cost of rice For food → ▲40% For feed → ▲50%	Development of super high-yielding rice（15 t/ha）with advanced ICT-Robotics cultivation practice
○Development of oversea market 2020 → Exports 1 trillion yen 2030 → Exports 5 trillion yen	Development rapid breeding technology for horticultural crops, and new cultivars with advanced traits

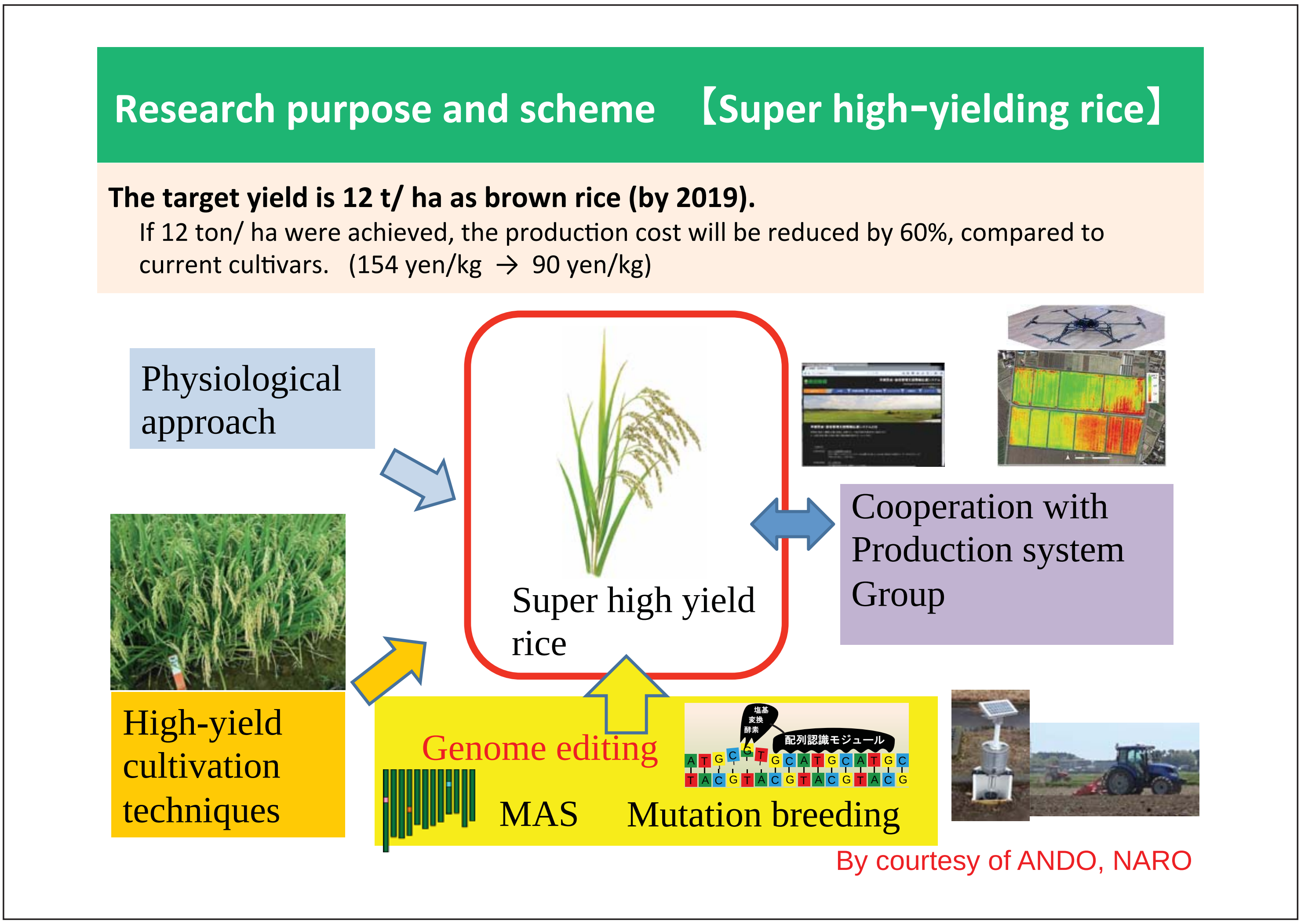
Offer agricultural products for a long healthy life

○Realization of a "society of health and longevity"	Development and supply of agricultural products with health promoting functionality

Sustainable use and stable supply of marine resources

○Sustainable ensure of marine resources such as bluefin tuna and eel	Establishment of advanced aquaculture technology such as development of farm-raised bluefin tuna


Establishment of New Breeding Techniques (RL: Ezura H)
Cross-ministerial Strategic Innovation Promotion Program(SIP), supported by CAO, Japan.
Technologies for Creating Next-Generation Agriculture, Forestry and Fisheries.
Social Implementation
Group4/Social Implementation Consortium
RL: Ohsawa R (Univ. Tsukuba)
Generation of genome editing products
Genome editing techniques
Group3/Genome Editing Breeding Consortium
RL: Ezura H (Univ. Tsukuba)
Group1/Next-Generation Breeding Technology Consortium
RL: Hirose S (NARO)
Information on target genes
Group2/Omics Breeding Technology Consortium
RL: Abe T (RIKEN)
New products
SIP 戦略的イノベーション創造プログラム


Research purpose and scheme 【Super high-yielding rice】
The target yield is 12 t/ ha as brown rice (by 2019).
If 12 ton/ ha were achieved, the production cost will be reduced by 60%, compared to current cultivars. (154 yen/kg → 90 yen/kg)
Physiological approach
Super high yield rice
Cooperation with Production system Group
High-yield cultivation techniques
Genome editing
MAS
Mutation breeding
配列認識モジュール
By courtesy of ANDO, NARO

Research activities 【Super high-yielding rice】

Increase by genome editing technique
・the grain number
・the grain size

Current cultivar (*japonica*) (5 ton/ ha)

Selected line (crossed with *indica* line) (10 ton/ ha)

Genome editing →

Genome editing rice (Green house)

↓

We got null segregants bred by genome editing for the grain number or the grain size using the CRISPR/Cas9 system.

← Selected lines

Field trial (Isolated field in Tsukuba) 2017－2019

By courtesy of ANDO, NARO

Future research plans 【Super high-yielding rice】

1）Field evaluation on the yielding ability of the genome editing rice with enlarged sink size.

2）Development of super-high yield cultivation technology using the parental lines for genome editing.

3）Continuous research on mechanism and genes to improve the translocation and grain-filling ability.

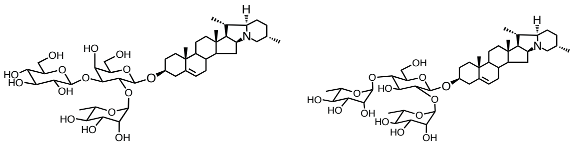
4）Development of growth prediction model for precise management and low cost production

By courtesy of ANDO, NARO

Potato

Breeding goal and its significance

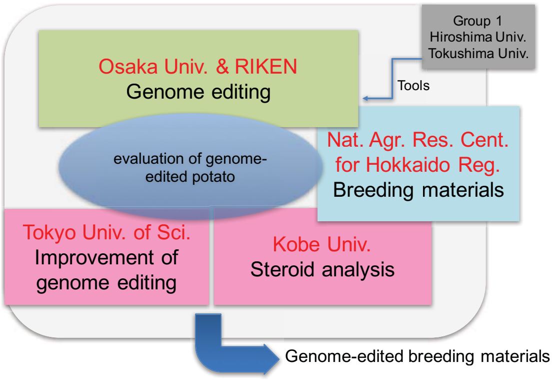
Toxin is abundant (~1% dry wt.) in sprouts and light-induced green tubers.



α-Solanine   α-Chaconine

Steroidal glycoalkaloid (SGA)

- SGA-free potato
  - Safety
  - Cost down for storage and breeding
  - Use of wild germplasms
- SGA-free and functional compound-rich potato
  - Phytosterol
  - Steroidal saponin



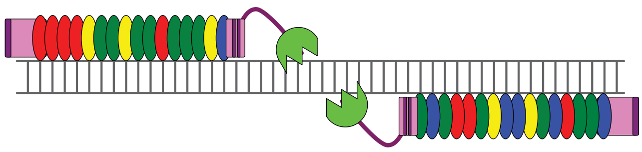
By courtesy of MURANAKA, Osaka Univ

Potato

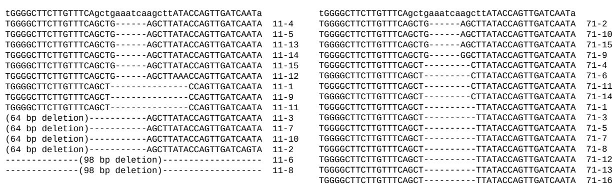
Current status of development

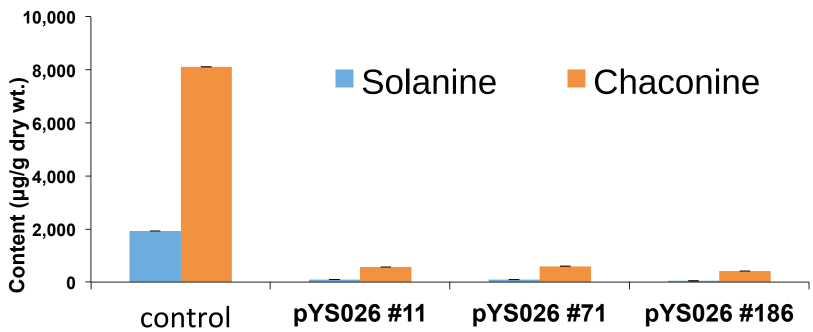
By courtesy of MURANAKA, Osaka Univ

- Platinum TALEN
  - Complete disruption of target gene (*SSR2*) in tetraploid potato
  - Drastic reduction of SGA in KO lines

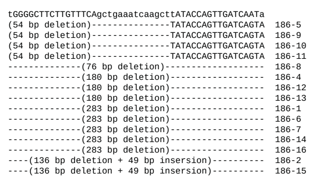


#11   #71





#186



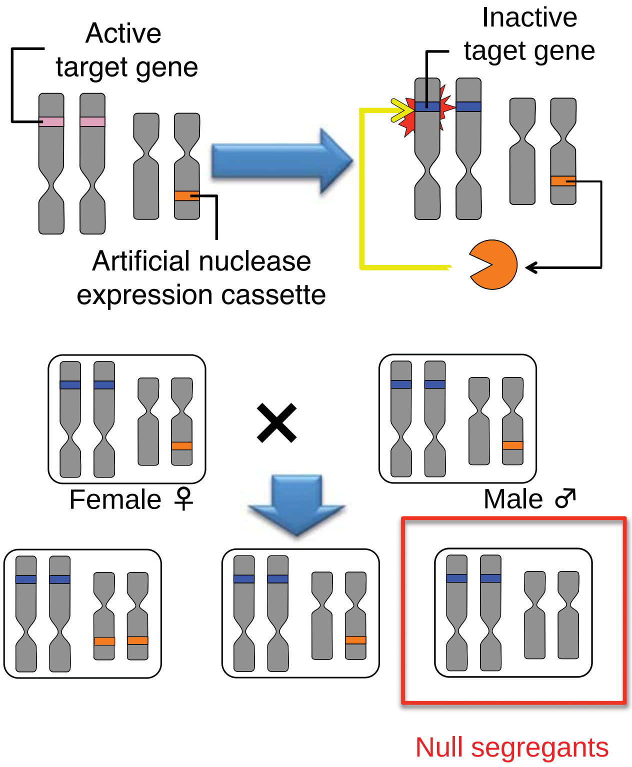
Potato

Future directions and issues

- Crossing transgenic *SSR2* KO lines to obtain "null segeregants"

Potato fruits on a self-fertile line

- ➢ Test in isolated field
- ➢ Establishment of DNA-free genome editing of potato



By courtesy of MURANAKA, Osaka Univ

To alleviate the pressure on the wild fishery of the bluefin tuna and aid in its conservation, the domestication of this fish and the development of a sustainable industry are necessary

Wild bluefin tuna

Completely farm-raised bluefn tuna

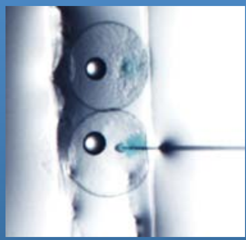
However, the occurrence of collision death of bluefin tuna during the seed production, other rearing, storage or transportation. It has imposed huge economic losses on tuna farmers. Tuna can be easily frightened by sudden flashes of bright light, which can startle them and cause them to panic and bump into the cage walls.

The final goal is to produce improved tuna varieties with targeted genetic modifications that are difficult to obtain through traditional breeding methods

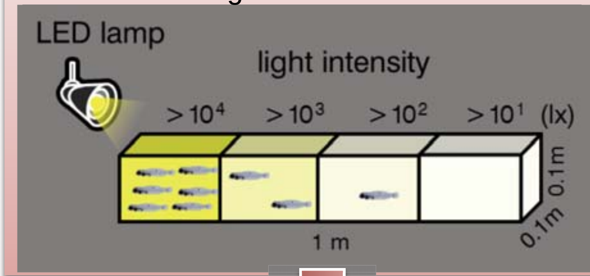
By courtesy of GEN, FRA

Identification of candidate genes associated with collision death of bluefin tuna (light response, fast moving etc.)

Development of Platinum TALEN system for high efficient genome editing in bluefin tuna



Construction of character evaluation device and method for genome-edited bluefin tuna



Development of land-based seed production systems of genome-edited bluefin tuna

Target gene : light response-related gene

Research in Progress !!

Phenotypic analysis of genome-edited bluefin tuna

By courtesy of GEN, FRA

Future Research Plans

Technical Challenges

- Expand the reliable target genes
- Pyramiding multiple genes
- Off-target cleavage prediction and prevention
- Development of a new approach for targeted gene editing in zygotes

Public Acceptance

- Guideline for regulatory compliance of genome editing technology in fish
- Possibility that escaped fertilized eggs and larvae may have genetic and ecological effects on wild populations
- Food safety (allergenicity studies etc.)

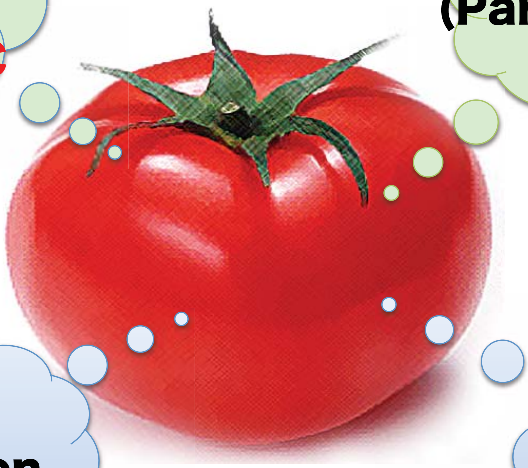
Development and application of gene editing technologies and the associated regulatory frameworks in aquaculture

By courtesy of GEN, FRA

Breeding Traits by Genome Editing Technique 【High added-value tomato】

Self-life
For P, V, C

Fruit setting (Parthenocarpy)
For P



Sugar accumulation
For C

Nutrition (GABA, etc.)
For C

P: Producer
V: Vender
C: Consumer

Tomato Breeding by Genome Editing Techniques 【High added-value tomato】

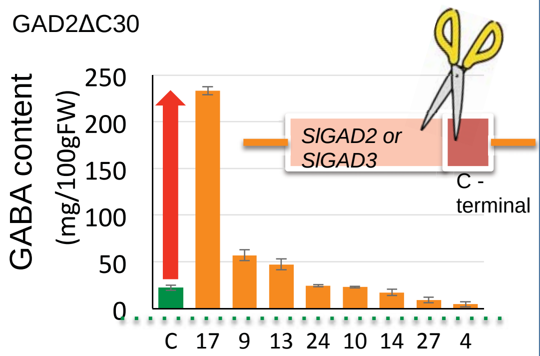
For exploiting domestic and overseas markets,

- Better transportability and high storability→ Long-distance transport for venders
- Labor-saving ability with pathenocarpy→ Low cost production for producers
- High quality (High sugar/Health-promoting materials)→ High added-value for consumers

High Storable/ High Sugar/ Pathenocarpy/ Health-promoting

Genome editing tomato

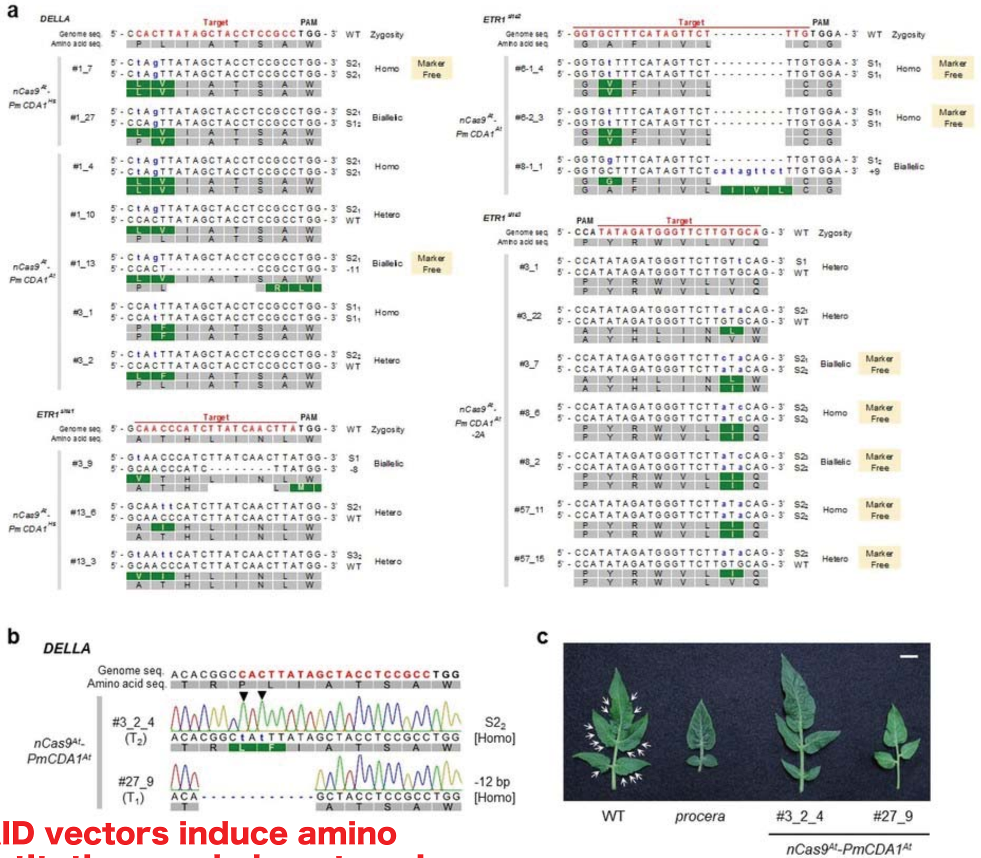
High GABA tomato



*GABA can lower blood pressure.

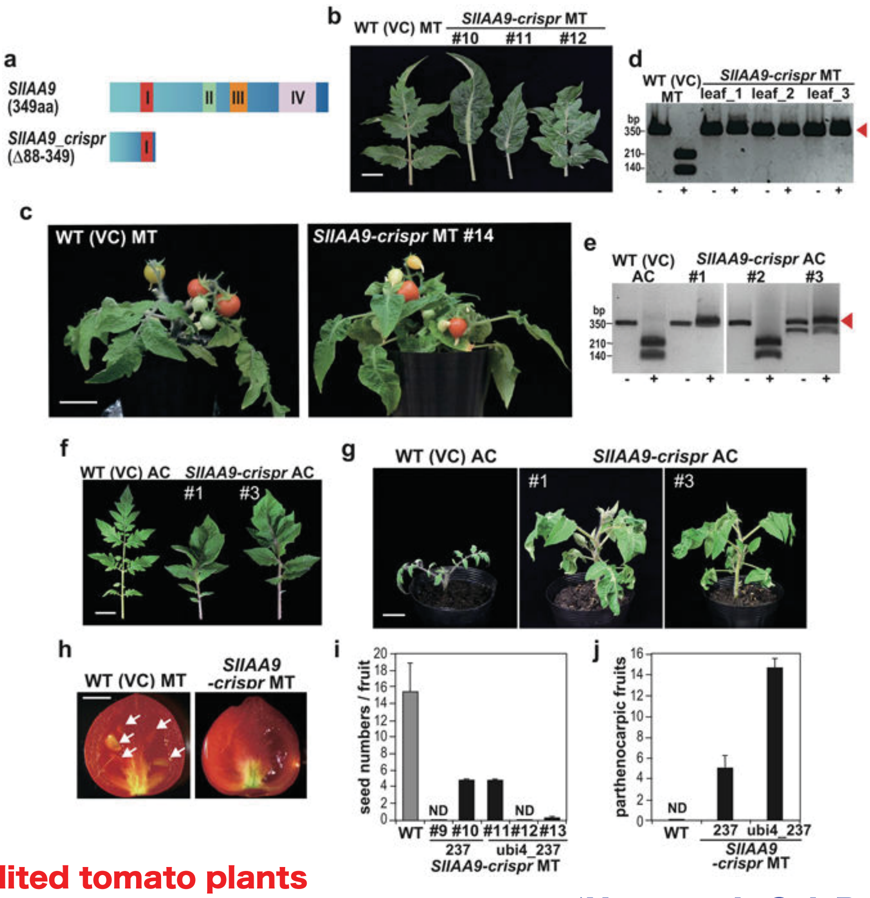
(Nonaka et al., In press., 2017)

Close Cooperation with Plant Factory Group and Food Functionality Group
→ Reduce production cost, Create new domestic and overseas markets.



Target-AID vectors induce amino acid substitutions and phenotype in genome-edited tomato plants.

(Shimatani et al., Nature Biotech., 2017)



Genome-edited tomato plants showed parthenocarpy.

(Ueta et al., Sci. Rep., 2017)

Evaluation of Genome Editing Tomato (on-going) 【High added-value tomato】

1. Validation of the absence of transgene in genome editing tomato (proof of null-segregant)- development of a standard method

2. Food safety assessment-development of a standard method

3. Off-target and on-target mutations, and its significance

4. Assessment of the performance as a parental line of F1 hybrid cultivars

5. Strategy for establishing public acceptance

SIP program ends in 2018. We would like to commence the social implementation at least in 2019.

Future challenges for practical application of genome editing in breeding program

○ Expand the applicable cultivars and crops and fishes

○ Expand the reliable target genes

○ Accumulation of development examples that can withstand the social implementation

○ Strategy for intellectual property

筑波大学 University of Tsukuba

COI Disclosure Information

Hiroshi Ezura

I have no financial relationships to disclose.