Outline of the Report by the New Plant Breeding Technique Study Group

- Towards the development and practical application of crops using new plant breeding techniques (NPBTs) such as genome editing -

September 2015

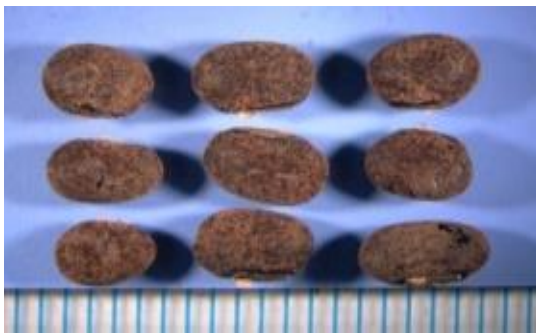
Agriculture, Forestry and Fisheries Research Council Secretariat

History of Plant Breeding and Improving

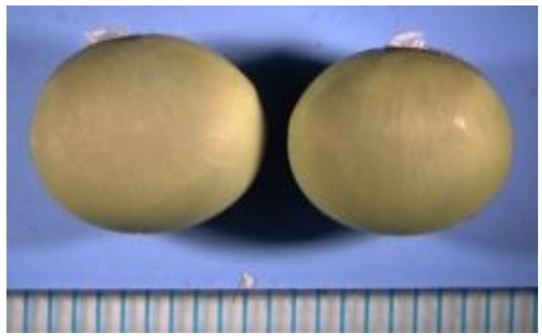
- Breeding of crops has a long history of humans modifying wild or native species. In recent years, the development and use of breeding techniques such as crossing and mutation have greatly contributed to the improvement of agricultural productivity and the stable supply of food, etc.

Ancient → ***Modern*** → ***Several decades ago*** → ***Present*** (History of breeding)

- Selection of useful species (mutants) from nature
- Crossing of useful species
- (Use of radiation, chemicals) Artificial induction of mutation
- Elucidation of genetic information (genome)
- Use of genetic modification technique
- Use of genome editing

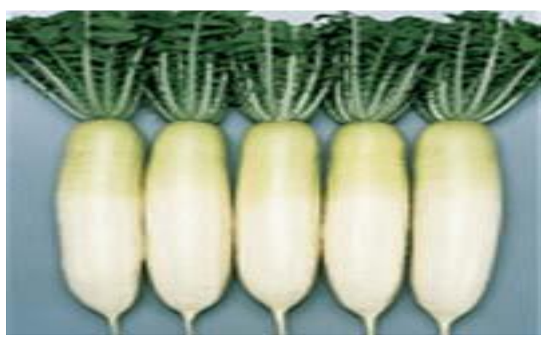


Glycine soja

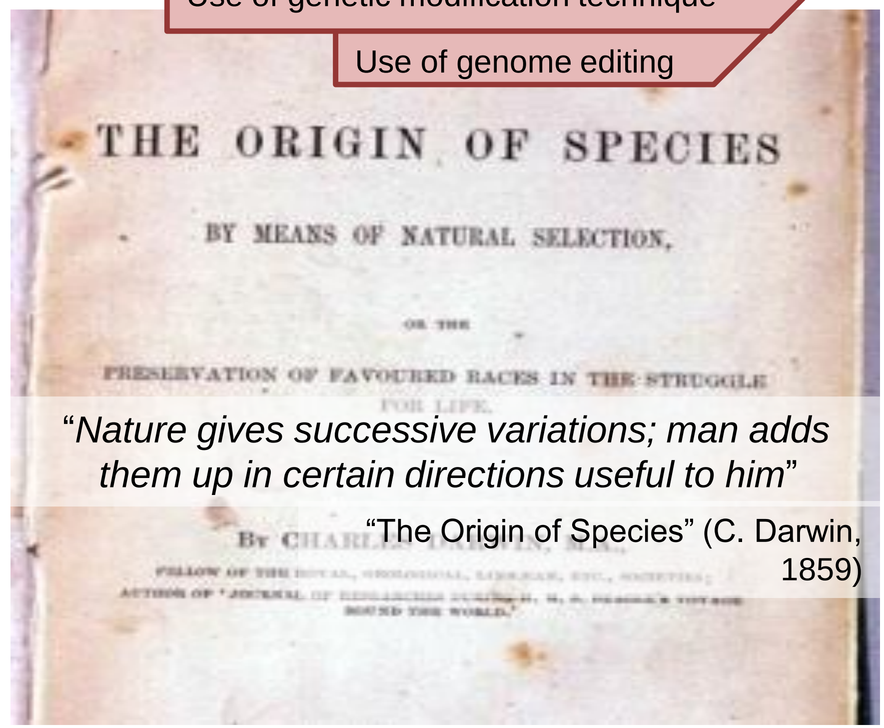


Soybeans

Japanese wild radish



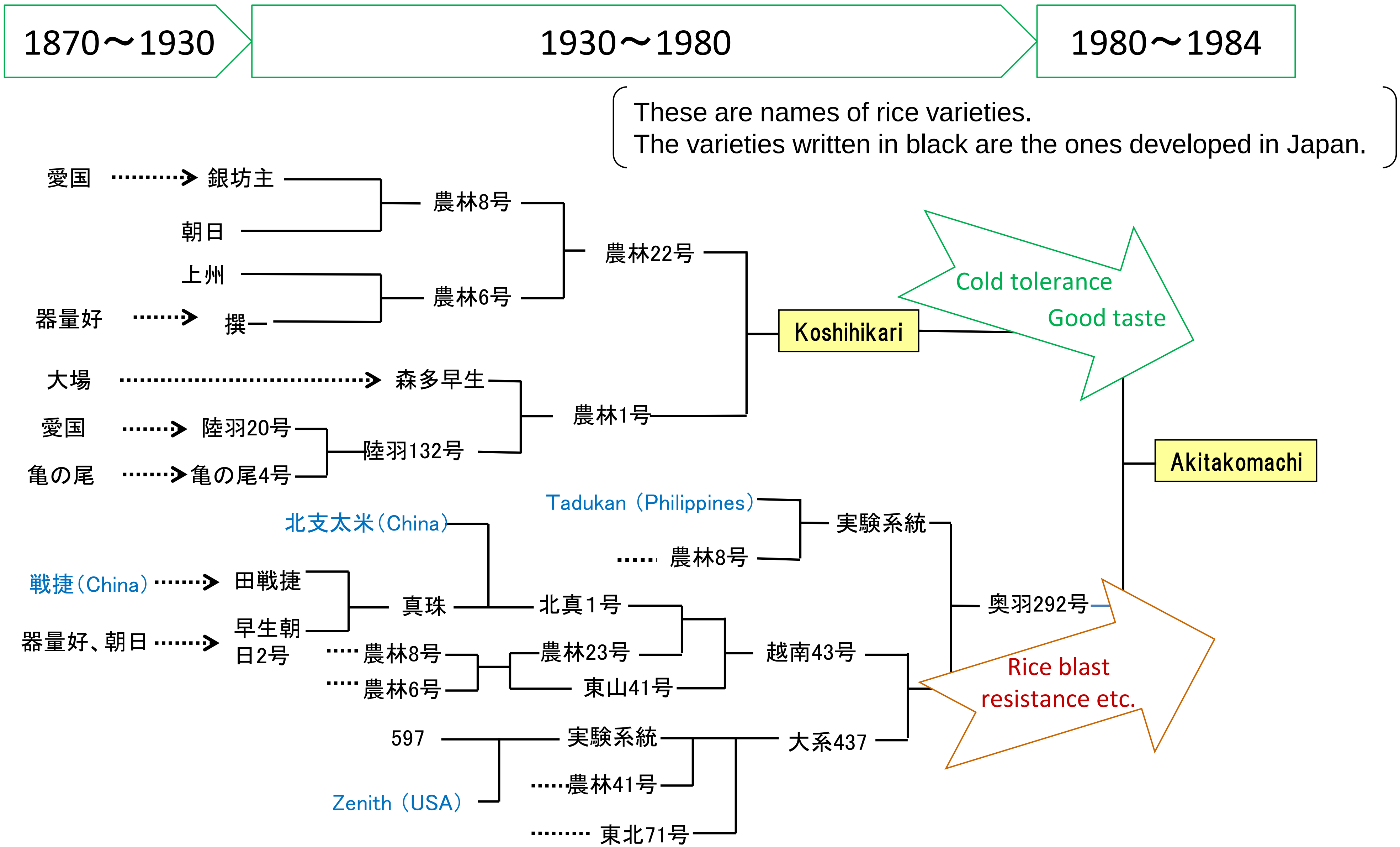
Japanese radish



"Nature gives successive variations; man adds them up in certain directions useful to him"

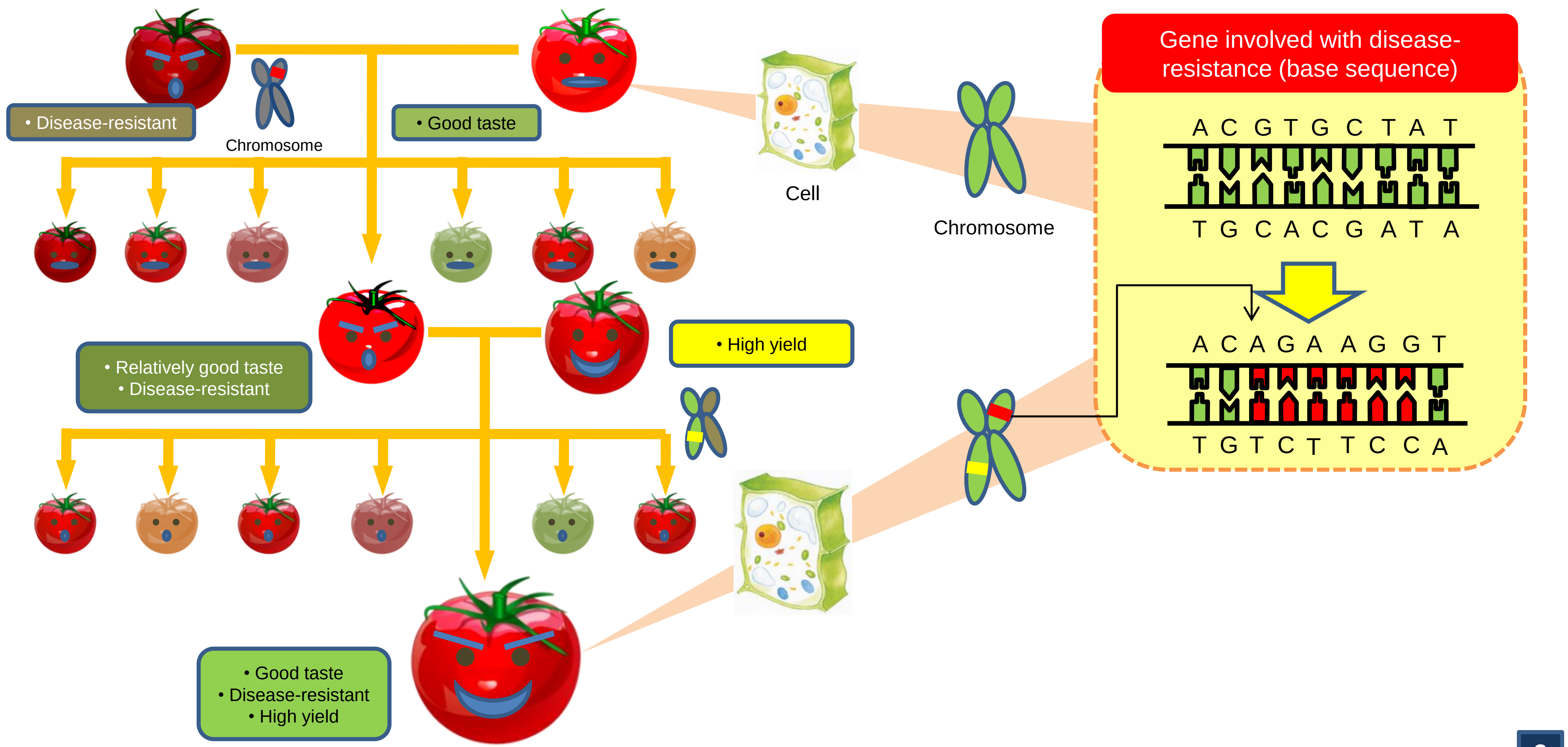
"The Origin of Species" (C. Darwin, 1859)

Breeding History of Koshihikari and Akitakomachi

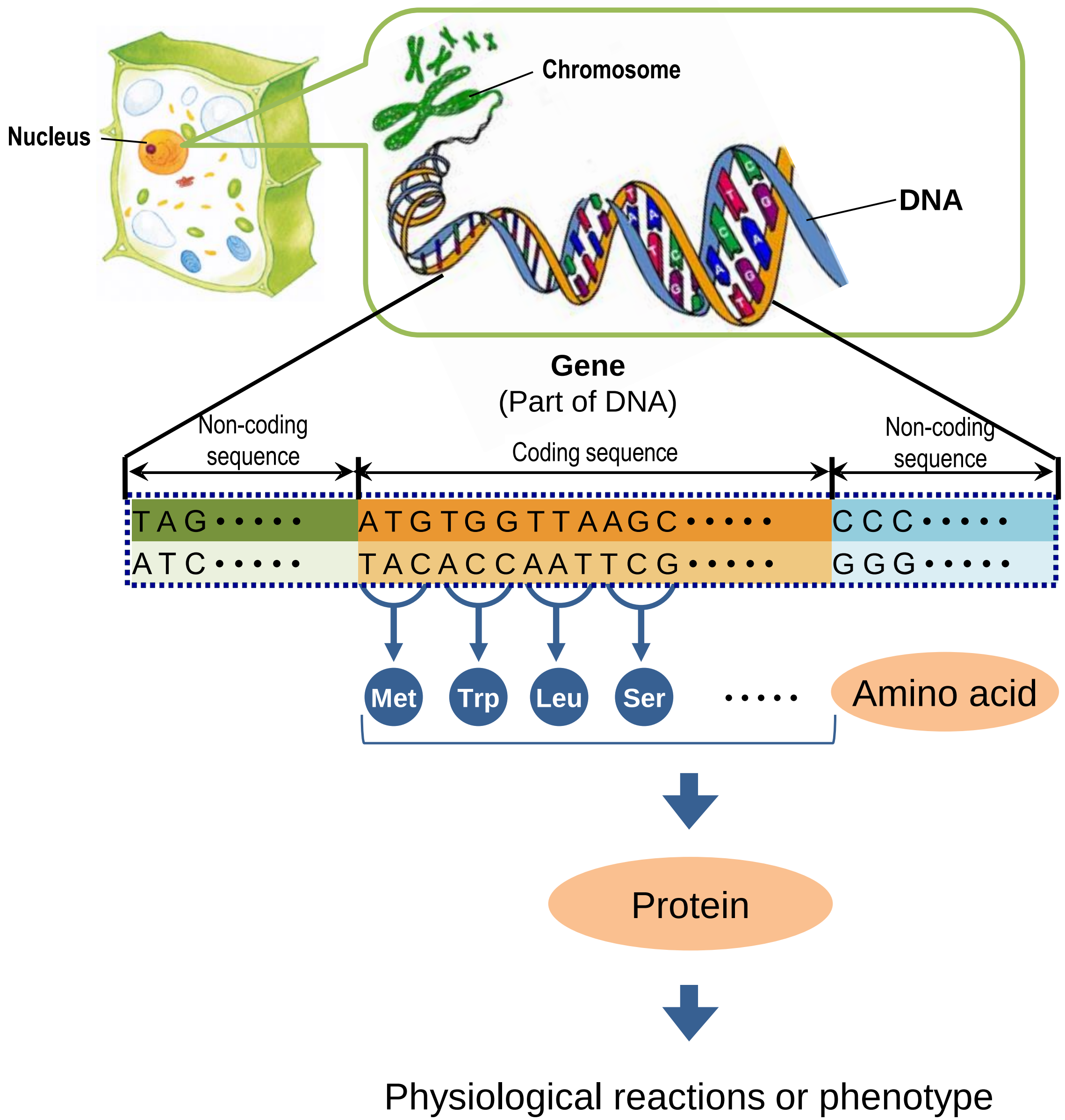


Conventional breeding

○ It is widely known nowadays that the principle of breeding basically relies on the genes in organisms, and that the combinations of four bases constituting DNA control the traits of any organism.



Gene expression in plant



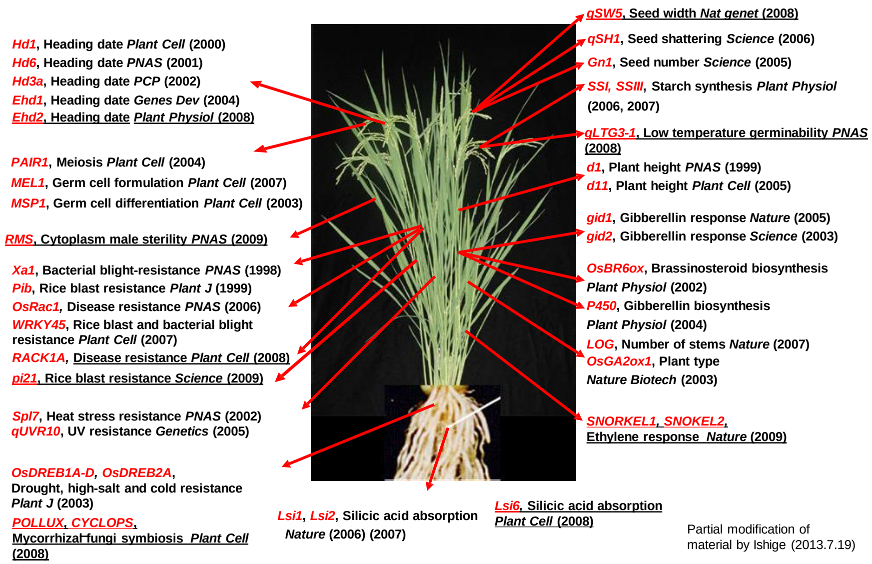
○ Genome size and gene number on genome of some organisms

Organism	Genome size (million base pairs)	Number of genes
Rice (*Japonica*)	400	32,000
Maize	2,200	45,000
Human	3,200	20,000
Drosophila melanogaster	180	15,000
Escherichia coli (K12 strain)	5	4,000

Decoding the Genome Information of Rice

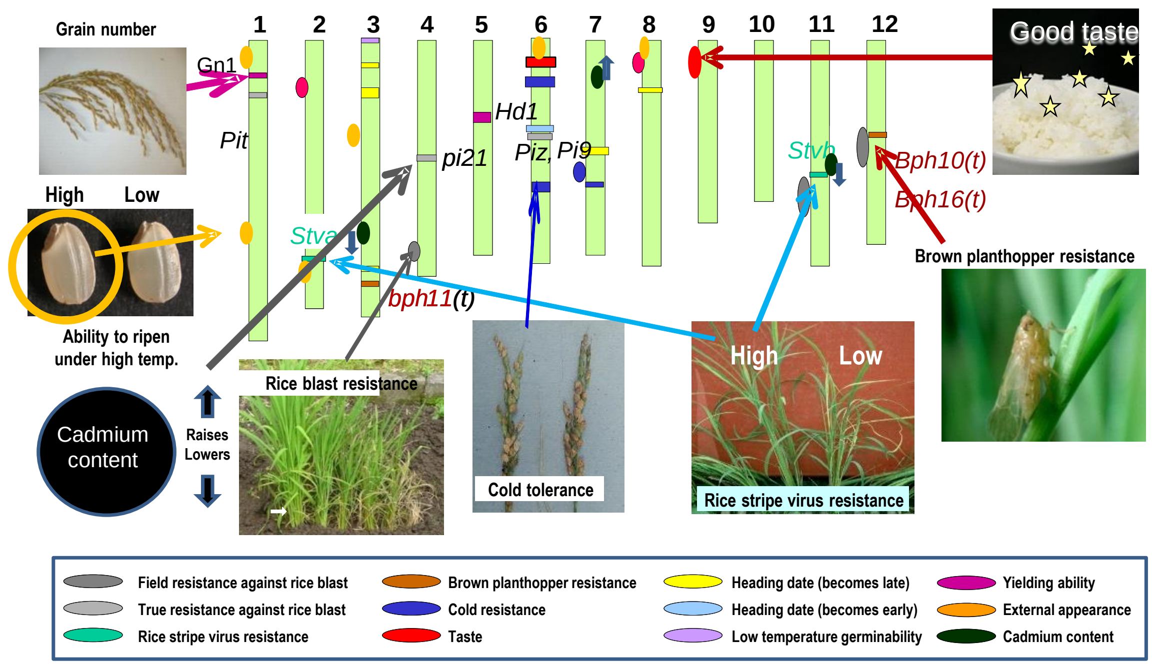
○ Recently, genes involved with traits useful for agricultural production have been identified one after another by decoding the genomic information of crops (e.g., rice).

○ Examples of genes identified in rice



Partial modification of material by Ishige (2013.7.19)

○ Genetic blueprint for breeding purpose (rice)

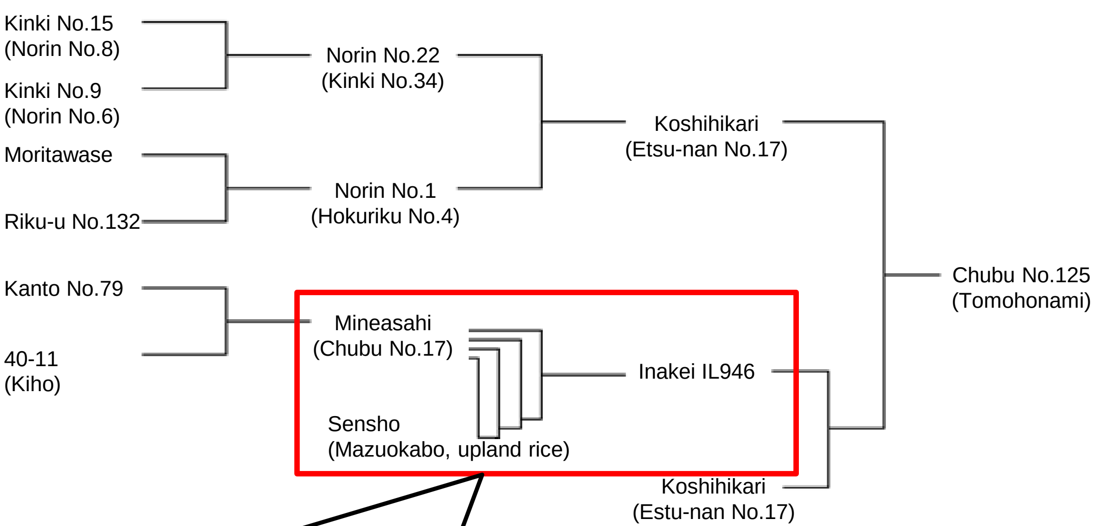


Note: Ellipses written on the chromosome shown above are some of the genes identified by their function.

Example of Creating New Species by DNA Marker Assisted Selection

- Under such a situation, in recent years, a new technique called DNA marker assisted selection was developed, which allowed to identify base sequence information of genes involved with traits useful for agricultural production and to use such information as a marker in the selection of a new useful varieties. This technique is applied for breeding of various crops including rice and vegetables.
- For instance, to breed blast-resistant rice, conventional techniques required many years of experiments including actual cultivation of various crossed varieties and examination of their disease resistance. However, by using DNA marker, rice with disease resistance can be examined and selected in a short period of time simply by extraction of DNA from the leaves.

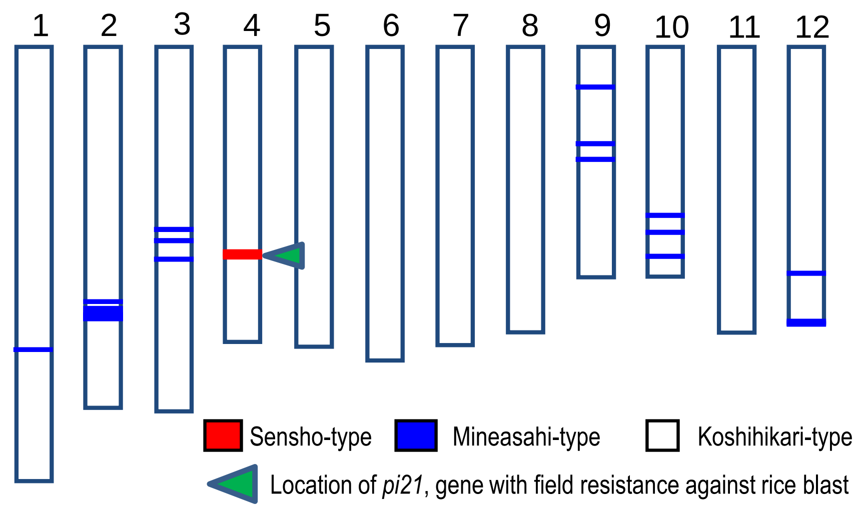
○ Breeding record of blast-resistance rice "Tomohonami"



While it has been known that a strong rice blast resistance gene (*pi21*) exists in upland rice, introducing it by crossing into a rice variety normally grown in Japan (paddy rice) would lead to worsen the taste of the rice as *pi21* is highly linked to genes involved with taste.

Therefore, the National Institute of Agrobiological Sciences and the Aichi Agricultural Research Center jointly solved this problem by taking advantage of DNA marker. They crossed paddy rice Mineasahi with the upland rice Sensho and backcrossed the descendants with Mineasahi, then they selected a plant with only the rice blast resistance gene (*pi21*) (Inakei IL946).

○ Gene structure of genome (chromosome) of "Tomohonami"



Rice growth state in an area with frequent rice blast infection

Characteristics of New Plant Breeding Techniques (NPBTs)

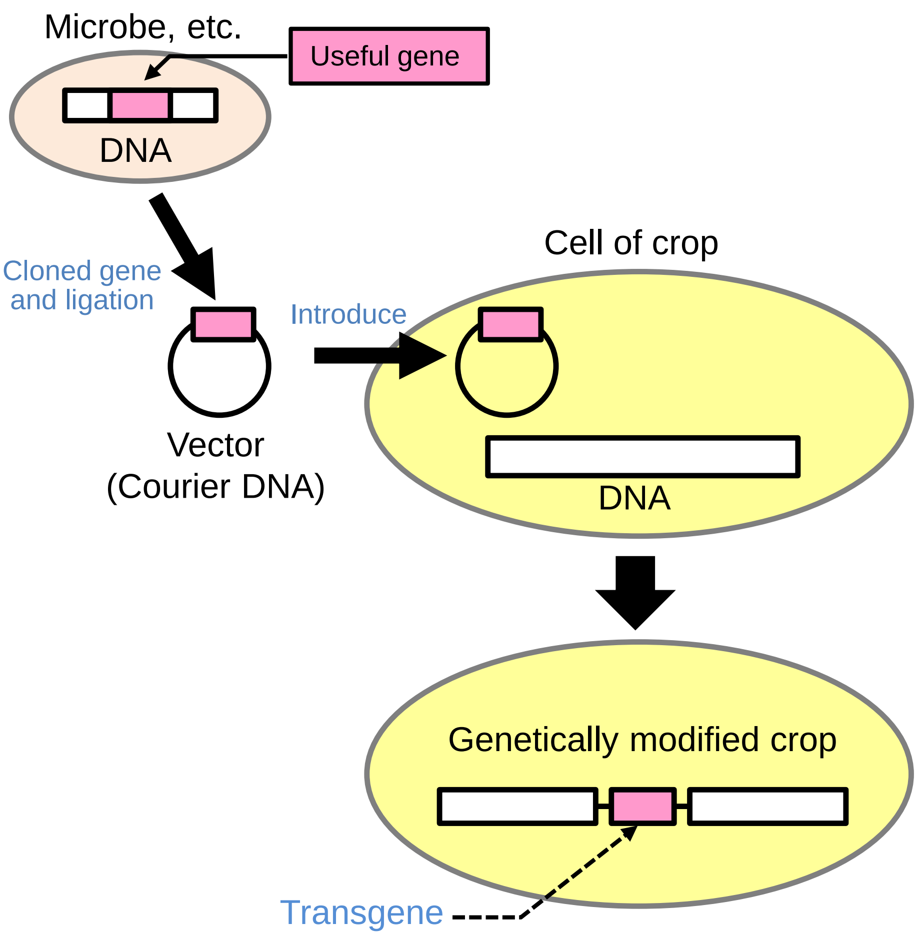
- In addition to DNA marker assisted selection, progress has been made on the development of "new plant breeding techniques (NPBTs)" in some countries that enable accurate and efficient introduction of traits useful for agricultural production, where genetic modification technologies are applied to a part of the breeding process of the conventional breeding.
- There are various techniques for NPBTs, including (1) those aiming at improving the efficiency of conventional mutation breeding and (2) those aiming at shortening the breeding term for conventional breeding by crossing, etc.
- The common characteristic to these techniques is that while genetic modification technologies are applied to a part of the breeding process, the final crop products are free from transgenes used for genetic modification. Therefore, some of the crops produced by NPBTs can be considered the same as those selected from the natural diversity or produced by conventional breeding.
- For this reason, when such crops become commercialized, we will have difficulty differentiating them from crops produced by conventional breeding techniques. There arises a question of whether regulations on genetic modification shall be applied to such crops. Currently, in several countries, discussions are being held on the regulatory framework for NPBTs under GM regulations concerning the food and feed safety and the environmental impact.

Techniques aiming at improving the efficiency of conventional mutation breeding	Techniques aiming at shortening the breeding term of conventional breeding by crossing, etc.	Other techniques
• Genome editing (e.g., ZFN, TALEN, CRISPR/Cas) • Oligonucleotide-directed mutagenesis (ODM) • RNA-dependent DNA methylation (RdDM)	• Cisgenesis/Intragenesis • Grafting using genetically modified rootstock • Agro-infiltration • Accelerated breeding of fruit plants utilizing early flowering genes • Recurrent selection of inbreeding crops such as rice	• Reverse breeding • Seed Production Technology (SPT)

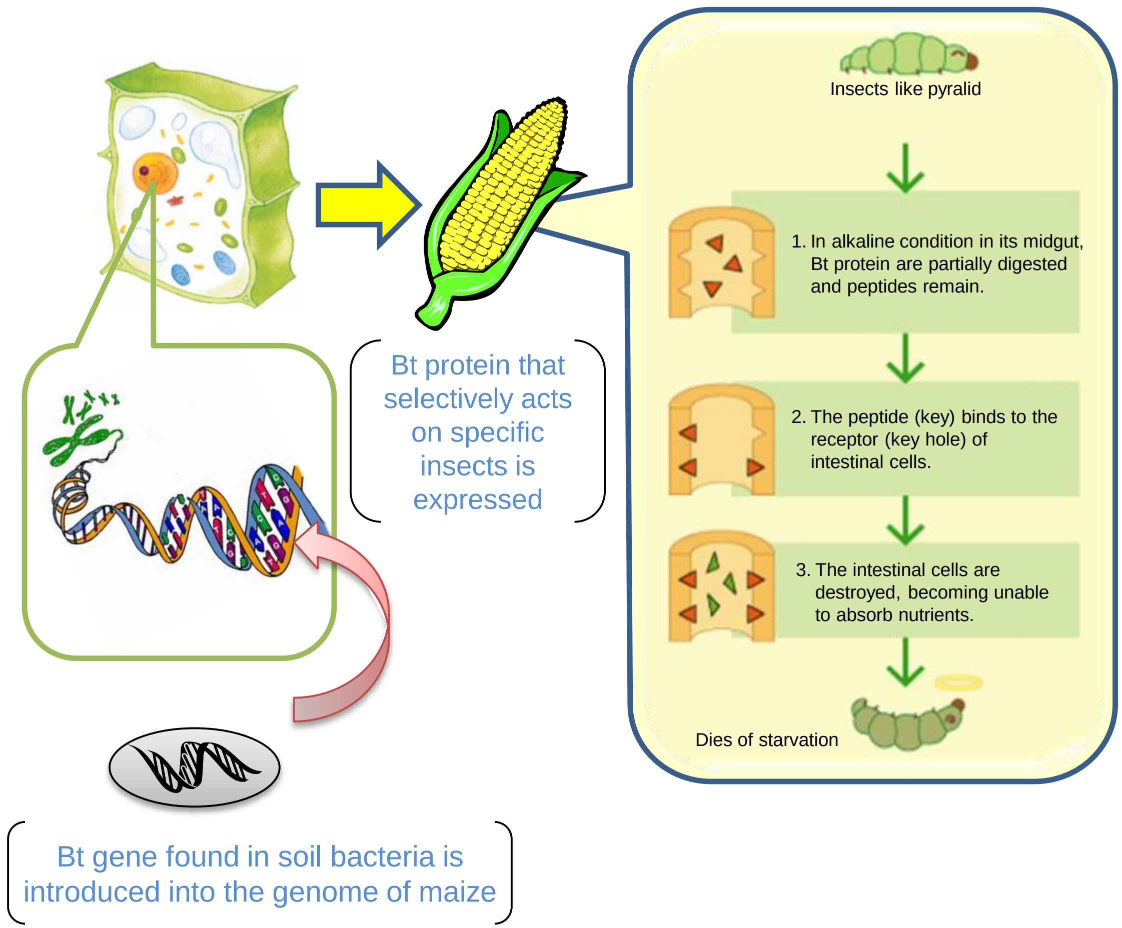
Conventional Use of Genetic Modification Technologies in Breeding

○ Conventionally, when genetic modification technologies were used for crop breeding, the common purpose was to bestow traits that cannot be acquired by natural crossing or conventional breeding techniques. For instance insecticide genes that form microbes have been introduced into some crops.

○ Method for creating genetically modified crops

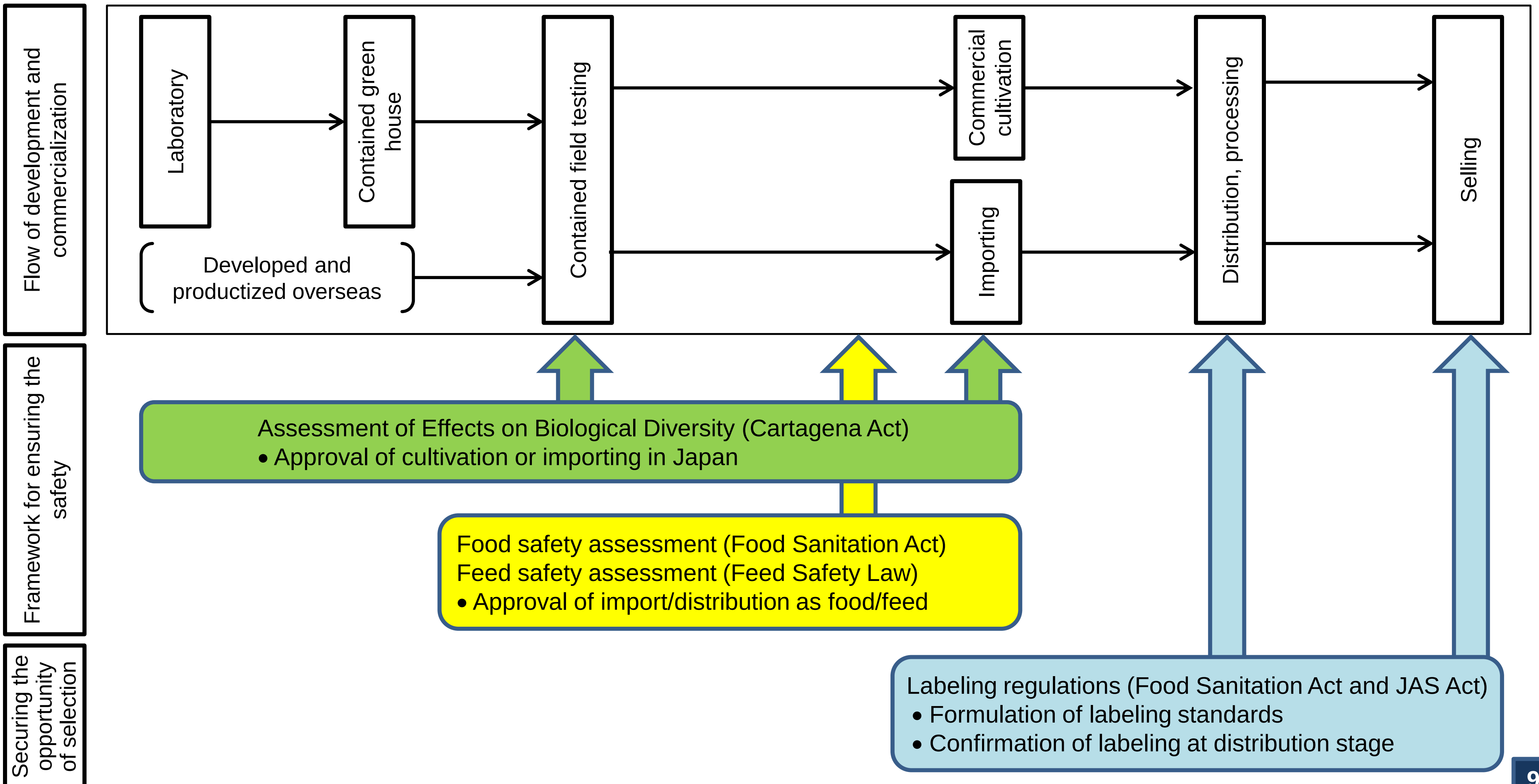


○ Mechanism of insect-resistant (Bt) maize



Framework of Regulations on Genetically Modified Crops in Japan

- GMOs possess transgenes originating from microbes etc. and new substances expressed by the transgenes, becoming organisms that humans have never eaten or cultivated before. Therefore GMOs have a possibility of unexpected adverse effects on human health or wildlife.
- in order to prevent such harmful effects, regulations have been established requiring the Japanese government to conduct a safety assessment for each individual case. Only those approved as safe by the government can be cultivated, imported, etc..



Background of Establishing a New Plant Breeding Technique Study Group

- The Ministry of Agriculture, Forestry and Fisheries (MAFF) is now accelerating the development of new innovative varieties that generate “advantages” in Japanese agricultural and livestock products as a part of its policy to realize “Aggressive Agriculture, Forestry and Fisheries”. Additionally, amid the situation where the transformation of agriculture, forestry and fisheries industries into growing industries is placed as an important political task for the Japanese government, the “Comprehensive Strategy on Science, Technology and Innovation 2015” developed in June 2015 indicates the development of next-generation breeding systems (e.g., NPBTs) as a priority R&D initiative, and relevant ministries are jointly promoting R&D under the Strategic Innovation Promotion Program (SIP).
- On practical application of the results of such research, the important task in Japan is how to develop social understanding, because there still remain strong concerns among consumers over the safety of crops and food to which GM techniques are applied.
- Additionally, in case in which discussions on the regulatory framework for NPBTs under GM regulations started in the EU or elsewhere, the Japanese Government needs to promote the collection and analysis of scientific knowledge and experience that can form a basis for discussions on NPBTs, and to hold discussions with diverse stakeholders in the future. Further, there is a necessity to conduct activities towards international regulatory harmonization.
- Based on such background, in October 2013, a study group consisting of expert scientists was established within the Agriculture, Forestry and Fisheries Research Council Secretariat, for gathering domestic and overseas information on NPBTs, compilation of scientific findings on the effect on the biological diversity, proper promotion of related R&Ds, smooth social implementation of research results, etc.

Name of expert scientists	Position	Specialty
Ryo Osawa	Professor, Faculty of Life and Environmental Sciences, the University of Tsukuba (Member of the Committee on Assessment of Adverse Effect on Biological Diversity, Ministry of the Environment (MOE) and MAFF)	Thremmatology
Hiroshi Kamada	Professor, Faculty of Life and Environmental Sciences, Gene Research Center, the University of Tsukuba Member of the Expert Committee on Genetically Modified Foods, Food Safety Commission of Japan (Member of the Committee on Assessment of Adverse Effect on Biological Diversity, MOE and MAFF)	Molecular biology, GM risk communication
Masakazu Shimada	Professor, Department of General Systems Studies, Graduate School of Arts and Sciences, the University of Tokyo (Member of the Committee on Assessment of Adverse Effect on Biological Diversity, MOE and MAFF)	Conservation ecology
Masashi Tachikawa	Professor, Department of Regional and Environmental Science, College of Agriculture, Ibaraki University	International GMO policy
Masahiro Nakagawara	Vice Chairman of Working Group on Harmonisation of regulatory Oversight in Biotechnology, OECD	Thremmatology
Nobuyoshi Nakajima	Head of Ecological Genetics Research Section, Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Japan (Member of the Committee on Assessment of Adverse Effect on Biological Diversity, MOE and MAFF)	Plant physiology
Akihiro Hino	Vice General Manager of the Central Laboratory, Nippon Flour Mills Co., Ltd. (Member of the Committee on Assessment of Adverse Effect on Biological Diversity, MOE and MAFF)	Genetic biochemistry, GM detection technology, risk communication

Trend of Research and Development Overseas

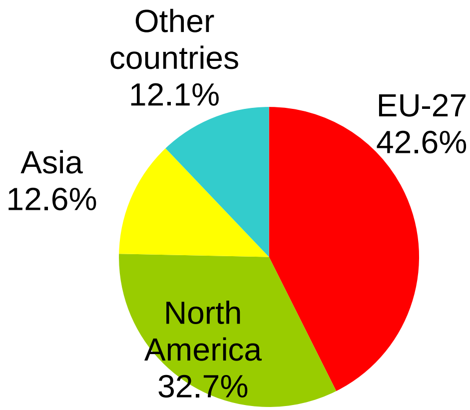
○ According to "New plant breeding techniques. State-of-the art and prospects for commercial development" reported by the European Commission, Joint Research Centre, Institute for Prospective Technological Studies in 2011.

(1) EU leads the world in the number of related scientific papers and the USA leads the world in the number of patent applications.

(2) As a result of a questionnaire survey given to major biotechnology enterprises, NPBTs "have been adopted by commercial breeders, and the most advanced crops could reach the stage of being commercialization in the short medium term (2-3 years)," if the crops produced by these techniques are not classified as GMOs.

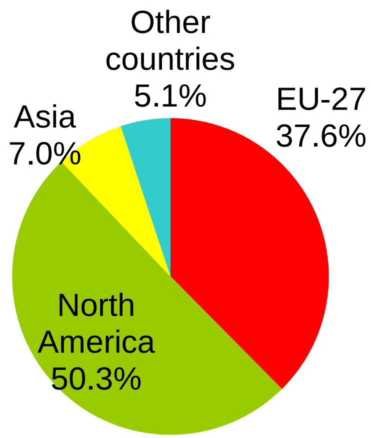
(3) All techniques are undergoing preparations for practical application, placing emphasis on the fact that in the final commercial products there is an absence of transgenes which is used for the developments and only transiently exist in the plants during the breeding process. Also, it is focused those higher efficiency than conventional breeding and substantial reduction in the cost for developing new products.

[Scientific publications]



Sector	N. publications	%
Academia	143	77%
Industry	24	13%
Joint	19	10%
Total	**186**	

[Related patents]



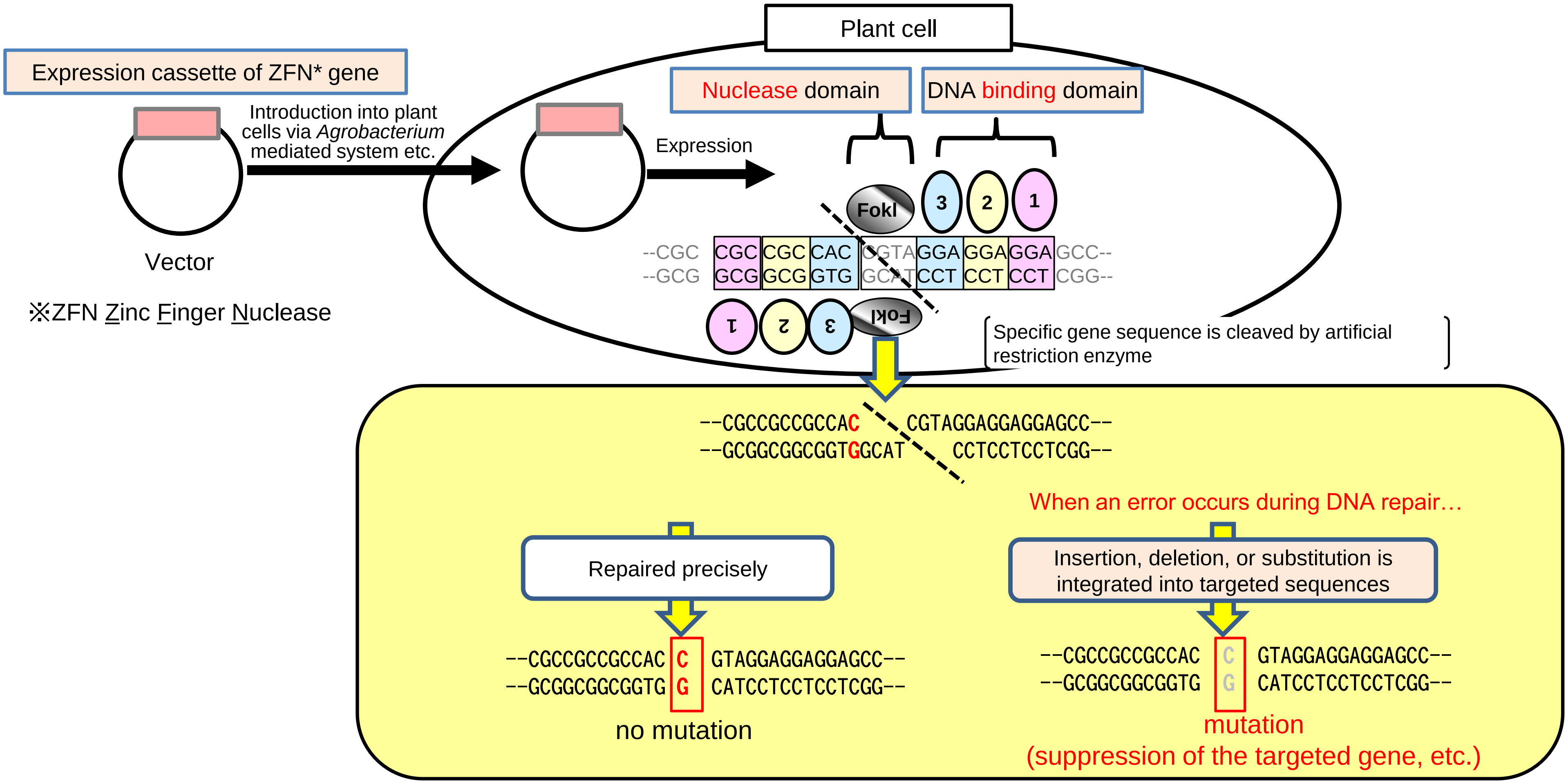
Sector	N. patents	%
Academia	27	20%
Industry	104	76%
Joint	6	4%
Total	**137**	

Source: Material from a lecture by Dr. Emilio Rodríguez Cerezo of JRC, Institute for Prospective Technological Studies (Feb 2014)

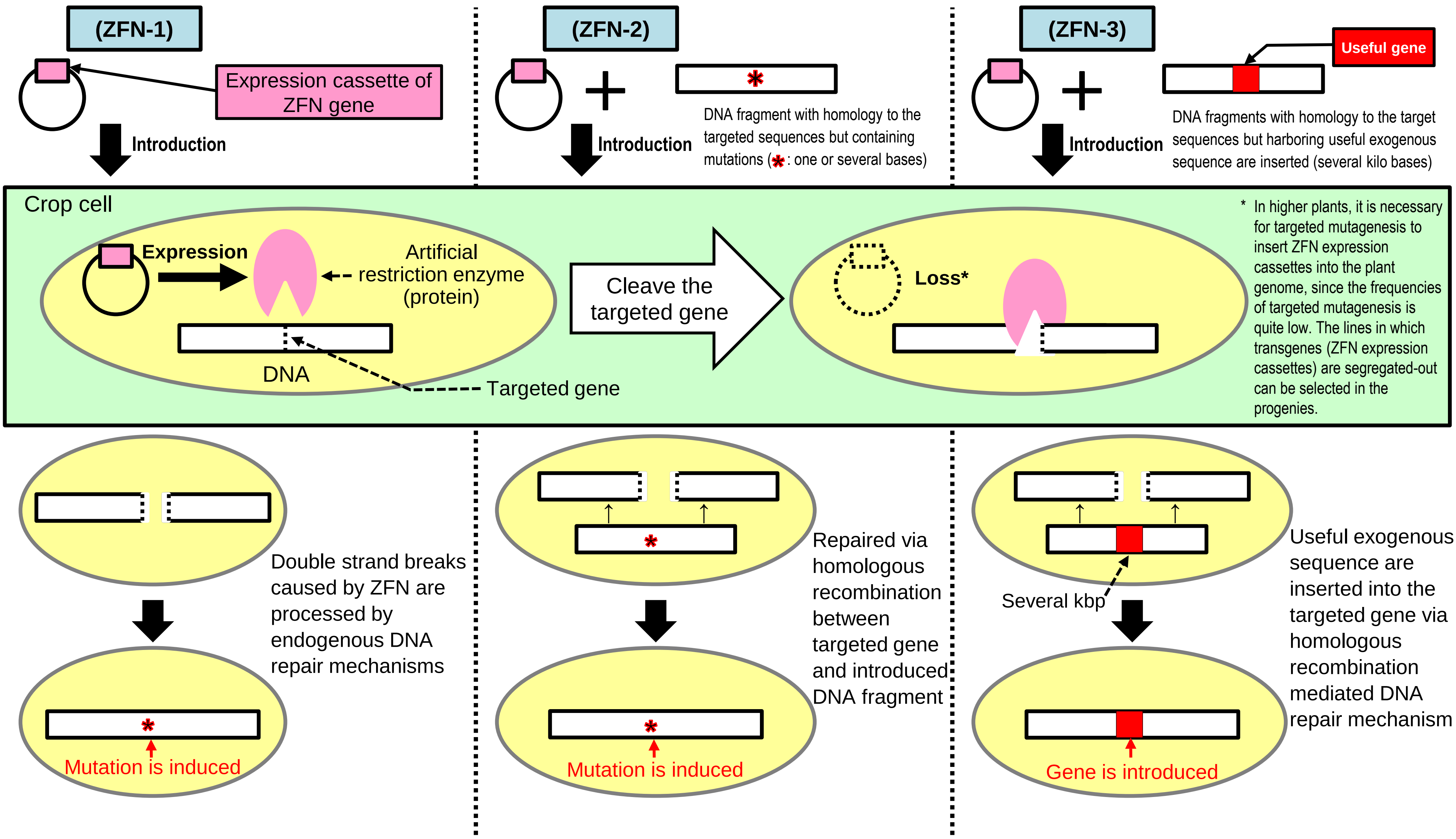
Note: All the data is for the period from year 1991 to 2011.

NPBT: Case 1 Genome Editing using Artificial Restriction Enzyme (ZFN)

- Lately, “artificial restriction enzymes” that allow for accurate cleavage of specific base sequence domains of target genes (DNA) have been developed, and it has become possible to arbitrarily induce gene modification (deletion, substitution or insertion) to the targeted domain on a genome (genome editing).
- By applying the genome editing to plant breeding, it would be possible to arbitrarily modify an endogenous gene that affects the flower color, plant height, etc. and thereby develop new innovative varieties in a short period of time.
- The chance of the same base sequence existing on a genome is calculated, for instance, as 1/(4^18) (maximum of 1 in 70 billion), if the number of bases in the DNA binding domain is 18. Considering that plant genomes consist of billions of base pairs, as long as special attention is paid to the specificity of the base sequence of the DNA binding domain at the time of designing the artificial restriction enzyme, the possibility of cleavage on the non-targeted base sequence (so-called “off-target”) is extremely low.



Type of DNA Mutation by ZFN

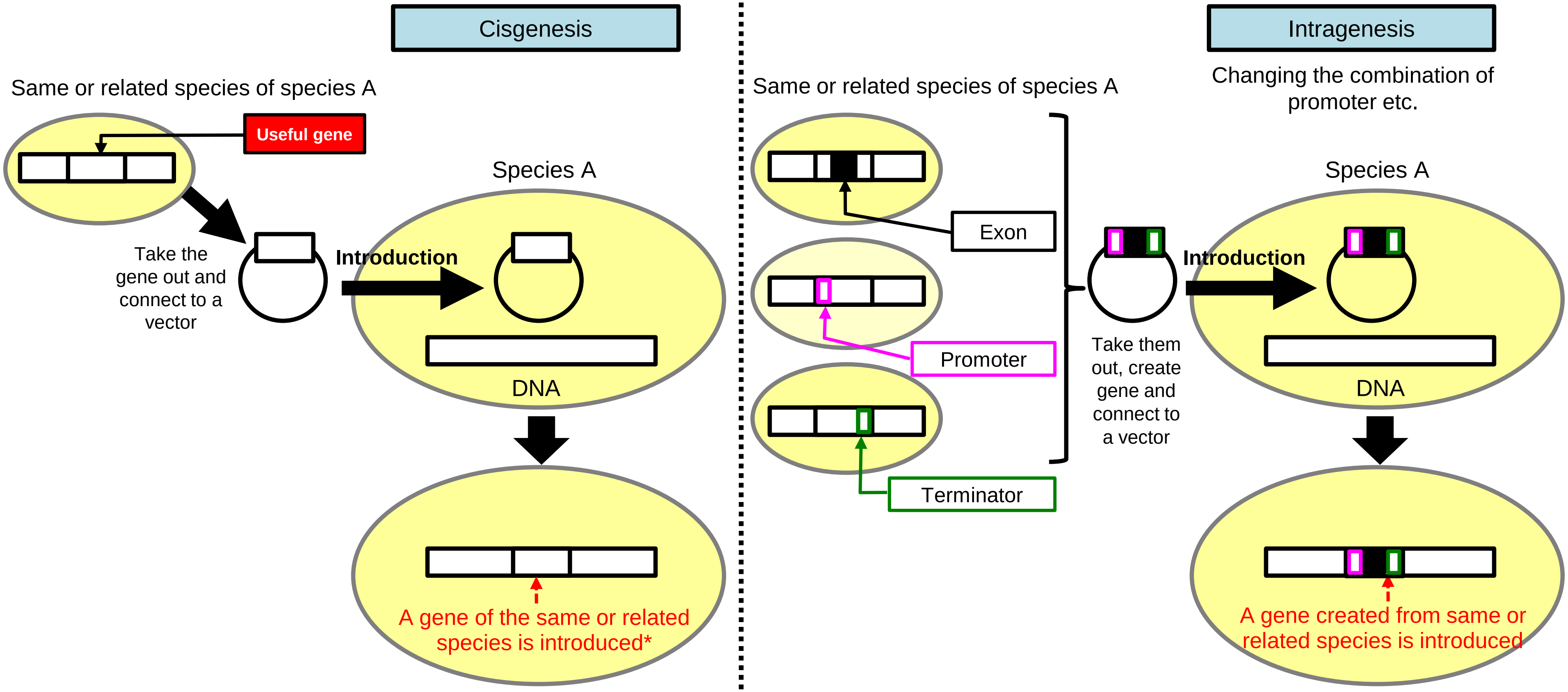


Comparison between Conventional GM Techniques and Genome Editing

	Technical advantage	Speed	Accuracy	Range of variation	Directionality of breeding application
Conventional GM techniques	Able to introduce useful genes of different species (e.g., microbes) where crossing is difficult	○	○	◎	1) Addressing global environmental changes (e.g., global warming, desertification) 2) Smartization of agriculture by introducing disease- and pest-resistance 3) Production of useful substances (e.g., drugs) in crops
Genome editing	Able to modify sequence information of an organism targeting a specific nucleic acid sequence (gene)	◎	◎	○	1) Addressing the needs for longevity by developing crops rich in functional ingredients 2) Improving the self-sufficiency rate by developing rice high in feed nutritive value 3) Development of new species with various traits that have not been actualized (e.g., disease- and pest-resistance) etc.

NPBT: Case 2 Cisgenesis and Intragenesis

- Cisgenesis is a method to introduce a gene of the same or related compatible varieties (cis-gene) to crops using GM technologies. It may be an effective method to breed vegetative propagation crops such as fruit plants, potatoes and sugar cane.
- Intragenesis also uses the same or related varieties as the source of gene in a similar manner as cisgenesis. However, intragenesis is used to control the expression amount of a specific gene by partially modifying the promoter or terminator that constitute the gene.



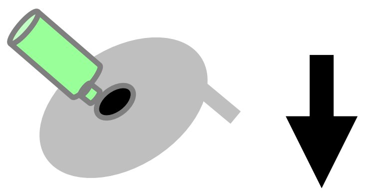
* There is a possibility of multiple genes randomly introduced to the host genome and the remaining T-DNA border sequence of the vector.

The genotypic composition is basically the same as species A

The genotypic composition is different from species A

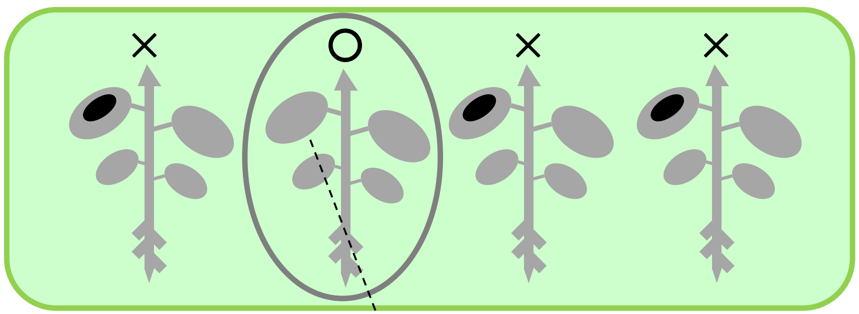
Case 3 Agro-infiltration

- ○ Agro-infiltration is a technique to locally infect a plant with *Agrobacterium* (bacteria) to which specific genes are incorporated, and, then, to select plants with disease resistance etc. by examining the expression of the relevant gene.
- ○ Genes originating from the *Agrobacterium* do not remain in the body of the selected plants, as long as the infected domain (e.g., leaf) is removed.



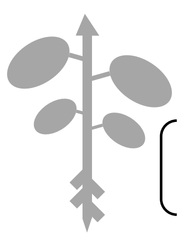
Inoculate GM microbe having genes involved with disease of crop* and make the leaf locally infected

(* Especially for the cases such as if a gene derived from unculturable pathogenic virus is introduced. etc.)



Disease-resistant plant does not exhibit symptoms

GMO or non-GMO?

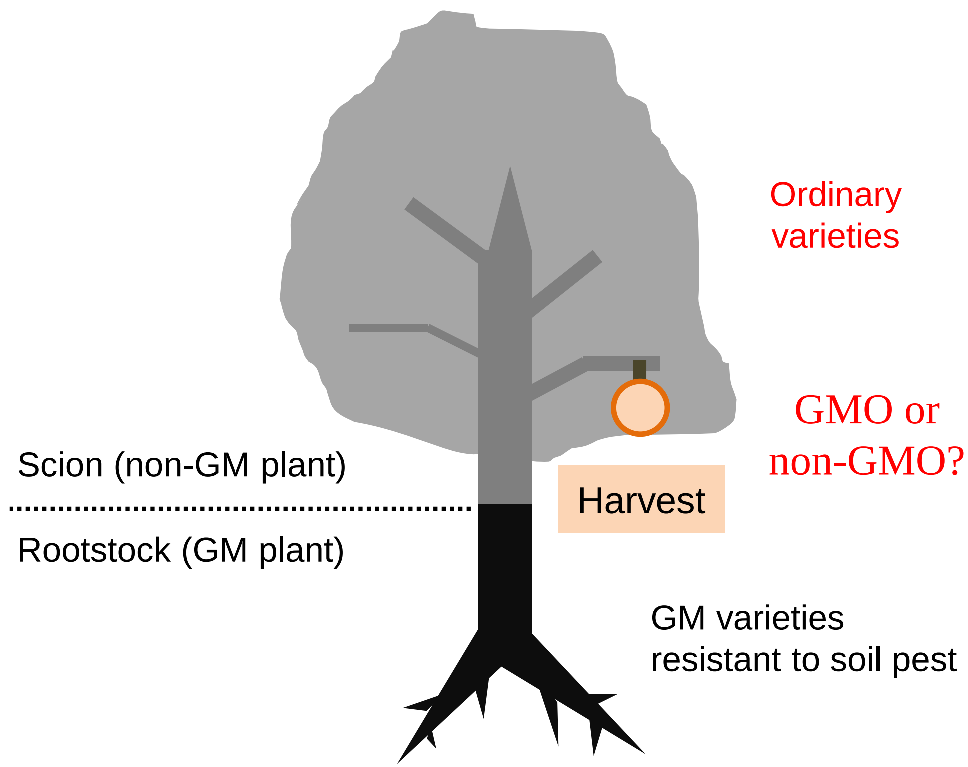


Select and cultivate healthy (disease-resistant) plant

(Since it is a local infection to the leaf, the GM microbe does not remain in seeds.)

Case 4 Grafting using GM rootstock

- ○ When a GM rootstock resistant to a specific soil pest is developed and a non-GM varieties is grafted to the rootstock, it is possible to cultivate crops without changing the quality of harvest from the scion with avoiding the effects of the soil pest.
- ○ Generally, transgenes (e.g., disease resistance gene) integrated into the genome of rootstock do not migrate to the scion.



Consideration on Effects on Biological Diversity, etc.

○ The Cartagena Act defines a GMO as "an organism that possesses nucleic acid, or a replicated product thereof, obtained through use of a technology, as stipulated in the ordinance of the competent ministries, for the processing of nucleic acid extracellularly". Therefore, the Study Group decided to;

(1) Deliberate the possibility of a foreign nucleic acid used for GM or its replicated product thereof remaining in the plant ("possessed" or not); and,

(2) Summarize the general ways of thinking on adverse effects on the biological diversity through the comparison with crops created by conventional breeding techniques, since there are rules to exclude organisms obtained through so-called "self-cloning" and "natural occurrence" from organisms subject to the regulation (enforcement ordinance of the Act).

○ Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No. 97 of 2003)

(Definitions)

Article 2

(2) In this Act, "living modified organism" shall mean an organism that possesses nucleic acid, or a replicated product thereof, obtained through use of the any of the following technologies:

(i) Those technologies, as stipulated in the ordinance of the competent ministries, for the processing of nucleic acid extracellularly

(ii) Those technologies, as stipulated in the ordinance of the competent ministries, for fusing of the cells of organisms belonging to different taxonomical families.

○ Enforcement Ordinance of the Act (MOF, MEXT, MHLW, MAFF, METI & MOE Ordinance No. 1 of 2003)

Article 2 Technologies stipulated in the ordinance of the competent ministries under Article 2 paragraph 2 subparagraph 1 of the Act shall be the technology for processing nucleic acid extracellularly for the purpose of introducing the nucleic acid into cells, viruses or viroids to transfer or replicate the nucleic acid, while excluding those mentioned in the following:

(i) Technology for processing by using, as nucleic acid to be introduced into cells, only the nucleic acid shown in the following:

A. The nucleic acid of living organism belonging to the same species as that of the living organism which the cells originate from

B. The nucleic acid of living organism belonging to the species that exchanges nucleic acid with the species of the living organism which the cells originate from in natural conditions

(ii) Technology for processing by using, as nucleic acid to be introduced into viruses or viroids, only the nucleic acid of viruses or viroids that exchanges nucleic acid with the viruses or viroids in natural conditions

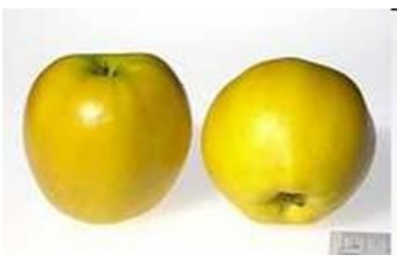
Example of NPBT Development in Japan (1)
– Accelerated breeding of fruit trees utilizing early flowering genes –

- Seedlings of fruit trees usually take 5-10 years to flower and fruit, which is a major obstacle to the development of new varieties.
- For instance, when the apple scab resistance gene (*Vf* gene) was introduced to apple cultivar (Rome Beauty) from a related varieties (*Malus floribunda*), it took 7 generations over 68 years to achieve the desired result (Gold Rush).

Golden Delicious
(6) Crossed in 1972
Coop 38
(PRI 2750-6
Illinois #2
('Winesap' open pollinated)
Co-op 17
(PRI 1689-100)
(5)
Melrose
PRI 668-100
(4)
Golden Delicious
PRI 14-126
(3)
9433-2-2
Rome Beauty
F_2 26829-2-2
(2)
(1) Crossed in 1926
9433-2-8
Malus floribunda 821


Malus floribunda


\`Rome Beauty'


Gold Rush Released in 1994

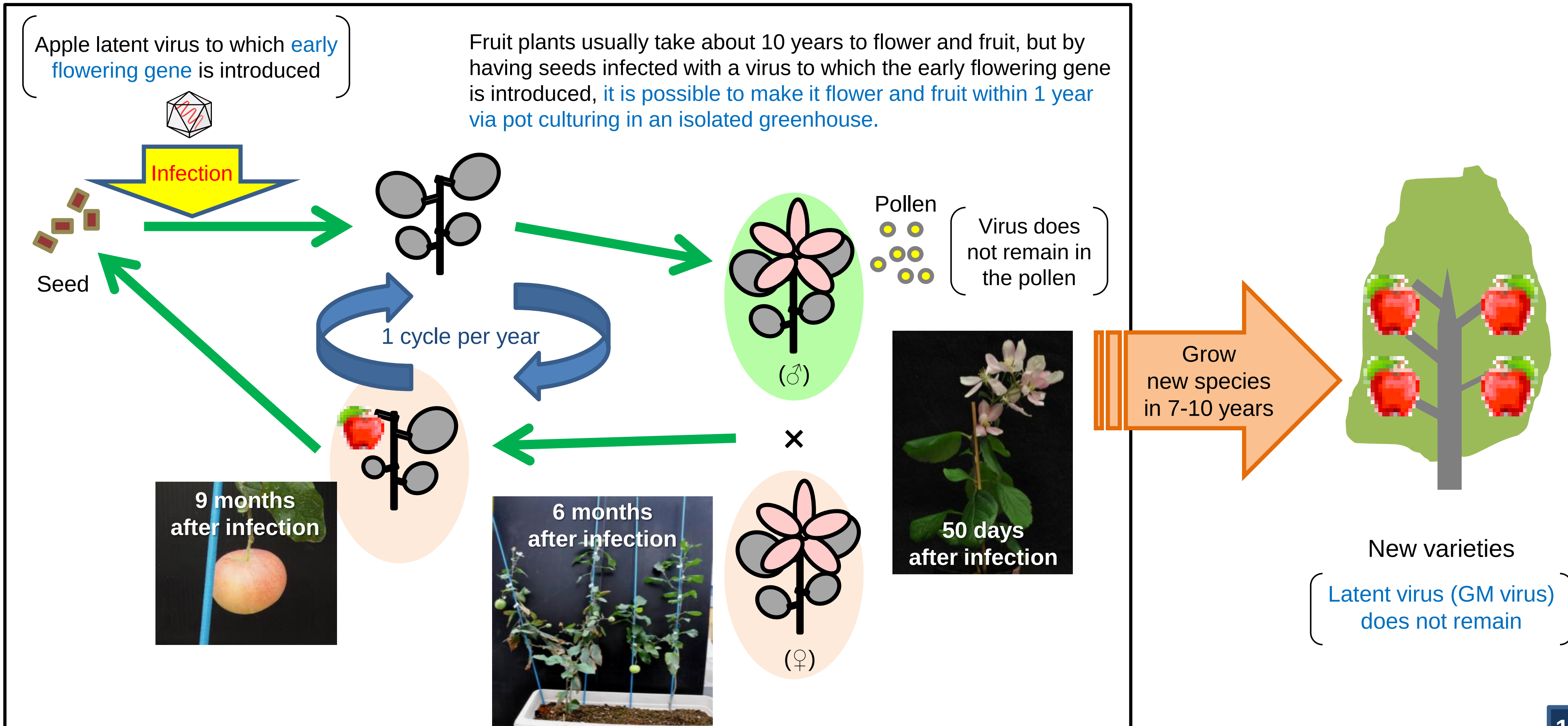
Prima
Released in
1970

- A certain quality was finally achieved after 7 generations over 68 years

- If the generation can be shortened to 1-2 years, breeding will advance drastically

– Accelerated breeding of fruit trees utilizing early flowering genes – (contd.)

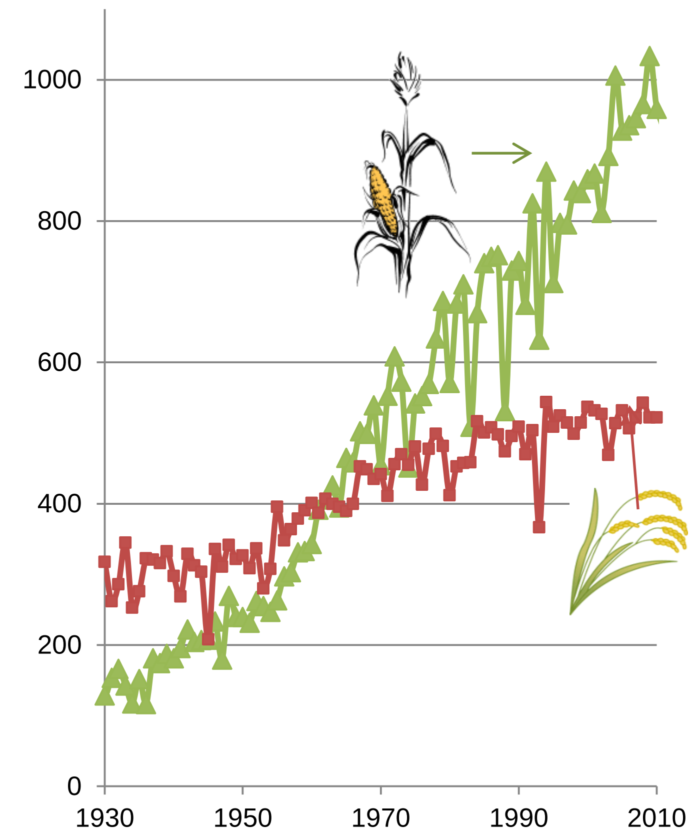
- In 1999, the *FT* gene that controls the flowering of plants was discovered by Prof. Araki *et al.* of Kyoto University. Prof. Yoshikawa of Iwate University developed a flowering promotion technique for apple by introducing the *FT* gene by using one of the latent viruses, "Apple Latent Spherical Virus (ALSV)".
- This technique is applicable to various crops including other fruits, soybean and vegetables. Development of accelerated breeding techniques for grapes, pears, etc. is currently in progress at Iwate University etc., under the Strategic Innovation Promotion Program (SIP) of the Cabinet Office, aiming at practical application with one crossing generation is reduced to one year or less.



Example of NPBT Development in Japan (2)
– Recurrent selection of inbreeding crops such as rice –

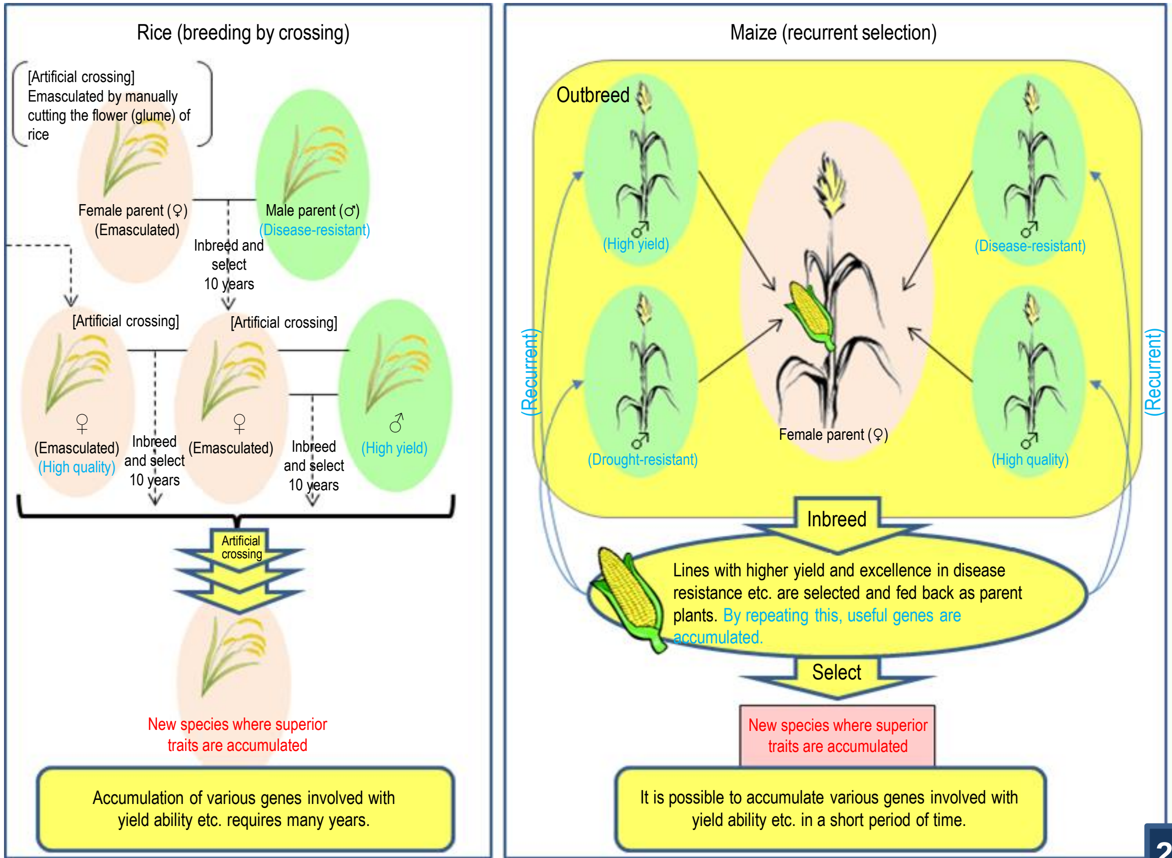
- Usually, rice is bred by crossing two varieties. However, this technique has a limitation in the number of usable breeding materials, and it takes a substantial amount of time to improve the agricultural traits such as yield ability that involve multiple genes related.
- The National Agriculture and Food Research Organization, Institute of Crop Science, etc. undertook the development of a breeding system for applying the recurrent selection, which is generally used for breeding outbreeding crop such as maize nowadays, to inbreeding crops such as rice (utilization of male sterility gene to make rice to outbreed), taking into account the fact that the unit crop yield of maize has been steadily improved over years through recurrent selection.

○ Transition in unit crop of maize (US) and rice (Japan)



Note: Green is the actual unit crop of maize in the US (USDA) and red is that of rice in Japan.

○ Difference in breeding techniques between maize and rice



Consideration on Effects of Abovementioned Cases on Biological Diversity

(1) Possibility of transgenes remaining

- ✓ At the initial stage of breeding (experimental research stage), plants emerge in which transgenes or recombinant viruses remain.

↓

Appropriate use based on the Cartagena Act (application for the approval to use in a closed system or to test at an isolated field) is required

- ✓ Final commercial varieties can be selected from those with no relevant transgenes remaining (Null Segregant)

↓

There is a possibility of being exempted from Cartagena Act regulations if it is confirmed to be free from transgenes by appropriate methods such as PCR

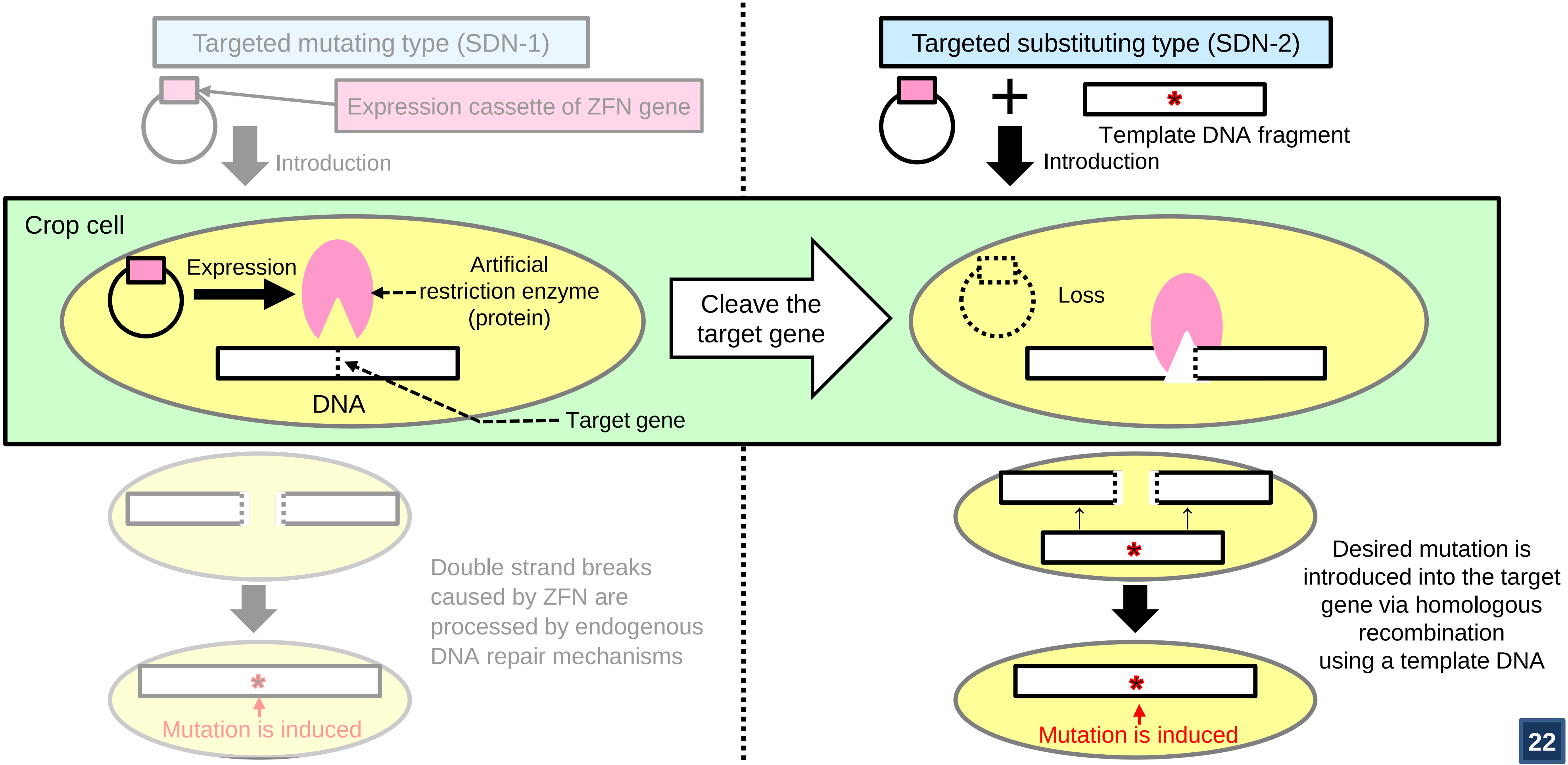
(2) Comparison with conventional breeding, etc.

- ✓ Transgenes and recombinant viruses are used to improve the efficiency of cross breeding or selection process, but no transgenes and other related DNA remain in the final commercial varieties.
- ✓ Therefore, newly produced crops (new varieties) can be regarded as the ones created by conventional breeding.
- ✓ In breeding of crop plants, usually, there is a process to eliminate undesirable plants from the line groups engineered. As a result, only the plants with good qualities and ease of growth are selected as commercial varieties.

From the above understanding, if it is confirmed that transgenes are not included in the final crop, the crop can be regarded the same as those produced by conventional breeding. Therefore, the Study Group considered that there are no matters that call for specific concerns over the effects on biological diversity.

Genome Editing utilizing Artificial Restriction Enzyme

- Genome editing is an epoch-making mutation breeding techniques that allows for inducing mutation to specific genes in crops. However, the induction of mutation requires introduction of a transgene that produces an artificial restriction enzyme into the genome of the crop.
- The National Institute of Agrobiological Sciences is developing techniques to completely remove artificial restriction enzyme genes introduced to the genome after mutagenesis, a method to improve the occurrence rate of mutation (utilization of SDN-2), etc.
- It will become possible to suppress genes that generate allergens in rice or toxic substances in potatoes (e.g., glycoalkaloids). It will also be able to develop feed rice rich in a specific amino acid, sweeter crop rich in oligosaccharide, tomato with high rate of flowering and high yield, tomato having parthenocarpy, tomato and capsicum that ripen even under low temperature in winter, grass that is easily digested by livestock, pollen-free Japanese cedar, etc., in a very short period of time.



Consideration on Effects of Abovementioned Cases on Biological Diversity

(1) Possibility of transgenes remaining

✓ Usually, an artificial restriction enzyme gene (transgene) will be incorporated into the plant genome.

⇩

Until the artificial restriction enzyme gene (transgene) is completely removed, appropriate use based on the Cartagena Act is required.

✓ (With transgene removing,) deletion, substitution, or insertion of bases can occur in nature or conventional mutation breeding. In the existing Cartagena Act, "natural occurrence" is exempted from the regulations.

⇩

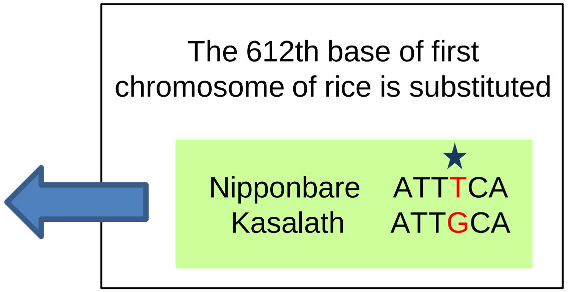
(1) **SDN-1**: There is a possibility to be exempted from the Cartagena Act regulations.
(2) **SDN-2**: It is important for developers to request for judgment on a case-by-case basis, with submitting related information including the characteristics of mutated trait to the regulatory.

(2) Comparison with conventional breeding, etc.

✓ SDN-1 or 2 induces deletion, substitution or insertion of bases. Such mutations of several bases occurs naturally or in conventional breeding such as mutation breeding unintendedly.

✓ Compared to conventional mutation breeding, SDN-1 and 2 allow arbitrary modification of the target gene only, so there is a smaller chance of unintended mutation occurrence, which means they may reduce the risk of effects on biological diversity etc.

○ Mutation of gene that controls seed shattering of rice



Picture on the left:
Left: Nipponbare (Japonica)
Right: Kasalath (Indica)

Source: Saeko Konishi *et al.*, Science (2006)
(Institute of the Society for Techno-Innovation of Agriculture, Forestry, and Fisheries)

From the above understanding, regarding SDN-1 and SDN-2 that induce mutation to several bases, if it is finally confirmed that no artificial restriction enzyme is included in the final crops, the crops can be regarded as the same as those produced by conventional breeding techniques. Therefore, the Study Group considered that there are no specific concerns over the effects on biological diversity. However, currently, there may be cases where sufficient knowledge is not available on the mutated target genes. In such cases, it is appropriate to provide the regulatory authorities with the related information in advance and to receive scientific consideration from experts as necessary.

Promotion of Future R&D

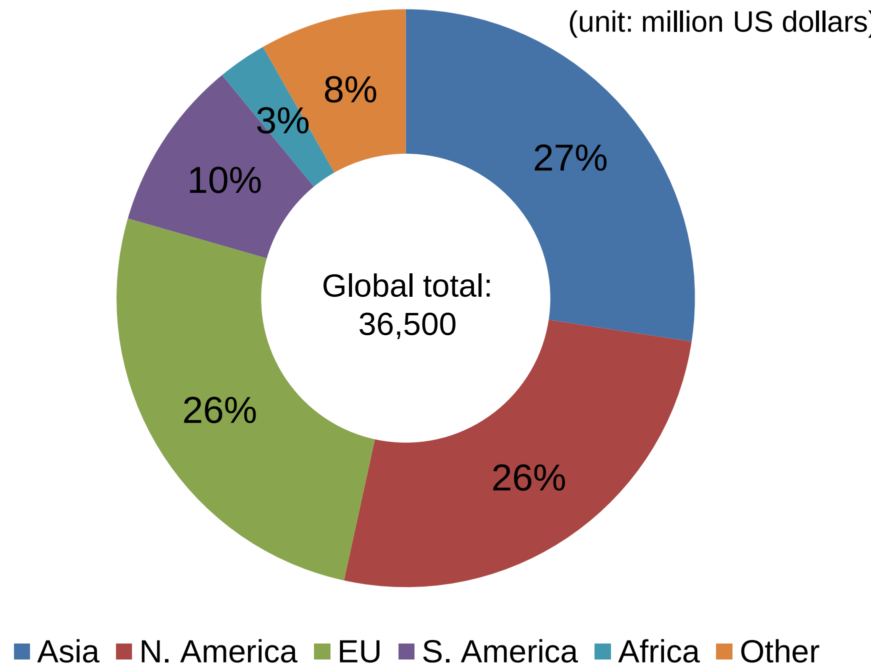
- In Japan, along with the increasing difficulty in practical cultivating GMOs domestically, the options of techniques usable for breeding main crops such as rice are limited, and it takes many years to develop new varieties.
  Regarding vegetables and flowering plants, personal breeding is actively performed and the export of seedlings is steadily increasing in recent years, especially to Asia. However, it has become difficult to obtain useful plant genetic resources from overseas as breeding materials, and there is a concern over stagnancy in the development of new varieties.
- NPBTs are techniques that can draw out the maximum potential of agriculturally useful genetic traits that exist in the same or related species, efficiently improve the quality, functionality, yield, etc. of crops, and thereby enable creation of unprecedented epoch-making varieties in a short period of time.
  Since the engineered crops do not possess transgenes at the end and may have the same gene organization as crops produced by conventional breeding, risks on the food/feed safety and the effects on biological diversity can be suppressed. Additionally, NPBTs may allow for reduction of development cost involved with the compliance with GM regulations, and there is a possibility for Japanese private companies etc. to apply NPBTs to breeding and improving various crops including vegetables and flowering plants.

○ Regulatory compliance cost for insect-resistant maize

Cost categories	Range of cost incurred ($)		
Preparation for hand-off of events into regulatory	20,000	-	50,000
Molecular characterization	300,000	-	1,200,000
Compositional assessment	750,000	-	1,500,000
Animal performance and safety studies	300,000	-	845,000
Protein production and characterization	162,000	-	1,725,000
Protein safety assessment	195,000	-	853,000
Nontarget organism studies	100,000	-	600,000
Agronomic and phenotypic assessment	130,000	-	460,000
Production of tissues	680,000	-	2,200,000
ELISA development, validation and expression analysis	415,000	-	610,000
EPA expenses for PIPs (e.g., environmental use permit)	150,000	-	715,000
Environmental fate studies	32,000	-	800,000
EU import (detection methods, fees)	230,000	-	405,000
Canada costs	40,000	-	195,000
Stewardship	250,000	-	1,000,000
Toxicology (90-day rat) – when done	600,000	-	4,500,000
Facility & management overhead costs	71,850	-	538,875
Total	**7,060,000**	**-**	**15,440,000**

Source: Nature Biotechnology, Volume 25, Number 5, May 2007

○ Scale of global seedling market (2009)



Source: Website of the Society of American Archivists

Promotion of Future R&D

○ Under the Strategic Innovation Promotion Program (SIP) of the Cabinet Office, the government (research institutions under MAFF, MEXT, etc.), academia (universities) and industry (related private enterprises such as those in the breeding industry) shall collaborate in actively implementing initiatives in the development of Japanese NPBTs, converting them into intellectual properties, creating innovative varieties of various plants including grains, vegetables and flowers through application of NPBTs, enhancing regulatory science and promoting direct dialogue with the citizens with developing science communication, etc.

○ R&D period: FY2014-2018

○ Fiscal budget: 810 million yen (FY2015)

○ Outline of the Program

1 Improvement and development of new plant breeding techniques (NPBTs)

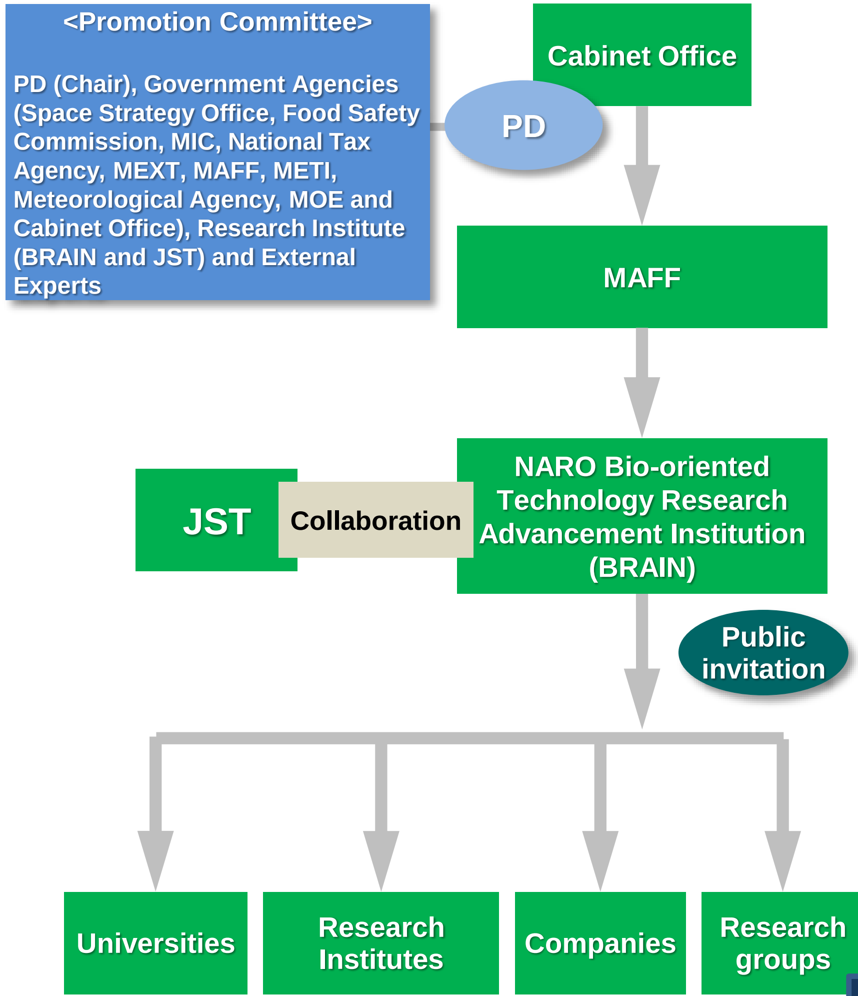
- To improve genome editing techniques such as TALEN and CRISPR/Cas9 so that they become applicable to various plants including rice and vegetables
- To newly develop Japan's genome editing
- To improve the early flowering technique for apples so that it becomes applicable to other fruit plants and trees

2 Development of epoch-making agricultural and fishery products

- To develop ultra-high-yield rice varieties
- To develop vegetables (e.g. tomato) rich in functional ingredients
- To develop tuna suited for culturing

3 Scientific research on social implementation

- To develop techniques to prove there are no transgenes and other related DNA remaining
- To accumulate scientific findings on natural mutation and artificial mutation at the nucleic acid level
- To establish methods to provide citizen with relevant information and promote communication



Initiatives towards Promoting Social Understanding

<Compliance with GM regulations>

○ Appropriate control based on the Cartagena Act is required in R&D stages, because foreign genes have been introduced to agricultural products during their breeding process even if transiently.

○ Applicants are required to consult with competent authorities prior to cultivation of new varieties and prior to its use in foods and feeds, so as to make sure whether the final products is subjected to the regulations. .

<The way of providing information and communicating with the nations>

○ It is important to promote interactive communication with various interested parties from the R&D stages and to reflect the voices of anticipation, anxiety and concerns from such parties on the R&D and the process of practical application, as many NPBTs use the latest molecular biological findings.

○ Additionally, considering the lingering social anxiety over the agricultural products and food utilizing GM techniques, the key point will be how to disseminate information and communicate in a convincing manner on:

(1) The significance of introducing NPBTs for increasing the speed of plant breeding to address the issues including the global environmental changes and food shortage; and

(2) Similar plants can be produced naturally or through conventional breeding techniques.

These information sharing should be along with showing the actual crop plants (new species). The development of new epoch-making species that brings tangible benefits to the farmers and consumers in Japan should be promoted.

○ For the reason above, it is important to continue to steadily collect related scientific findings and formulate opinions about the effects on biological diversity etc., and promote dialogue with various interested parties including a broad range of experts, consumer organization, mass media, farmers, industries etc. so as to generate a support and trust from a wide society.

<Promotion of international harmonization pertaining to regulatory framework>

○ Currently each country or region is considering the regulatory framework for NPBTs, and there is a possibility that differences among the countries/regions could cause confusion in the trade of agricultural products in the future.

○ International harmonization of regulatory oversight and scientific view in NPBTs in fora such as OECD-WG is important, as well as accelerating the activities such as the formulation of scientific opinions in Japan.