Advanced Mutation Breeding by Ion Beams

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20 March 2014


100 km
100 miles
CHINA
NORTH KOREA
SOUTH KOREA
SEA OF JAPAN
Akita
Send
TAKASAKI
TSURUGA
JAPAN
TOKYO
Yokohama
Nagoya
Mt Fuji
Hiroshima
Kobe
Kyoto
Osaka
Kitakyushu
Fukuoka
Shikoku
Mt Aso
Kyushu
EAST CHINA SEA
NORTH PACIFIC OCEAN
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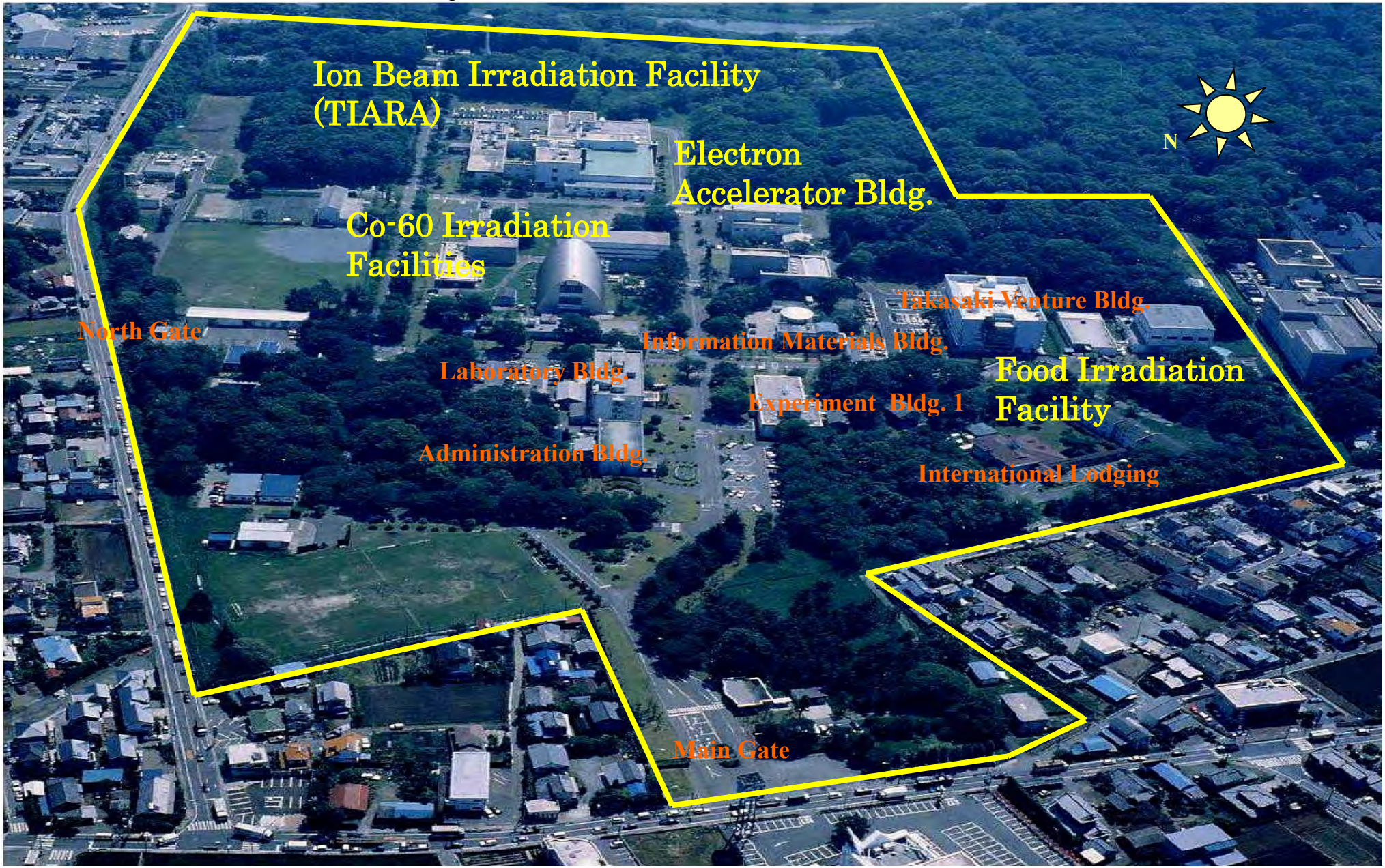
Bird's Eye View of JAEA-Takasaki



Irradiation Facilities in Takasaki

Gamma-ray Irradiation Facility

- Japan's First Large-scale Gamma-ray Irradiation Facilities
- Wide Range of Six-digit Adsorption Rate
- 8 Irradiation Rooms in 3 Facilities

Electron Beam Irradiation Facility

- Japan's First Electron Accelerator for Industrial Scale (2 MV, 30 mA)

TIARA (Takasaki Ion Accelerators for Advanced Radiation Application)

- Rapid Beam Stabilize & Exchange
- High-energy Heavy Ion Microbeam

- Heavy Ion Microbeam & Single Ion Hit
- Swift Cluster Ions

- Wide Variety of Ions Including Fullerene
- Combination Use with SEM

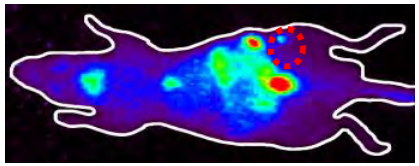
- High Spatial Resolution Light Ion Microbeam
- Micro-PIXE Analysis & Proton Beam Writing

World's First Facility Specialized for R&D of Materials and Biotechnology

- More than 80 Joint Research Activities: collaborated with Private companies, Public research institutes, Universities, etc.
- Irradiation service: 250 subjects（65 millions yen Revenue ）

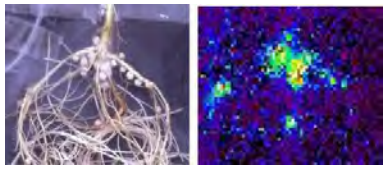
Recent Activities in Takasaki

Observe



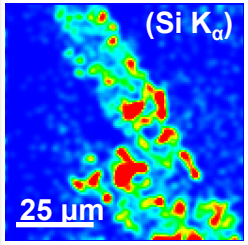
Small Tumors

Nobel RI-labeled compound



Nitrogen fixation

Imaging of plant functions using Positron Imaging



White lung

Detection of elements using Micro-PIXE

Create（Modify）

New features

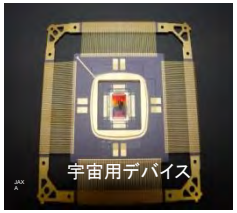
Fuel cell electrolyte membrane

Adsorbent for Scandium

Hydrogen separation membrane

Biodegradable dummy lens

Resistance evaluation to radiation



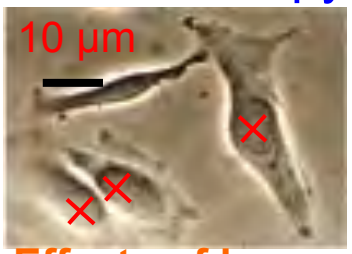
Semiconductors for aerospace

Evaluation of materials used in Accelerator

Radiation grafting & cross-linking

Cure

Cancer therapy



Effects of heavy ion micro beams

New mutants (low cadmium rice)

Ion beam breeding

FNCA Mutation Breeding Project

IAEA Technical Meeting

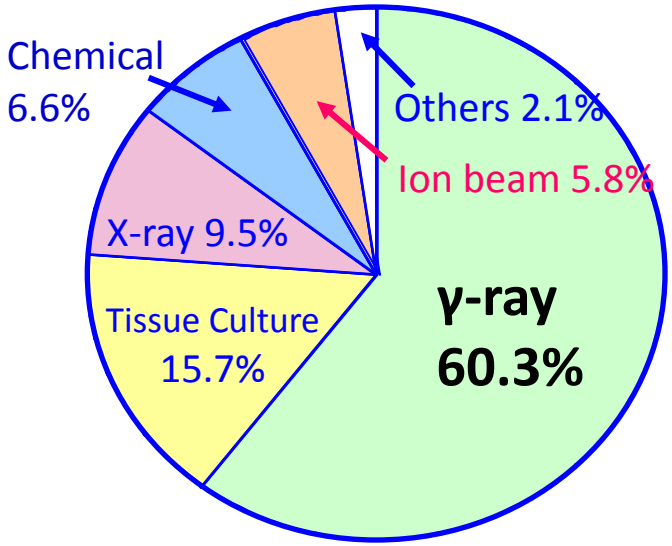
13-16 August 2013, Mongolia

Mutation Breeding in Asia

Researched by A. Tanaka (FAO/IAEA Database, October 2011)

Country	No. of cv. Total	Rice	Barley	Wheat	Maize	Soybean	Chrysanthemum
All countries	3212	815	304	252	89	170	277
China	808	290	7	162	47	79	21
Japan	481	222	10	7	0	30	56
India	329	59	13	4	0	7	46
Russia	215	6	29	36	5	9	17
Netherland	176	0	1	0	0	0	80
Germany	171	0	66	2	0	1	34
USA	139	36	13	4	0	0	1

Bangladesh	44
Indonesia	29
Korea	35
Pakistan	53
Thailand	20
Viet Nam	55



More than half varieties with ion beams created by using TIARA

Mutagen (Japan)

Nakagawa, TechnoInnovation (2007)

Mutation induced by Ion Beams

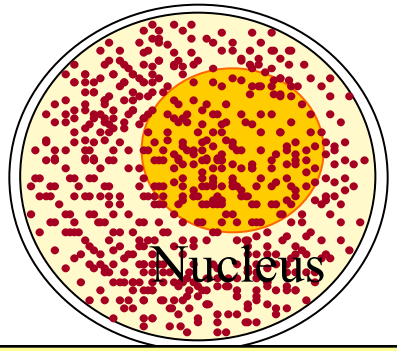
Energy deposition

γ-rays: low LET radiation

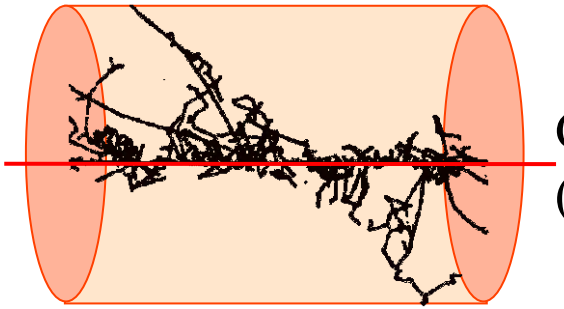
300nm

spur (~3nm)

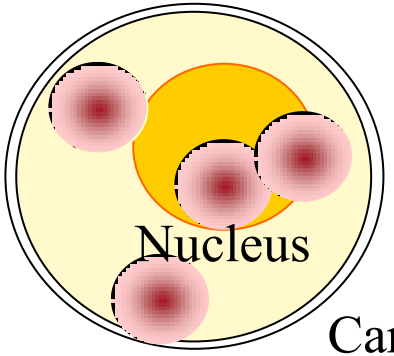
Irradiation to cell



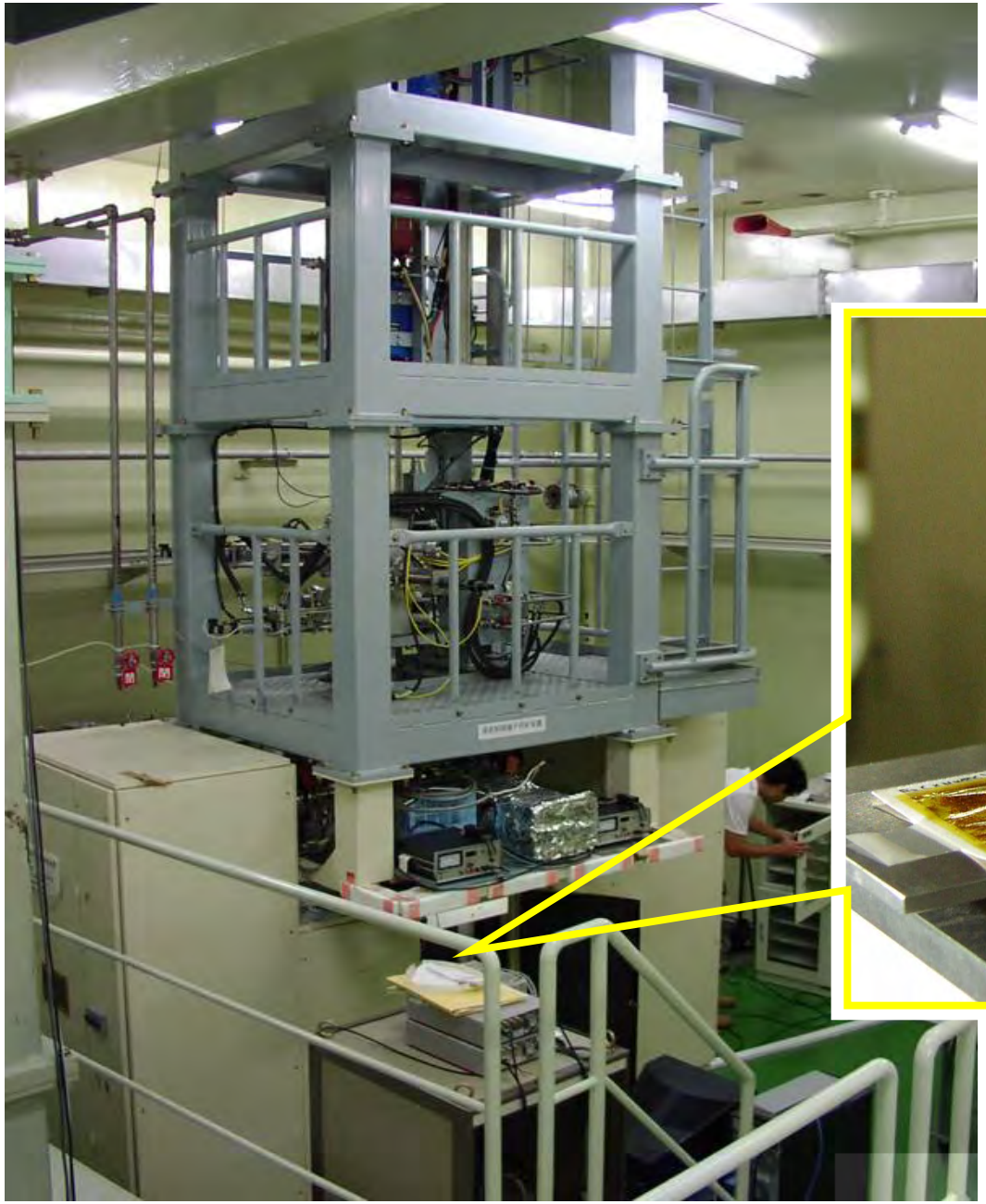
Can ion beams cause different mutation?



Core
(~3nm)

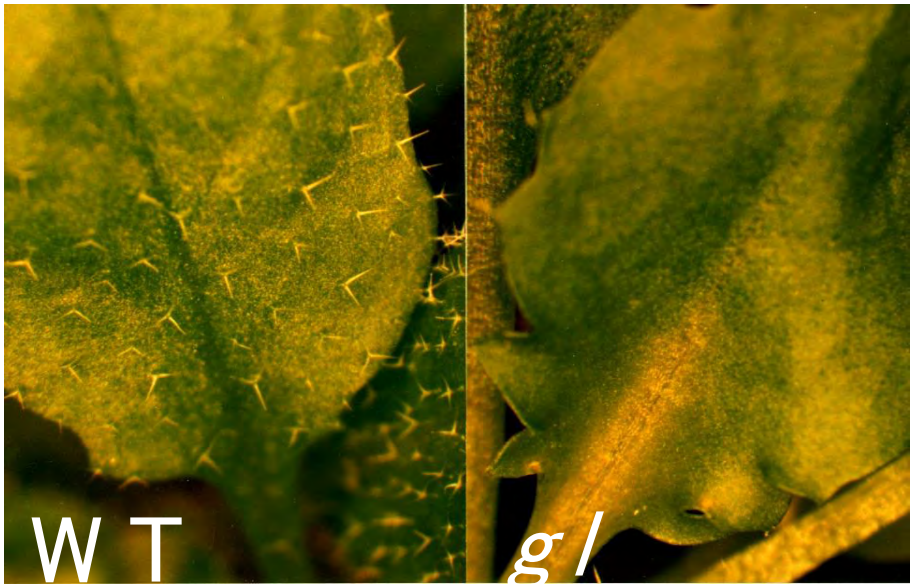


Carbon ions: 4 tracks/Gy



Started from 1991
by using TIARA

Arabidopsis *tt* and *gl* mutant phenotypes



Mutation frequency

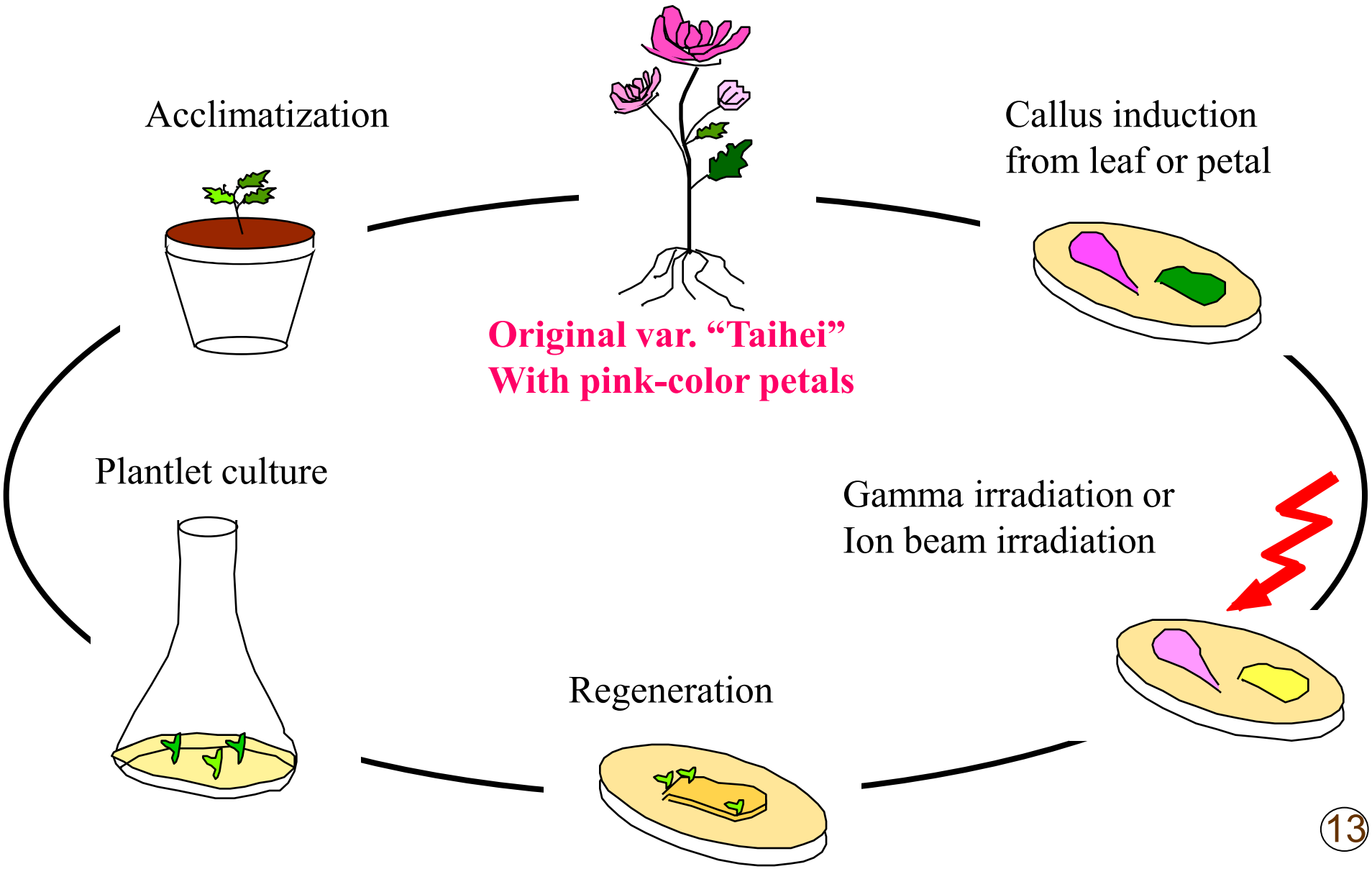
Mutation frequencies for mutant groups induced by carbon ions and electrons

Mutagen (Dose)	No. of M1seeds sown	No. of M2 plants	Mutant group (loci)	No. of mutants in M2	Mutation frequency/ locus/ diploid cell/ dose(Gy) ($\times 10^{-6}$)
Carbon ions (150Gy)	26,200	104,088	*tt3, tt4, tt5, tt6, tt7, tt18,tt19 gl1, gl2, gl3, ttg1, ttg2*	88	1.9
Electrons (750Gy)	c.a.15,600	80,827	*tt3, tt4, tt5, tt6, tt7, tt18,tt19 gl1, gl2, gl3, ttg1, ttg2*	18	0.097

- 20 times higher per dose, and
  4 times higher per plant (seed)

Shikazono et al. Genetics(2003)
Shikazono et al. JXB (2005)

Mutation breeding system in chrysanthemum



Mutation spectrum

Table Flower mutation of regenerated plants from floral organ culture induced by carbon ions and γ rays

Mutagen	Mutation frequency of mutated character (%)					
	White	Light pink	Dark pink	Orange	Yellow	Complex/Stripe
Unirradiated	0	0.3	0	0	0	0
γ rays	0	27.7	2.1	0	0	0

First cultivation of new chrysanthemum varieties using ion beams (1998)

Flower mutation of carnation regenerated from leaf cultures treated by mutagen

Mutagen	Mutation frequency (×10^{-1} %)										
	Flower color									Shape	
	Light pink	Pink	Dark pikn	Red	Salm-on	Yel-low	Cream	Stripe	Com-plex	Round petals	Dianthus type petals
EMS	0	5.2	0	1.0	0	0	0	3.1	0	0	0
Soft X-rays	1.7	8.4	0	3.4	0	0	0	0	0	0	0
Gamma-rays	1.7	9.6	0	1.7	0	0	0	0	0	0.9	0
Carbon ions	3.5	4.7	1.2	3.5	2.4	1.2	1.2	3.5	2.4	4.7	2.4

Mutation spectrum is much broader

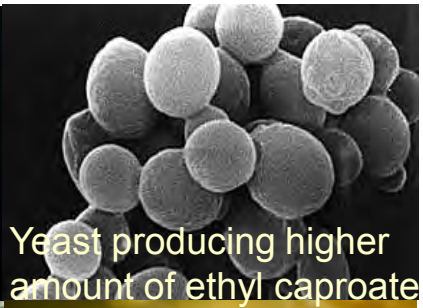
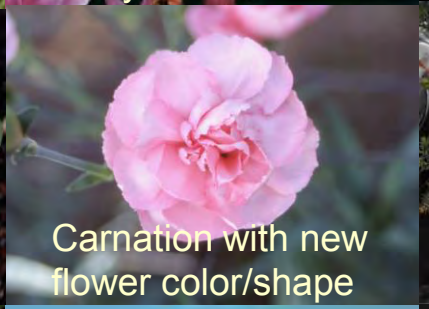
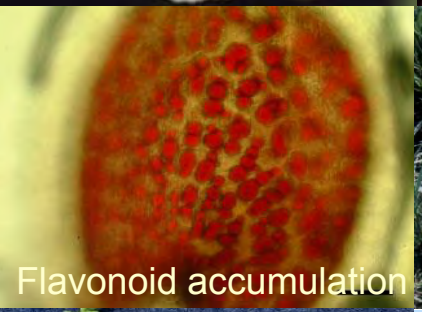
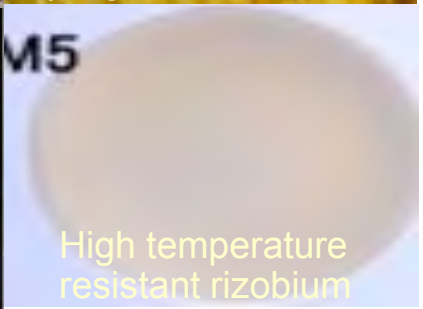
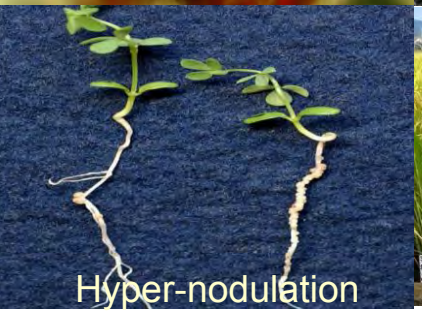
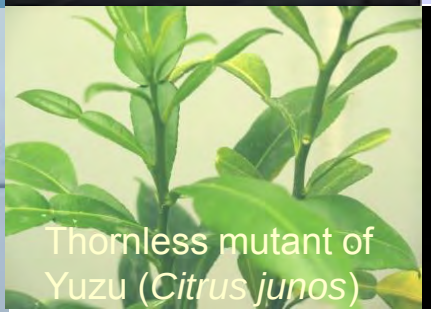
Okamura et al. NIMB (2003)

New varieties of carnation induced by ion beams

Commercialized in 2002 EU & Japan ($ 13 million in 2010)

with KIRIN Brewery Co.Ltd.

Novel mutants and varieties

Model plants	Crops	Ornamentals	Trees & fruit	Microorganism
 UV-B resistant	 Blast resistant rice Bacterial-Leaf-Blight resistant rice	 New flower-color chrysanthemum	 Ficus with high NO_2 assimilation	 Yeast producing higher amount of ethyl caproate
 Frilled flower	 Potato Virus Yellow resistant tobacco	 Carnation with new flower color/shape	 Wax –rich conifer	 Protease high-producing *Aspergillus*
 Flavonoid accumulation	 Yellow Mosaic Virus resistant barley	 Chrysanthemum with reducing axillary buds	 Melon with good growth in low temperature	 High temperature resistant rizobium
 Hyper-nodulation	 Low-nitrogen-fertilizer grown rice	 Osteospermum with new pastel coloration	 Thornless mutant of Yuzu (*Citrus junos*)	 Oyster mushroom with new character

with Kagoshima Biotechnology Institute

Greatest goal in chrysanthemum: reducing axillary buds

Original Var.

Ion beams

Reduced Axillary Buds

Ion beams

Reduced Axillary Buds
+
Early Flowering

- Products: 22 Million flowers (2012)
- Requested license from more than 35 marketing cooperatives

Ueno et al. JJSHS (2013)

Saving energy: Netted melon with a large fruits under low temperature (cut down on heating expense)

Shizuoka prefecture

静岡新聞

イオンビーム育種活用

省エネ・温室メロン誕生へ

低温でも玉伸び上々

磐田の県農試

「重油節約」農家も期待

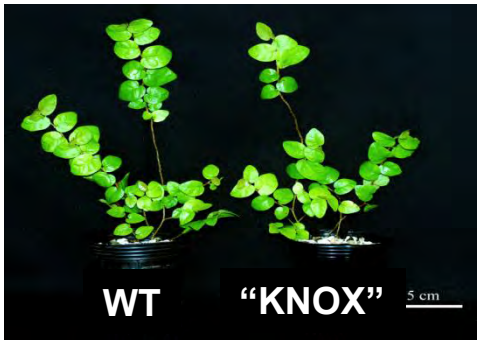
New variety as “Shizuiku No.1 (静育 1 号)” was established in 2011.

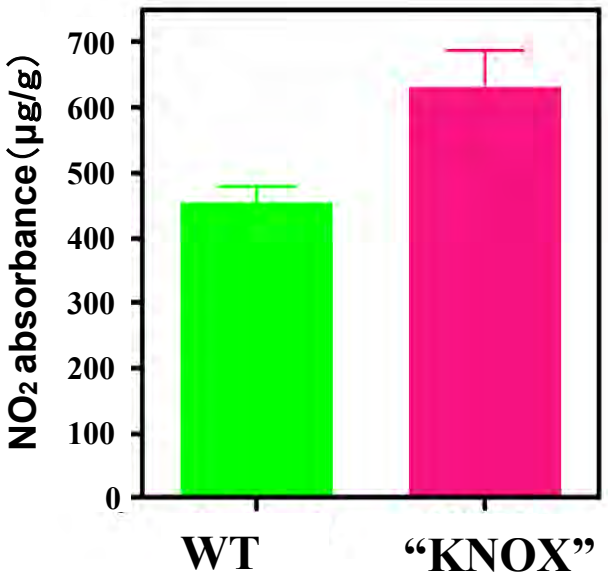
- Grown in 2℃ below & 20 % cost saving

New Variety

Phytoremediation of air pollution by greening

with Hiroshima University



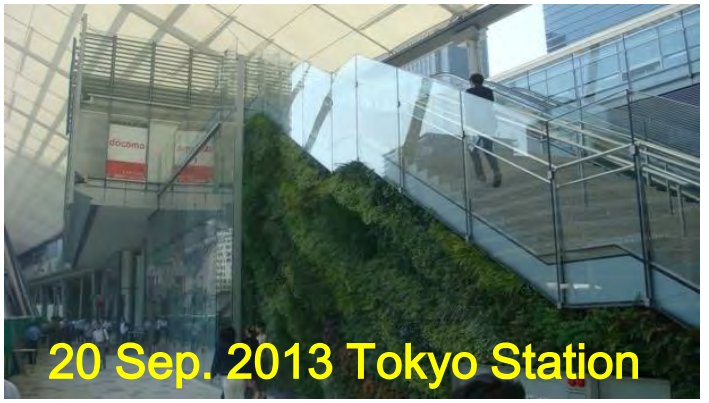


40-80% Up of the capability to assimilate atmospheric nitrogen dioxide (NO_2)

Press release (Aug. 2007)

Takahashi et al. J. Phytoremediation (2012)

Technology transfer (2009) & Commercialized (2010)



Utilized for wall greening, green roof, etc.

A Solution to Eutrophication
- Rice breeding suitable for low nitrogen input -

with Shiga Prefecture

Original var.
"Akino-uta"

Low-nitrogen-fertilizer grown rice mutant var.

2008.10.1 Press Announcement

New Variety

Food safely & security: development of low-cadmium Koshihikari rice

NIAES

東京大学 THE UNIVERSITY OF TOKYO

2012.3 Press release

elease

Conclusive evidences

1. **The same yield and good taste as original Koshihikari**
2. **Three independent mutants on the same gene:** *transporter responsible for manganese*

Ion beam breeding is definitely suitable for staple grain !

allowed
cent.

Ishikawa et al. Proc. Natl. Acad. Sci.USA (2012)

Microbial Ion Beam Breeding in Yeast

Collaborative research with the Gunma Industrial Technolgy Center

Aims

Development of a new variety superior to the Gunma prefecture original yeast「Gunma KAZE Yeast」

New variety No.277

Target phenotype :

- Improvement of alcohol fermentation property
- High production of good flavor component

(Increase of ethyl caproate → Fruity flavor)

Current status

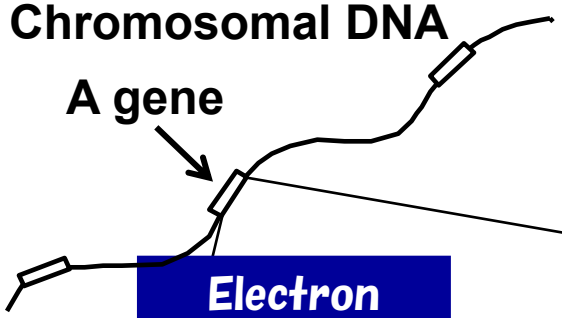
2012.12 Press release

Success in obtaining a new mutant variety that produces a higher amount of ethyl caproate than 「Gunma KAZE Yeast」

Now on sale! (from April 2013)

Intragenic point-like mutation

TT4 locus (395 a.a.)



Electron
TC ⇒AA
Ser^{196}⇒Asn^{196}

Carbon ion
A deletion
at 259 a.a

Carbon ion
8 base deletion ⇒A
at 104 a.a

Intron

AAATACACCTAACTTGTTTAGTACACAACAGCAACATCAAACTCTAATAAACCCAAGTTGGTGTATACTATAATGGTGAT
GGCTGGTGCTTCTTCTTTGGATGAGATCAGACAGGCTCAGAGAGCTGATGGACCTGCAGGCATCTTGGCTATTGGCACTGCT
AACCCTGAGAACCATGTGCTTCAGGCGGAGTATCCTGACTACTACTTCCGCATCACCAACAGTGAACACATGACCGACCTC
AAGGAGAAGTTCAAGCGCATGTGTACGTCTTATTAACTTCTACTTTCATTTCCTTTGGCATATATCTTCATTCACATAGTTT
AGCTAACAAGTATTTACTATTCACAGGCGACAAGTCGACAATTCGGAAACGTCACATGCATCTGACGGAGGAATTCCTCAA
GGAAAACCCACACATGTGTGCTTACATGGCTCCTTCTCTGGACACCAGACAGGACATCGTGGTGGTCGAAGTCCCTAAGCT

Exo

TCGTTGTCTGCTCTGAGATCACAGCCGTTACCTTCCGTGGTCCCTCTGACACCCACCTTGACTCCCTCGTCGGTCAGGCTCTT
TTCAGTGATGGCGCCGCCGCACTCATTGTGGGGTCGGACCCTGACACATCTGTCGGAGAGAAACCCATCTTTGAGATGGTGT
CTGCCGCTCAGACCATCCTTCCAGACTCTGATGGTGCCATAGACGGACATTTGAGGGAAGTTGGTCTCACCTTCCATCTCCT
CAAGGATGTTCCCGGCCTCATCTCCAAGAACATTGTGAAGAGTCTAGACGAAGCGTTTAAACCTTTGGGGATAAGTGACTG
GAACTCCCTCTTCTGGATAGCCCACCCTGGAGGTCCAGCGATCCTAGACCAGGTGGAGATAAAGCTAGGACTAAAGGAAGA
GAAGATGAGGGCGACACGTCACGTGTTGAGCGAGTATGGAAACATGTCGAGCGCGTGCGTTCTCTTCATACTAGACGAGAT
GAGGAGGAAGTCAGCTAAGGATGGTGTGGCCACGACAGGAGAAGGGTTGGAGTGGGGTGTCTTGTTTGGTTTCGGACCAGG
TCTCACTGTTGAGACAGTCGTCTTGCACAGCGTTCCTCTCTAAACAGAACGCTTGCCTTCTATCTGCCTACCTACCTACGCA
AAACTTTAATCCTGTCTTATGTTTTATATAATATAATCATTATATGTTTACGCAA TAATTAAGG

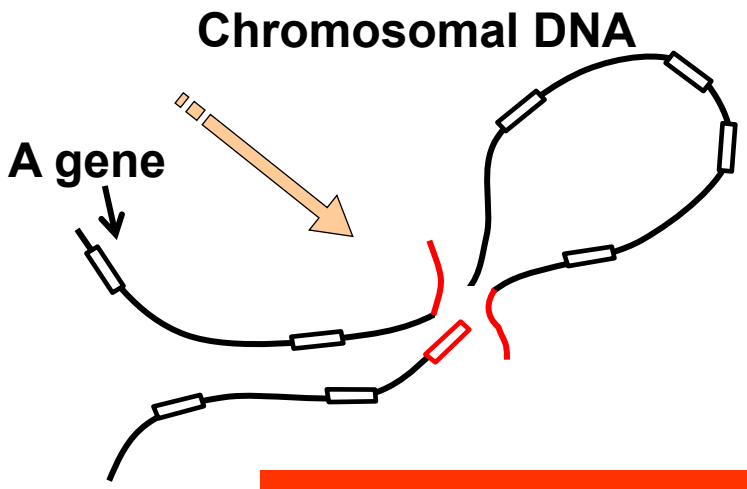
Ion beams induce '*deletion*', leading to '*null mutation*'

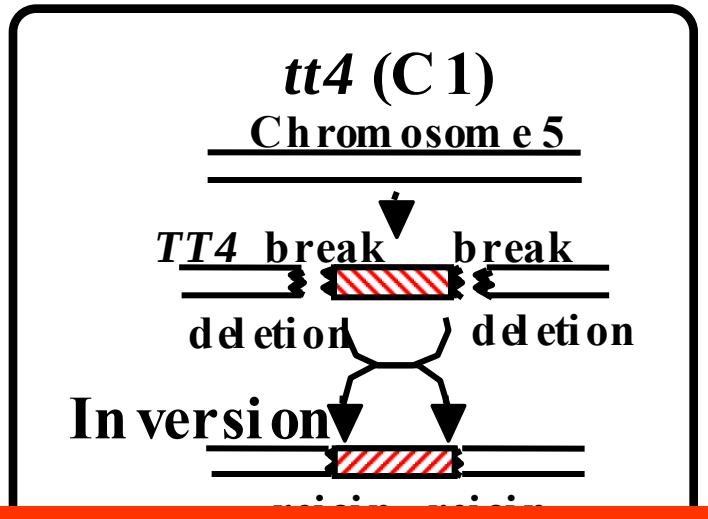
Electron
T ⇒A
$Ileu^{252}$⇒Asn^{252}

Carbon ion
A deletion
at 259 a.a.

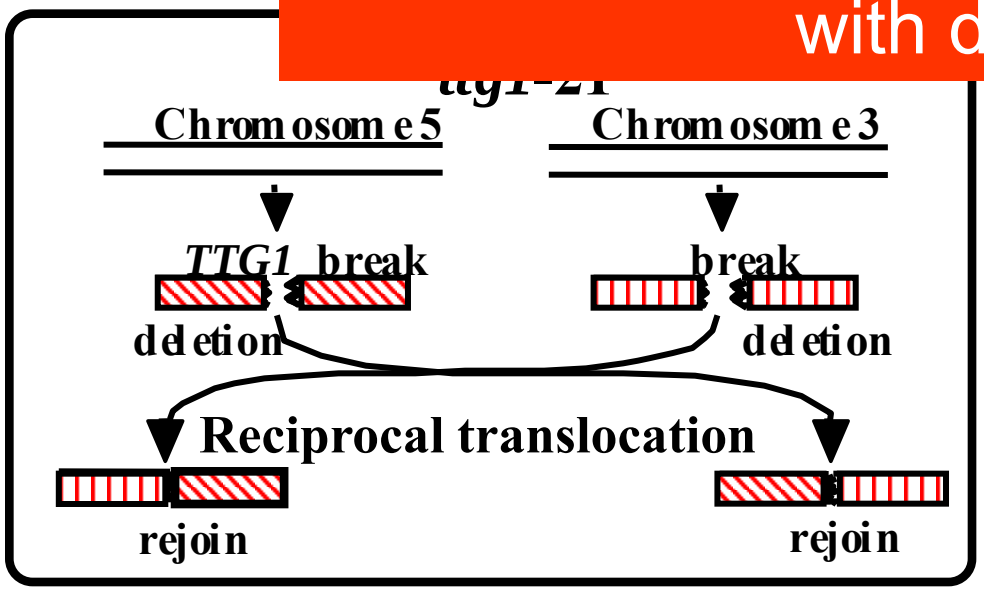
Carbon ion
22 base deletion
at 344 a.a.

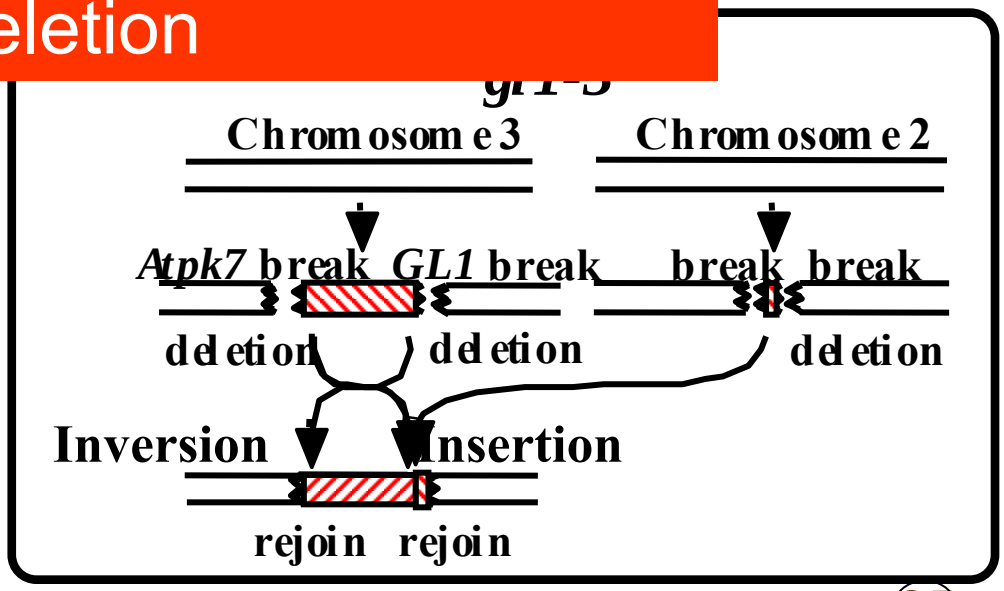
Large DNA alteration





Ion beams induce large DNA alteration with deletion

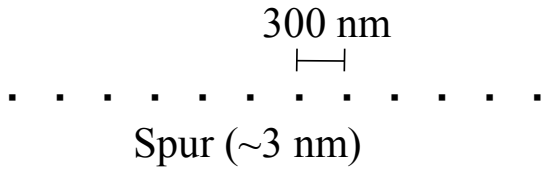




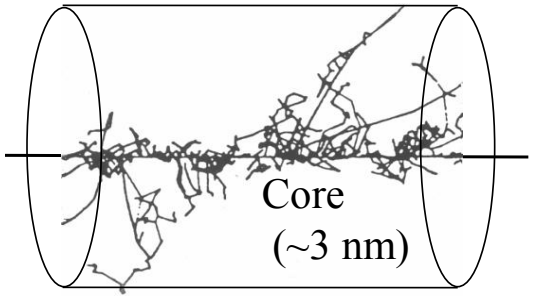
Effect of ion beams for mutation induction

Energy deposition

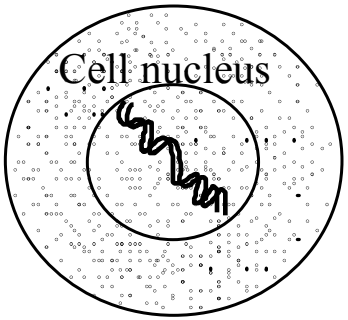
Gamma rays



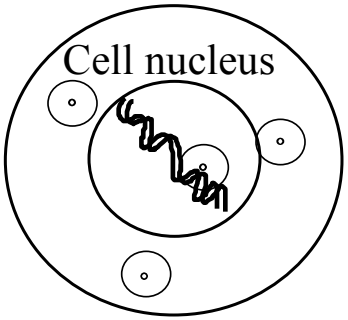
Ion beams



Irradiation to cell



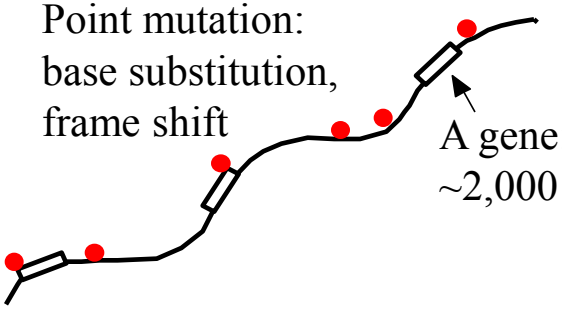
Gamma rays:
2,000 spurs/Gy



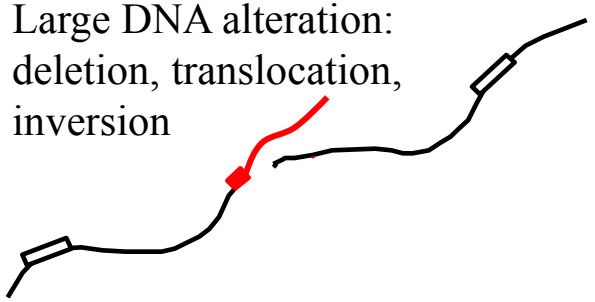
Carbon ions:
4 tracks/Gy

Mutation

Point mutation:
base substitution,
frame shift



Large DNA alteration:
deletion, translocation,
inversion

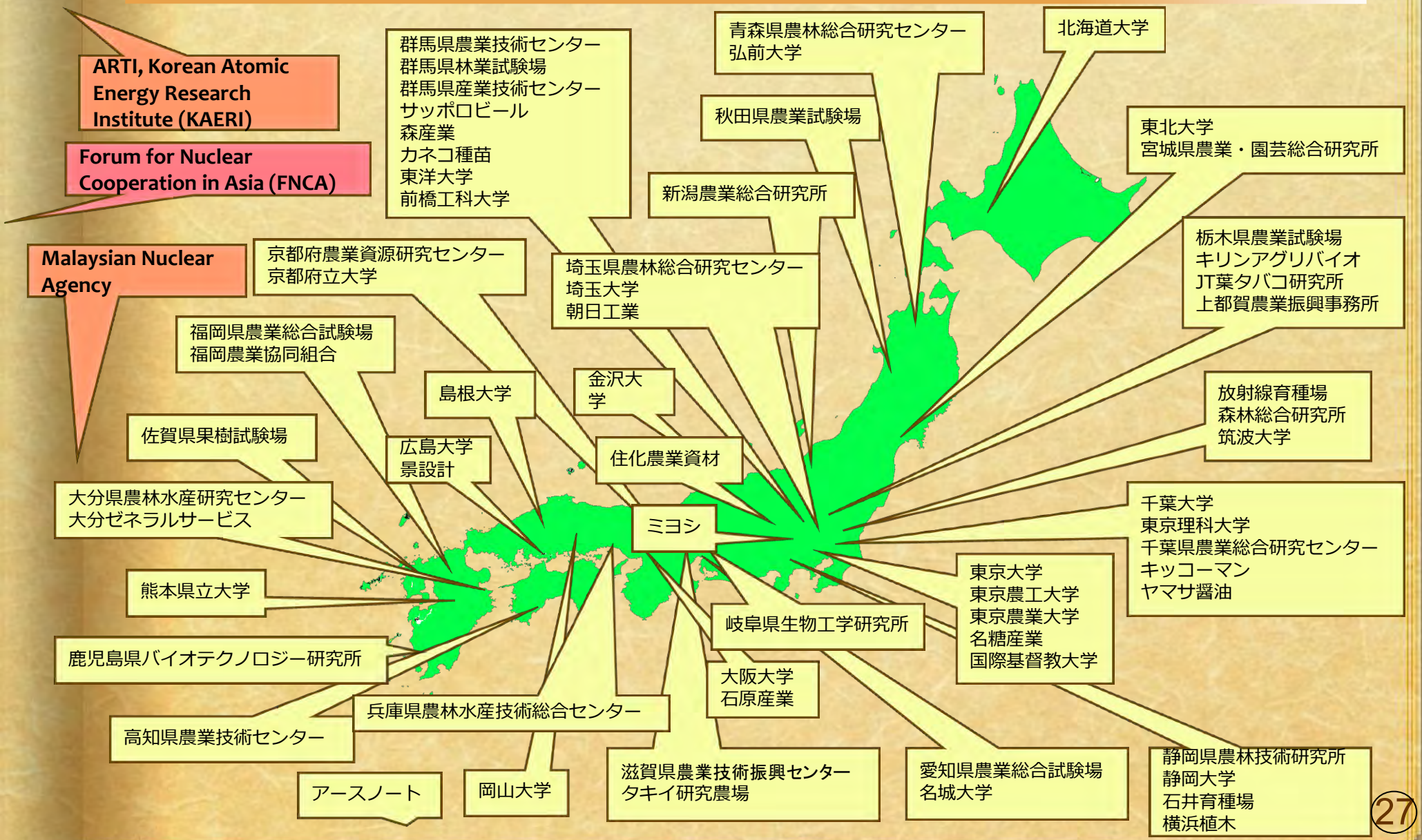


Ion-beam induced mutation for plant breeding

1. **High mutation rate** : Small samples & spaces for screening
2. **Broad mutation spectrum** : Producing new varieties and mutants
3. **Minimum No. of DNA damage** : Pinpoint-breeding without bad characters

Tanaka et al. JRR (2012)

TIARA Users for Ion Beam Breeding in Japan



Malaysia：gamma-rays, ion beams

D. jayakarta

Original Variety

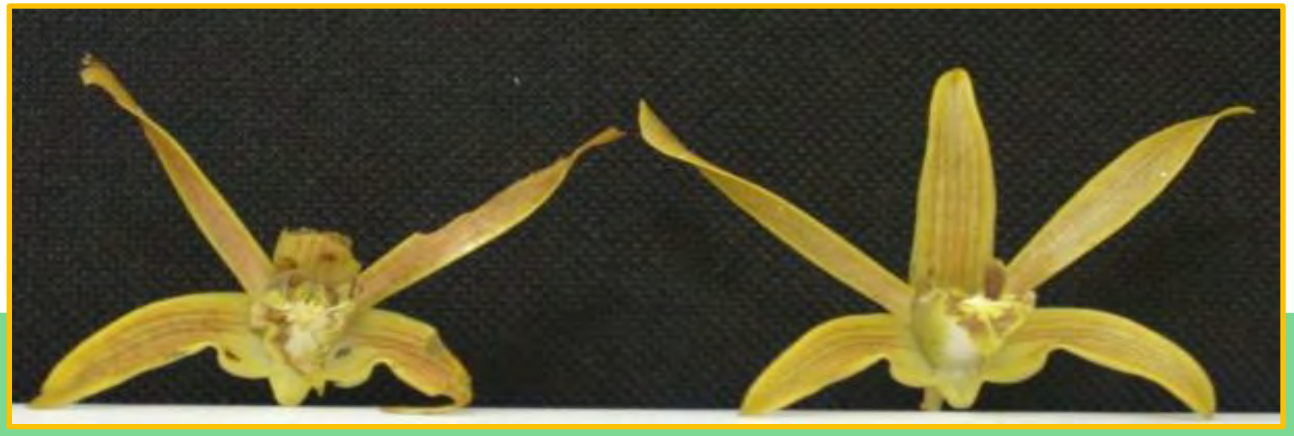
Mutant (Gamma-ray)

2 mutant lines resistant to thrips

D. mirbellianum

Original Variety

Mutant (Ion-beam)



1 mutant line resistant to both mites and thrips

technology transferred to commercial laboratory at Nuclear Malaysia

Economic Effect:
USD 160,000/year（2014-）

Vietnam

Original var. *Khang dan*

28 Sep. 2010

Lodging-resistant mutant (Gamma-rays)

Lodging-resistant mutant (Ion beams)

High yielding

Bangladesh

Salt tolerance & High yielding