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DCC, Korea

Start of Mutation Breeding Research Using Ion Beam in Korea

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KAERI Korea Atomic Energy Research Institute

RFT Road to Fine Tomorrow

Contents

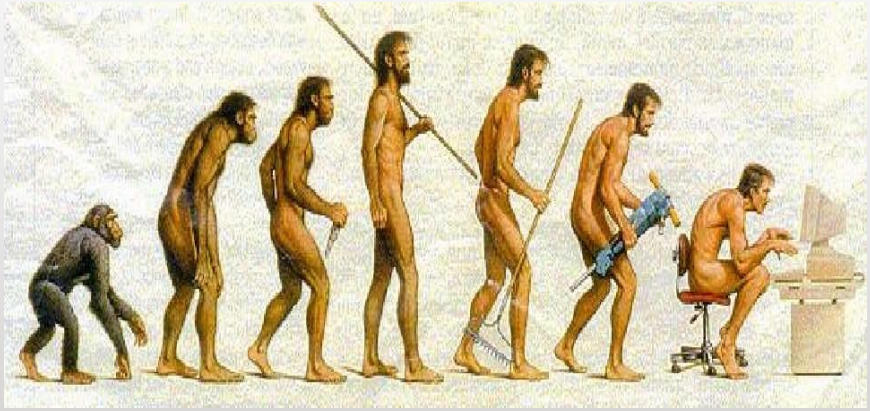
I. Mutation Breeding using Radiation

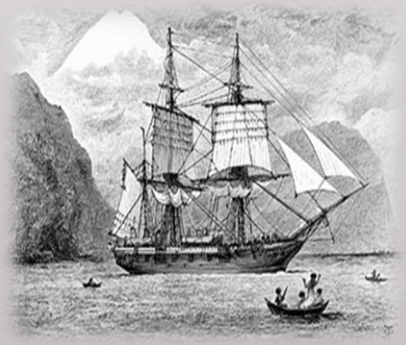
Evolution & Breeding

Darwin's Theory of Evolution

(The Origin of Species, 1959)

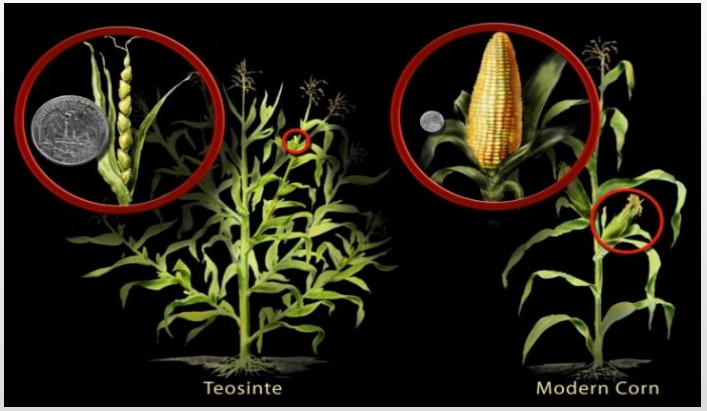
Natural variation (mutation, natural crossing) + Natural Selection…





Breeding

Natural variation+ **Artificial variation + Artificial selection, …**



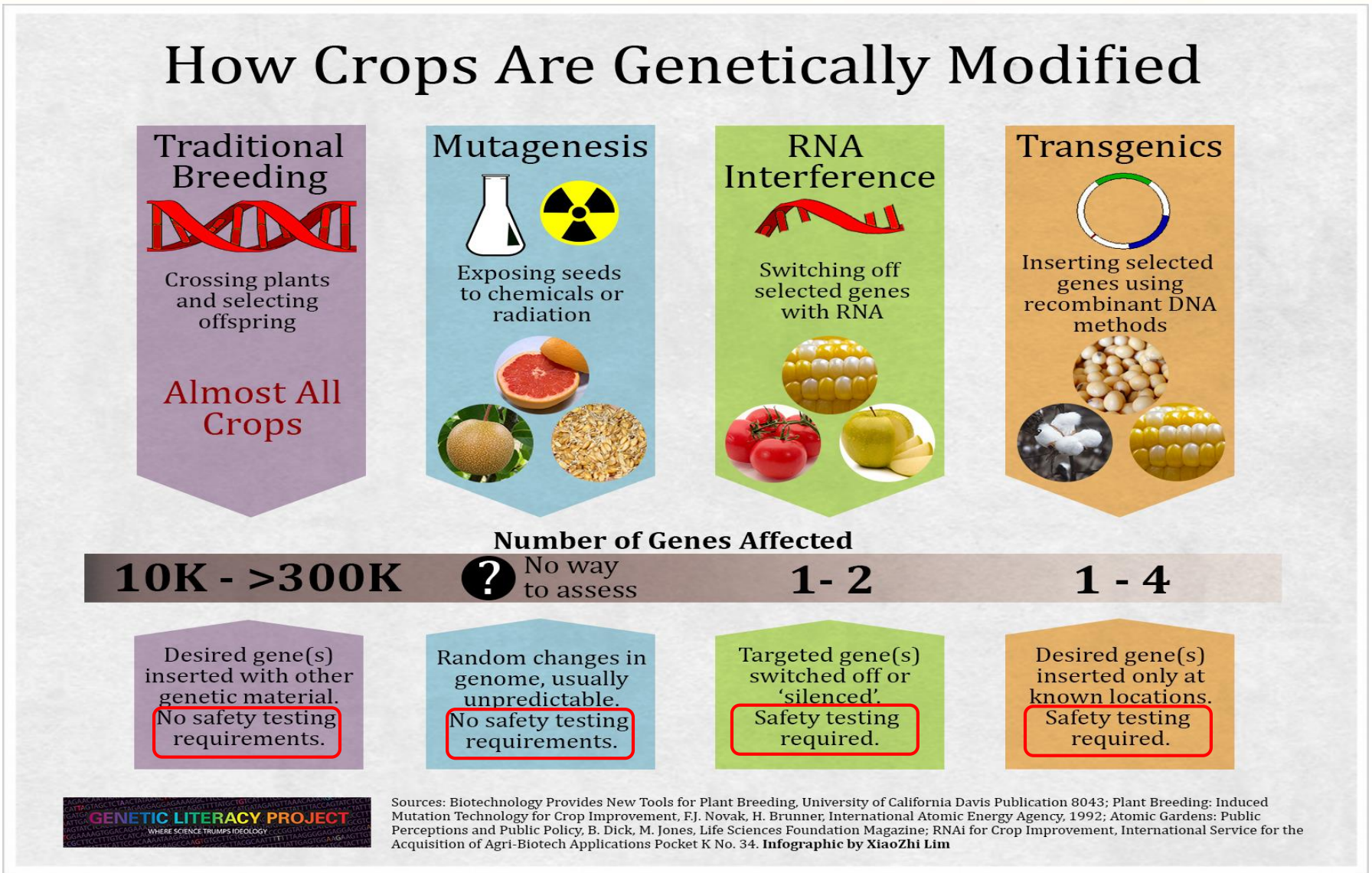
Mutation breeding

Breeding Techniques

Development new genetic resource by change of gene & chromosome

- **Cross breeding (+, –) :** Recombination of genes in a species by sexual reproduction (♀ + ♂)
- **Transgenic (GMO) (+) :** Introduce of some genes from other species by bio-engineering technology
- **Mutation Breeding (–) :** Self gene change (deletion) without genetic recombination by treatment of mutagen (radiation, chemical, etc)

Safety


How Crops Are Genetically Modified
Traditional Breeding
Crossing plants and selecting offspring
Almost All Crops
Mutagenesis
Exposing seeds to chemicals or radiation
RNA Interference
Switching off selected genes with RNA
Transgenics
Inserting selected genes using recombinant DNA methods
Number of Genes Affected
10K - >300K
?
No way to assess
1- 2
1 - 4
Desired gene(s) inserted with other genetic material.
No safety testing requirements.
Random changes in genome, usually unpredictable.
No safety testing requirements.
Targeted gene(s) switched off or 'silenced'.
Safety testing required.
Desired gene(s) inserted only at known locations.
Safety testing required.
GENETIC LITERACY PROJECT
WHERE SCIENCE TRUMPS IDEOLOGY
Sources: Biotechnology Provides New Tools for Plant Breeding, University of California Davis Publication 8043; Plant Breeding: Induced Mutation Technology for Crop Improvement, F.J. Novak, H. Brunner, International Atomic Energy Agency, 1992; Atomic Gardens: Public Perceptions and Public Policy, B. Dick, M. Jones, Life Sciences Foundation Magazine; RNAi for Crop Improvement, International Service for the Acquisition of Agri-Biotech Applications Pocket K No. 34. Infographic by XiaoZhi Lim

Development of Radiation Breeding

•1928: X-ray induced mutation in maize and barley proved by L. J. Stadler

Low LET

X-ray

Since 1920s

Low LET

γ-ray

Since 1950s

High LET

Heavy ion beam (Japan)

Since 1990s

High LET+ Microgravity..

Space breeding (China)

Since 1990s

•1936: First mutant variety released in Tabacco, Chloria

Radiation : Gamma Ray (acute & choric irradiation)

Gamma-Phytotron (KAERI)

Gamma Room (KAERI)

acute

Gamma field (JAPAN)

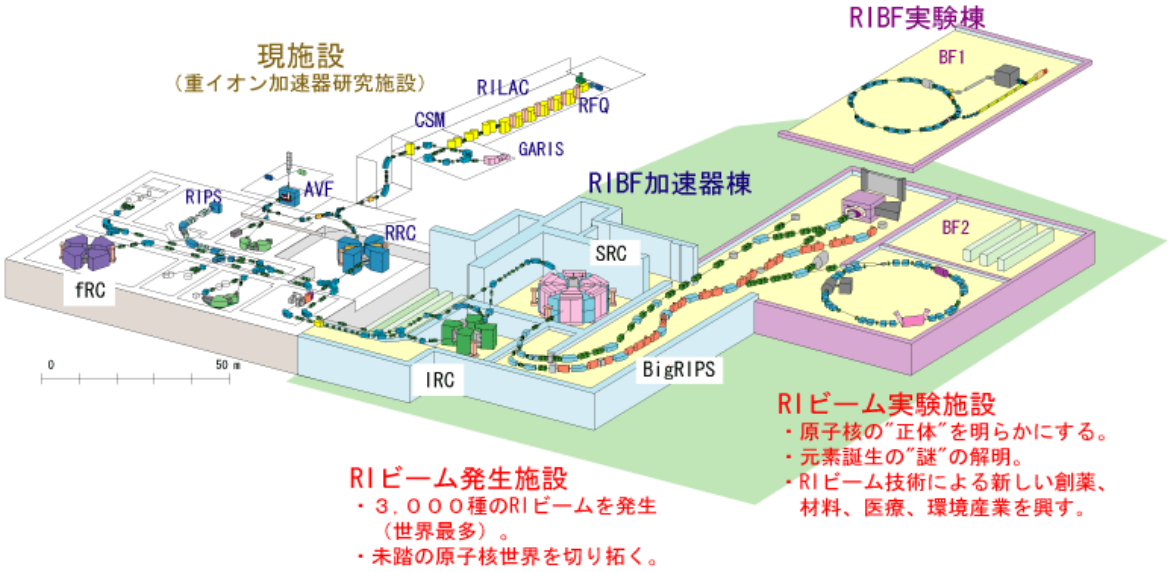
Gamma greenhouse (Malyasia)

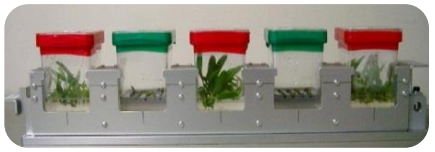
Chronic gamma irradiation house (Thailand)

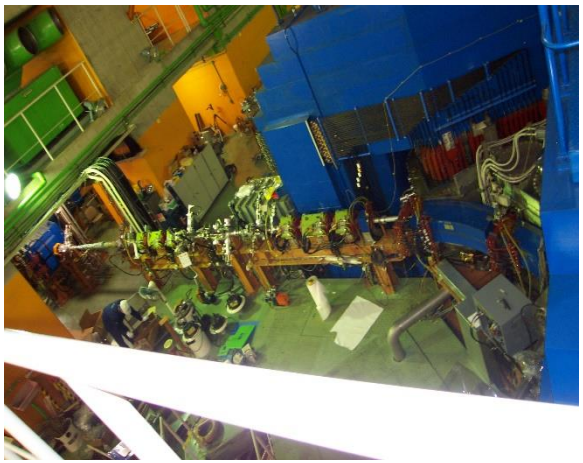
Gamma Cell (Thailand)

Radiation : Heavy Ion Beam Accelerator (Japan)

RIKEN- Ring Cyclotron



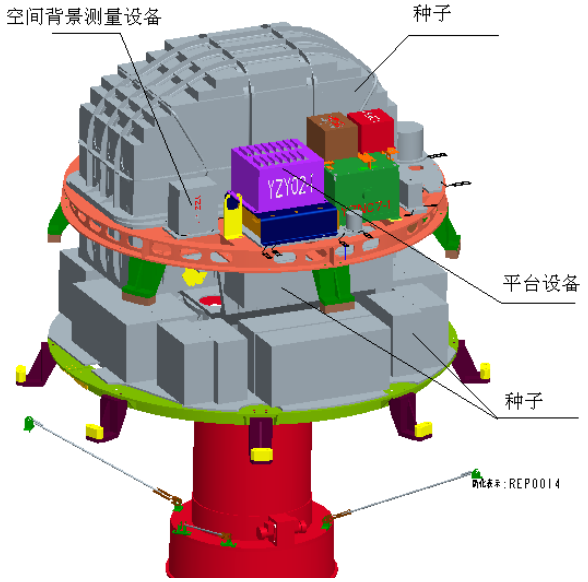




Space Breeding in China

- **Development of useful variations of plants induced in the space environment* that can be reached by the recoverable spacecraft to create new germplasms on the ground, then to develop new crop varieties**

  ***Cosmic radiation, Microgravity, Weak geomagnetic field, Supervacuum, Superclean**



Recoverable Part of Seed-loading Satellite after Space Flight (Shiqian No. 8, 2006)

Mutation Breeding Crop Varieties

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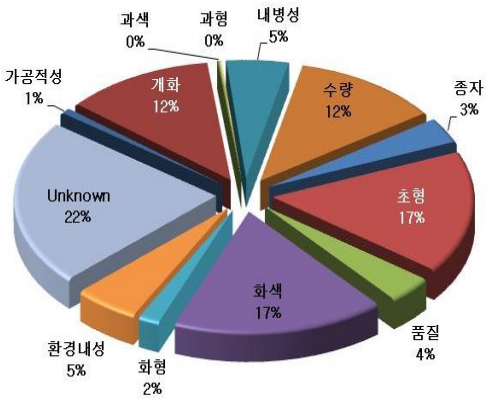
Joint FAO/IAEA Programme
Database of Mutant Variety and Genetic Stocks

(FAO/IAEA DB, October 2016)

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

Viet Nam	55
Pakistan	53
Bangladesh	44
Korea (12th)	40
Indonesia	29
Thailand	20



II. Heavy Ion Beam Application for Mutation Breeding (Japan)

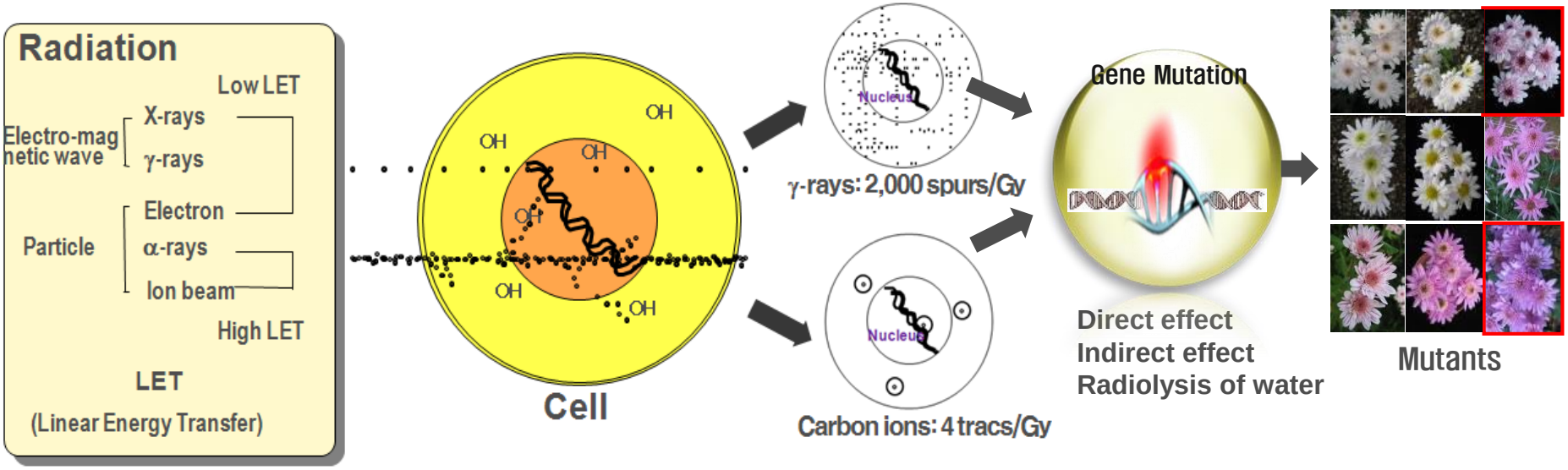
Accelerators for Heavy Ion Beam Breeding in Japan

	Facility	Ion Source	Energy (MeV/nucleon)	LET (keV/um)	Range in water (mm)	Plant material
	RIBF (RIKEN)	C N Ne	135 135 135	23 31 62	40 34 23	Arabidopsis (seed) Tobacco (seed) Chrysanthemum (cultured)
	TIARA (QST)	He C	100 18.3	8.9 113	6.2 2.2	Buckwheat (seed) Arabidopsis (seed)
	W-MAST (WERC)	H C	200 41.7	0.5 52	256 5.3	Chrysanthemum (cultured)

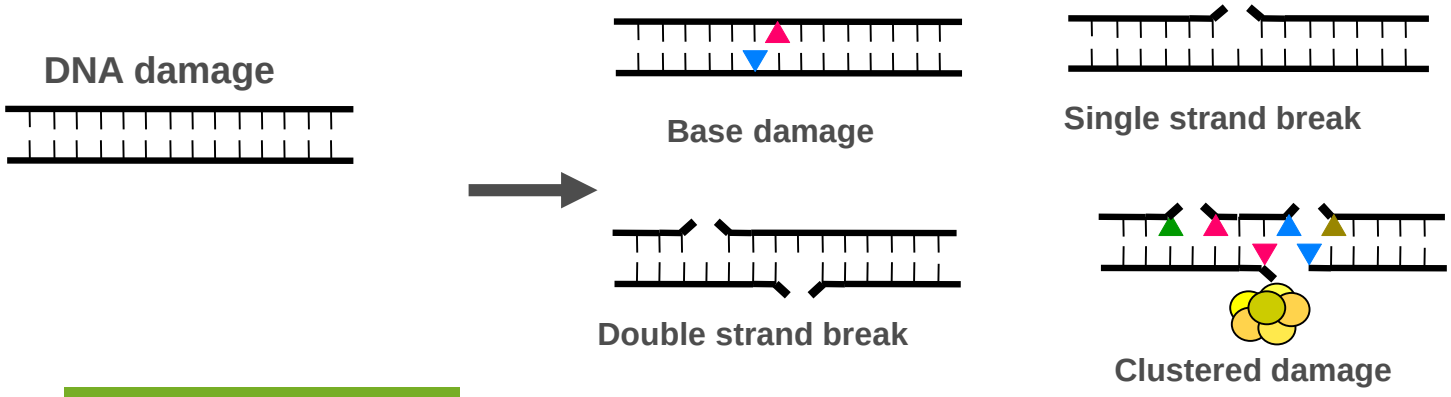
✓TIARA : Takasaki Ion Accelerators for Advanced Radiation Application (JAEA -> QST)
✓W-MAST : Wakasawan Energy Research Center, Multipurpose Accelerator System with Synchrotron and Tandem

Biological Effects of Radiation

Mutation by radiation

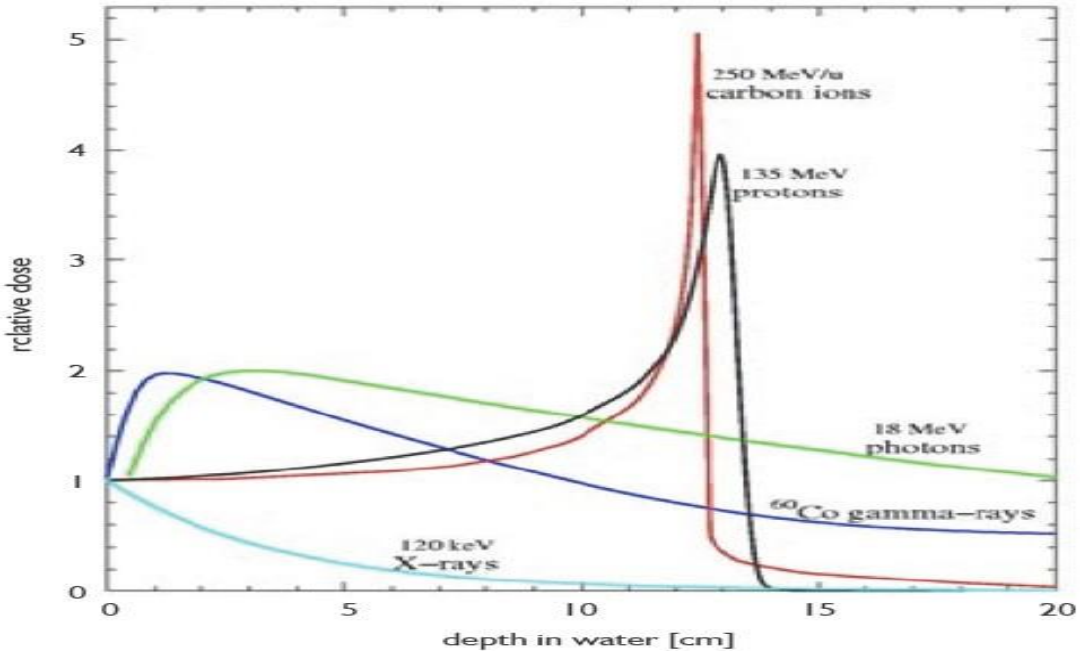


Gene Mutation

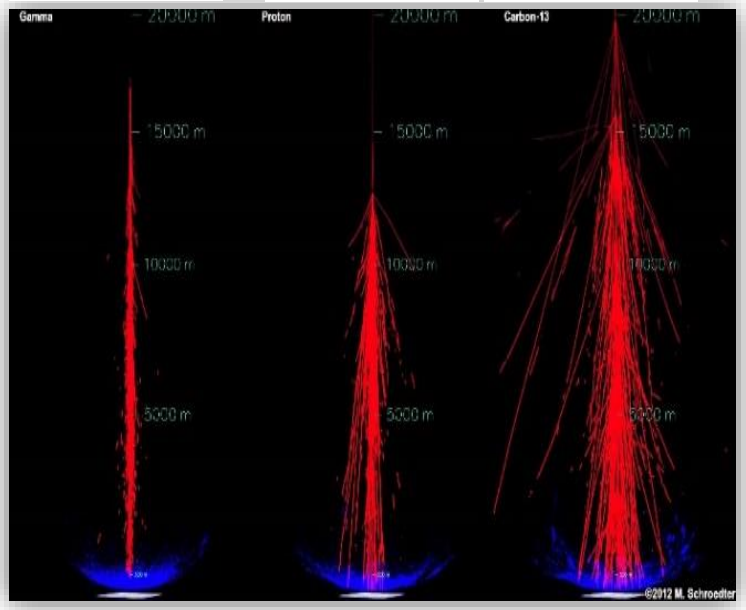


From Dr. Tanaka A. (JAEA)

Characteristics of Ion Beam (1) : Bragg Peak



Gamma ray | Proton | Carbon

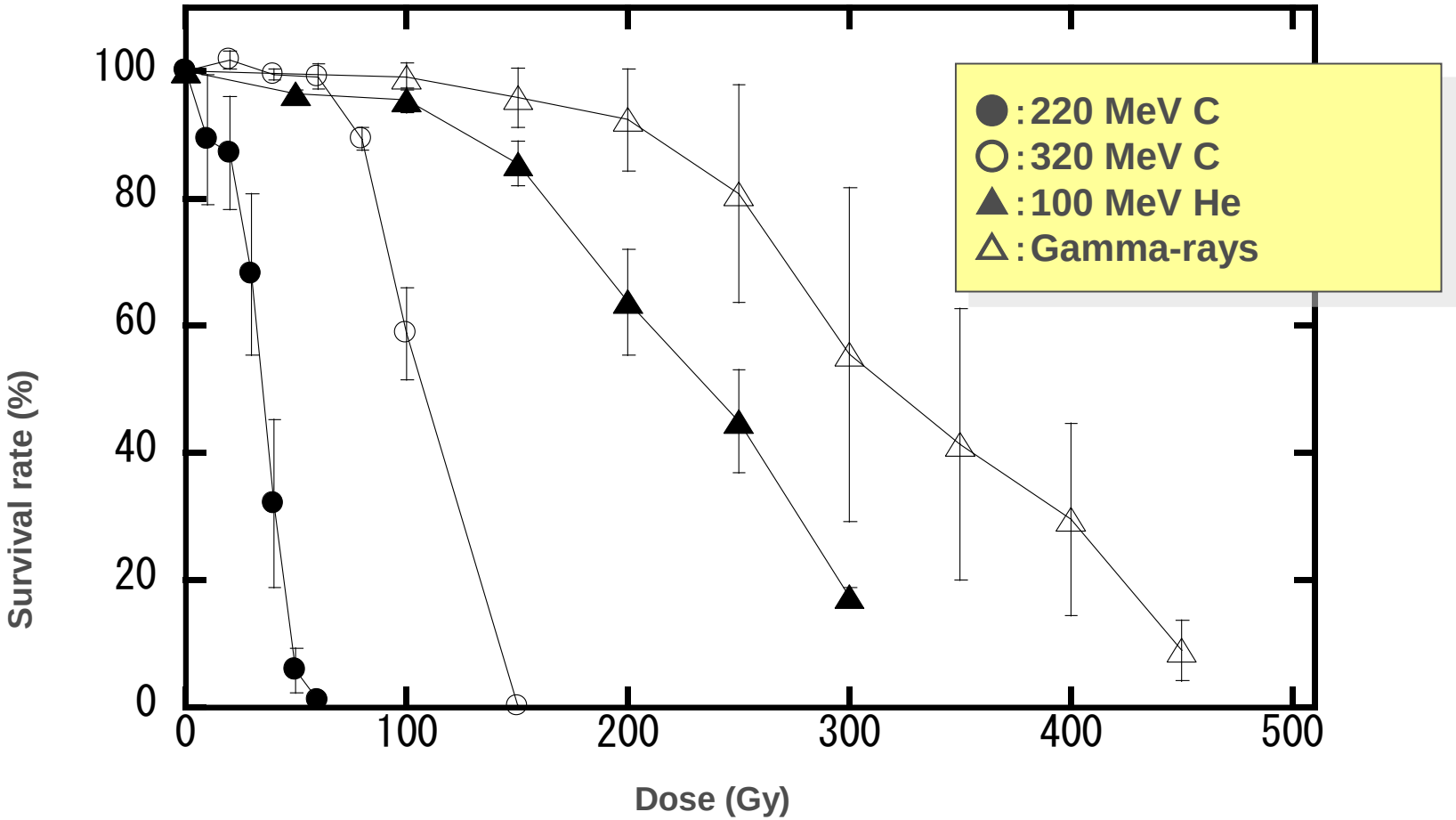


<Difference of energy transfer by γ-ray, proton and carbon heavy ion beam>

Ion-beam

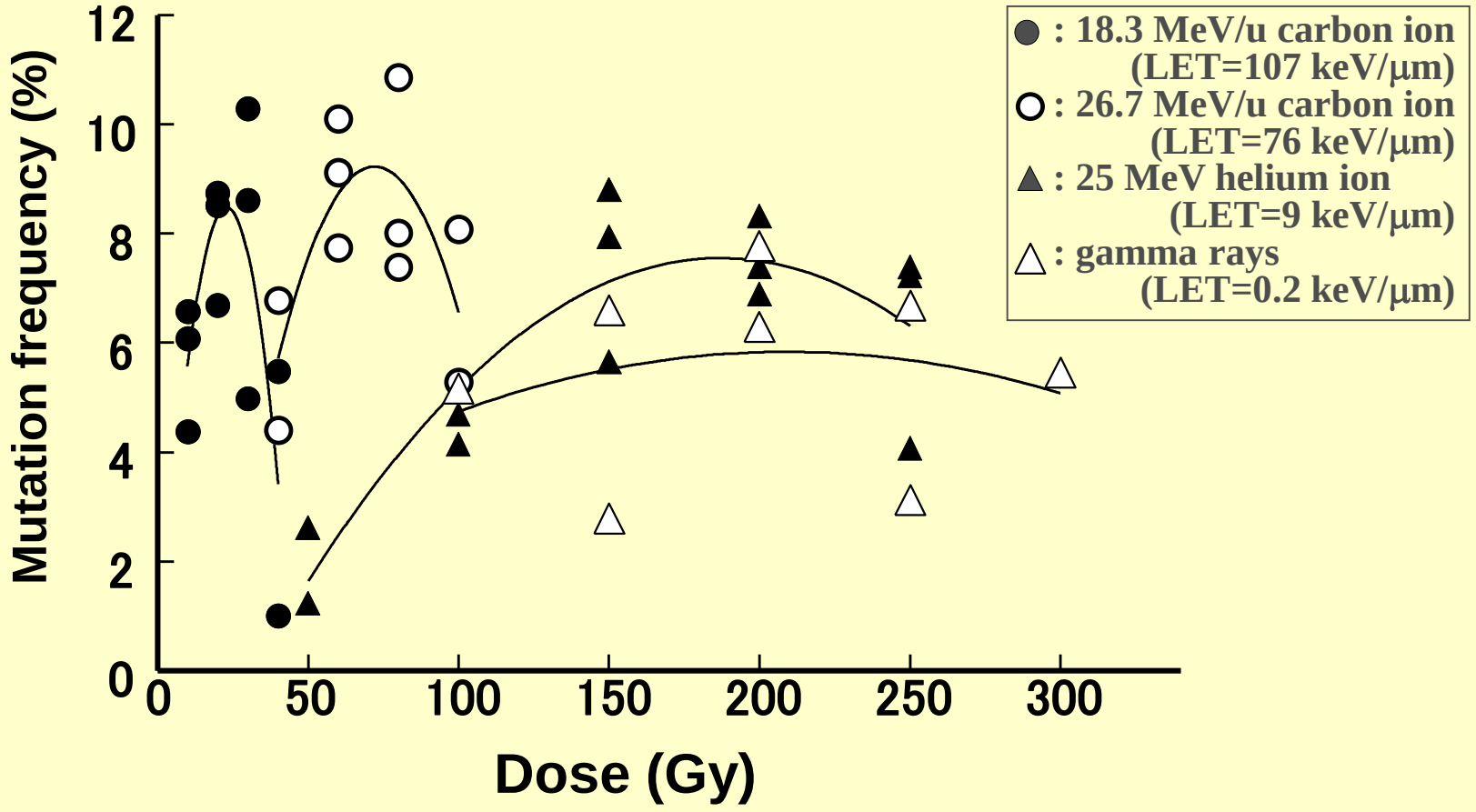
UV, γ-ray

Biological Effects of heavy ion-beam



Yamaguchi et al (2009)*Breeding Science* 59:169-177

Mutation Frequency



Mutation frequency of chlorophyll mutants

Mutation frequency was calculated as the number of M1 plants that produced chloro phyll mutants in their progeny (M2 plant) divided by the number of sown M1 seeds.

Yamaguchi et al (2009)*Breeding Science* 59:169-177

Mutation spectrum

Flower mutation of carnation regenerated from leaf cultures treated by mutagen

Mutagen	Mutation frequency (×10^{-1} %)											
	Flower color										Shape	Dianthus type petals
	Light pink	Pink	Dark pikn	Red	Salm-on	Yel-low	Cream	Stripe	Minute striped	Com-plex	Round petals	
EMS	0	5.2	0	1.0	0	0	0	3.1	0	0	0	0
Soft X-rays	1.7	8.4	0	3.4	0	0	0	0	0	0	0	0
Gamma-rays	1.7	2.6	0	1.7	0	0	0	0	11.3	0	0.9	0
Carbon ions	2.4	4.7	2.4	3.5	2.4	2.4	1.2	3.5	0	2.4	4.7	2.4

Original var."Vital"

From Dr. Tanaka A. (JAEA)

Mutation analysis

Summary of mutation induction

	Carbon ions			Electrons		
tt,gl locus	Point-like mutation	Large DNA arrangement		Point-like mutation	Large DNA arrangement	
Mutation	48%	52%		75%	25%	
Deletion	79%			44%		
Base substitution	14%			44%		
Insertion	7%			11%		
Breakpoint						
Deletion		65%	C,G,CAT, CCAAAAC, 17,29bp,etc.		13%	A,CC,AGT, TATTC,etc.
Duplication		24%			75%	

Ion beams preferentially induce 'deletion'

Shikazono et al. (2005) JXB, 56, 587

Mutants and varieties induced by ion beams (TIARA, QST + others)

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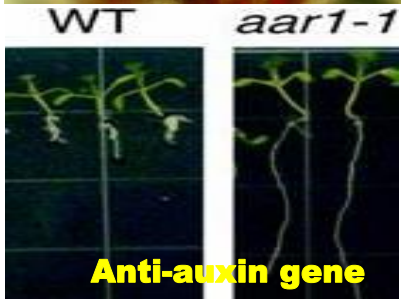
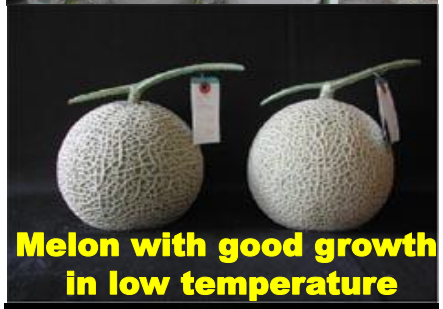
New genes



Crops

Ornamentals

Trees, fruit & mushroom



Mutants and varieties induced by ion beams (RIKEN + others)

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New Color

Hiroshima City

Hokko Chemical Co. & Kaneko Seeds Co.

JFC Ishii Farm

Tsunoda Nursery

Dwarf

Iwate Pref.

Nebarikko No.2

Everblooming

Rose

Fuji Chemical Industry Co.

Hajime

RIKEN has released 21 cultivars for sales since 2001~2012 (ca □ 2 billion/year).

Sterile

Suntory Flowers Ltd. (Temari series)

Temari Bright Pink

Temari Sakura Pink

Temari momo

New Color

Suntory Flowers Ltd.

Surfinia Rose

Advantages and Disadvantages of Ion Beam Breeding

❖ **Advantages;**

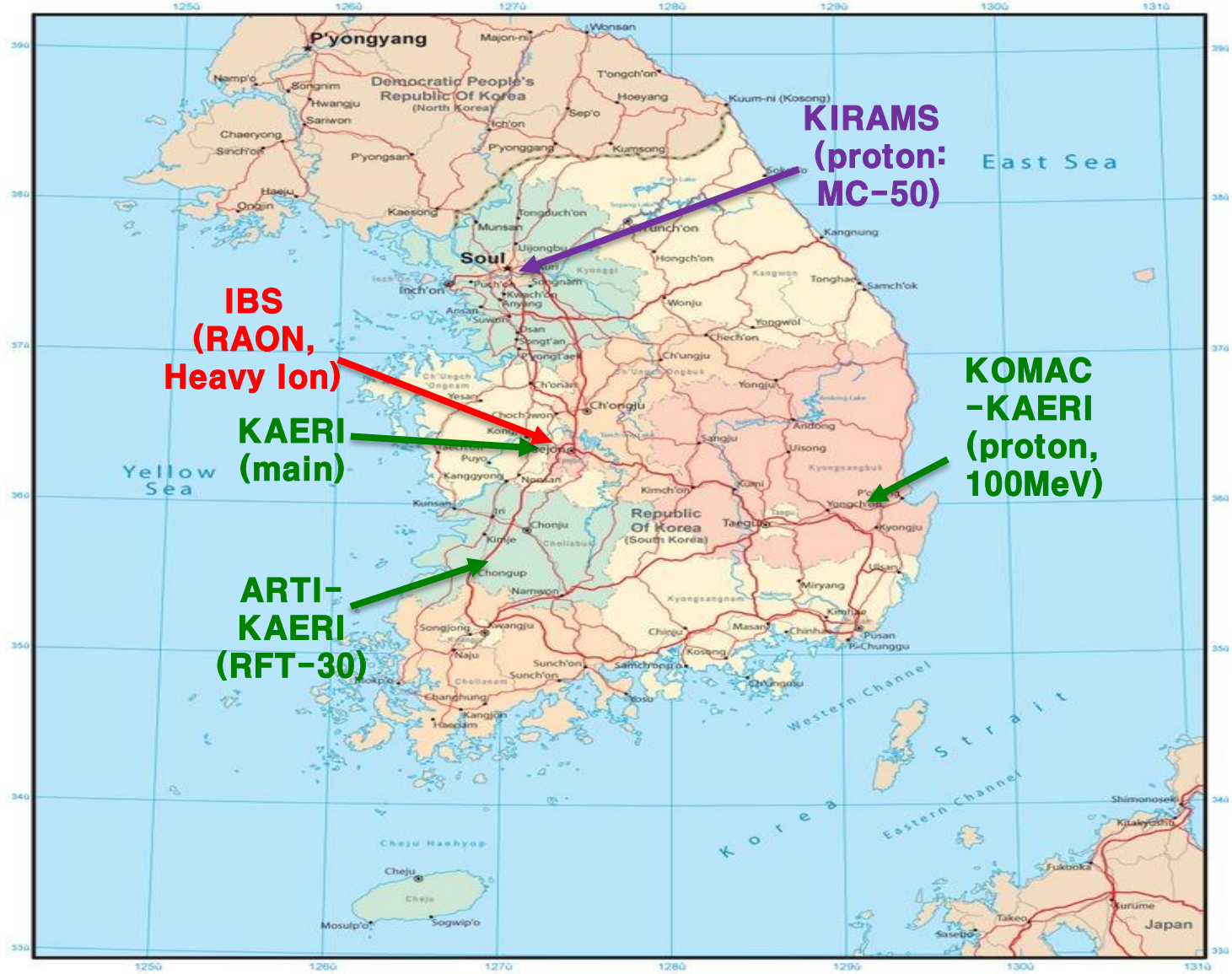
1. range (depth) in biological materials is very short, but RBE is much higher
2. low doses, but high mutation frequency and mutation spectrum
3. minimum numbers of DNA damage: pinpoint-breeding without bad characters

- **Dis-advantages;**

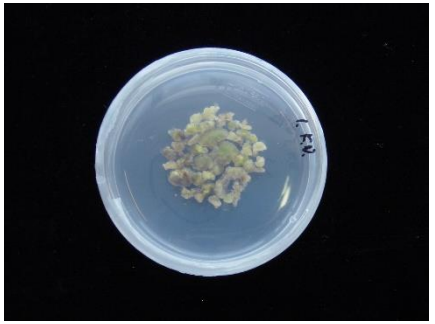
1. limited on irradiation material and amount per one time
2. high cost for construction and operation of an ion beam accelerator

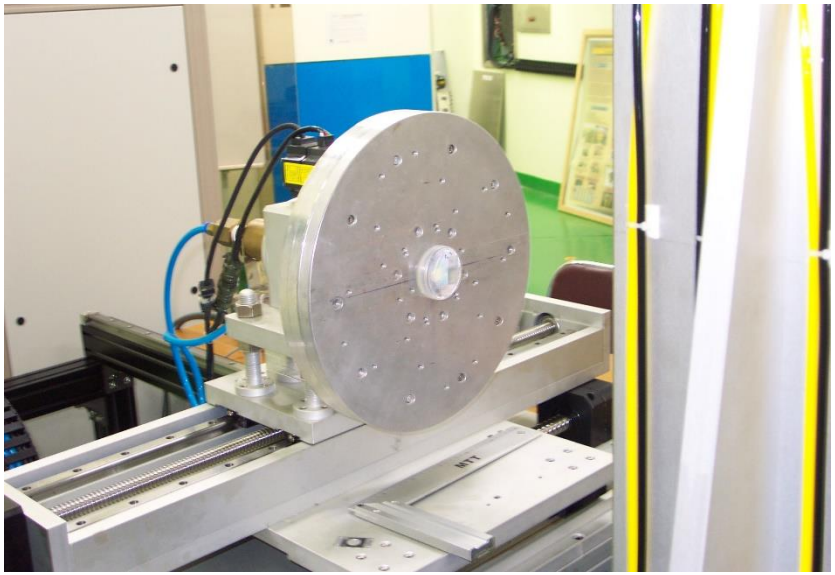
III. Current Status and Future Plans of Ion Beam Breeding in Korea

Ion-beam irradiation Facilities in Korea



Proton beam irradiation using MC-50 cyclotron of the KIRAMS





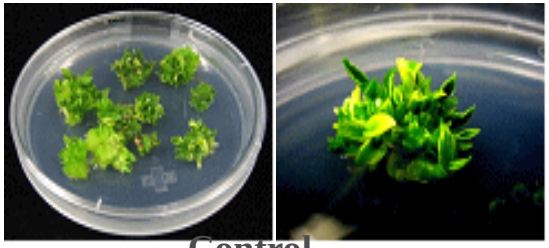
Bioeffects of proton beam irradiation by MC-50: radiash

Dose (Gy)	Sprouting (%)	Shoot length (cm)	Root length (cm)	Dry weight (mg)
Cont.	93.3	12.4	6.2	191
100	95.0	11.4	5.7	188
200	95.0	11.1	4.6	153
300	95.0	10.6	5.1	147
500	93.3	9.1	5.1	150
1000	91.7	8.2	4.8	165
1500	86.7	7.2	3.9	134
2000	83.4	6.2	2.8	137

Bioeffects of proton beam irradiation by MC-50: perilla

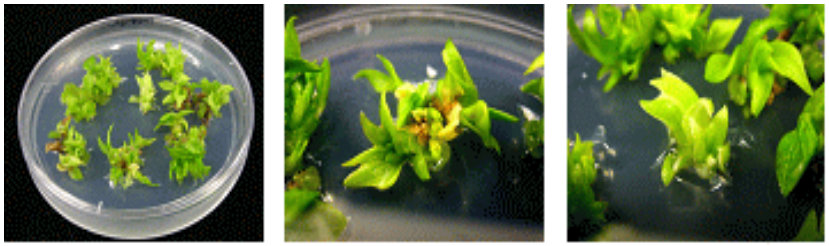
Proton beam irradiation dose (Gy)	Emergency rate (%)		Shoot length (cm)	
	Baiksang	Yangsang	Baiksang	Yangsang
Cont.	90.0	86.7	2.70	1.64
25	73.3	80.0	0.83	0.52
50	33.3	3.3	0.53	0.20
75	6.7	0	0.20	-
100	0	0	-	-
200	0	0	-	-

Bioeffects of proton beam irradiation : orchid

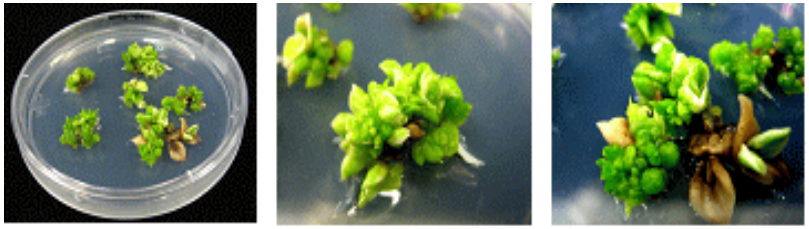


Control

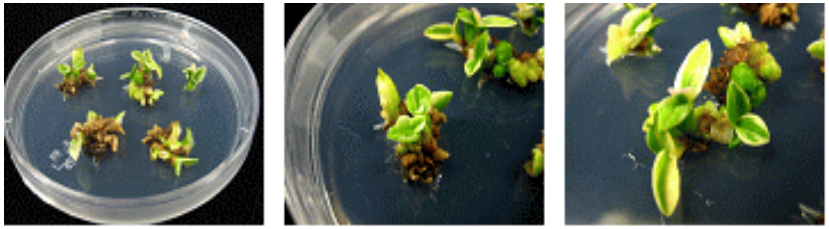
10 Gy



25 Gy



50 Gy

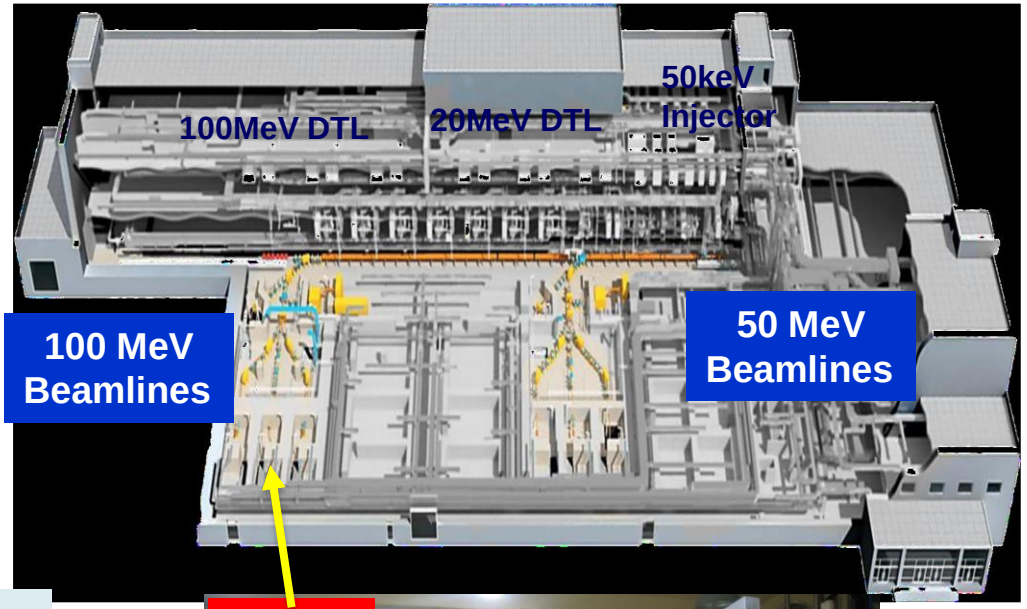


100 Gy

Generated tissue cultured orchids at 30 days after irradiation of proton beam

100 MeV Proton Beam, KOMAC

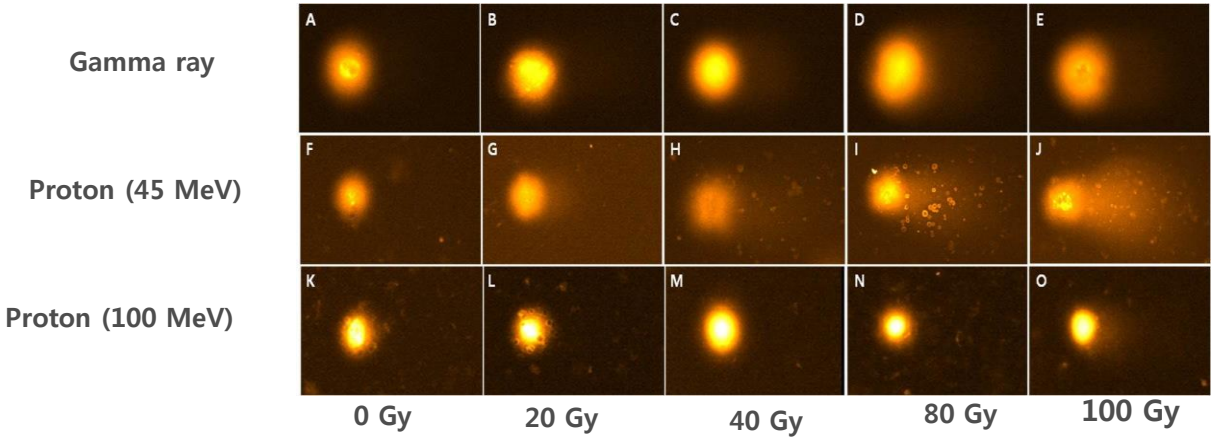
- Proton beam service in TR103 target room for breeding material was started from July 2013 at the KOMAC (Korea Mutiple-Purpose Accelerator Complex, KAERI)
- But irradiation condition for mutation breeding is not settled

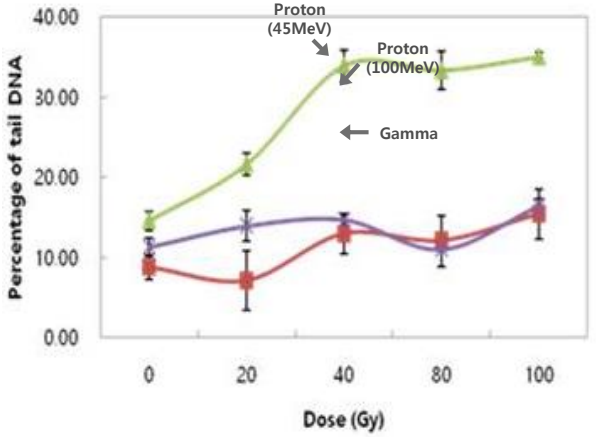


Energy (MeV)	100
Peak Current (mA)	0.1 ~ 20
Max. Duty (%)	8
Max. Ave. Current (mA)	1.6
Pulse Width (ms)	0.05 ~ 1.33
Max. Repetition Rate (Hz)	60
Max. Beam Power (kW)	160
Emmitance (mm-mrad)	0.3 / 0.3

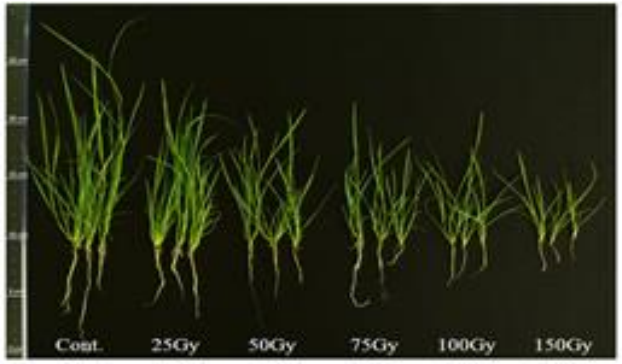
Bioeffect of proton beam irradiation

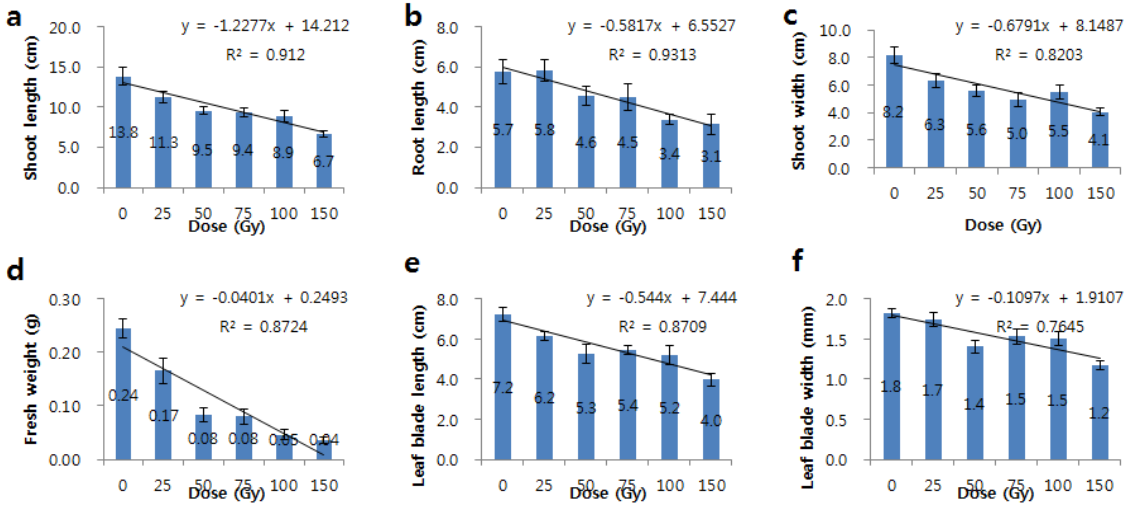
◆ DNA damage of orchid tissue by comet assay irradiated proton beam





◆ Effects of proton beam doses irradiated seeds of bentgrass





Bioeffects of proton beam irradiation : Arabidopsis

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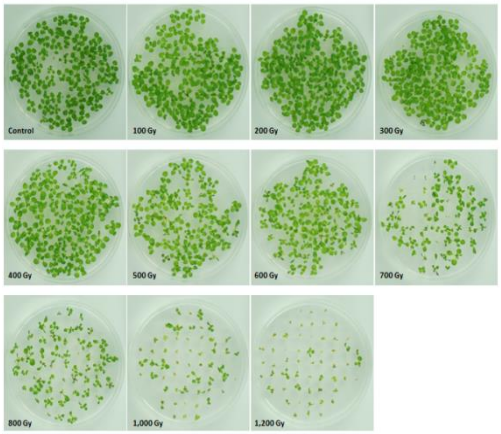
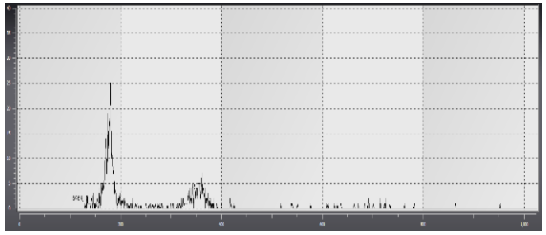
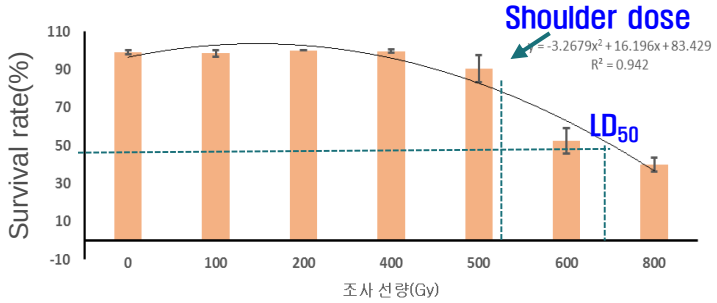
Analysis of bioeffect and construction of mutant pool using proton beam irradiation

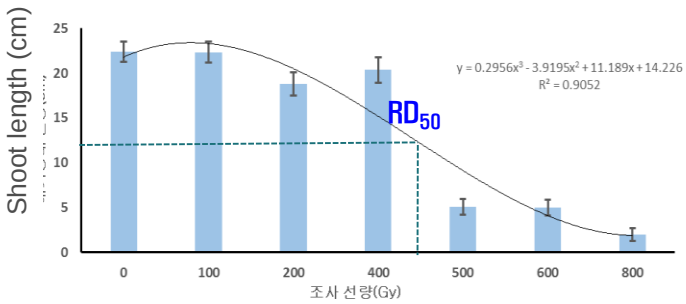


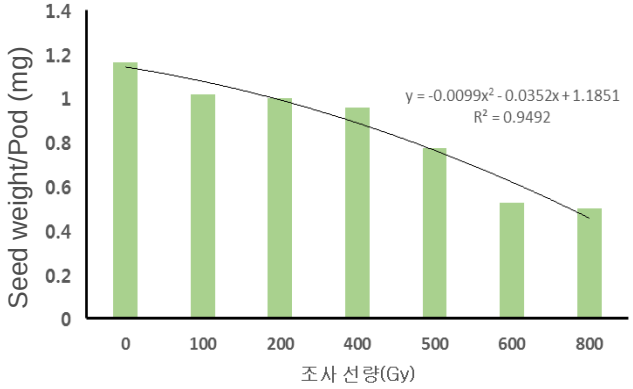
Bioeffect of irradiation dose



Flow cytometry analysis







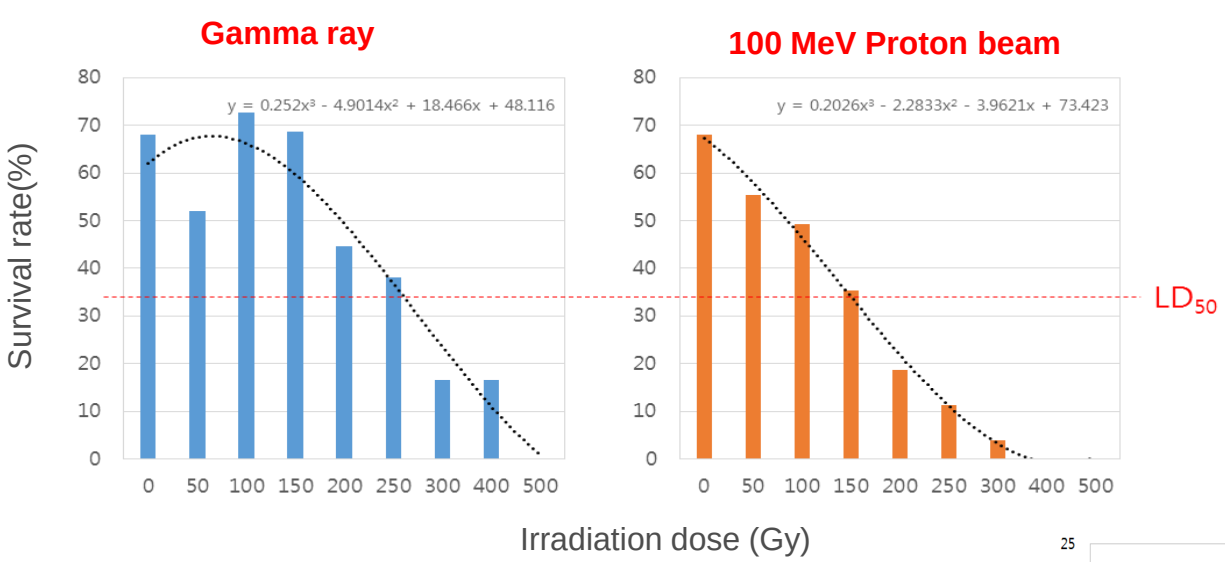
Determination of proper irradiation dose

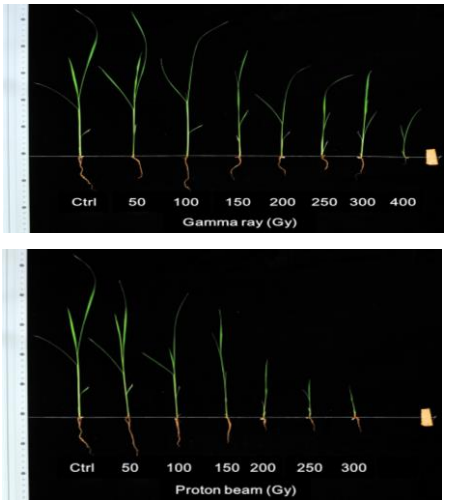
Irradiation doses (Gy)	No. of selected mutants
100	396
200	398
300	200
400	398
500	381
600	304
700	185
800	201
1,000	115
1,200	80
1,400	0
총합	2,658

Selected mutants pool at M2 generation

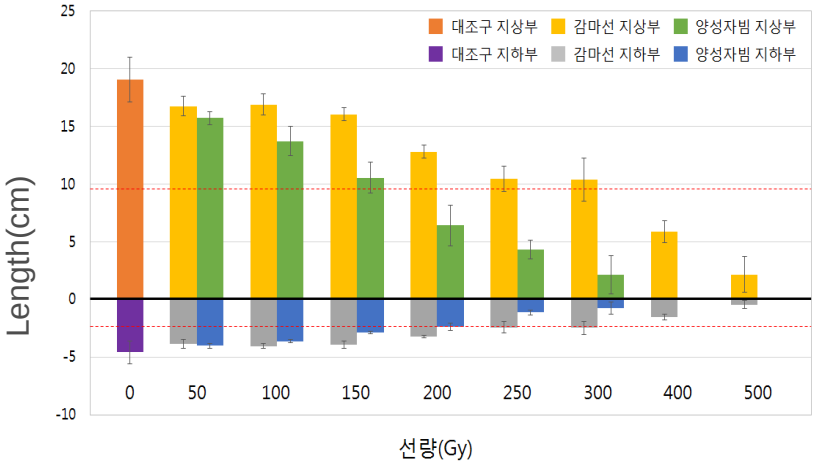
Bioeffects of proton beam irradiation : rice

◆ Survival rate of irradiated rice seed by proton beam and gamma ray at 28 DAS





- ✓ Proton beam LD_{50} ≈ 150 Gy
- ✓ Proton beam RD_{50} ≈ 175 Gy

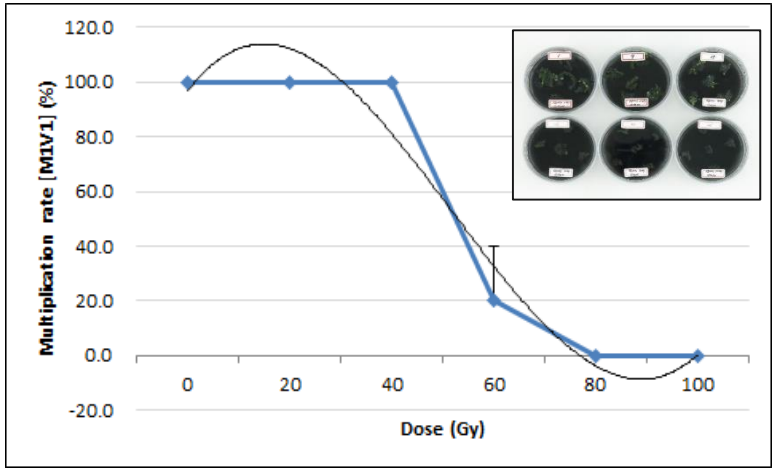


Shoot and loot length irradiated rice at 28 days after seeding

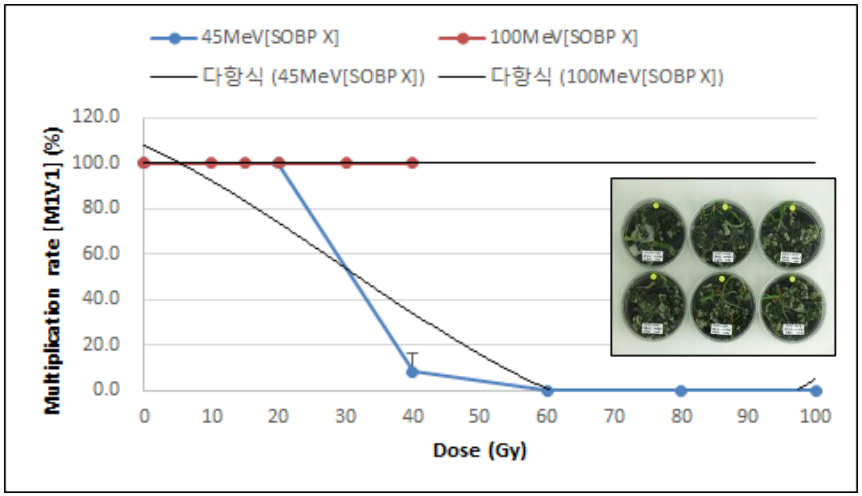
Bioeffects of proton beam irradiation : orchid

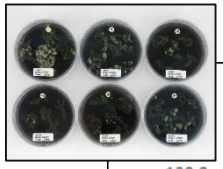
- LD_{50} & RD_{50} of a Cymbidium hybrid (*C. sinensis* x *C. goeringil*) irradiated 100 MeV & 45 MeV proton beam (TR103)

Gamma ray

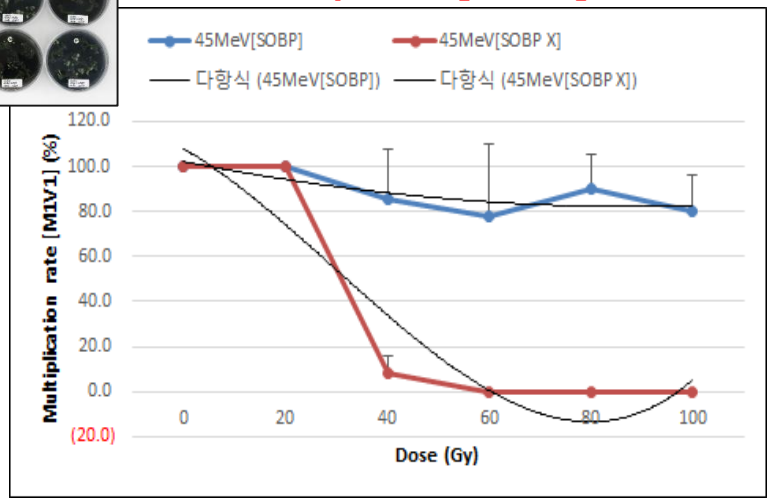


100MeV proton

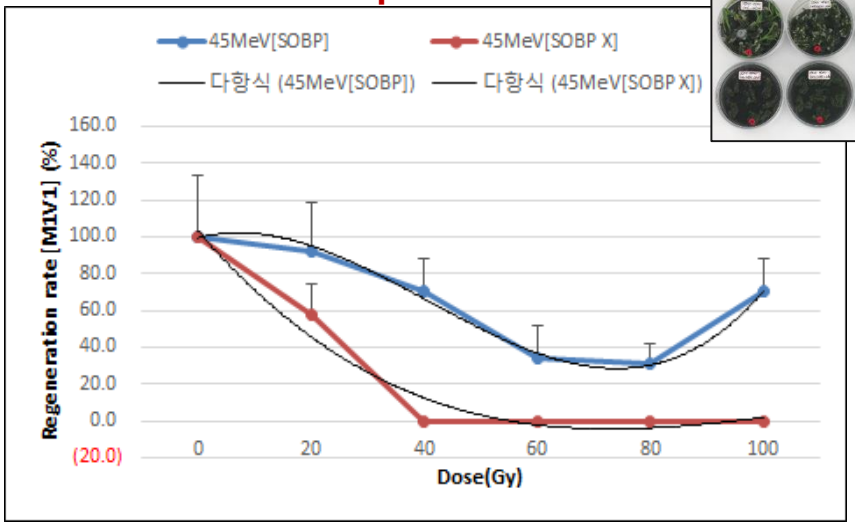




45MeV proton [SOBP]



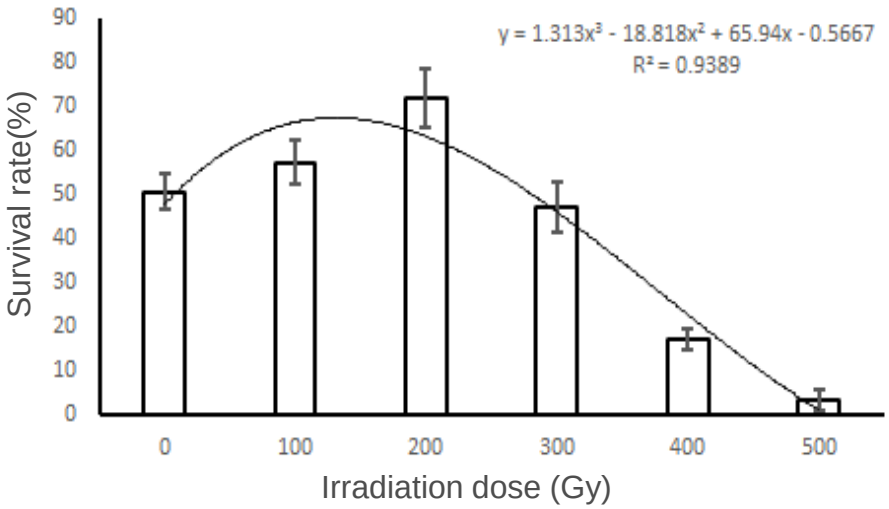
45MeV proton

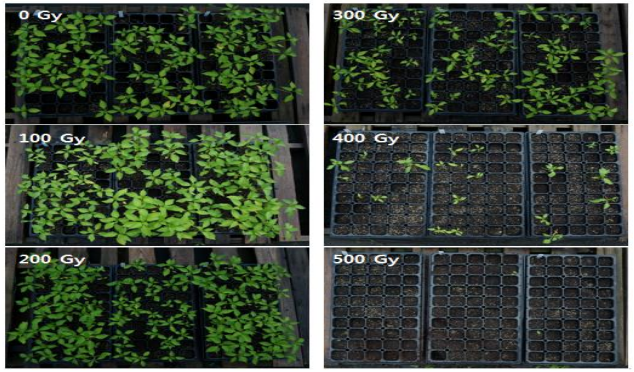


Bioeffects of proton beam irradiation : hot pepper

KAERI

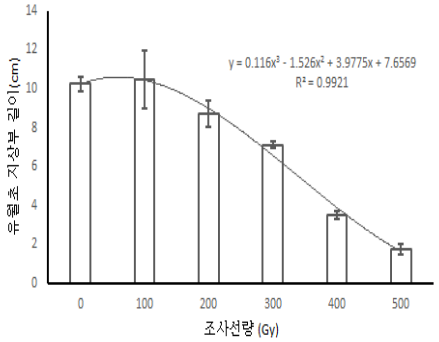
Survival rate and growth of hot pepper irradiated by 100 MeV proton beam at 36 days after seeding



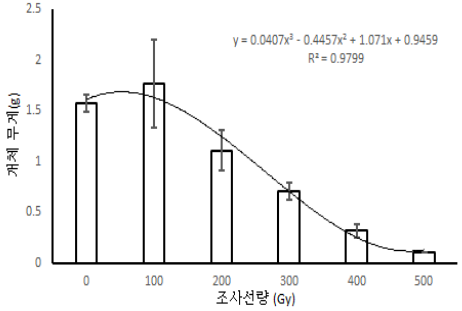


- ✓ Proton beam LD_{50} ≈ 350Gy
- ✓ Proton beam RD_{50} ≈ 300Gy

Shoot length



Shoot weight



RFT-30 : 30 MeV Cyclotron in ARTI-KAERI

- Research of RI production & Targetry (PET, SPECT)

- Research of proton beam application in biology

Accelerator Application

30Mev Cyclotron(RFT-30)

20Mev Electron Accelerator

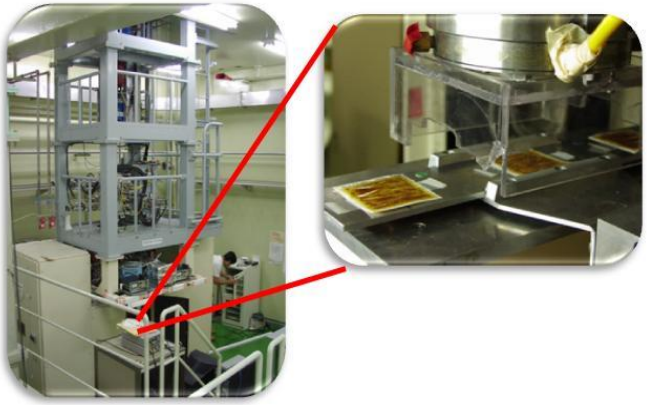
International Joint Research with Ion-beam Breeding

KAERI

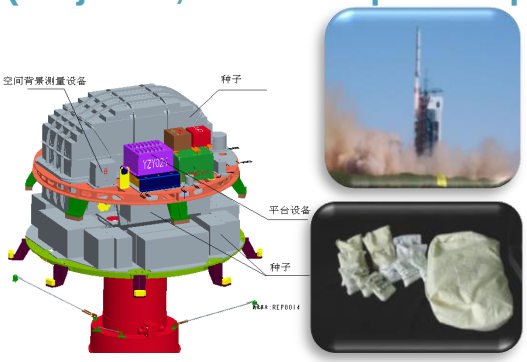
Seeds treatment with different radiation sources and spaceship loading through international cooperation

- Physiological analysis of monocot model plant (rice) in response to ionizing radiations (heavy ion-beam and gamma-ray) and space environment
- High throughput trancriptomic and bioinfomatic analysis of irradiated plants

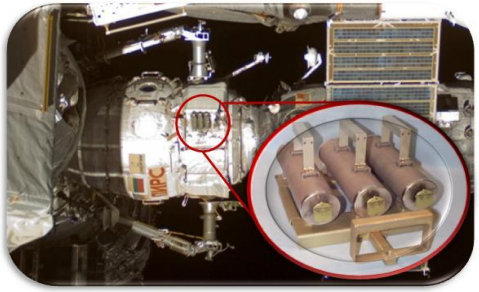
Heavy Ion-beam(C)
(JAEA-Takasaki, Japan)



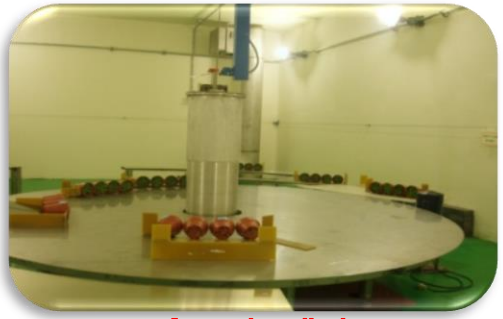
Cosmic environment
(Shijian-8, Chinese spaceship)



Seed loading test at the ISS
(IBMP, Russia)



Gamma-rays (KAERI)



Acute Irradiation

Chronic Irradiation

Research with heavy ion-beam irradiated rice

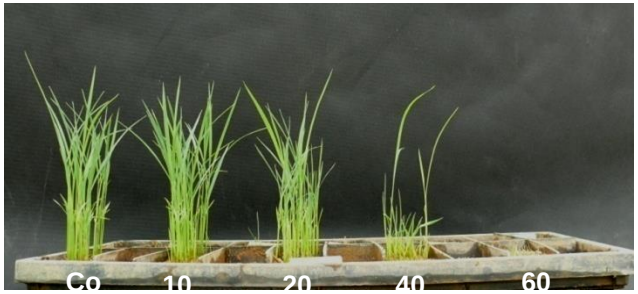
KAERI

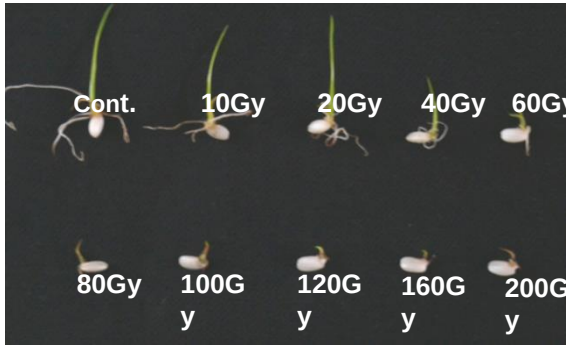
<Germination rate of rice irradiated with gamma-ray and heavy ion-beam>

	cont.	10Gy	20Gy	40Gy	60Gy	80Gy	100Gy	120Gy	160Gy	200Gy
Gamma-ray	99.3±0.7	100.0	98.3±1.7	98.3±1.7	100.0	100.0	100.0	98.3±1.7	100.0	95.0±2.9
Heavy Ion-beam	71.3±1.8	74.0±6.4	68.0±1.2	55.3±5.5	2.0±2.0	0.0	0.0	0.0	0.0	0.0

<Plant growth of 5-weeks grown rice irradiated with heavy Ion-beam>

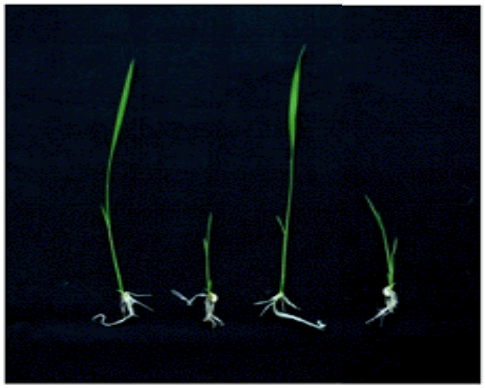
	Plant heights(㎜)	Length of roots(㎜)	No. of roots
cont	402.0±6.5	145.0±17.7	23.9±1.8
10 Gy	395.5±9.2	135.0±8.9	21.3±1.0
20 Gy	395.0±7.8	119.0±5.8	23.2±1.4
40 Gy	293.0±12.3	91.0±6.3	13.7±0.6



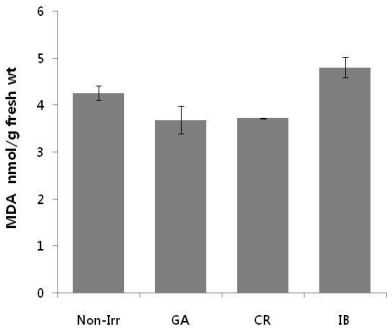


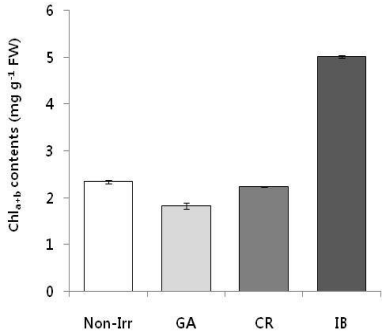
Biological effects by different treated rice seedling

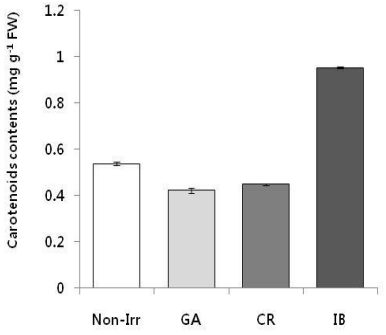
Plant Growth



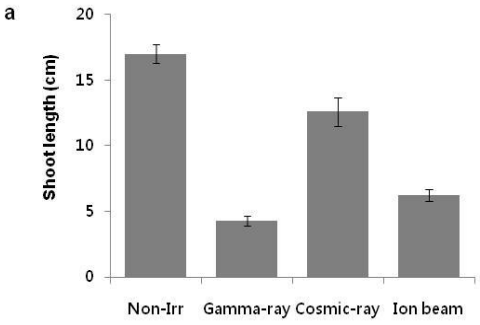
MDA, Chlorophyll, Carotenoid contents

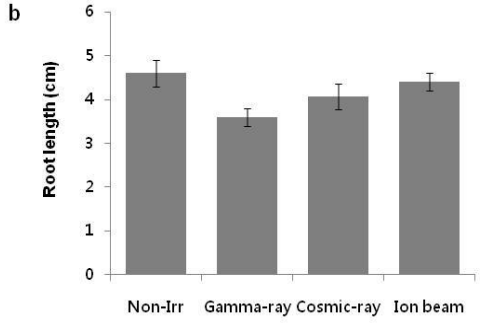


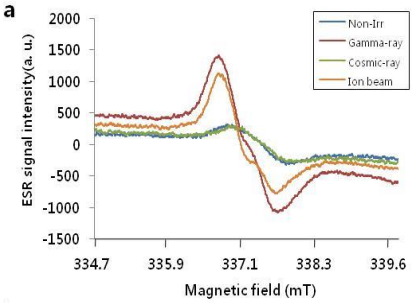


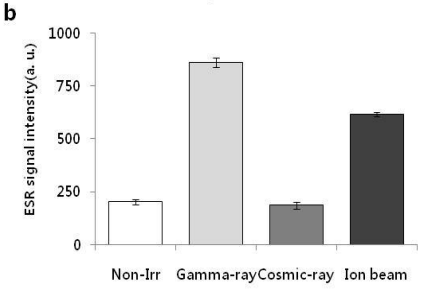


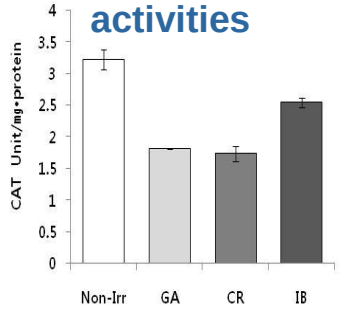
ROS contents and Antioxydant enzyme activities

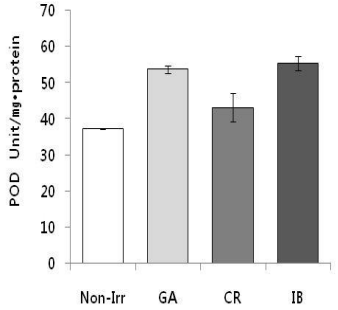


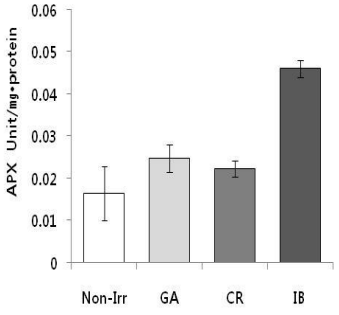


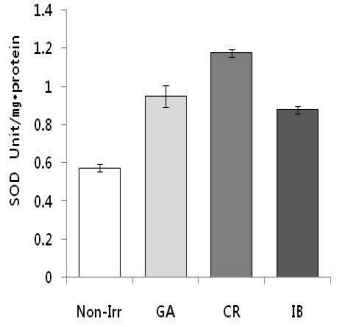












Transcriptome profiling – Microarray

Microarray design (Affymetrix oligo chip)

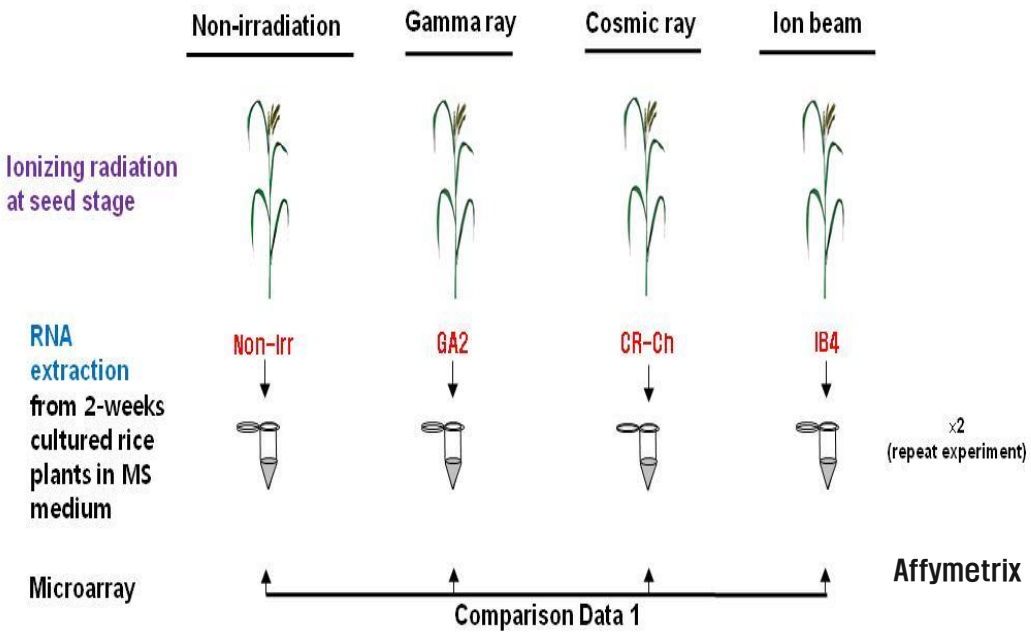
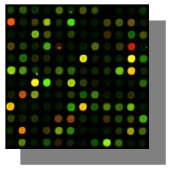
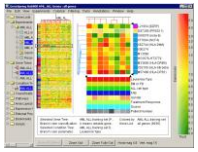


Image processing & signal calculation



Affymetrix GeneChip Scanner 3000 7G

Data Mining



RMA(Quantile) Normalization

Filtering: Affymetrix Command Console1.1

Student' s T-test : p-value < 0.05

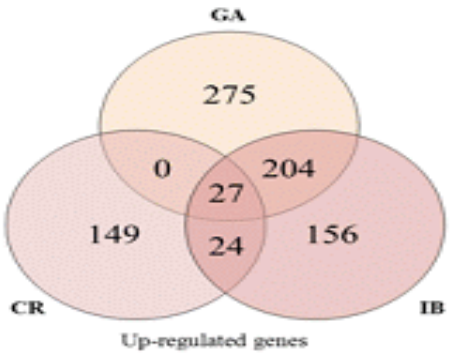
Clustering of DEGs: TMEV 4.4

Differentially expressed genes (DEGs)

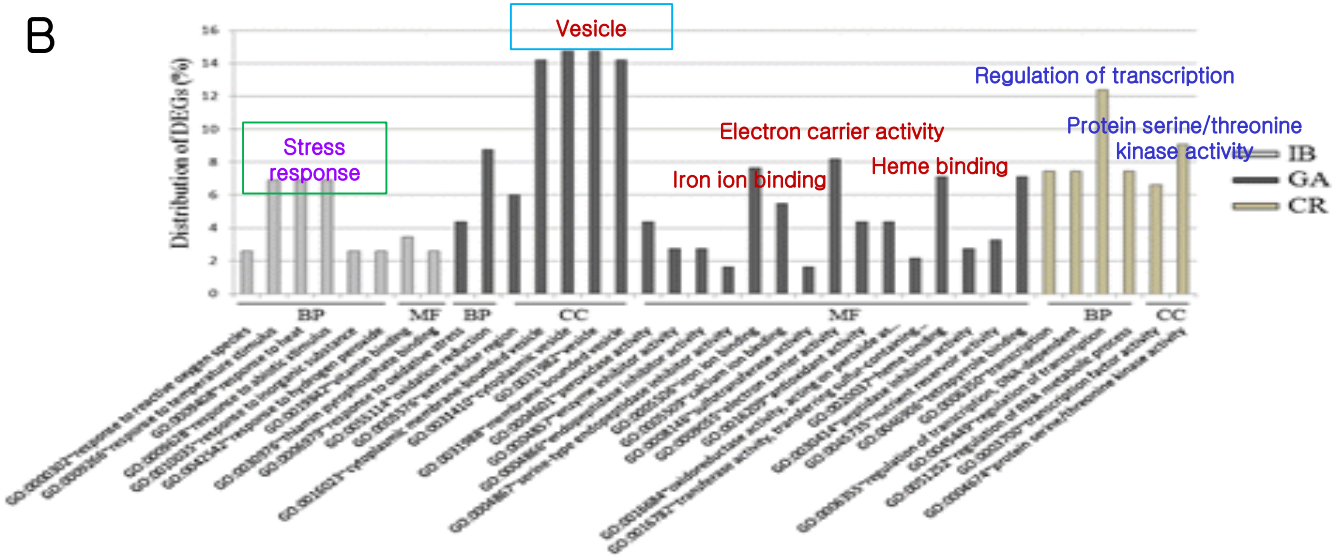
KAERI

<DEGs grouping by functional enrich test>

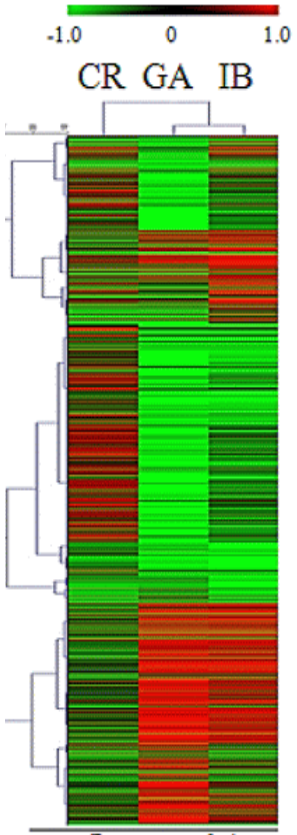
A



B



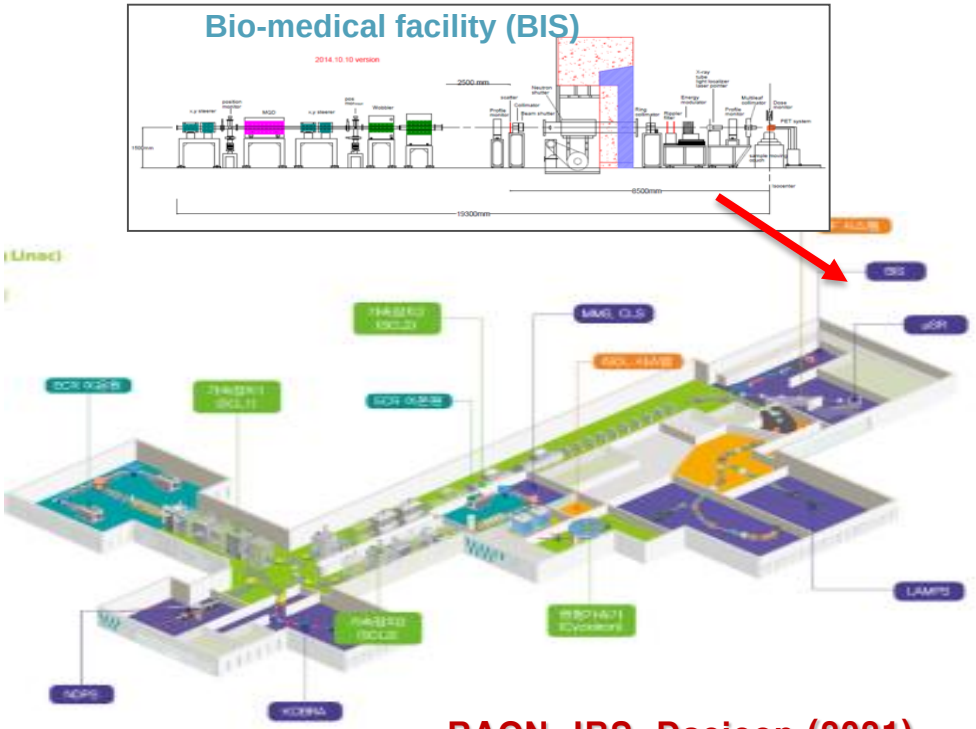
Pearson correlation coefficient analysis



Pearson correlation coefficient			
CR	1		
GA	0.35	1	
IB	0.54	0.80	1

In future : Heavy Ion Beam Research

- Construction of RAON, heavy ion-beam accelerator project, will be finished in 2021, at Sindong, Daejeon, IBS.
- Suggested to make a beam line for breeding



RAON, IBS, Daejeon (2021)

- Comparison of mutation induction rate with other radiations
- Identify of irradiation condition of new heavy ion beam for each plant
- Development of useful new varieties and genetic resources

Conclusions

- Economic effects of mutant varieties is huge. Establishment of new creating methods of useful mutants using various radiation sources is important for the development of genetic resources.
- Japanese research groups have been succeeded to develop various useful genetic resources using heavy ion beam
- It is necessary to elucidate differences in the effects on mutations among gamma ray, heavy ion and proton radiations.
- To increase of application of ion beam accelerators (e.x, 100 MeV proton, KOMAC & 200 MeV heavy ion, LAON) for mutation breeding in Korea, it is necessary to make special beam line for irradiation of plant materials as well as to set up irradiation condition for each of crops.

History of Nuclear Energy & Radiation Research Institutes in Korea

Radiation Agriculture Research Institute
(1966~1973)

Radiation Breeding

Advanced Radiation Technology Institute (ARTI-KAERI, 2006~)

Radiation Research

Nuclear Agency
(Nuclear Power Plant)
(1959~ 1973)

Korea Atomic Energy Research Institute
Nuclear Power Plant, Radiation Technology,
(Medical Sciences)
(1973 ~ Present)

Nuclear Science
(4th Generation Nuclear Power Reactor & New Research Reactor)

Radiation Medicine

Medical Science

Radiation Medicine Research Institute
(1963~1973)

Korea Institute of Radiological & Medical Sciences (KIRAMS, 2007~)

1960 1970 1980 1990 2000 2010

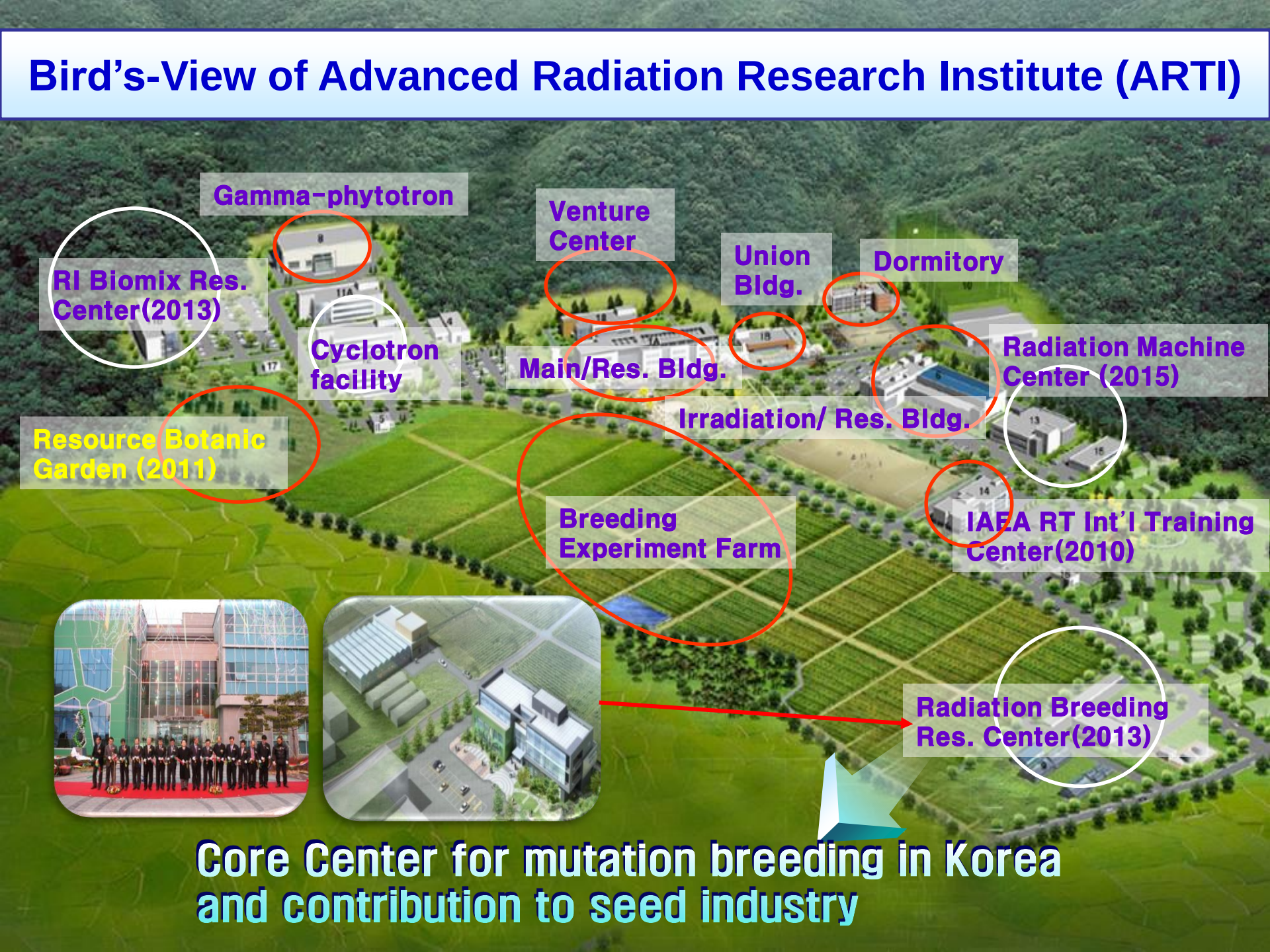
ARTI & RFT

Advanced Radiation Technology Institute (ARTI), KAERI

- Establishment: 2006 (371,736 m^2)
- A Branch organ of the Korea Atomic Energy Research Institute (KAERI)
- Location: Jeongeup, Jeonbuk Province
- Utilization of various radiations
- R&D on radiation fusion technology

Radiation Fusion Technology (RFT)

Bird's-View of Advanced Radiation Research Institute (ARTI)



Mutation Breeding Researches at the KAERI

- **Main crops for breeding**
  - highly functional crops: rice, soybean, wheat, perilla, blackberry.
  - ornamental plants: chrysanthemum, orchid, turfgrass, hibiscus, wild flowers, etc.
  - biomass & industrial plants: kenaf, sorghum, etc.
  - mushroom, algae (micro-algae)

- **Creation of mass useful mutants, and promotion of genebank project and functional genomic researches**

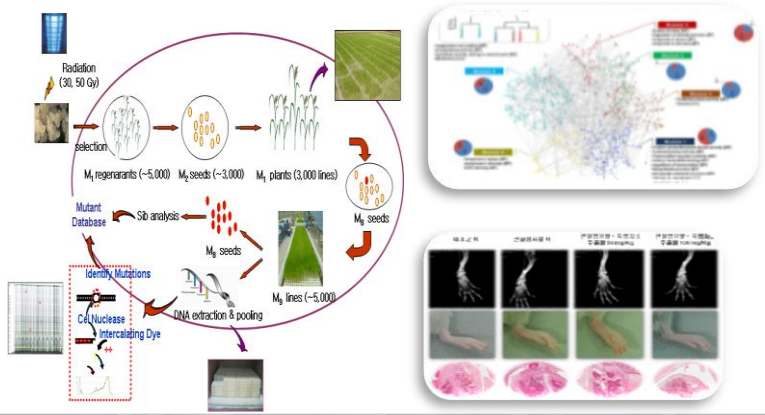
- **Development of new mutation techniques using ion-beam, spaceship and chronic gamma ray irradiation facility and also combined with biotechnologies.**

Main Achievements of Mutation Breeding in KAERI

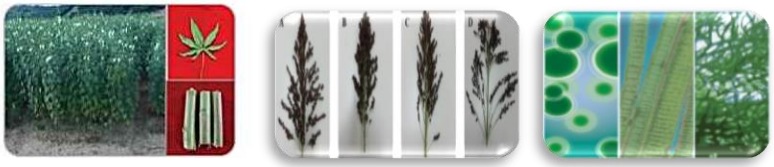
KAERI

Development of new crop varieties with high function (rice, soybean, perilla, blackberry, etc) and their commercialization

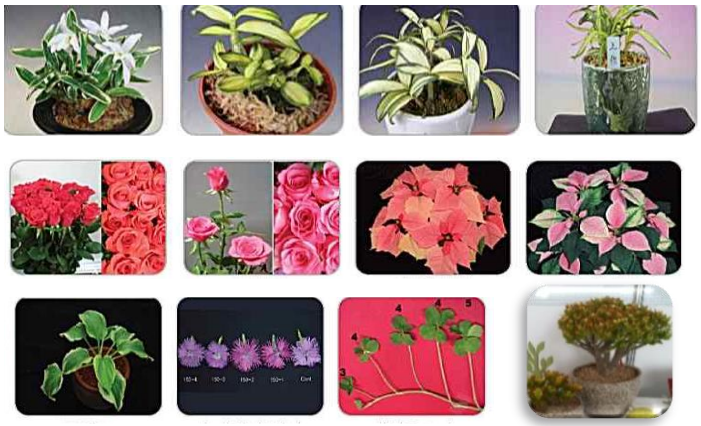
Functional genomics and metabolomics study using various mutant genetic resources



Breeding of bio-energy plants (kenaf, rape, sorghum, algae, etc)



Ornamental plants (orchid, rose, poinsettia, wild flowers, succulent plants, etc) breeding jointed with other research organs



Breeding of chrysanthemum & Korean national flower “hibiscus “

Radiation Breeding and Irradiation Facility

Gamma Room (acute)

Gamma-phytotron (chronic irradiation)

Low temperature seed storage facility

Culture room

Mushroom & Phytotron

Radiation Breeding Research Center

Greenhouse

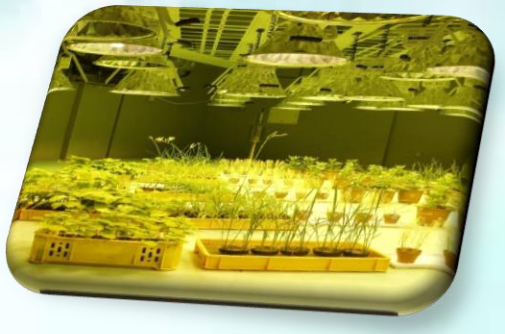
Upland & paddy field

Resource botanic garden

Other activities to promote mutation breeding

- National Training Course on Mutation Breeding Techniques since 2012 at the ARTI-KAERI
  - ✓ 20 trainees per year from Company, private breeder and institute
  - ✓ 17 lectures from KAERI, university and others
  - ✓ One week annual course
- Organizing of international and national symposium on mutation breeding
  - ✓ IAEA-RCA, FNCA etc
- Support of radiation breeding techniques and irradiation service
- Attending the exhibition at the bio- or seed expo
- Public relations via mass media

Thank You !



RFT
Road to Fine Tomorrow