Ion beam mutagenesis and mutagenomics

Tomoko Abe

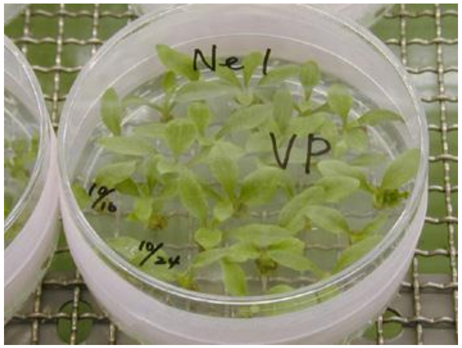
RIKEN Nishina Center
Radiation Biology Team

One point improvement

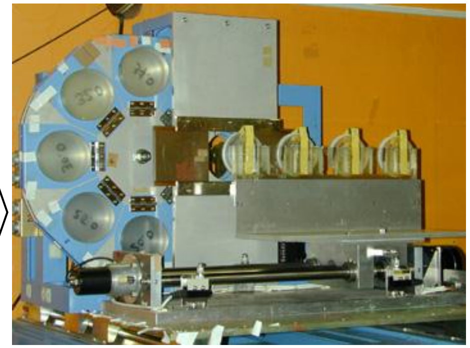
Original(Petunia)
Surfinia Purple

Tissue culture (In-Vitro Condition)

Multiple Propagation

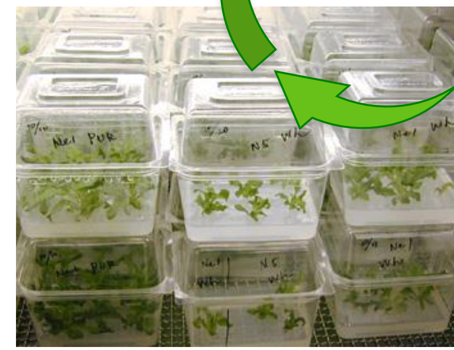


Lateral bud Culture



Heavy-ion Beam Irradiation

Cutting



Shoot Development

Selection and Purification

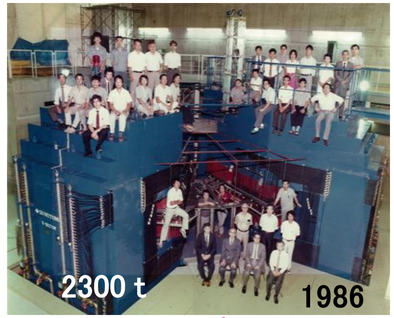
Green House Condition

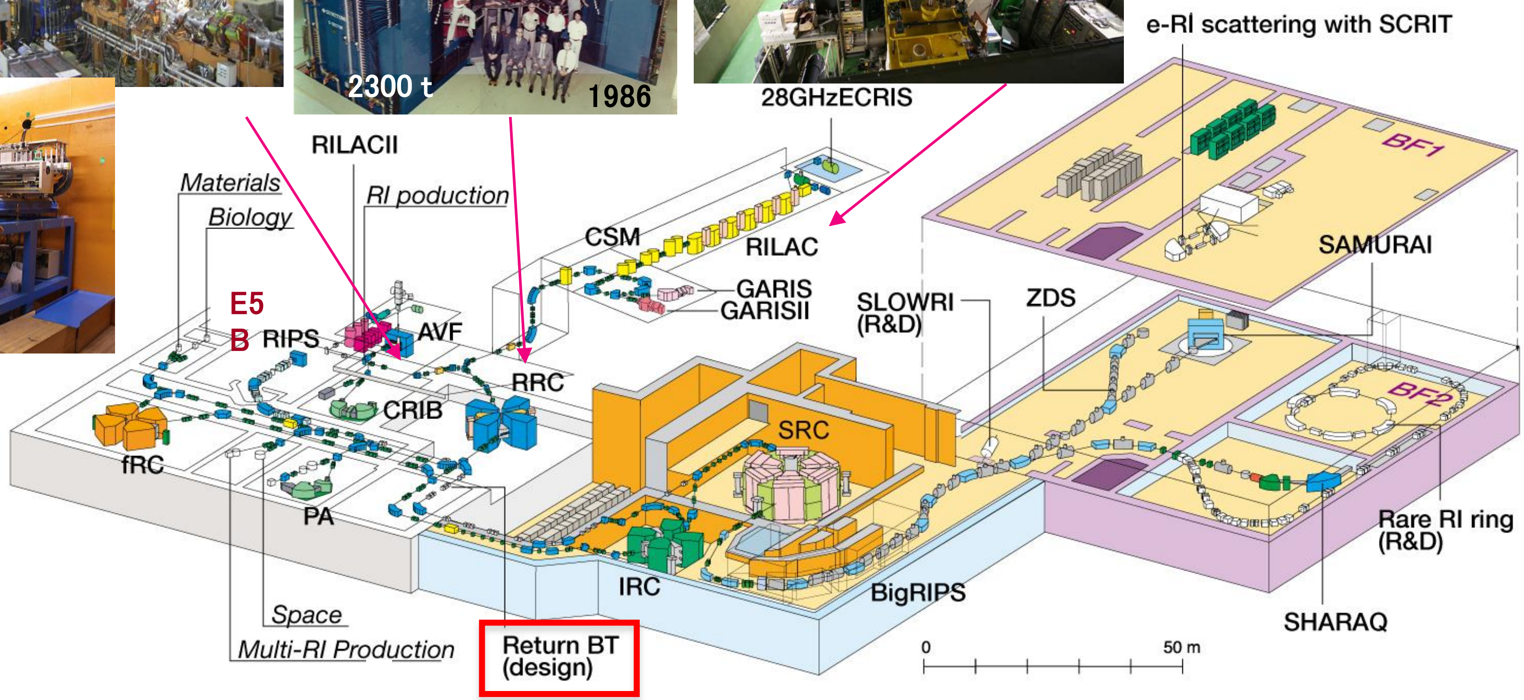
New cultivar
Surfinia Rose Veined

New Cultivar (candidate)
Variegated Mutant

Selection and Purification

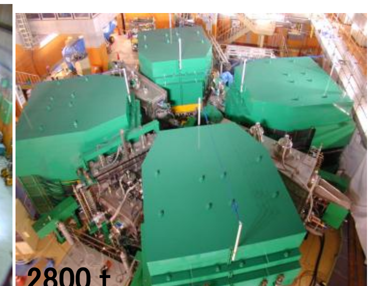
Overview of the RI-Beam Factory (RIBF)

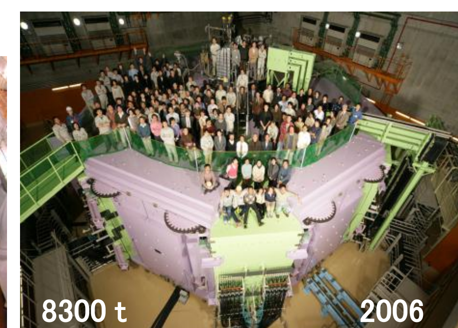




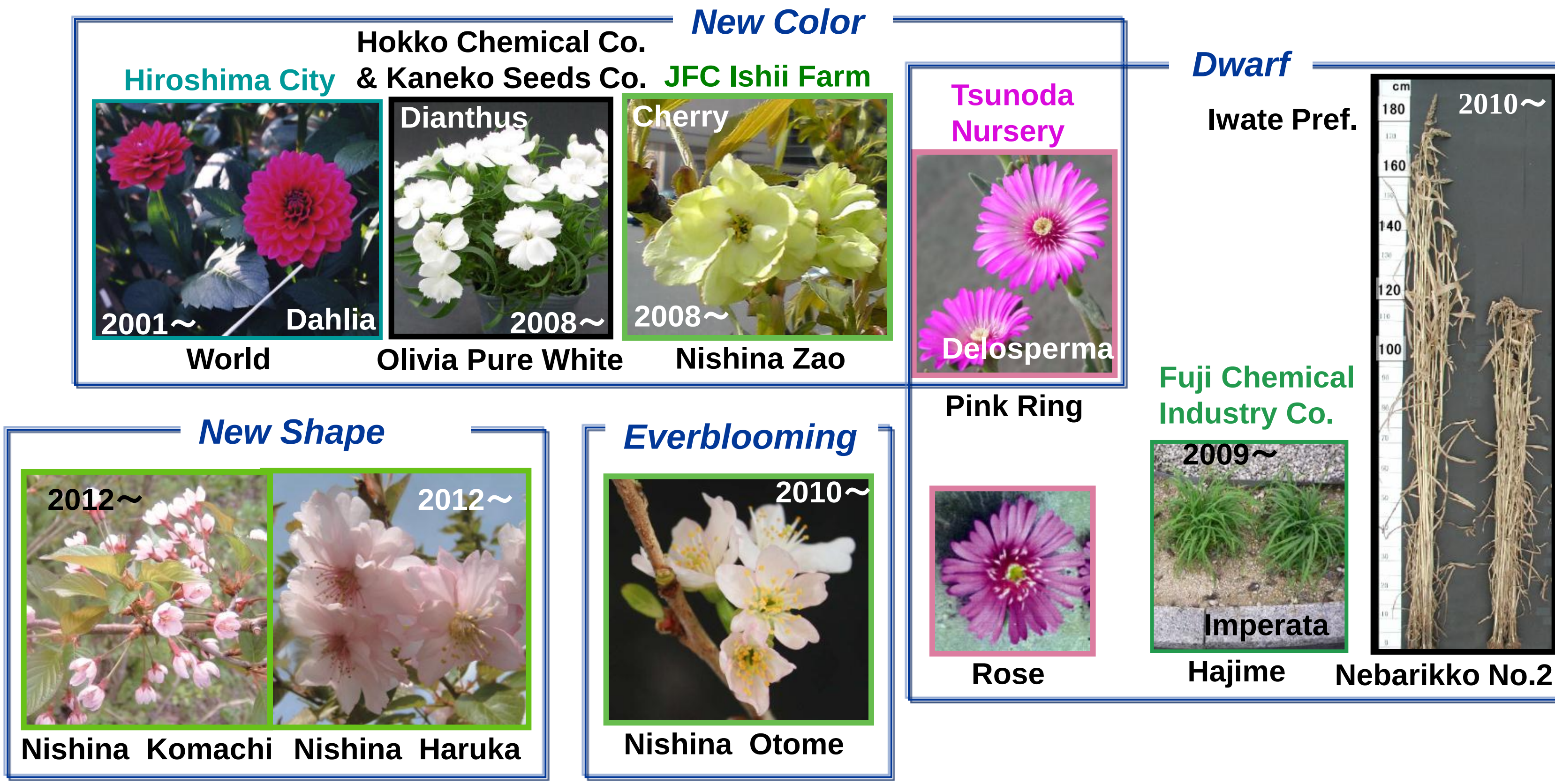
Plant breeding with heavy-ion beams is a technology unique to Japan.

It is achieved through an efficient synergistic link between agricultural science and accelerator physics





Ion-beam Makes New Plant Business at RIKEN

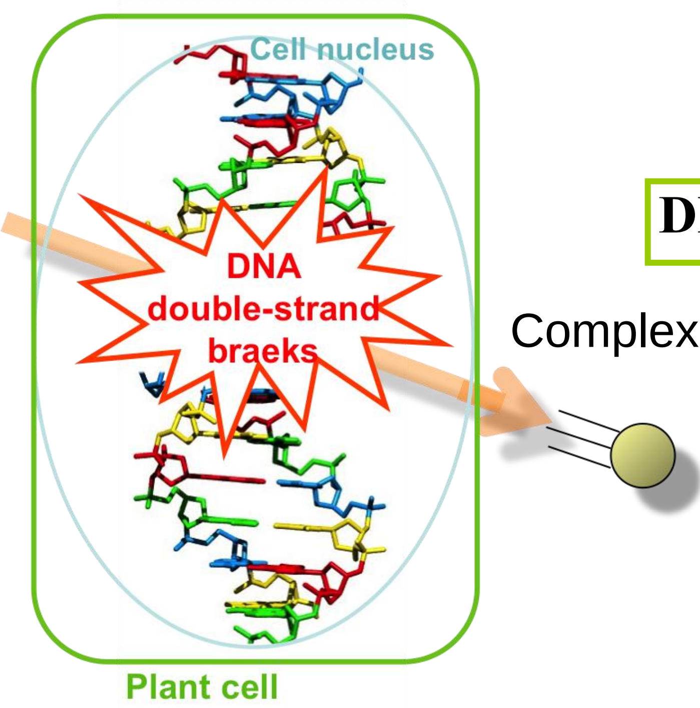


Advantages of ion-beam breeding

- Low dose, high mutation rates, and wide variation.
- A mutant lacking just a single gene to the target characteristic.
- The time span for breeding can be shortened to 2 years.

22 plant cultivars and 2 microbes on sale since 2001 (ca 2 billion Yen /year).

Ion-beam Mutagenesis



DNA repair

Complex, clustered damage

Original(dianthus)
Olivia White Eye

Difficult to repair.
Cell which fail complete repair may still survives

Mutants

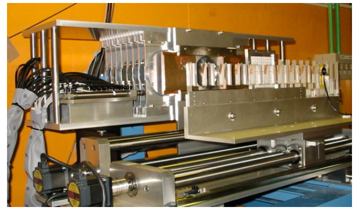
In mutagenesis, genes are artificially damaged using mutagenes, and a variety with the desired characteristics is selected form among the resulting mutants.

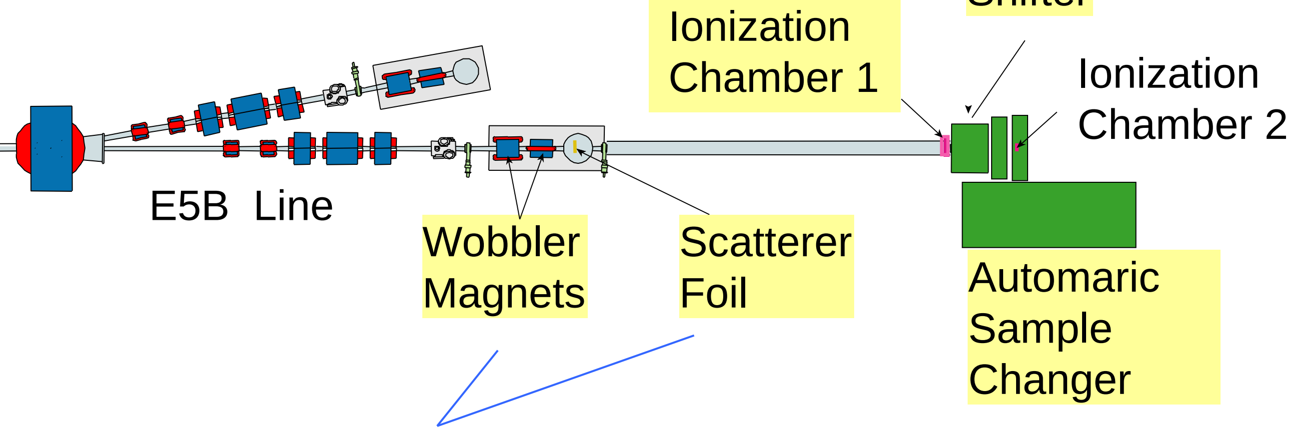
Mutagenesis using radiation is based on the same principle as naturally.

New cultivar

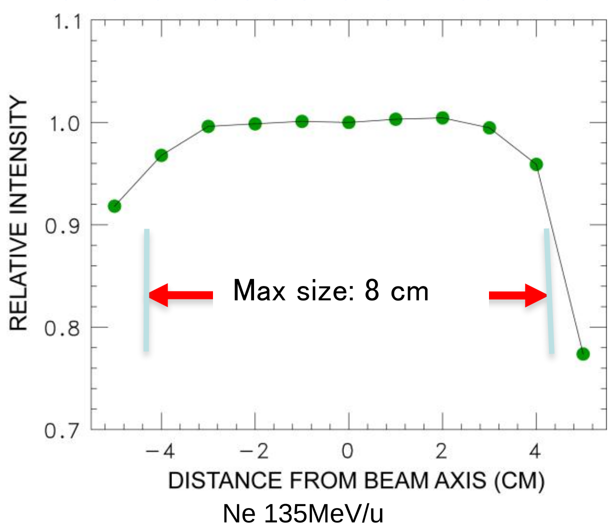
Olivia Purele White
(Hokko Chemical Industry Co.LTD.)

E5B Line for Biological Experiments



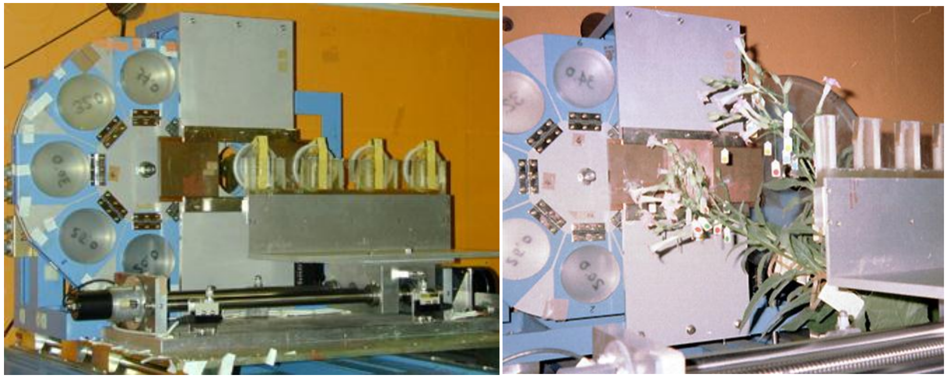


Lateral dose distribution



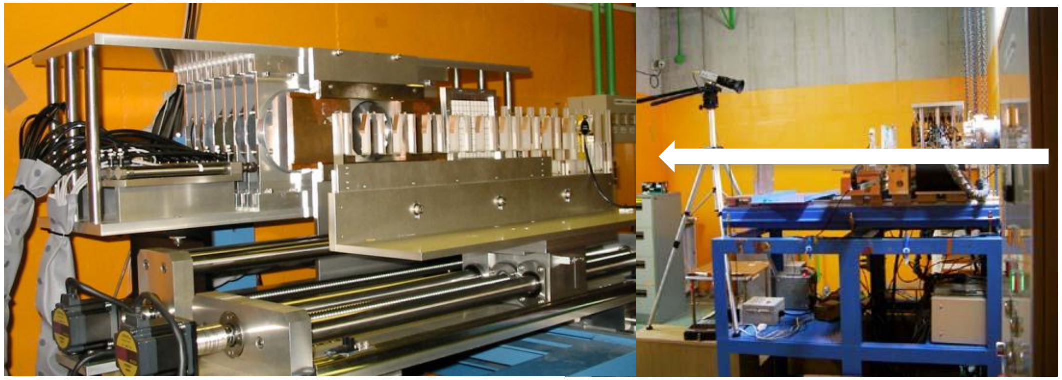
Development of Automatic Sample Changer

The original model (12 samples/h)



Ver. 3 model of Apr. 2004 (40 samples/h)

Ver.2 model of Apr. 2003 (12 samples/h)
with the range shifter (LET adjuster)



Beam

Ryuto H. et.al., J. Biomed. Nanotech. **2**, 88-93 (2006)

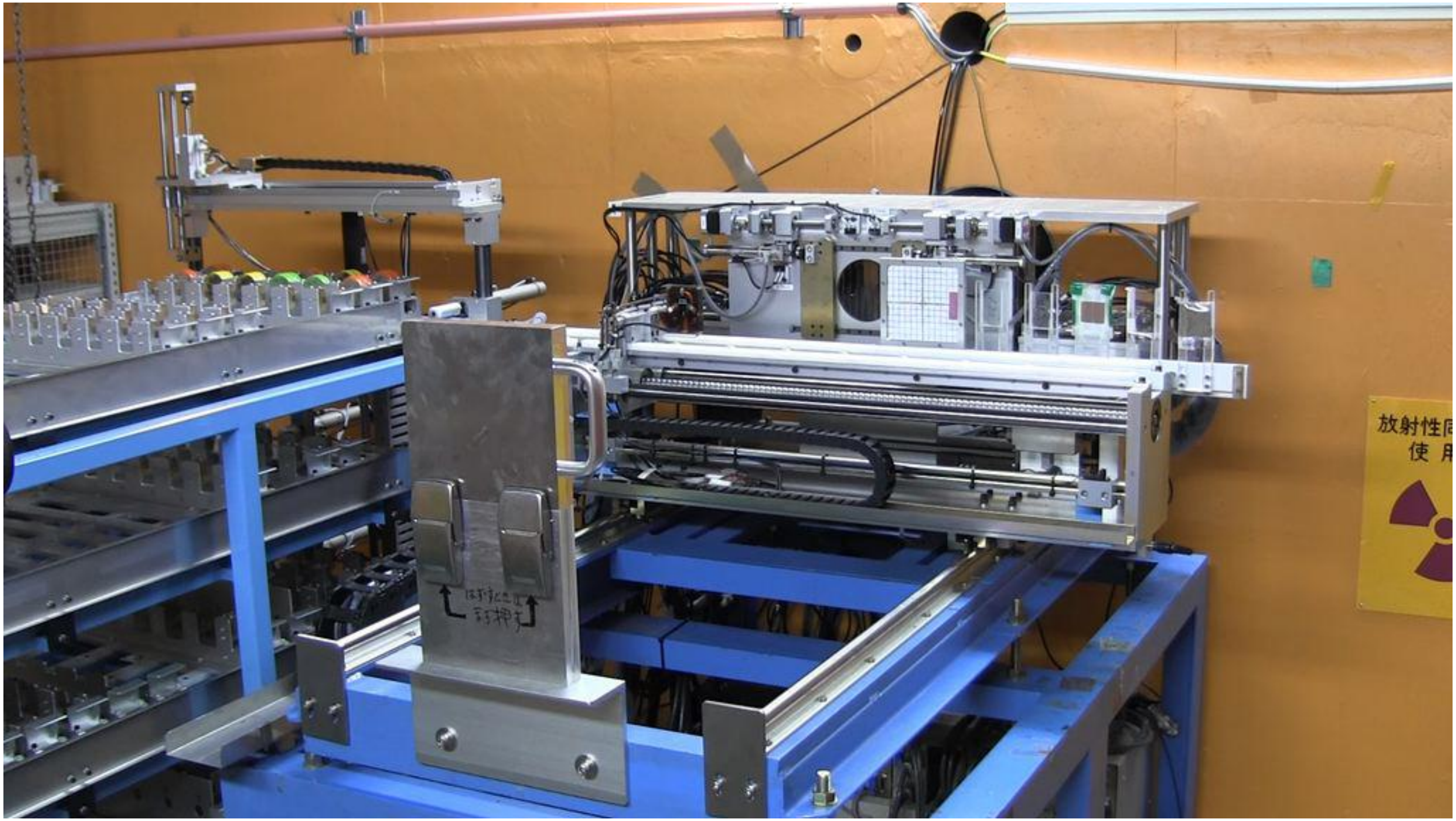
Various Plant Targets Are Available for Irradiation

Dry seeds Wet seeds Branchs

Tissue culture materials
Callus, Stem, shoot, leaf

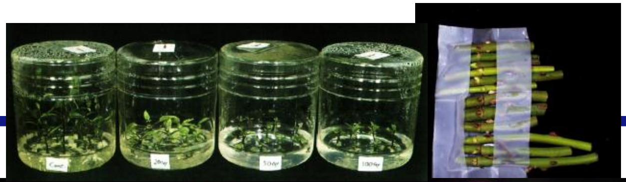
Ryuto H. et.al., Plant Biothec. **25**, 119-122 (2008)

Plant organs to be irradiated

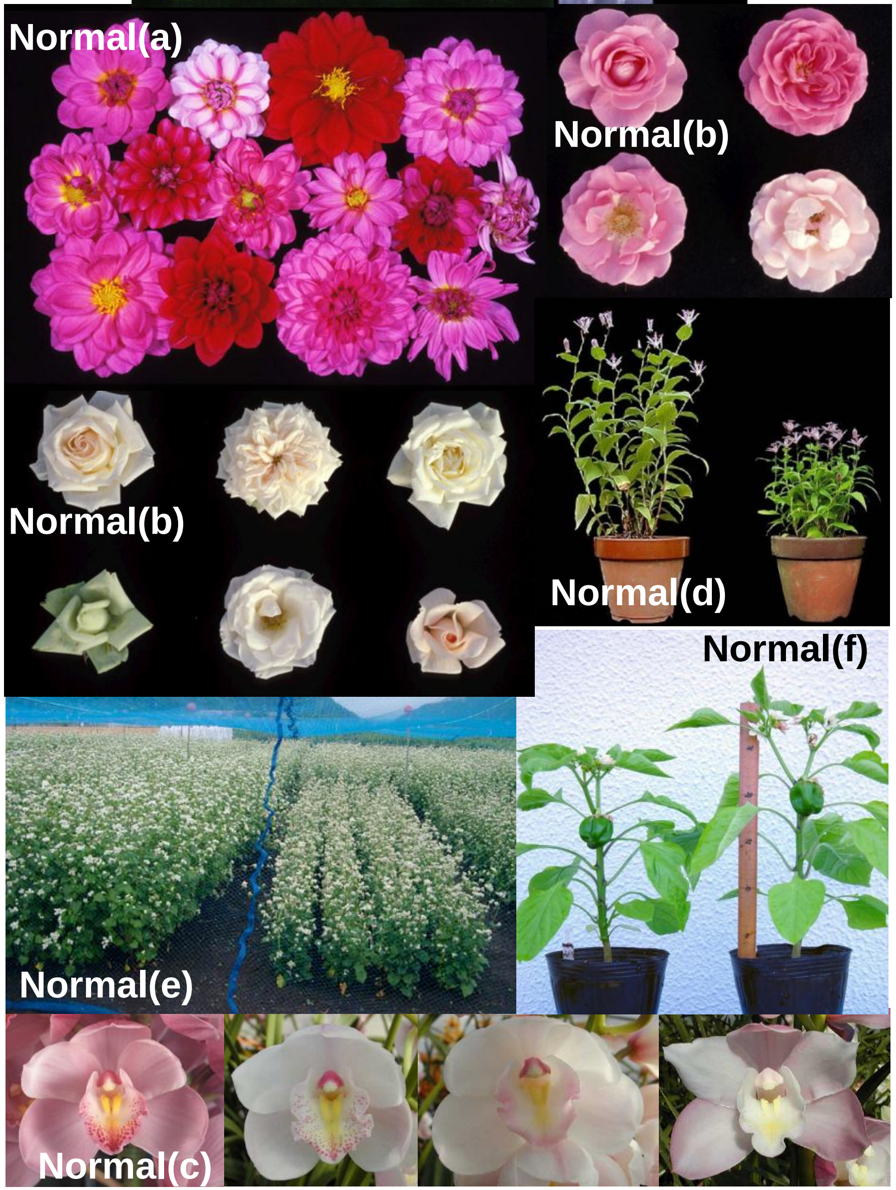


Ryuto H. et.al., J. Biomed. Nanotech. **2**, 88-93 (2006)

Mutants Developed from the RIKEN Beams



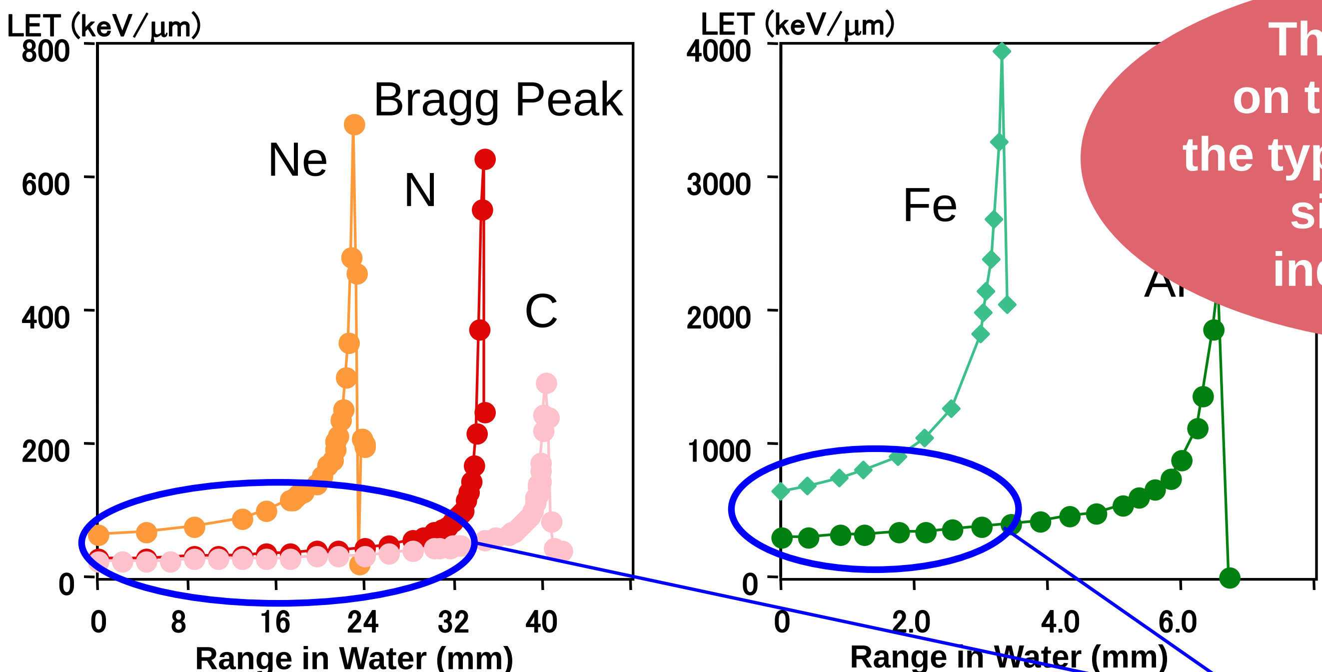
Mutant phenotype	Plant material	Mutation rate (%)
Sterile		
Verbena	Stem	09-2.8
Cyclamen	Tuber	6.7
Eucalyptus	Shoot primordia	9.3
Color and shape		
Petunia	Ovary	1.0
Dahlia[a]	shoot	20.3-50.1
Rose[b]	Dormant scion	43.1-51.7
Chrysanthemum	Stem	4.5-14
Torenia	Leaf and stem	1.6-18.8
Orchid[c]	shoot	5.0-6.3
Variegation		
Petunia Hybrida	Stem	1.8
Dwarf		
Tricyrtis hirta[d]	Embryogenic callus	2.4
Millet	Dry seed	0.1
Buckwheat[e]	Dry seed	0.6
Pepper[f]	Dry seed	1.3
Salt tolerance		
Rice	Wet seed	1.2



Heavy-ion beam Mutagenesis :
From plant breeding
to gene function analysis

A Photo of Mutant flowers made the cover of BUTSURI Vol 67, Oct. 2012, Membership Journal of the Physical Society of Japan.

Ion Beams for Biological Experiments at RIBF



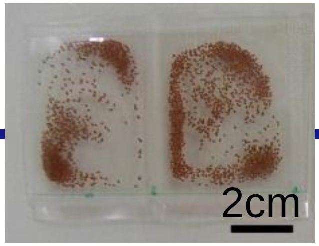
The effects of LET on the mutation rate, the type of DNA mutation, size of deletion, induced in plants.

Ion	Energy MeV/u	Range in H_2O (mm)	LET keV/μm	No. of * Particles
^{12}C	135	39	22.5	277
^{14}N	135	33	30.6	204
^{20}Ne	135	22	61.5	101
^{40}Ar	95	6	280.0	22
^{56}Fe	90	3	624.0	10

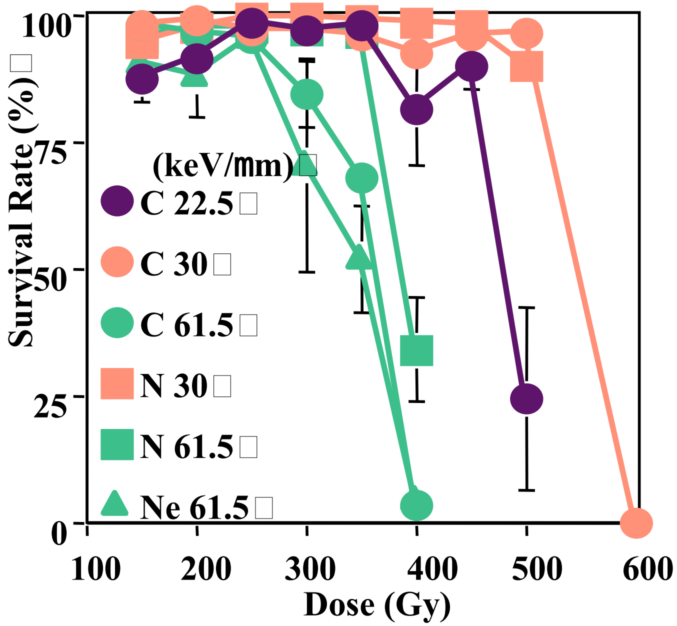
*in $(10\mu m)^2$ at 10 Gy

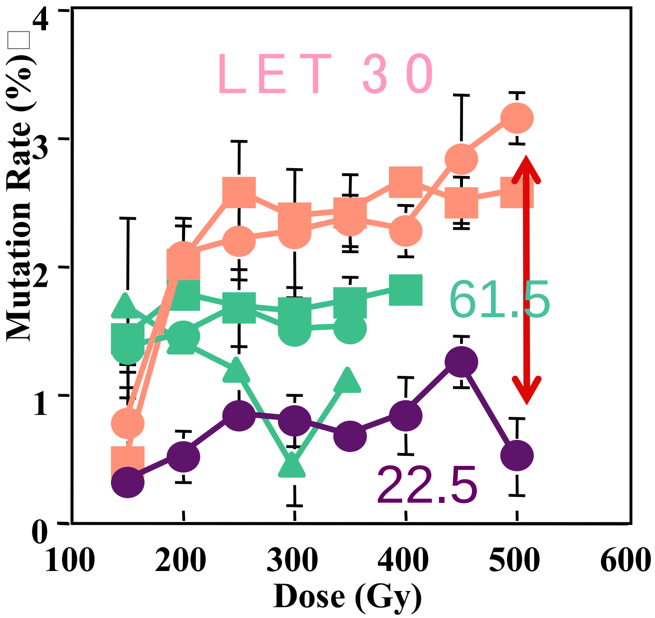
LET below Bragg peak is mainly used for mutation. Ions pass through the samples. A uniform dose distribution is a key to the systematic study,

Optimum LET (LET_{max}) in Arabidopsis-Dry Seeds



Arabidopsis: Model plant used for genetics





LET of
22.5 keV/μm is not enough to induce mutation,
30 keV/μm is the best for mutation,
61.5 keV/μm is not enough particle no. for induce mutation,because those dose reduces survival rate.

Kazama Y. et.al., Plant Biothec. **25**, 113-118 (2008)

Comparison of Mutation Rate in *Arabidopsis*

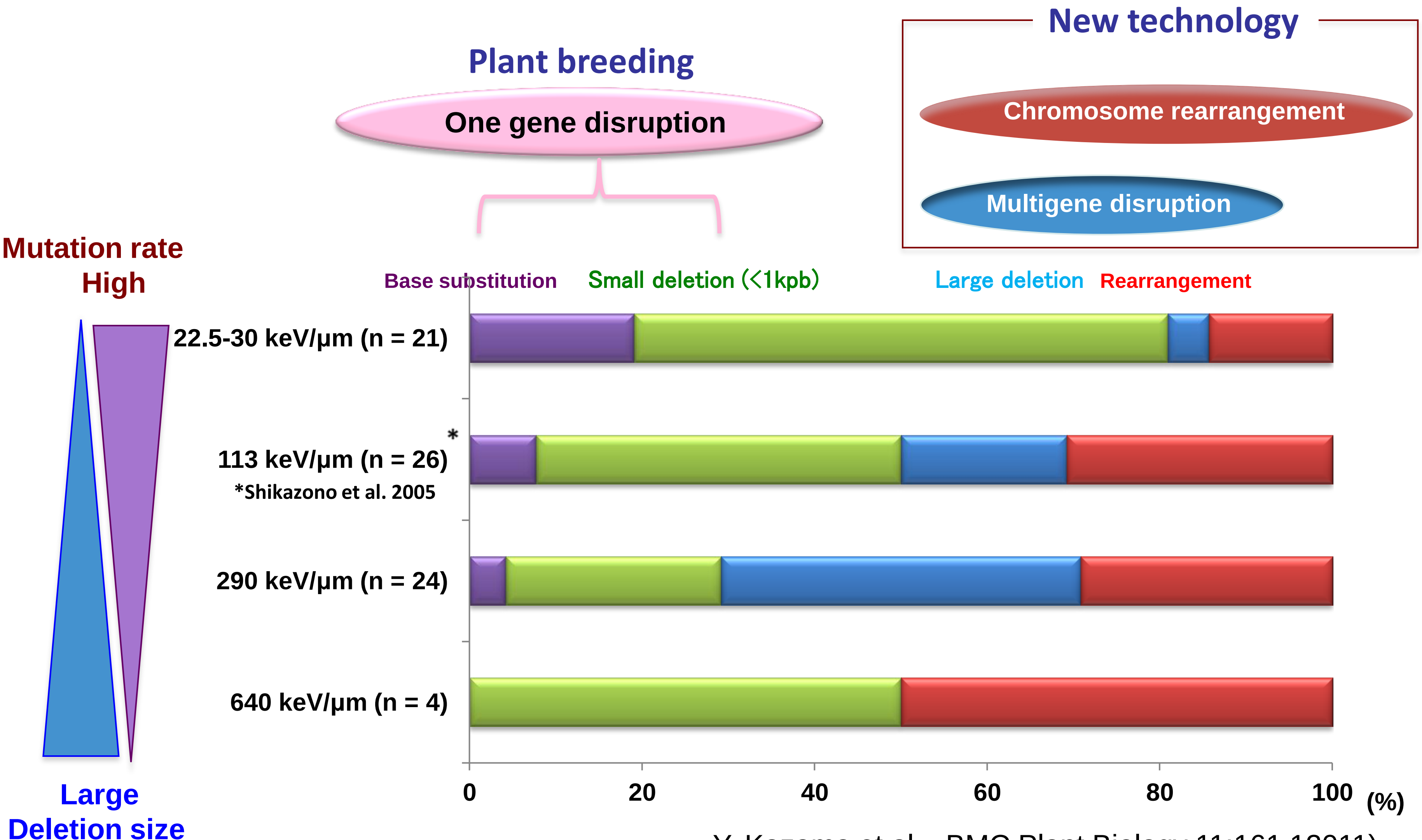
Ion species	LET (keV/µm)	No. of M_1 plant	No. of M_2 plant	No. of mutants (‰) ← *hy + gl* mutants
C	22.5	3,734	27,765	11 (0.40)
	30.0	3,056	29,595	23 (0.78) ← LET_{max}
	290	5,863	57,771	23 (0.40)
Ar	290	5,726	51,686	27 (0.52)

Mutation frequency (‰) was calculated by dividing the number of mutants by the total number of M_2 plants.

EMS⇒ 0.87
ethyl methanesulfonate
X-ray⇒ 0.32
Koornneef et al. (1982)

C-ions at LET_{max} (30keV/µm) has same mutation rate as chemical mutagens.

Is Damaged Regions in DNA Selectable?



Y. Kazama et.al., BMC Plant Biology 11:161 12011)

T. Hirano et.al., Mut. Res. 735, 19-31(2012)

Mutagenomics

Wheat, Morning Glory, Arabidopsis, Rice, etc.

Resequencing ↓

Plants & Microbes ↓

Bioresource → Mutant → Mutation Breeding

↓ Gene analysis ↓ New Cultivars

Gene function

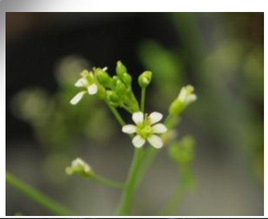
DNA replication and repair

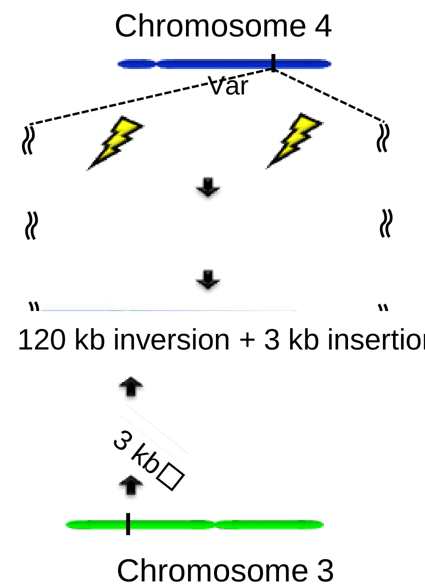
Evolution

Gene Expression and Regulation

The discovery of genes

Mutants collection (Mutant panel) of *Arabidopsis*



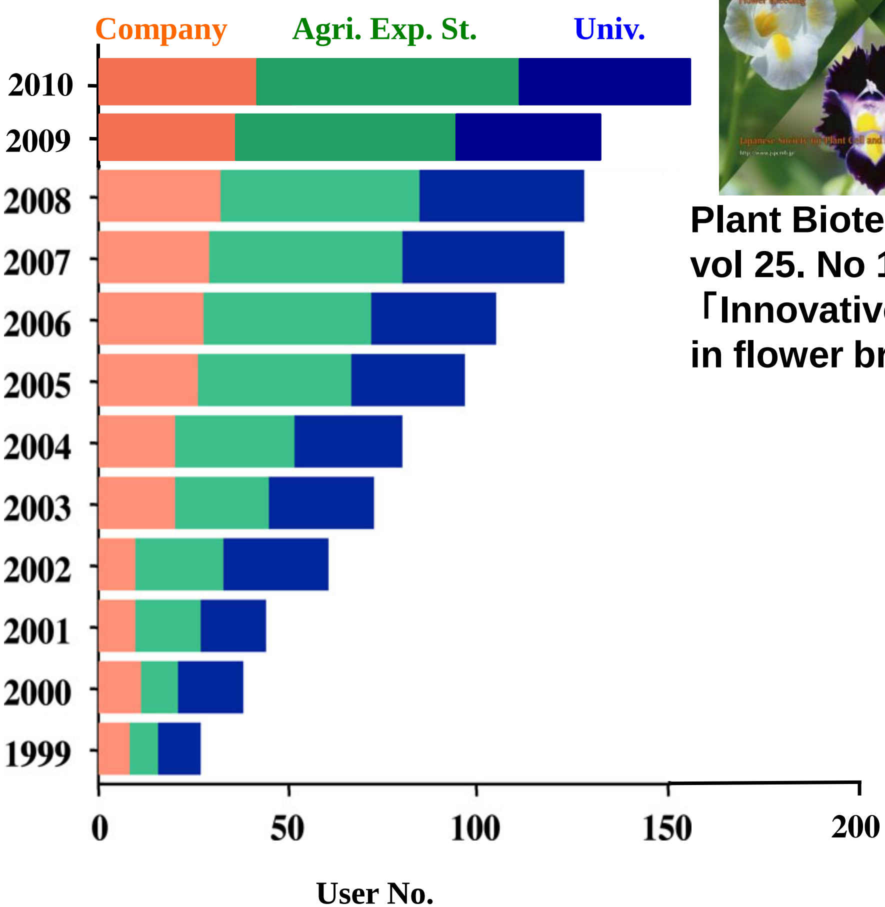


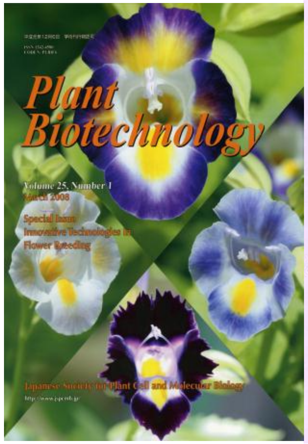
Mutants have become more and more useful and important in modern genetic studies.

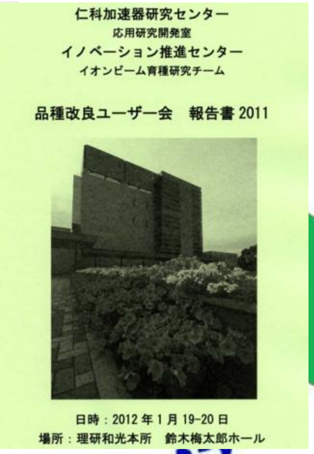
The discovery of genes using mutants may lead to the new field in biology, “Mutagenomics”.

Consortia on ion beam breeding

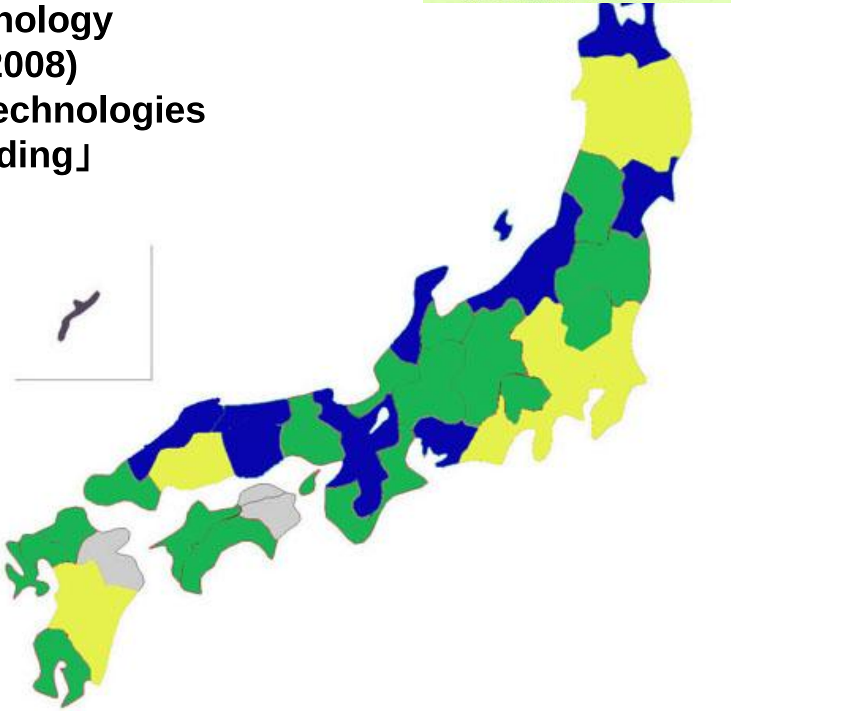
(156 Japanese groups, 15 oversea groups)





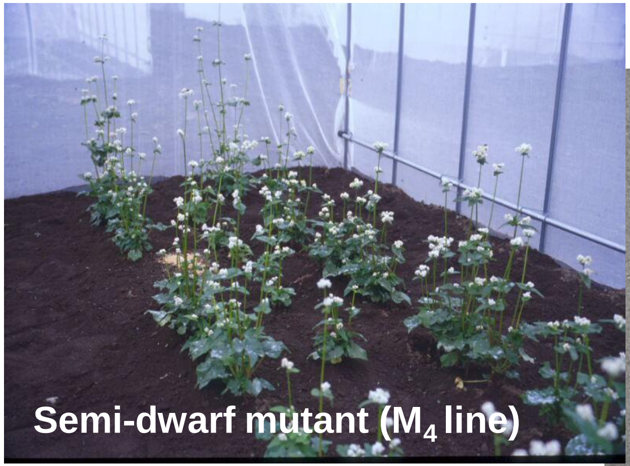


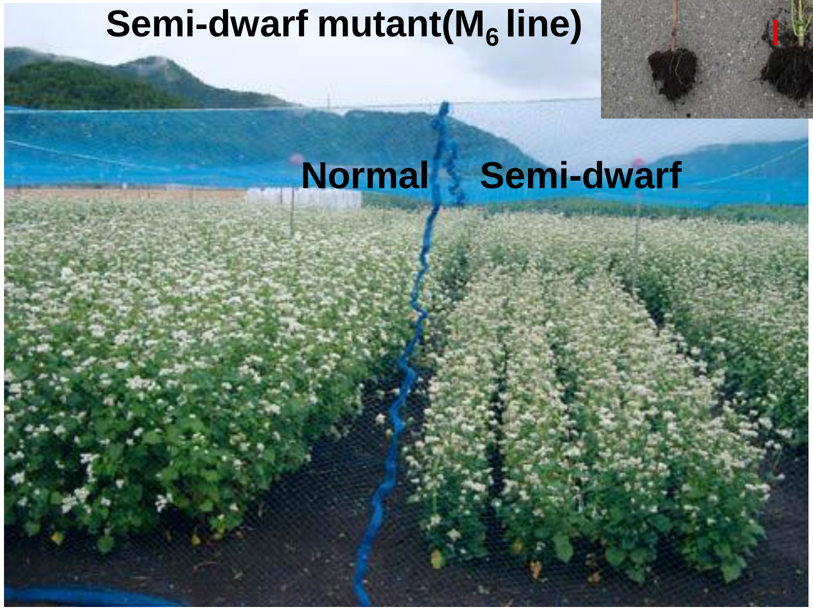
Plant Biotechnology
vol 25. No 1 (2008)
「Innovative technologies
in flower breeding」



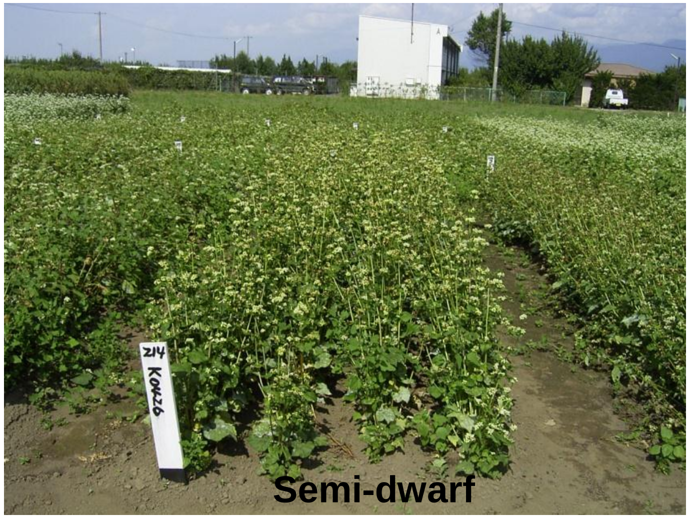
Semi-dwarf buckwheat (2001-2004)

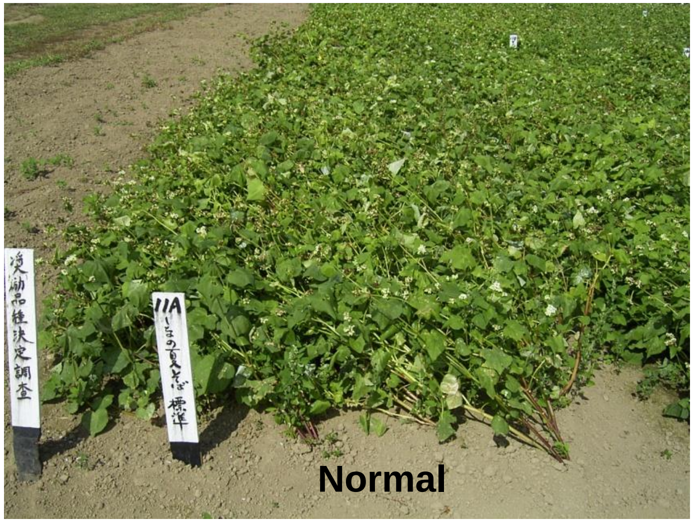
Two semi-dwarf mutants were isolated from 324 M_3 lines.





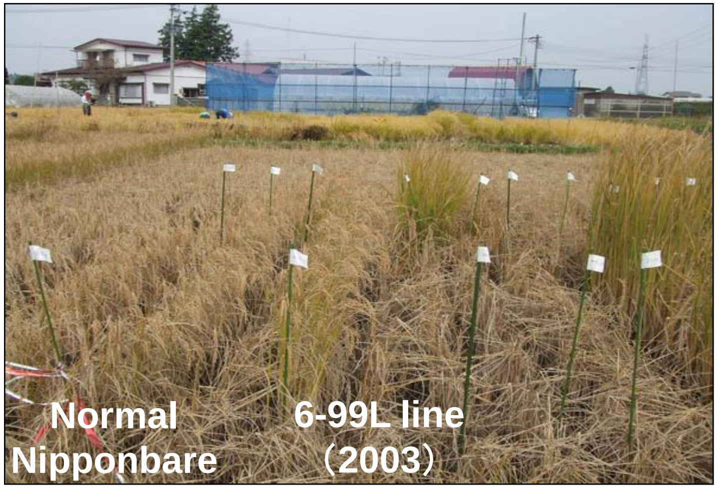
After typhoon No.14 in 2005

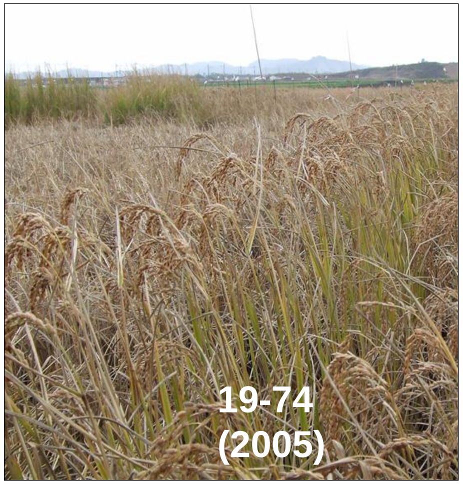


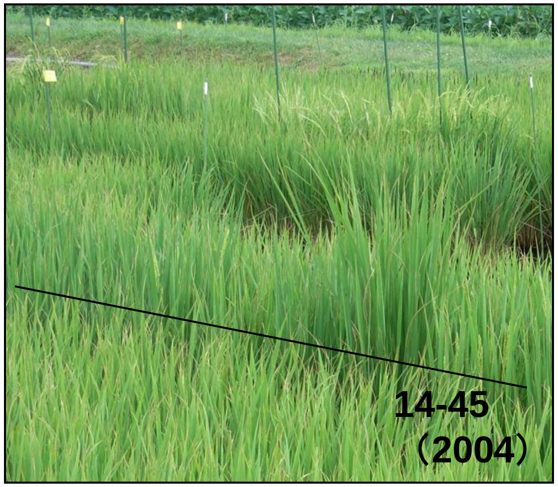


Nagano Chushin Agric. Ext. st.

Selection of the Salt-resistant Rice (2003-)







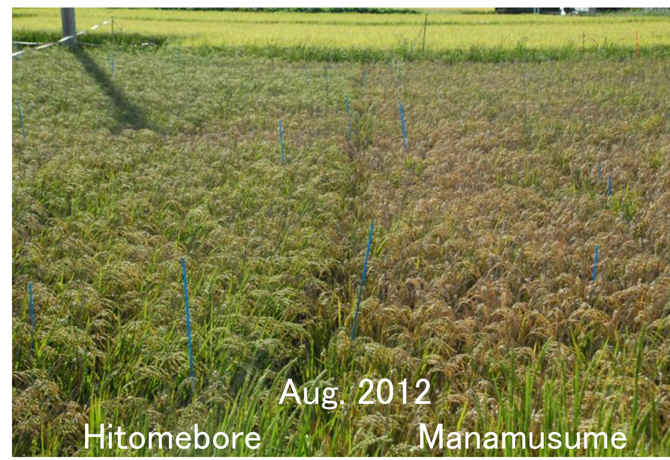
Saline paddy field is maintained at ¼ of sea water concentration.
We isolated 4 salt resistant lines in saline paddy field from 325 M_2 progeny lines.
Compared to the yields of the control plants, 6-99L and 14-45 plants were 1.16- and 1.21-fold higher, respectively.

Line	Mutation rate(%)	Plant height (cm)		Grain yield in saline paddy field(g/m^2)
		Normal	Saline	
Nipponbare		114.9	85.0	402.9
6-99L (40Gy,23keV/μm)	1/91 (1.0)	124.4	102.5	466.5
19-74 (20Gy, 23keV/μm)	1/82 (1.2)	124.2	97.6	454.2
18-36 (15Gy, 60keV/μm)	1/75 (1.3)	124.0	95.1	451.2
14-45 (20Gy, 40keV/μm)	1/77 (1.3)	130.3	102.2	487.8

Selection of the Salt-resistant Rice in Miyagi Prefecture

C ion beam irradiation at April 2011.

The seedlings were grown in a paddy field at the Miyagi Pregectural Furukawa Agricultural Experiment Station. We obtained 368 M_2 lines for Hitomebore and 351 for the Manamusume.



368 lines X16=5888 plants 351 linesX16=5616 plants

We isolated 73 salt-resistant candidate lines from 719 lines.

We will select again salt-resistant plants from 73 lines in 2013.

Newspaper

International Herald Tribune April, 2012

SPECIAL REPORT: BUSINESS OF GREEN

Rice Farmers Seek to Save Their Crops From Salt

Tomohiro Ohsumi/Bloomberg News

A farmer drying harvested rice in a field in Miyagi Prefecture. In total, 11 percent of the prefecture's farmland was damaged last year.

By YURIKO NAGANO
Published: April 22, 2012

TOKYO — Toshiharu Ota, a rice farmer in Miyagi Prefecture, in northeastern Japan, survived the earthquake, tsunami and nuclear disaster last year. But his fields were devastated by the salt deposits left behind when the tsunami's floodwaters receded. Now, to help farmers like Mr. Ota, a research team is working to develop a new salt-tolerant variety of rice.

Related
Advocate Helps Track Polluters on Supply Chain (April 23, 2012)
Shale Gas Search Divides Romania (April 23, 2012)

The tsunami's waves, up to 40 meters, or 130 feet, high engulfed the coastline around Ishinomaki City, where Mr. Ota lives, devastating hundreds of thousands of lives and washing away whole sections of towns, neighborhoods and farmland.

Miyagi Prefecture has estimated the cost of damage to agricultural land and facilities at ¥381 billion, or $4.6 billion, making it one of the prefectures hardest hit in economic terms by the disaster.

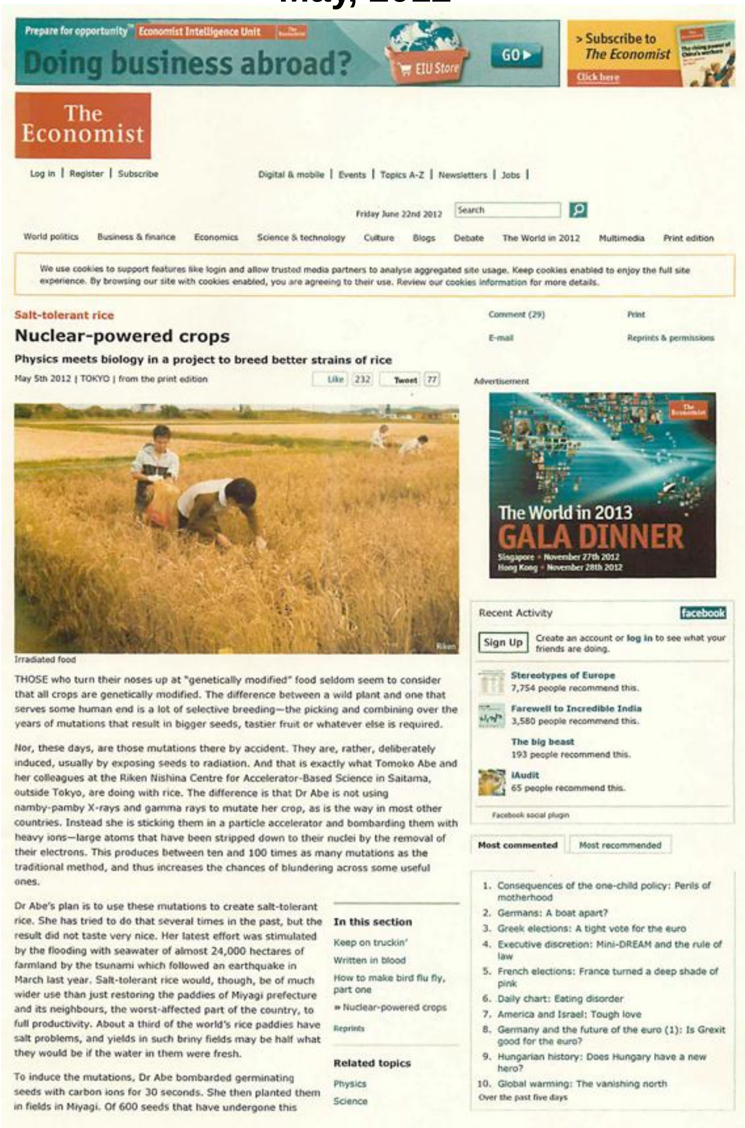
Rice has traditionally been a leading crop in northeastern Japan. Miyagi Prefecture's 2010 harvest fetched $818 million. But last year the harvested rice acreage fell short of target by 4,600 hectares, or 11,400 acres, according to the agriculture ministry. In total, 11 percent of the prefecture's farmland was damaged.

More than a year after the disaster, many farmers like Mr. Ota are still struggling to cope with the economic consequences. Even leaving aside the widespread fallout of radioactive elements released from the Fukushima Daiichi Nuclear Power Plant, the tsunami left the soil of coastal farmlands damaged by sodium chloride from the sea water.

Mr. Ota, who farmed 11 hectares of rice paddies, said nearly half were flooded. Local workers have labored hard to remove salt from the soil in the past year. Still, nothing has been the same since.

"Even with desalination, the yield has dropped," Mr. Ota, 56, a sun-tanned, fifth generation farmer with graying hair, said during a recent interview.

The Economist May, 2012



Salt-tolerant rice

Nuclear-powered crops

Physics meets biology in a project to breed better strains of rice

May 5th 2012 | TOKYO | from the print edition

Irradiated food

THOSE who turn their noses up at "genetically modified" food seldom seem to consider that all crops are genetically modified. The difference between a wild plant and one that serves some human end is a lot of selective breeding—the picking and combining over the years of mutations that result in bigger seeds, tastier fruit or whatever else is required.

Nor, these days, are those mutations there by accident. They are, rather, deliberately induced, usually by exposing seeds to radiation. And that is exactly what Tomoko Abe and her colleagues at the Riken Nishina Centre for Accelerator-Based Science in Saitama, outside Tokyo, are doing with rice. The difference is that Dr Abe is not using namby-pamby X-rays and gamma rays to mutate her crop, as is the way in most other countries. Instead she is sticking them in a particle accelerator and bombarding them with heavy ions—large atoms that have been stripped down to their nuclei by the removal of their electrons. This produces between ten and 100 times as many mutations as the traditional method, and thus increases the chances of blundering across some useful ones.

Dr Abe's plan is to use these mutations to create salt-tolerant rice. She has tried to do that several times in the past, but the result did not taste very nice. Her latest effort was stimulated by the flooding with seawater of almost 24,000 hectares of farmland by the tsunami which followed an earthquake in March last year. Salt-tolerant rice would, though, be of much wider use than just restoring the paddies of Miyagi prefecture and its neighbours, the worst-affected part of the country, to full productivity. About a third of the world's rice paddies have salt problems, and yields in such briny fields may be half what they would be if the water in them were fresh.

To induce the mutations, Dr Abe bombarded germinating seeds with carbon ions for 30 seconds. She then planted them in fields in Miyagi. Of 600 seeds that have undergone this

In this section
Keep on truckin'
Written in blood
How to make bird flu fly, part one
» Nuclear-powered crops
Reprints

Related topics
Physics
Science

Beam Time in 2011 (conclusions)

Number of Samples

Ion LET(keV/μm) Energy (keV/u)	^{12}C 22.5 135	^{20}Ne 61.5 135	^{40}Ar 290 95	^{56}Fe 640 90	Total
April	357	171			528
June	356				356
Sep	582			70	70
Feb	382		84	24	490
Total	1677	171	84	94	2026

Plant breeding
LETmax=30-70 keV/μm

New Technology

Microbial breeding

Seed/shoot/branch
Seasonal crop
Field Green house
Spring

Autumn
Tree

In vitro cultured
Plant &
microbial

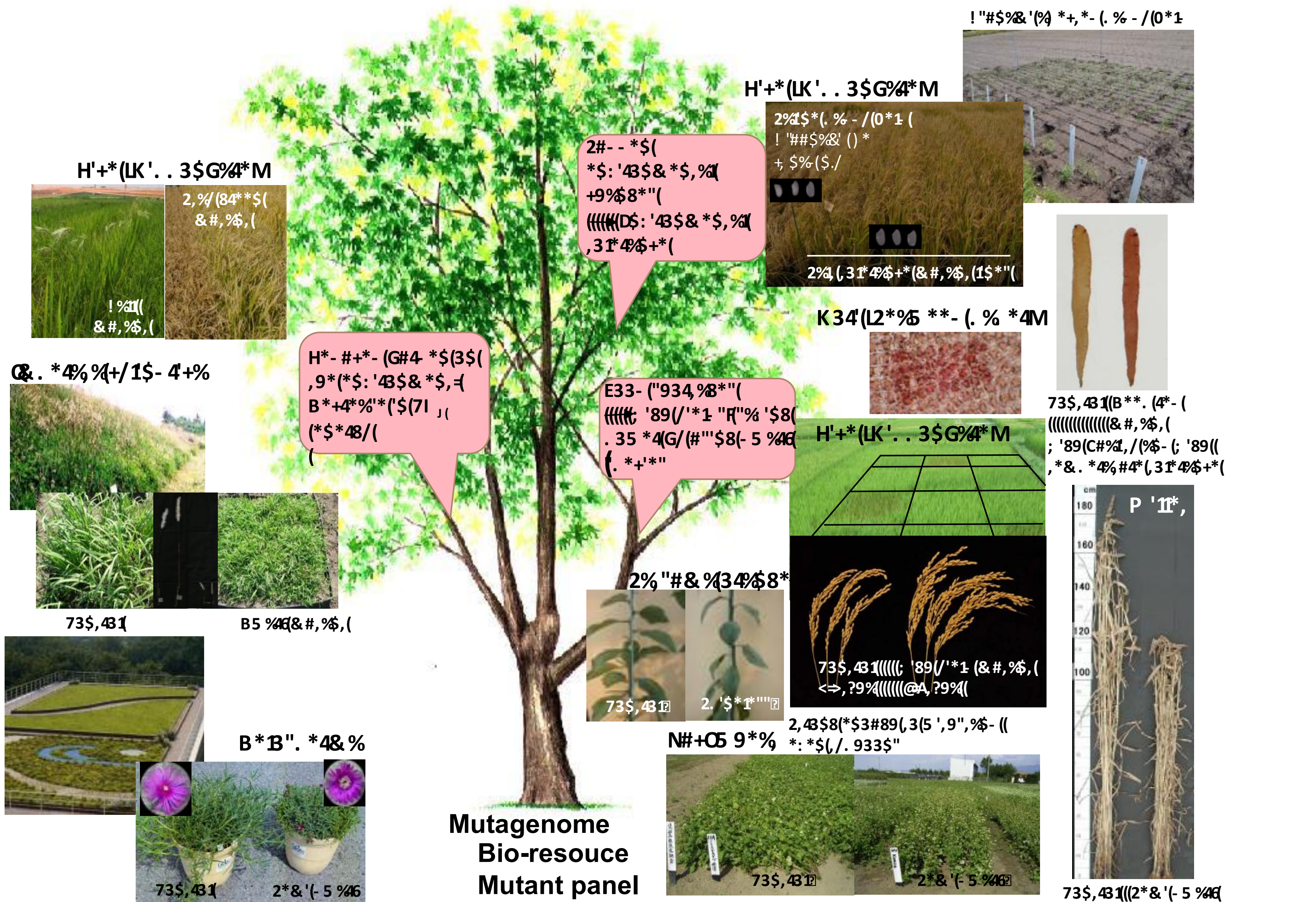
Total Beam time is about 48 hrs

Surfinia White

Saitama Yesat G strain

"Nishina Homare"(in honor of Nishina), named after Yoshio Nishina, the father of nuclear physics in Japan and one of RIKEN's most eminent scientists.

Growing A Peaceful World through Breeding



Mutagenome
Bio-resouce
Mutant panel
Nano cutter□

Thanks

装置開発
照射シミュレーション
物理班

共同研究
品種改良
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国内156団体
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林　祐子


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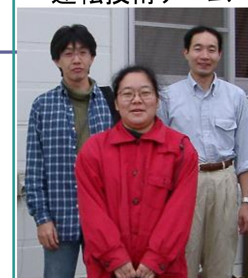
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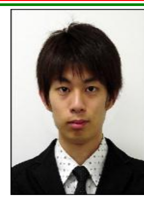
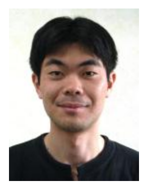
鈴木賢一
（サントリー
フラワーズ）

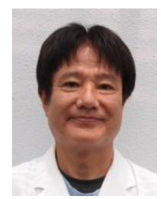

馬　立秋
（埼玉大学）
JRA (D3)
微生物変異解析


風間裕介


石井公太郎

雌雄異株植物変異解析


林　依子


一瀬勝紀


森田竜平


杉山正夫
（北興化学）


茶谷正孝
（長崎県）

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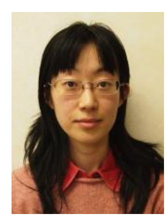
保倉明子


増山文博
（東京電気大）　M1


白川侑紀


平野智也


若菜妙子


小暮祥子

イネ系統保存
・変異解析


佐藤陽一


笠原　諒
（理研食品）

育種技術開発・変異解析

放射光を用いた植物の重金属観察技術開発

Thanks for your attention!