Attempt to generate banana/plantain resistant to *Fusarium oxysporum* f. sp. *cubense* by irradiation-induced mutagenesis

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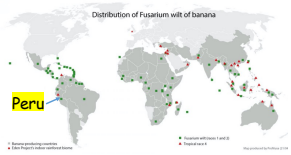
Abstract

Fusarium wilt disease of banana/plantain is caused by *Fusarium oxysporum* f. sp. *cubense* (*Focb*). Recently, *Focb* race TR4, which causes wilt disease on the resistant cultivar 'Cavendish', has become a devastating threat to banana production worldwide. In the international collaborative SATREPS project between Japan and Peru, we are attempting to obtain TR4-resistant banana/plantain plants through random mutagenesis. *In vitro* tissue-cultured buds of three cultivars (Isla, Bellaco Harton, and Bellaco Plantano) in addition to Cavendish, were irradiated with the heavy-ion beam in Japan, while Isla was used for gamma ray irradiation in Peru. The irradiated buds are grown *in vitro* and selected to obtain progeny generations through the chimera dissolution process. Race TR4-resistant lines will be screened after propagation and acclimatization of the plantlets from selected buds.

Background: Pandemic of TR4 banana wilt disease

Fusarium oxysporum f. sp. *cubense* (*Focb*) is an important pathogen that causes banana wilt disease over the world. In recent years, tropical race 4 (TR4), a new race of *Focb* that infects the *Focb*-resistant cultivar 'Cavendish', has spread from Asia to Australia, Africa, and, by 2019, South America, raising concerns about its impact on the global market. In March 2021, TR4 was found in northwestern Peru, Piura.



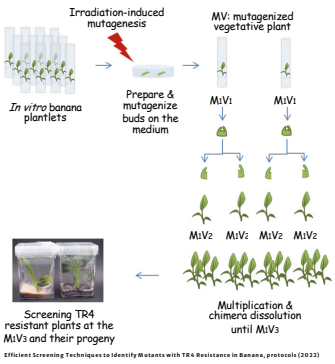
Banana wilt symptoms by Fusarium infection

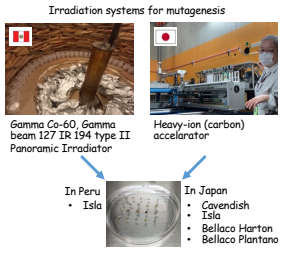
SATREPS project and goal of this study

SATREPS is a JST/JICA program for research projects targeting global issues and involving partnerships between researchers in Japan and developing countries.
From 2022, we started the SATREPS project between Japan and Peru to establish a preventative system to stop further invasion and spread of TR4 in Peru.

This study is one of the project activities and aims to generate TR4-resistant banana/plantain plants through irradiation-induced mutagenesis using the heavy-ion beam and gamma ray.

Experimental design





Isla and Bellaco cultivars, which are widely grown in Peru and distributed throughout Peruvian markets, were selected as targets for mutagenesis.

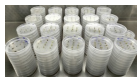
Result 1: Gamma ray-induced mutagenesis in Peru

1-1. Preliminary experiments of gamma ray irradiation with Isla buds.

Dose (Gy)	Survival rate (%)	Explant height	No. of shoots	No. of roots	Root length	Leaf color
0	98	22.70	1.42	1.67	16.41	green
10	84	23.69	1.23	1.68	22.97	green
20	98	18.93	1.14	1.32	10.92	green
30	84	12.83	1.03	0.36	3.38	light green
40	60	10.49	0.37	0	0	light green
50	42	8.36	0.34	0.13	1.05	light green
60	22	5.41	0.00	0	0	light green

40 Gy irradiation resulted in a 60% survival rate.
↓
40 Gy was decided to be used for mutagenesis.

1-2. Isla buds were irradiated with gamma ray at 40 Gy.



2,040 buds were used.

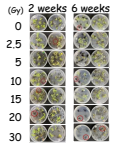
The shoot survival rate was 92%.

For TR4-resistant line screening
- 355 explants irradiated (M_1V_2)
- 40 explants (0 Gy)

For second irradiation at 40 Gy
- 1,600 M_1V_2 explants

Result 2: Carbon beam-induced mutagenesis in Japan

2-1. Preliminary experiments of heavy-ion (carbon) irradiation with Cavendish buds.



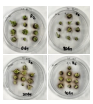
Weeks after irradiation	0 Gy n=28	2.5 Gy n=29	5 Gy n=31	10 Gy n=29	15 Gy n=32	20 Gy n=14	30 Gy n=13
1 week	100%	100%	100%	97%	100%	100%	92%
2 weeks	100%	93%	100%	97%	91%	79%	92%
3 weeks	100%	76%	100%	97%	75%	79%	85%
4 weeks	100%	76%	90%	86%	81%	71%	62%
5 weeks	100%	76%	84%	86%	75%	50%	46%
6 weeks	100%	76%	84%	86%	75%	36%	31%

Survival rate after 6 weeks of carbon beam irradiation
20 Gy = 36%
30 Gy = 31%

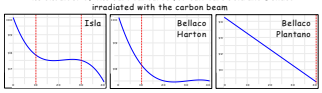
2-2. Isla and Bellaco buds were irradiated with carbon beam at different Gy doses.

Carbon beam irradiation (0, 20, 30, and 40 Gy)

Select surviving buds

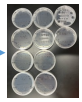


M_1V_1



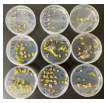
Isla is more tolerant to carbon beam irradiation than two Bellaco cultivars.

Divide the meristem into two (30 and 40 Gy)



M_1V_2

Cut the bud halfway down



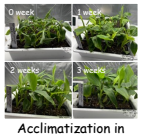
M_1V_3

No. of surviving buds		
Isla	Bellaco Harton	Bellaco Plantano
125	15	16

The M_1V_3 buds will be multiplied and inoculated with TR4.

Result 3: How to screen TR4-resistant lines

3-1. Cavendish plantlets were successfully acclimatized from mericlone seedlings and inoculated with TR4.



Acclimatization in a growth chamber

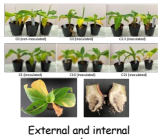
Transplanted to new pots

Inoculated with TR4

10^6 cells/mL × 10 mL per pot

Grown for one month

Disease analysis



One treatment was not enough to cause the disease efficiently.

Additional inoculation

Disease rate 66-70%

Inoculation system should be improved.

Future challenges

To more efficiently screen TR4-resistant plants, we need to establish a system to inoculate a large number of mutagenized plantlets and effectively induce the disease in susceptible ones. To this end, we are trying a direct soaking inoculation at acclimatization.