

Nurturing rich and beautiful forests for the future generation



# Forestry and Forest Products Research Institute

Incorporated Administrative Agency

Forestry and Forest Products Research Institute  
Forest Tree Breeding Center  
Forest Bio-Research Center

# Professionals in forest and development



Plus-tree Saeki No. 3

**Forest Tree Breeding Center (FTBC) and Forest Bio-Research Center (FBRC), both have been engaged in forest tree breeding by development of genetically superior varieties, speed-up breeding process through high-tech application, collection and conservation/preservation of genetic materials, and international technical cooperation on forest tree breeding in the world.**

## Features

### **【The largest institute of forest tree breeding in Japan】**

- FTBC, the largest center of forest tree breeding in Japan, has developed more than 2,400 new genetically superior varieties of forest tree species.
- FTBC is also serving as a core organization for the distribution of new varieties of clones to the local government seed/scion orchards which are functioning as the authorized regional seed/scion supply centers for private nurseries.
- There are 5 Breeding Regions in Japan. Breeding regions are set up considering climate conditions, tree species and administration areas of the national forest and prefectural governments.
- FBRC has conducted studies to develop the techniques necessary for shortening breeding periods of forest trees by using biotechnologies.
- As one of the world's prime research institute in forest tree breeding, FTBC is leading in the research and development of pine wilt nematode resistant varieties and pollen free / less pollen varieties, and snow damage tolerant varieties, which other countries have not developed yet.
- FTBC is carrying out its R/D taking due consideration of regional climate differences and associated species diversity with four regional breeding offices, i.e. Hokkaido, Tohoku, Kansai and Kyushu, and Iriomote tropical forest tree breeding garden where tropical and sub-tropical forest tree species are main targeted species.

### **【The only forest tree gene bank in Japan】**

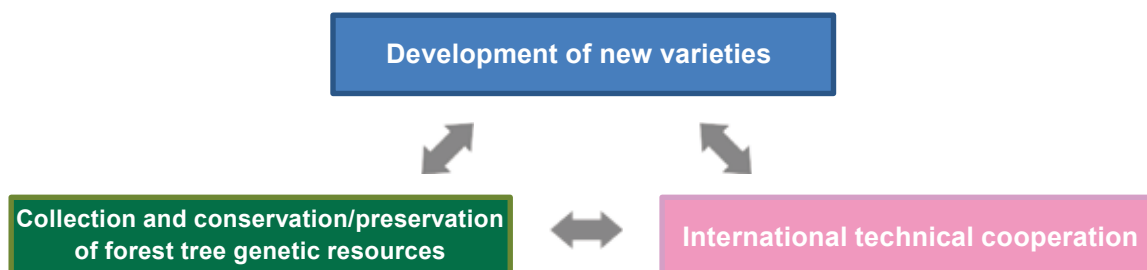
- FTBC is the only forest tree species gene bank, and the scale of gene stock in forest tree species is the largest in Japan.
- Our conservation/preservation forms in national forests are classified into three types, i.e. standing tree (30,000 varieties), seed (12,000 varieties), pollen (4,200 varieties) and DNA (400 varieties).

### **【Advanced research facilities in leading forest tree breeding R/D】**

- Advanced research for speed-up breeding process development of genetically superior varieties by using genetic engineering research facility.
- Developing 3<sup>rd</sup> generation plus-tree by using crossing (hybridization) greenhouse.
- Conservation/preservation of seeds and pollen in forest tree gene bank storing facilities have been investigated.
- The largest specific net house and GMO isolation field have been utilized for genetic engineering.

Content

# tree breeding research



Main tasks

## 01

### 【Development of new varieties】

- Varieties superior in growth and wood quality (fast growth, trunk straightness and strength)
- Varieties of pollen allergies (pollen free and less pollen)
- Varieties superior in resistance against damages (pine wilt nematode and snow damage tolerance)

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## 02

### 【Collection and conservation / preservation of forest tree genetic resources】

- Breeding materials for new varieties of cedar, cypress, etc.
- Endangered tree species and natural heritage trees (part of the National Biodiversity Strategy)

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## 03

### 【International technical cooperation】

- International technical cooperation and collaborative research for climate change adaptation, etc.
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e n t s



**Development strategy of new varieties and their outputs**

**Varieties superior in growth and wood quality**

- 9,000 individuals from cedar, cypress, pine and others were selected as plus-trees and have been conserved / preserved
- 287 individuals out of plus-trees have been further selected as new varieties with good growth and trunk straightness
- 646 cedar clones, 311 cypress clones and 134 larch clones, 50 sakhalin fir clones and 4 gmelini larch clones have been selected by crossing between plus-tree clones
- 229 less twisted larch varieties have been selected
- 226 cedar clones, 58 cypress clones, 94 larch clones (including 1 gmelini larch clone) and 29 Sakhalin fir clones have been designated as 'Specified Mother Tree' by Minister of Agriculture, Forestry and Fisheries of Japan

**Varieties superior in environmental and other services**

- Pollen free cedar :24 varieties
- Less pollen cedar(less than 1% pollen production) : 147 varieties
- Less pollen cypress :55 varieties
- Low pollen cedar(low amount of male strobilus) : 16 varieties
- Cedar with pollen free gene :2 varieties

**Varieties superior in resistance against diseases/pests attack or severe weather conditions**

- Pine wilt nematode resistance :590 varieties
- Snow damage tolerance :46 varieties

**Application of high technology to forest tree breeding**

- Development of genetic engineering techniques for genetic recombination, genome editing and tissue culture



**Status of dissemination of the improved varieties :**  
**About 70% of seedlings / saplings for forest planting are from the improved varieties ( cedar, cypress, red pine and black pine, )**

**Forest tree breeding and its history**

Nurturing rich and beautiful forests through artificial planting will require genetically superior seedlings / saplings which can grow under severe natural environments over several decades.

In Japan, preliminary trials related to tree breeding will be found in Obi and Hita areas in Kyusyu.

People in those areas have selected plus-trees and propagated saplings from plus-trees scions for more than 400 years. The national-based tree breeding program started around 60 years ago.

In 1954, Forestry Agency launched a nationwide project of plus-trees selection for timber production increase and enhancement of multiple functions from planted forests.

As a core implementing organization for tree breeding in Japan, the first Forest Tree Breeding Stations were established under Forestry Agency in 1957.

As a part of the structural reform of the central government, Forest Tree Breeding Center became an incorporated administrative agency under the Ministry of Agriculture, Forestry and Fisheries in 2001.

Furthermore, in 2007, Forest Tree Breeding Center merged into Forestry and Forest Products Research Institute which is also an incorporated administrative agency. Forest Tree Breeding Center (FTBC) and Forest Bio-Research Center (FBRC) were established under Forestry and Forest Products Research Institute (FFPRI) in 2017.

The tree breeding program has been initiated from plus-trees selection project, then with the reflections of emerging damages/problems inside and outside forests, pine wilt nematode resistant varieties, snow damage tolerant varieties, pollen free / less pollen varieties have been also developed.

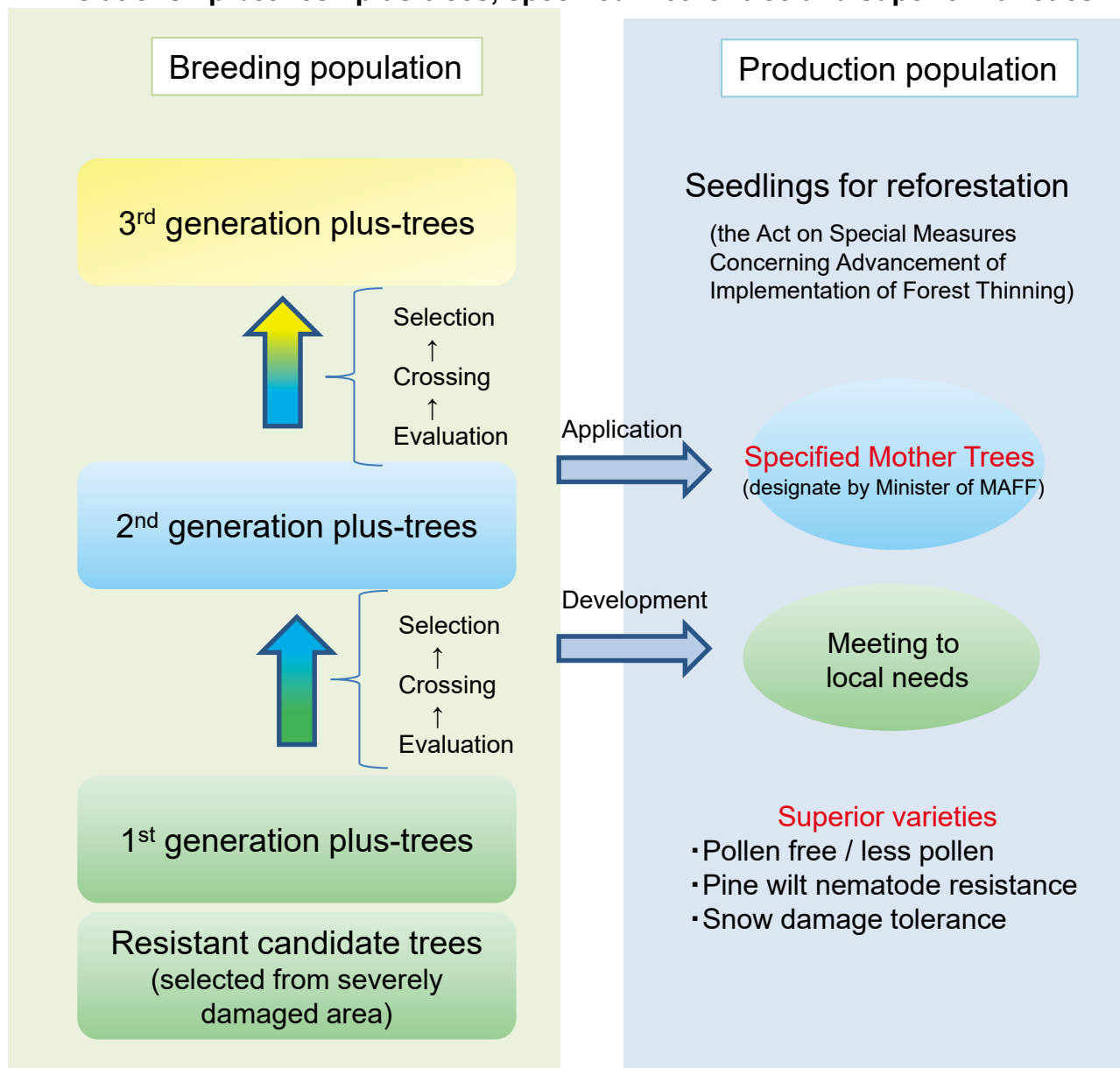
## Procedure of forest tree breeding projects

To sustain the efforts of tree breeding and make a good forest planting for several generations, forest tree breeding projects are promoted by two groups, “Breeding population” for mainstream tree breeding and “Production population” to produce seedlings for reforestation.

“Breeding population” is composed of plus-tree clones that are superior in character such as form, growth, etc. Extensive crossing and selection are performed for the selection of second and later generation of plus-trees.

“Production population” is composed of trees selected from “Breeding population” and they are good varieties for forest maintenance. ‘**Specified Mother Tree**’ which is superior in growth, is designated by Minister of MAFF, in according with a law “the Act on Special Measures Concerning Advancement of Implementation of Forest Thinning” for future forest improvement. **Superior varieties** are developed for local needs including pine wilt nematode resistance, snow damage tolerance and pollen free / less pollen.

### Relationship between plus-trees, specified mother tree and superior varieties

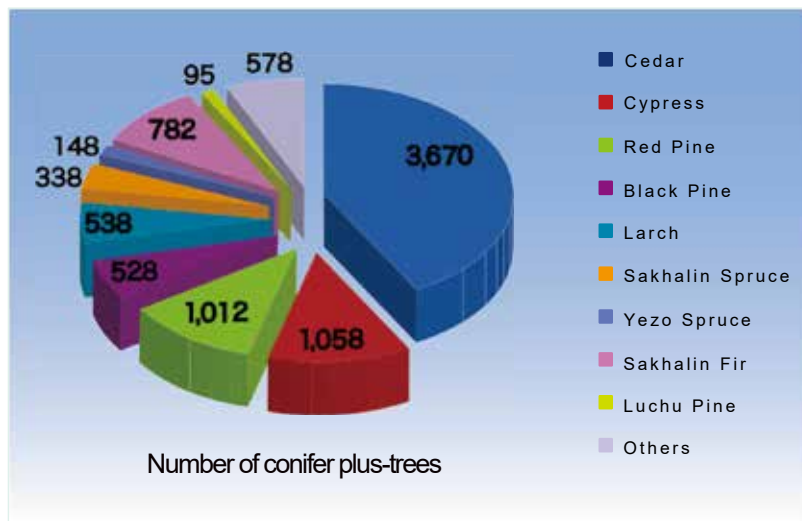


**Development of varieties superior in growth and quality**

**1. Selection of plus-trees**

Approximately 9,000 trees have been selected as plus-trees from the national forests, which are 30% or more superior in volume and growth compared to adjacent three large trees.

In the plus-trees, there are some trees that include individual of fast growth, trunk straightness and good wood quality that support forest breeding projects. The number of conifer plus-trees in Japan is as follows.



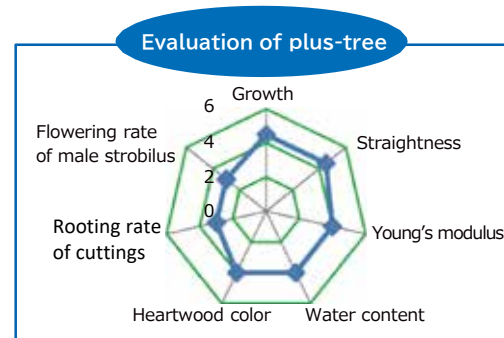
**2. Evaluation of plus-trees trait**

Progeny test forests are examinations of how plus-trees are genetically superior. Clones and seedlings of plus-trees are reforested. Currently, 1,800 progeny test forests, (Each one is from 1ha to 6ha, 2,400ha in total) are maintained and monitored nationwide.

FTBC is conducting periodical monitoring (each 10, 20 and 30 years) of the progeny test forest. Collected data such as stem growth, stem trunk straightness and wood quality are compiled and evaluated, then published as “Plus-tree trait tables”.



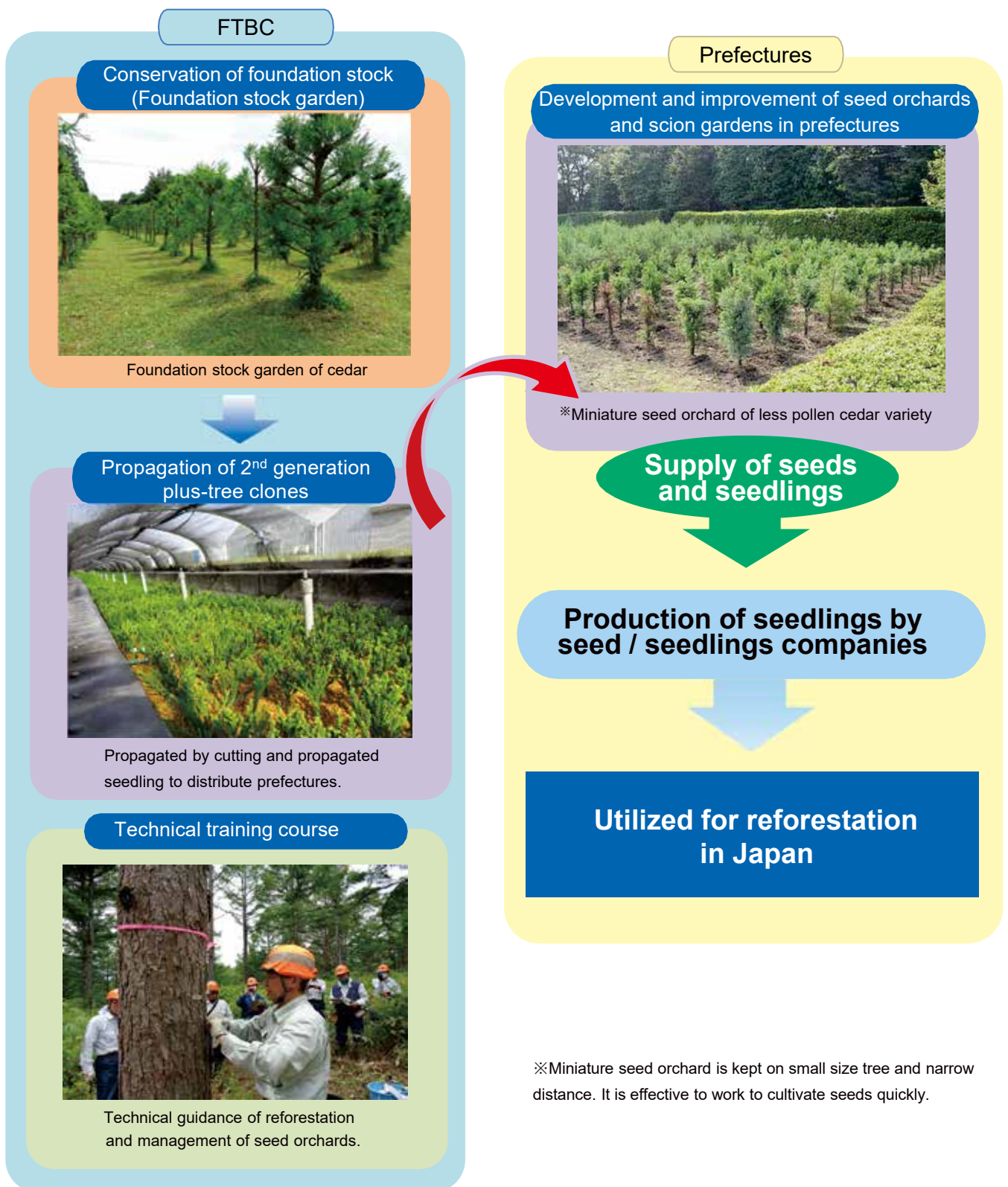
General progeny test forest of cedar



In plus-tree trait tables, the trait-wise standard score is calculated from the measured data. Such standard score is converted into a five-degree index. Degree 5 will include 7% of the total.

### 3. Dissemination and propagation of second generation plus-tree clones

Developed plus-trees are preserved as foundation stock under clones management at FTBC. These clones are propagated and provided to prefectures that establish seed orchards and scion gardens. FTBC also implements training courses for prefectures on how to produce and manage seed orchards and scion gardens.

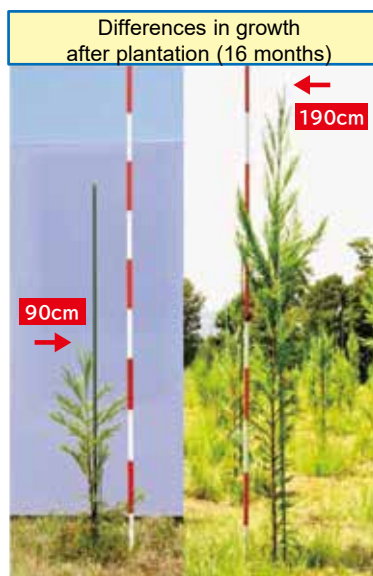


※Miniature seed orchard is kept on small size tree and narrow distance. It is effective to work to cultivate seeds quickly.



#### 4. Selection of 2<sup>nd</sup> generation plus-trees

FTBC has reforested progeny test forests (136 sites, 81ha. in total) to select 2<sup>nd</sup> generation plus-trees (2G-PT), which have superior traits such in growth performance, breeding populations have been established by crosses or open pollination of the 1<sup>st</sup> generation plus-tree clones. More than 9,000 crosses have been made, and the breeding populations are comprised of more than 200,000 trees. As the 2G-PT is superior in growth and can reduce the number of trees planted and weeding, it is expected to reduce initial investment in reforestation.

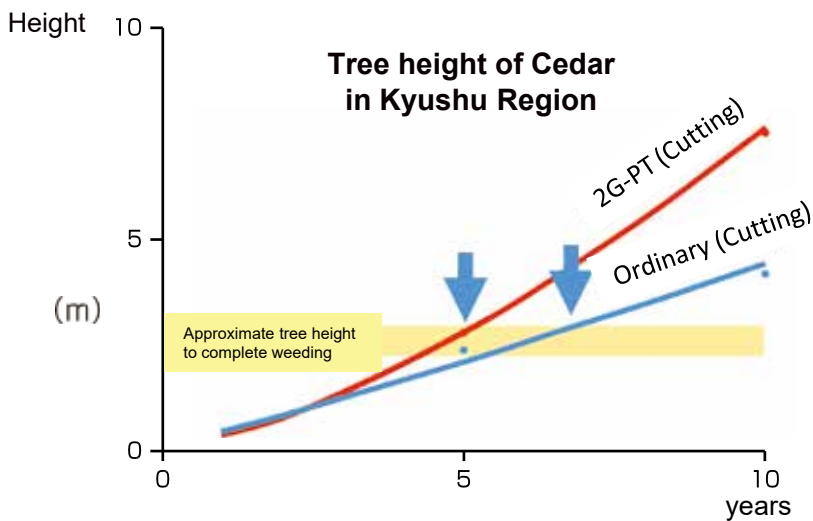


Ordinary seedling

2G-PT clone seedling



2G-PT (5 years old, 8m in height)



(Renovated Hoshi ·Kuramoto(2013))

Left figure shows data from test sites for the top 10 superior 2G-PT cedar clones in the Kyushu region. 2G-PT clones are particularly superior in growth compared to previous varieties. So weeding management can be reduced 1 or 2 times and be expected to reduce initial investment in reforestation.



## 5. Dissemination of Specified Mother Tree(SMT)

To increase the carbon dioxide absorption capacity of forests, Minister of Agriculture, Forestry and Fisheries (MAFF) has designated a superior tree of growth as a “Specified Mother Tree (SMT)” on the basis of the revised Act in 2013.

Apart from cedar and cypress, larch and other trees are designated as SMT.

MAFF encourages the use of seedlings obtained from SMT for afforestation.

FTBC has selected superior trees mainly based on 2nd generation plus-trees (2G-PT) and is prompting dissemination of SMT to distribute from the foundation stock.

### SMTs in Japan



**Sakhalin Fir 2G-PT**  
(30years old)  
Height: 19.9m  
Diameter: 26cm  
Hokkaido



**Cedar 2G-PT**  
(31years old)  
Height: 18.4m  
Diameter: 28cm  
Tohoku



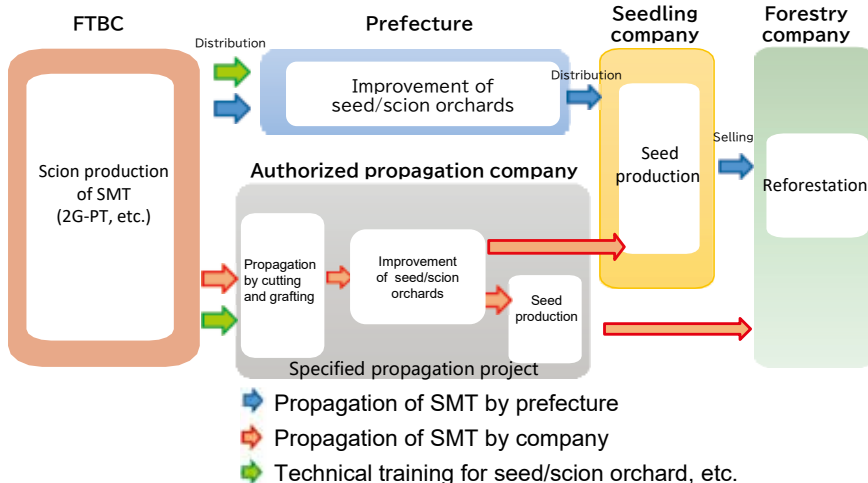
**Larch 2G-PT**  
(30years old)  
Height: 15.5m  
Diameter: 23cm  
Kanto



**Cypress 2G-PT**  
(31years old)  
Height: 17.7m  
Diameter: 24cm  
Kansai

- 4years after plantation -

### Dissemination system of SMT



Left: Cedar SMT



Right: Ordinary cedar  
(Less pollen)

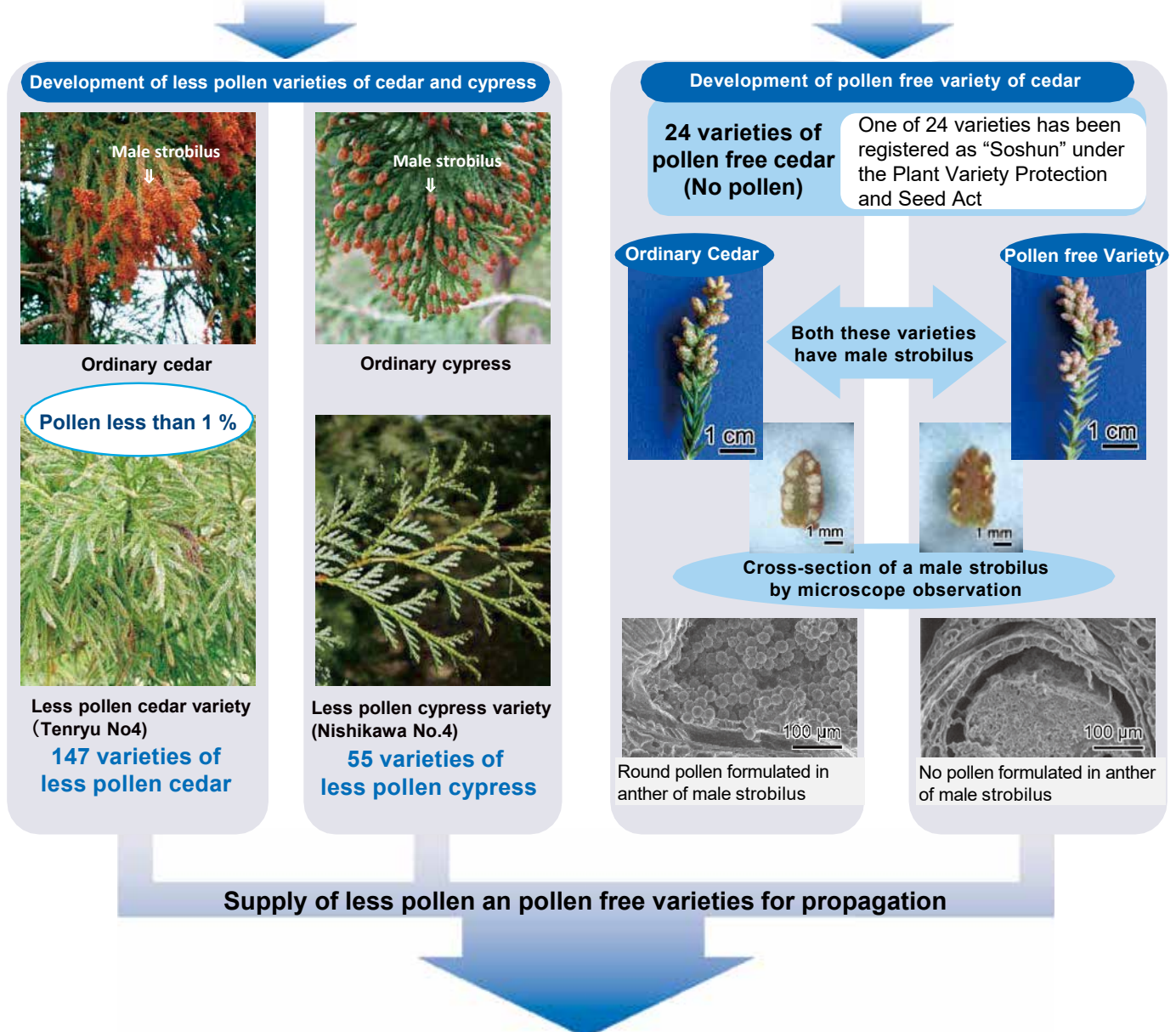
## Development of pollen free and less pollen varieties

### 1. Status on the research and development

Pollen allergy has been one of the socio-medical issues in Japan. FTBC in collaboration with prefectures are carrying to develop less pollen varieties of cedar with approximately 1 % less pollen and cypress, compare to ordinary tree from plus-tree which are superior in growth and straightness. FTBC is also developing pollen free cedar as following which produce male strobilus like ordinary cedar but no pollen at all.

Pollen free variety with straightness called "Soshun" is developed in 2004 and with equivalent quality to 1<sup>st</sup> generation plus-tree clones called "Sugi-Mie-Hunen (Kansai) No.1" with developed in 2006. Additionally, FTBC performed artificial crossings between Soshun and 1<sup>st</sup> generation plus-tree clones, FTBC developed new pollen free varieties called "Rin-iku-Hunen No1" in 2016 and called "Rin-iku-Hunen No2" in 2017.

FTBC in collaboration prefecture developed 3 pollen free varieties from 2018 to 2019. In addition, 17 more pollen free varieties were developed from 2020 to 2022.



## 2. Improvement of pollen free cedar by crossing with plus-tree clones

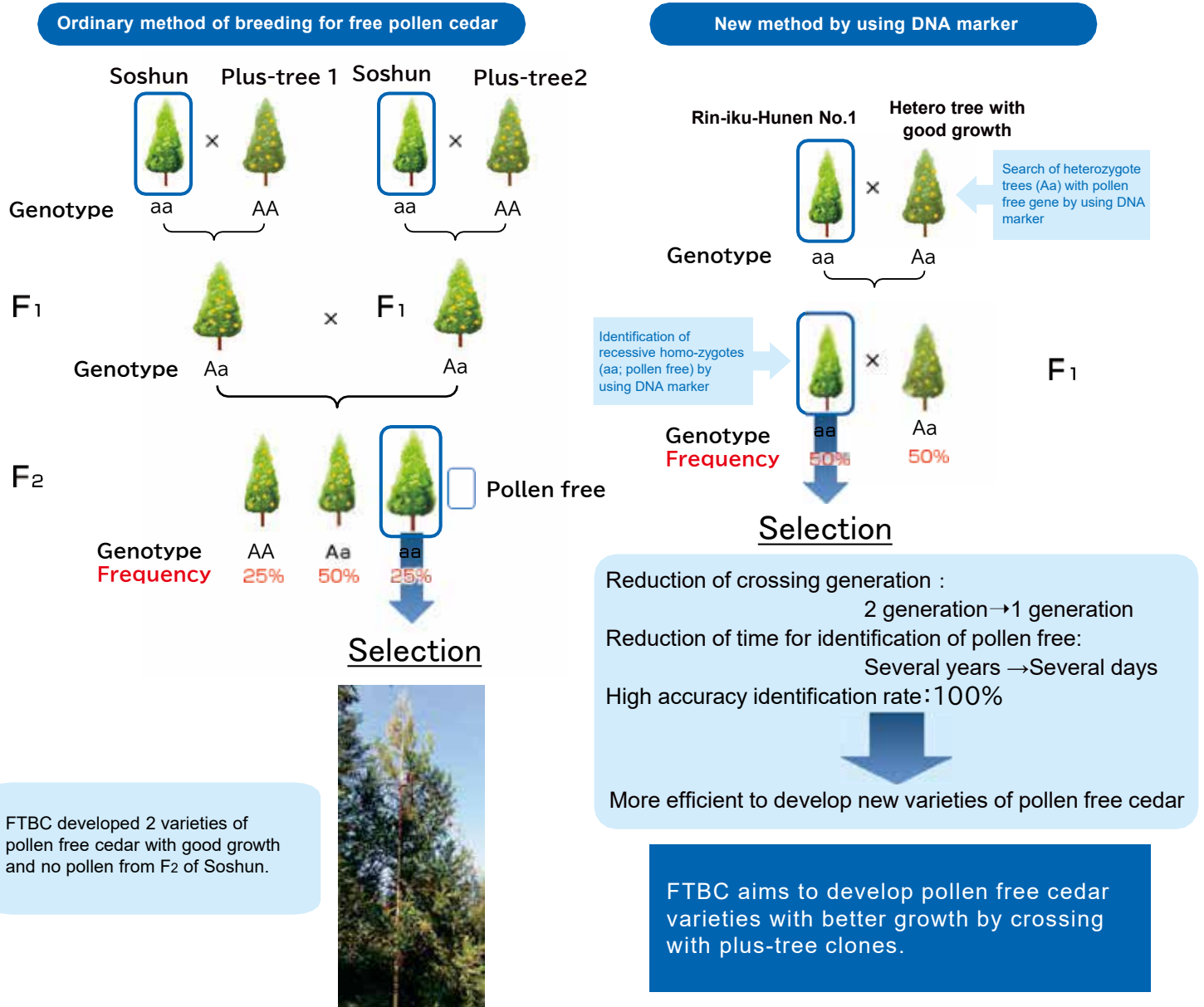
FTBC processes developing new varieties of pollen free cedar by artificial crossing between female strobilus of Soshun (pollen free cedar) and male strobilus of 2<sup>nd</sup> generation plus-tree clones (2G-PT) with good growth and quality.

Pollen free genome is inferior. As indicated by Mendel's Law, the first generation (F<sub>1</sub>) makes hetero type (Aa) with pollen free genome. Crossing the same hetero type at the second generation (F<sub>2</sub>) appears pollen free type (aa).

FTBC planted F<sub>2</sub> and examined for pollen, growth and wood property in the progeny test site. From these F<sub>2</sub> individuals, FTBC developed 2 varieties of pollen free cedar superior growth named "Rin-iku-Hunen No.1" and "Rin-iku-Hunen No.2".

FTBC also developed two varieties of pollen free cedar with stakeholders in 2019. They are expected to be used as a pollen parent for future variety development and seed production in seed orchards.

Additionally, the development of DNA markers for pollen free genetic detection of Soshun has helped search hetero genome (Aa) and develop a new variety of pollen free cedar.



Pollen free cedar "Rin-iku-Hunen No.1"  
6 years old, 6.6m in height  
※Almost the same as plus-tree clones



## Development of pine wilt nematode resistant varieties


### 1. Situation of pine wilt nematode


Pine wilt nematode (*Bursaphelenchus xylophilus*) is serious infections decease of pine trees caused by nematodes which are about 1mm in length carried by longhorn beetles.

This disease was found around 1900, and damages have been serious since the late 1965s, throughout western Japan.


Currently, pine wilt diseases are now being reported throughout Japan without Hokkaido. FTBC has been carrying out research and development of pine wilt nematode resistant varieties and 590 new varieties have been developed by 2022.

**Situation of pine wilt disease**





**Longhorn beetle**  
Longhorn beetles carry pine wilt nematodes and fly from one pine tree to another one.

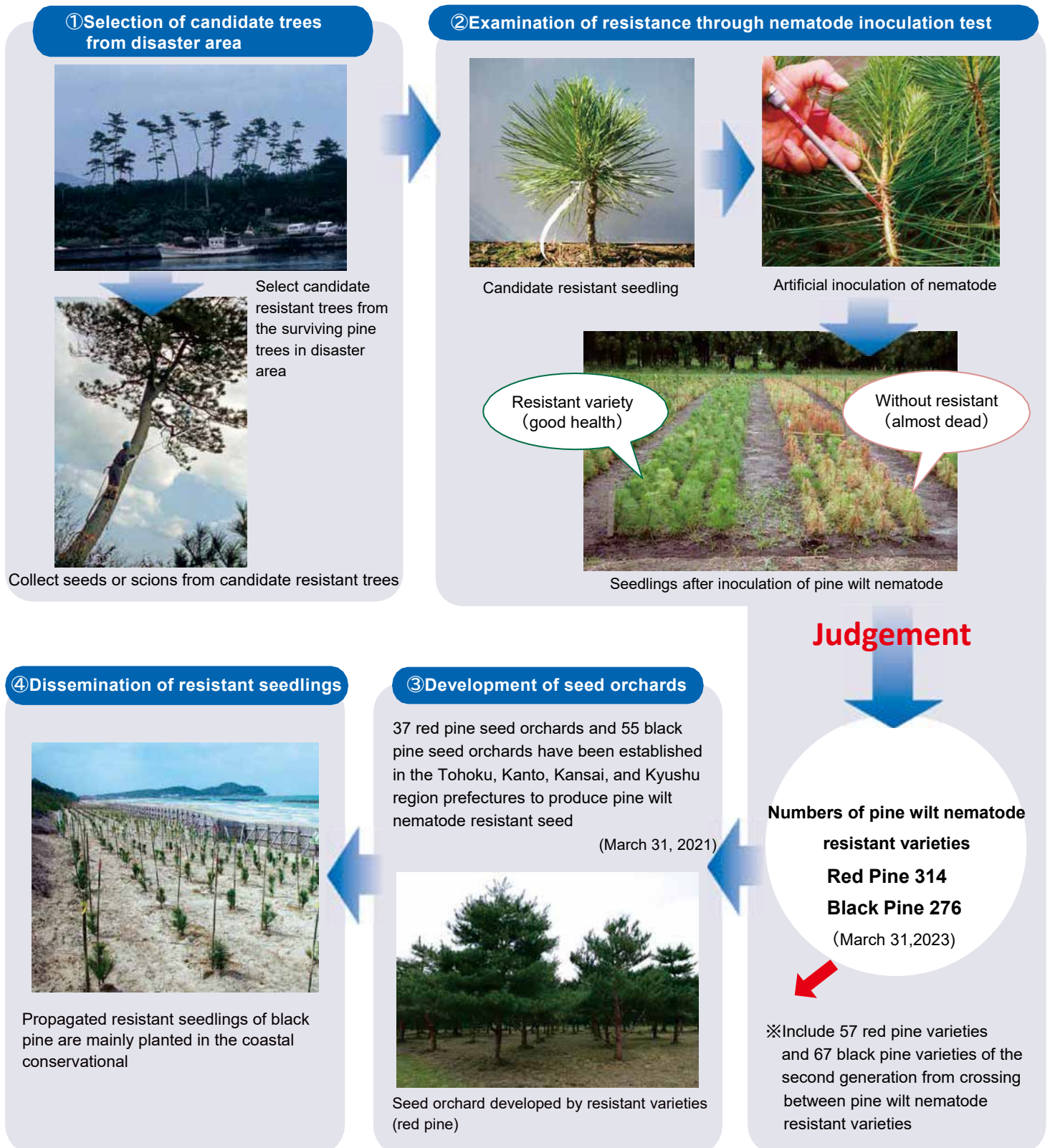


**Pine wilt nematode**  
(From 0.6mm to 1.0mm in length)  
Pine wilt nematodes invade the xylem of pine trees from an open score of the young needle caused by a longhorn beetle bite. The propagated nematodes in the xylem will cause pine wilt soon.

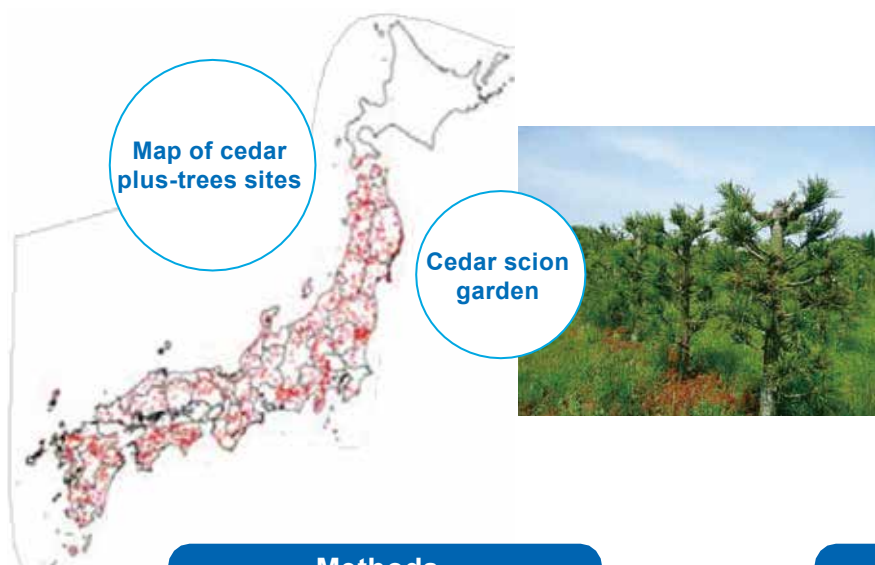
### <History of pine wild nematode and FTBC's activities>

- 1905 First pine wilt disease reported in Nagasaki prefecture
- 1971 FFPRI researchers revealed that nematode causes pine wilt diseases
- 1978 The pine wilt nematode resistant varieties development project started
- 1985 FTBC developed 92 pine wilt nematode resistant varieties of *Pinus densiflora*(red pine) and 16 pine wilt nematode resistant varieties of *Pinus thunbergii*(black pine)
- 2010 FTBC developed 2<sup>nd</sup> generation of pine wilt nematode resistant varieties for black pine
- 2017 FTBC developed 2<sup>nd</sup> generation of pine wilt nematode resistant varieties for red pine
- 2018 FTBC improved resistance testing technology and applied it for new variety development

## 2. Development methodologies of pine wilt nematode resistant varieties



## DNA-based traceability system of plus-trees



More than 3,000 clones of cedars have been selected as plus-trees nationwide. Those plus-trees have been clonally propagated, and planted in progeny test forests as well as seed/scion orchards. As it is difficult to distinguish these clones from the outside, FTBC is using DNA to promote more accurate clone management.

### Methods



Collecting DNA sample

1



Processing by liquid nitrogen



DNA extraction

2



Polymerase Chain Reaction (PCR)

3



DNA preservation (some parts)



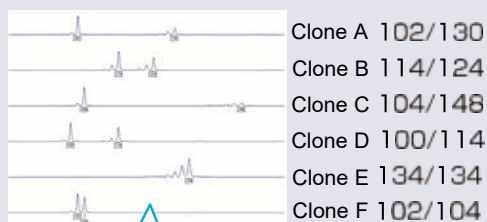
Polymerase Chain Reaction (PCR)

4



Confirmation of PCR result

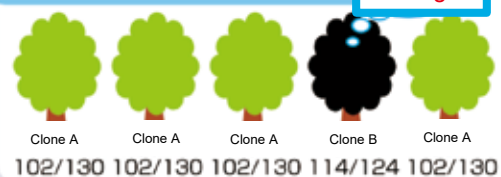
### Results



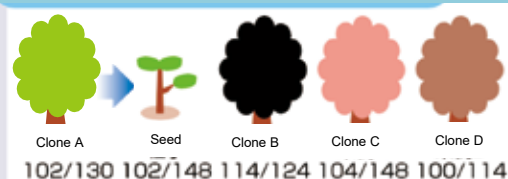
The length of DNA amplified varies from clone to clone. We can discriminate the part as a difference in the degree of mobility by using a sequencer.

Clone management; in case of Clone A

Wrong!



Seed orchards management; in case of seed from Clone A



When seeding from Clone A has a genotype of 102/148, the candidate pollen donor of this seedling will be very likely Clone C.

DNA analysis enables us to do not only high-accuracy clones but also parentage of breeding materials.



# Bio-technology in forest tree breeding

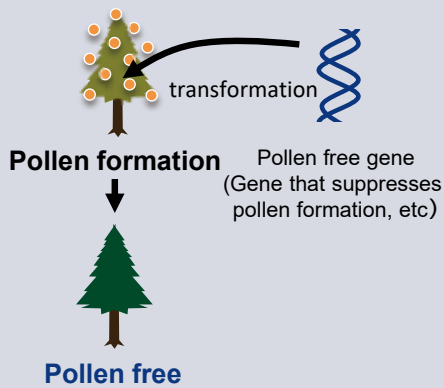
## 1. Development of pollen free cedar through genetic recombinant and genome editing

Genetic recombinant is a technology that introduces only the target gene, enabling efficient and rapid breeding. Genome editing technology, which can modify the function of a plant's own genes, has also attracted attention in recent years.

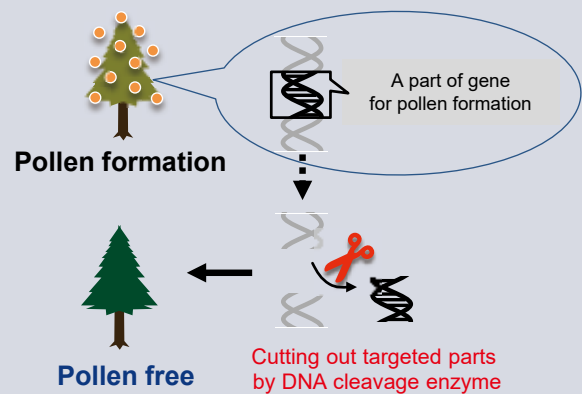
FTBC is researching and developing pollen free cedar varieties with superior growth and wood quality by using these advanced biotechnologies.

### Genetic recombinant and genome editing

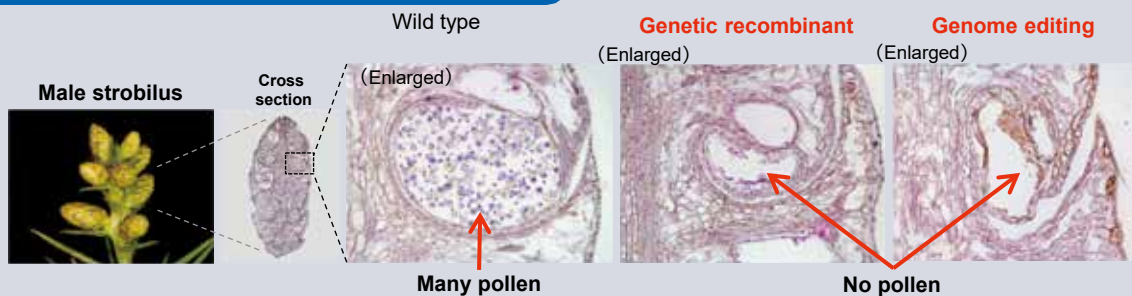
#### Forest tree breeding by genetic recombinant



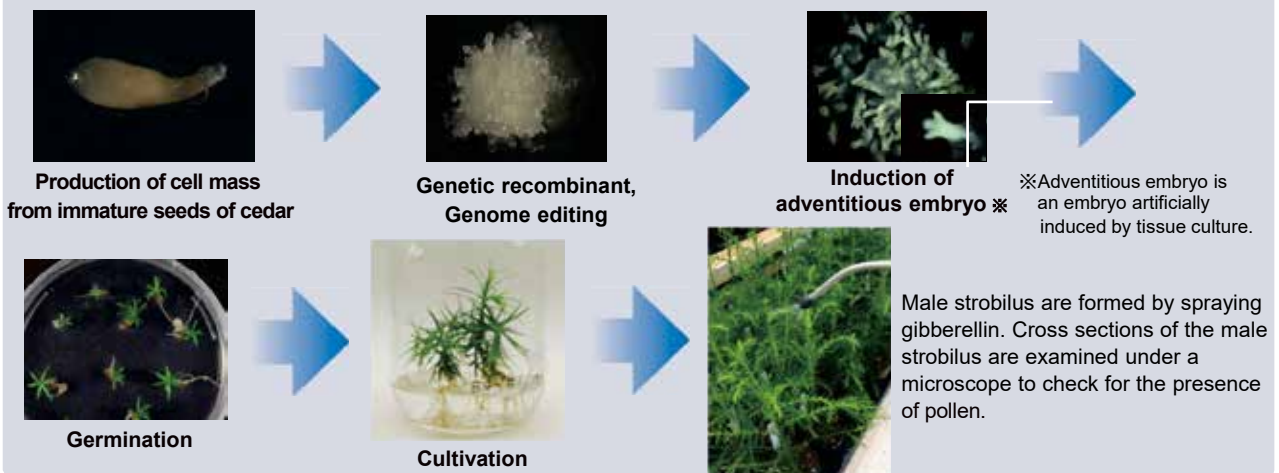
#### Forest tree breeding by genome editing



### Inside of male strobilus in pollen free cedar



### Actual procedure for pollen free cedar production



### The only forest tree gene bank in Japan

#### Necessity of forest tree genetic resources conservation

Forest tree genetic resources have been providing various forest products on which human beings depend since ancient times. These genetic resources will be expected to provide various services in the future with science and technology development.

Also, conservation of forest biodiversity has been strongly called in recent years. If these genetic resources were lost, the same genetic resources cannot be reproduced.

Therefore, these genetic resources have to be conserved and inherited to the next generations.

#### Outline of forest tree gene bank program

FTBC is the only forest tree gene bank program in Japan and has the largest stock of woody plants.

With taking due consideration to the application value of genetic resources, needs and priority of conservation, FTBC is systematically conducting exploration and collection of genetic resources, and Conserving / preserving such resources by seeds, pollens, DNA, and propagated individuals.

FTBC is also conducting trait analysis of collected genetic resources for identification of potential use as well as distributing such resources for research purposes.

### Status of conservation / preservation of forest tree genetic resources

#### Method and form of conservation / preservation

Conservation and preservation of forest tree genetic resources are performed by combining "In-situ" and "Ex-situ" from the characteristics of the target tree are many varieties, long-lived, extremely large plants, and so on.

#### In-situ conservation / preservation

In-situ conservation is a method of preserving the forest genetic resources that make up the forest as a whole by preserving the forest, or habitat, in which the trees originally reside.

Conservation is carried out in protected forests set aside in national forests, etc.

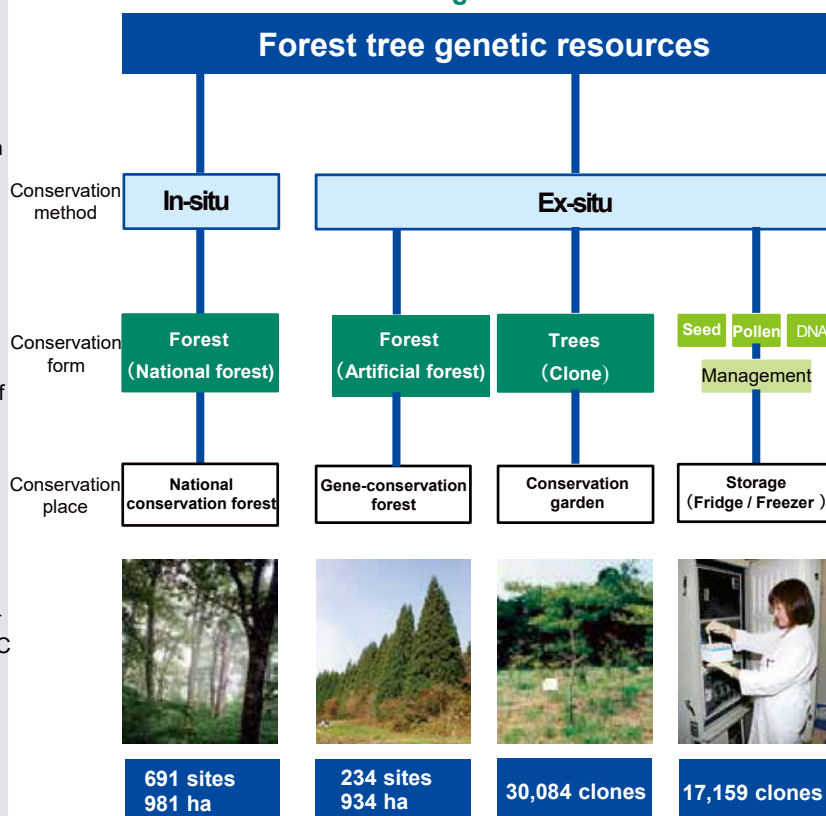
#### Ex-situ conservation/preservation

Ex-situ conservation is a method of preservation that involves taking genetic resources outside their original habitat.

They are conserved by a combination of the three methods as follows.

1. Methods to preserve genetic resources in the form of forests as a population by creating planted forests with genetic resources taken from the habitat.
2. Methods of preserving of genetic resources by mother tree from the habitat area and storage in adult form as clones or strains in a conservation forest on the FTBC premises.
3. Methods of preserving germplasm such as seeds and pollen collected from inside and outside the habitat, or as DNA, which is stored in facilities in freezers or other storage facilities.

#### Method and form of conservation/preservation on forest tree genetic resources



(March.31,2023)

# Outline of forest tree gene bank program

**Exploration/Collection**  
Exploration and collection of scions and seeds of genetic resources



Collection of scions from "Katsura in Ousen's fall", one of "Hundred Giant Trees in Japan" designate by Forestry Agency

**Propagation/Conservation**  
Propagation by grafting and cutting.  
Conservation in gene stock garden or storage



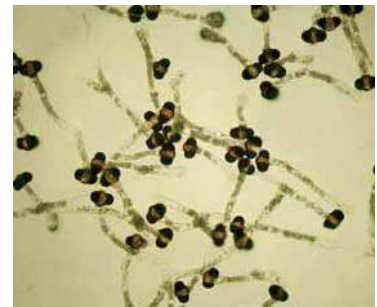
Propagation by cutting of *Acer pycnanthum*, which is endangered species

**Evaluation of various traits**

Traits analysis and evaluation on the gene stock



Collection of various trait such as growth



Trait analysis on pollen germination rate and ecological/morphological features

**Data management**

Establish a database of information on the collection location, history information, conservation form, and traits evaluation of forest tree genetic resources



Data input into the database



Database

**Distribution**

To meet the demand for research purposes, forest tree genetic resources will be distributed in various forms upon the request



Seeds



Pollen



Scions



Seedlings

**Outcomes**

**New varieties**

Distribution of pollen for artificial crossing

**New Products**

Distribution of pollen for the development of pollen allergy products

**Academic advances**

Distribution of scions for elucidation of genetic variation



## International technical cooperation and collaborative research

FTBC is developing forest tree breeding technologies to adapt to climate change through international technical cooperation and collaborative research.

### Technical cooperation

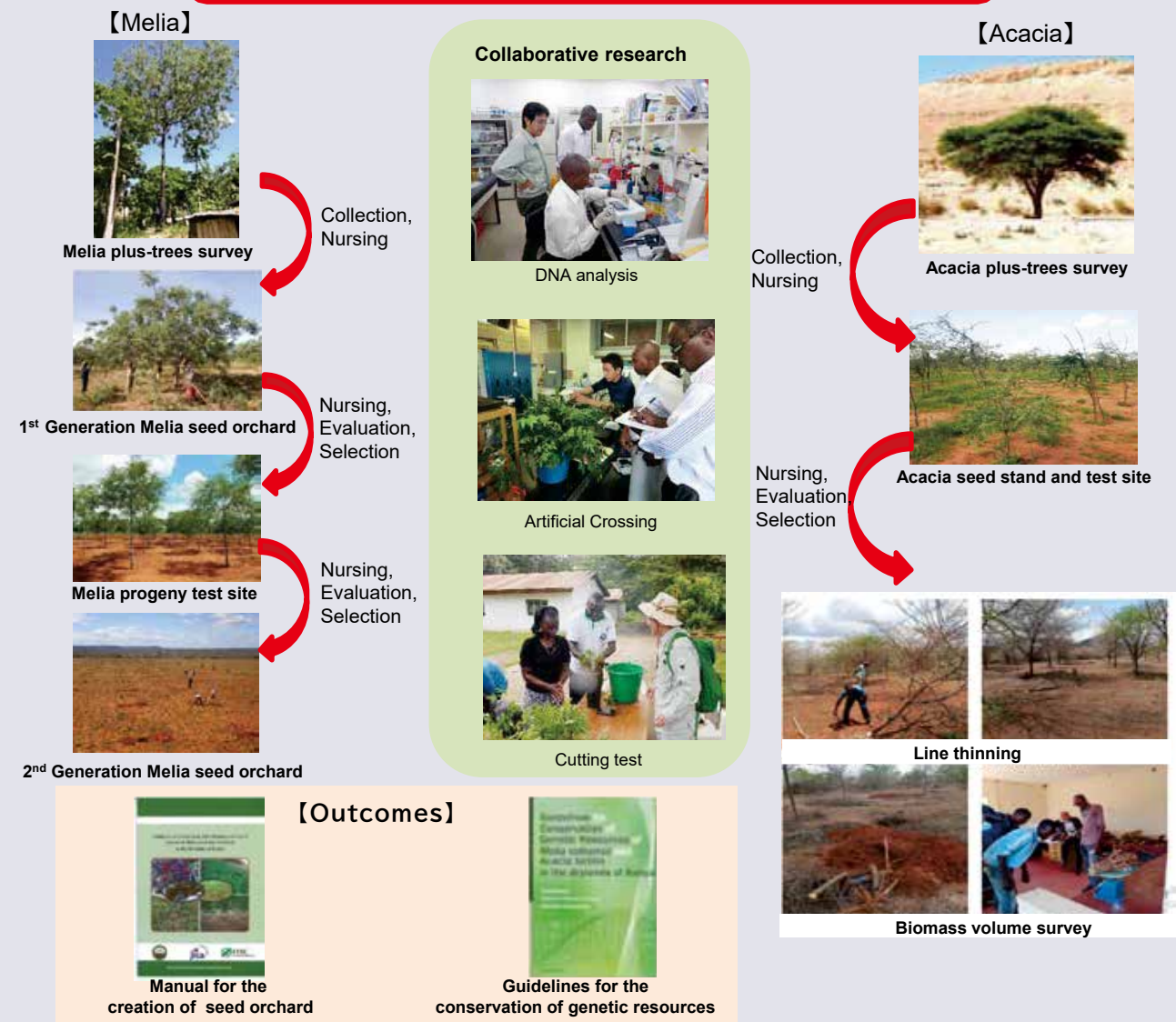
#### Forest tree breeding project in Kenya

Partner: Kenya Forestry Research Institute (KEFRI)

Duration: 2012-2026

Outline: FTBC has been supporting and guiding research on the breeding of Kenya's native species *Melia volkensii* (Melia) to utilize furniture and interior and *Acacia tortilis* (Acacia) to use fuel wood and livestock through a JICA technical cooperation project since 2012.

- 1<sup>st</sup> Phase**      **Project on Development of Drought Tolerant Trees for Adaptation to Climate Change in Drylands of Kenya**  
2012-2017
- 2<sup>nd</sup> Phase**      **Capacity Development Project for Sustainable Forest Management in the Republic of Kenya (Tree Breeding)**  
2017-2021
- 3<sup>rd</sup> Phase**      **Project for Strengthening Forestry Sector Development and Community Resilience to Climate Change through Sustainable Forest Management and Landscape Restoration (Tree Breeding)**  
2022-2026



## Collaborative research

### Breeding research on *Calophyllum inophyllum* with superior wind and high tide protection

Partner : Taiwan Forest Research Institute (TFRI)  
 Duration : 2011-2028  
 Species : *Calophyllum inophyllum*  
 Outline : *Calophyllum inophyllum* planted as coastal disaster protection on the Sakishima Islands in Okinawa prefecture are ocean-dispersing trees and are widely distributed in tropical and subtropical areas of the Pacific ocean.  
 FTBC is analyzing genetic variants of *Calophyllum inophyllum* in various regions and developing varieties that grow fast and have good quality to cope with the severe coastal disasters like wind and high tides associated with global warming.



Test site in Iriomote from UAV



Meeting in TFRI



Test site in Taiwan



Test site in Iriomote

### Demonstration test of artificial crossing technique of Acacia Hybrid in Vietnam

Partner : Private company (Oji Green Resources Co.Ltd.)  
 Duration : 2013-2022  
 Species : Acacia hybrid (*Acacia mangium* × *Acacia auriculiformis*)  
 Outline : FTBC developed efficiently superior growth and good quality Acacia hybrids through artificial crossing and selected clonal candidates in the test forests.



Efficient artificial crossing technique



Established test site for selection of plus-trees



Evaluation of growth potential In test site



Established seed orchards of superior clones

## Information on advanced breeding techniques from oversea

### Indoor seed orchard in Ireland

Crossing between superior Sitka Spruce individuals to prevent contamination by external pollen, and developing even better next-generation varieties.



Indoor seed orchard with open and shut roof for Sitka Spruce (Photo:2018)



### Efficient seedling production in New Zealand

This private entities produce 7 million seedlings of *Pinus radiata* (including graft seedlings) per year. They have efficient seedling production technologies, such as automated covering of seedling boxes.



Automatic soil covering machine (photo:2019)



Moving bench (photo:2019)



## 【 Advanced research facilities in leading forest tree breeding R/D 】

### Crossing (hybridization) greenhouse



This is a greenhouse with closed hybridization booth that can shut out pollen from the outside and automatic watering and composting.

### Specific net house



This is a greenhouse for growing and evaluating genetically modified forest trees that have been tested in laboratory or closed greenhouse. Fine-meshed nets are fully equipped to avoid invasion of insects carrying pollens.



### Nursing facility



This facility is able to control temperature, day length and carbon dioxide concentration to promote seedlings' growth in all seasons.





## 【 Advanced research facilities in leading forest tree breeding R/D 】

### Genetic engineering research facility



This facility is a laboratory for evaluating on testing, growing, and safety of genetically modified forest trees.



### Forest tree genetic resources conservation facility



This is the only gene bank in Japan that collects and preserves forest tree genetic resources to contribute to species diversity and genetic diversity within species.

Seeds and pollens are stored in ultra-low temperature freezers for long-term safe storage.



### Genetically Modified Organism (GMO) isolation field



GMO isolation field is an evaluation field after a specific net house. This is the largest specific in Japan and is isolated by 60m x 54m in space with 8m height fences, 1m depth sealed concrete fence, with invasion sensors and cameras.

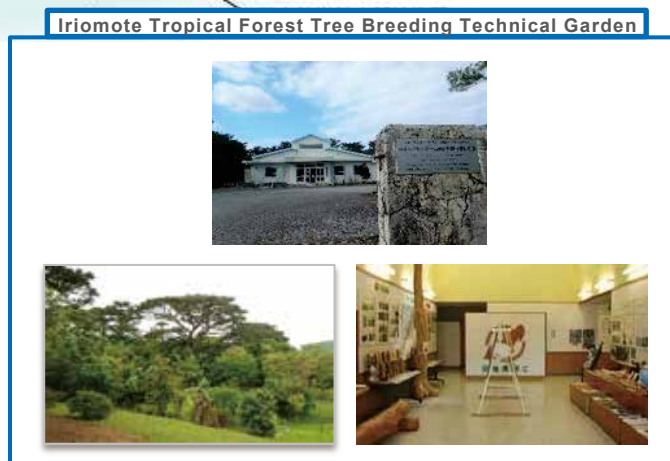
## 【 Location of FTBC/FBRC, regional office and others 】

Breeding region (Office)	Breeding tree species
Hokkaido(HBO)	Larch, Hybrid larch, Sakhalin Fir, Yezo spruce
Tohoku(TBO)	Cedar (snow area), Larch, Red pine, Black pine
Kanto(FTBC/FBRC)	Cedar (less pollen), Larch, Cypress, Black pine
Kansai(KBO)	Cypress, Cedar (less pollen), Red pine, Black pine
Kyushu(KBO)	Cedar (clonal forestry), Cypress, Black pine, Luchu pine



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