No. A-8-1E

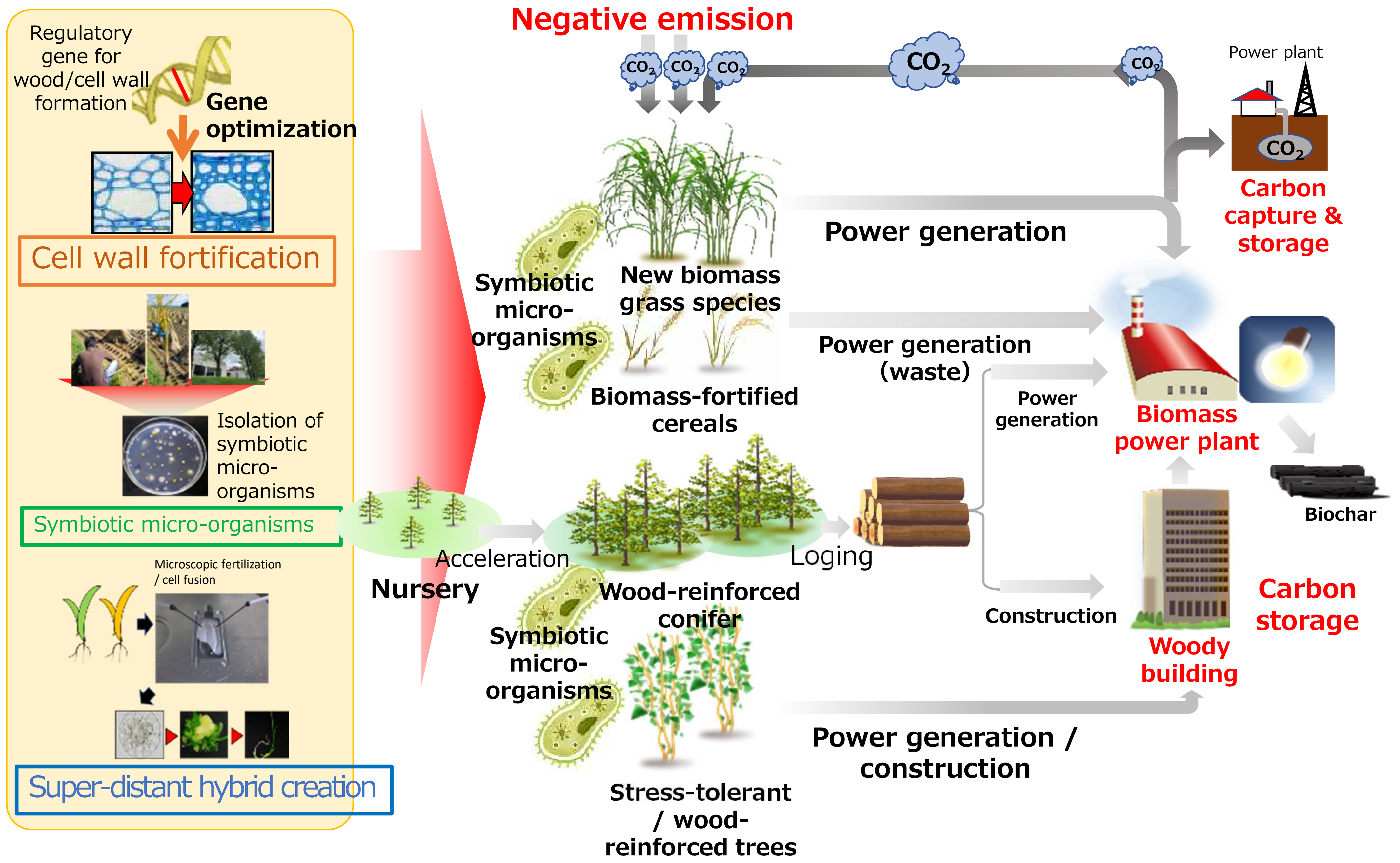
PJ: Development of Next-Generation CO_2-Fixing Plant Through the Gene Optimization, Distant Hybrid, and Microbial Symbiosis

Organization: Natl. Inst. Adv. Ind. Sci. Tech. (AIST), Tokyo Metr. Univ., SUMITOMO FORESTRY Co., Ltd.
Contact: Nobutaka MITSUDA (AIST) nobutaka.mitsuda@aist.go.jp

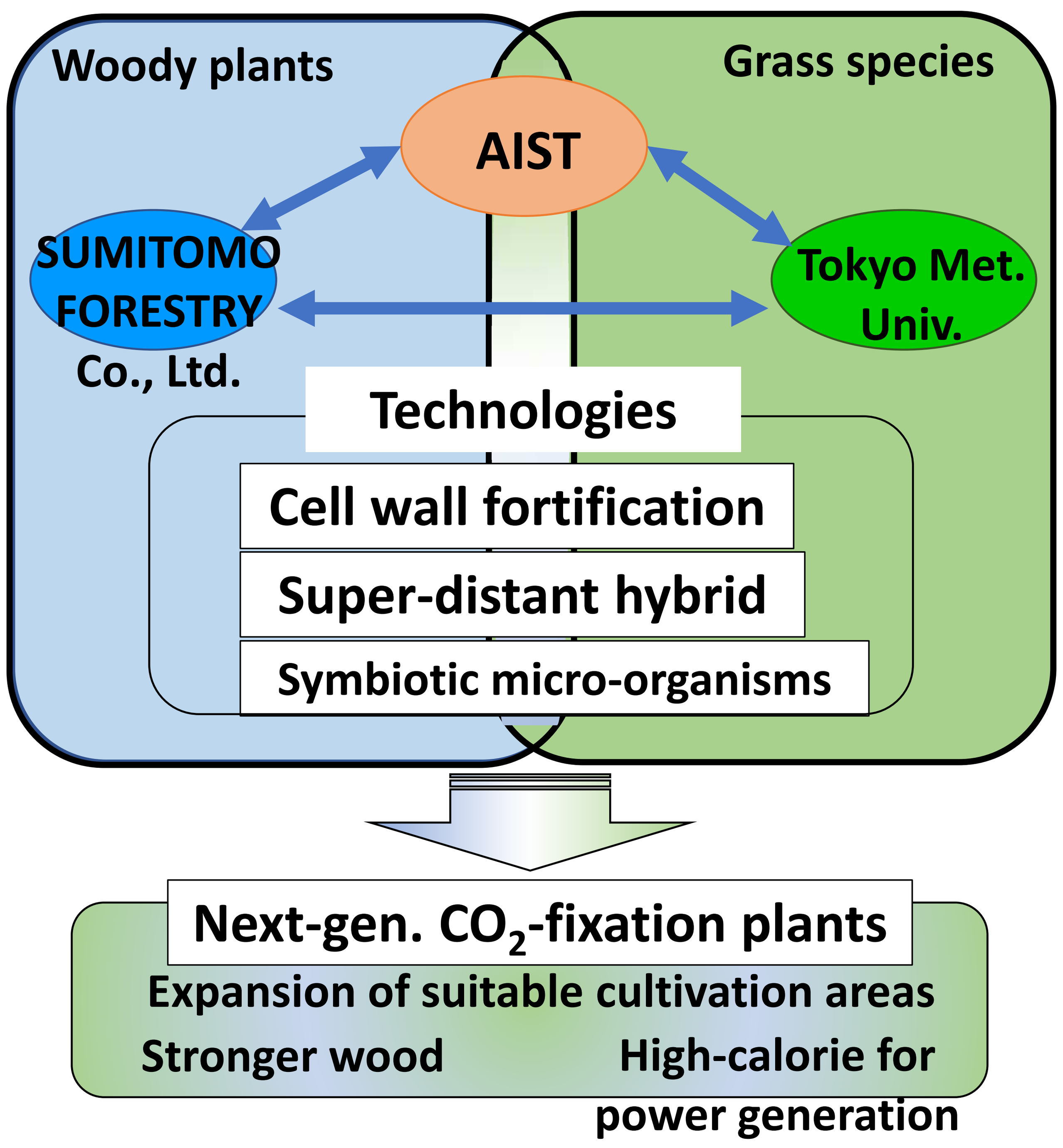
1. Abstract

In order to fix CO_2 at a low cost and on a large scale, the development of plants (and technologies to support their growth) that can be grown in a wide area, fix more CO_2, and produce higher-performance products (woody biomass) is desired. In this research project, we are going to create next-generation CO_2-fixation woody plants and grass species with enhanced CO_2 absorbing capacity. For the development of woody plants, we will mainly apply gene optimization (gene edit) technology to enhance wood formation. In grass species, we will employ super-distant hybrid creation and the gene optimization technologies. In both cases, optimization of symbiotic micro-organisms will be applied in the early growth stage. By combining these elemental technologies, we will establish next-generation CO_2 fixation plants and their utilization strategies.

2. Graphical abstract



3. Team building

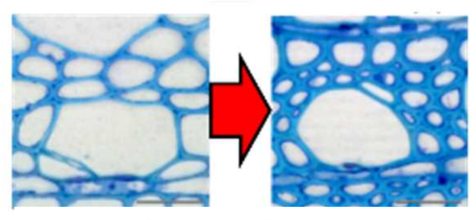


4. Three major technologies

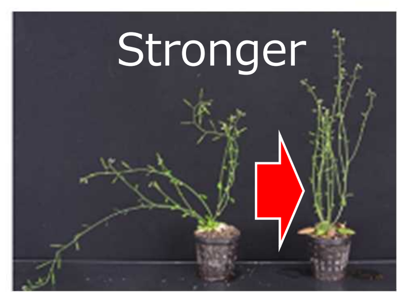
4–1. Gene optimization

◆**Three strategies**
Based on gene edit technology

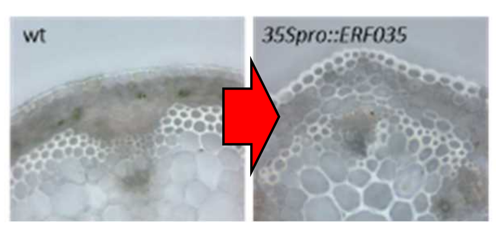
1. Wood reinforcement by NST hyperactivation


Wood reinforcement

2. Increased strength
   - Beneficial trait in addition to higher wood productivity



3. Primary cell wall enhancement (only for grass)



PCW enhancement

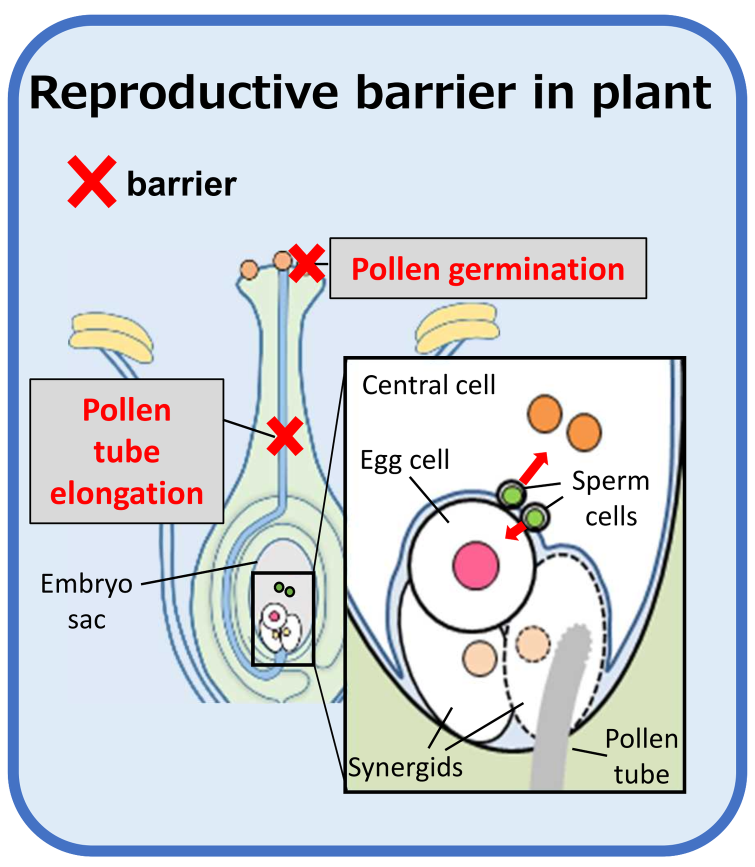
NST transcription factor

Master regulator of wood / secondary cell wall formation. Loss of function lost wood formation. Gain of function can fortify wood formation

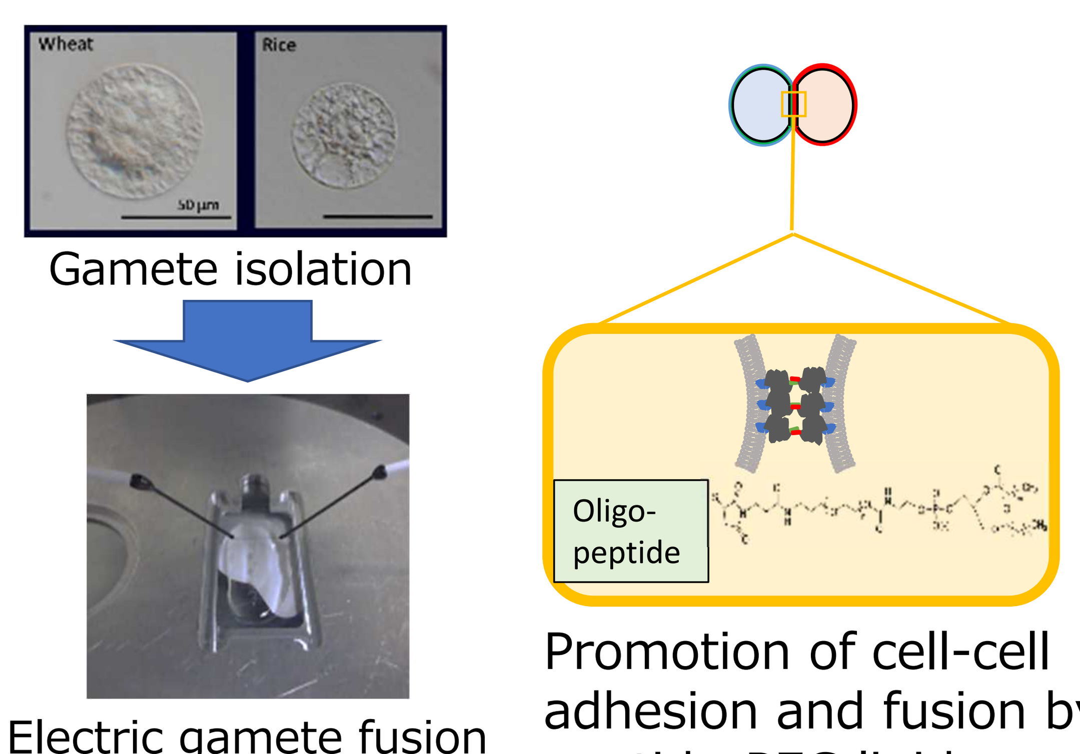
Takata et al., *Tree Physiol.* **39**, 514-525 (2019)

4–2. Super-distant hybrid

◆**Super-distant hybrid creation technology**
Technology to overcome reproductive barrier between different species through "*in vitro* fertilization" or "cell fusion".



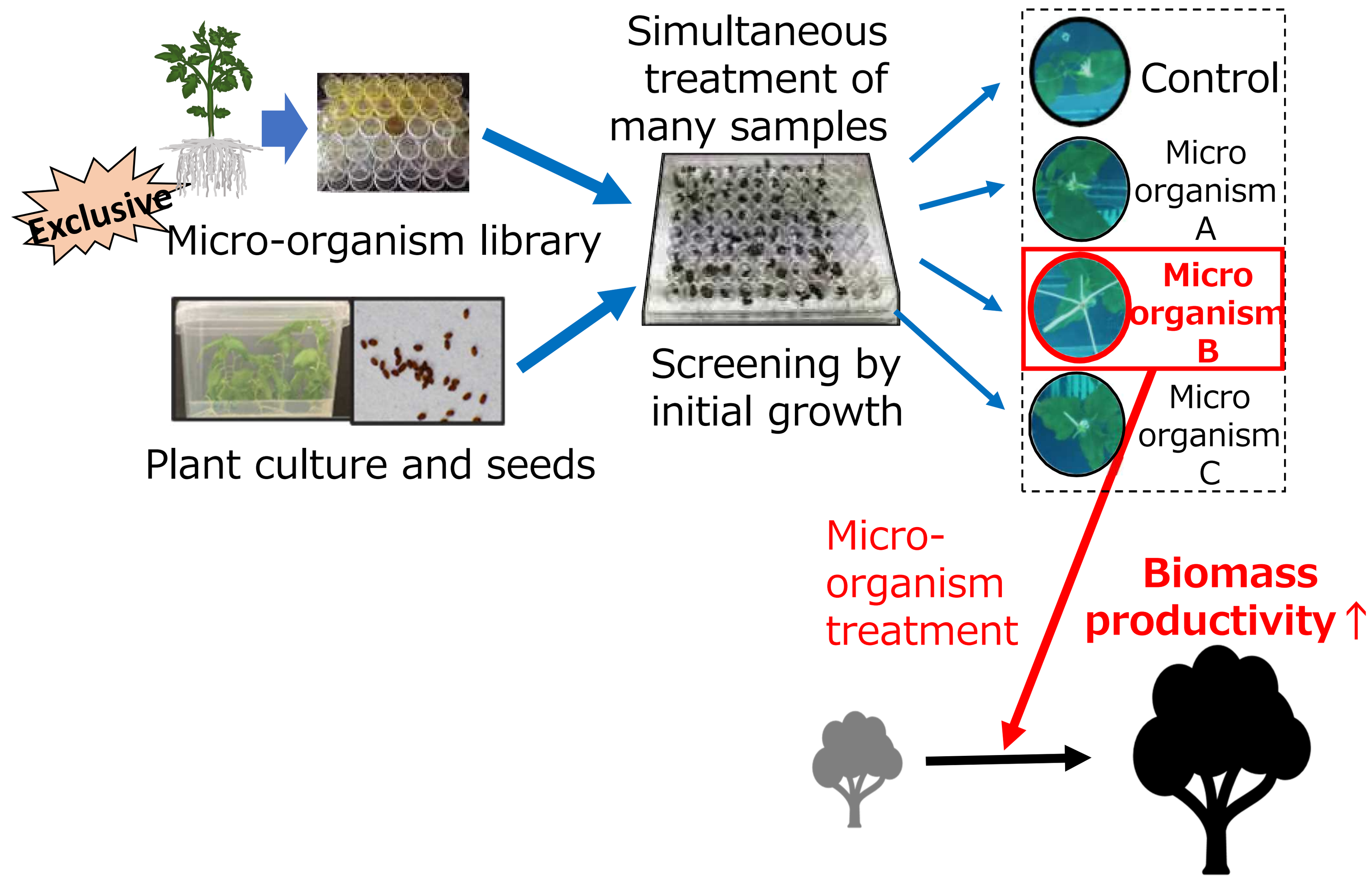
1. *In vitro* fertilization 2. Promotion of cell fusion



➢ Towards higher biomass production and/or expansion of suitable cultivation areas

4–3. Symbiotic micro-organisms

◆**Exploration of symbiotic micro-organisms**



No. A-8-2E

PJ : Development of Next-Generation CO_2-Fixing Plant Through the Gene Optimization, Distant Hybrid, and Microbial Symbiosis

Theme: Discovery of a symbiotic microorganism with unprecedented growth-promoting abilities across diverse plant species, including grass species and woody plants

Organization: National Institute of Advanced Industrial Science and Technology

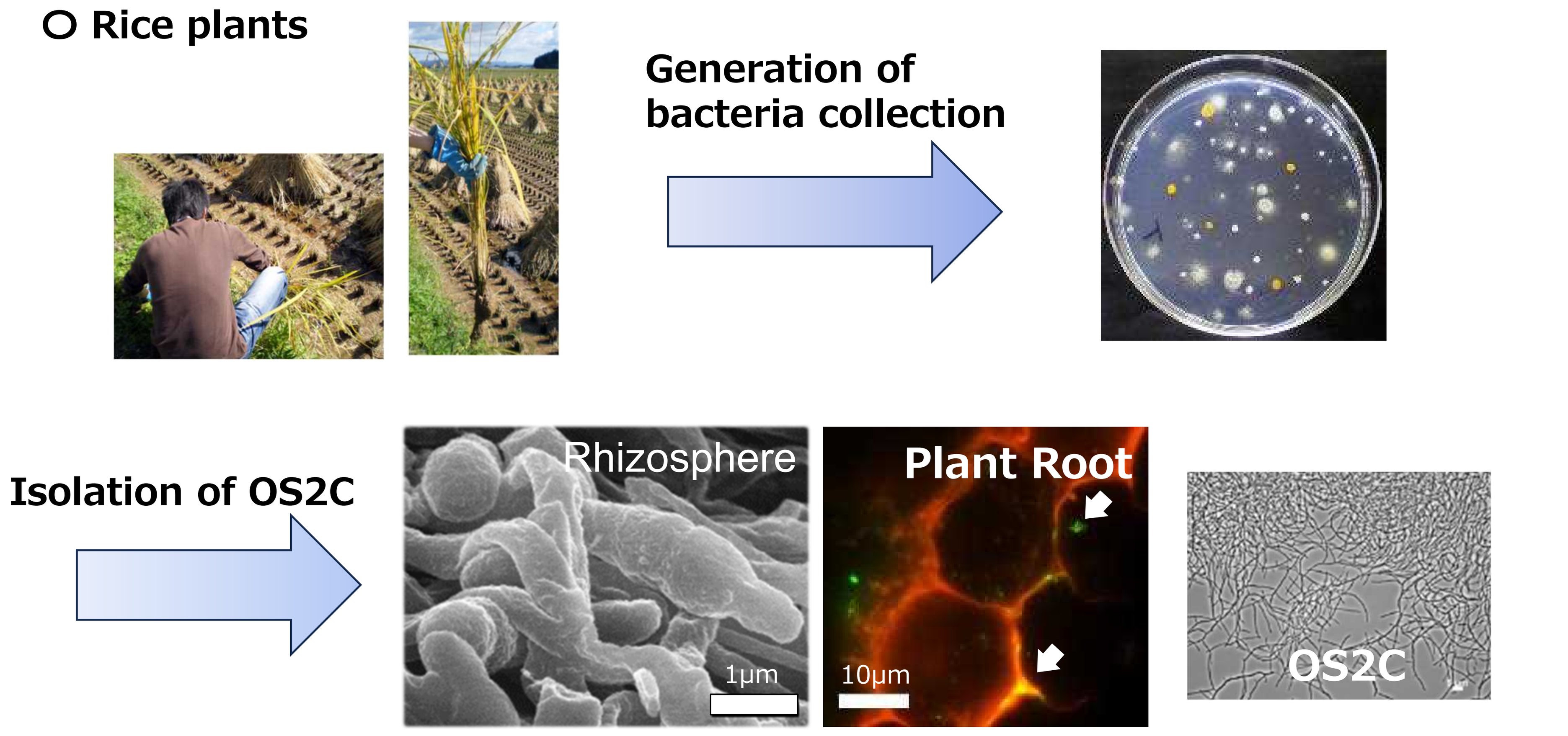
Contact: Hideyuki TAMAKI（AIST）tamaki-hideyuki@aist.go.jp

Abstract

Among symbiotic microorganisms, Plant Growth-Promoting Rhizobacteria (PGPR) represent beneficial bacteria residing in the rhizosphere, the soil region directly influenced by plant roots. Increasing emphasis is being placed on harnessing the potential of these bacteria to enhance sustainable plant industries, given their demonstrated ability to improve productivity, mitigate environmental impact, and reduce reliance on chemical pesticides and fertilizers.

In our research project, we revealed unprecedented plant growth-promoting (PGP) abilities of an endophytic actinobacterium, *Streptomyces thermocarboxydus* strain OS2C, isolated from rice. The strain OS2C significantly promoted the growth of rice biomass, and further enhanced the final grain yield. Besides, this strain highly promoted the growth not only of Erianthus biomass as grass species but also of woody plants such as poplar and Eucalyptus. Collectively, our findings suggest that OS2C is a promising PGPR for achieving substantial biomass across diverse plant species (patent application completed: 特願2023-192519). In this presentation, we will share the clear evidence for the PGP abilities of OS2C and provide the comparative gene expression data for rice with and without OS2C, unveiling the molecular mechanisms underlying its PGP effects.

OS2C, an endophytic actinobacterium belonging to the genus *Streptomyces* was isolated from rice (*Oryza sativa* L.) shoots.

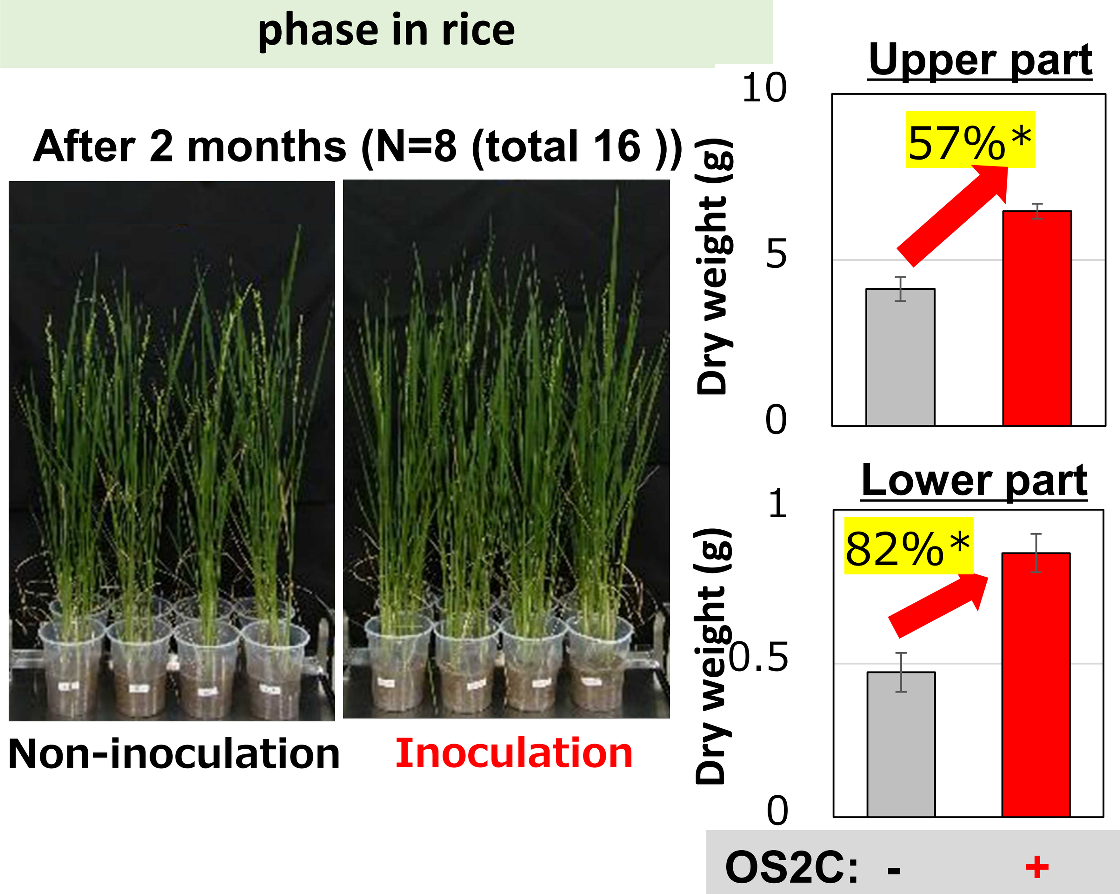


(Kanno *et al*., Environ. Microbiol. 2016)

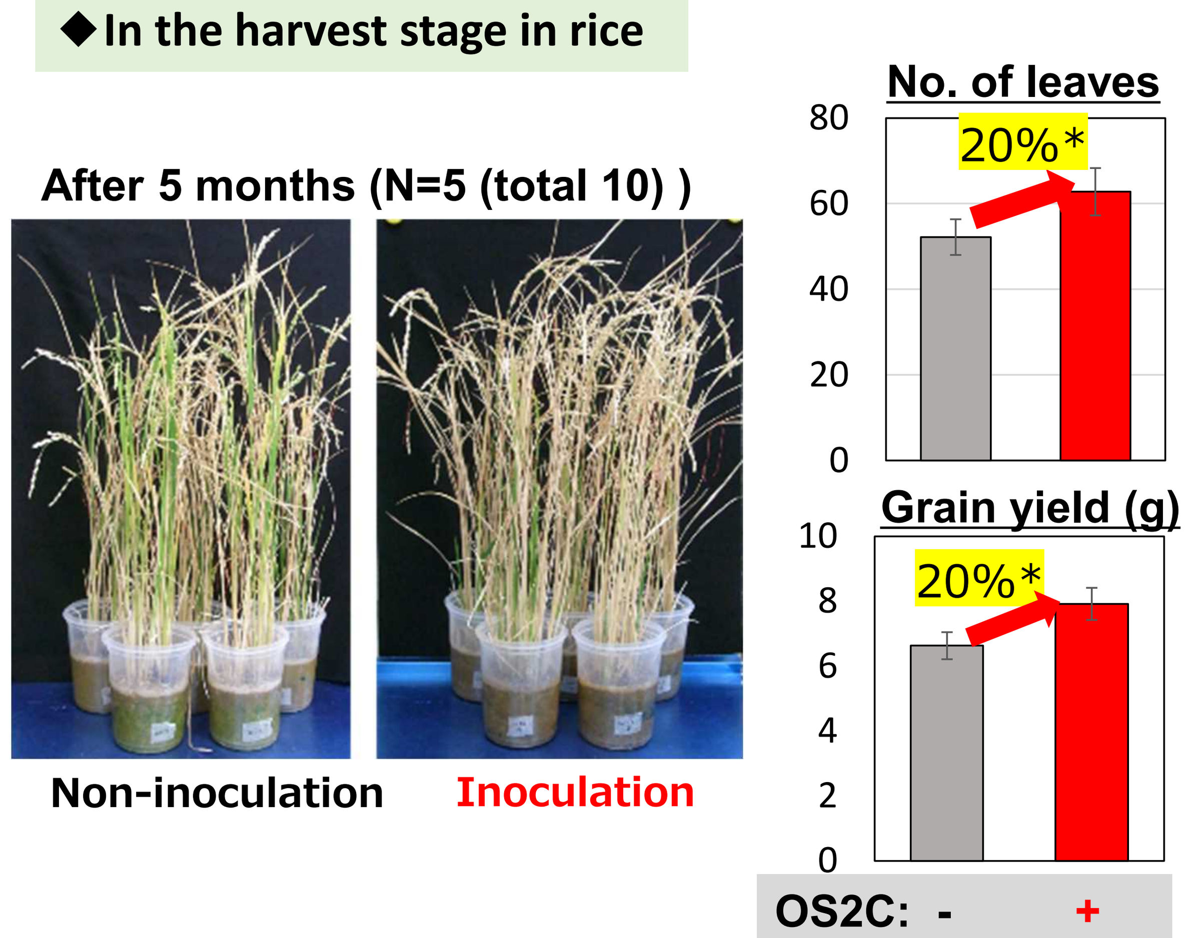
1. Growth-promoting effects of OS2C on grass species: Rice & Erianthus

OS2C promotes the growth of rice biomass during the vegetative growth phase and significantly increases rice grain yield.

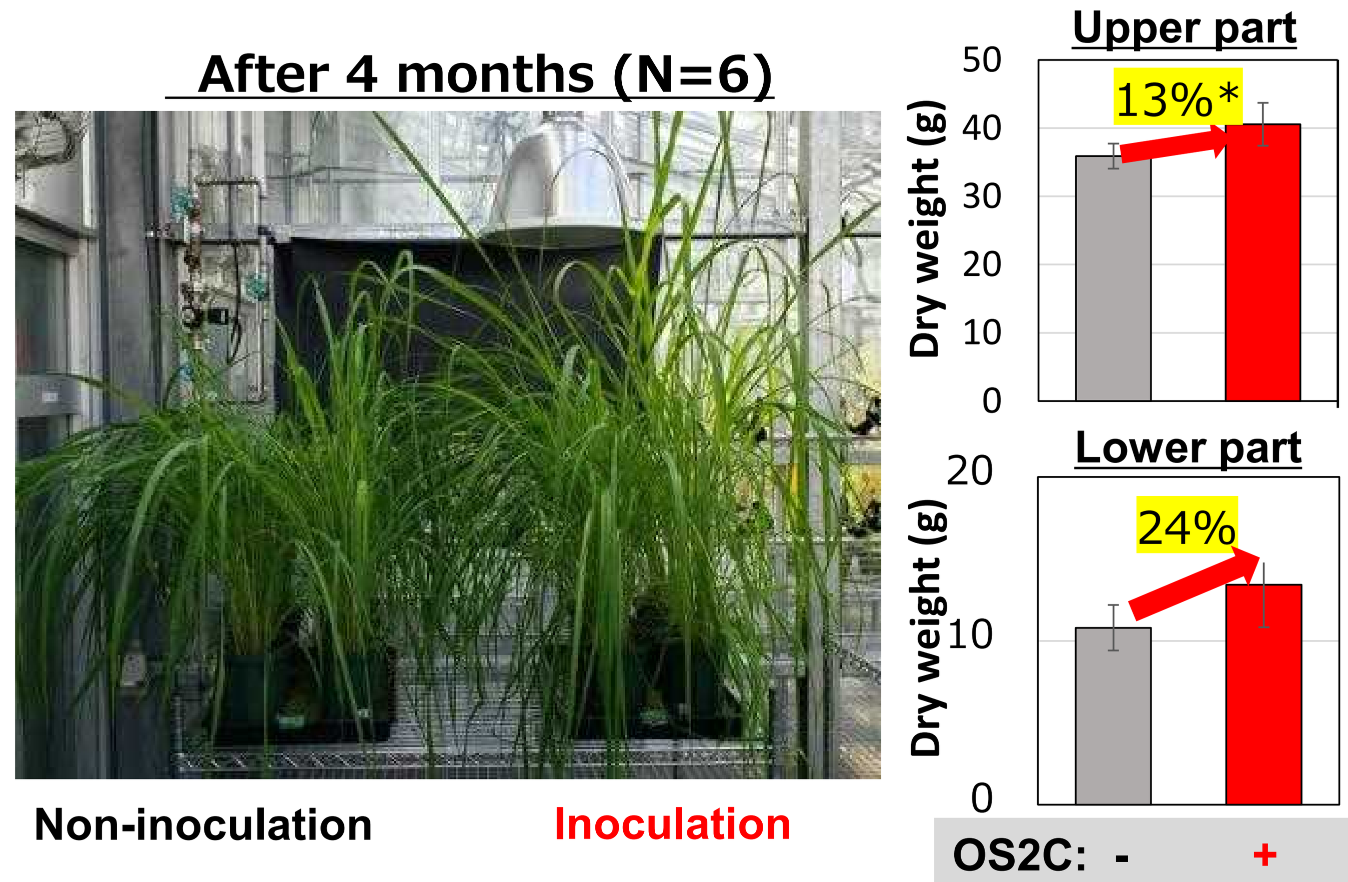
◆During the vegetative growth phase in rice



◆In the harvest stage in rice

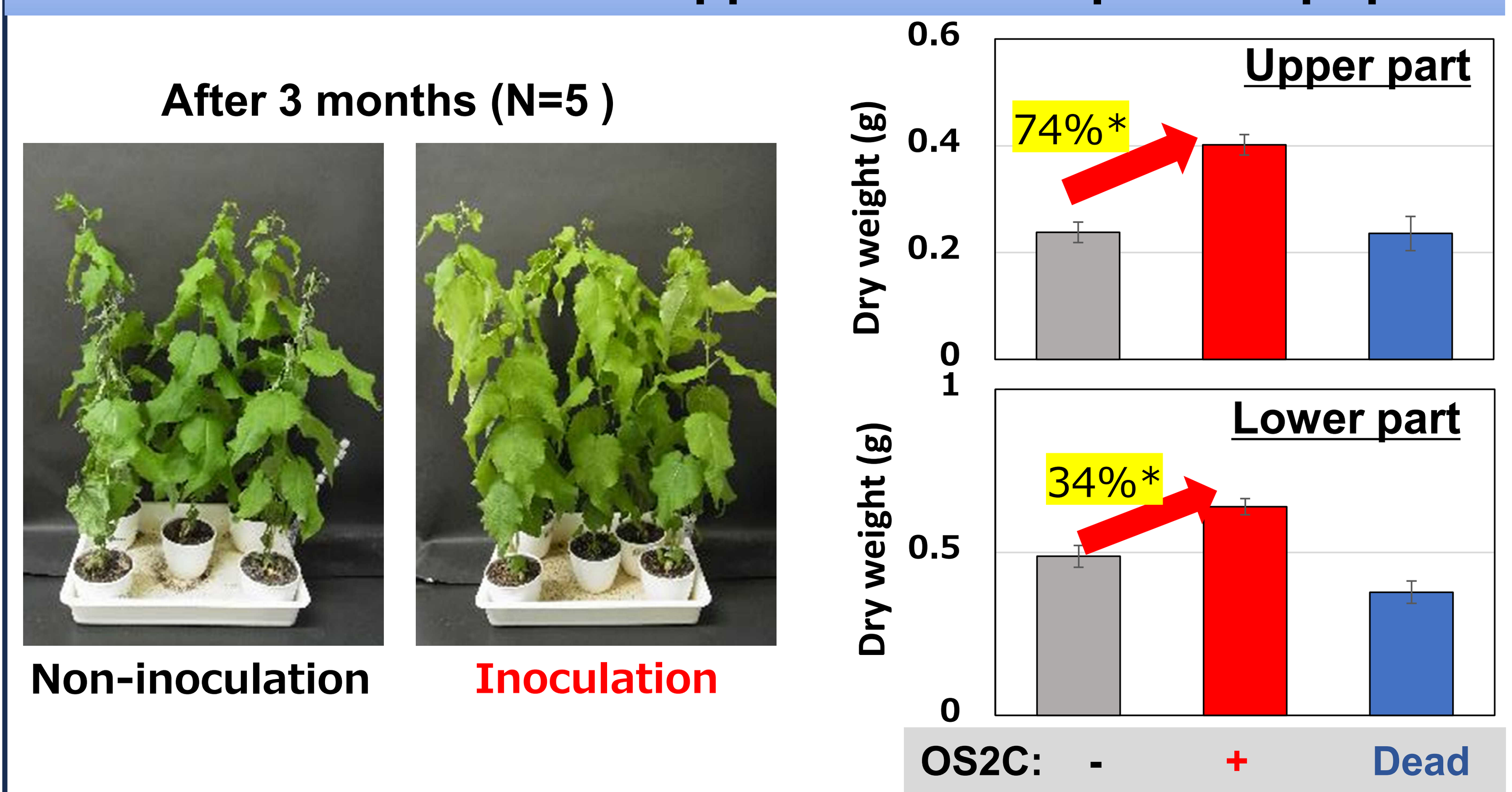


OS2C also enhances the growth of Erianthus biomass.

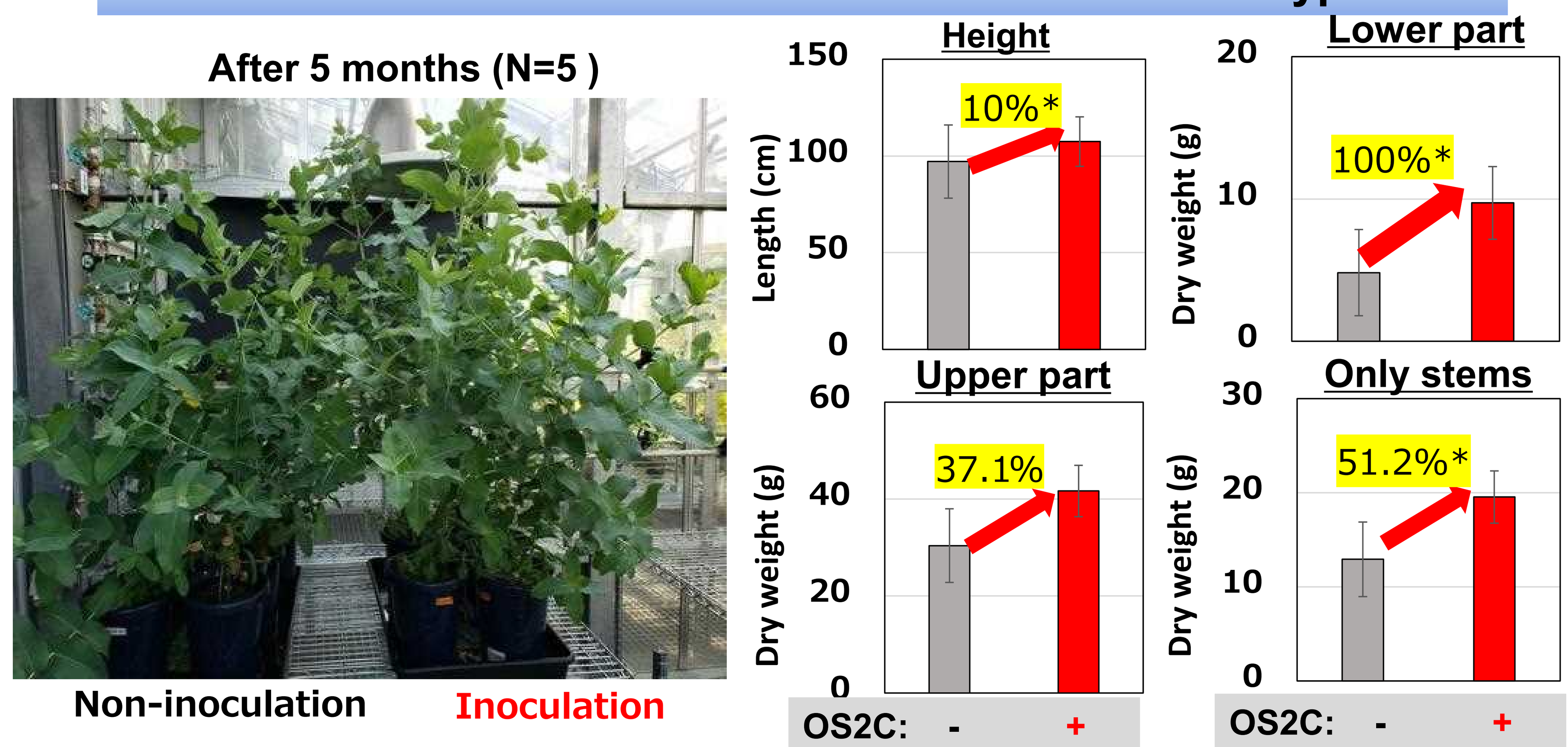


2. Growth-promoting effects of OS2C on woody plants: Poplar & Eucalyptus

OS2C promotes the growth of upper and lower parts in poplar.

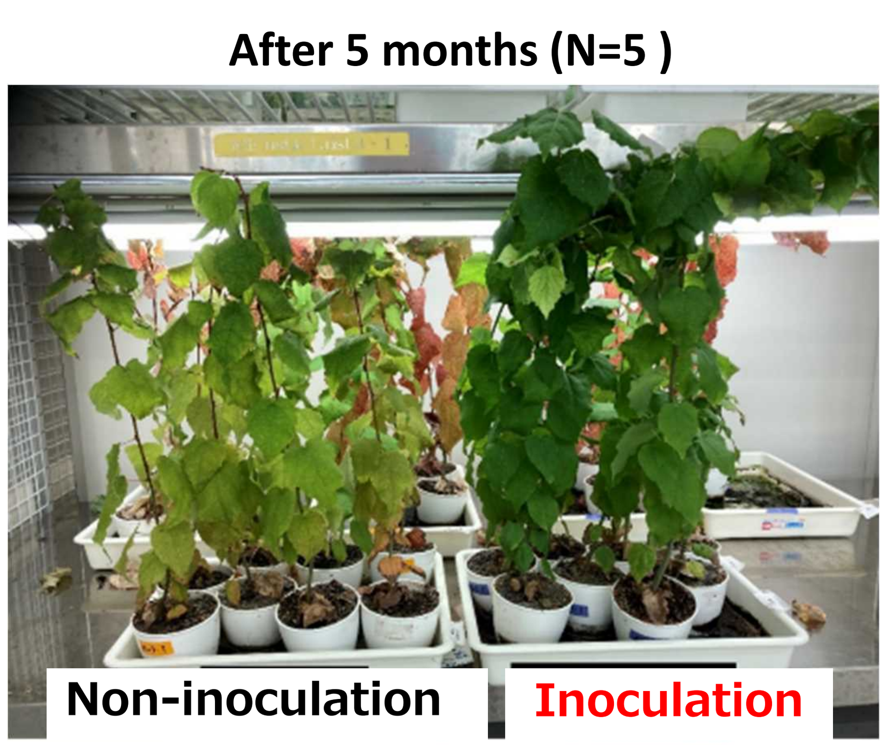


OS2C also stimulates the overall biomass of Eucalyptus.

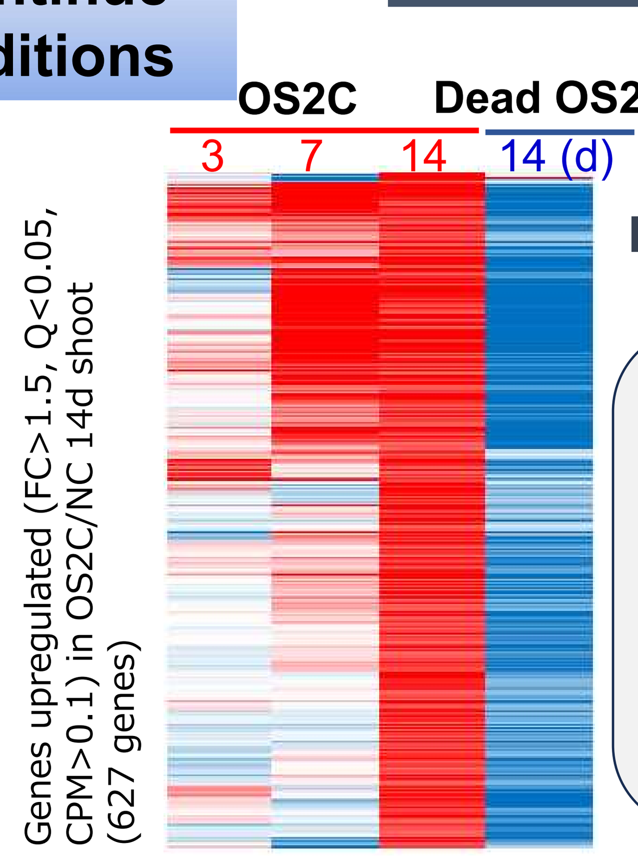


3. Additional beneficial effect of OS2C on plants

OS2C-inoculated poplar trees can continue to grow even in unfavorable soil conditions



4. RNA-seq analysis for understanding OS2C-mediated growth promotion mechanisms in rice

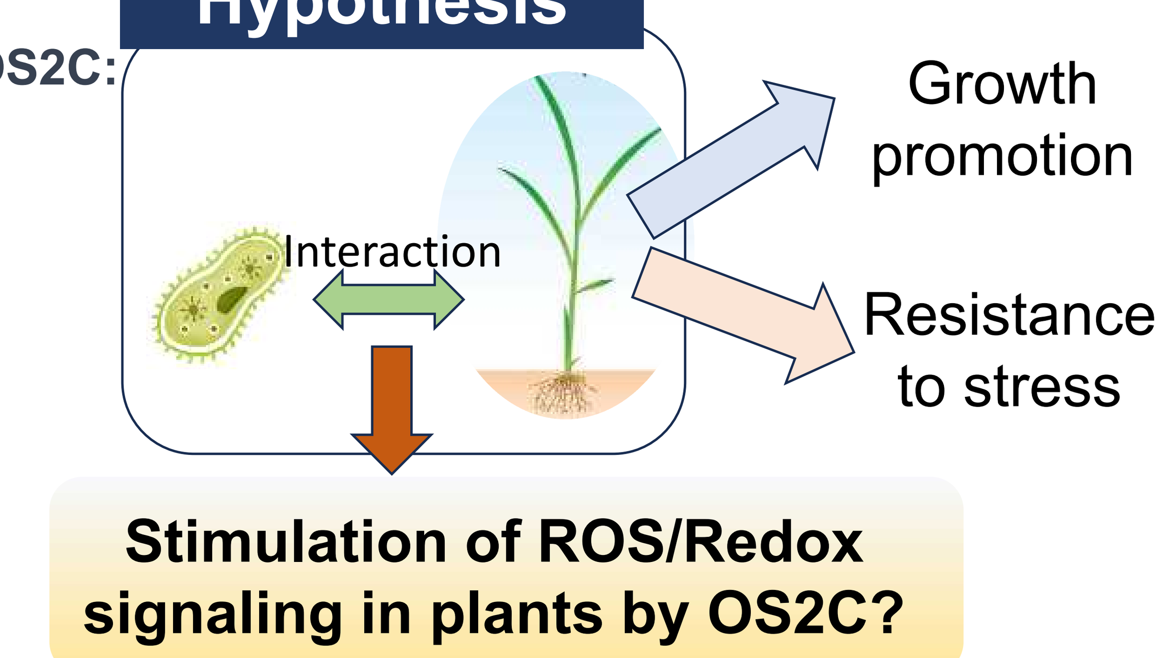


Redox-related genes are more highly and specifically expressed in OS2C-inoculated rice plants compared to dead-OS2C inoculated plants.

Enrichment analysis results using the up-regulated genes induced by OS2C:

- plant-type cell wall organization
- peroxidase activity
- response to oxidative stress
- metal ion transport
- transporter activity
- hydrolase activity, hydrolyzing ...
- zinc ion binding
- carbohydrate metabolic process
- oxidation-reduction process

Hypothesis



Stimulation of ROS/Redox signaling in plants by OS2C?

No.: A-8-3E

PJ:Development of Next-Generation CO_2-Fixing Plant Through the Gene Optimization, Distant Hybrid, and Microbial Symbiosis

Project:Development of new crops and biomass plants through distant hybridization

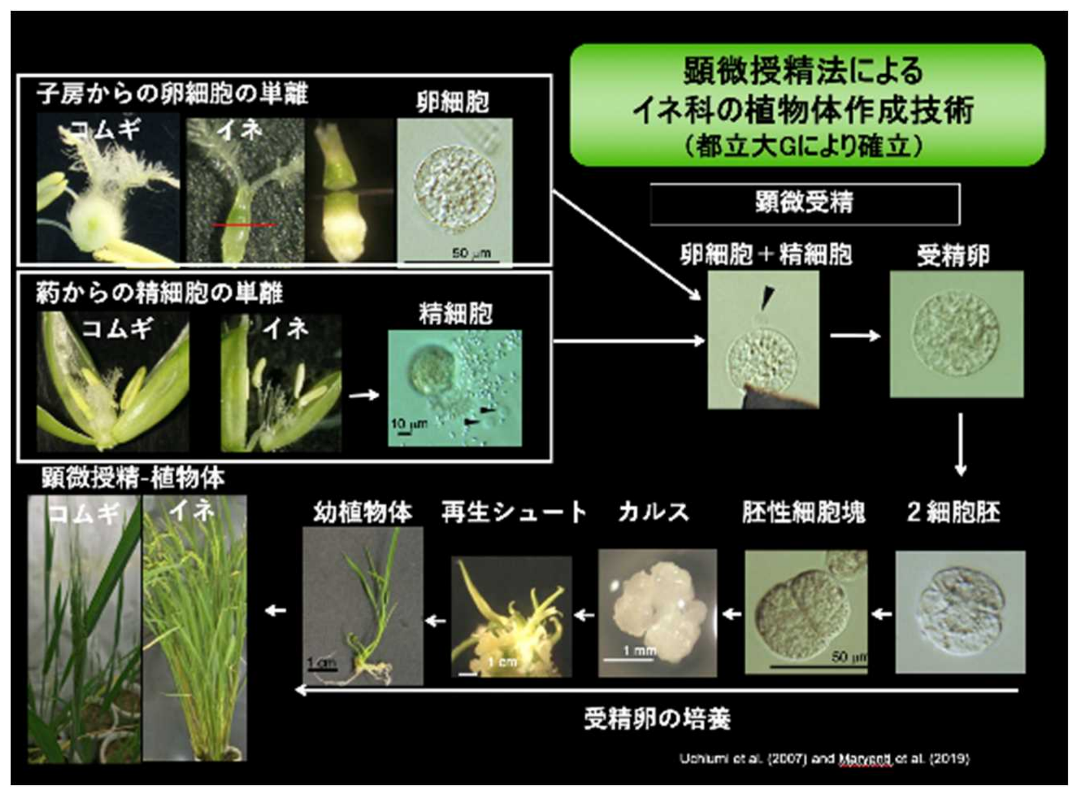
Institution: Tokyo Met. Univ./Tottori Univ./JIRCAS/AIST
Contact: Takashi OKAMOTO (Tokyo Met. Univ.) okamoto-takashi@tmu.ac.jp

Research Summary

One of the most realistic means which absorb and fix CO2 in the atmosphere at low cost is the utilization of plants (crops) that are widely cultivated. As the remaining biomass of rice and wheat, which account for approximately 60% of the world's cereal production, has not been effectively utilized, it is important to provide rice and wheat with traits suitable for effective utilization of biomass, that is, traits that efficiently fix CO2.

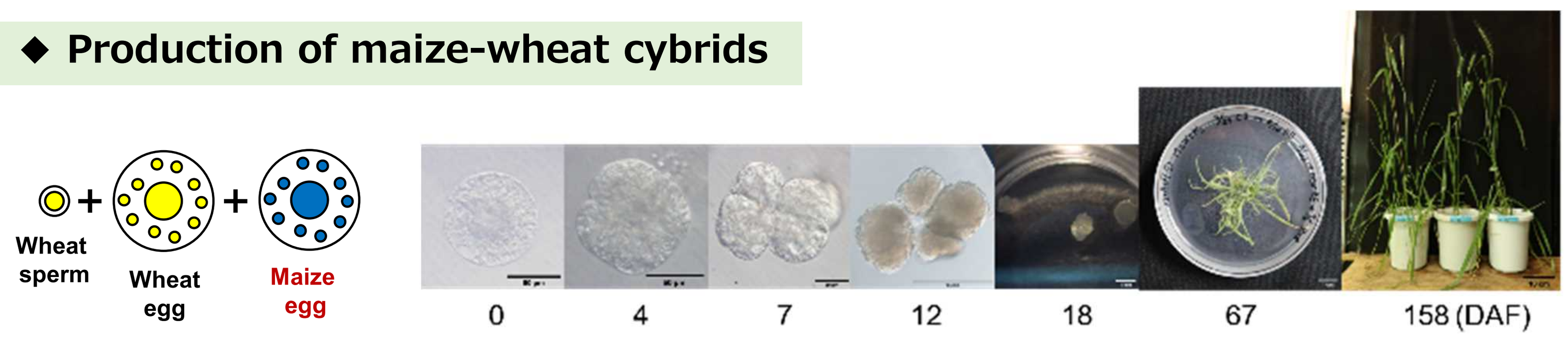
In addition, biomass plants such as Erianthus are tropical and subtropical origin and do not fully demonstrate their biomass production potential in the temperate zones, indicating that the creation of new biomass plants with cold tolerance will lead to the creation of highly efficient CO2 resource-utilizing biomass plants.

In this project, we will establish technological elements for the creation of highly efficient CO2 resource-utilizing crops and plants which are produced by in vitro fertilization system using isolated gametes across inter-subfamily. In addition, the development of novel cell surface modification materials that promote selective fusion of heterologous cells (gametes) will be promoted as a technological element for the creation of new distant hybrids.

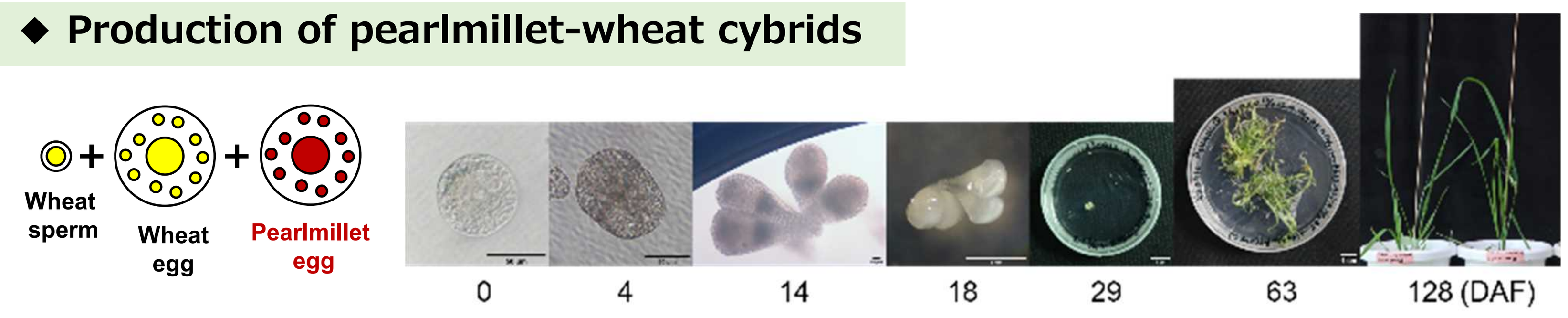


Establishment of elemental technologies for the creation of new rice and wheat plants and verification of their potential

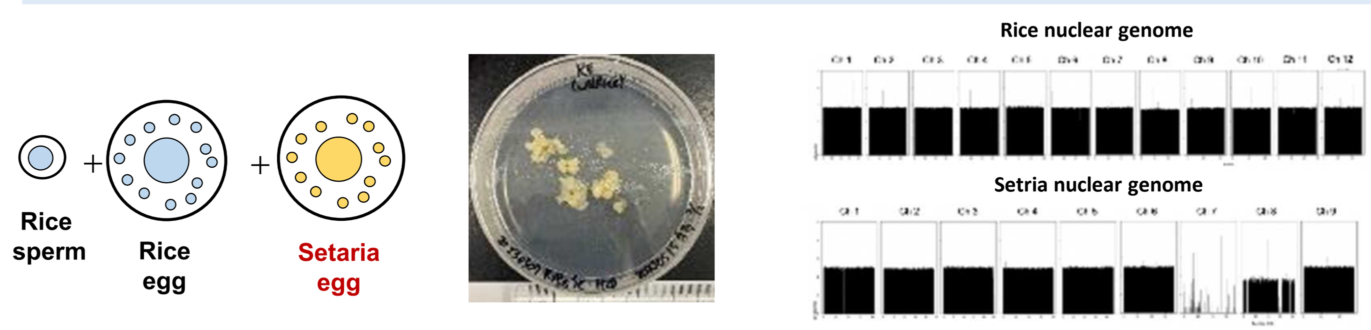
◆ Production of maize-wheat cybrids



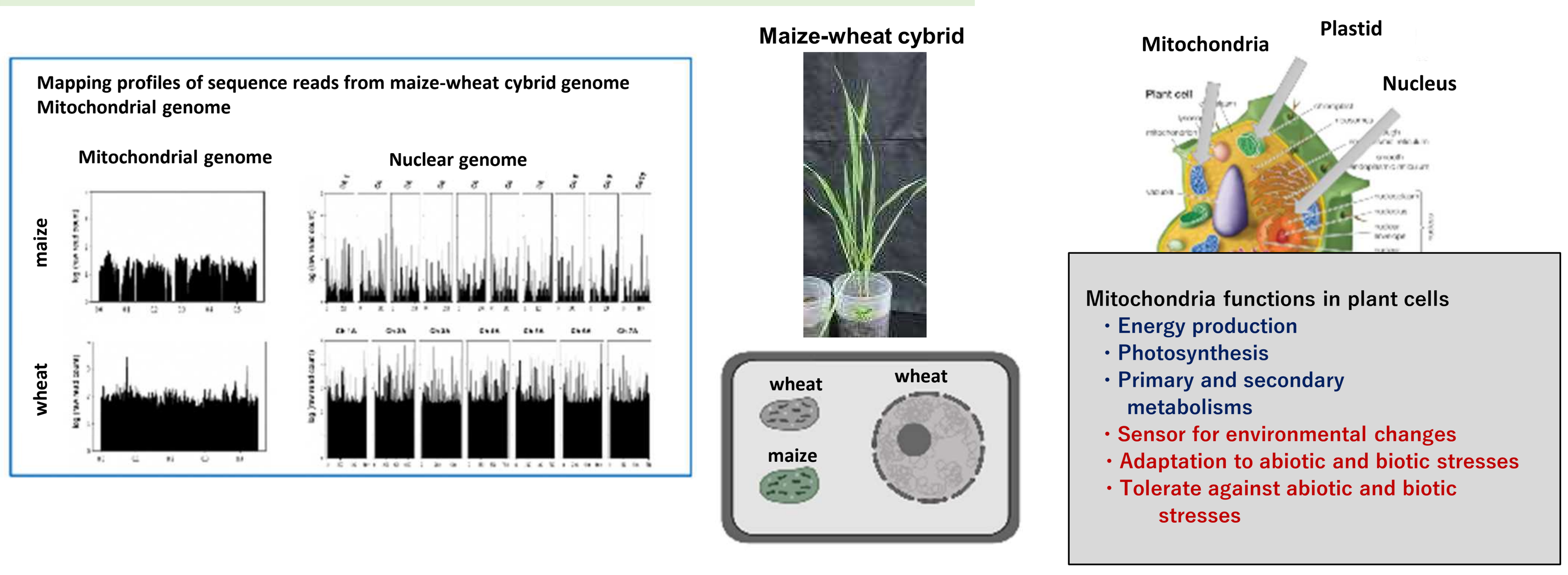
◆ Production of pearlmillet-wheat cybrids



◆ Calli from rice-setaria hybrid zygotes and their genome composition

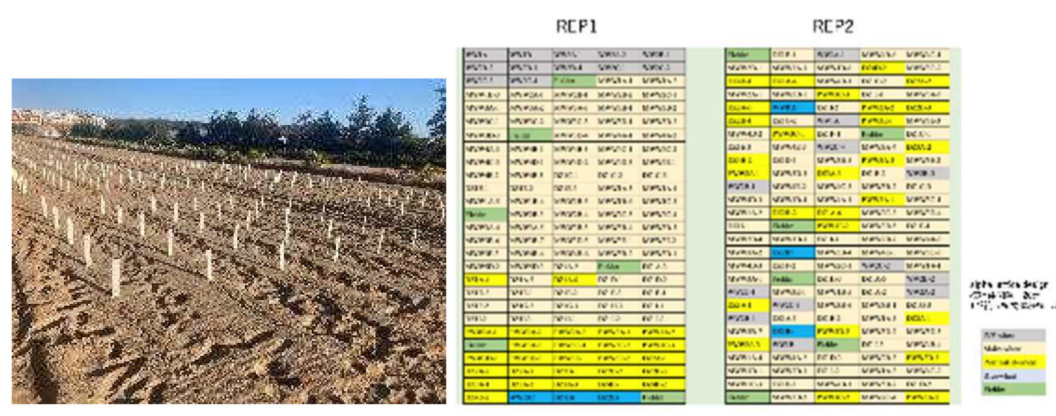


◆ Genome composition of maize-wheat cybrids



《Future plans》

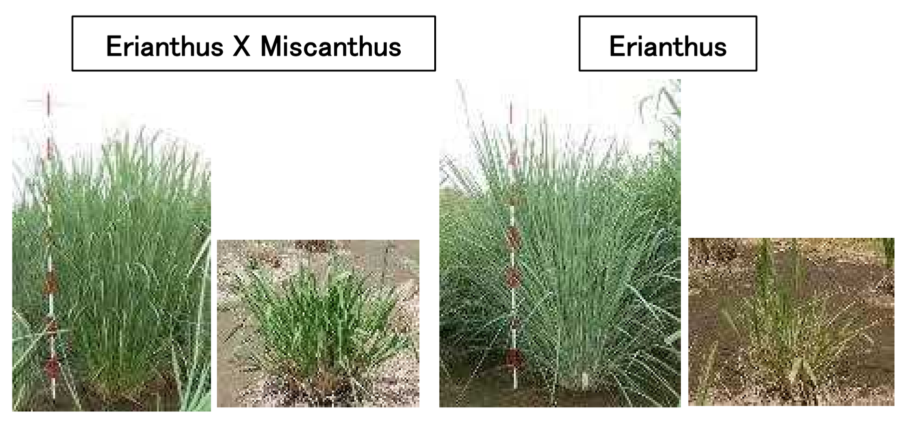
- Agronomic and physiological traits
  - Maize-wheat seeds, propagated
  - Photosynthetic potential and mode
  - Characteristics of cell wall
- Regeneration calli from rice-C4 plant hybrid zygotes and their genome



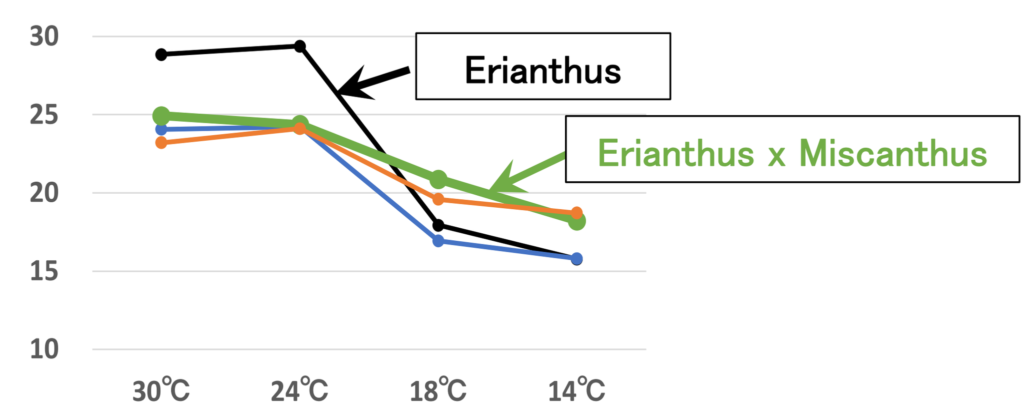
Establishment of elemental technologies for the creation of new biomass plants and verification of their potential

◆ Erianthus-Miscanthus hybrid via pollination

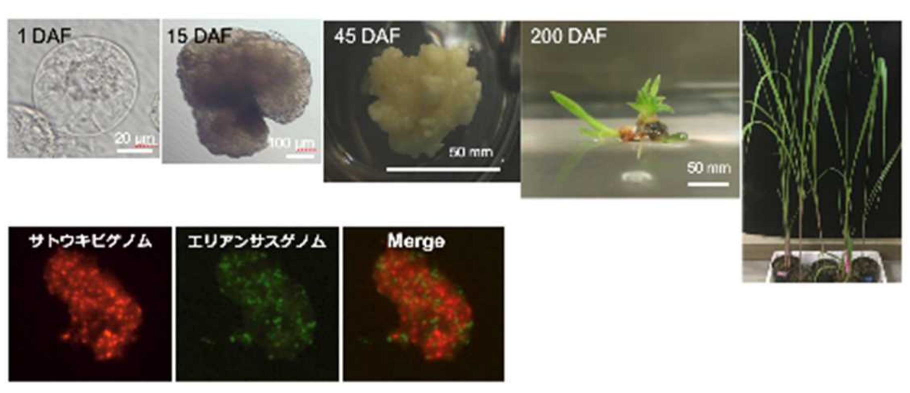
◆Growth and overwintering in northern region

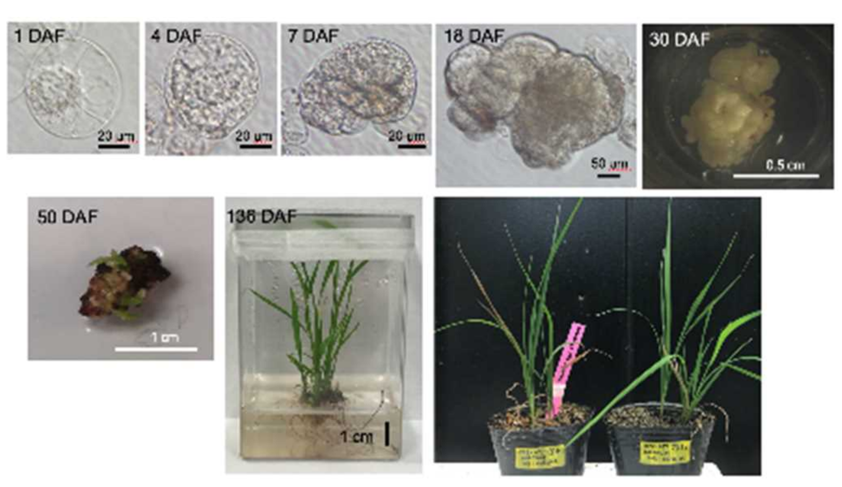


◆ Photosynthetic activity at various temperatures



◆ Erianthus-Miscanthus hybrid (left panels) and possible Erianthus-Sugarcane/Miscanthu hybrid (Right panels)



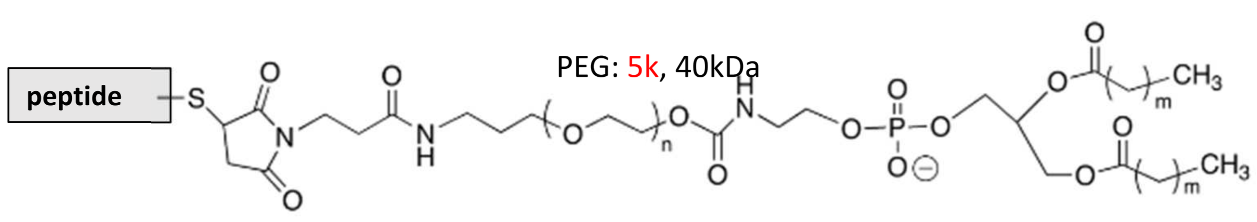


《Future plans》

- Trait evaluation for cold tolerance, biomass production capacity, and photosynthetic potential
- Propagation and basic growth characterization of Erianthus-Miscanthus hybrid and possible Erianthus-Sugarcane/Miscanthu hybrid

Development of highly efficient technology for heterologous cell fusion using peptide PEG-lipid conjugates

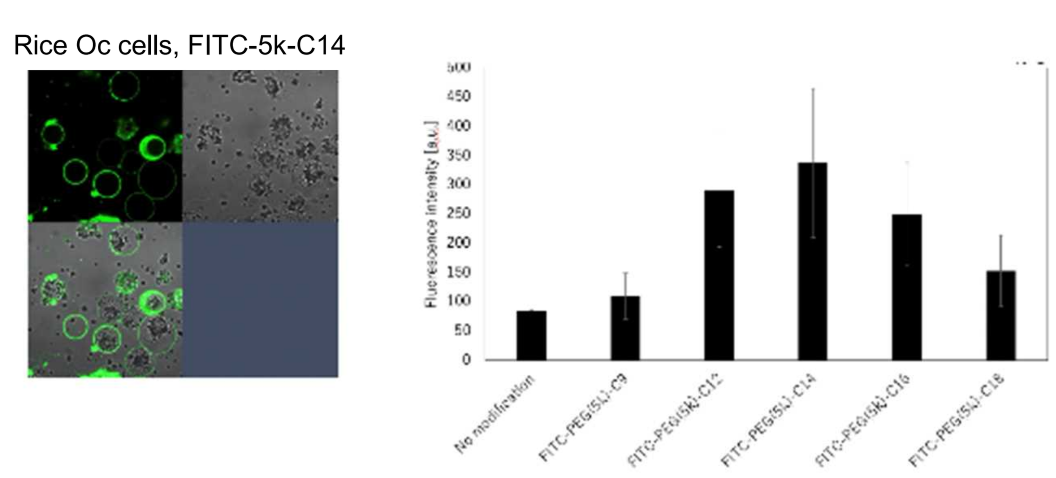
◆ Peptide PEG-lipid conjugates



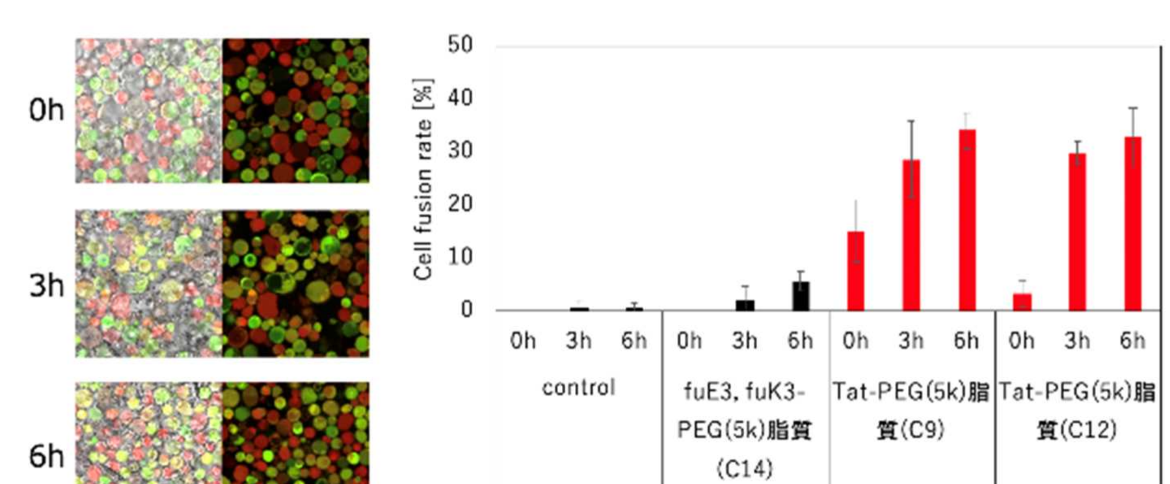
fuE3, fuK3 peptides:
EIAALEKEIAALEKEIAALEKGGGC (fuE3)
KIAALKEKIAALKEKIAALKEGGGC (fuK3)

Tat peptide:
YGRKKRRQRRR (Tat)
Patent (特願2023-192501)

◆ Optimization of carbon chain length



◆ Fusion efficiency of rice protoplasts



《Future plans》 **Improvement of cell surface modification materials (cell fusion promotion materials)**
Oligopeptide moiety is changed to oligo-DNA, and effectiveness in cell fusion is checked using cultured cells or leaf protoplasts. The new material will be applied to Eucalyptus protoplasts and grass gametophyte fusion.

No. A-8-4E

PJ : Development of Next-Generation CO_2-Fixing Plant Through the Gene Optimization, Distant Hybrid, and Microbial Symbiosis

Theme: Development of high-strength trees by gene optimization

Organization: Natl. Inst. Adv. Ind. Sci. Tech., Sumitomo Forestry Co., Ltd. (SFC), Inplanta Innovations, Inc.

Contact: Satoshi KOGAWARA (SFC) KOGAWARA_satoshi@star.sfc.co.jp

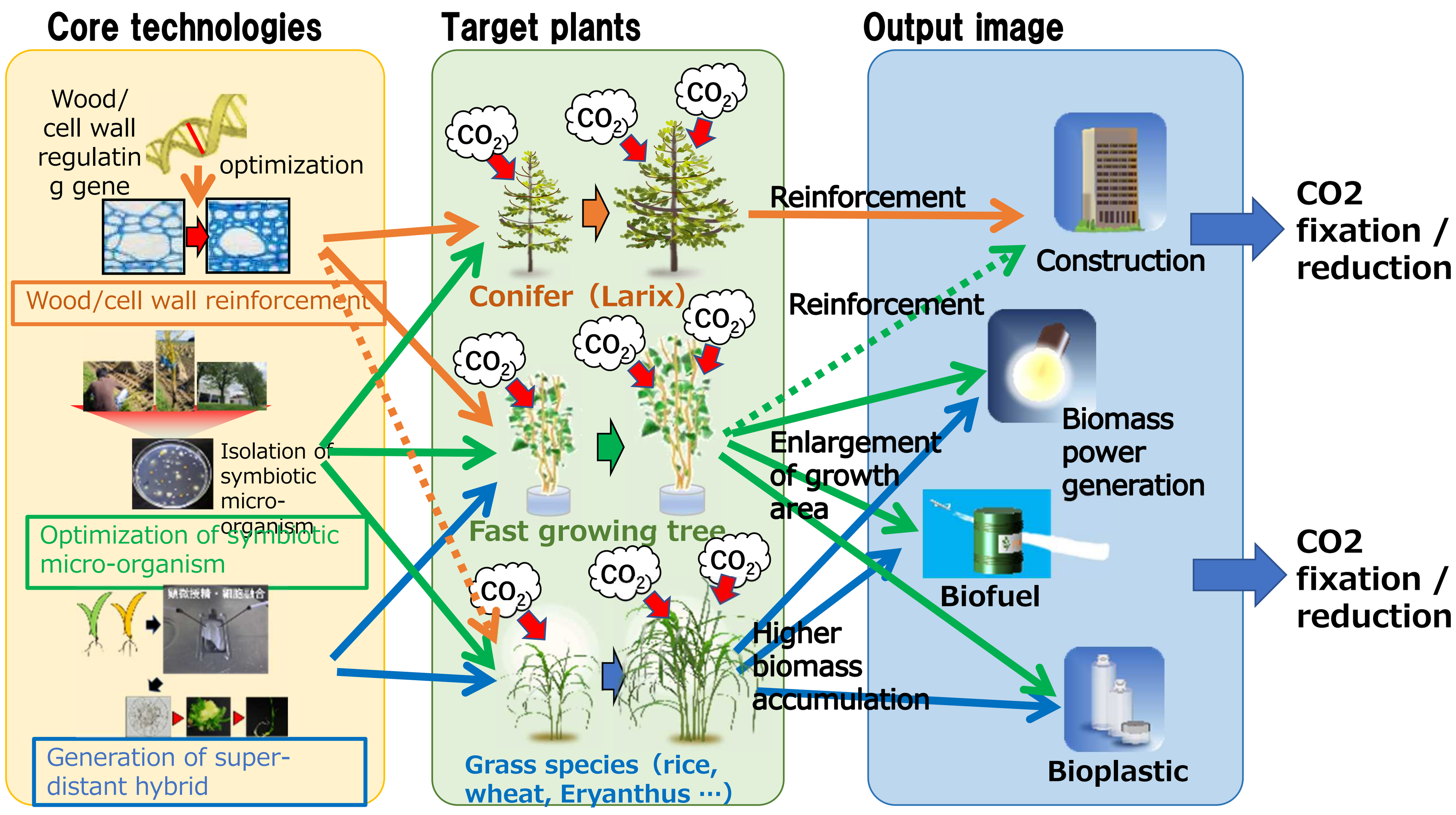
Research outline

This project aims to develop plants (and technologies to support their growth) that not only have high CO2 fixation capacity but also produce woody biomass with high value added for industrial use, by making full use of three core technologies.

In this theme, we are developing trees that have high CO2 fixation capacity and produce high-strength wood that can be used for wooden high-rise buildings through high activation of the NST transcription factor, a master regulator of wood formation (wood and cell wall strengthening technology). So far, we have comprehensively analyzed the activity of NST transcription factors with mutations in their genes and identified hyperactivating mutations. Furthermore, we have demonstrated that wood and cell walls are strengthened in plants expressing the NST transcription factors with the introduction of high-activating mutations (gene optimization).

In addition, with a view to social implementation of high-strength trees, we are aiming to establish a genome-editing system for larch, since we are considering applying genome editing with base editor rather than genetic recombination as the method of gene optimization.

Graphical abstract



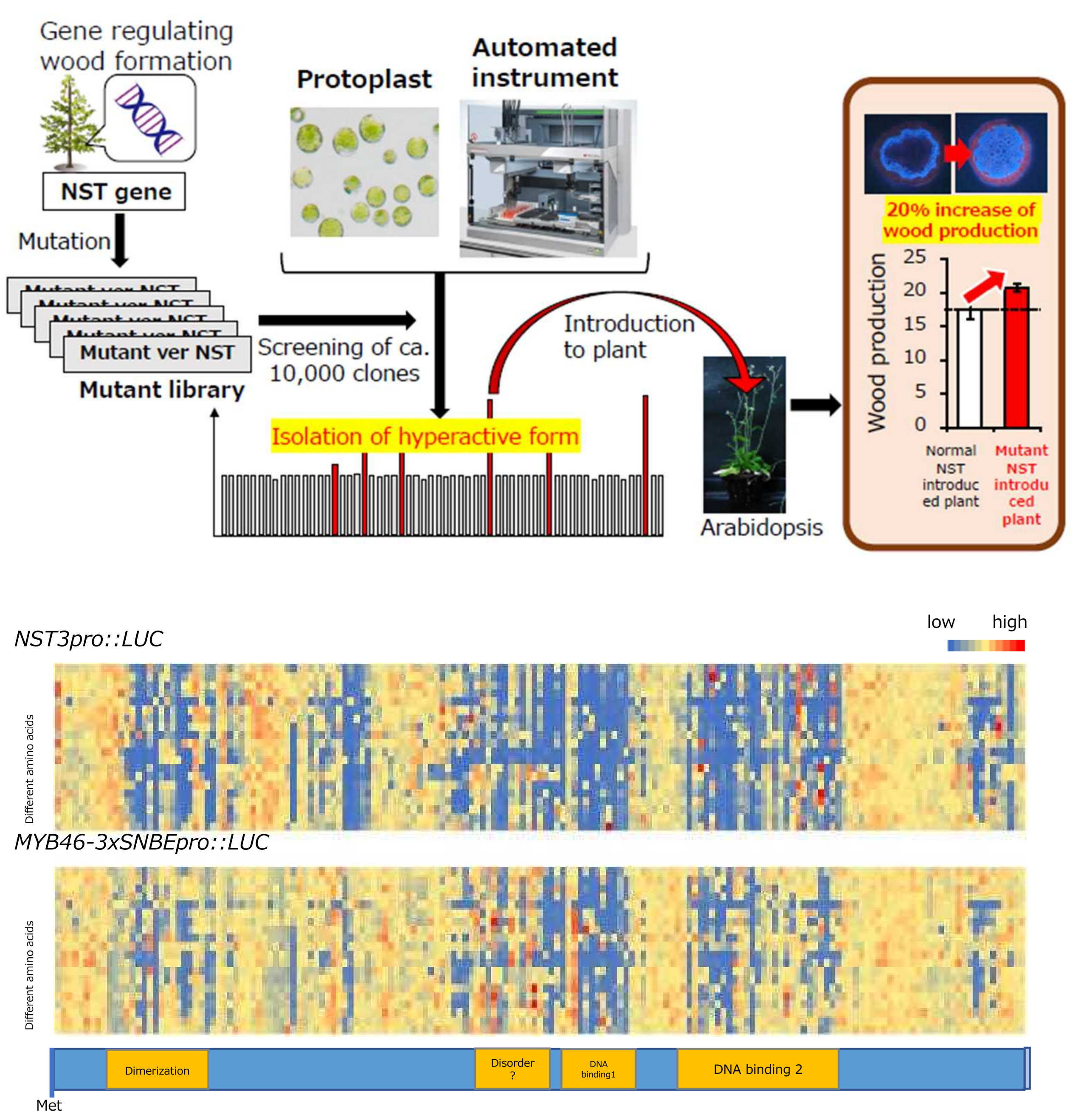
Results

- Identification of hyperactivating mutations in NST transcription factors
- Demonstration of cell wall-fortification effects of NST hyperactivating mutations
- Establishment of a genome editing system for larch

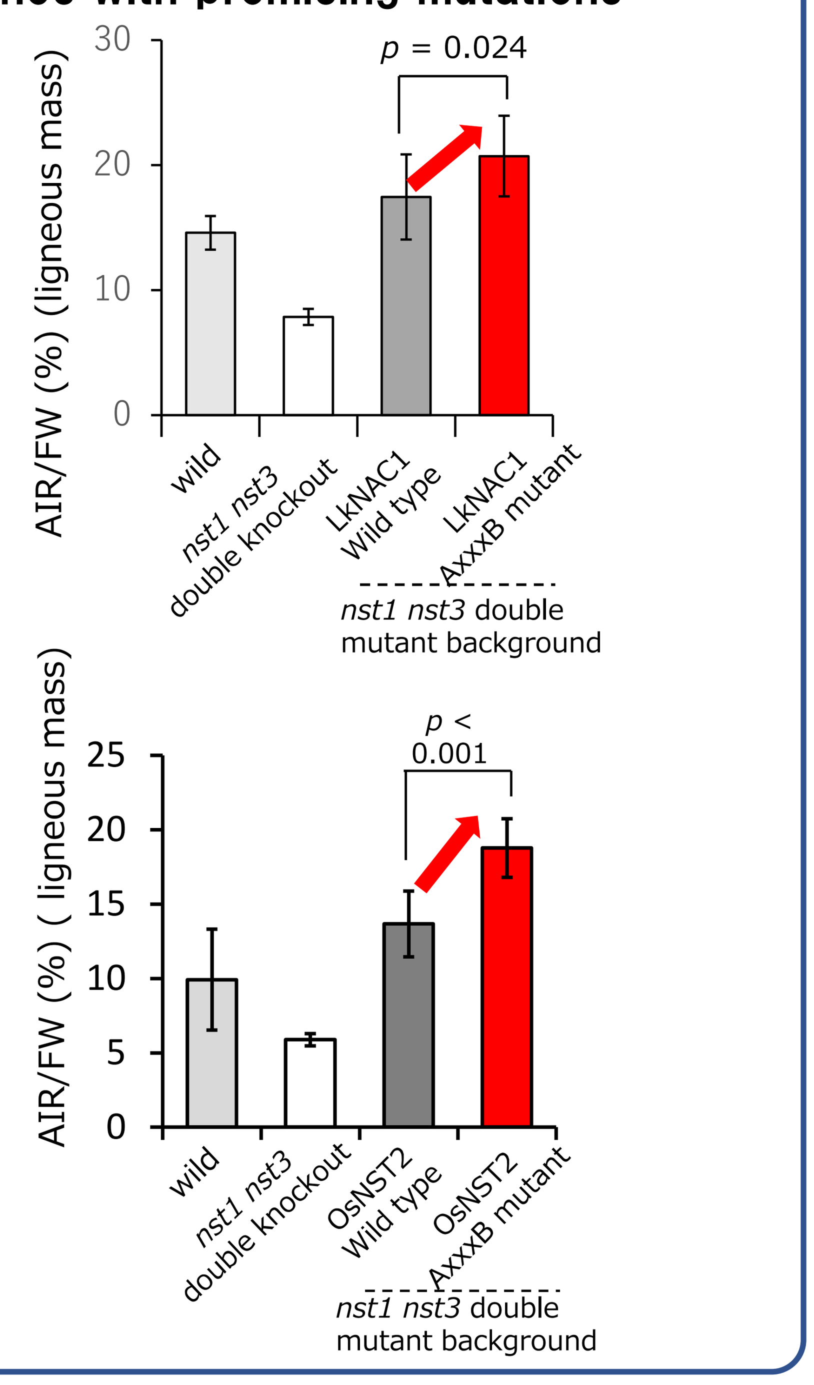
■ Identification of the NST transcription factor gene, a key regulator of xylem formation in plants (AIST)

Takata et al., Tree Physiol. 39, 514-525 (2019)

■ Identification of hyperactive NST transcription factor



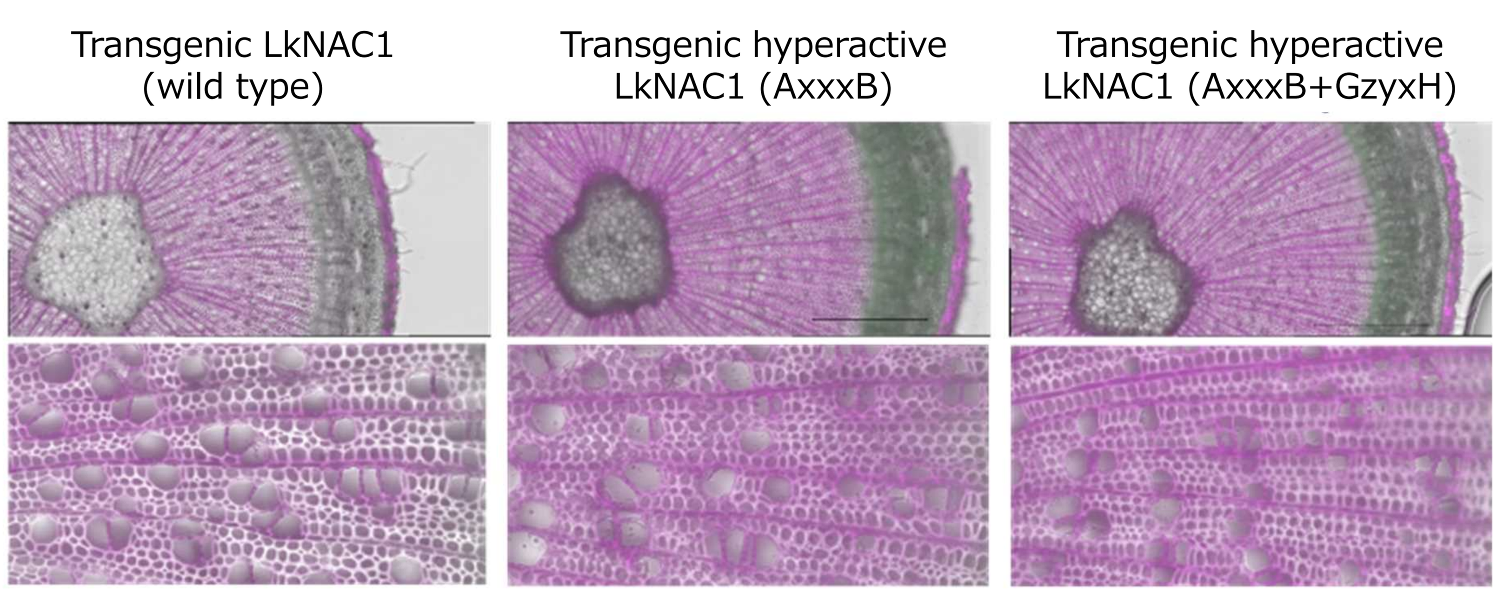
■ Woody mass of transgenic *Arabidopsis thaliana* for NST transcription factor genes with promising mutations



■ Transgenic poplar for NST transcription factor gene with promising mutations

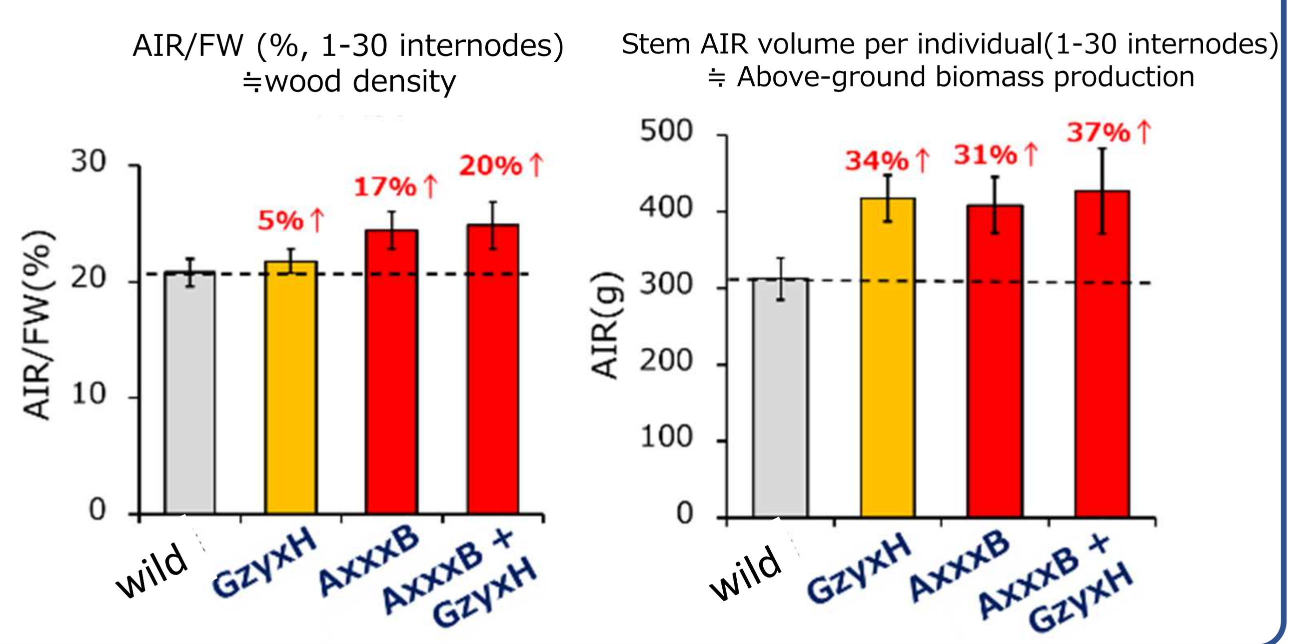
poplar

sectional view



Pink color : Lignin autofluorescence under UV irradiation in pseudo color

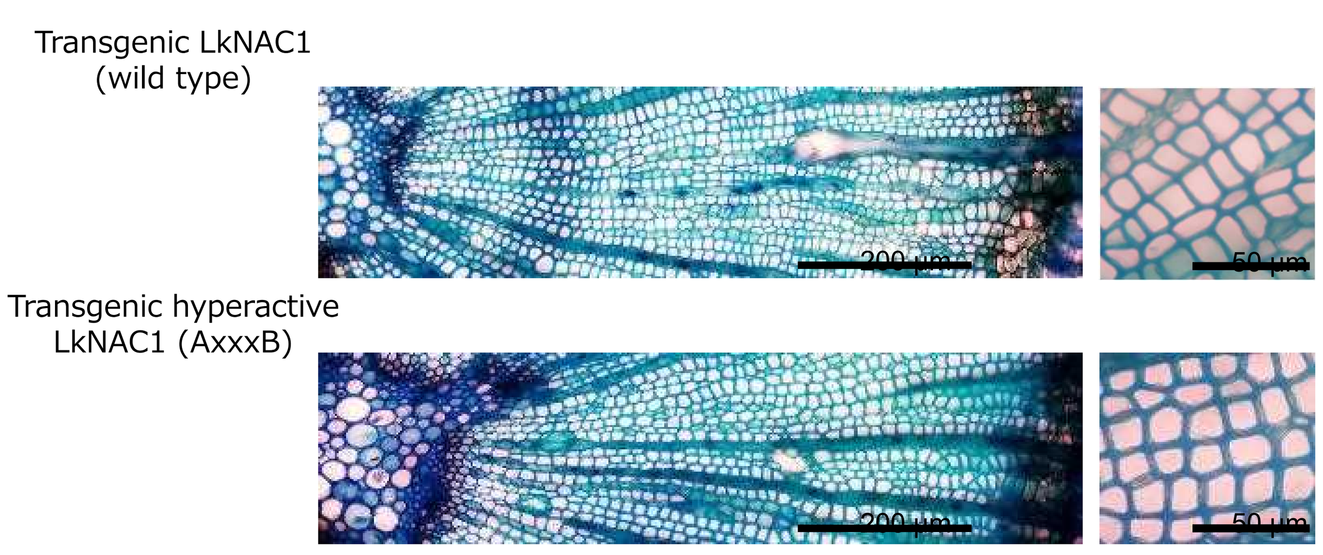
ligneous mass



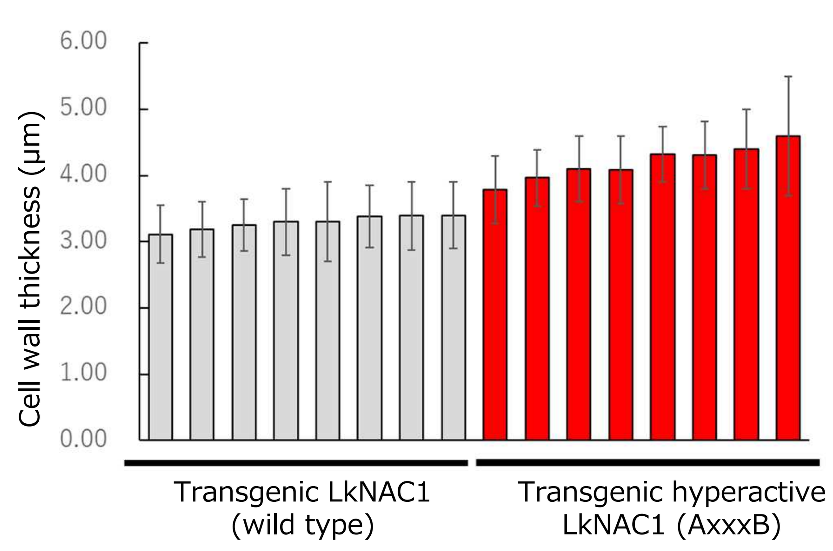
■ Transgenic larch for NST transcription factor gene with promising mutations

larch

sectional view



Cell wall thickness



■ Establishment of a genome editing system for larch