Development of Next-Generation CO2-Fixing Plant Through the Gene Optimization, Distant Hybrid, and Microbial Symbiosis

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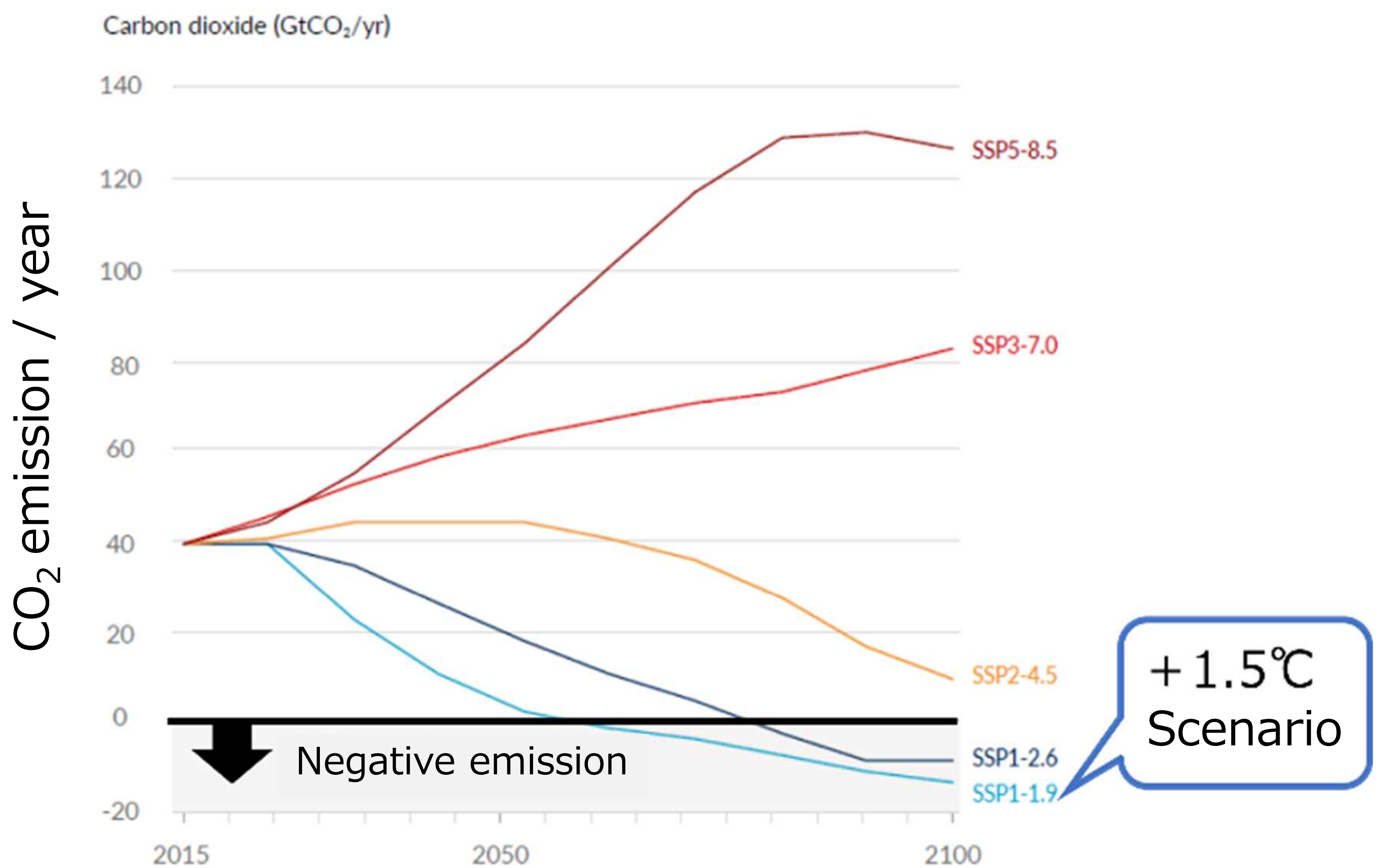
Background

- To stop global warming…

Five scenarios about transition of CO_2 emission（IPCC AR6）

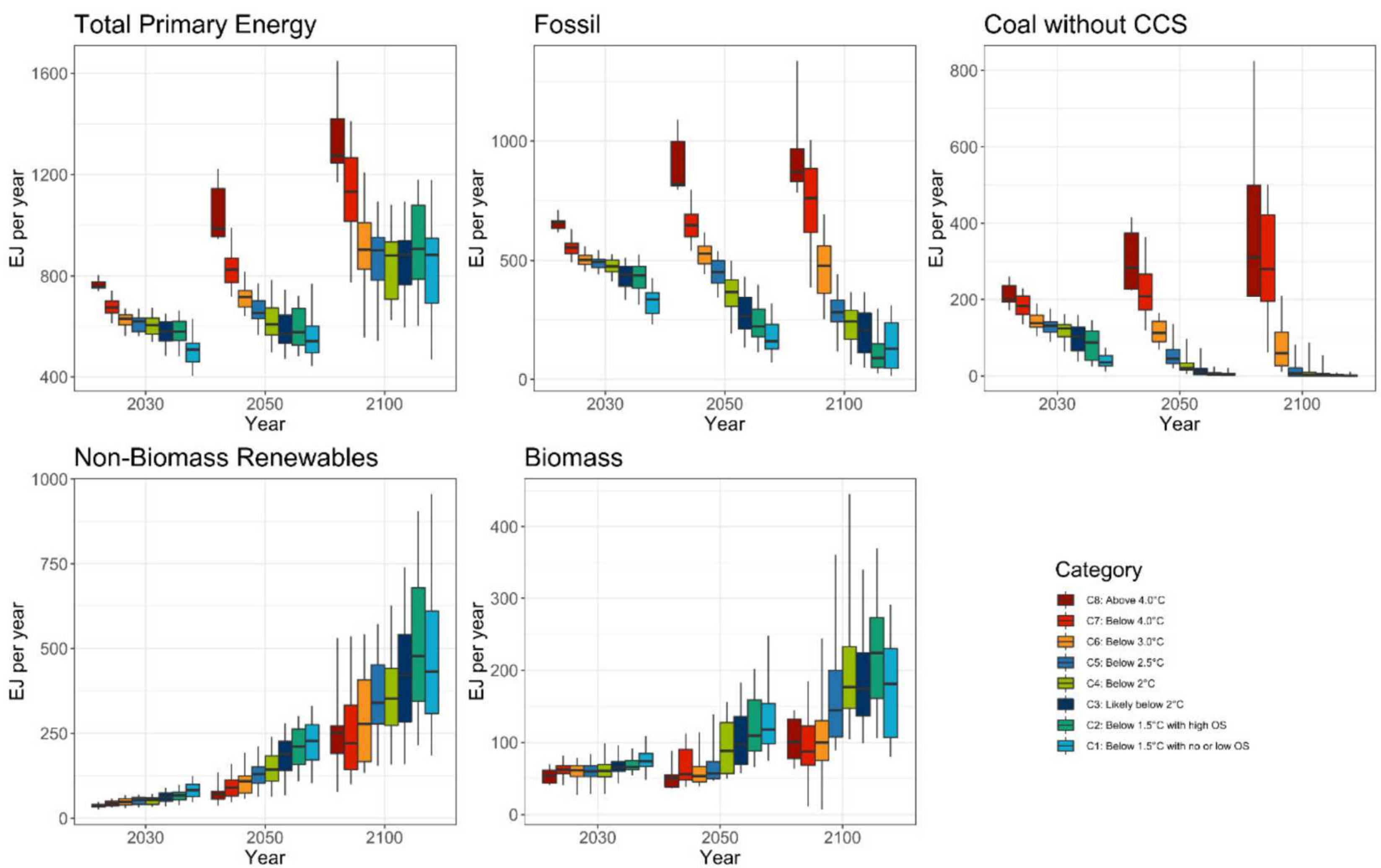


➢ To achieve the +1.5°C scenario, CO_2 emissions in NET must be reduced even from 2023, and negative emissions in NET must be achieved around 2055

Background

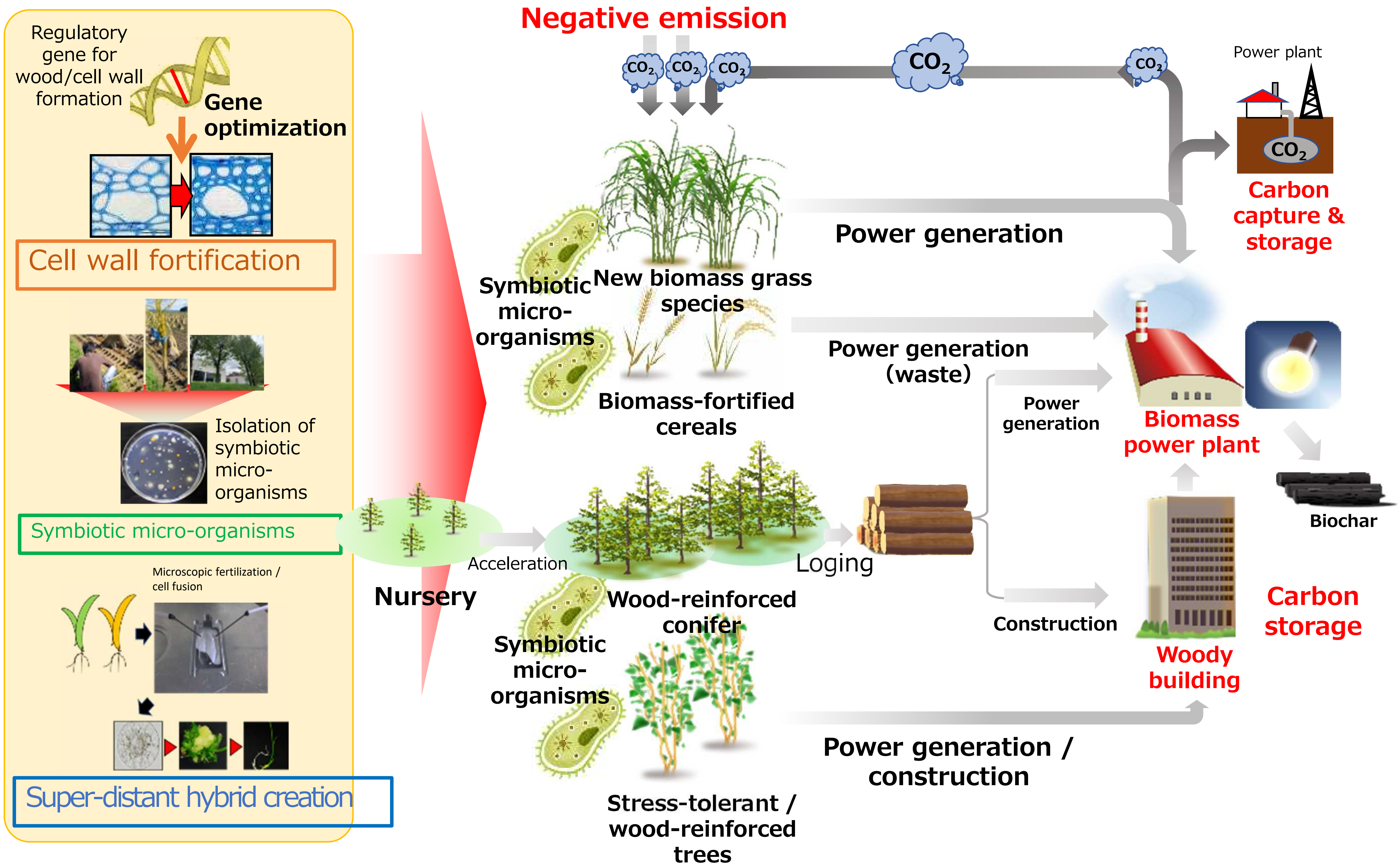
- To achieve ＋1.5℃ scenario …

Energy source transition in each scenario （IPCC AR6）

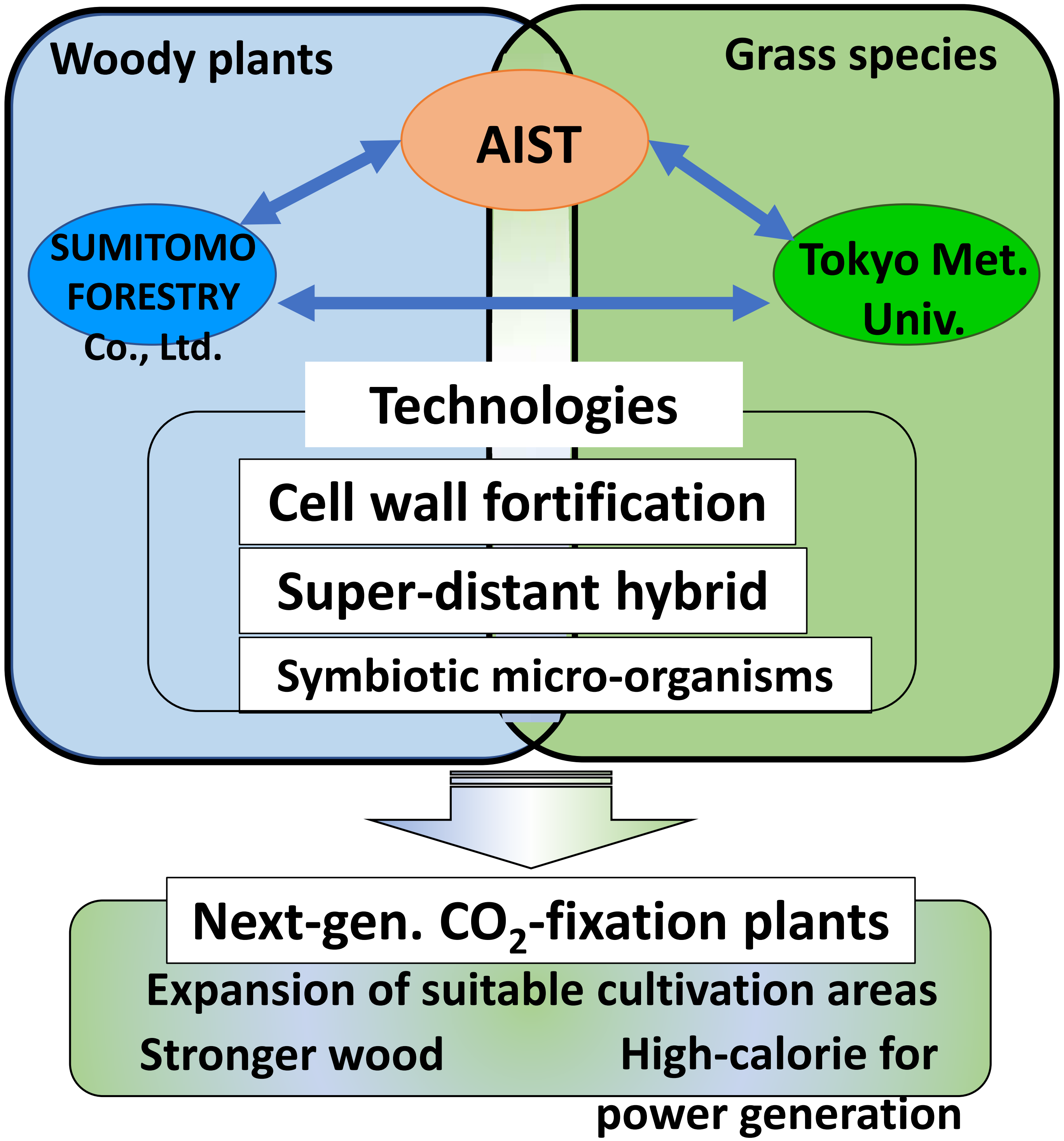


➢ For +1.5°C scenario, about a quarter of all energy consumption must come from biomass in 2100
➢ High expectations for biomass, not only for energy

Graphical abstract



Project's structure・Goals



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- Aiming to improve biomass productivity by 30% compared to conventional methods for genetic optimization, hyper distantly related hybrids, and symbiotic microorganism optimization, respectively.
  - Results can be used in combination with conventional breeding
- Using the optimization of symbiotic micro-organisms as a hub, combined with super distantly related hybrids in the short term and genetic optimization in the long term, aim to increase biomass productivity by 50% compared to conventional methods.

R&D schedule

	2022～2023FY	2024FY
1． Establishment of biomass enhancement strategy by gene optimization	Wood reinforcement in woody and herbaceous plants by NST hyperactivation → Examination of primary cell wall enhancement strategy in grass species →	→
2． Establishment of new biomass plant creation method by super-distant hybrid creation technology	Development of enhanced cell fusion system → Creation of new biomass plants by super-distant hybrid creation technology →	→ Evaluation of newly created plants →
3． Establishment of plant growth promotion system by symbiotic micro-organism	Exploration of symbiotic micro-organisms promoting plant growth → Evaluation of the effect of symbiotic micro-organisms on plant growth →	→

Three major technologies

- Gene optimization
- Super-distant hybrids
- Symbiotic micro-organisms

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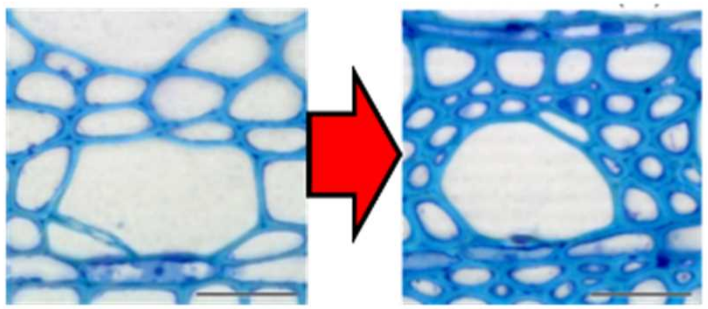
- Gene optimization
- Super-distant hybrids
- Symbiotic micro-organisms

Biomass fortification by gene optimization

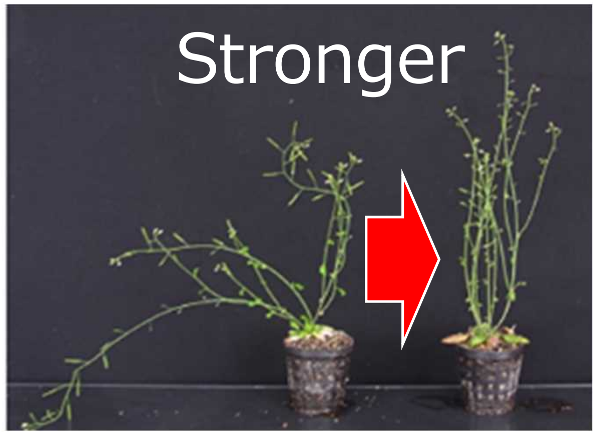
◆Three strategies

Based on gene edit technology

1． Wood reinforcement



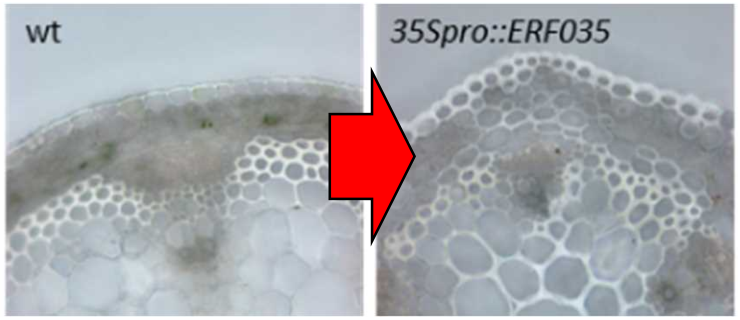
Wood reinforcement



2． Increased strength

- Beneficial trait in addition to higher wood productivity

3． Primary cell wall enhancement (only for grass)



PCW enhancement

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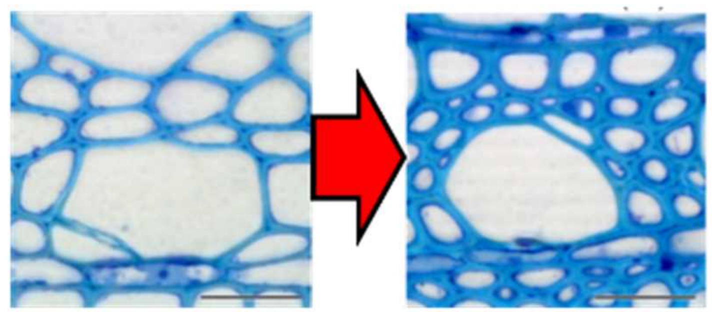
Biomass fortification by gene optimization

◆Three strategies

Based on gene edit technology

1． Wood reinforcement



Wood reinforcement

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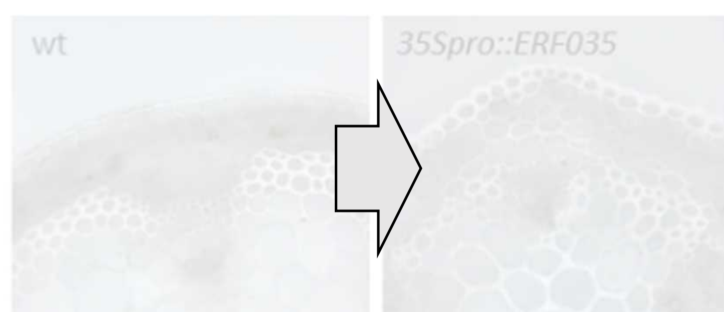

PCW enhancement

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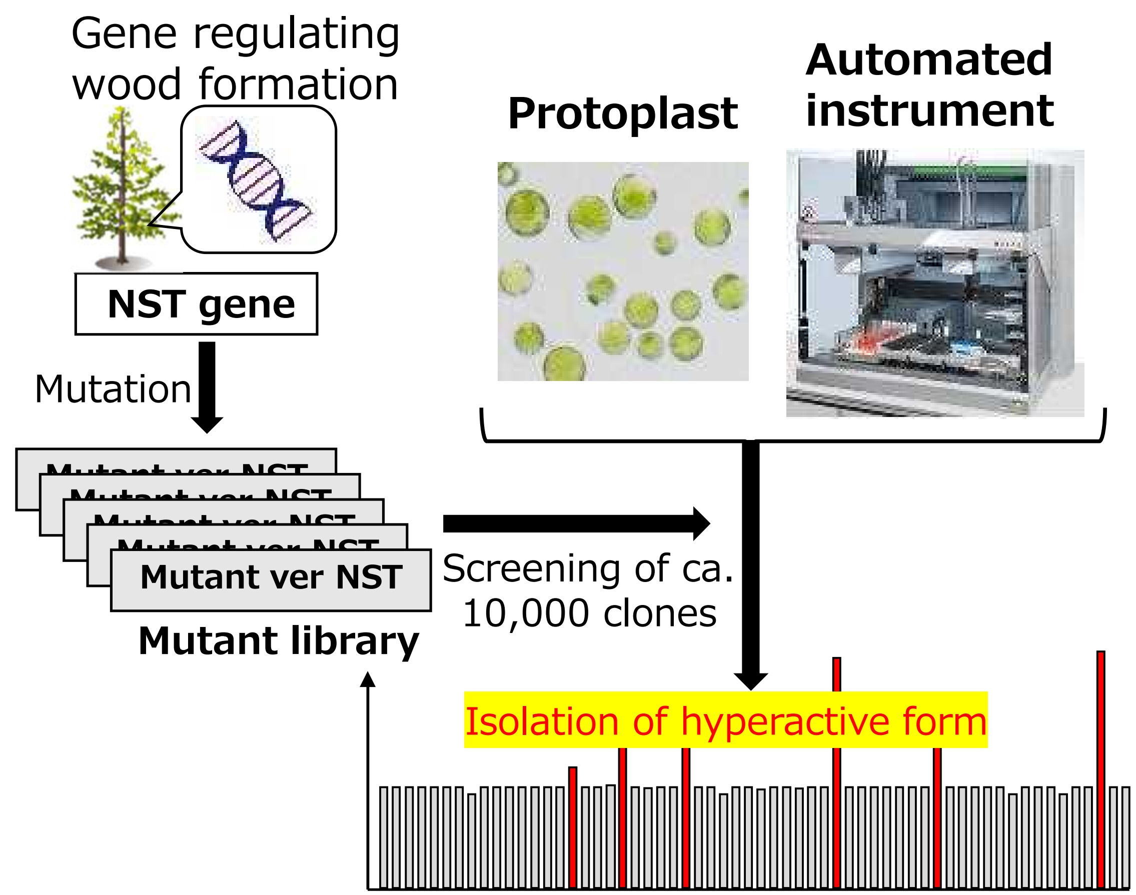
Wood reinforcement by NST hyperactivation

Hyperactivating NST transcription factor regulating wood formation

- NST transcription factor

WT NST knockout

- Getting hyperactive form of NST transcription factor

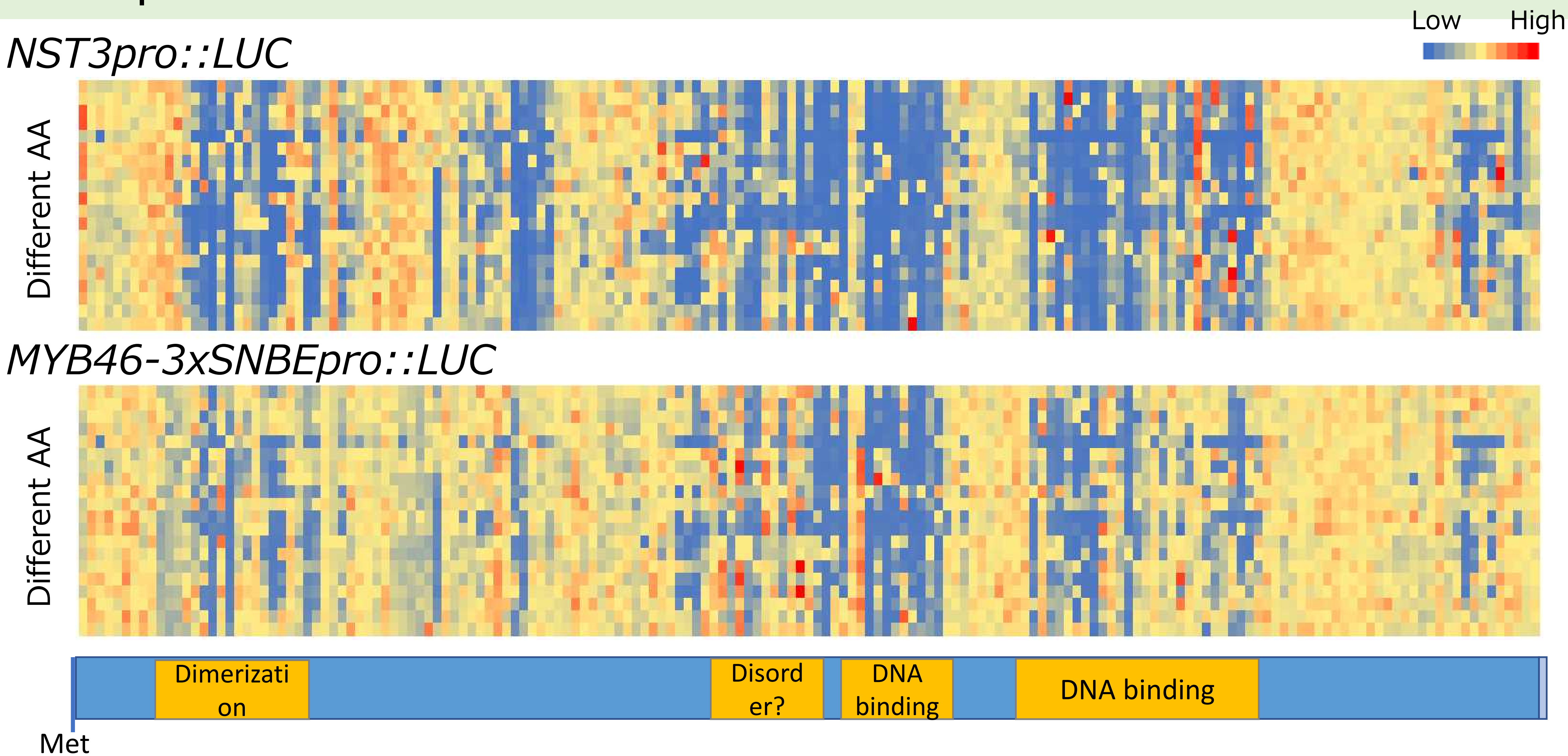


→ Developing a technology to apply the gene edit in practical plants

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

Wood reinforcement by NST hyperactivation

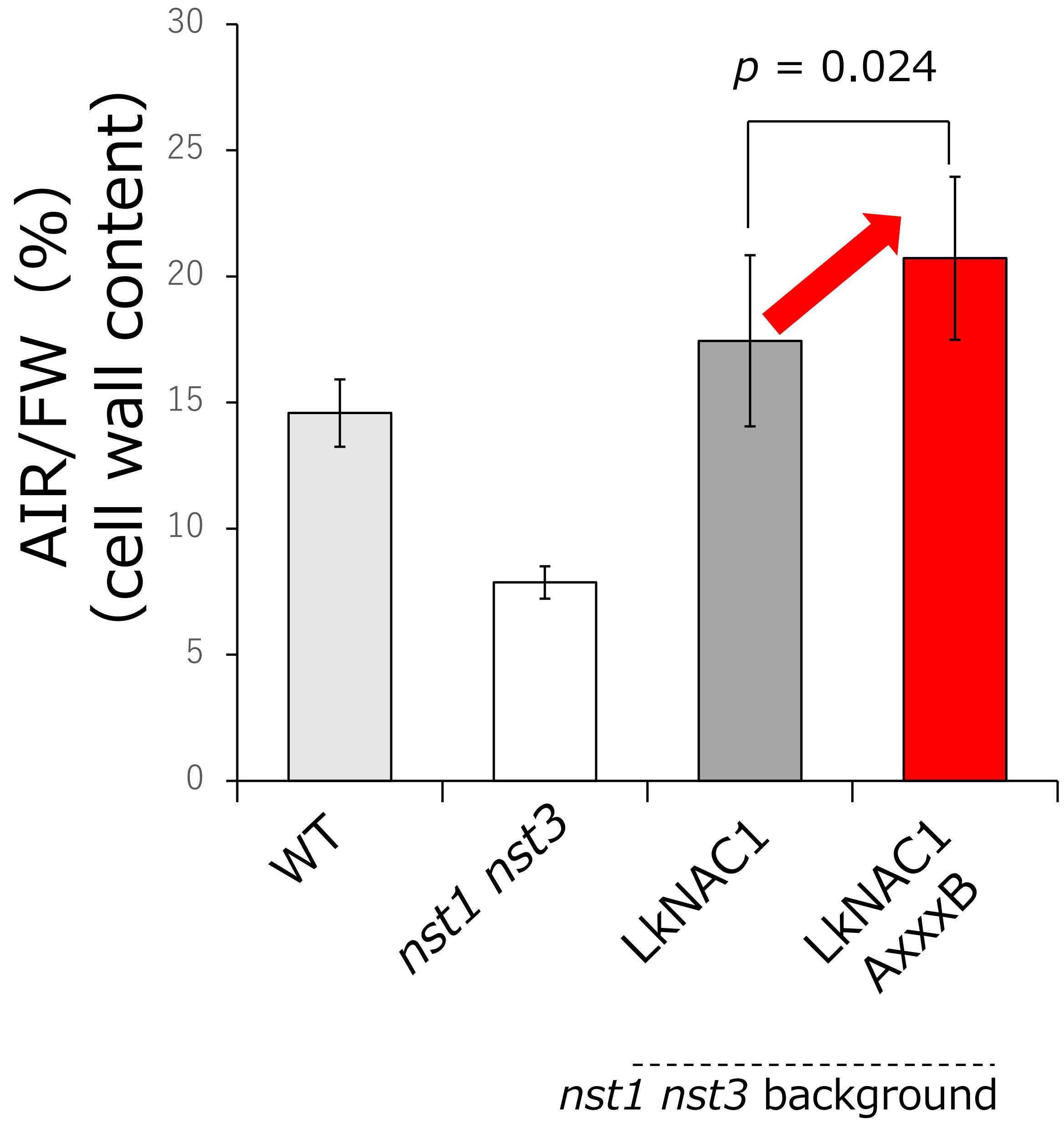
◆ Results of NST transcription factor activity measurements utilizing two reporters.

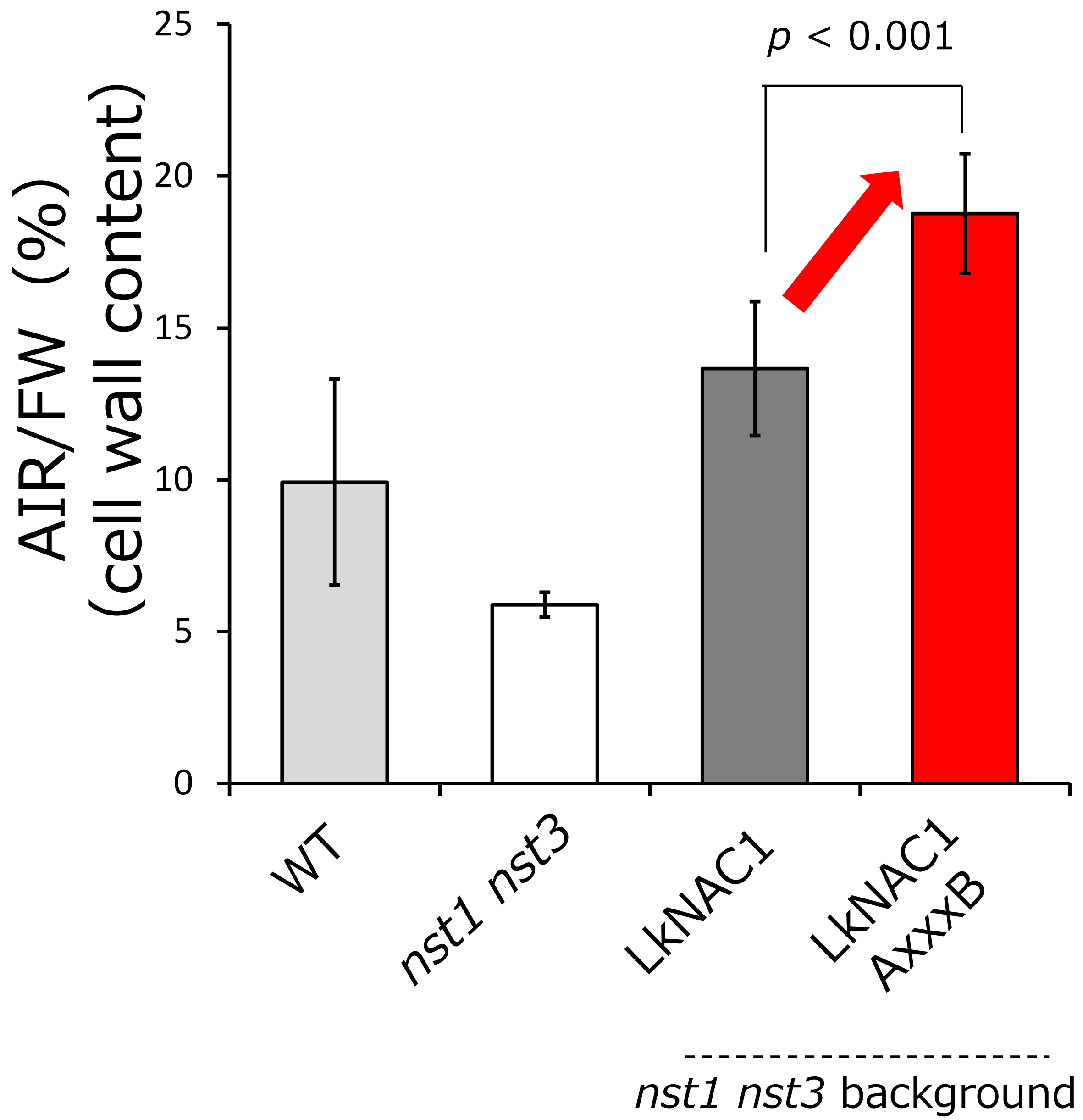


➢ NST転写因子を高活性化する変異を約60カ所同定（産総研ー住友林業BIP）

Wood reinforcement by NST hyperactivation

◆ Cell wall amount of transgenic *Arabidopsis thaliana* for NST transcription factor genes with candidate mutations.





➢ 有望変異が木質増強効果を有することをシロイヌナズナで確認（産総研ー住友林業BIP）

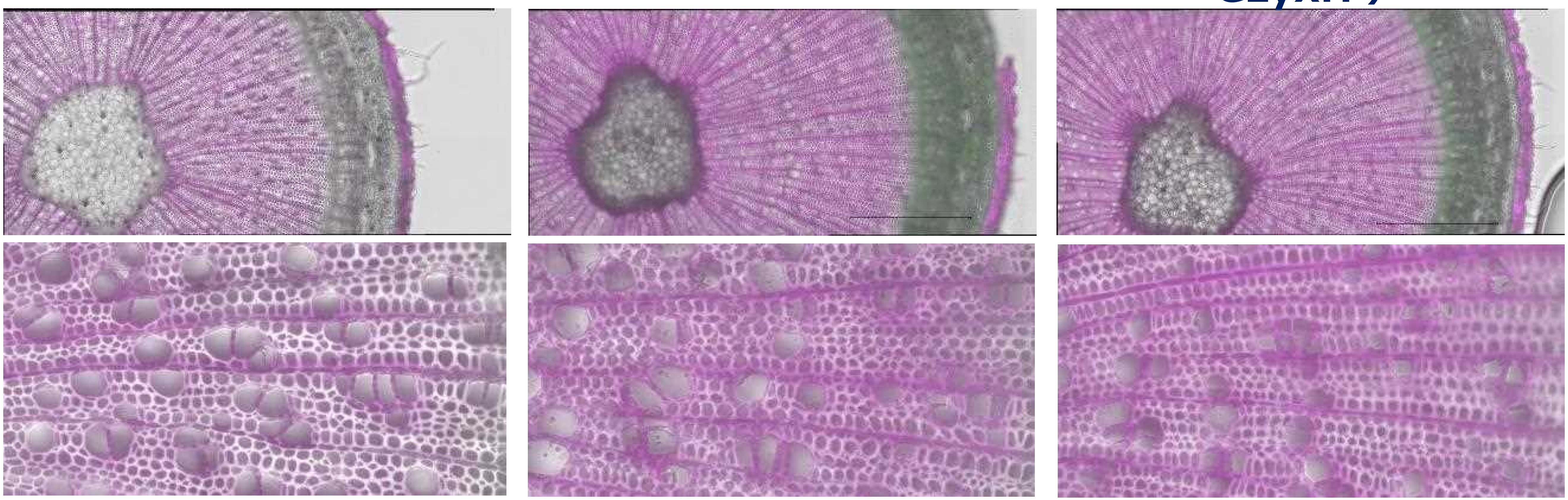
Wood reinforcement by NST hyperactivation

◆ Observation of cross sections of transgenic poplar for NST transcription factor gene with candidate mutations

LkNAC1 introduced

Hyperactive LkNAC1 introduced（AxxxB)

Hyperactive LkNAC1 introduced（AxxxB + GzyxH ）

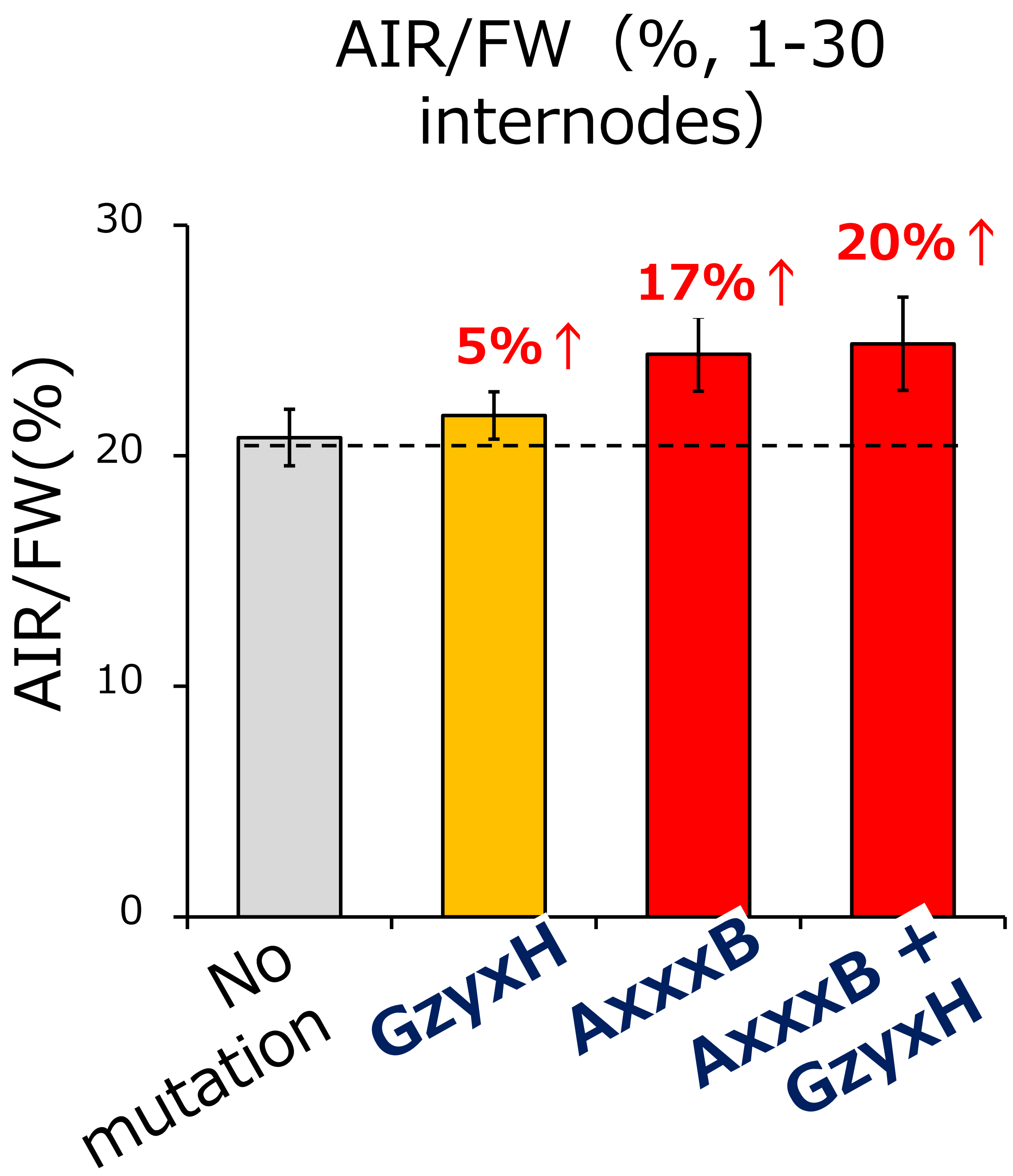


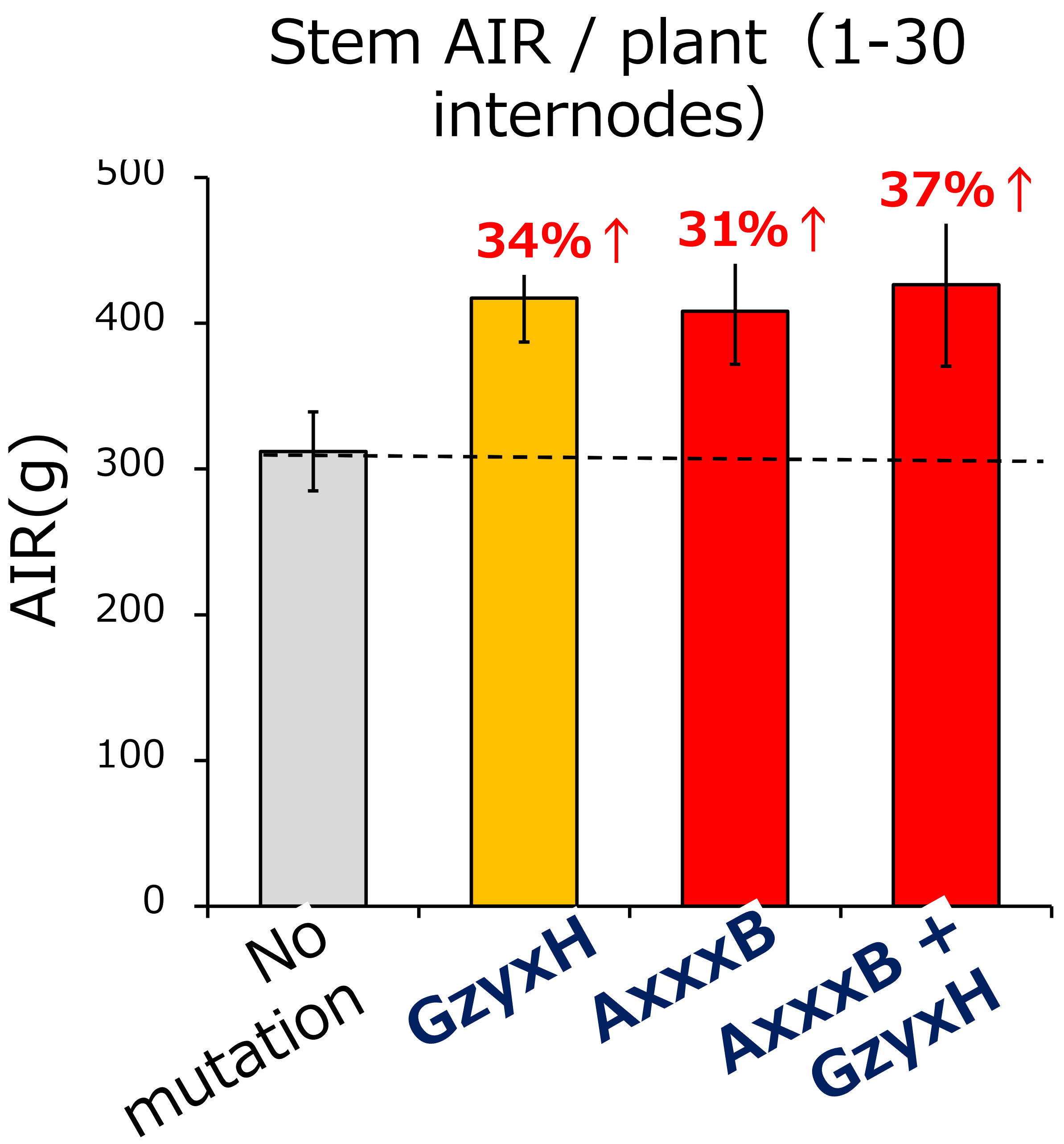
Pinkish color: Lignin autofluorescence under UV irradiation in pseudo color

➢ Candidate mutation has wood-enhancing effect also in poplar

Wood reinforcement by NST hyperactivation

◆ Cell wall amount of transgenic poplar for NST transcription factor genes with candidate mutations.





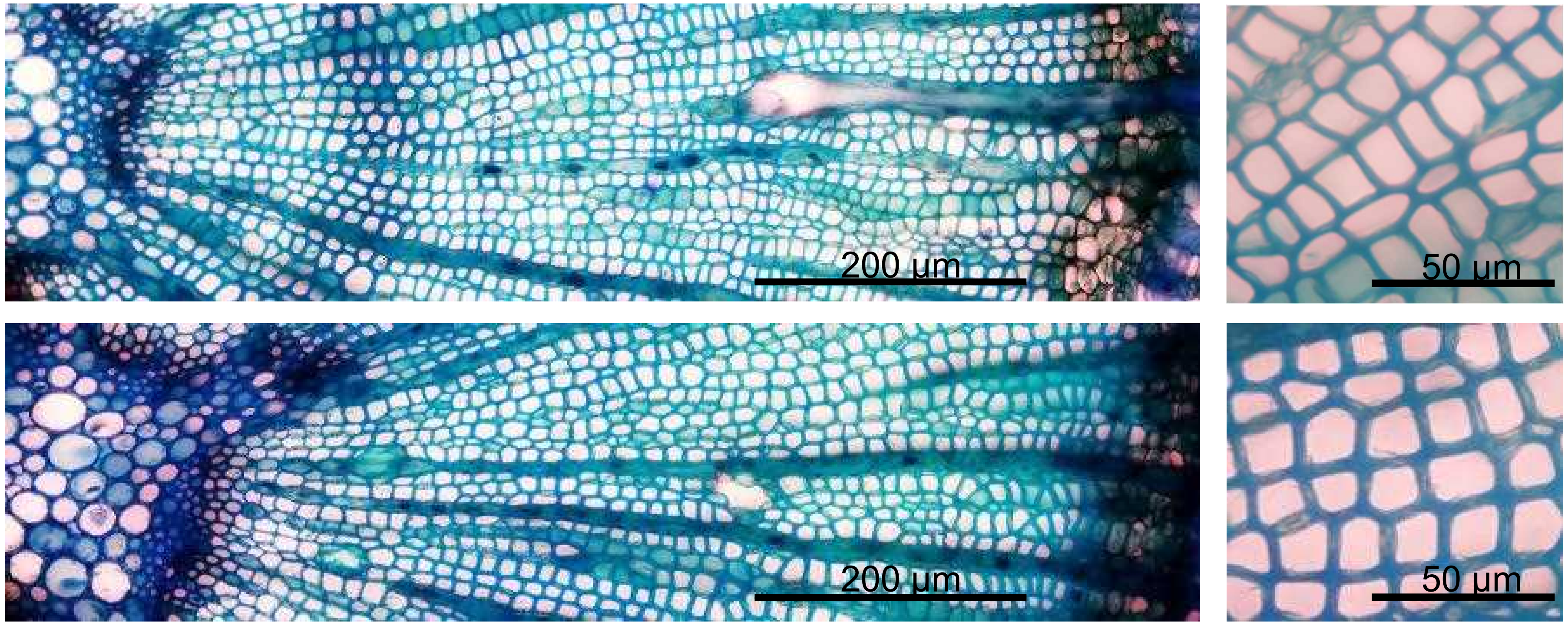
➢ Candidate mutation has wood-enhancing effect also in poplar

Wood reinforcement by NST hyperactivation

◆ Observation of cross sections of transgenic larch for NST transcription factor gene with candidate mutations

LkNAC1 introduced

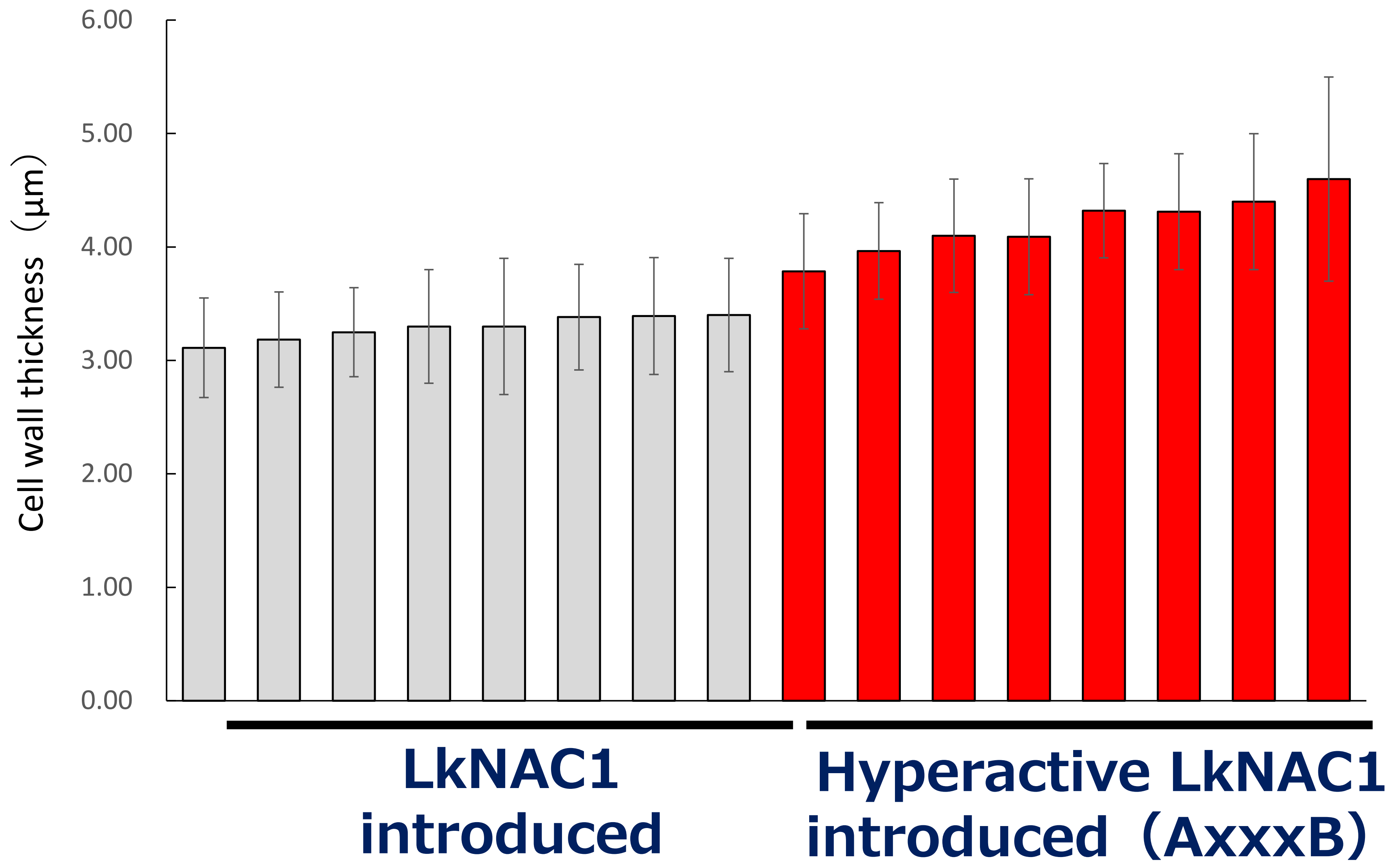
Hyperactive LkNAC1 introduced (AxxxB)



➢ Candidate mutation has wood-enhancing effect also in larch

Wood reinforcement by NST hyperactivation

◆ Larch cell wall thickness with NST transcription factor genes with candidate mutations.



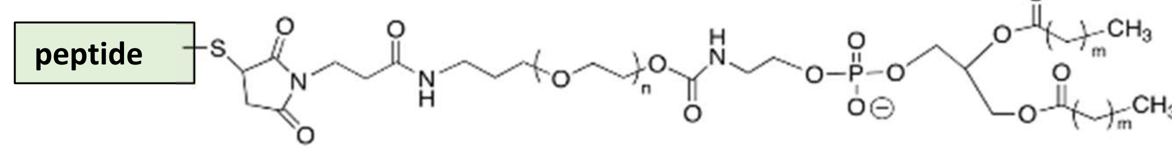
➢ Candidate mutation has wood-enhancing effect also in larix

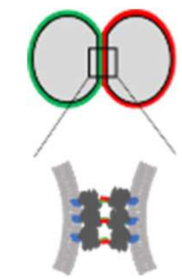
Three major technologies

- Gene optimization
- Super-distant hybrids
- Symbiotic micro-organisms

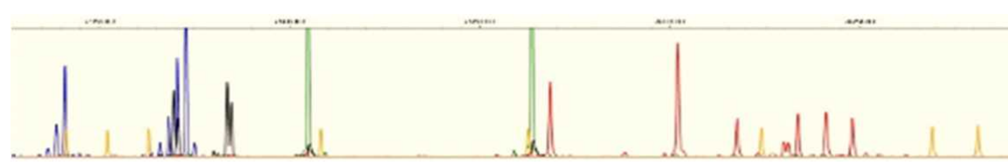
Development of new crops and biomass plants through distant hybridization

1. Development of efficient cell fusion technology using peptide PEG-lipid conjugates

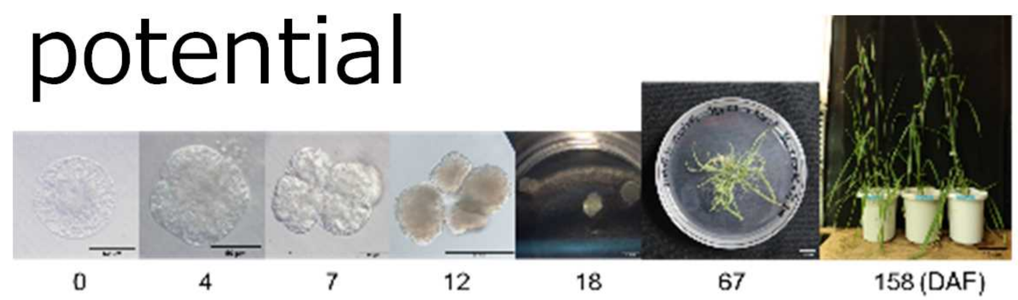


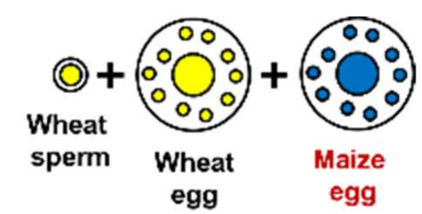


2. New hybrid Eucalyptus with expanded suitable cultivation areas

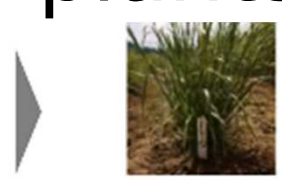


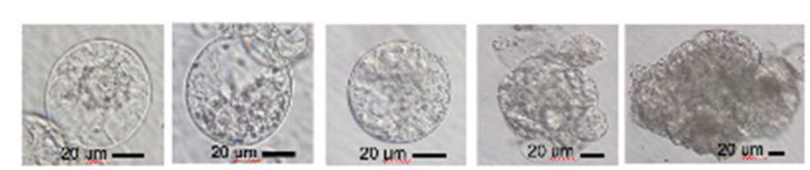
3. Creation of new rice and wheat plants and verification of their potential





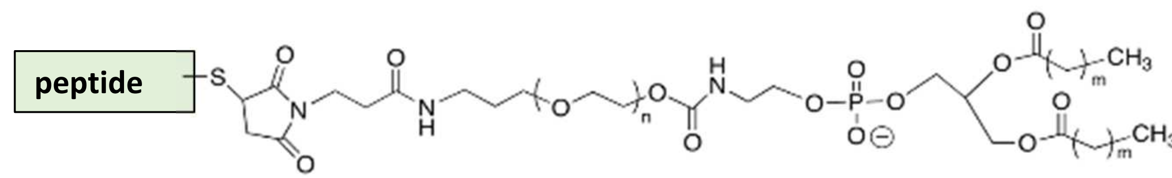
4. Creation of new biomass plants and verification of their potential

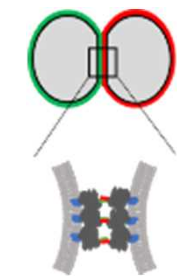




Development of new crops and biomass plants through distant hybridization

1. Development of efficient cell fusion technology using peptide PEG-lipid conjugates





2. New hybrid Eucalyptus with expanded suitable cultivation areas

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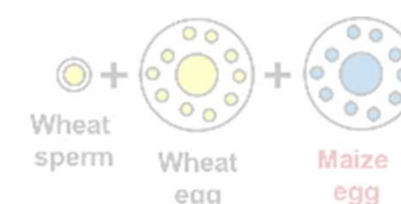
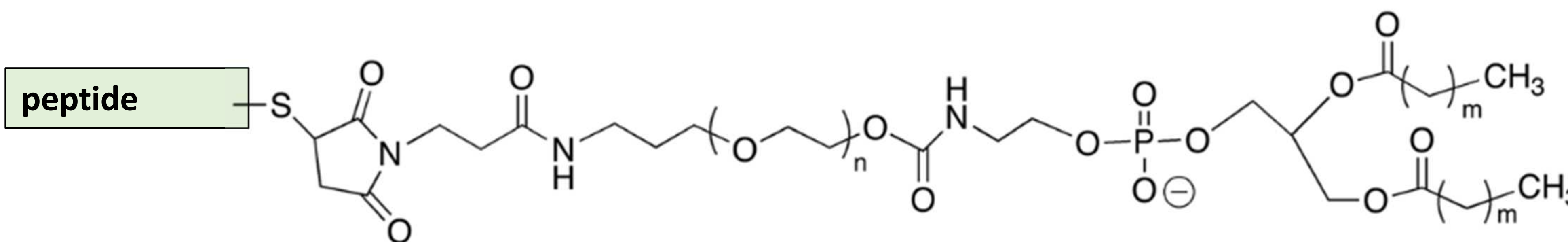


4. Creation of new biomass plants and verification of their potential

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

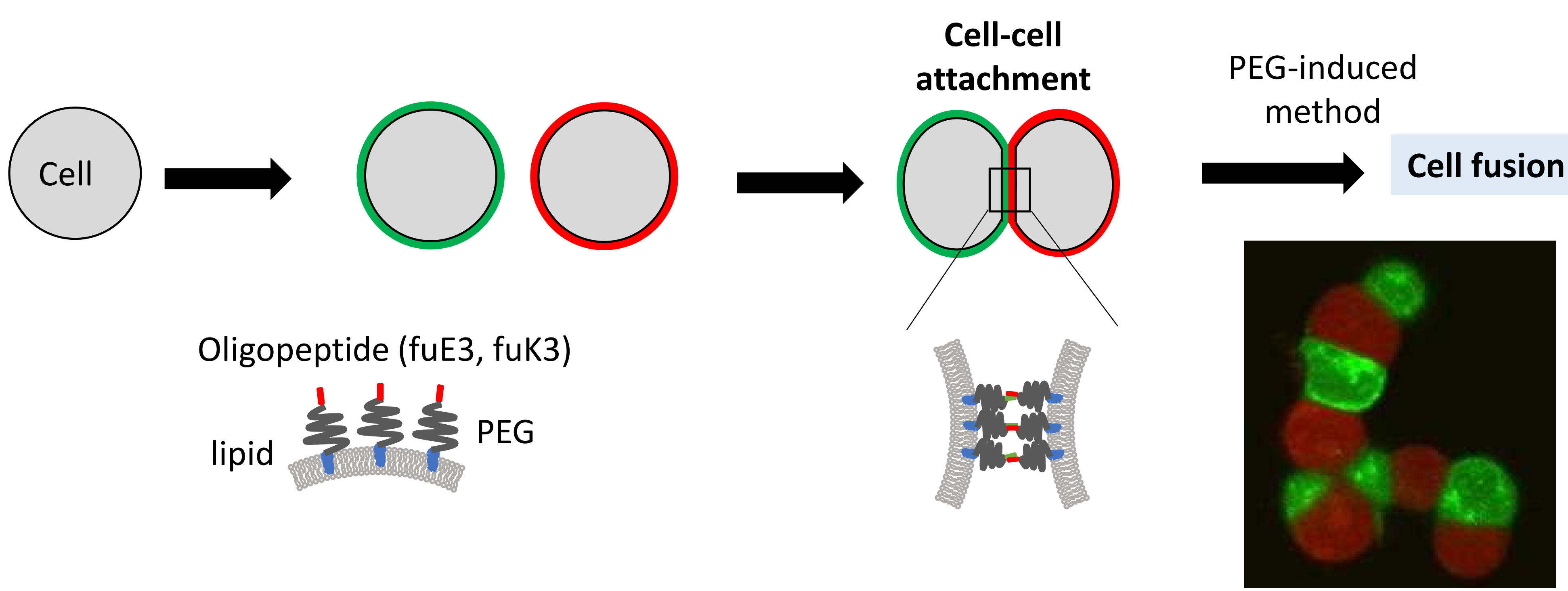


PEG: 5k, 40kDa

Lipids : C9 – C18

Peptide:
EIAALEKEIAALEKEIAALEKGGGC (fuE3)
KIAALKEKIAALKEKIAALKEGGGC (fuK3)

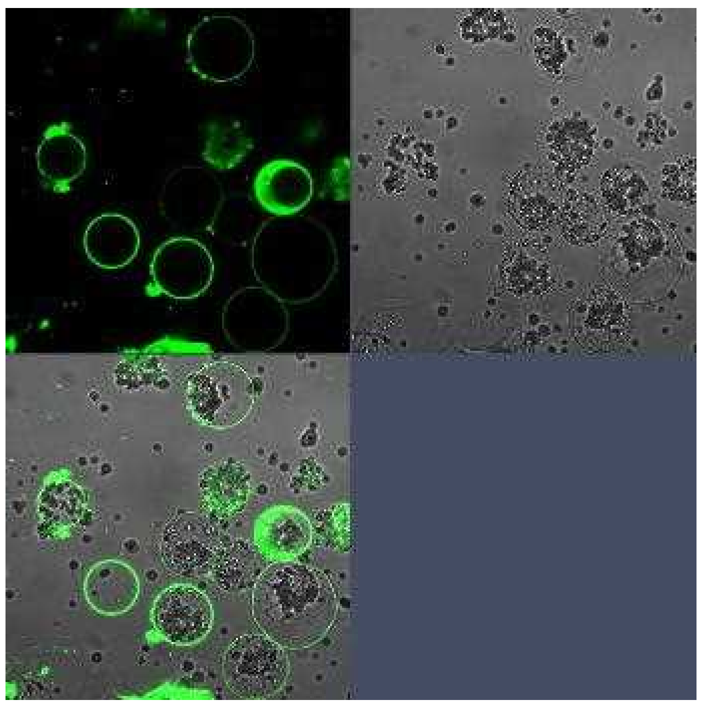
} These attract each other

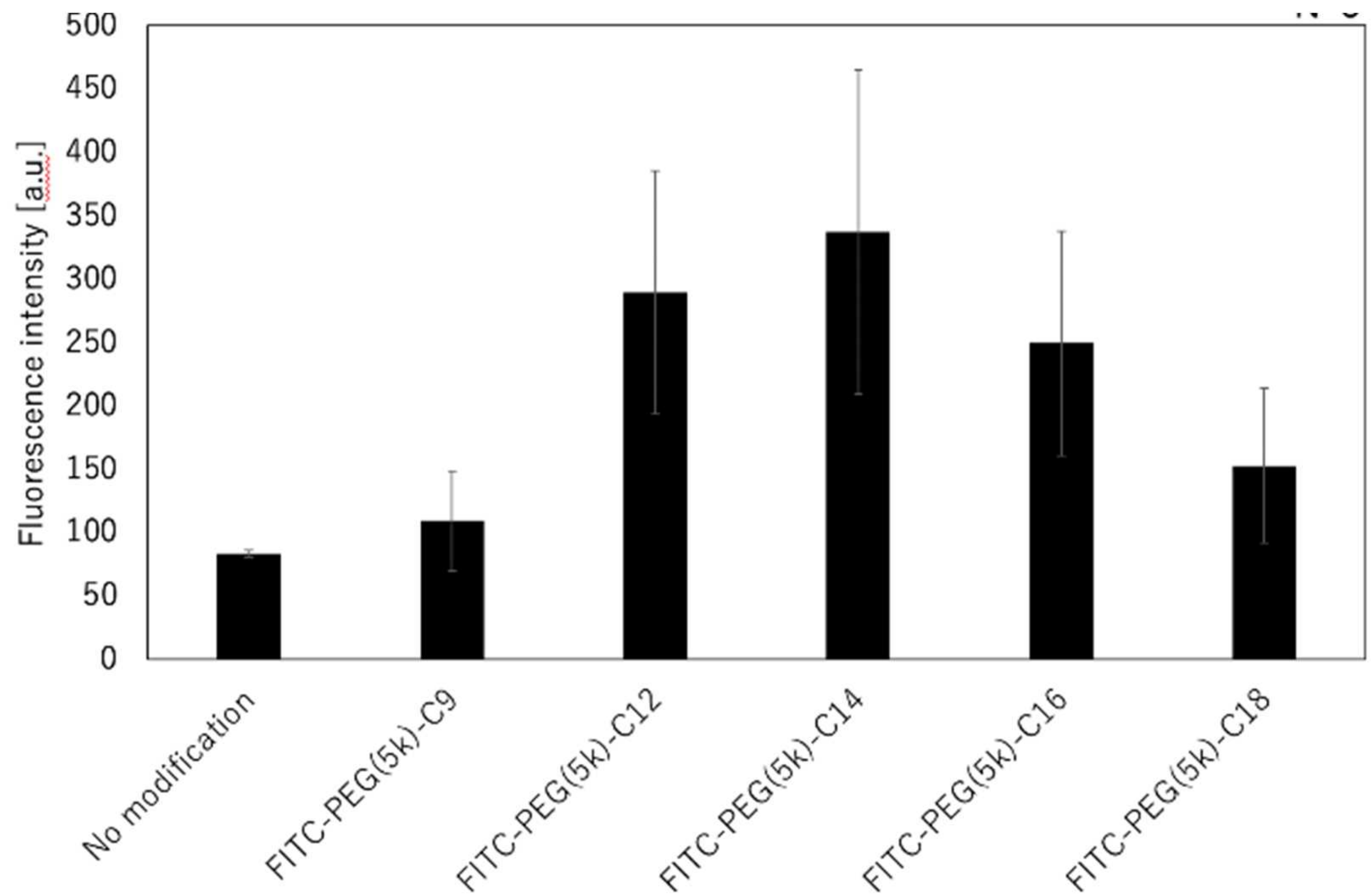


Development of efficient cell fusion technology using peptide PEG-lipid conjugates

◆ The length of carbon chain was optimized in rice Oc cells.

Rice Oc cell, FITC-5k-C14

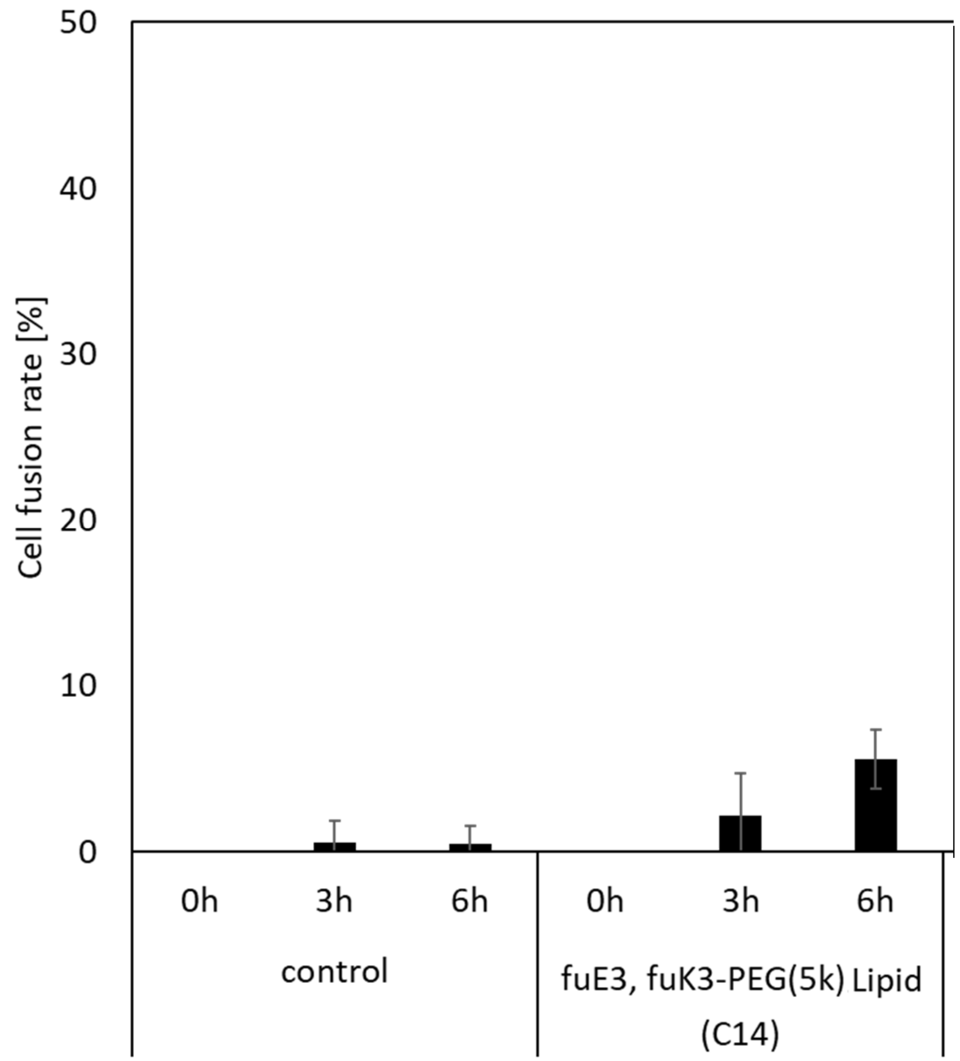




➢ Determine optimal structure for plant cells

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

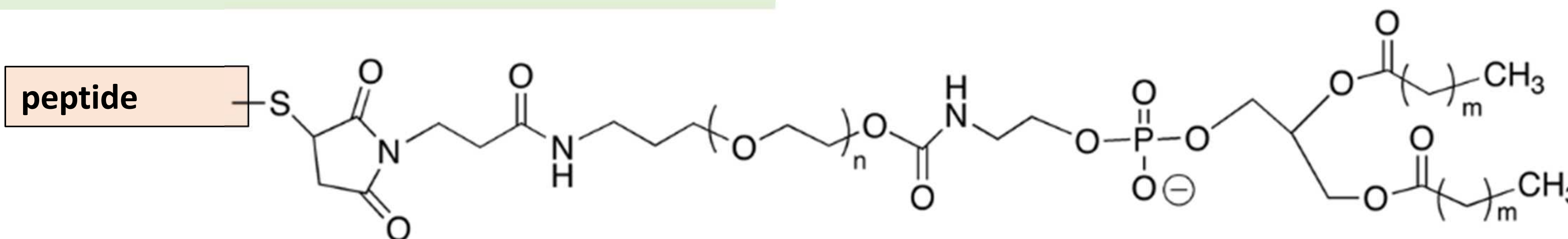
◆ Examination in rice protoplast



➢ Improvement of cell fusion was observed but the effect was limited

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

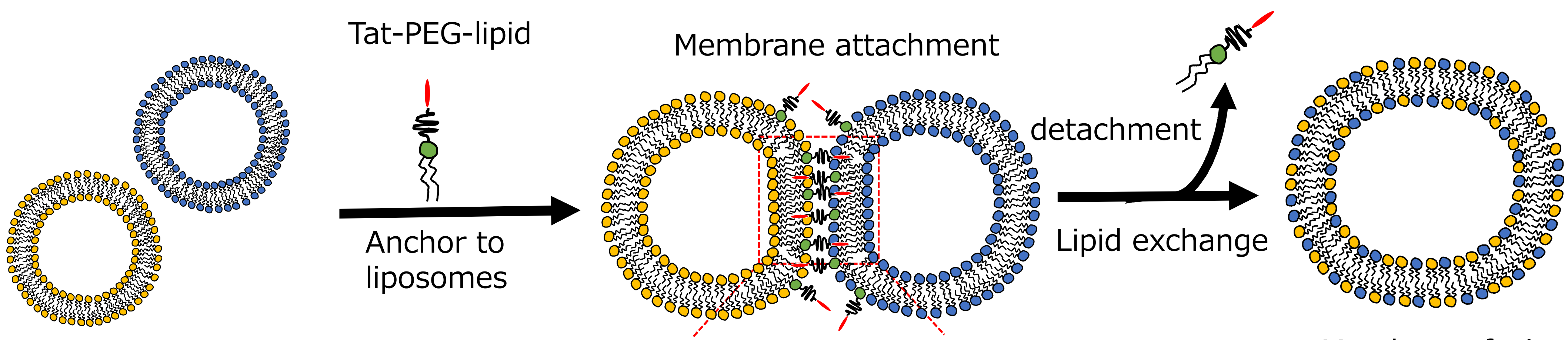
◆ Examination of new material



PEG: 5k, 40kDa

Lipids : C9 – C18

Peptide:
YGRKKRRQRRR (Tat: Trans-Activator of Transcription Protein)… Membrane-permeable peptides but with no selectivity



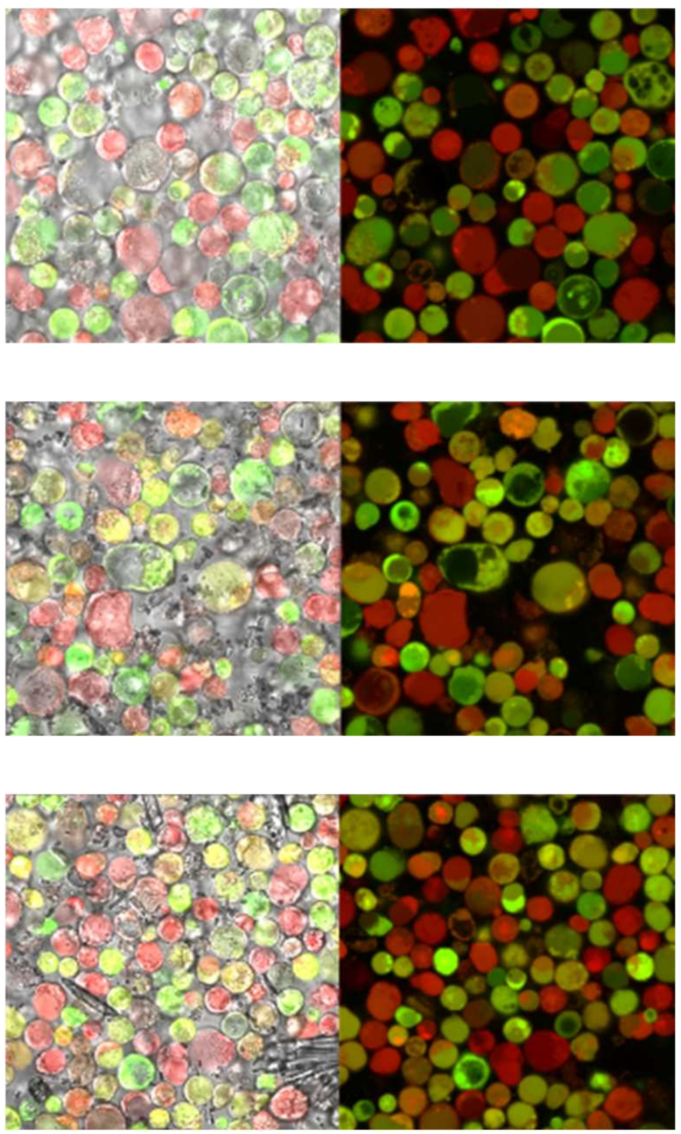
Development of efficient cell fusion technology using peptide PEG-lipid conjugates

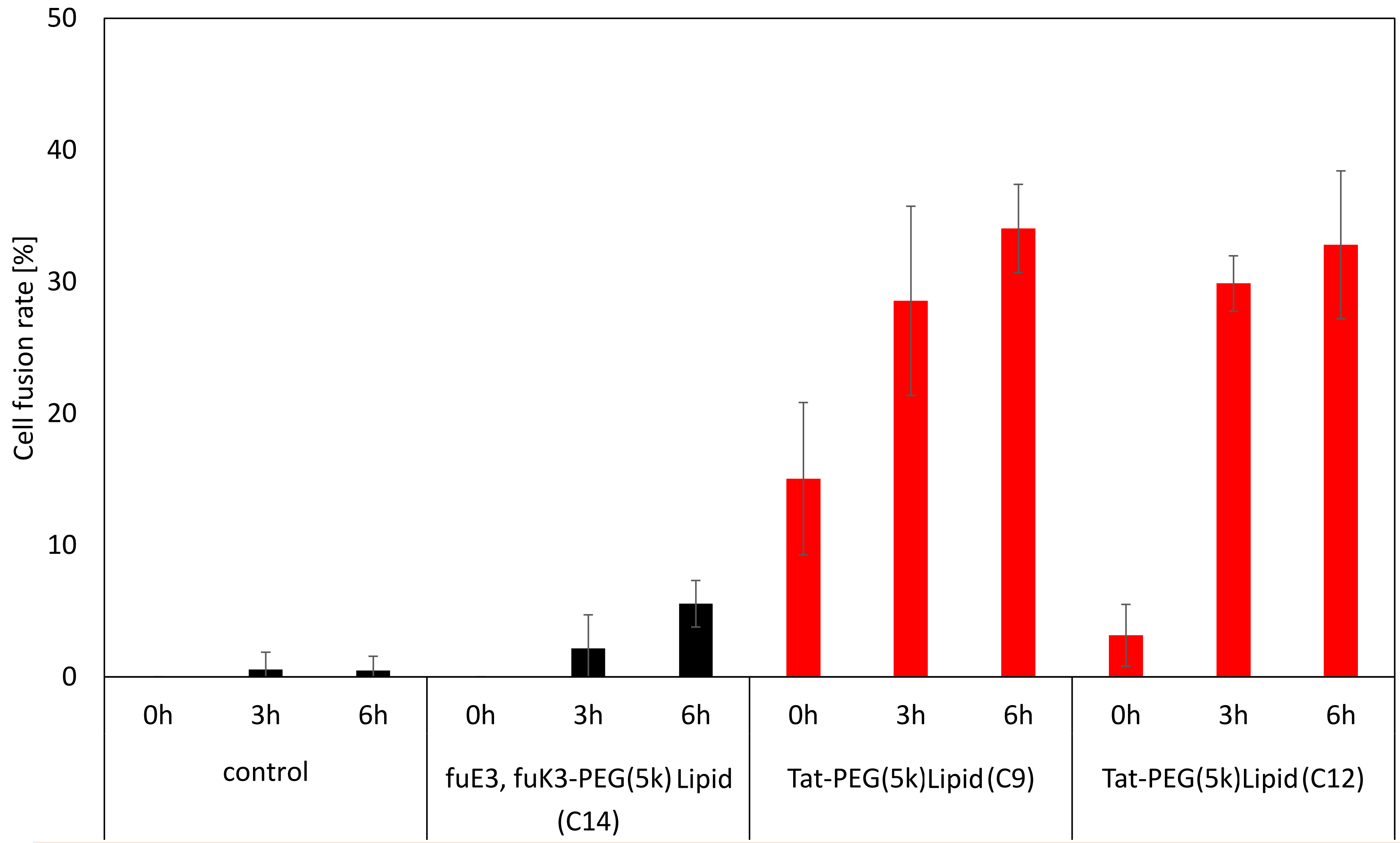
◆ Examination of new material in rice protoplast

0h

3h

6h

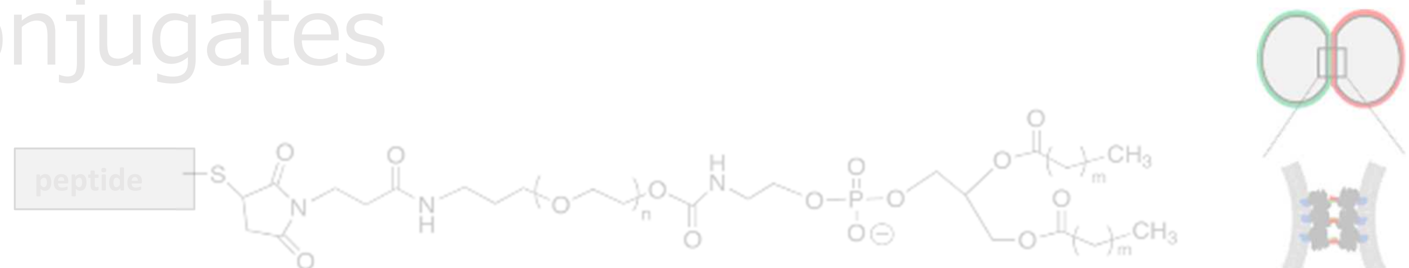




➢ The use of new materials lost selectivity but showed significant improvement

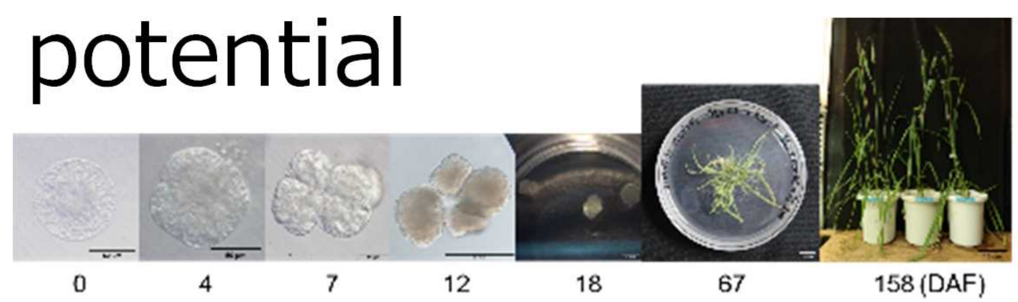
Development of new crops and biomass plants through distant hybridization

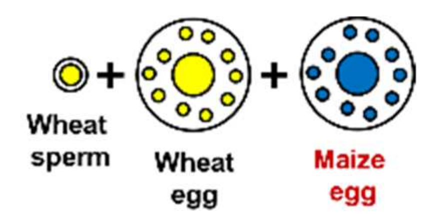
1． Development of efficient cell fusion technology using peptide PEG-lipid conjugates



2． New hybrid Eucalyptus with expanded suitable cultivation areas

3． Creation of new rice and wheat plants and verification of their potential

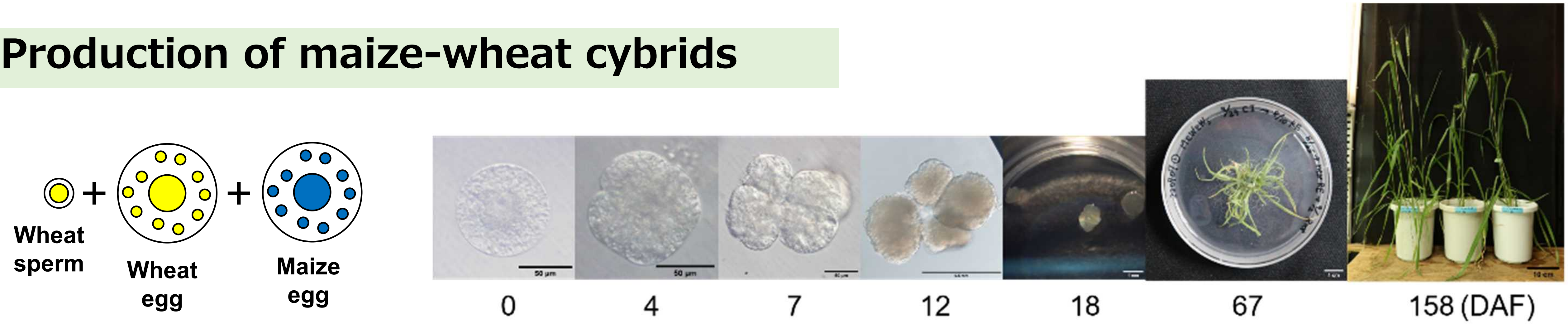




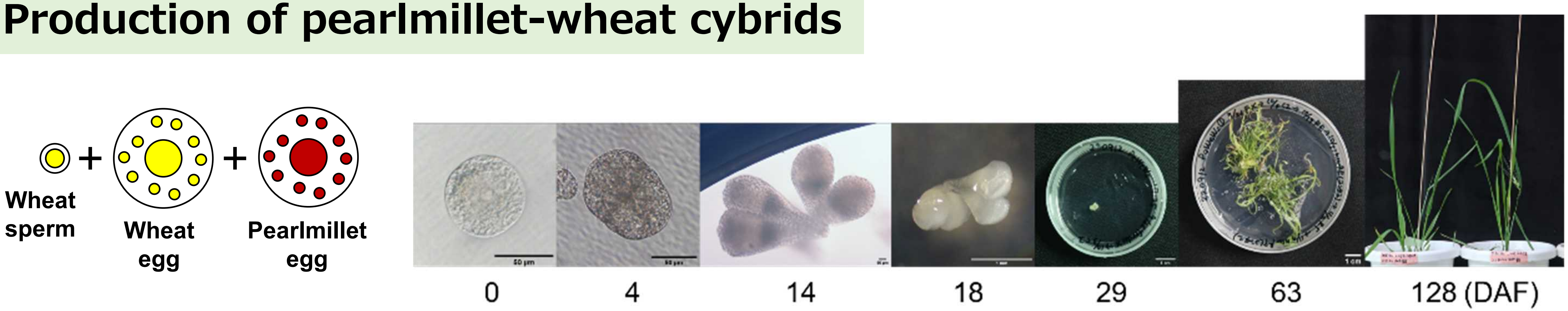
4． Creation of new biomass plants and verification of their potential

Creation of new rice and wheat plants and verification of their potential

◆ Production of maize-wheat cybrids



◆ Production of pearlmillet-wheat cybrids



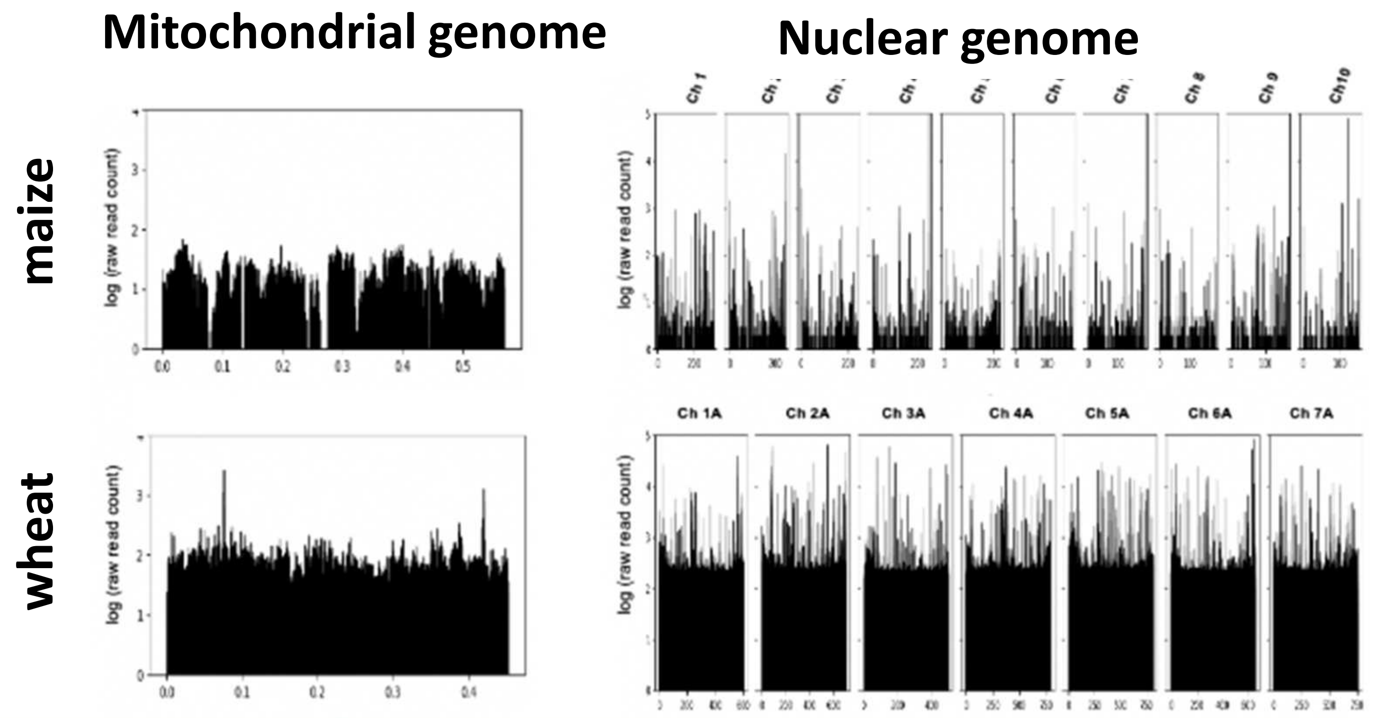
➢ Basic elemental technologies have been established.

Creation of new rice and wheat plants and verification of their potential

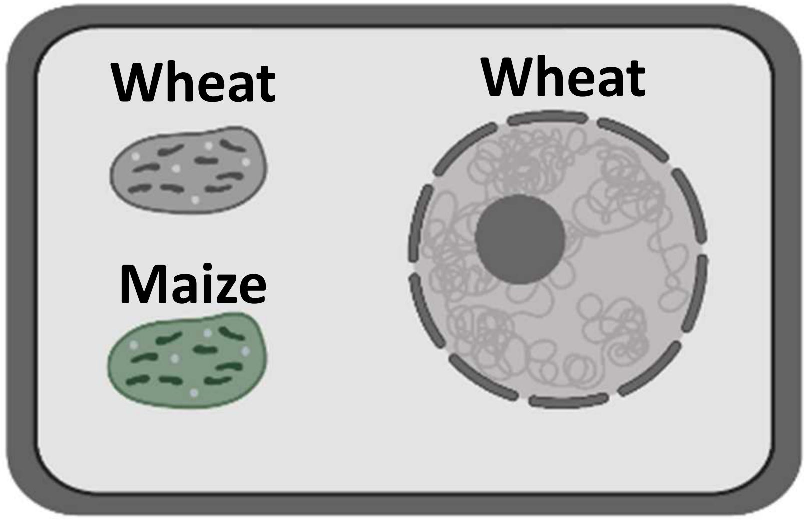
◆ Genome composition of maize-wheat cybrids

・Nuclear genome: almost wheat
・Mitochondria genome: Maize + wheat

Mapping profiles of sequence reads from maize-wheat cybrid genome Mitochondrial genome



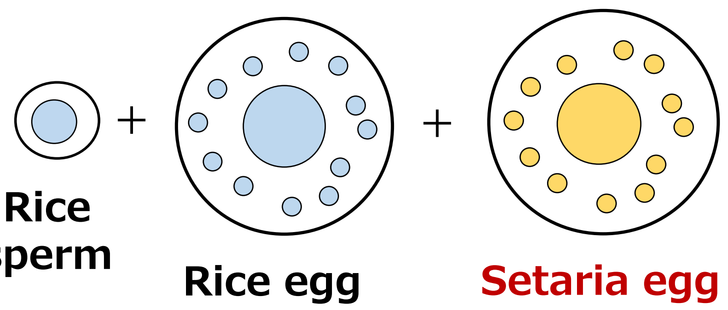
Maize-wheat cybrid

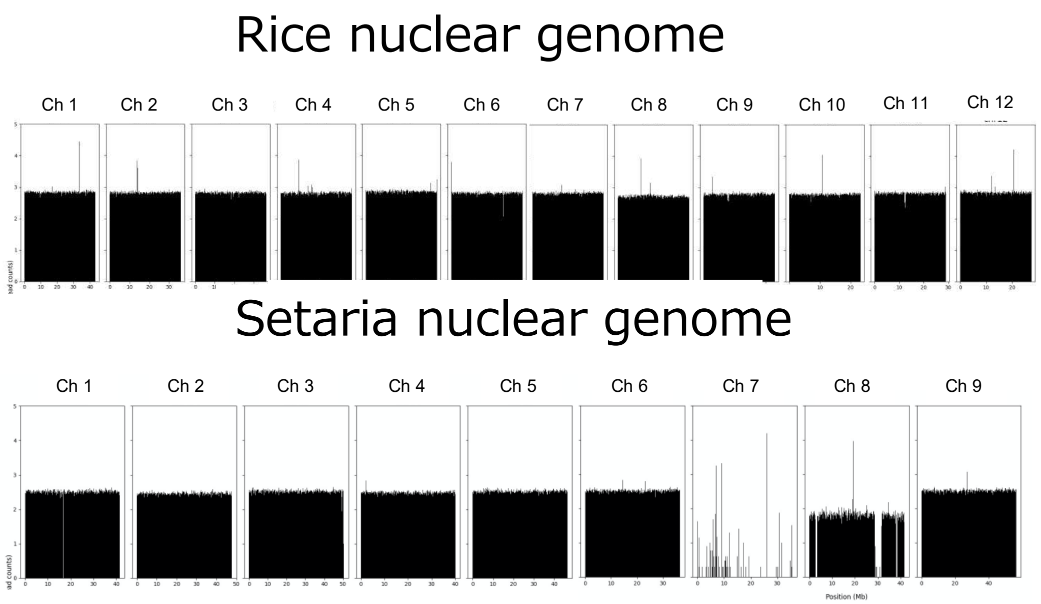


➢ Confirmed the intended cybrid is created.

Creation of new rice and wheat plants and verification of their potential

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





- ➢ The elemental technology to create up to a fusion callus could be established.

Creation of new rice and wheat plants and verification of their potential

《Future plans》

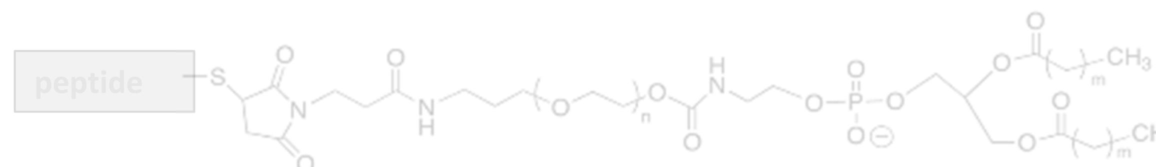
- **Analysis of nuclear and cytoplasmic genome abundance in hybrid plants**
  - Genome analysis
    - ・Maize × Wheat： Done for 6 lines
    - ・Pearlmillet × Wheat： Underway for 11 lines
      raw data obtained
  - FISH analysis

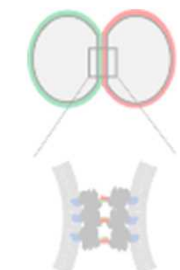
- **Examination of agronomic and physiological traits**
  - Maize-wheat seeds, propagated
  - Photosynthetic potential and mode
  - Characteristics of cell wall

- **Rice-C4 Plant callus redifferentiation and determination of genome composition**

Development of new crops and biomass plants through distant hybridization

1． Development of efficient cell fusion technology using peptide PEG-lipid conjugates





2． New hybrid Eucalyptus with expanded suitable cultivation areas

3． Creation of new rice and wheat plants and verification of their potential

4． Creation of new biomass plants and verification of their potential

Creation of new biomass plants and verification of their potential

◆Erianthus-Miscanthus hybrid via pollination

Erianthus ♀

×

Miscanthus ♂

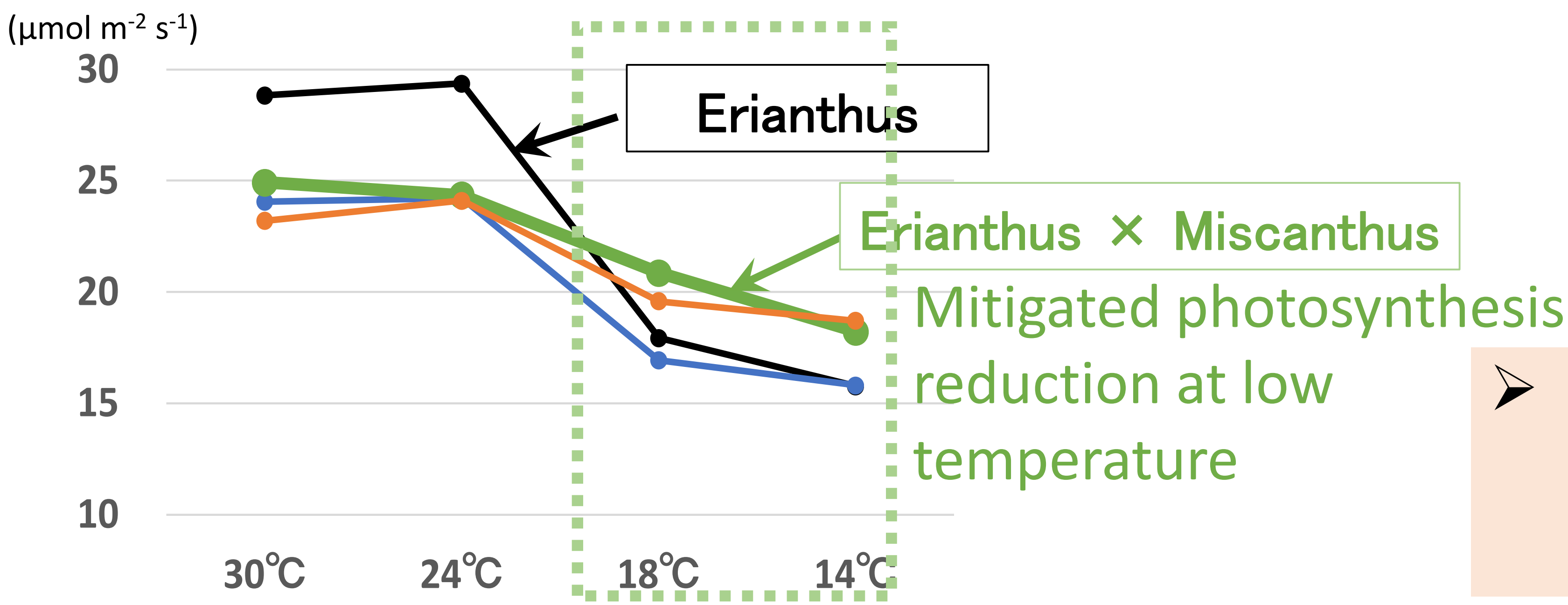
Hybrid (1 line)

◆ Growth and overwintering in northern region

Erianthus X Miscanthus

Erianthus

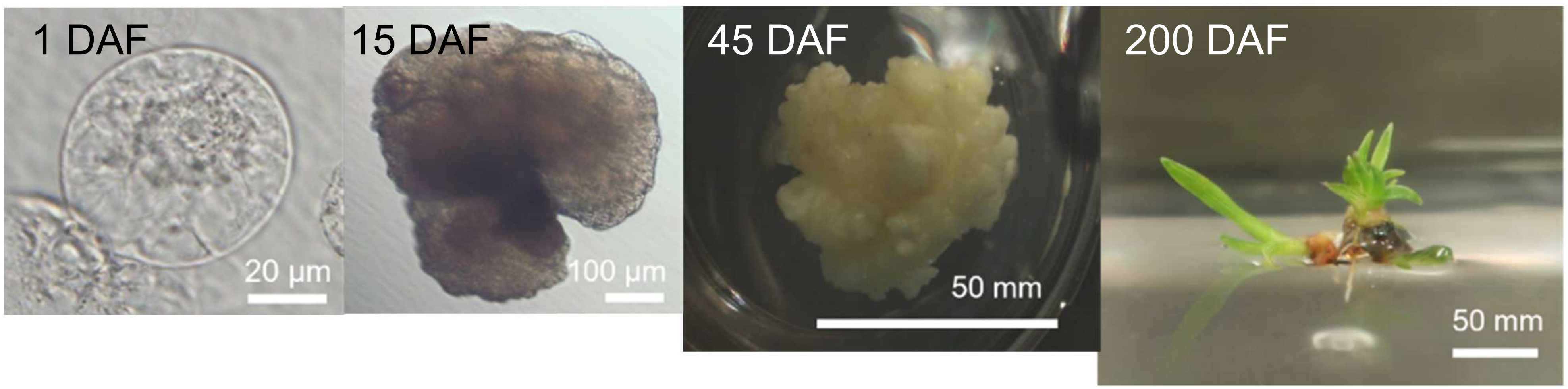
◆ Photosynthetic activity at various temperatures



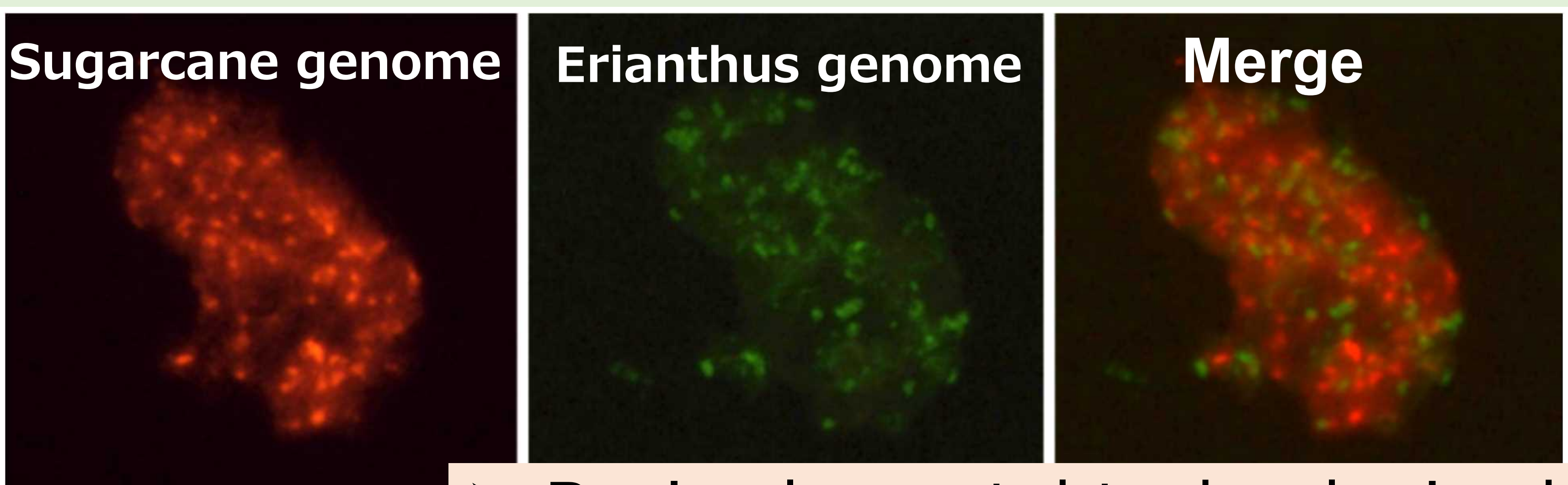
➢ New biomass plant (Erianthus x Miscanthus sinensis) shows enhanced cold tolerance

Creation of new biomass plants and verification of their potential

◆ **Erianthus × sugarcane by in vitro fertilization**



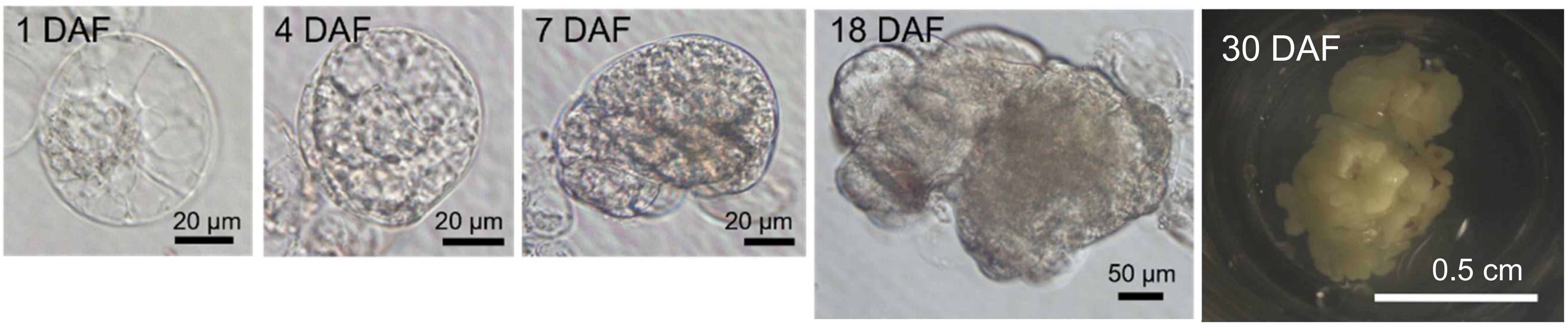
◆ **Confirmation that Elianthus x sugarcane has both genomes.**

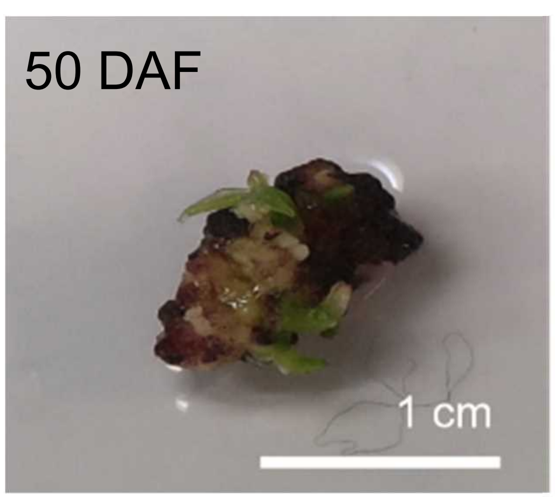


➢ Basic elemental technologies have been established.

Creation of new biomass plants and verification of their potential

◆Possible Erianthus-Sugarcane/Miscanthu hybrid by in vitro fertilization





- ➢ Basic elemental technologies have been established.

Creation of new biomass plants and verification of their potential

《 Future plans 》

- **Trait evaluation for cold tolerance, biomass production capacity, and photosynthetic potential**
  - Cultivation trials to evaluate cold tolerance, biomass traits, etc. of hybrids of Erianthus x Miscanthus will be conducted in Ishigaki Island, Tottori and Akita.

- **Propagation and basic growth characterization of Erianthus-Miscanthus hybrid and possible Erianthus-Sugarcane/Miscanthu hybrid**

Three major technologies

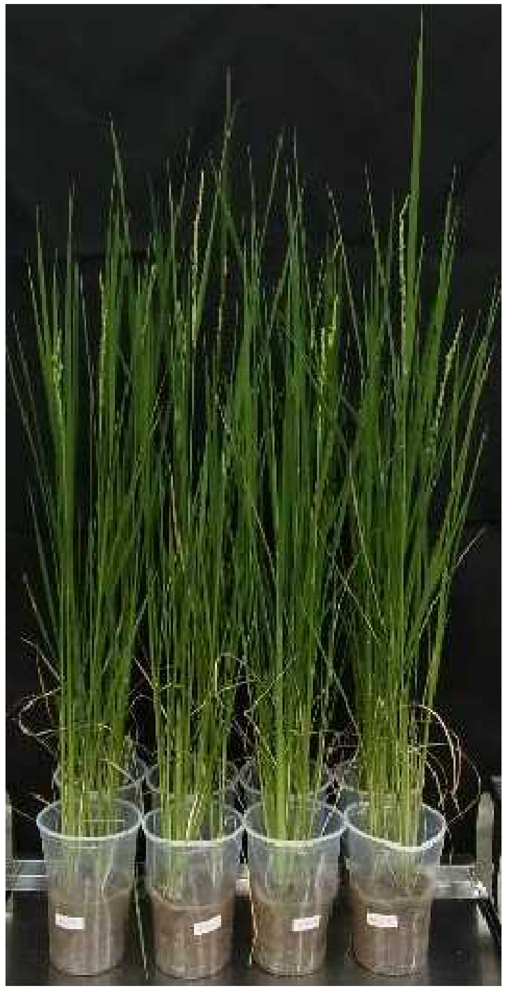
- Gene optimization
- Super-distant hybrids
- Symbiotic micro-organisms

Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

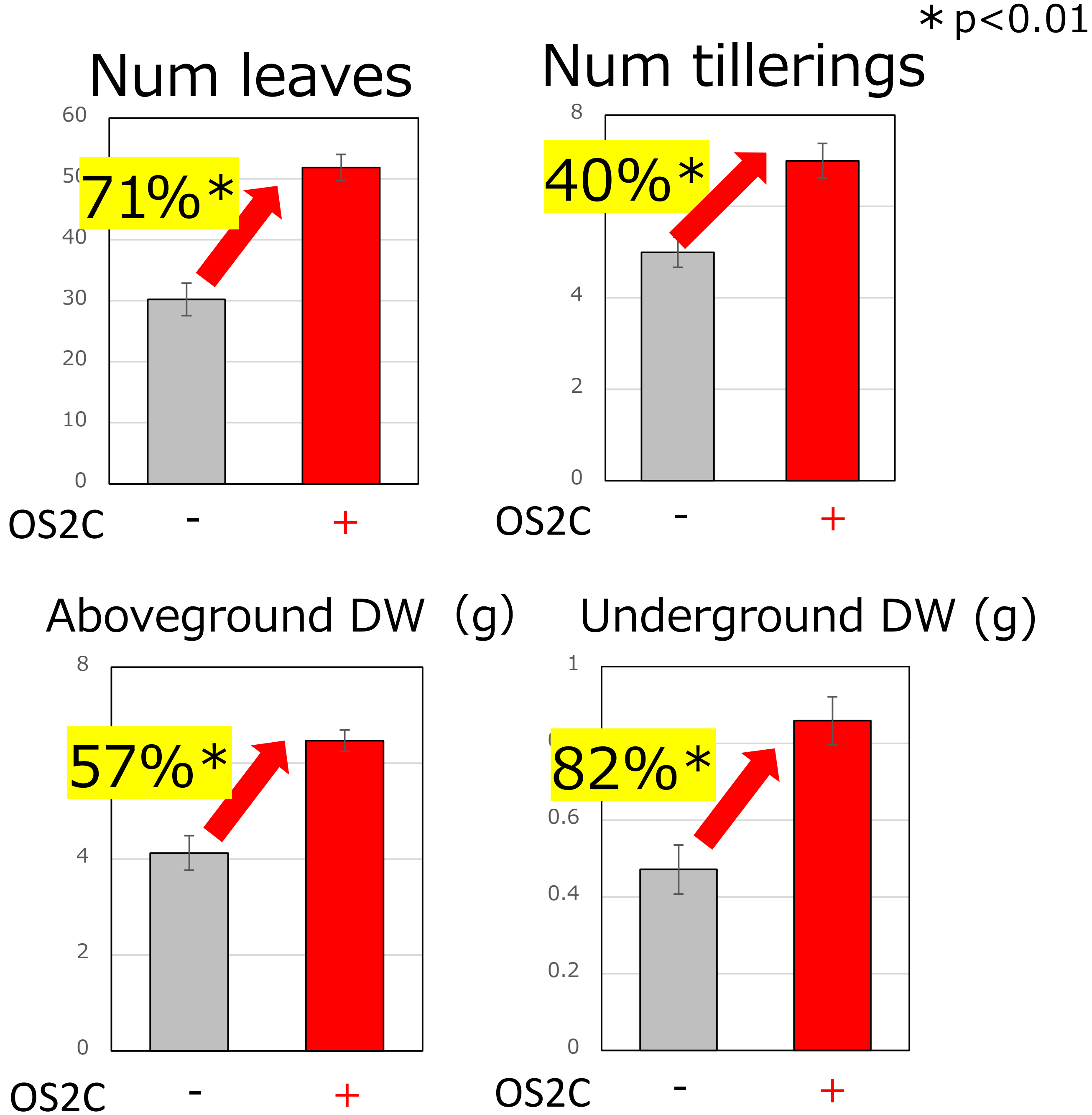
◆Growth promotion in rice (vegetative phase)

After two months（N=8）

No inoculation



OS2C inoculated



➢ Clear growth-promoting effect was confirmed in rice

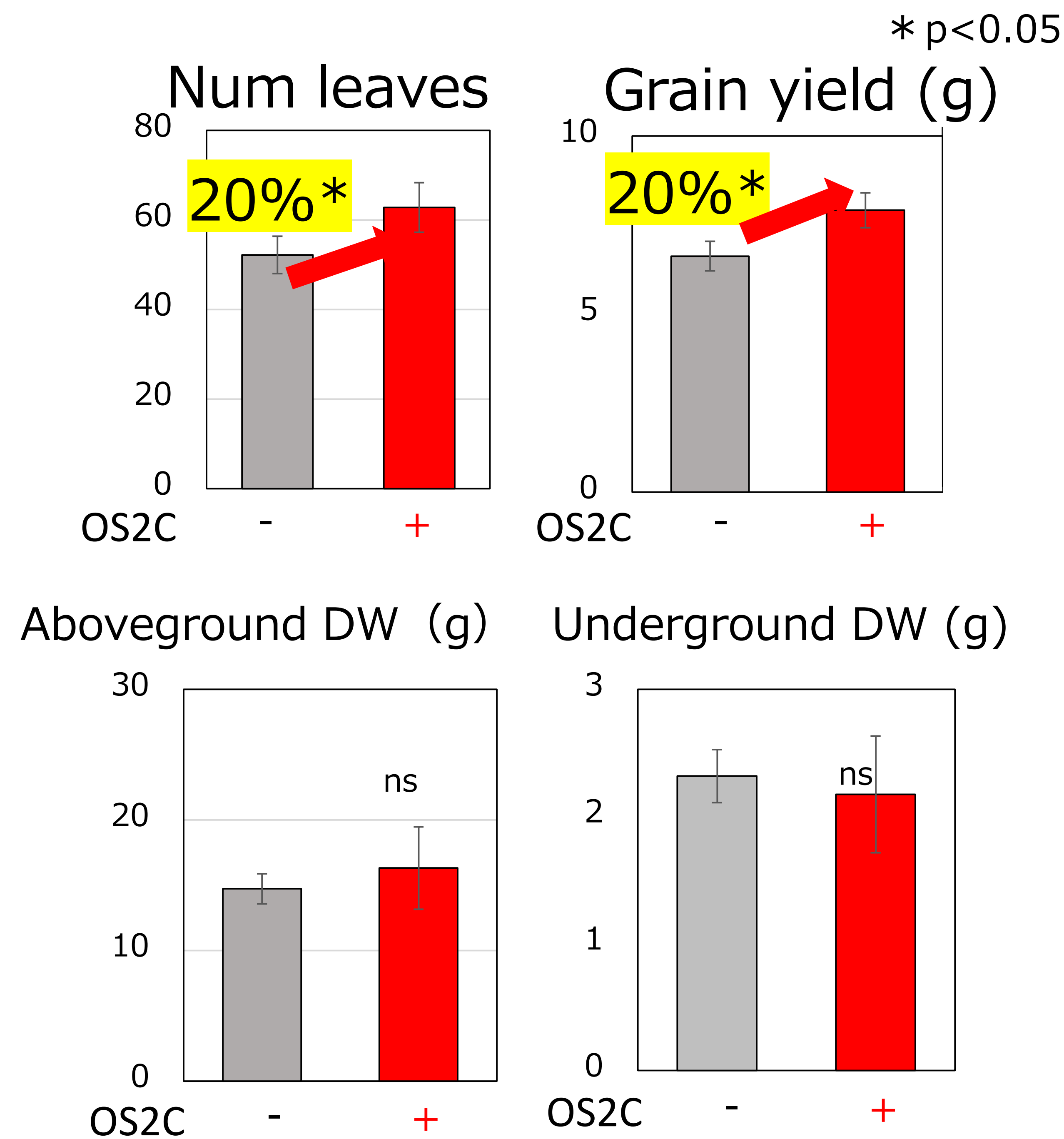
Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

◆Growth promotion in rice (harvest stage)

After 5 monts (N=5 [10 individuals])

No inoculation

OS2C inoculated

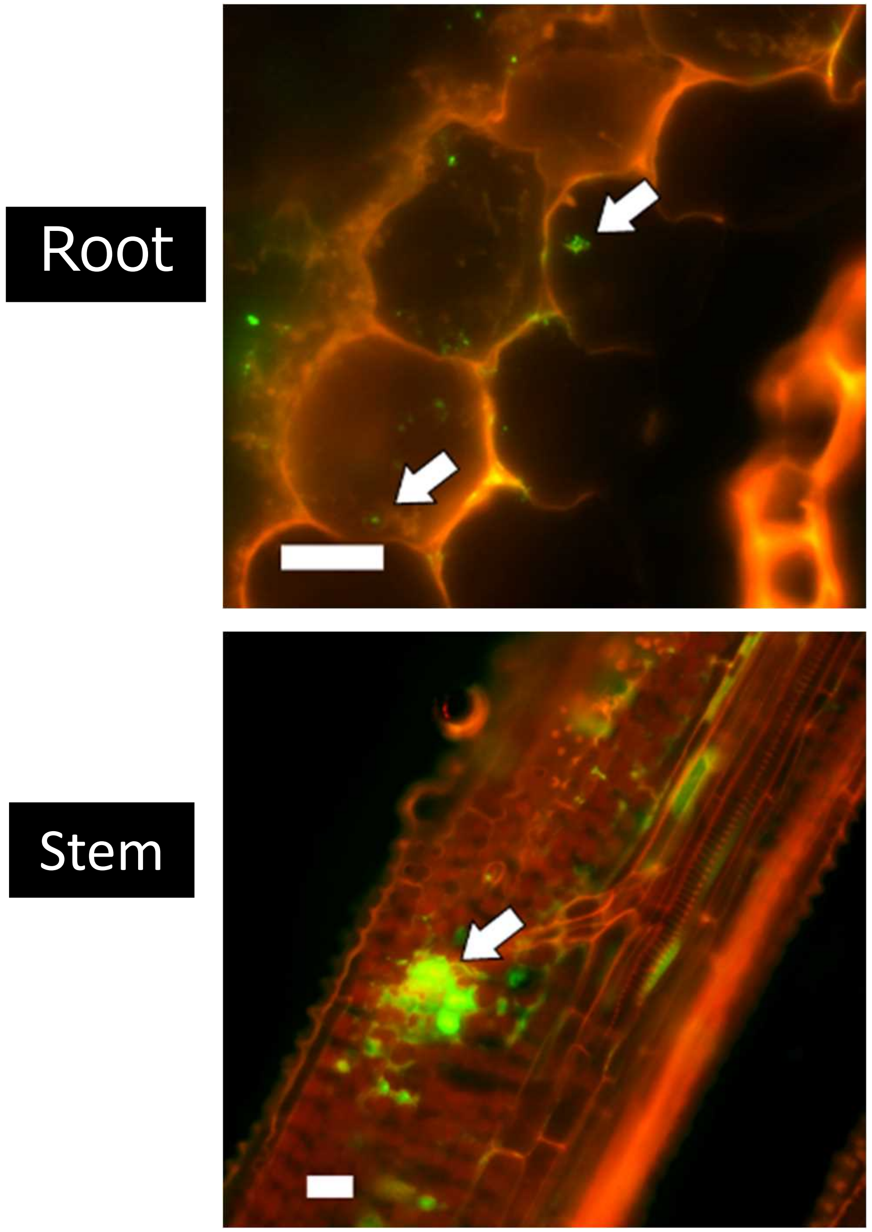


➢ Confirmation that rice grain yields increase

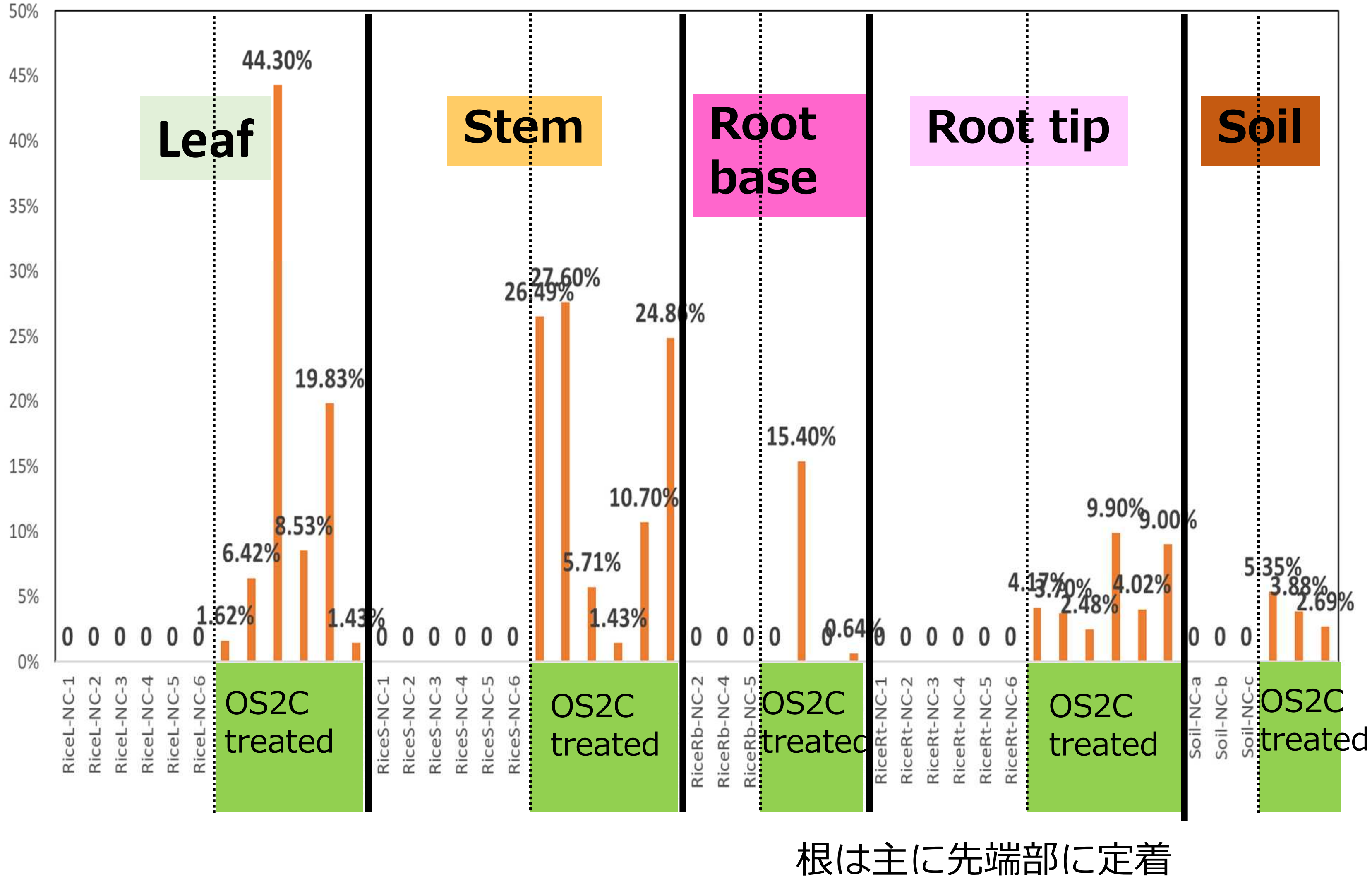
Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

◆ Confirmation of the survival of treated bacteria

FISH analysis



Occupancy of OS2C strain (16SrRNA gene sequence) in each sample



➤ Confirmation of treated bacteria on plants grown for approximately 3 months

Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

＊p<0.05

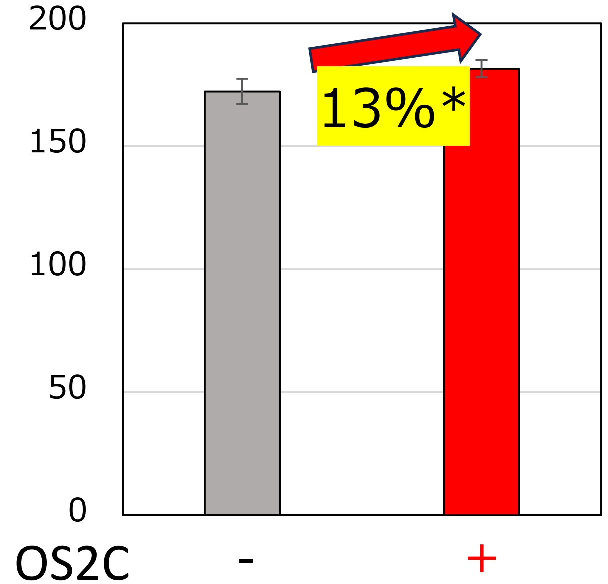
◆Growth promotion in Erianthus

After 4 months（N=6）

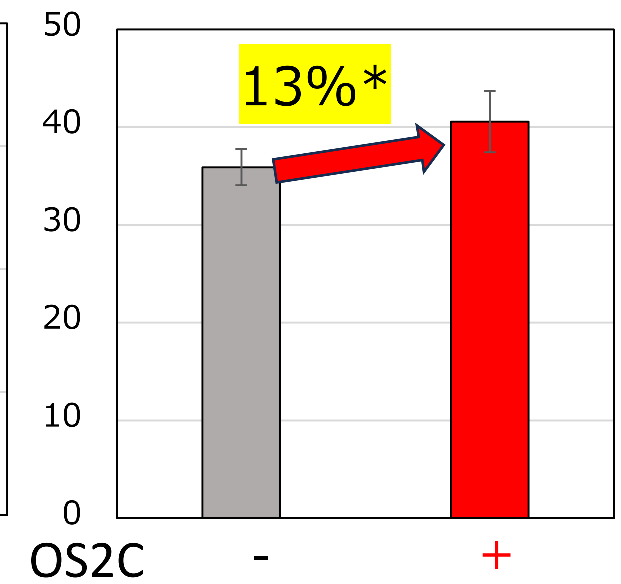
No inoculation

OS2C inoculated

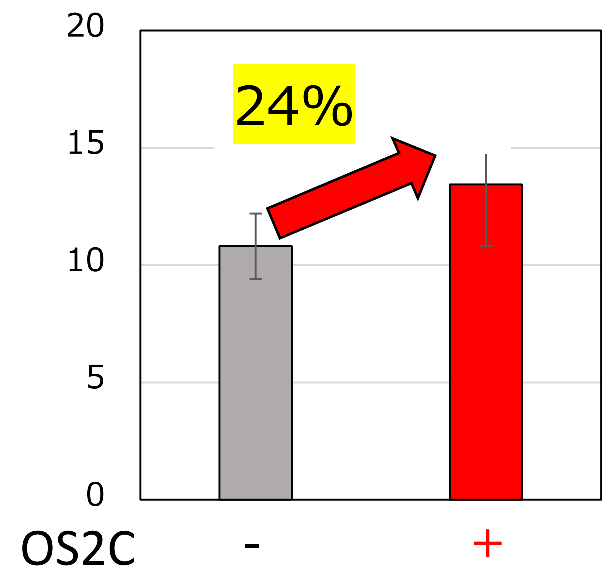
Height（cm）



Aboveground DW（g）



Underground DW（g）



- ➢ Effect was confirmed even in practical biomass plants

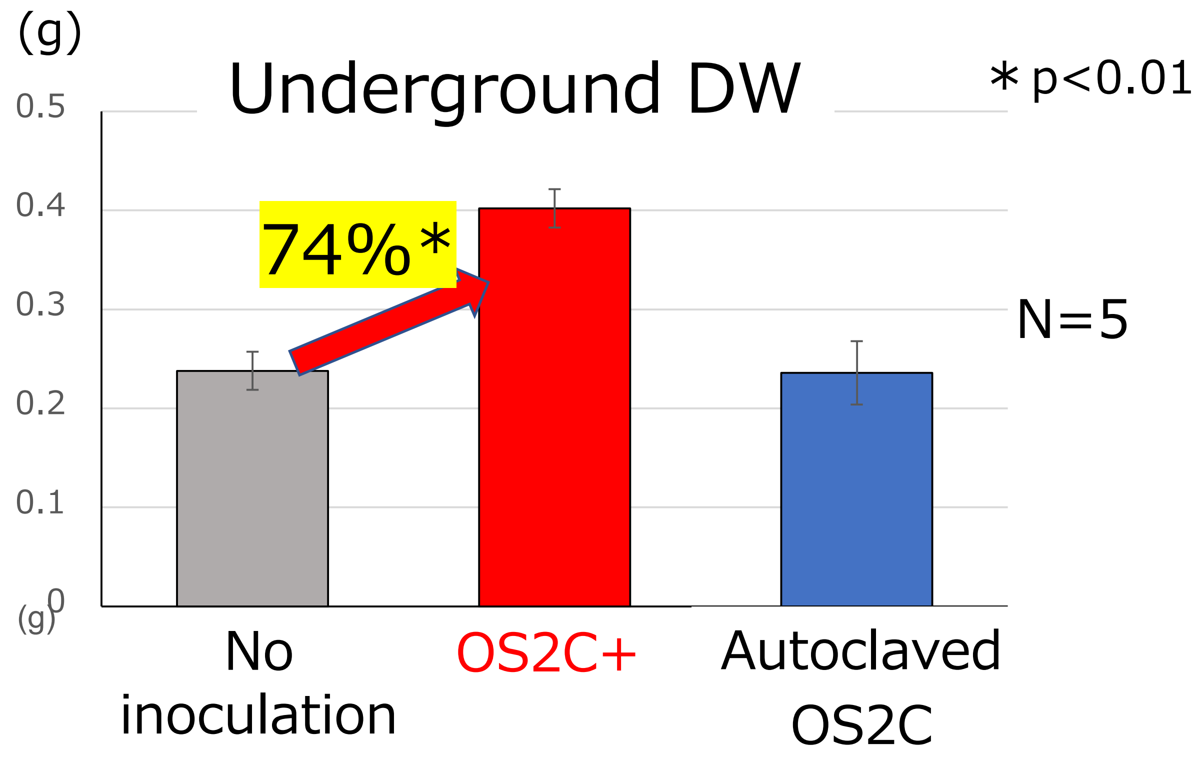
Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

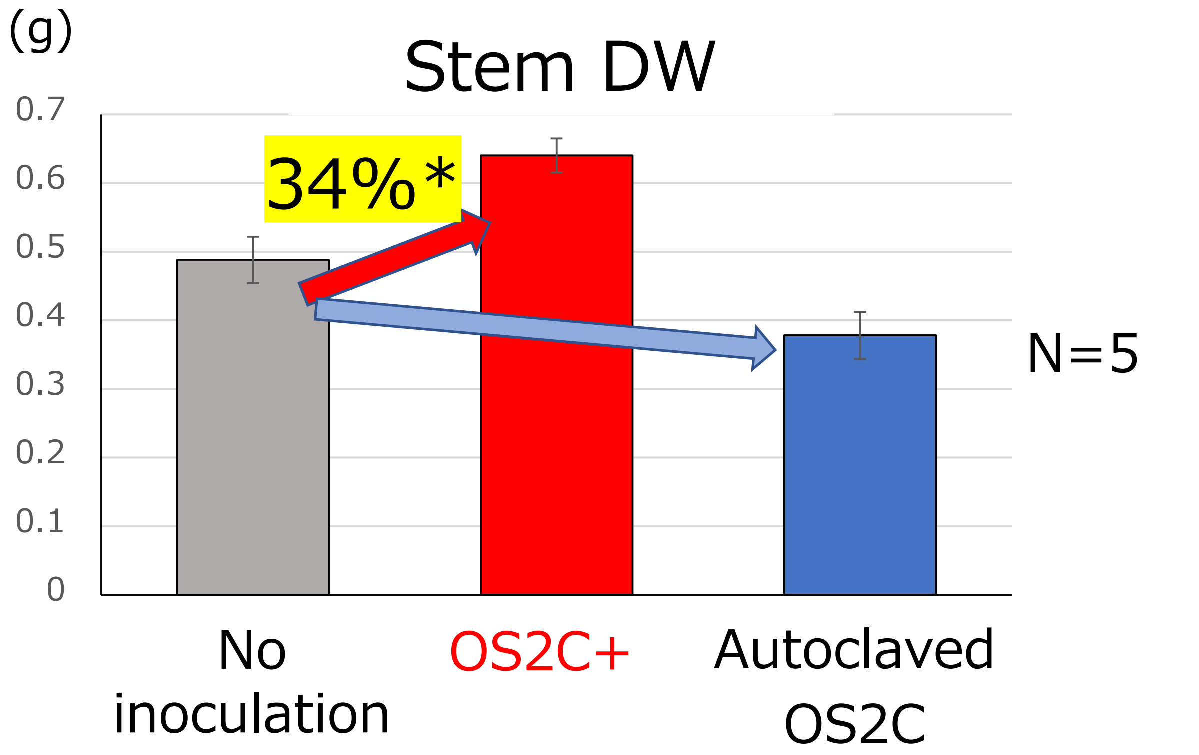
◆Growth promotion in poplar

After 3 months (N = 5)

No inoculation

OS2C inoculated

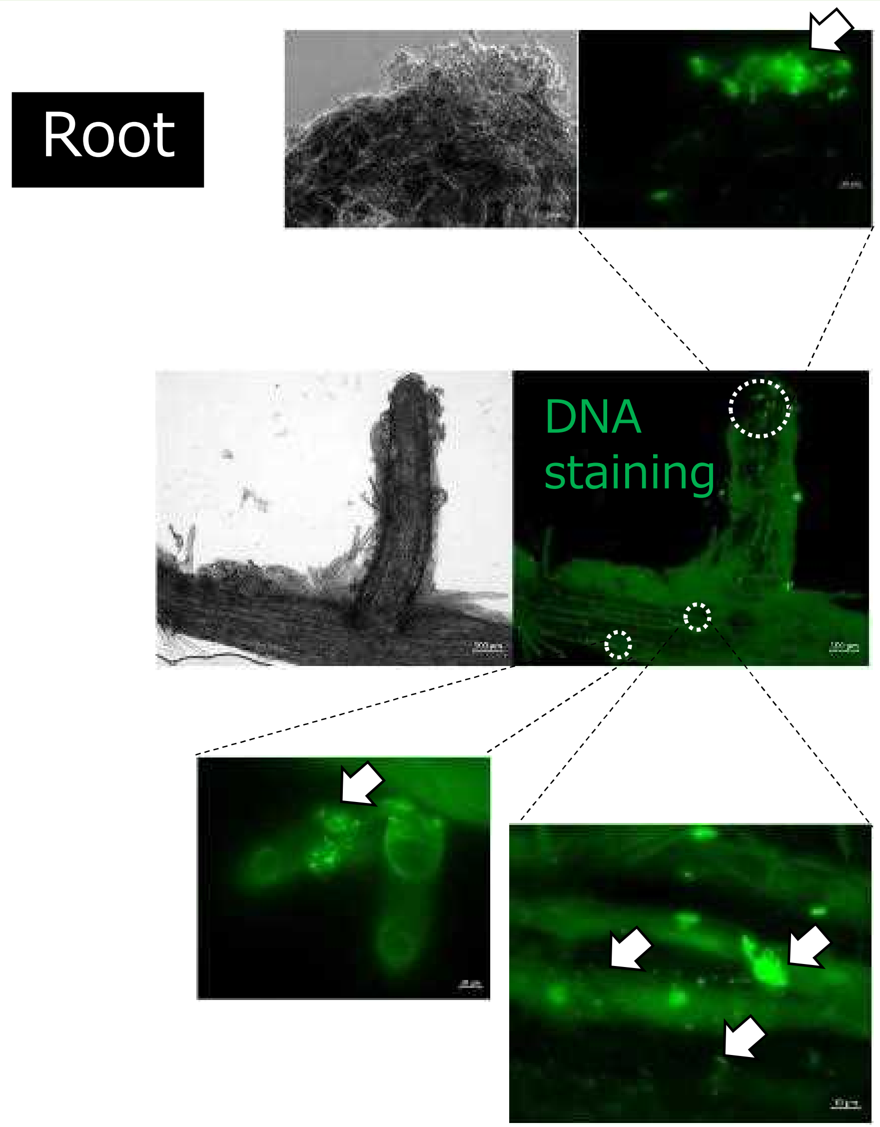


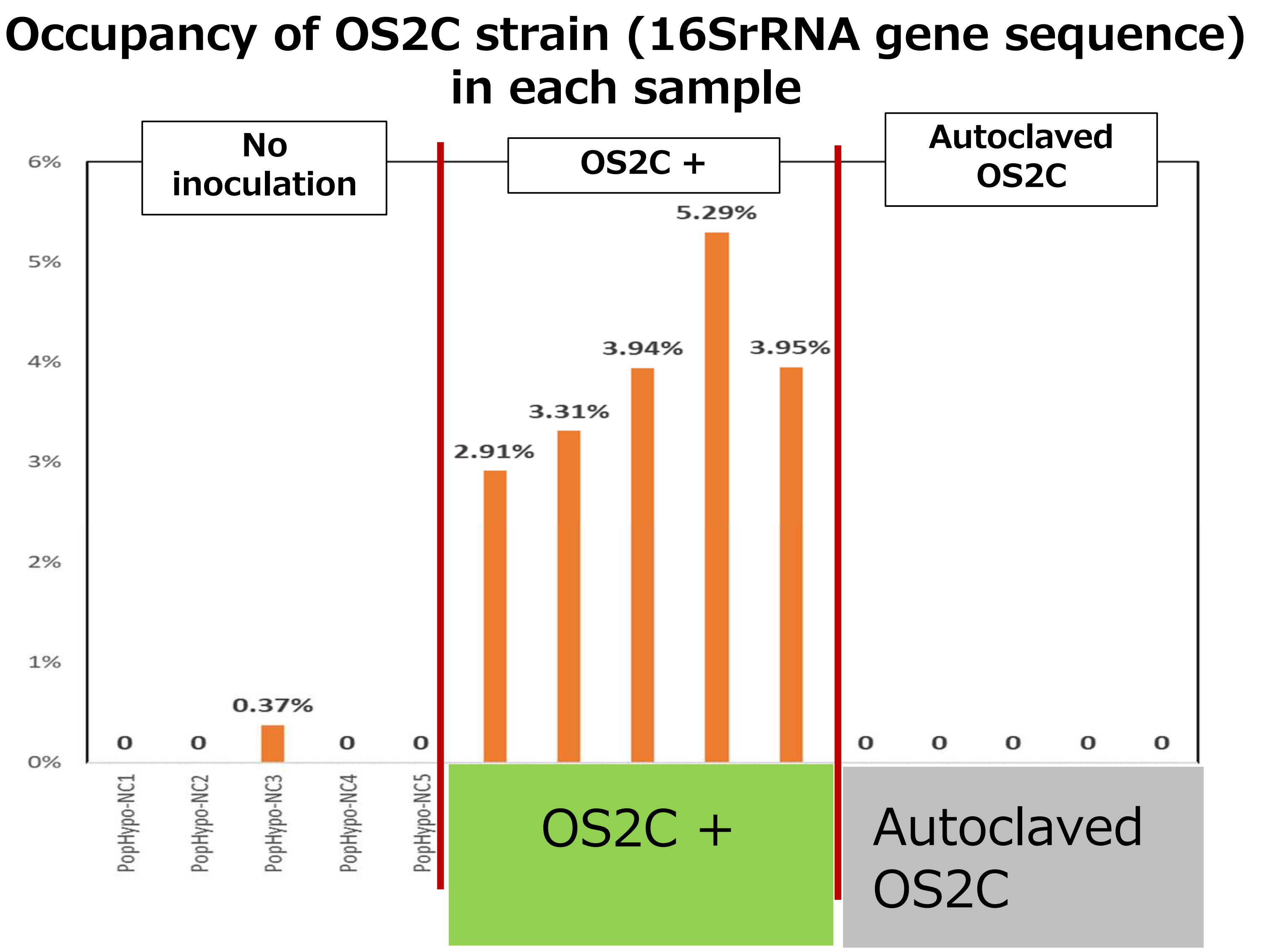


➢ Clear growth-promoting effect was confirmed even in trees

Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

◆ Confirmation of OS2C survival on poplar



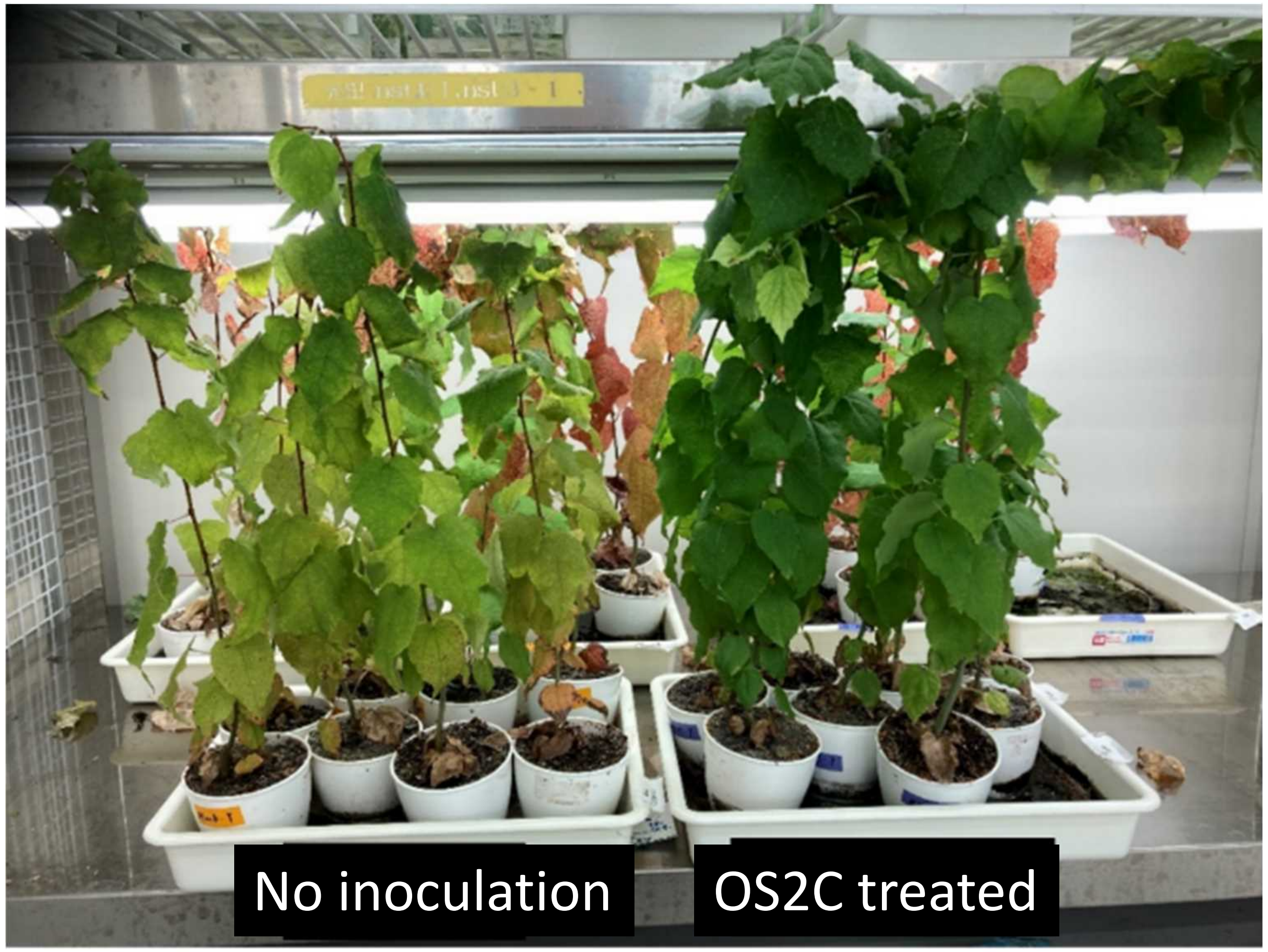


➢ OS2C survival was confirmed even in trees grown for about 3 months

Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

◆Effect of OS2C to suppress dormancy in poplar

After 5 months



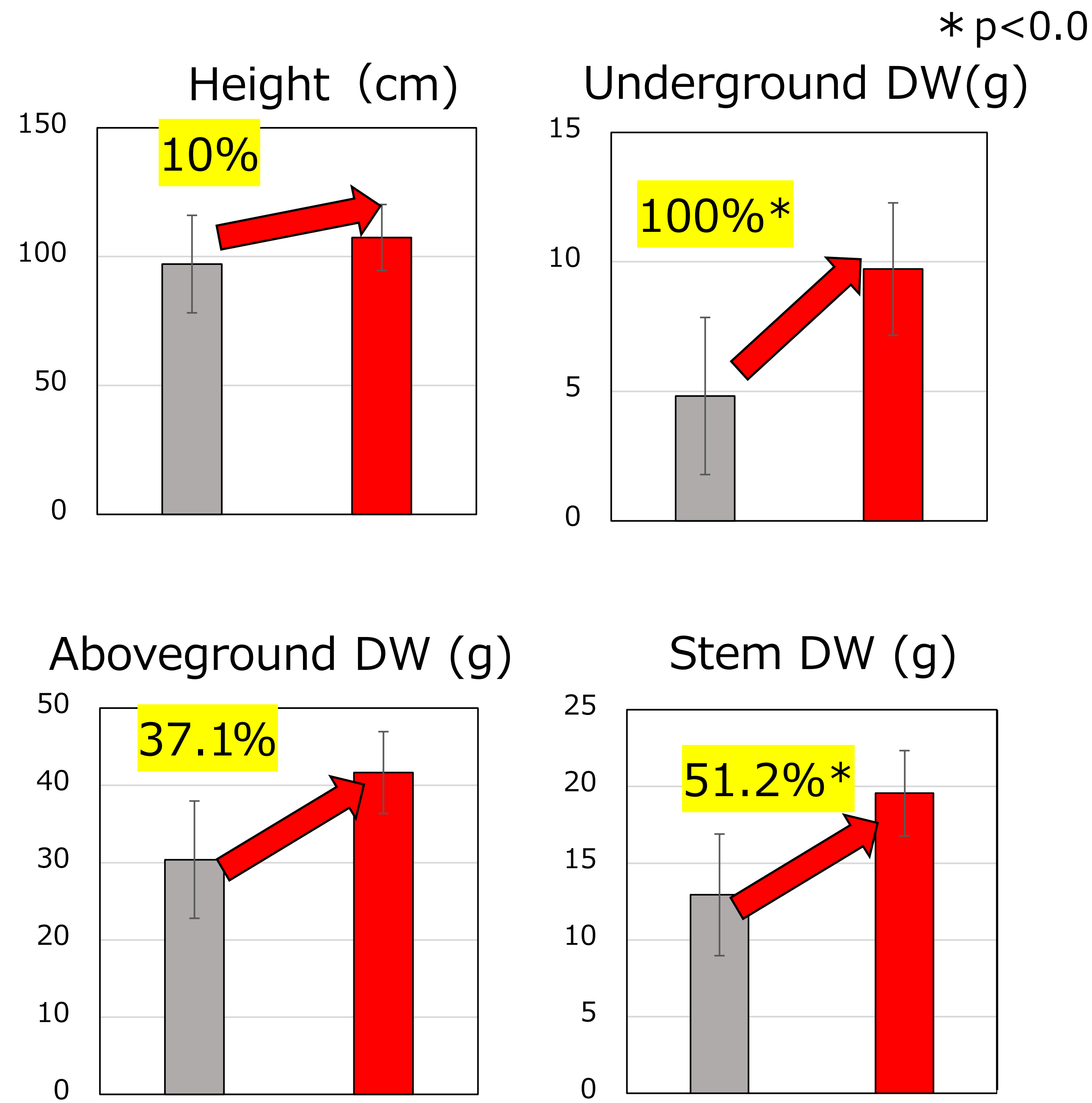
➢ OS2C might suppress dormancy of poplar. Study of this mechanism is underway.

Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

◆Growth promotion in Eucalyptus

After 5 months（N=5）

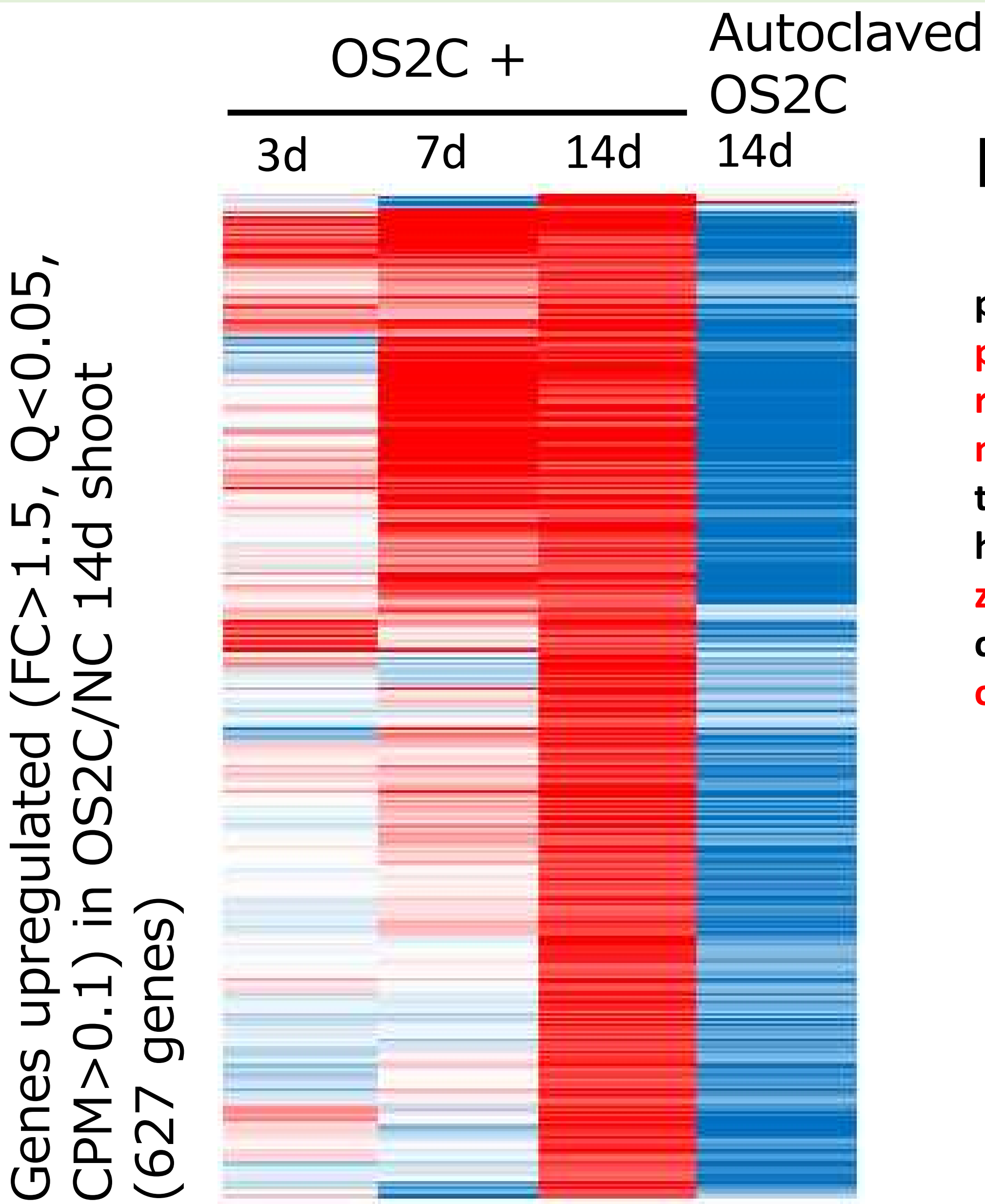
No inoculation　　OS2C inoculated



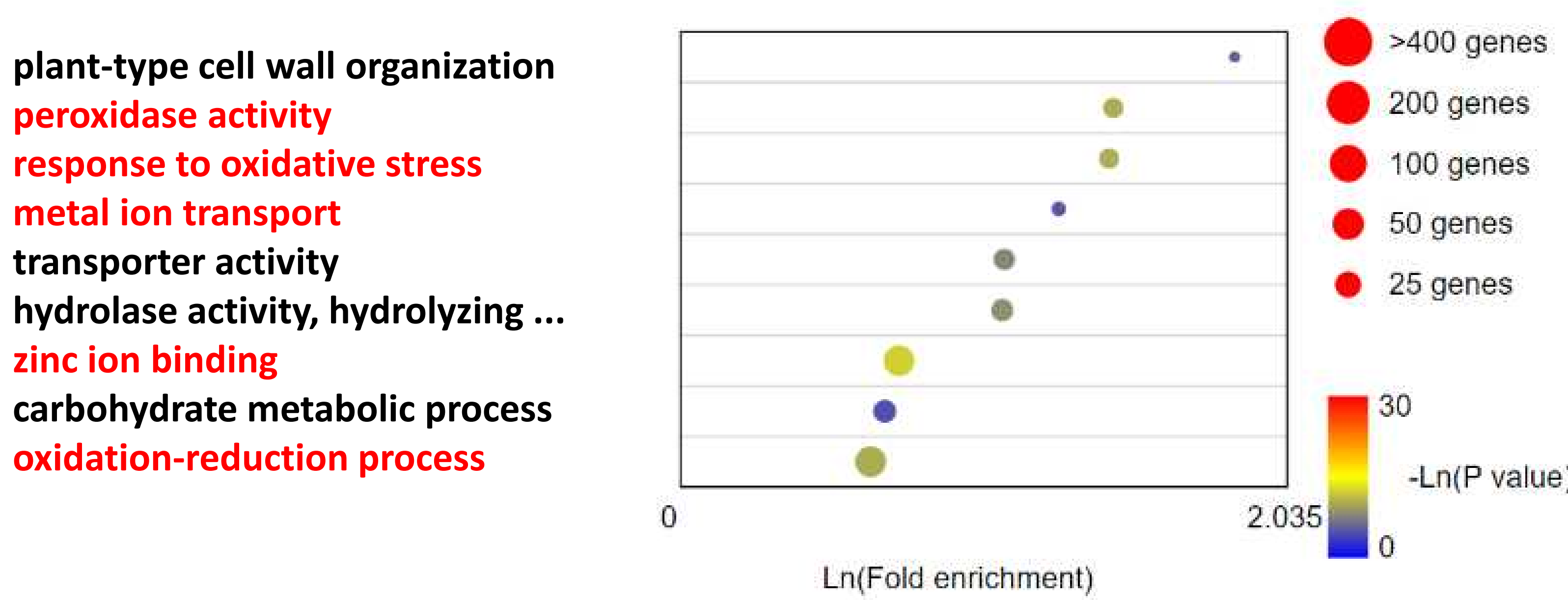
➢ 実用樹木でも明らかな生育促進効果を確認

Discovery of a symbiotic microorganism with growth-promoting abilities across diverse plant species

◆**RNA-seq analysis in rice to elucidate the mechanism of growth promotion**



Enrichment analysis for upregulated genes



➢ OS2C may activate reactive oxygen sequestration in plant

Summary

- Approximately 30% wood enhancement in larch introduced with an hyperactive NST transcription factor
- New cell fusion-promoting material showed breakthrough high effectiveness
- Erianthus x Miscanthus hybrid showed improved cold tolerance compared to Erianthus
- Partial elucidation of the mechanism of the growth-promoting effect of OS2C plant symbiotic microorganisms