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# Development of a **Non-Vernalization-Grafting** Floral Induction Method and Suitable Rootstocks for Rapid Breeding of Brassica Crops

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# Rapid floral induction of Brassica crops by grafting technique

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- **Key factors for the floral induction**
- **Potential application to breeding of Brassica crops**



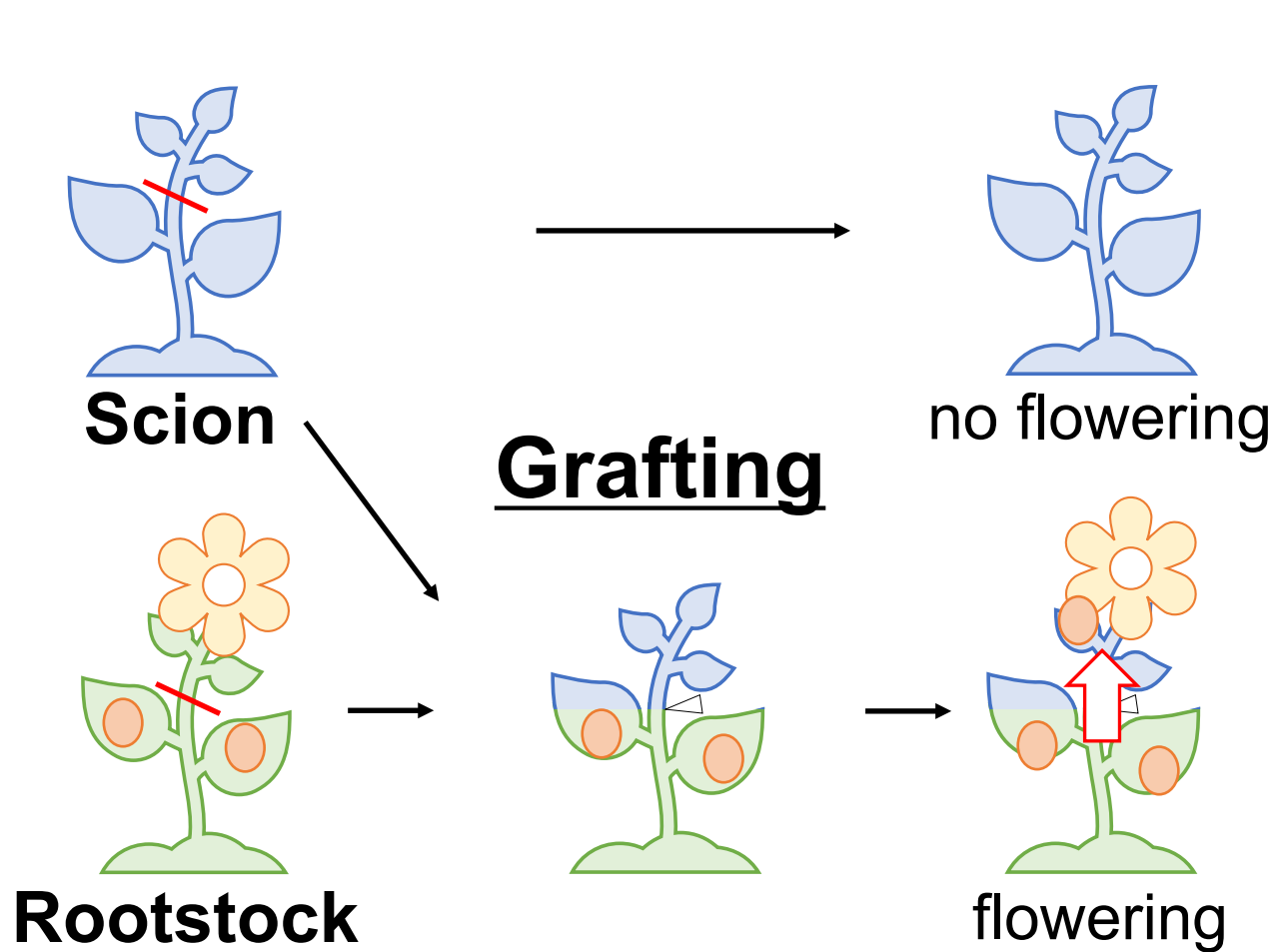
↑ **Cabbage**

↓ **Radish**

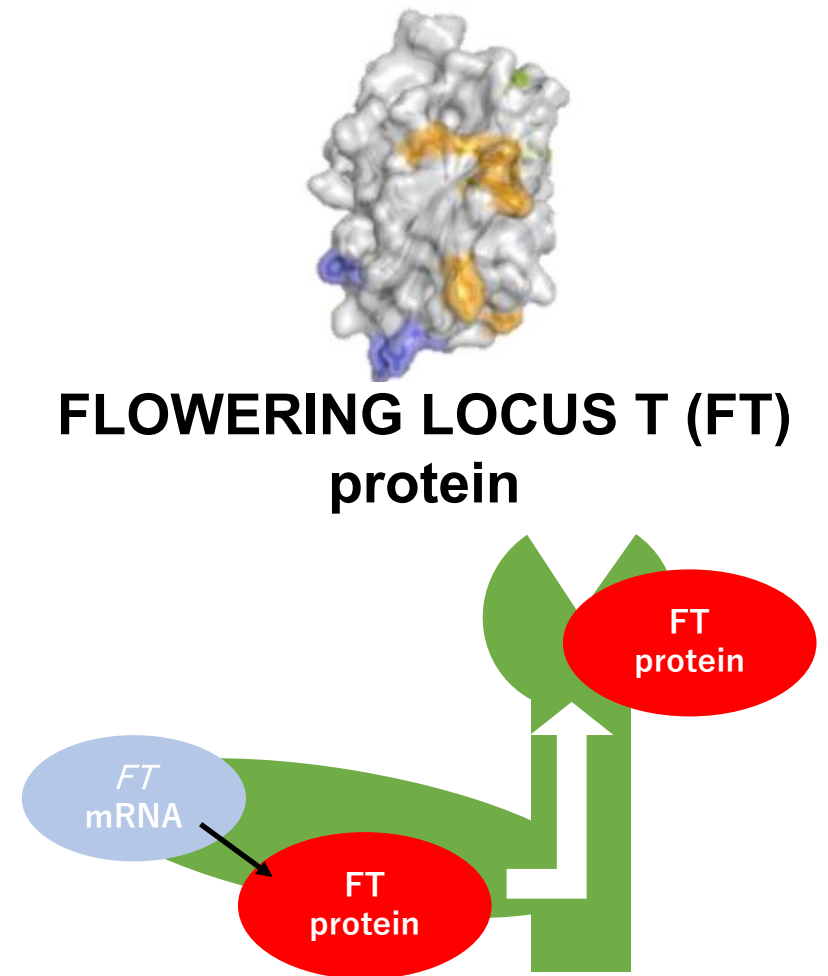


# Floral induction caused by grafting

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● Flowering hormone (Florigen)



(Lifschitz et al., 2006; Lin et al., 2007; Notaguchi et al., 2008)

# Floral induction of non-flowering cabbage mutant by grafting

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'nfc' 4 years after cutting propagation

- 'nfc' (non-flowering cabbage)
- Rarely flowers even after ov



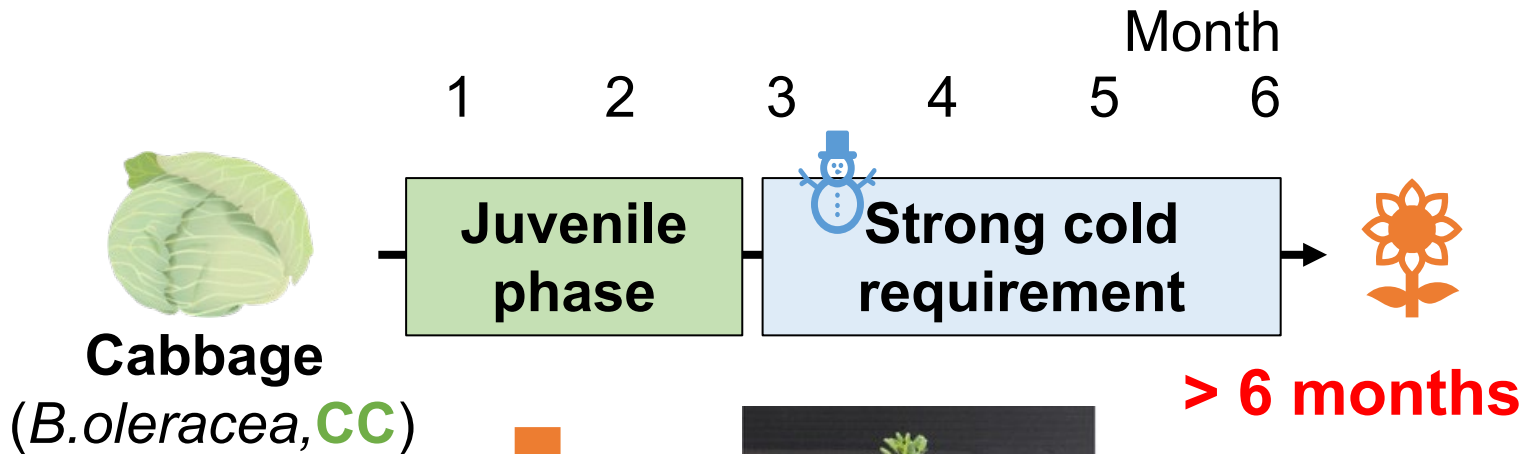
Ornamental kale (*B. oleracea*)  
rootstock (Non-flowering)



Radish (*R. sativus*)  
rootstock (Flowering)

# Application to the breeding of Brassica crops

Vernalization requirement can be a problem in the breeding of Brassica crops



Grafting



Induce flowering rapidly, without vernalization

# Flowering response of grafted cabbage significantly varies depending on rootstocks

## Rootstocks

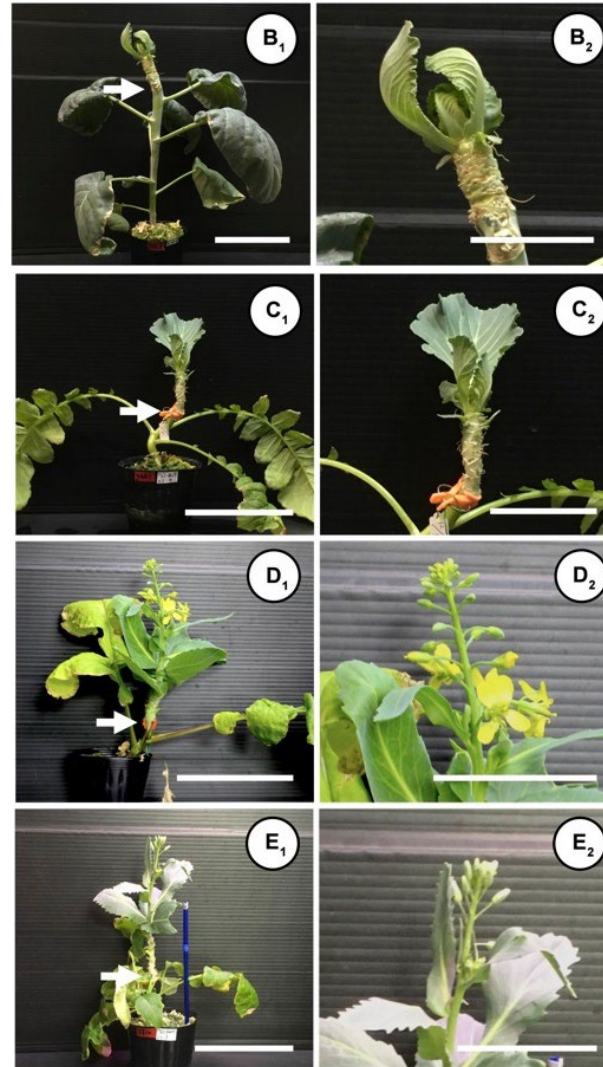
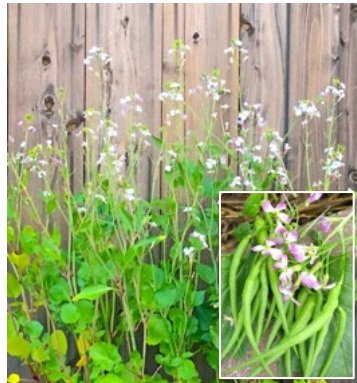
Rapid flowering type **cabbage**



Root harvesting type **radish**



Rapid flowering type **radish**



Flowering response of grafted cabbages

**No flowering**  
(0%)

**Weakly flowered**  
(0-17%)

**Intra-specific variation within radish**

**Moderately flowered**  
(0-75%)

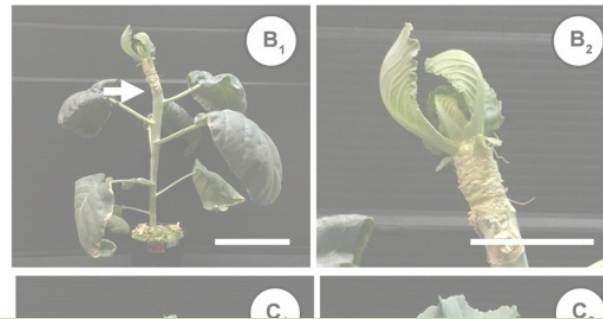
(Kagawa, 1957; Motoki et al., 2019.)

# Flowering response of grafted cabbage significantly varies depending on rootstocks

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## Rootstocks

Rapid flowering type **cabbage**



Flowering response of grafted cabbages

**No flowering**  
(0%)

**Reveal the cause of this variability by the rootstocks to develop a reliable floral induction technique.**

Rapid flowering type **radish**



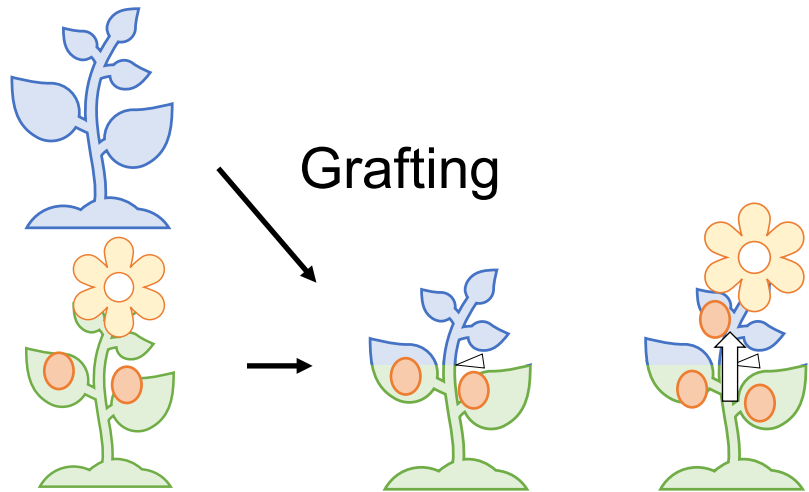
Intra-specific variation within radish

**Moderately flowered**  
(0-75%)

(Kagawa, 1957; Motoki et al., 2019.)

# Hypothesis on the involvement of florigen in the variability of flowering induction

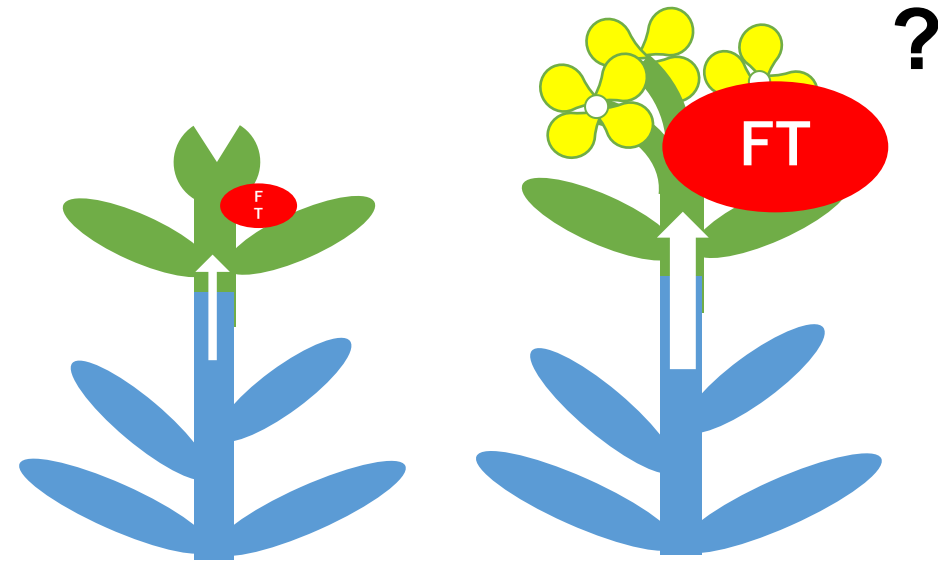
FT protein may somehow involve in the different flowering response?



○ Florigen (FT protein)

Comparison of FT protein sequence between **cabbage** and **radish**

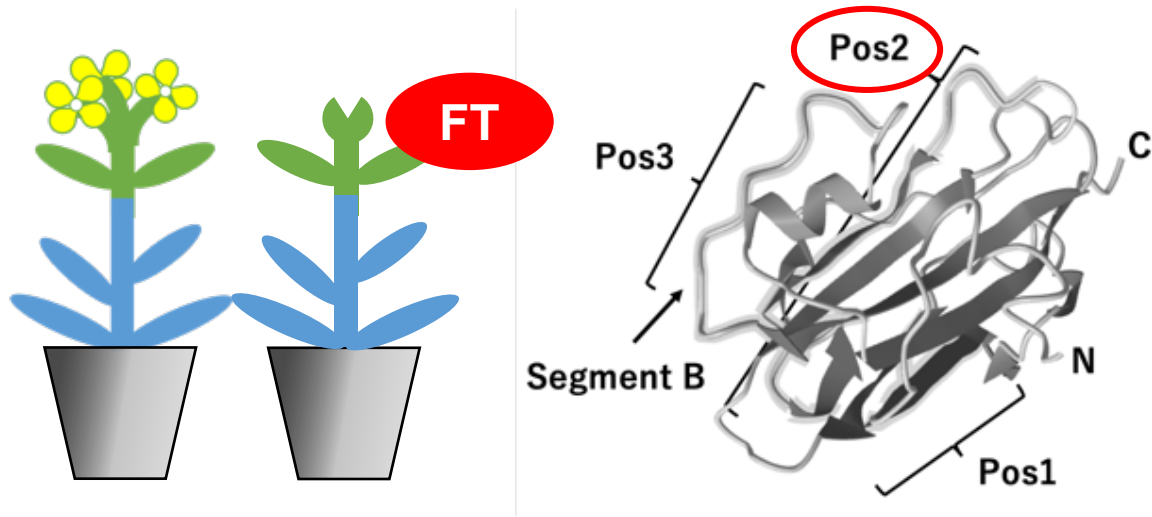
→ **No major difference** related to FT function or mobility was found



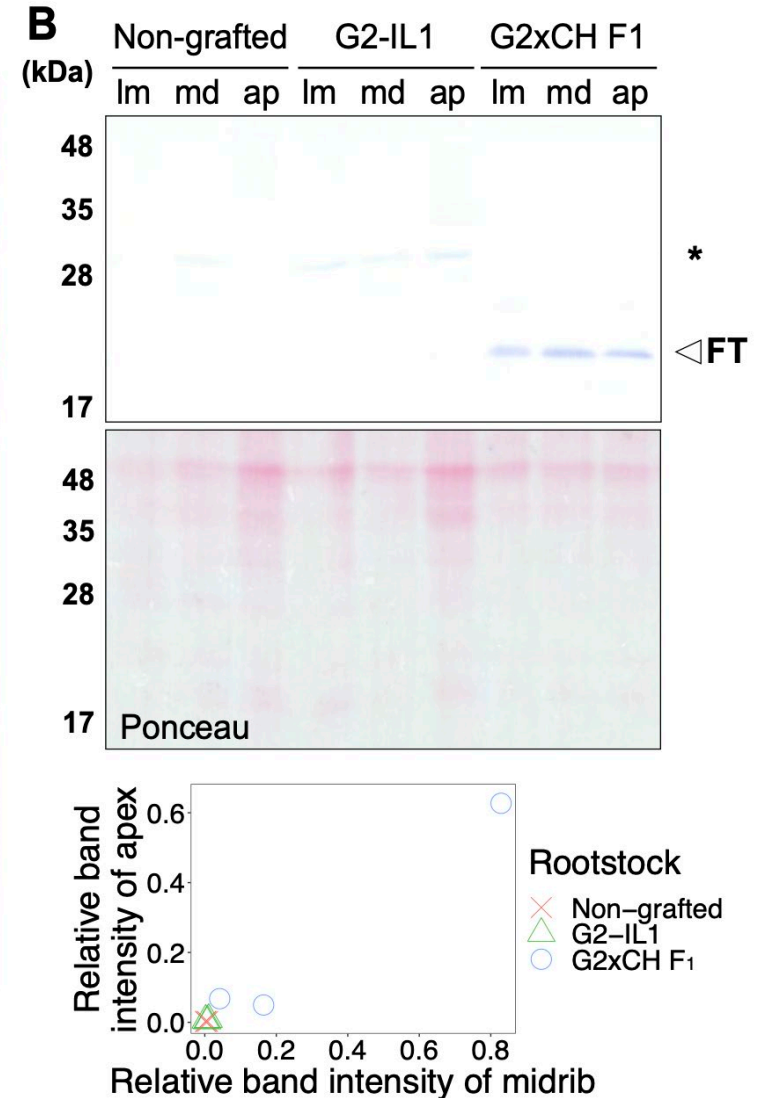
**Compared FT protein accumulation level** in the grafted cabbage

# Establishment of a method to detect FT protein

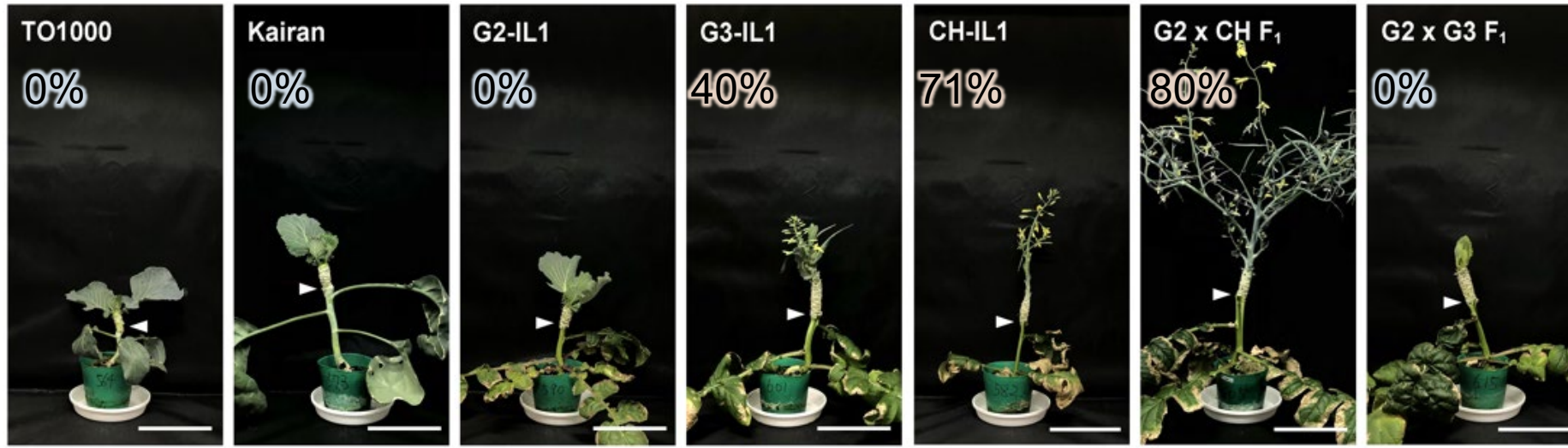
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- New antibody for cabbage and radish FT proteins was developed
- Established a method to quantify FT protein accumulation in the grafted cabbage by immunoblotting



# FT protein accumulation level is associated with flowering response of grafted cabbage

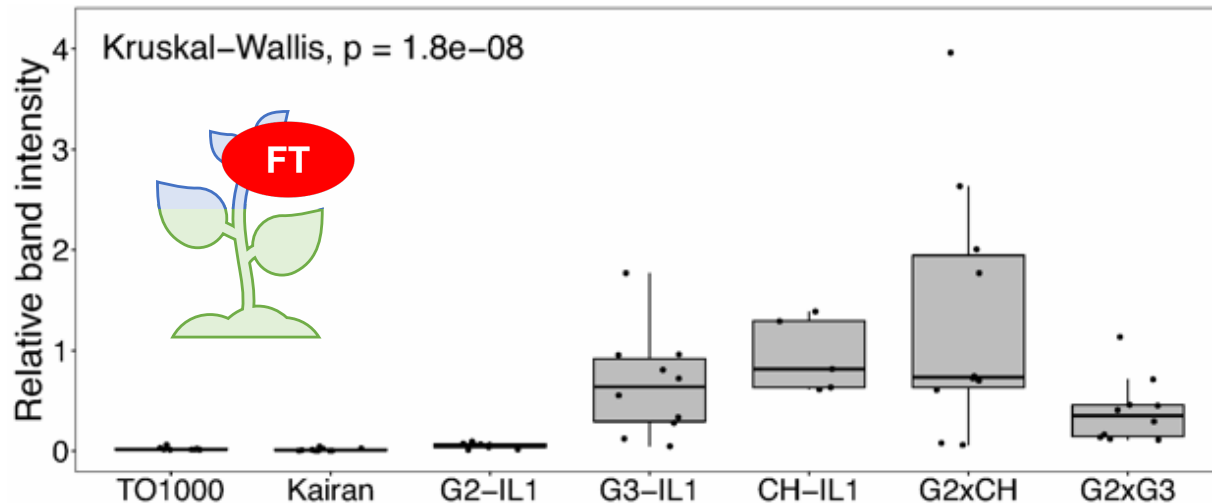


Percentage of the flowered plants

Cabbage rootstocks

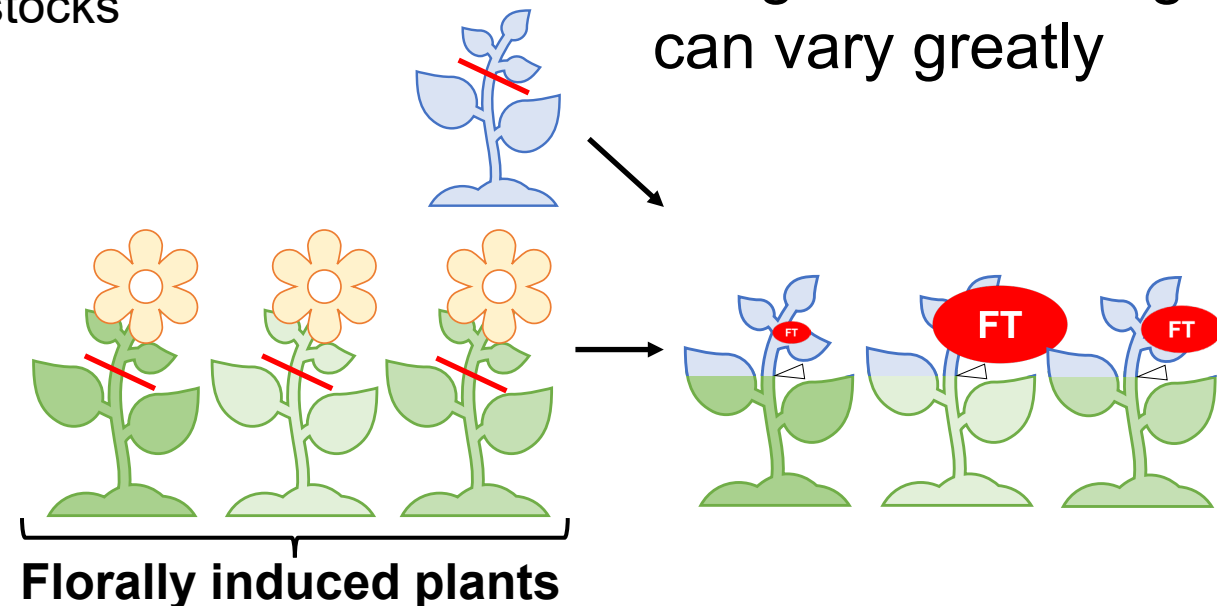
Radish rootstocks

FT accumulation in the grafted cabbage can vary greatly



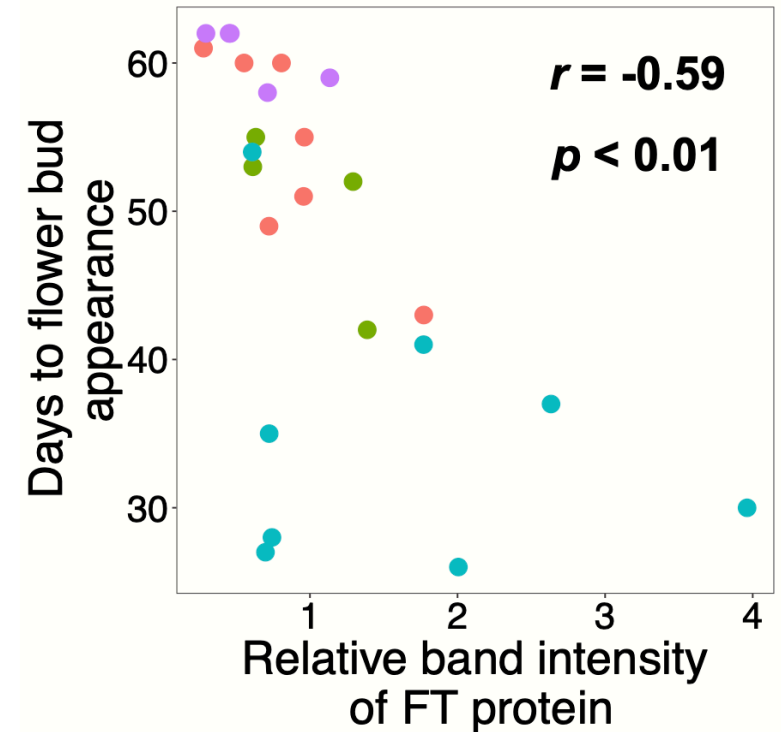
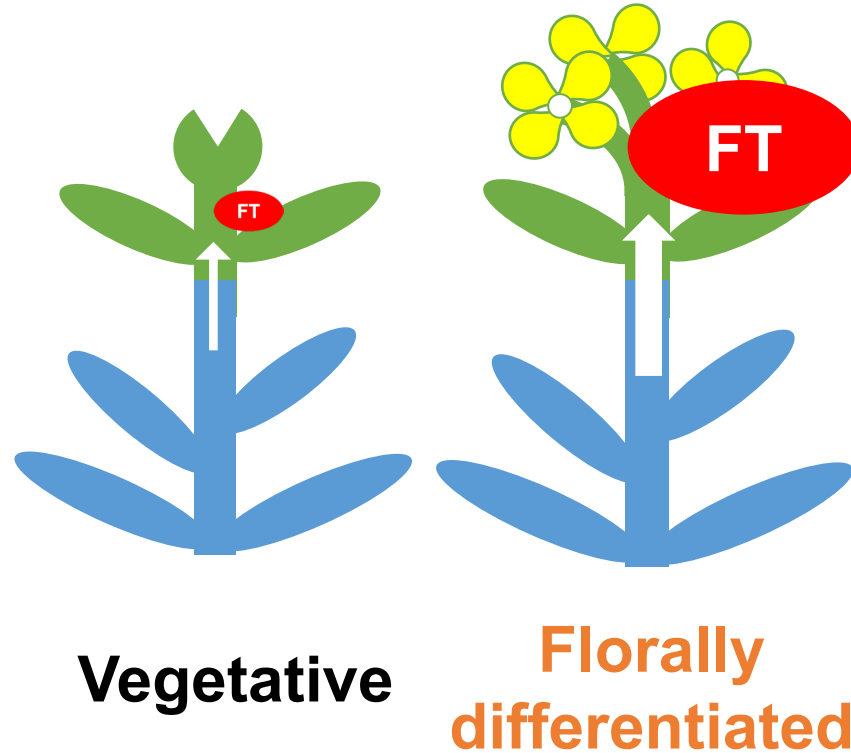
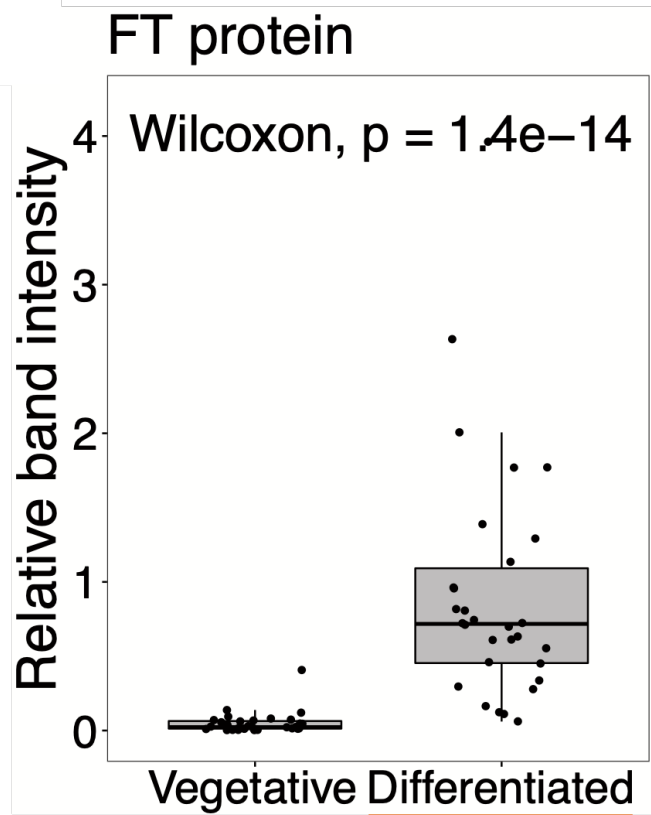
Cabbage rootstocks

Radish rootstocks



# FT protein accumulation level is associated with flowering response of grafted cabbage

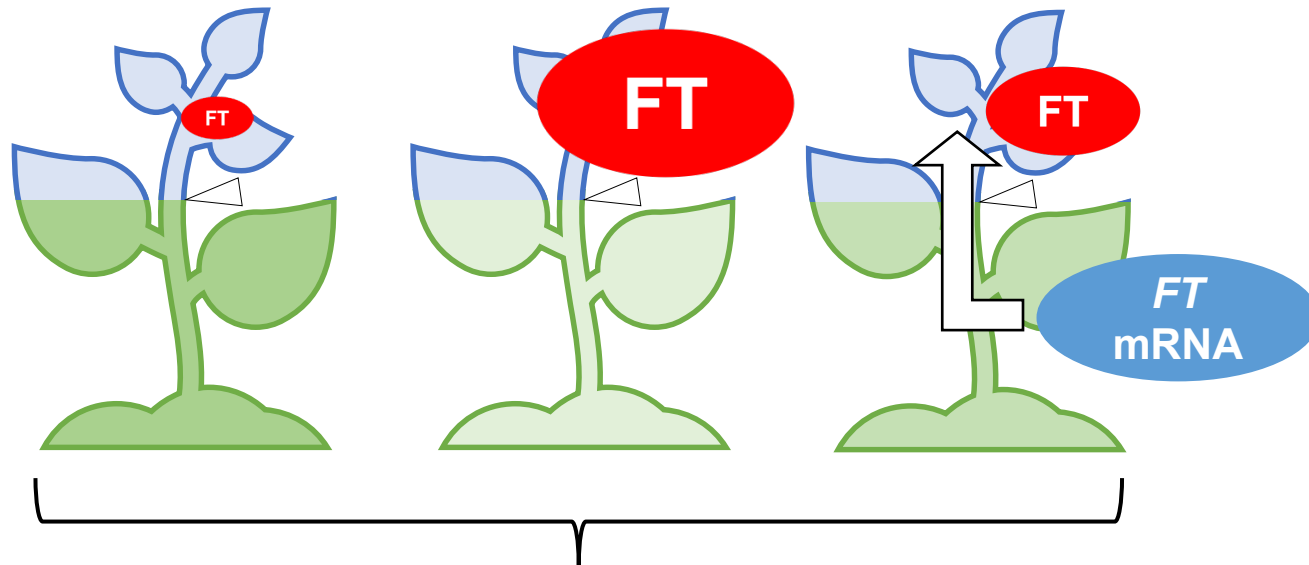
11



The variations in flowering responses of the grafted cabbage was assumed to be caused by **differing levels of FT protein supplied by the rootstocks.**

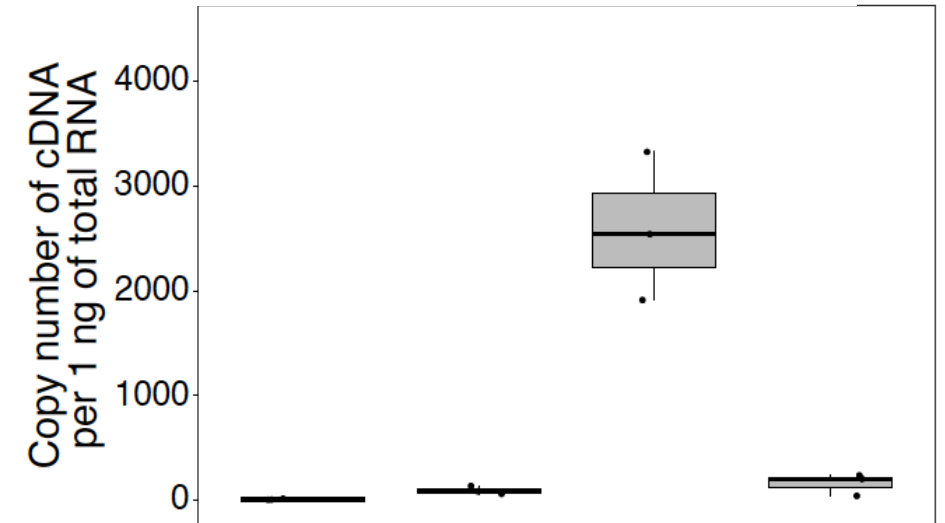
# Factors responsible for the different FT protein supply from the rootstock

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Explored the factors responsible for the different FT protein supply from the rootstock

## FT mRNA / rootstock

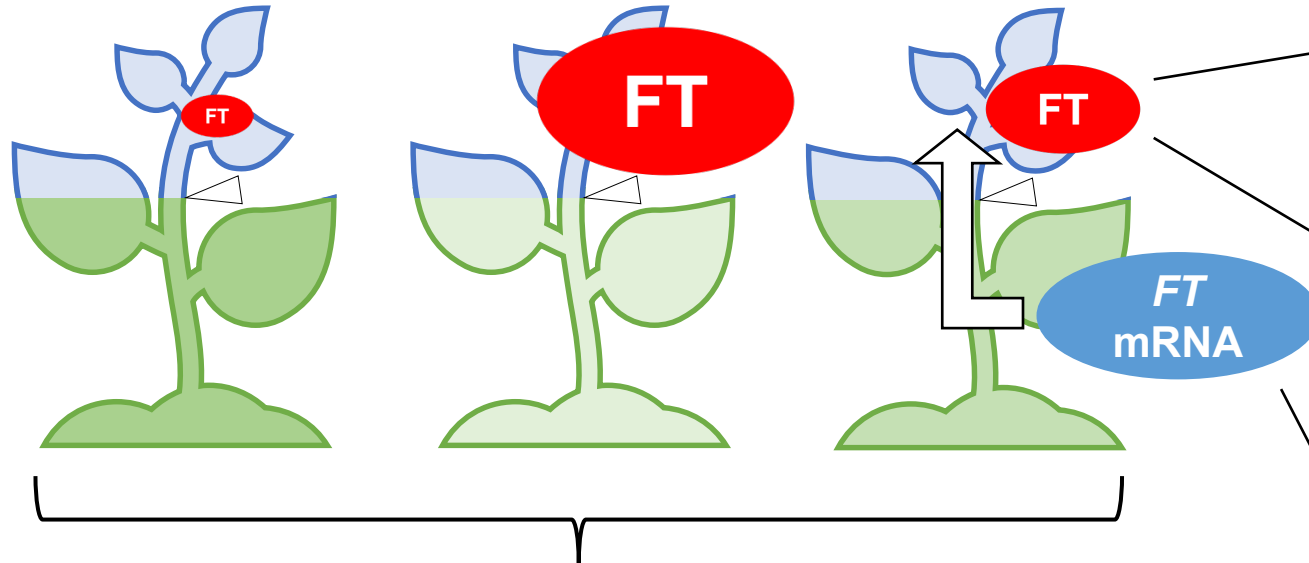


Floral induction ability  
Kairan Low Cabbage  
TO1000 Low Cabbage  
G2xCH High Radish  
G2-IL1 Low Cabbage

Much higher *FT* gene expression were observed in radish compared to cabbage rootstocks.



# Factors responsible for the different FT protein supply from the rootstock

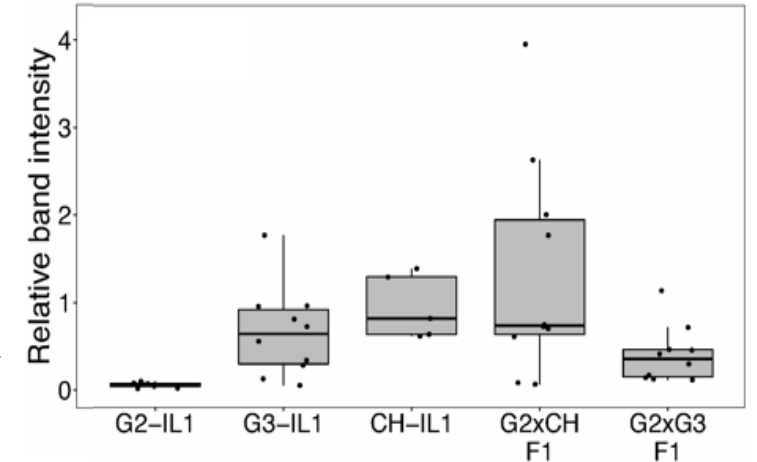


Explored the factors responsible for the different FT protein supply from the rootstock

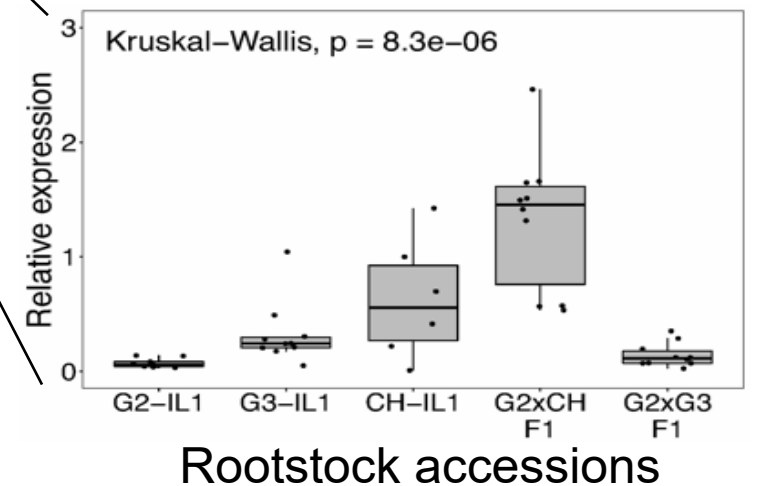
*FT* gene expression level was also different among radish rootstocks



### FT protein / scion



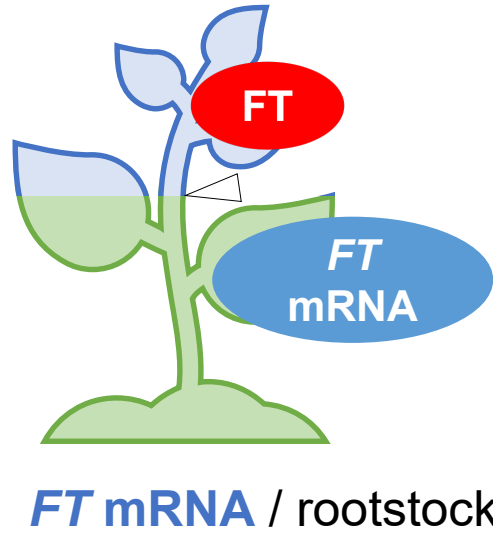
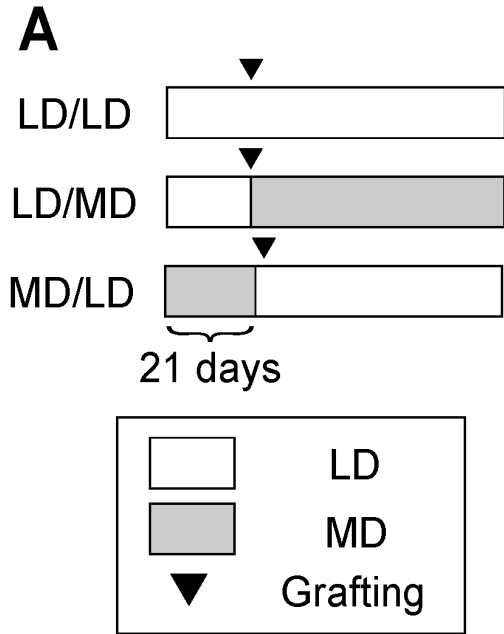
### FT mRNA / rootstock



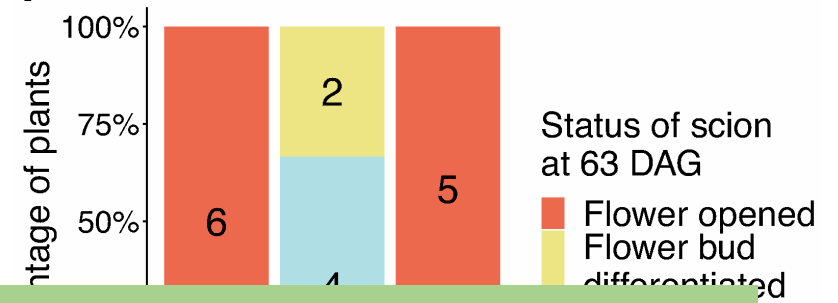
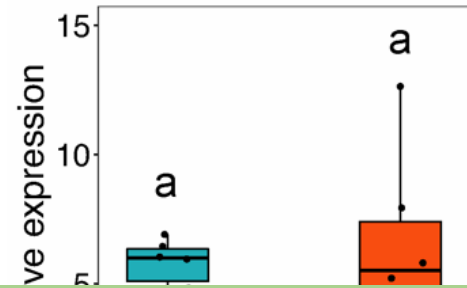
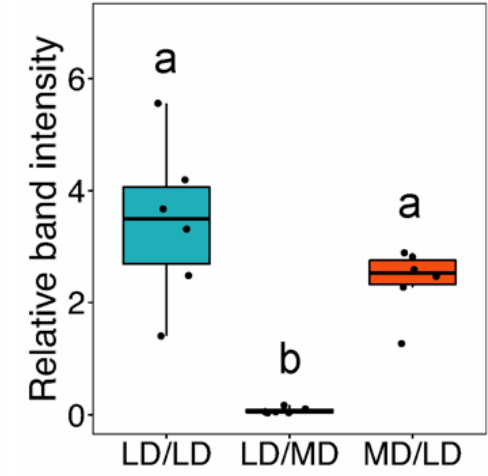
Rootstock accessions

# Factors responsible for the different FT protein supply from the rootstock

## Day length treatment



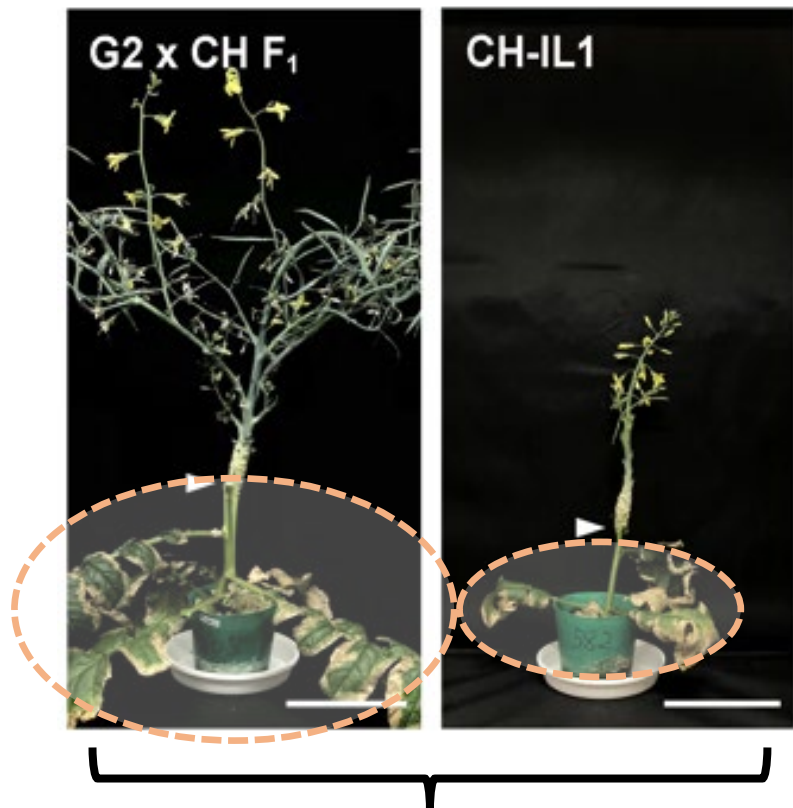
FT protein / scion



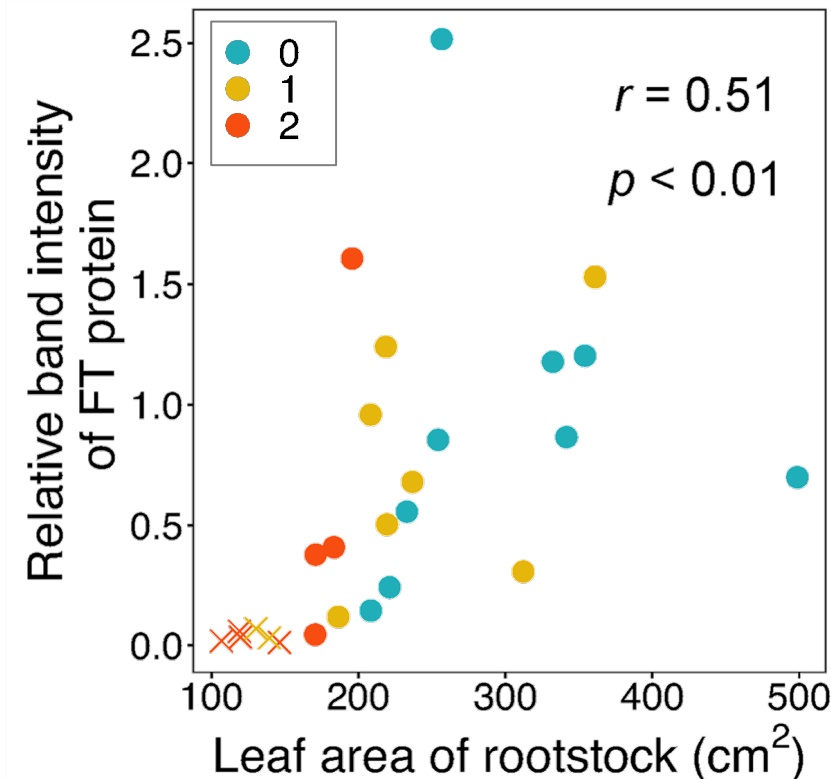
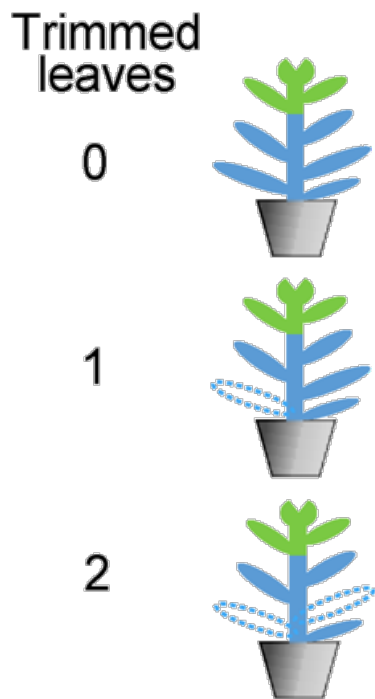
**Difference in the *FT* gene expression level was suggested to be the primary factor affecting the floral induction by grafting**

# Factors responsible for the different FT protein supply from the rootstock

## The effect of the rootstock plant size



Showing different flowering response though their *FT* gene expression levels were almost same



**The plant size of the rootstock is another crucial factor**

# Key findings and implications for stable floral induction by grafting



Cabbage rootstocks

Radish rootstocks

FT protein accumulation

Low

High

*FT* gene expression

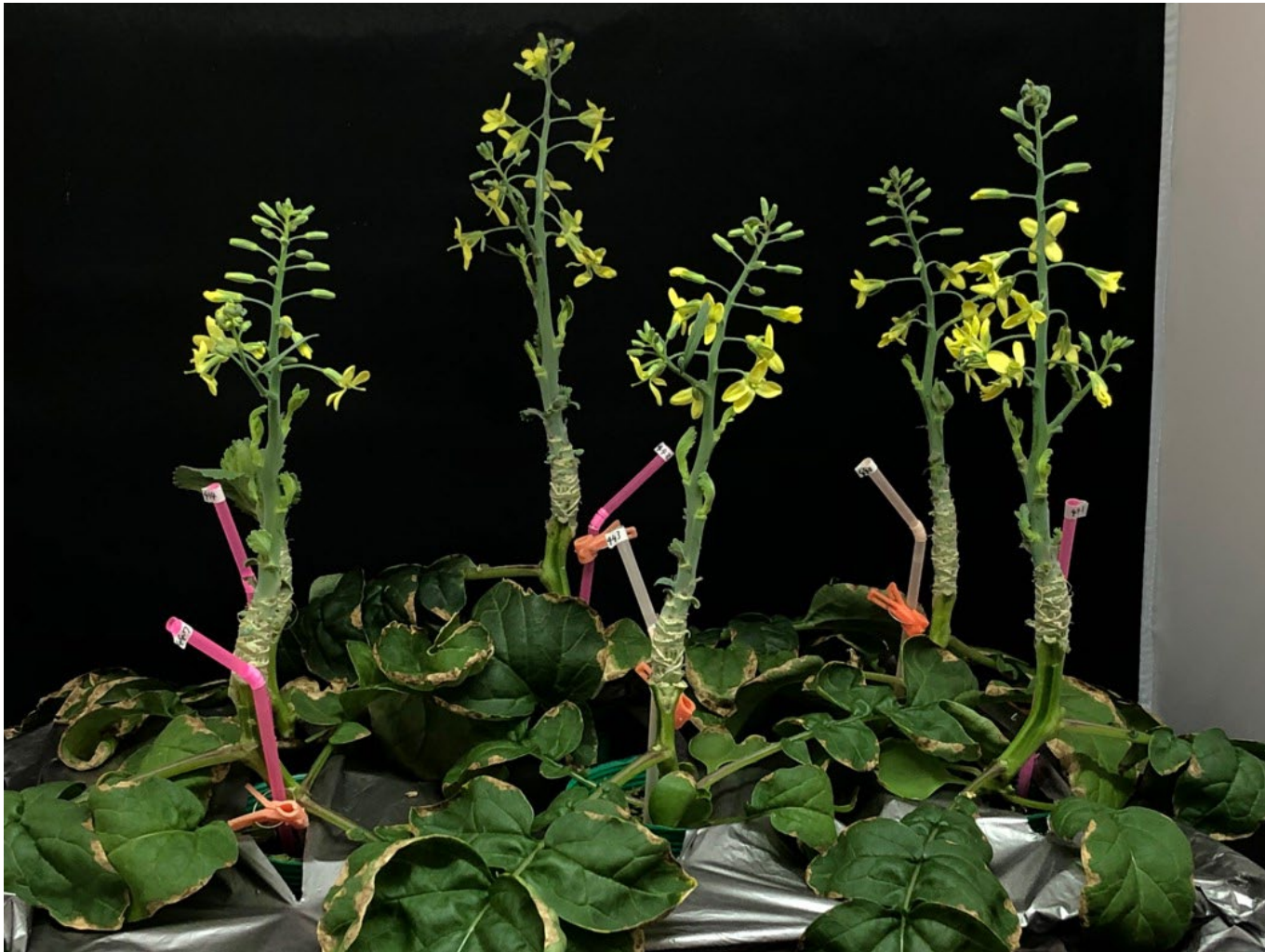
Plant size

Selecting rootstocks high in both factors

### Future work

Genetic and environmental elements controlling these two factors of the rootstocks

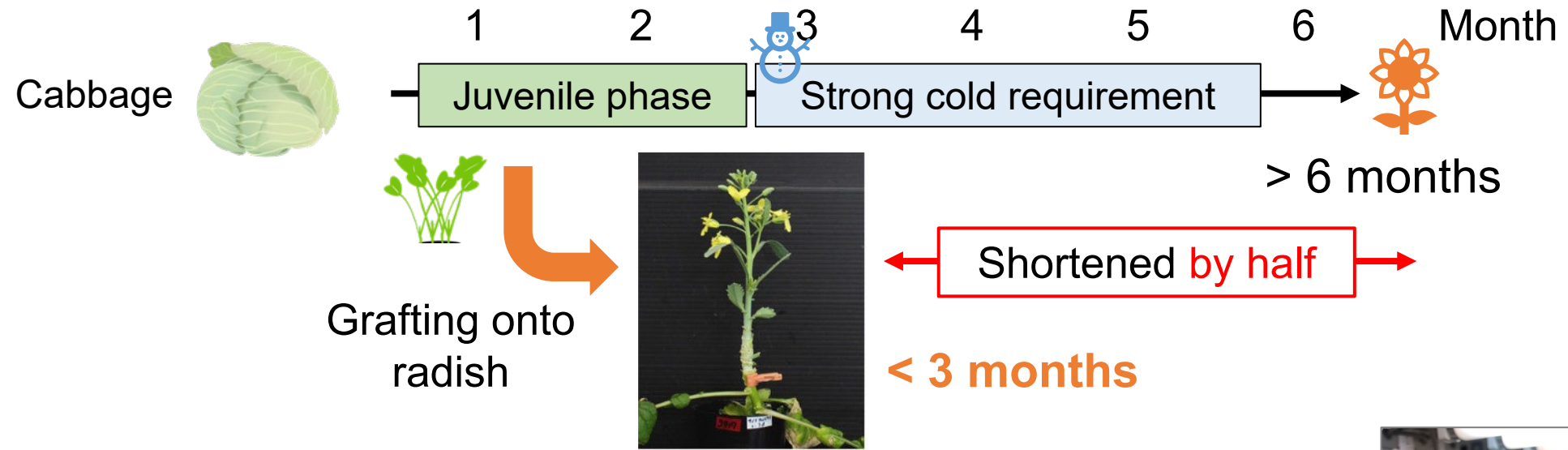
# Application to the breeding of Brassica crops 17



Selected radish rootstocks with high flowering induction ability, and became able to stably induce flowering of cabbage

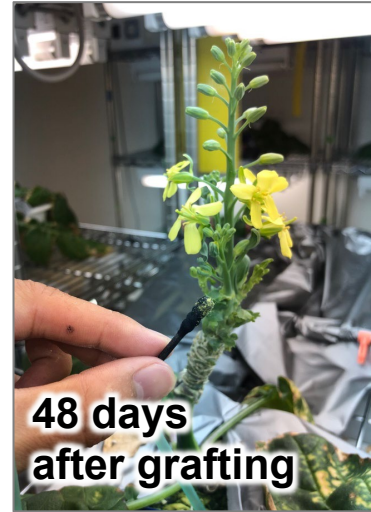
# Application to the breeding of Brassica crops 18

## Speeding up the breeding process



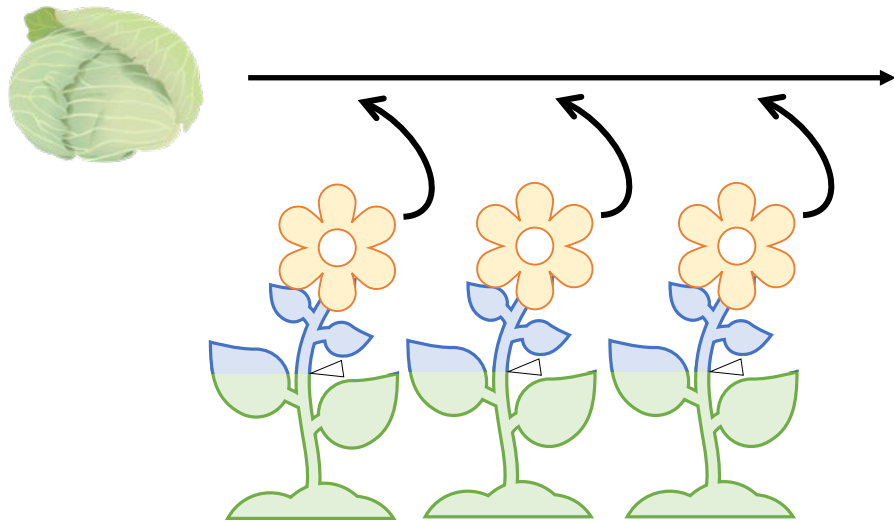
The procedure can be conducted **indoors**, under **warm conditions** with a **small spaces**.

- Breeding lines
- Transgenic plants, Genome-edited plants



# Application to the breeding of Brassica crops 19

Japanese seed companies have started using our method



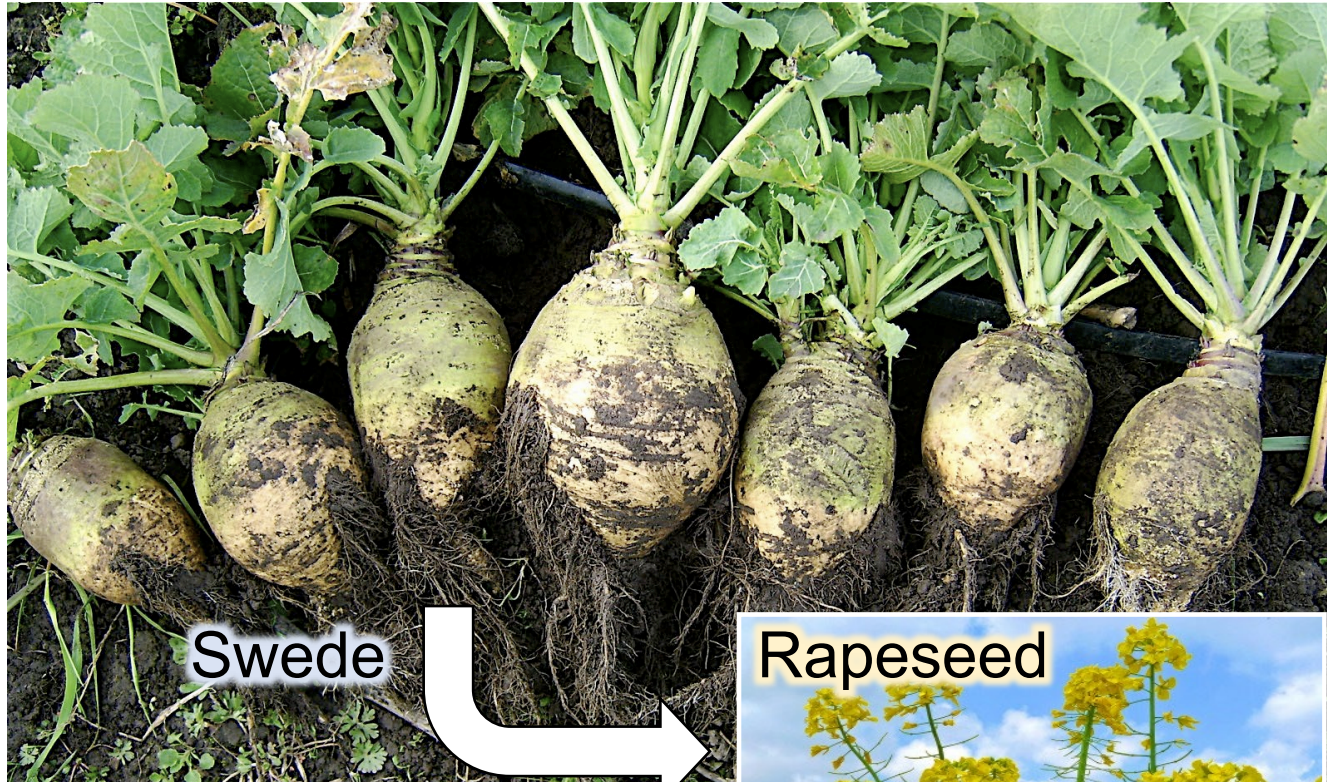
Suitable for  
**recurrent backcrossing**



# Applicable to other Brassica crops



# Application to the breeding of Brassica crops 21



**We are open to collaboration with researchers and providing technical assistance to companies**

# Acknowledgement

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## Providing information on grafting-induced flowering

Dr. Susumu Yazawa (Professor Emeritus, Kyoto University)

## Plant materials

Original seeds of G2-IL1 and G3-IL1 were kindly provided by the Genebank Project, NARO, Japan (JP No. 76703 and JP No. 76704, respectively).

## Mass spectrometry analysis

The Medical Research Support Center, Graduate School of Medicine, Kyoto University.

## Funding

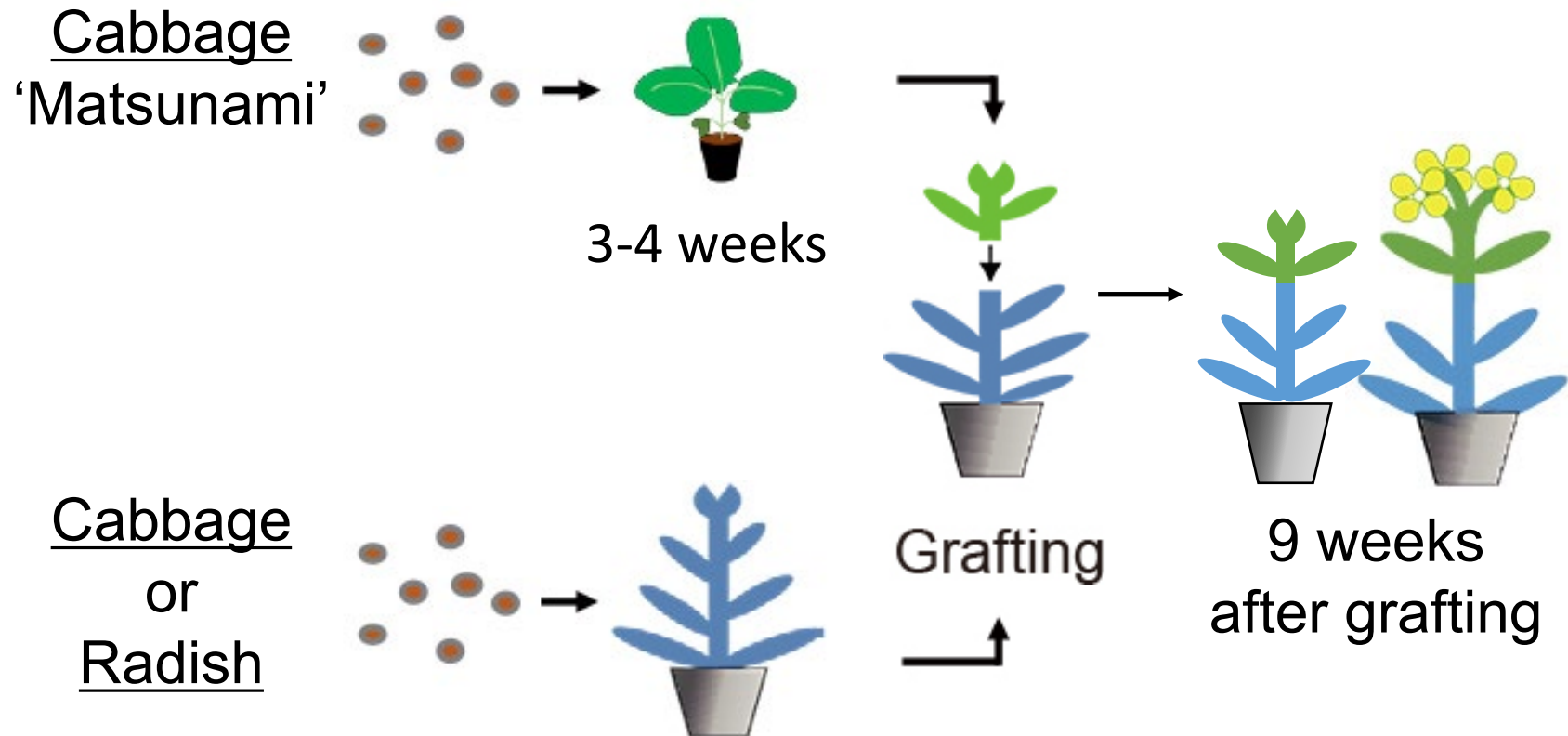
A Grant for Scientific Research from the Faculty of Agriculture, Kindai University, Grant-in-Aid for JSPS Fellows 19J15038, grant-in-aid for Young Scientists (B) 20K15518]  
Kyoto University start-up grant



- 1) Motoki, et al. □ Non-vernalization flowering and seed set of cabbage induced by grafting onto radish rootstocks. *Front. Plant Sci.* 9, 1967 (2019)  
(<https://doi.org/10.3389/fpls.2018.01967>)
- 2) Motoki, et al. □ Quantitative analysis of florigen for the variability of floral induction in cabbage/radish inter-generic grafting. *Plant Cell Physiol.* 63, 1230–1241 (2022)  
(<https://doi.org/10.1093/pcp/pcac098>)
- 3) Motoki, et al. □ Investigation of the field performance of progenies obtained by a non-vernalization-grafting floral induction method in terms of application to cabbage breeding and seed production. *Hort. J.* 92, 66-76 (2022)  
(<https://doi.org/10.2503/hortj.QH-018>)

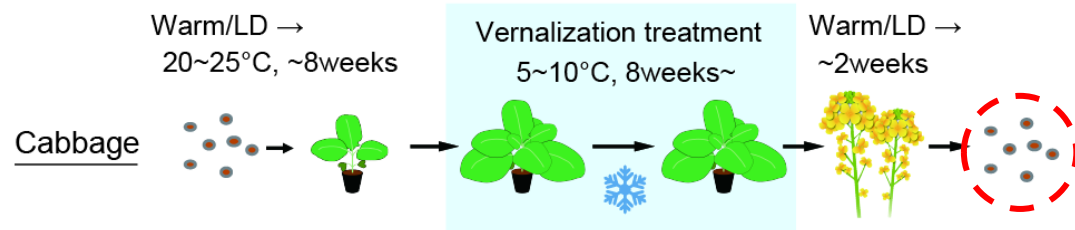
# (Suppl.) Materials and methods

Growth room  
22°C, Long-day (16 h day length)

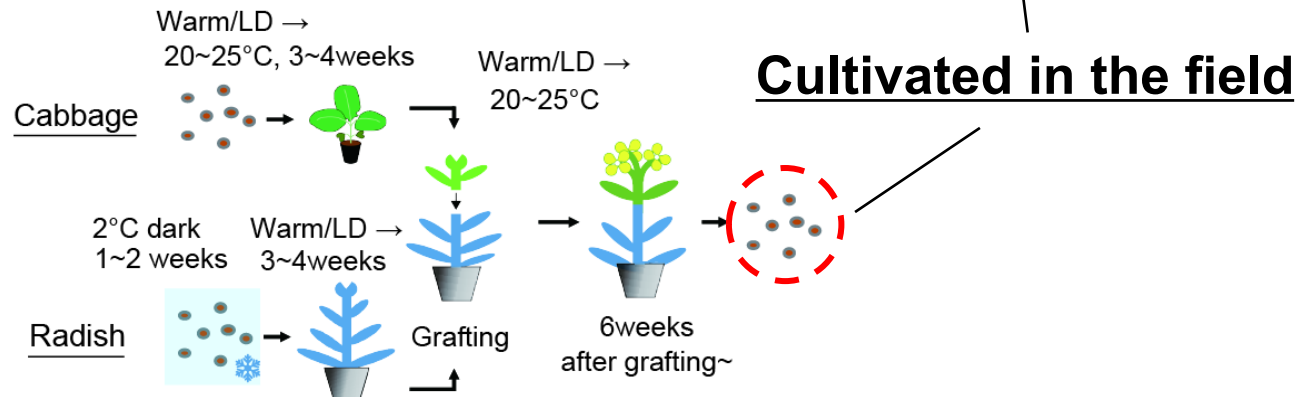


# (Suppl.) Investigation of seed traits obtained by grafting method in the field trials

## ○ Conventional seed production by vernalization



## ○ Seed production by grafting



No differences in major agronomic traits were found between the two seed production methods

(Motoki et al., 2023. *Hort. J.*)