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# Development of a non-vernalization-grafting floral induction method and suitable rootstocks for rapid breeding of Brassica crops

***Ko Motoki*[1]**

Yu Kinoshita[2]
Ryohei Nakano[2]
Munetaka Hosokawa[3]
Tetsuya Nakazaki[2]

[1] Okayama University, Okayama, Japan

[2] Kyoto University, Kyoto, Japan

[3] Kindai University, Nara, Japan

**Background:**

Floral induction by grafting, in which the flowering of grafted plants is directly induced by the transmission of florigen from the rootstock plants, has the potential to accelerate and diversify crop breeding and seed production process. Nonetheless, only a few reports exist regarding this floral induction method in relation to Brassica crops. Furthermore, the flowering responses of the grafted scions were unstable in these reports.

**Objective:**

The objective of this study was to develop a rapid and stable floral induction method by grafting without vernalization treatment for Brassica crops, especially for cabbage (Brassica oleracea L. var capitata), which has the strong vernalization requirement for its flowering. To achieve this, rootstock factors causing the variability in the flowering response of the grafted cabbage were analysed, and rootstock accessions suitable for this method were developed.

**Methods:**

Plants were grown in a growth room maintained at 20-25°C for LD (16 h/8 h, light/dark) or MD (12 h/12 h, light/dark) conditions. Several B. oleracea accessions and radish (Raphanus sativus) accessions were grown after certain days of seed vernalization treatment and used as rootstocks. Grafting was conducted according to a previously published protocol (Motoki et al. 2019). A seedling of 'Matsunami' cabbage grown for 3-4 weeks after sowing was used as a scion. Flowering responses of the grafted scions were investigated and accumulation levels of FLOWERING LOCUS T (FT) protein, the main component of florigen, in the grafted scions were measured by immunoblotting.

**Results:**

Significant variation in the flowering response of grafted scions were observed when different rootstocks were used. The earliest flower opening of the grafted cabbage was observed 37 days after grafting. As previously reported, B. oleracea rootstocks did not induce flowering in grafted cabbage scions, whereas certain radish rootstocks did. The immunoblotting analysis of the FT protein showed that floral induction was quantitatively correlated with the accumulation of FT protein in grafted scions. Two rootstock factors, FT transcript levels and leaf area, were altered by vernalization, daylength and leaf trimming treatments, and then grafting experiment were conducted using these rootstocks. As a result, the two rootstock factors were identified to cause variation in the accumulation level of FT and the flowering response in grafted scions.

**Conclusions:**

It was concluded that increasing the total amount of FT protein produced in a rootstock is important for stable floral induction in grafted cabbage, and this can be achieved by increasing both FT transcription level and the leaf area of the rootstock. Using the developed method, the generation time of cabbage could be shortened up to 4 months in the shortest time. The results of this study will accelerate breeding not only in cabbage, but also in other Brassica crops.

**References:**

1) Motoki, et al. (2019). Non-vernalization flowering and seed set of cabbage induced by grafting onto radish rootstocks. Front. Plant Sci. 9, 1967

2) Motoki, et al. (2022). Quantitative analysis of florigen for the variability of floral induction in cabbage/radish inter-generic grafting. Plant Cell Physiol. 63, 1230–1241