

Research Paper

Far-red-enriched ultra-long-day conditions induce constitutive *FT* expression and rapid flowering in radish rootstocks, promoting graft-mediated floral induction in Brassicaceae crops

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ABSTRACT

Efficient floral induction is essential for breeding and seed production in Brassicaceae crops, particularly for late-bolting cultivars and plant-vernalization-type species such as cabbage (*Brassica oleracea* L.), which require substantial time and labor for artificial flower induction. A graft-mediated floral induction method was recently developed for cabbage, enabling flowering without vernalization treatment by grafting cabbage scions onto radish (*Raphanus sativus* L.) rootstocks. Although high expression of florigen gene *FLOWERING LOCUS T (FT)* in the rootstocks is a key determinant of success, environmental conditions capable of inducing strong *FT* expression in radish have remained unclear. Here, we demonstrate that a far-red-enriched ultra-long-day photoperiod (ULD-FR) markedly upregulates expression of radish *FT* homolog *RsFTa* and greatly enhances graft-mediated floral induction in cabbage. Under the ULD-FR condition, *RsFTa* expression remained constitutively high throughout the day, with daily transcript abundance increasing more than tenfold compared with standard high red/far-red (R/FR) ratio long-day conditions that employed fluorescent lamps. *FT* protein accumulation in cabbage scions grafted onto radish rootstocks was also strongly elevated, resulting in rapid flowering approximately 30 days after grafting. ULD-FR also promoted flowering in rapid-cycling *Brassica rapa* and *B. oleracea* accessions, and induced flowering in a vernalization-requiring *R. sativus* cultivar without low temperature treatment, suggesting that the response may be broadly conserved across Brassicaceae. Because ULD-FR can be implemented using standard lighting equipment by adding an FR light source, it presents potential utility as a versatile tool for breeding-related applications, including generation advancement and flowering synchronization among divergent accessions.

1. Introduction

The rapid development of crop cultivars resilient to diseases and environmental stresses, together with the establishment of stable and reliable seed production systems, is critical for farmer livelihoods and global food security. Climate change is intensifying abiotic stresses and pest pressures, increasing the demand for resilient cultivars (Prado et al., 2025; Rivero et al., 2022), while also destabilizing seed production through altered flowering phenology and elevated risks of disease and abnormalities during seed maturation and harvest (Maity et al., 2023). In response to these challenges, technological innovations aimed at accelerating breeding and improving production efficiency have attracted increasing attention. Controlled-environment approaches and

precise selection techniques that shorten breeding cycles have been developed in several crops (Chaudhary and Sandhu, 2024; Watson et al., 2018). In parallel, advances in agricultural technologies, including machine-based monitoring and physiology-based crop management, are being actively explored (Ahmed and Shakoor, 2025; Sharma and Shivandu, 2024) and are also expected to be applicable to seed production systems (Maity et al., 2023). Together, these developments highlight the importance of technologies that enable precise and efficient management of plant growth and development.

Leafy and root-type Brassicaceae vegetables such as cabbage (*Brassica oleracea* L.), Chinese cabbage (*Brassica rapa* L.), and radish (*Raphanus sativus* L.) are economically important crops cultivated and consumed worldwide. During production, premature bolting and

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flowering markedly reduce market value by deteriorating head or root quality and reducing yield. Therefore, repression of flowering during cultivation is essential to ensure stable production, which is accomplished by choosing the appropriate transplanting timing (Takada et al., 2021), or by selecting late-bolting cultivars (Kitamoto et al., 2017). On the other hand, in breeding and seed production, reliable and timely flowering is indispensable for advancing generations and for efficient F₁ hybrid seed production. Thus, flowering control in leafy and root-type Brassicaceae vegetables represents a dual challenge: its suppression is essential for commercial production, whereas its promotion is indispensable for breeding and seed production.

Many Brassicaceae species require exposure to low temperatures for floral induction, followed by warm long-day conditions to promote flowering. This low-temperature-induced promotion of flowering is known as vernalization. Plants can be classified into two types according to their developmental stage of cold responsiveness: the seed-vernalization type, in which sensitivity begins upon seed imbibition, and the plant-vernalization type, in which sensitivity appears only after the seedlings reach a certain developmental stage (Chouard, 1960). In plant-vernalization-type species such as cabbage, artificial induction of flowering for crossing or seed production requires the cultivation of large plants and prolonged cold treatment, thereby demanding significant time and space. Consequently, breeding and seed production of such species are labor- and time-intensive. To facilitate the floral induction of cabbage, researchers have employed artificial low-temperature treatments (Wang et al., 2000), gibberellin application (EL-Eslamboly AASA, 2021; Nyarko et al., 2007), or drought treatments (EL-Eslamboly AASA, 2021).

Recently, a graft-mediated floral induction technique has been established for cabbage to shorten the time required for flower initiation (Motoki et al., 2022, 2018). In this method, young cabbage seedlings can be induced to flower without vernalization by grafting onto florally induced radish rootstocks. This phenomenon, in which scions are induced to flower under non-inductive conditions by grafting onto florally induced rootstocks, has also been reported in several plant species (Lang, 1952; Zeevaert, 1976). Molecular genetics approaches revealed that the transmission of the small globular protein FLOWERING LOCUS T (FT) and its orthologs from donor rootstocks to recipient plants is the major factor underlying graft-mediated floral induction (Corbesier et al., 2007; Lifschitz et al., 2006; Lin et al., 2007; Notaguchi et al., 2008). FT is a member of the phosphatidylethanolamine-binding protein (PEBP) family, expressed in leaves under inductive conditions, and transported through the phloem to the shoot apical meristem, where it activates downstream floral integrator genes to initiate flower bud differentiation (Abe et al., 2005; Kardailsky et al., 1999; Kobayashi et al., 1999; Wigge et al., 2005). Graft-mediated floral induction is expected to facilitate breeding in cabbage and other Brassicaceae crops by enabling rapid flowering and thereby shortening the breeding cycle (Motoki et al., 2024). This grafting-based floral induction system is hereafter referred to as the “GRAFT” method (Grafting onto Relatives capable of Abundant Florigen Transmission).

This naming reflects the fact that the key to developing this technique was selecting radish rootstocks with a high capacity to supply FT protein, thereby enabling greater FT accumulation in the grafted cabbage (Motoki et al., 2024). Previous studies identified two major factors determining the FT-supplying capacity of radish rootstocks: the level of FT expression and the leaf area of the rootstock (Motoki et al., 2022). Using radish rootstocks with both high FT expression and large leaf area was shown to be crucial for achieving stable floral induction (Motoki et al., 2022). Although genetic variation in FT-supplying capacity among radish accessions has been identified (Motoki et al., 2025), little is known about the effects of environmental factors.

FT expression is known to respond to various environmental and endogenous cues, thus integrating them into the regulation of flowering (Song et al., 2015). In the Brassicaceae model plant *Arabidopsis thaliana*, FT expression is induced under long-day conditions (Kobayashi et al.,

1999) and is further modulated by light intensity (King et al., 2008), the red/far-red (R/FR) light ratio (Kim et al., 2008; Wollenberg et al., 2008), ambient temperature (Jung et al., 2012; Lee et al., 2007), and endogenous gibberellin (Galvão et al., 2012). Similarly in radish, FT expression has been reported to be influenced by photoperiod (Motoki et al., 2022) and exogenous gibberellin treatment (Jung et al., 2020).

Light conditions are of particular interest because they play a central role in regulating FT expression and can be easily manipulated in practical cultivation environments. Under long-day conditions with artificial light sources, FT expression in *Arabidopsis* exhibits a single peak at the end of the light period, whereas under long-day outdoor conditions, two distinct peaks, one in the morning and another at dusk, are observed (Song et al., 2018). This difference has been attributed to the lower R/FR light ratio in natural sunlight (approximately 1) compared with that of artificial light sources (greater than 2), which leads to the stabilization of the CONSTANS (CO) protein. CO, the major transcriptional activator of FT, is stabilized in the morning through phytochrome A (*phyA*) and EARLY FLOWERING 3 (*ELF3*) dependent signaling (Lee et al., 2023; Song et al., 2018). In addition, under long-day conditions, exposure to canopy-shade-like low R/FR light (R/FR < 0.5) increases FT expression and accelerates flowering through increased CO abundance. This low R/FR-mediated response has been suggested to act primarily through the light-stable phytochromes *phyB*, *phyD*, and *phyE* rather than *phyA* (Kim et al., 2008; Wollenberg et al., 2008). The R/FR ratio alters the proportion of the two photo-absorbing forms of phytochrome, P_r (red-light-absorbing form, λ_{max} = 660 nm) and P_{fr} (far-red-absorbing form, λ_{max} = 730 nm), thereby modulating downstream gene expression (Kevei et al., 2007).

In breeding programs that rely on artificial illumination—either to grow plants indoors or to extend day length through supplemental lighting to accelerate generation advancement—the artificial light sources commonly used, such as fluorescent lamps or LEDs, often lack FR wavelengths. However, the impact of this spectral deficiency on FT expression in radish and on graft-mediated floral induction has not been fully elucidated. In this study, we investigated how the R/FR ratio and its combination with an ultra-long-day photoperiod (longer than conventional long-day conditions, e.g. >18 h) affect FT expression and the flowering response in radish. We further examined whether the resulting increase in FT expression enhances graft-mediated floral induction.

2. Materials and methods

2.1. Plant materials

The *R. sativus* F₁ hybrid, G2 × CH F₁, which exhibits high FT expression levels (Motoki et al., 2022), was used in the following experiments to ensure genetic uniformity while avoiding inbreeding depression often observed in advanced inbred lines. In our previous study, G2 × CH F₁ showed the highest FT expression among the five early-flowering radish accessions examined and induced flowering in the grafted cabbage scions at a rate of 80–100% within two months (Motoki et al., 2022). The hybrid was generated by crossing *R. sativus* G2-IL1 (an inbred line of the seed-pod-harvesting type radish isolated from accession no 76703, Genebank Project, NARO, Japan) with CH-IL1 (an inbred line of the seed-pod-harvesting type radish isolated from the cultivar ‘Rat-tailed’, Chiltern Seeds, United Kingdom). In addition, *B. oleracea* TO1000DH3 (accession no CS29002, Arabidopsis Biological Resource Center, The Ohio State University, USA), *Brassica rapa* ‘40 DAYS RAPE’ (accession no 37407, Genebank Project, NARO, Japan), and *R. sativus* ‘Hayabutori-shogin’ (Takii Seed Co., Japan) were used in the experiments described below.

2.2. Growing conditions

Seeds of each plant species were germinated in darkness at 23°C for one day and then sown into 6.0-cm pots filled with granular rockwool

(Nippon Rockwool Corp., Japan) for Experiments 2.4.1–2.4.3, or into 6.0-cm rockwool cubes (Nippon Rockwool Corp.) for Experiments 2.4.4–2.4.5, to initiate cultivation under the designated conditions. The air temperature was maintained at 22°C in all incubators and growth rooms. Watering and fertilization were performed by bottom irrigation using half-strength Enshi-shoho nutrient solution (formulated by the National Horticultural Research Station, Japan).

2.3. Light sources and adjustment of the R/FR ratio

Light sources with different R/FR ratios (0.05–2) were generated by combining daylight-type fluorescent lamps (FL10EX-D-X, Panasonic Corp., Japan) with far-red LEDs (peak wavelength 730 nm; Shenzhen

Chanzon Technology Co., Ltd., China). Fig. 1 shows representative spectral distributions of the light sources with R/FR ratios of 2, 1, and 0.3. The R/FR ratio was measured using a light analyzer (LA-105; NK System, Japan) and was calculated as the sum of photon flux density (PFD) between 640 and 680 nm divided by the sum of PFD between 710 and 750 nm. The light sources with adjusted R/FR ratios were installed in incubators maintained at 22°C. The photosynthetic photon flux density (PPFD) at the pot surface was 50–60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For the growth of cabbage scions used in grafting experiments, daylight-type LED lamps (40 W-type, 6000 K; NISSIN LUX, Japan) were used, providing an R/FR ratio of approximately 6 and a PPFD of 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The difference in light intensity between the rootstock/post-graft treatments (50–60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and scion growth conditions (120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was due to technical limitations of our growth facility. Photon flux density profiles of representative light conditions used in this study are summarized in Table 1.

2.4. Experimental design

2.4.1. Effect of the R/FR ratio on FT expression patterns in radish

Pots containing germinated G2 $\times$ CH F₁ radish seeds were transferred to incubators set at R/FR ratios of 2, 1, 0.3, 0.1, and 0.05 under a 16-h photoperiod (16 h/8 h, light/dark). Fourteen days after transfer, leaf discs were sampled from the largest fully expanded leaf for gene expression analysis at 0, 4, 8, 12, 16, 20, and 24 h after the start of the light period (Zeitgeber time; ZT0, 4, 8, 12, 16, 20, and 24). For plants grown under R/FR ratios of 0.1 and 0.05, sampling was conducted only at ZT4 and ZT16.

2.4.2. Effects of the combination of low R/FR ratio and photoperiod on FT expression levels in radish

Pots containing germinated G2 $\times$ CH F₁ radish seeds were transferred to an incubator set at an R/FR ratio of 0.1 and grown under photoperiods of 16-h (16 h/8 h, light/dark), 20-h (20 h/4 h, light/dark), or 24-h (continuous light). Fourteen days after transfer, leaf discs were sampled from the largest fully expanded leaf for gene expression analysis at ZT0, 4, 8, 12, 16, 20, and 24. Cultivation was continued until the first flower opened, and the number of true leaves developed before the formation of the terminal inflorescence was recorded at the end of the experiment.

2.4.3. Comparison of the far-red-enriched ultra-long-day (ULD-FR) with the end-of-day far-red (EOD-FR) condition

Pots containing germinated G2 $\times$ CH F₁ radish seeds were transferred to incubators programmed for a 20-h photoperiod (20 h/4 h, light/dark) under the following light conditions: (1) R/FR = 2 throughout the 20-h light period (ZT0–ZT20); (2) R/FR = 2 for the first 17 h (ZT0–ZT17) followed by R/FR = 0.1 for three hours during ZT17–ZT20 (EOD-FR condition); and (3) R/FR = 0.1 throughout the 20-h light period (ZT0–ZT20) (ULD-FR condition). Plants were grown until flowering, and the number of days to the first flower opening and the number of true leaves developed before the formation of the terminal inflorescence were recorded for each plant.

2.4.4. Effects of the ULD-FR condition on graft-mediated floral induction in cabbage grafted onto radish

Pots containing germinated G2 $\times$ CH F₁ radish seeds were transferred to incubators set at an R/FR ratio of 2 under a 16-h/8-h (light/dark) photoperiod. Germinated seeds of cabbage ‘Matsunami’ were sown to 128-cell trays and grown in a growth room equipped with daylight-type LED lamps (R/FR = 6, PPFD = 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Plants at 4–5 weeks after sowing were used as scions for grafting. When radish rootstocks had bolted, cabbage scions were wedge-grafted onto the radish flowering stems following the method described by Motoki et al. (2022).

After grafting, plants were grown under three light conditions: (1) R/FR = 2, 16-h photoperiod (conventional method, control); (2) R/FR =

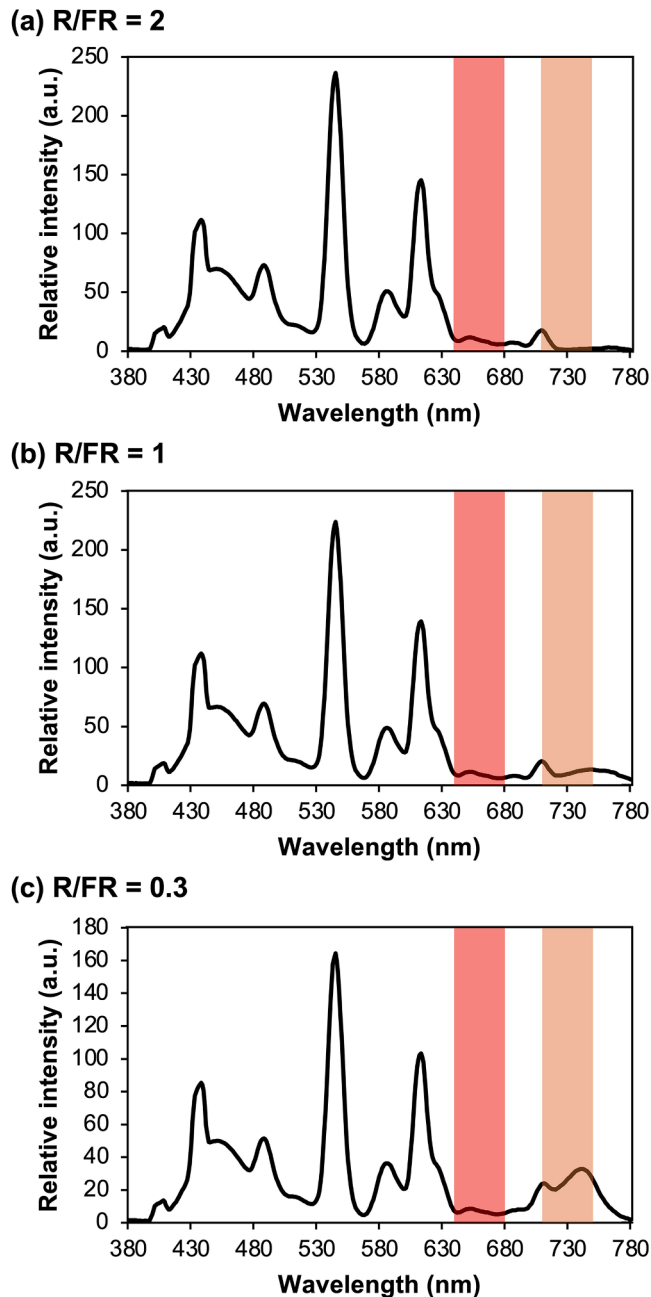


Fig. 1. Spectral distributions of light sources used in the experiments. The R/FR ratio was adjusted by adding FR-LEDs to daylight fluorescent lamps. Spectra of the light sources are shown for (a) R/FR = 2, (b) R/FR = 1, and (c) R/FR = 0.3.

Table 1Photon flux density ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) profiles of the representative light conditions used in this study.

Light conditions	Blue (400–500 nm)	Green (500–600 nm)	Red (600–700 nm)	Far-red (710–750 nm)	R/FR ratio*	Total PPFD
Fluorescent lamps (R/FR = 2)	23	23	14	1	2	60
Fluorescent lamps with far-red LEDs (R/FR = 1)	23	23	14	2	1	60
Fluorescent lamps with far-red LEDs (R/FR = 0.3)	23	23	14	7	0.3	60
Daylight-type LED lamps (R/FR = 6)	33	57	30	1	6	120

*The R/FR ratio was calculated as the sum of photon flux density (PFD) between 640 and 680 nm divided by the sum of PFD between 710 and 750 nm, whereas the “Red (600–700 nm)” column represents total red photons contributing to photosynthetically active radiation.

0.1, 16-h photoperiod (LD-FR); and (3) R/FR = 0.1, 24-h photoperiod (ULD-FR). Defoliation of the scion leaves began when seven leaves exceeding 2 cm in length had expanded, and leaves larger than 5 cm in length were continuously removed thereafter to maintain the sink activity of the scion. On day 21 after grafting, the midribs of the upper leaves (1.5–2.5 cm in length) of the cabbage scions were sampled for FT protein quantification. Plants were grown for up to 56 days after grafting.

To examine the combined effects of ultra-long-day and low R/FR ratio, an additional experiment was conducted under three post-grafting light conditions: (1) R/FR = 2, 16-h photoperiod (conventional method, control); (2) R/FR = 2, 24-h photoperiod (ULD); and (3) R/FR = 0.1, 24-h photoperiod (ULD-FR). The conditions for seedling growth, grafting, and cultivation were the same as described above. Plants were grown for 56 days after grafting, and leaf blades of the largest expanded leaves of the radish rootstocks were sampled at ZT4 and ZT16, two weeks after grafting, for analysis of FT gene expression.

2.4.5. Effects of the ULD-FR condition on flowering in other Brassicaceae species

Pots containing germinated seeds of *B. rapa* ‘40DAYS RAPE’ and *B. oleracea* TO1000DH3 were transferred without vernalization treatment to the following light conditions: (1) R/FR = 2, 16-h photoperiod (control); (2) R/FR = 0.1, 16-h photoperiod (LD-FR); and (3) R/FR = 0.1, 24-h photoperiod (ULD-FR). Each plant was cultivated for 41 days after sowing, and flowering responses were evaluated based on (i) the number of true leaves developed before the formation of the terminal inflorescence and (ii) the presence or absence of flowering at the end of cultivation.

For *R. sativus* ‘Hayabutori-shogoin’, pots containing germinated seeds were transferred without vernalization treatment to the following light conditions: (1) R/FR = 2, 16-h photoperiod (control); (2) R/FR = 2, 24-h photoperiod (ULD); (3) R/FR = 0.1, 16-h photoperiod (LD-FR); and (4) R/FR = 0.1, 24-h photoperiod (ULD-FR). Each plant was cultivated for 37 days after seed sowing. Days to first flower opening after seed sowing were monitored continuously, and flower bud differentiation was examined under a microscope at the end of the cultivation period.

2.5. Gene expression analysis

Total RNA was extracted from the sampled leaf tissues using a modified version of the Direct-TRI method described by Ujibe et al. (2021), in which powdered frozen leaf tissue was homogenized in Sepasol RNA I Super G (Nacalai Tesque, Inc., Japan), and RNA was subsequently purified using Econospin™ for RNA (Epoch Life Science, USA). Purified RNA was reverse-transcribed using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Japan) according to the manufacturer’s instructions.

One microliter of fivefold-diluted cDNA was used as a template for quantitative reverse transcription-PCR (RT-qPCR) in a total reaction volume of 10 μL . RT-qPCR was conducted using the THUNDERBIRD® Next SYBR™ qPCR Mix (Toyobo Co., Ltd.) in accordance with the manufacturer’s instructions, with amplification performed on a Thermal Cycler Dice® Real Time System II (Takara Bio Inc., Shiga, Japan). The

thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s. Amplification specificity was confirmed by melting curve analysis. RT-qPCR primers were designed for *RsFTa* (Forward: 5'-GGACGAAGAAGTTAGATGAGCCTC-3'; Reverse: 5'-CATTGCTCGTATAGTAAAAGGCTTC-3'), and for *RsActin* (Forward: 5'-ACAA-CACCATGCTCAATAGG-3', Reverse: 5'-ATCATGGTGTGTCATGGTTGGG-3'). Among the two FT homologs in radish (*RsFTa* and *RsFTb*; (Mitsui et al., 2023)), *RsFTa* was analyzed because it exhibits higher expression levels than *RsFTb* (Motoki et al., 2025).

2.6. Immunoblotting analysis

Protein extraction and immunoblotting were performed following the methods described by Motoki et al. (2022) with minor modifications. For total protein extraction, tissues were frozen in liquid nitrogen in microcentrifuge tubes, ground by bead beating using a TissueLyser II (Qiagen, USA), and homogenized in approximately five volumes of extraction buffer [50 mM Tris-HCl (pH 9.0), 2% (w/v) sodium dodecyl sulfate (SDS), 5 mM ascorbic acid, 0.1% (v/v) 2-mercaptoethanol, and 1 $\times$ protease inhibitor cocktail (P9599; Sigma-Aldrich Co. LLC, USA)]. The homogenate was centrifuged twice at 12,000 $\times$ g for 10 min, and the resulting supernatant was used for subsequent analysis. The total soluble protein concentration was determined using an XL Bradford assay (Intégrale Co., Ltd., Japan).

Extracted proteins were mixed with an equal volume of 2 $\times$ SDS sample buffer [125 mM Tris-HCl (pH 6.8), 4% (w/v) SDS, 30% (w/v) glycerol, bromophenol blue, and 10% (v/v) 2-mercaptoethanol] and heated at 95°C for 3 min. A total of 15 μg of protein was loaded onto a 15% (w/v) polyacrylamide gel (e-PAGEL E-R15L; ATTO Corp., Japan) and electrophoresed for 40 min at a constant current of 20 mA per gel. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Trans-Blot Turbo RTA Transfer Kit, Bio-Rad Laboratories, Inc., USA) using the Trans-Blot Turbo system for 7 min (Mixed MW protocol). After transfer, membranes were stained with Ponceau S solution (Beacle, Inc., Japan) and scanned to quantify total protein loading for normalization. Membranes were blocked with PBST (phosphate-buffered saline containing 0.1% Tween 20) containing 1% skim milk for 1 h at room temperature (20–25°C), then incubated with an anti-FT antibody (Motoki et al., 2022) diluted to 300 ng mL⁻¹ in Can Get Signal® Immunoreaction Enhancer Solution 1 (Toyobo Co., Ltd.). Subsequently, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:20,000 dilution; Cell Signaling Technology, Inc., USA) in Can Get Signal® Solution 2 (Toyobo Co., Ltd.) at room temperature.

For chemiluminescent detection, ECL™ Start Western Blotting Detection Reagent (RPN3243, Cytiva, Japan) was applied to the membranes, and signals were visualized using a ChemiDoc™ Touch Imaging System (Bio-Rad Laboratories, Inc.). The FT protein band intensity was normalized to total protein loading quantified from the corresponding Ponceau S-stained membrane (Romero-Calvo et al., 2010) and further normalized to a positive control (a protein extract mixture from the leaf petiole of a cabbage grafted onto radish showing a high level of FT accumulation) included on the same blot.

2.7. Statistical analysis

Tukey's HSD post hoc tests were conducted for continuous data following one-way ANOVA using the 'TukeyHSD' function from the 'stats' package when the assumptions of normality and homogeneity of variance were satisfied. Normality of residuals and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. For data that did not meet the assumptions of normality or homogeneity of variance, including discretized flowering data, pairwise Wilcoxon comparisons with Bonferroni adjustment were performed using the 'pairwise_wilcox_test' function from the 'rstatix' package. For Fig. 4, to test the a priori hypothesis that ULD-FR conditions increase *RsFTa* expression and promote flowering relative to the conventional 16-h photoperiod, planned one-sided Wilcoxon tests were conducted, comparing the 20-h and 24-h photoperiods individually against the 16-h control with Bonferroni correction for the two comparisons. All analyses were conducted in R (version 4.3.2; R Core Team, 2023).

3. Results

3.1. Effect of R/FR ratio on the diurnal expression pattern of *RsFTa*

Under light conditions with an R/FR ratio of 2, *RsFTa* expression showed a single peak at the end of the light period (ZT16) (Fig. 2). When the proportion of far-red light increased (R/FR = 1.0 or 0.3), an additional expression peak appeared at ZT4, shortly after the onset of the light period. Under the lowest R/FR condition (R/FR = 0.3), *RsFTa* expression remained elevated throughout the daytime. These results show that reducing the R/FR ratio induces an additional *RsFTa* expression peak in the early light phase and increases its overall expression levels in radish, consistent with previous observations in *Arabidopsis* (Song et al., 2018).

To further investigate the effects of lower R/FR ratios, *RsFTa* expression was examined under additional light conditions (R/FR = 0.1 and R/FR = 0.05). The expression level at ZT4 increased as the R/FR ratio decreased and reached a plateau at R/FR = 0.1 (Fig. 3). Although some variations were observed at ZT16, *RsFTa* expression tended to be higher under lower R/FR ratios at this time point, with the maximum level also detected at R/FR = 0.1.

3.2. Effects of the combination of low R/FR ratio and photoperiod on *FT* expression levels in radish

The effect of photoperiod length on *RsFTa* expression was examined under R/FR ratio of 0.1, which maximized its induction in the previous experiment. Compared with the commonly used long-day condition in

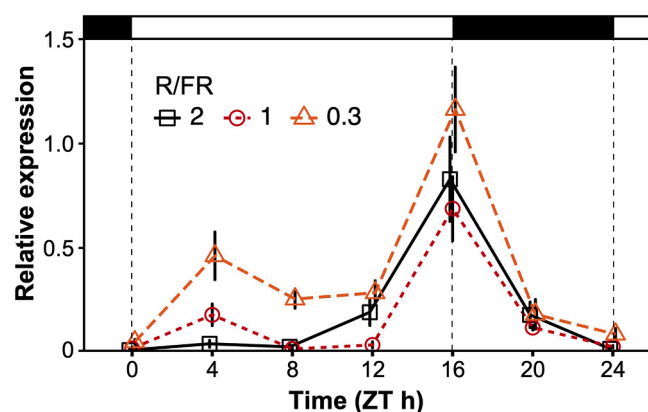


Fig. 2. Diurnal expression pattern of the *RsFTa* gene in radish grown under light sources with different R/FR ratios (R/FR = 2–0.3). Bars represent mean $\pm$ SE (n = 3–4).

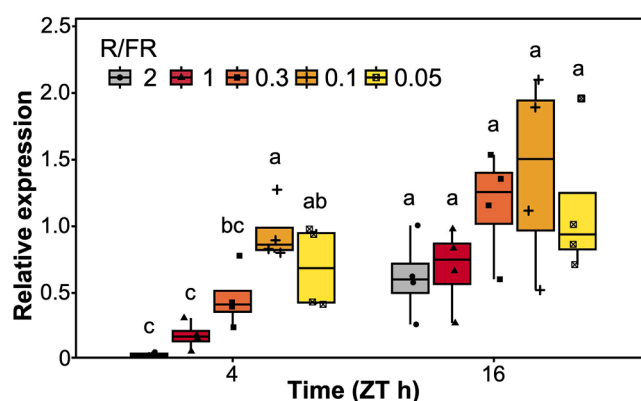


Fig. 3. Relative expression levels of the *RsFTa* gene in radish grown under light sources with different R/FR ratios (R/FR = 2–0.05) at ZT4 and ZT16 (n = 4). Note that the data for R/FR = 2, 1, and 0.3 are the same as those shown in Fig. 2. Different letters indicate significant differences among light sources at each time point based on one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Brassicaceae (16-h photoperiod), longer photoperiods markedly affected the temporal pattern of *RsFTa* expression (Fig. 4a). As the photoperiod increased, the period during which *RsFTa* was expressed extended, and under the 24-h photoperiod, *RsFTa* expressed at high levels throughout

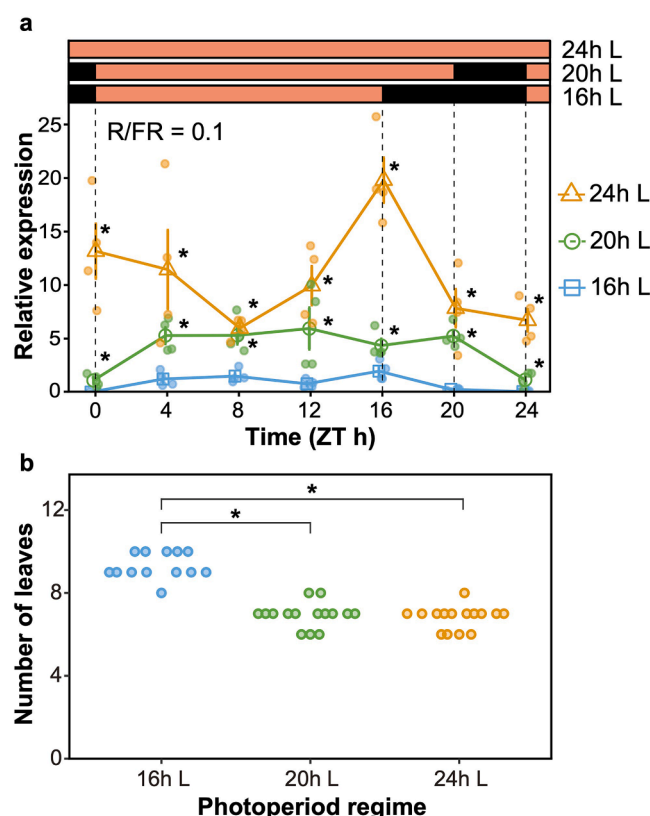


Fig. 4. Diurnal expression pattern of the *RsFTa* gene and flowering response of radish grown under ultra-long-day photoperiods with an R/FR ratio of 0.1. (a) Expression pattern of the *RsFTa* gene. Bars represent mean $\pm$ SE (n = 4). Asterisks indicate statistically significant differences compared with the 16-h photoperiod at the same time point based on planned one-sided Wilcoxon tests with Bonferroni correction (16-h vs. 20-h and 16-h vs. 24-h, $p < 0.05$). (b) Number of leaves produced before the terminal inflorescence (n = 13–15). Brackets with asterisks indicate statistically significant differences compared with the 16-h photoperiod based on planned one-sided Wilcoxon tests with Bonferroni correction (16-h vs. 20-h and 16-h vs. 24-h, $p < 0.05$).

the day. Remarkably, the overall *RsFTa* transcript abundance also increased substantially under the 24-h photoperiod, with mean daily expression levels more than tenfold higher than those under the 16-h photoperiod (Fig. 4a). When data from all time points (ZT0–ZT24) were pooled, *RsFTa* expression levels under the 20-h and 24-h photoperiods were significantly higher than those under the 16-h photoperiod (planned one-sided Wilcoxon tests with Bonferroni correction, < 0.05). In addition, the variation in *RsFTa* expression among individuals at the same time point was larger under the 24-h photoperiod than under shorter photoperiods, which may partly contribute to the apparent fluctuations observed in the time-course data. Nevertheless, the difference between conventional and ultra-long-day photoperiods (16 h vs. 20 h and 24 h) was clear, and *RsFTa* expression levels were consistently higher under the 20-h and 24-h photoperiod than under the 16-h photoperiod at the individual level (Fig. 4a).

Consistent with these gene expression data, plants grown under the 20-h and 24-h photoperiods at an R/FR ratio of 0.1 developed markedly fewer true leaves before the formation of the terminal inflorescence compared with those under the 16-h photoperiod (Fig. 4b). This flowering promotion supported the interpretation that a low R/FR ratio combined with ultra-long-day conditions enhances *RsFTa* expression.

We hereafter refer to this far-red-enriched ultra-long-day photoperiod as the “ULD-FR” condition (Ultra-Long-Day with Far-Red enrichment).

3.3. Comparison of the ULD-FR with the EOD-FR condition

In several Brassicaceae species, shade-avoidance responses can be induced by FR light irradiation at the end of the light period, known as the end-of-day FR (EOD-FR) treatment (Franklin and Whitelam, 2005; Takemura et al., 2020). To compare the effect of EOD-FR irradiation with that of ULD-FR, radish plants were grown under a 20-h photoperiod with either (1) no FR addition (control) (2) a 3-h low R/FR light period (R/FR = 0.1) at the end of the light phase (EOD-FR) or (2) continuous low R/FR light (R/FR = 0.1) throughout the light phase (ULD-FR) (Fig. 5a). The flowering response was evaluated as a proxy of *RsFTa* expression levels. Flowering was more strongly promoted in plants under the ULD-FR condition, as indicated by both a shorter time to flowering and a reduced number of true leaves formed before inflorescence initiation (Fig. 5b, c). These results suggest that the enhancement of *RsFTa* expression and the promotion of flowering observed under the ULD-FR condition cannot be fully achieved by EOD-FR treatment alone; instead, prolonged exposure to low R/FR light appears to be crucial for maximum induction.

3.4. Effects of the ULD-FR on graft-mediated floral induction in cabbage grafted onto radish

Based on the above findings, we next examined whether 24-h-ULD-FR conditions, that maximized *RsFTa* expression in the rootstock, could promote flowering in graft-mediated floral induction of cabbage (Fig. 6a). The cabbage cultivar ‘Matsunami’ was used because it is a vernalization-requiring cultivar and is therefore difficult to induce flowering without cold treatment. This cultivar was also used in our previous grafting study (Motoki et al., 2022), allowing direct comparison with earlier results. Under the control condition (R/FR = 2, 16-h photoperiod), none of the grafted plants flowered by the end of the cultivation period (56 days after grafting), although some plants initiated flower bud formation (Fig. 6b, c). This response was weaker than in our previous study (Motoki et al., 2022), where 80–100% of grafted plants flowered by 63 days after grafting. The difference is likely due to the lower light intensity used in the current experiment (PPFD 50–60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) compared with the previous conditions (PPFD 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). In contrast, plants grown under LD-FR condition (R/FR = 0.1, 16-h photoperiod) flowered on average 44.0 days after grafting, and flowering was further accelerated under ULD-FR condition (R/FR = 0.1,

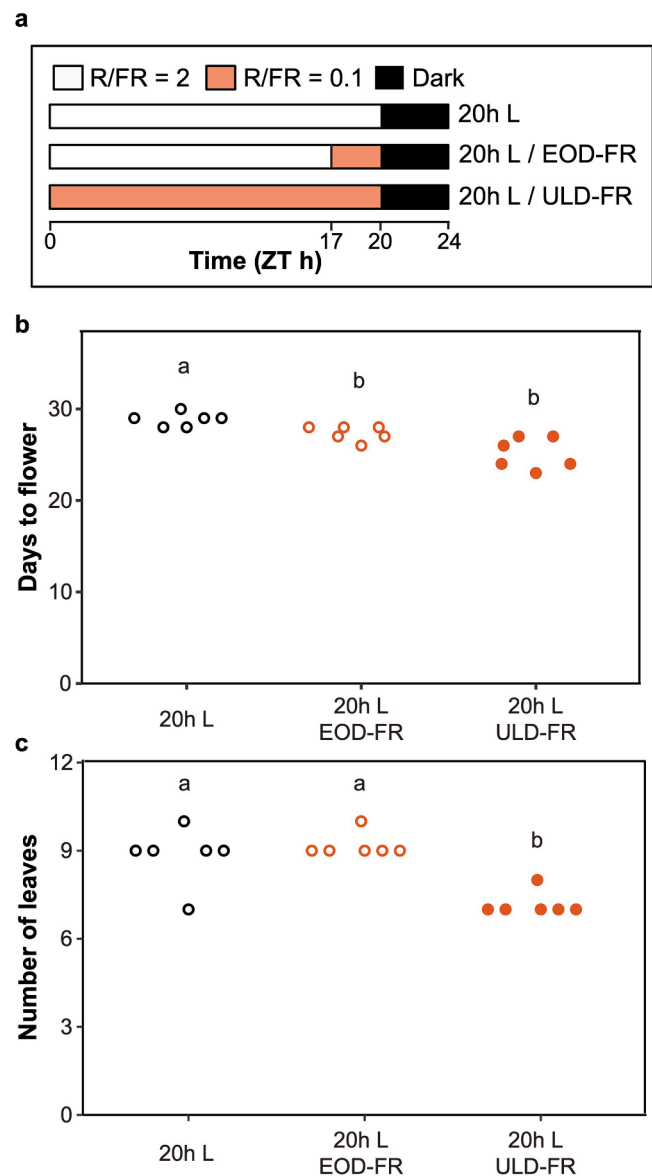


Fig. 5. Comparison of a far-red-enriched ultra-long-day (ULD-FR) with the end-of-day far-red (EOD-FR) condition for the floral promotion. (a) Light irradiation schedules of each condition. (b) Days from sowing to the first flower opening ($n = 6$). (c) Number of leaves expanded before the formation of terminal inflorescence ($n = 6$). Different letters in panels (b) and (c) indicate significant differences among light conditions based on pairwise Wilcoxon tests with Bonferroni correction ($p < 0.05$).

24-h photoperiod), occurring on average at 30.0 days after grafting (Fig. 6b, c). Correspondingly, the amount of FT protein accumulated in the scion at 21 days after grafting increased markedly in the order: control < LD-FR < ULD-FR (Fig. 6d, e). These results demonstrate that low R/FR light, and particularly its combination with an ultra-long-day photoperiod, greatly enhances the supply of FT protein from radish rootstocks to cabbage scions, thereby markedly promoting graft-mediated floral induction. It should be noted, however, that a minor carry-over effect of the scion pre-growth light environment (R/FR = 6) cannot be completely excluded in our experiment; nevertheless, the spectral shift after grafting was larger in the ULD-FR treatments than in the control, making it unlikely that the control condition was selectively disadvantaged by changes in light quality.

To further verify whether this effect was specifically due to the combination of low R/FR light and ultra-long day, we investigated the

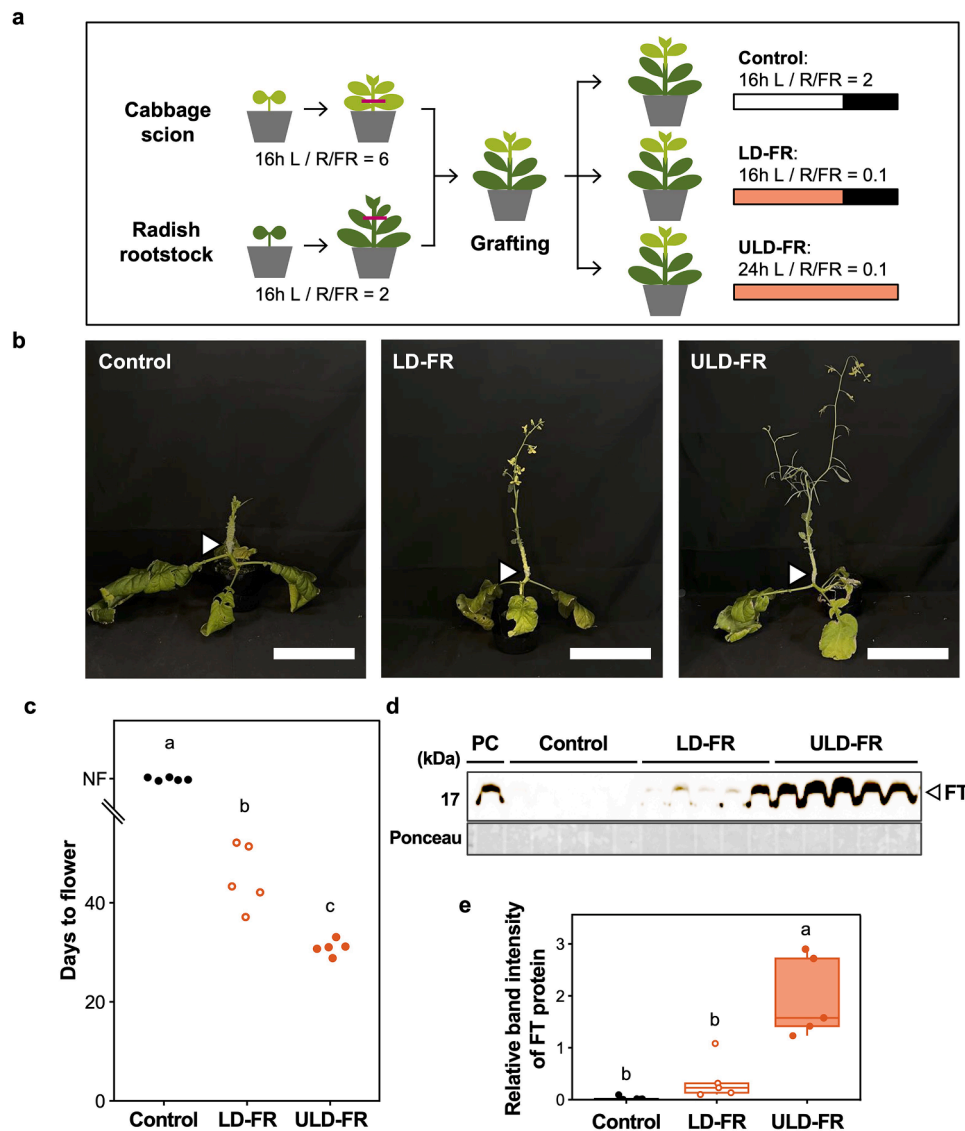


Fig. 6. Effect of the ULD-FR condition on the graft-mediated floral induction in cabbage. (a) Overview of experimental conditions. (b) Representative grafted plants at the end of cultivation (56 days after grafting). White triangles indicate graft unions. Scale bars = 10 cm. (c) Days from grafting to the opening of the first flower (n = 5). NF, no flowering. Different letters indicate significant differences among light conditions according to the pairwise Wilcoxon tests with Bonferroni correction ($p < 0.05$). (d) Immunoblotting analysis of FT protein accumulation in scions 21 days after grafting. “PC” denotes the positive control. (e) Quantification of the results shown in (d) (n = 5). Different letters indicate significant differences among light conditions based on one-way ANOVA followed by Tukey’s HSD post hoc test ($p < 0.05$).

flowering response of grafted plants grown under ultra-long day with high R/FR light (ULD condition, Fig. 7a). Under both the control condition (R/FR = 2, 16-h photoperiod) and the ULD condition (R/FR = 2, 24-h photoperiod), all plants did not flower by 56 days after grafting, although floral bud initiation was occasionally observed (Fig. 7a, b). In contrast, plants exposed to the ULD-FR condition (R/FR = 0.1, 24-h photoperiod) exhibited early flowering, with a mean time to flowering of 34.6 days (Fig. 7a, b). Gene expression analysis of the radish rootstocks revealed that *RsFTa* transcript levels remained relatively low under both the control and ULD conditions, whereas *RsFTa* expression was markedly upregulated only under the ULD-FR condition (Fig. 7c). These findings confirm that ultra-long day alone is insufficient to induce strong *RsFTa* expression or promote flowering in the grafted cabbage; rather, the combination of a low R/FR ratio and ultra-long day is essential to upregulate *FT* expression in the rootstock and achieve efficient graft-mediated floral induction.

3.5. Effects of the ULD-FR conditions on flowering in other Brassicaceae species

To examine whether the ULD-FR condition also promote flowering in other Brassicaceae crops, we evaluated the flowering responses of rapid-cycling lines of *B. rapa* and *B. oleracea*. In both species, the number of true leaves produced before the formation of the terminal inflorescence decreased under low R/FR conditions (R/FR = 0.1, 16-h or 24-h photoperiod) compared with the control condition (R/FR = 2, 16-h photoperiod) (Fig. 8a, b). Moreover, the proportion of plants that flowered was markedly higher under the ULD-FR condition (R/FR = 0.1, 24-h photoperiod). Representative plants of *B. rapa* grown for approximately one month after sowing are shown in Fig. 8c, illustrating vigorous flowering under the ULD-FR condition and showing that flowering was strongly accelerated.

We also examined the effect of the ULD-FR condition on the vernalization-requiring plants. Japanese root-type radish ‘Hayabutori-shogoin’, which does not flower without overwintering under summer-

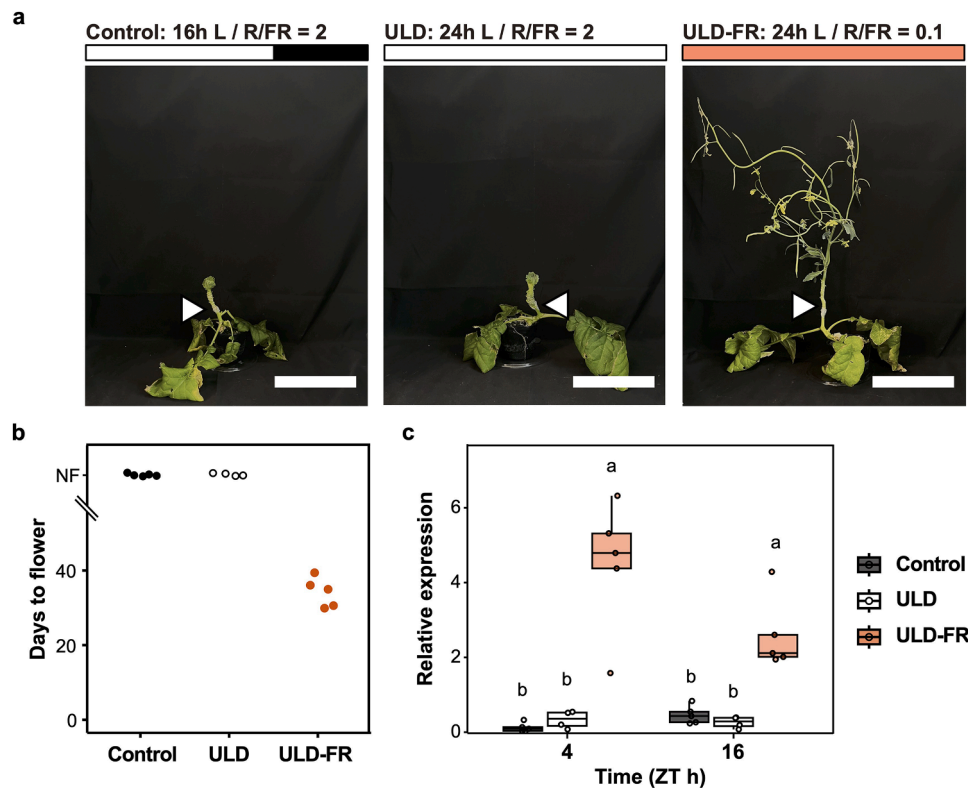


Fig. 7. Comparison of the effects of an ultra-long-day photoperiod combined with high or low R/FR light on the graft-mediated floral induction in cabbage. (a) Representative grafted plants at the end of cultivation (56 days after grafting). White triangles indicate graft unions. Scale bars = 10 cm. (b) Days from grafting to the first flower opening ($n = 4-5$). Different letters indicate significant differences among light conditions according to the pairwise Wilcoxon tests with Bonferroni correction ($p < 0.05$). (c) Relative expression levels of the *RsFTa* gene in radish rootstock leaves 14 days after grafting ($n = 4-5$). Different letters indicate significant differences among light conditions at the same time point based on pairwise Wilcoxon tests with Bonferroni correction ($p < 0.05$). Sample size: $n = 5$ for the control and ULD-FR conditions, and $n = 4$ for the ULD condition, where one individual was excluded due to grafting failure.

sown field conditions, was grown without seed vernalization treatment under different combination of R/FR ratio and photoperiod (Fig. 8d). Plants under ULD-FR condition began flowering as early as three weeks after seed sowing (Fig. 8d, e). In contrast, plants grown under control, ULD, and LD-FR conditions remained vegetative at the end of the cultivation period (five weeks after seed sowing), except for one plant under the LD-FR condition that initiated flower bud differentiation (Fig. 8d, e). These results indicate that the combination of a low R/FR ratio and ultra-long day markedly promotes flowering not only in specific radish genotypes but also in other Brassicaceae species.

4. Discussion

4.1. A far-red-enriched ultra-long-day condition induces a constitutively high *FT* expression in radish

Our results demonstrate that the combination of a low R/FR ratio and an ultra-long-day photoperiod synergistically induces a constitutively high *RsFTa* expression pattern that has not been previously described in radish or other Brassicaceae crops. *RsFTa* expression increased progressively as the R/FR ratio decreased, and an additional peak appeared in the early light phase (Fig. 2-3). These responses are consistent with earlier studies in Arabidopsis, where an R/FR ratio around 1.0 induces a morning *FT* peak (Song et al., 2018), and with reports showing that even lower R/FR ratios can enhance *FT* expression (Kim et al., 2008; Wollenberg et al., 2008).

A key finding of this study was that, under such low R/FR conditions, extending the photoperiod beyond the commonly used 16-h long day not only prolonged the period during which *RsFTa* was expressed but also dramatically increased its overall expression level (Fig. 4).

Importantly, *RsFTa* expression levels were consistently higher under the 20-h and 24-h photoperiods than under the conventional 16-h photoperiod at the individual level during the light phase (ZT4–ZT20), supporting a consistent inductive effect of ULD-FR. It was also observed that *RsFTa* expression under the 24-h photoperiod showed increased inter-individual variability compared with shorter photoperiods (Fig. 4a). This variability may reflect multiple, non-exclusive factors, including partial loss of circadian coordination under continuous light, developmental heterogeneity, or stochastic gene expression, which remain to be directly tested. Although the underlying regulatory mechanisms remain to be clarified, the observed responses may result from the combined effects of low R/FR light irradiation and the ultra-long-day condition on the light signaling pathway. In Arabidopsis, *FT* expression normally declines during the dark phase due to CO degradation and Polycomb-mediated chromatin repression (Jang et al., 2008; Luo et al., 2025). Loss of Polycomb factors such as *CLF-PRC2* or *EMF1* leads to strong constitutive *FT* de-repression (Luo et al., 2018; Wang et al., 2014), resembling the *RsFTa* expression pattern observed under ULD-FR in this study (Fig. 4a). The decrease of dark-induced repression under continuous light may contribute to the sustained *RsFTa* expression. This possibility is proposed here as a testable hypothesis, and molecular validation will be required to confirm this mechanism. In particular, examining the expression of Polycomb factors and histone modifications at the *RsFTa* locus, using techniques such as chromatin immunoprecipitation sequencing (ChIP-seq), would provide direct evidence.

However, an ultra-long-day photoperiod alone was insufficient to induce high *RsFTa* expression; even under a 24-h photoperiod, *RsFTa* levels remained low when the R/FR ratio was high (Fig. 7c). This indicates that, in addition to the decrease in dark-phase repression, mechanisms associated with low R/FR are essential for strong *RsFTa*

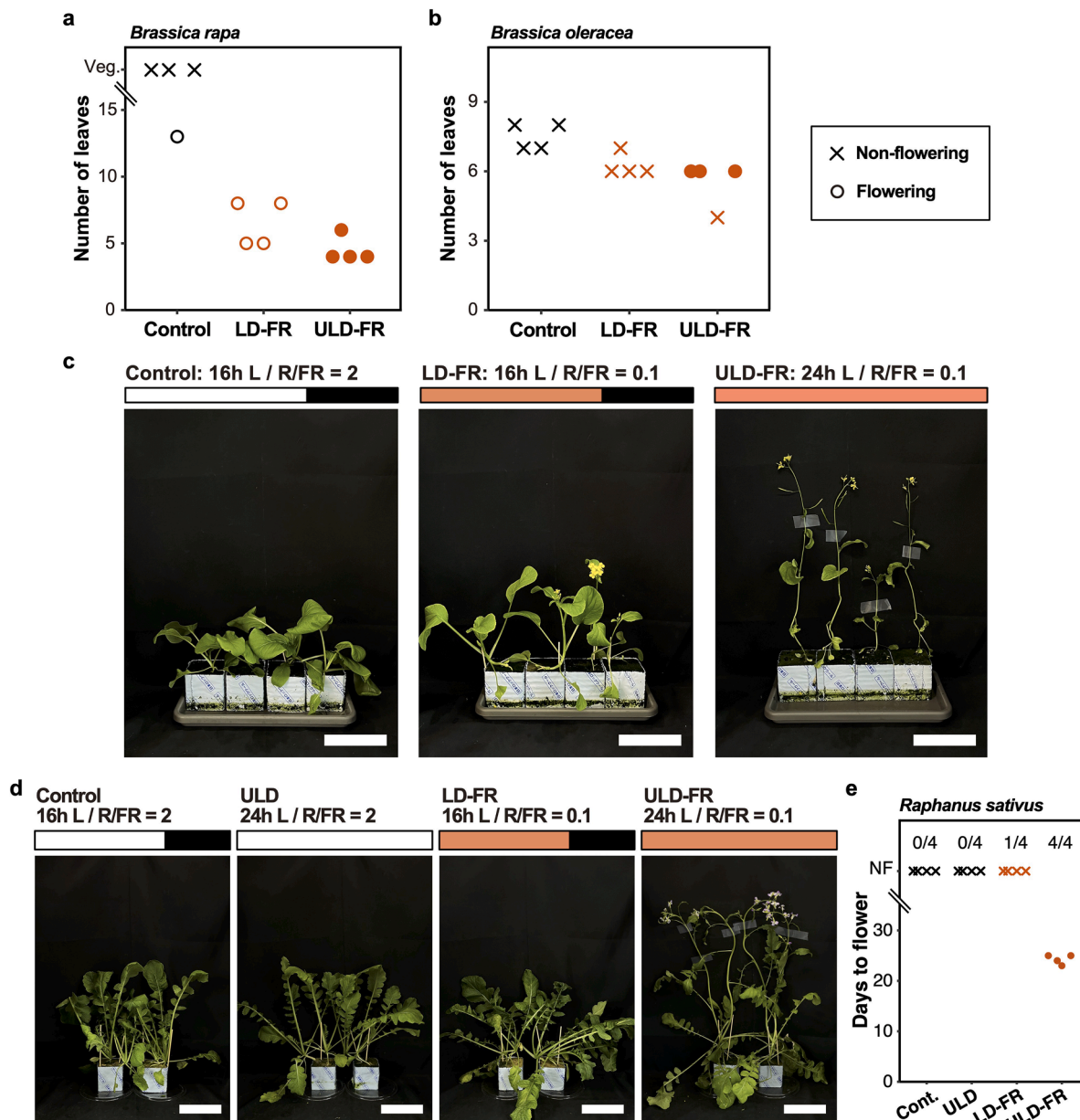


Fig. 8. Effects of the ULD-FR conditions on flowering in several Brassicaceae species. (a) Number of leaves produced before the terminal inflorescence in *B. rapa* '40DAYS RAPE' ($n = 4$) and (b) *B. oleracea* 'TO1000DH3' ($n = 4$). Circles and crosses represent plants that flowered or did not flower, respectively, within 41 days after sowing. Veg: plants that continued vegetative growth without visible flower buds within 41 days after sowing. The non-flowering individual of *B. oleracea* in the 24 L, R/FR = 0.1 condition showed early flower bud formation but exhibited poor growth and did not open flowers. (c) Photograph showing flowering status of *B. rapa* '40DAYS RAPE' 31 days after sowing. Arrows indicate flowers at anthesis or post-anthesis. Scale bars = 10 cm. (d) Photograph showing flowering status of the vernalization-requiring *R. sativus* cultivar 'Hayabutori-shogoin' grown under non-vernalized conditions at 26 days after seed sowing. Scale bars = 10 cm. (e) Days to first flower opening after seed sowing in 'Hayabutori-shogoin' ($n = 4$). Circles and crosses indicate plants that flowered or did not flower, respectively, within 37 days after sowing. NF, no flowering. Numbers above the data points indicate the number of plants that had initiated flower bud differentiation by 37 days after sowing.

induction. A recent study in *Arabidopsis* demonstrated that the *FT* expression pattern under long-day conditions is regulated by two distinct mechanisms: the external coincidence mechanism, which contributes to the dusk peak, and a phyA-mediated high-irradiance response (HIR), which accounts for the morning peak (Lee et al., 2023). The dusk *FT* peak is defined by the circadian clock and its magnitude is affected by phyB (Song et al., 2015). Consistent with this mechanism, the dusk *FT* peak can be enhanced by a 2-h FR pulse in the late afternoon (Lee et al., 2023), a condition analogous to EOD-FR treatment, which is suggested to be mediated primarily by phyB (Bagnall and King, 2001). In contrast, the morning *FT* peak requires cumulative exposure to low R/FR through a phyA-dependent HIR, in which PHYTOCHROME-INTERACTING

FACTOR 7 (PIF7), together with PIF4 and PIF5, mediates *FT* induction (Lee et al., 2023). Because ULD-FR induced stronger flowering promotion than EOD-FR (Fig. 5), the strong and constitutive *RsFTa* activation observed under ULD-FR may reflect the combined contribution of the phyB-related external coincidence mechanism and sustained activation of the phyA-HIR pathway. This interpretation is based on indirect evidence, and the involvement of these pathways has not been directly tested in this study. Future validation could be achieved by examining the responses of mutants in genes involved in these pathways, either in radish or closely related model species.

The effect of light intensity on ULD-FR-induced *FT* expression remains to be determined. All experiments in this study were conducted

under a relatively low light intensity (PPFD 50–60 $\mu\text{mol m}^{-2} \text{s}^{-1}$), due to technical limitations of our facility in setting up independent growth environments for multiple photoperiod and spectral conditions. Although the strong effects of ULD-FR on *FT* expression and flowering promotion were clearly observed even under this relatively low light intensity, *FT* expression is known to be influenced by light intensity (King et al., 2008). At the same time, previous studies in *Arabidopsis* have also reported enhanced *FT* expression and accelerated flowering under low R/FR conditions at higher light intensities (PPFD 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Kim et al., 2008; PPFD 90–110 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Lee et al., 2023). These findings highlight the importance of systematically examining how the magnitude of the ULD-FR effect depends on light intensity. In particular, determining the range of effective light intensities, including whether a minimum and/or a maximum light intensity threshold exists for this response, will be essential for both mechanistic understanding and practical optimization of this approach.

4.2. ULD-FR markedly enhances the *FT*-supplying capacity of radish rootstocks, improving graft-mediated floral induction

Application of the ULD-FR condition markedly enhanced graft-mediated floral induction of cabbage grafted onto radish rootstocks, accompanied by substantially higher accumulation of FT protein in the grafted scions (Figs. 6,7). Cabbage scions grafted under ULD-FR flowered approximately 30 days after grafting, and considering that graft union formation requires about one week, this represents flowering as early as is developmentally feasible. These results highlight the importance of FR enrichment and demonstrate that ULD-FR is highly effective for graft-mediated floral induction. They also reinforce the central concept of the GRAFT method—that the abundance of FT supplied from the rootstock is a key determinant of the success of floral induction (Motoki et al., 2024). Manipulating the light environment therefore provides a powerful strategy to enhance FT-supplying capacity, complementing approaches that exploit genetic variation in radish rootstocks to increase *FT* expression levels (Motoki et al., 2025).

When combined with the GRAFT method, ULD-FR enables cabbage to reach flowering approximately two months after sowing (about one month for seedling growth and one month for flowering after grafting), resulting in an estimated seed-to-seed cycle of around four months when seed maturation is included. In contrast, conventional vernalization-based approaches typically require a longer seedling growth period followed by prolonged low-temperature treatment and subsequent flowering induction, leading to a total cycle of approximately six to seven months for moderately vernalization-requiring cabbage cultivars. Thus, the ULD-FR-based grafting system has the potential to shorten the breeding cycle of cabbage by roughly two to three months, with the magnitude of this advantage possibly varying with genetic background. In addition, this approach reduces space requirements by eliminating the need for large low-temperature facilities, although labor inputs may increase due to the grafting process.

Our findings suggest a potential two-step strategy for optimizing the GRAFT method in combination with ULD-FR conditions. When ULD-FR is applied from the beginning of rootstock development, bolting is triggered extremely rapidly (Fig. 4b; Fig. 8c), resulting in rootstocks that are too small at the time of grafting. Previous studies have shown that a reduction in donor plant size prevents graft-mediated floral induction (Suge, 1984; Zeevaert, 1958), and this inhibition is associated with marked decreases of the amount of FT protein transported to the scion in case of cabbage/radish grafting (Motoki et al., 2022). Accordingly, in this study, ULD-FR was applied only after grafting to avoid insufficient vegetative growth of the rootstocks. Building on this experimental design, we propose a two-step protocol in which rootstocks are first cultivated under more repressive conditions, such as shorter photoperiods without FR enrichment, to promote vegetative growth, and are then transferred to ULD-FR conditions after grafting (Fig. 9). This strategy, involving sufficient vegetative growth followed by strong FT

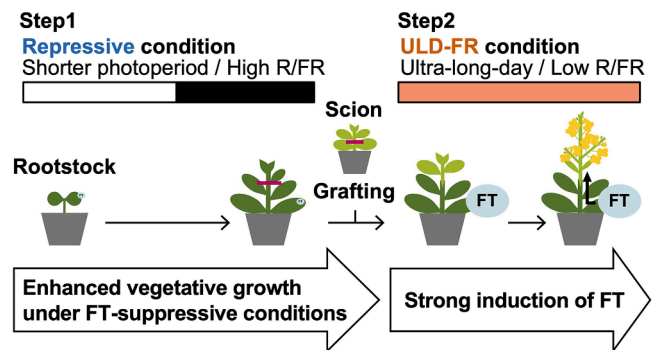


Fig. 9. Proposed two-step protocol for optimizing graft-mediated floral induction in combination with ULD-FR conditions. In the first step, rootstocks are grown under repressive conditions, such as shorter photoperiods with a high R/FR ratio, to promote vegetative growth. After grafting, plants are transferred to ULD-FR conditions to induce strong FT expression, thereby enhancing the overall FT supply capacity of rootstocks and improving graft-mediated floral induction.

induction, could in principle enhance FT supply capacity and improve the efficiency of graft-mediated floral induction. Future comparative studies between this phased protocol and continuous ULD-FR exposure will be valuable to quantitatively evaluate its performance and practical applicability.

4.3. ULD-FR may broadly promote flowering in Brassicaceae species with implications for flowering control in breeding contexts

The flowering of *B. rapa* and *B. oleracea* rapid-cycling accessions, as well as the vernalization-requiring *R. sativus* cultivar was accelerated under ULD-FR (Fig. 8), indicating that the response is not restricted to specific genotypes of radish but may be broadly conserved across Brassicaceae crops. Although further verification using diverse germplasm is required, the strong acceleration observed in multiple species suggests that ULD-FR may have broader applicability. Given the conservation of the FT-dependent flowering pathway across angiosperms (Wickland and Hanzawa, 2015), it is possible that similar responses occur in other long-day plants, although this remains to be tested.

It is intriguing that ULD-FR has potential to overcome the vernalization requirement without low-temperature treatment (Fig. 8d, e). It remains an open question how the ULD-FR-induced upregulation of *FT*, as discussed above, relates to the vernalization flowering pathway, particularly in vernalization-requiring genotypes. *FLOWERING LOCUS C* (*FLC*) is a key regulator of the vernalization pathway in *Arabidopsis*, which represses the expression of floral integrator genes including *FT* under warm conditions, and is epigenetically silenced after prolonged low temperature exposure (Helliwell et al., 2006; Michaels and Amasino, 1999). It has been reported that shade-like conditions created by low R/FR light irradiation can induce *FT* upregulation and early flowering even in vernalization-requiring accessions with active *FLC* expression. Notably, this floral promotion occurs without changes in *FLC* expression levels (Kim et al., 2008; Wollenberg et al., 2008). On the other hand, a subsequent genetic study showed that stronger *FLC* alleles weaken the floral induction effect by low R/FR light conditions (Adams et al., 2009). These findings suggest that low R/FR light irradiation can promote *FT* expression by partially bypassing *FLC*-mediated repression through activation of the light signaling pathway, while also interacting with the vernalization pathway depending on the strength of *FLC* activity. Accordingly, the response to ULD-FR conditions may also vary with *FLC* activity and other genetic backgrounds. Clarifying this potential variation will require future studies using more diverse germplasm.

While we observed that plants induced to flower under ULD-FR conditions produced pollen and set seeds (data not shown),

quantitative evaluation of fertility and seed quality was not performed in this study. A tendency toward stem elongation and reduced biomass was observed in plants grown under ULD-FR conditions (Fig. 8c, d), which is consistent with classical shade-avoidance responses and potentially affects seed production performance. Previous studies in *Arabidopsis* and *B. rapa* have shown that reduced R/FR conditions can decrease seed production, for example by reducing ovule number, while still allowing seed formation to occur (Procko et al., 2014; Roig-Villanova et al., 2025). These findings indicate that flowering induction by ULD-FR conditions can be accompanied by quantitative changes in reproductive performance, highlighting the need for quantitative evaluation in future studies. The proposed two-step strategy (Fig. 9) or optimization of the ULD-FR irradiation duration may help mitigate these effects. At a conceptual level, potential applications may include accelerated generation advancement during breeding, enhanced flowering of late-bolting genotypes, and synchronization of flowering times among genetically divergent accessions. As the ULD-FR condition is technically simple in terms of lighting control, it may represent a supplementary approach in breeding programs, though its effectiveness requires further validation under diverse cultivation settings.

A potential limitation of the ULD-FR condition is the increased energy cost associated with ultra-long-day lighting, which poses a challenge for large-scale or long-term application. The time, space, and energy savings relative to alternative approaches, such as EOD-FR or cold treatments, should be evaluated and optimized on a crop-specific basis. In the present study, continuous ULD-FR (24-h L) was employed as a proof-of-concept experimental condition to maximize and stabilize FT induction, enabling a clear demonstration of the physiological potential of this light regime. However, it is plausible that comparable FT induction could be achieved with lower energy input by using shorter ultra-long photoperiods (e.g. 18–20 h), intermittent light supplementation, or developmental stage-specific light treatment. Future studies aimed at defining the minimal light requirements for inducing constitutive FT expression under ULD-FR (as discussed in the following section) will be important for improving energy efficiency and facilitating broader practical application.

4.4. Future perspectives

Several questions remain regarding both the mechanistic basis and the practical optimization of the ULD-FR-induced flowering response. A first issue is to clarify the molecular mechanisms that lead to constitutive FT expression under ULD-FR, including the signaling pathways involved in this response, how it interacts with other flowering pathways such as the vernalization pathway, and the extent to which it is conserved across species. Addressing these questions will help elucidate how ULD-FR-induced constitutive FT expression is established, how it may relate to adaptive traits, and what its evolutionary origin might be.

A second issue is to define the minimal light requirements for inducing constitutive FT expression. Key unresolved factors include the contribution of specific wavelength components beyond far-red, the absolute FR intensity needed to trigger the response, its interaction with total light intensity, and the extent to which ULD-FR remains effective under natural sunlight or mixed-light environments. Systematic evaluation of these parameters will deepen our understanding of light-dependent FT regulation and help identify cultivation settings in which ULD-FR can be incorporated efficiently and reproducibly.

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Declaration of competing interests

The findings described in this manuscript are included in a Japanese patent application (application number: JP 2025–108196 in Japan) related to the enhancement of the florigen expression and graft-mediated floral induction. KM, NK and KY are listed as applicants on this patent. The authors declare that there are no other potential conflicts of interest.

Data availability

The plant materials and datasets used in this study will be available by the corresponding author upon request.

CRediT authorship contribution statement

Ko Motoki: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft. **Nami Kakita:** Investigation, Writing – original draft. **Tanjuro Goto:** Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Ken-ichiro Yasuba:** Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ko Motoki has patent #JP 2025–108196 pending to Okayama University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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