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RESEARCH ARTICLE

Photoreceptors and Circadian Clock Regulators Control Growth and Flowering Under Ionizing Radiation Stress

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Abstract

Plants have evolved mechanisms to optimize flowering time in response to adverse environments, ensuring reproductive success and survival. Although diverse regulatory networks controlling flowering have been identified, the molecular mechanisms by which environmental stresses influence flowering remain poorly understood. In particular, little is known about the impact of ionizing radiation (IR) such as γ-rays. Here, we show that photoreceptors and circadian clock components are crucial for growth and flowering regulation of *Arabidopsis thaliana* under IR stress. Exposure to 125 Gy of γ-radiation accelerated flowering in wild-type plants, primarily through elevated expression of the floral activator *FT*, rather than its upstream regulator *CO*. Plants lacking the blue-light photoreceptor *FKF1* exhibited heightened sensitivity to IR. At the same time, the *cry1 cry2* mutant, in which a different type of blue light photoreceptor is impaired, exhibited promotion of flowering by IR, although weaker than that observed in *fkf1*. Unlike *fkf1* and *cry1 cry2*, the *cry1 cry2 fkf1-2* mutant showed an IR-insensitive flowering phenotype. Similarly, the flowering time of *ZTL* mutant and overexpression plants was unaffected by IR. The *cca1 lhy* mutant lost IR-induced flowering promotion and accumulated high H_2O_2 levels, implicating ROS regulation in IR-responsive flowering. Interestingly, the *phyA-211* mutant exhibited extreme growth sensitivity, with severe retardation and anthocyanin accumulation. In contrast to *phyA-211*, the *phyB-9* mutant showed no significant differences in growth or anthocyanin accumulation compared with the wild type. These findings reveal distinct roles of photoreceptors and circadian clock regulators in coordinating flowering and growth under IR, providing insights into plant adaptation to radiation stress.

Keywords Ionizing radiation · Flowering time · Growth regulation · Reactive oxygen species

Introduction

Plants are immobile organisms that cannot escape adverse environmental pressures. Consequently, they have evolved diverse adaptive mechanisms that allow them to survive and adapt under varied environmental conditions (Bandurska 2022). For example, plants can endure extremely low temperatures in tundra regions and tolerate intense ionizing radiation (IR) in areas such as Chernobyl (Myers-Smith et al. 2019; Mousseau and Møller 2020). Remarkably, plant resilience to IR has significant implications for ensuring stable food production in extraterrestrial environments such as space stations, the Moon, or Mars (Maffei et al. 2024). Thus, elucidating the regulators and mechanisms that define the anatomical, physiological, and biochemical responses of plants to IR is of both fundamental and applied importance.

Flowering represents a prime example of plant environmental adaptation, as its timing is tightly regulated

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by seasonal cues, particularly changes in photoperiod, as extensively characterized in the model plant *Arabidopsis thaliana* (Song et al. 2015; Takagi et al. 2023). In many plants, including *Arabidopsis*, flowering time is primarily determined by the expression of the floral integrator gene *FLOWERING LOCUS T* (*FT*) (Andrés and Coupland 2012; Song et al. 2013). Multiple environmental signals, including light quality, photoperiod, and temperature, modulate *FT* expression (Song et al. 2013). Notably, prolonged low-dose IR exposure has been reported to accelerate flowering through increased *FT* expression, suggesting that known flowering regulators may participate in IR-induced flowering responses (Horemans et al. 2023; Laanen et al. 2023). In *Arabidopsis*, *FT* transcription is primarily activated by the CONSTANS (CO) protein (Samach et al. 2000).

Arabidopsis is a facultative long-day plant, flowering earlier under long-day (LD) conditions and exhibiting delayed flowering under short-day (SD) conditions (Mouradov et al. 2002; Song et al. 2015; Takagi et al. 2023). The regulation of flowering in response to environmental changes is mediated by photoreceptors and circadian clock components, whose functions converge on *FT* expression (Mouradov et al. 2002; Song et al. 2015; Takagi et al. 2023). Photoreceptors play crucial roles in perceiving different radiation spectra, thereby fine-tuning flowering to habitat-specific conditions (Ito et al. 2012; Song et al. 2013). Ultraviolet-B (UV-B) radiation is sensed by the UV RESISTANCE LOCUS 8 (UVR8) photoreceptor and exerts photoperiod-dependent effects on flowering (Kliebenstein et al. 2002). UV-B delays flowering under SDs but does not alter flowering under LDs, although it increases *FT* expression under LD conditions (Takahashi et al. 2025).

In contrast to UV-B, other light spectra show clearer regulatory roles. Blue light promotes flowering through *FT* induction, mediated by photoreceptors such as ZEITLUPE (ZTL), FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1), and LOV KELCH PROTEIN 2 (LKP2) (Ito et al. 2012). These proteins contain LOV (Light, Oxygen, or Voltage) domains with E3 ubiquitin ligase activity and form complexes with GIGANTEA (GI), with the GI interaction being critical for their activity (Kim et al. 2007; Sawa et al. 2007; Takase et al. 2011). FKF1 functions as a photoperiod sensor, establishing multiple feedforward loops to determine evening-specific *FT* induction under LDs (Sawa et al. 2007; Song et al. 2012, 2015). Under LDs, FKF1 promotes degradation of CYCLING DOF FACTORs (CDFs), transcriptional repressors of *CO* and *FT* genes, thereby enhancing evening *CO* and *FT* expression (Imaizumi et al. 2005; Sawa et al. 2007; Song et al. 2012). FKF1 also increases the stability of CO protein by directly binding to the protein and inhibiting the activity of the RING-finger E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1), leading to *FT* activation (Song et al. 2012; Lee et al. 2017; Cho et al. 2024). In contrast, ZTL and LKP2 act as repressors of flowering: their overexpression severely reduces *CO* and *FT* transcript levels (Takase et al. 2011). ZTL represses *FT* by forming complexes with TARGET OF EATs (TOEs), *FT* repressors, and destabilizes CO through direct interaction (Song et al. 2014; Kim et al. 2024). Blue light–dependent complex formation with GI suppresses ZTL activity in the late afternoon, allowing FKF1 to stabilize CO and promote *FT* expression under LDs (Kim et al. 2007; Song et al. 2012; Hwang et al. 2019). Another class of blue-light photoreceptors, cryptochromes, particularly CRY2, contribute to CO stabilization by inhibiting the CO-degrading COP1–SUPPRESSOR OF PHYA-105 1 complex and, when activated by blue light, enhance *FT* transcription through interaction with CRY2-interacting bHLH1 (CIB1) that binds to CO (Liu et al. 2008, 2018).

Red/far-red light photoreceptors also shape flowering responses. Phytochrome B (phyB) represses *FT* by promoting CO degradation via the E3 ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (Lazaro et al. 2012, 2015). Conversely, phyA is activated by far-red light, counteracting phyB activity, stabilizing CO protein, and promoting afternoon *FT* expression (Valverde et al. 2004). Moreover, phyA-mediated high-irradiance responses are critical for morning-specific *FT* expression under natural long days (Song et al. 2018; Lee et al. 2023).

Circadian clock proteins also contribute to *FT* regulation. CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) represses *GI* and *FT* transcription by binding directly to their promoters, while LATE ELONGATED HYPOCOTYL (LHY) negatively regulates *FT* levels by altering rhythmic expression patterns of *FKF1* and GI (Lu et al. 2012; Park et al. 2016). EARLY FLOWERING 3 (ELF3) interacts with CO to promote its degradation and suppress *FT* expression (Song et al. 2018). In contrast, PSEUDO RESPONSE REGULATORS (PRRs) stabilize CO protein through direct interaction, thereby enhancing *FT* transcription (Hayama et al. 2017).

In this study, we aimed to identify key regulators of flowering and survival in *Arabidopsis* under intense IR stress by analyzing mutants and overexpression lines of critical photoreceptors and circadian clock components. Through flowering time analysis, gene expression profiling, and reactive oxygen species (ROS) assays, we show that blue- and far-red photoreceptors, as well as circadian clock components, act as key regulators of flowering or growth responses under IR exposure.

Materials and Methods

Plant Materials and Growth Conditions

All *Arabidopsis thaliana* plants—WT, *ztl-4* (Michael et al. 2003), *fkf1* null mutant, *fkf1-2* T-DNA insertion mutant (Imaizumi et al. 2003), *gi-2* (Sawa et al. 2007), *phyA-211* (Reed et al. 1994), *phyB-9* (Reed et al. 1993), *cry1 cry2* (Mockler et al. 1999), *cca1-1 lhy-20* (Blair et al. 2019), *35S:Myc*-ZTL (Song et al. 2014), *35S:HA/fkf1* (Sawa et al. 2007), *35S:HA-GI/gi-2* (David et al. 2006), *35S:PRR5* (Sato et al. 2002), *35S:ELF3-6H3F* (Song et al. 2018), *35S:3HA-CO #10* (Song et al. 2012)—used in this study are in the Columbia (Col-0) background. The *cry1 cry2 fkf1-2* triple mutant was generated by a genetic cross between *cry1 cry2* and *fkf1-2*.

Except for flowering time measurements, surface-sterilized seeds were sown on 0.5× MS agar medium supplemented with 1% sucrose, stratified at 4°C for 3 days, and transferred to a growth chamber at 22°C under long-day (16 h light/8 h dark) conditions with white LED illumination (90–100 µmol m^{-2} s^{-1}).

For flowering time measurements, seeds were stratified in water at 4 °C for 3 days, sown in soil, and cultivated under the same growth conditions. The number of rosette and cauline leaves on the primary shoot was recorded when the inflorescence reached 3–5 cm in height.

γ-Irrdiation

Ten-day-old Arabidopsis plants grown under long-day conditions were exposed to γ-rays at doses of 125, 250, 500, or 1000 Gy for 4 h at the Advanced Radiation Technology Institute (Jeollabuk-do, Korea). The absorbed radiation dose for each sample was determined using a 5-mm diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany).

RNA Isolation and Gene Expression Analysis

For gene expression analysis, wild-type plants with or without γ-ray treatment were harvested every 3 h from ZT1 to ZT22 at 11 days after germination for three consecutive days. Total RNA was isolated using the HiGene Total RNA Prep Kit (BioFact, Daejeon, Korea), and 500 ng of total RNA was reverse transcribed into cDNA with the PrimeScript RT Master Mix (Takara, Shiga, Japan). Quantitative real-time PCR (qRT-PCR) for *IPP2*, *CO*, and *FT* expression, as well as primer information, was described previously (Song et al. 2012). All expression data were normalized to *IPP2* values.

Histochemical Staining for Hydrogen Peroxide

To examine changes in H_2O_2 accumulation after irradiation, γ-ray—treated plants at 10 days after germination were sampled at ZT13 on the 1st, 2nd, and 3rd days of recovery under long-day conditions. H_2O_2 accumulation was visualized with 1 mg ml^{-1} 3,3′-diaminobenzidine (DAB, pH 3.8; Sigma-Aldrich, St. Louis, MO, USA). Chlorophylls in the DAB-stained plants were removed by subsequent incubation in bleaching solution (ethanol: acetic acid: glycerol = 3:1:1).

Anthocyanin Measurement

For anthocyanin quantification, plants were transferred to soil after γ-ray irradiation and sampled 2 weeks later. Harvested tissues were ground and incubated overnight in methanol acidified with 1% HCl. Absorbance of the extracts was measured at 530 nm and 657 nm using a spectrophotometer. Anthocyanin levels were determined as the optical density at 530 nm (OD_{530}) minus the optical density at 657 nm (OD_{657}) and normalized to the total fresh weight of the tissues in each sample.

Results and Discussion

PhyA is Involved in Tolerance Response to IR Stress and Anthocyanin Accumulation

To enable space exploration, it is essential to predict the physiological responses of plants in extraterrestrial environments and to identify the regulators underlying these changes. Among various factors, ionizing radiation (IR) represents one of the most severe stresses for plants, influencing survival and developmental processes such as flowering (Laanen et al. 2023). Previous studies have shown that IR interacts with specific light qualities to modulate plant physiology and metabolite accumulation, suggesting that photoreceptors may play important roles in IR stress responses (Maffei et al. 2024). Therefore, we examined the responses of Arabidopsis photoreceptor mutants and overexpression lines to IR exposure. Given that circadian clock components also participate in the perception of environmental changes and in regulating plant physiology, selected clock mutants and overexpression lines were included in the analysis.

Ten-day-old Arabidopsis plants grown under long-day conditions were irradiated with γ-rays, a form of IR, at doses of 125, 250, 500, or 1000 Gy, and their phenotypes were subsequently monitored for 3 weeks. Visible phenotypic changes began to appear approximately 2 weeks after irradiation. At doses above 500 Gy, all plants failed

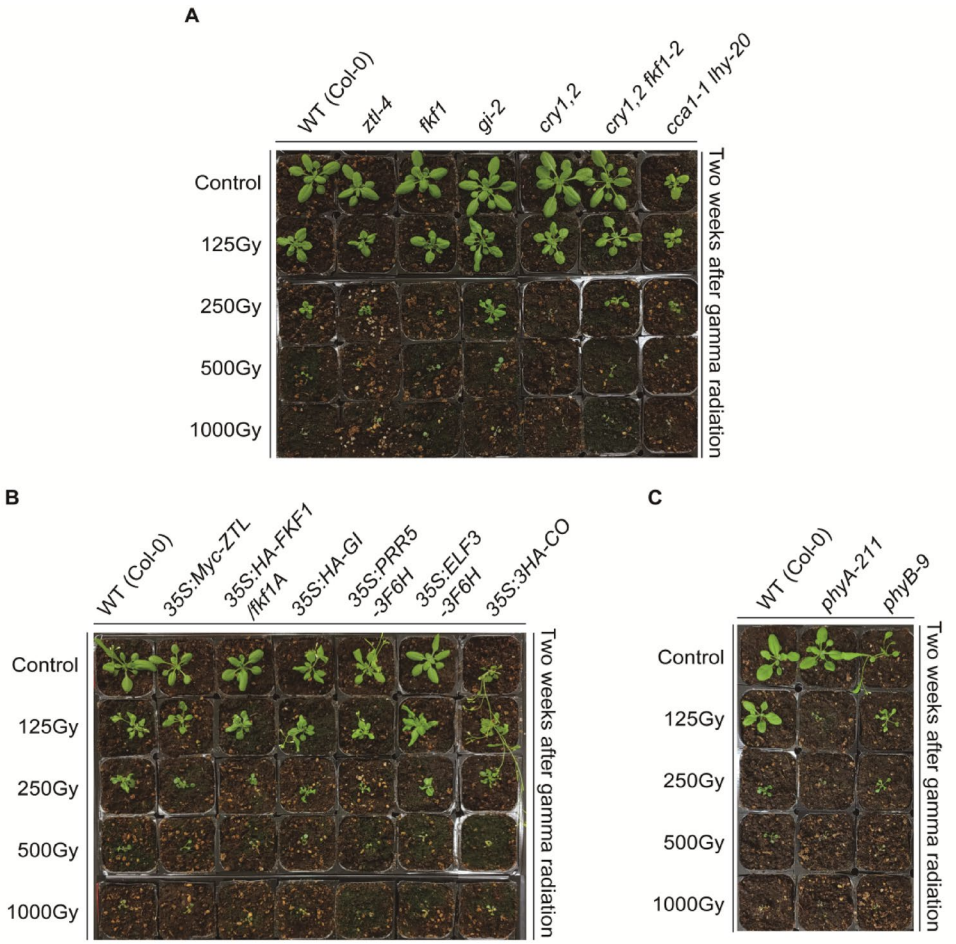


Fig. 1 Phenotypic analysis of Arabidopsis plants under ionizing radiation (IR). Ten-day-old plants grown under long-day conditions (LDs) were irradiated with 0, 125, 250, 500, or 1000 Gy of γ-rays and observed for phenotypic changes during a 3-week recovery period. Shown are representative phenotypes 2 weeks after irradiation. **A** Wild type (Col-0), *ztl-4*, *fkf1* null mutant, *gi-2*, *cry1 cry2*, *cry1 cry2 fkf1-2*, and *cca1-2 lhy-20*. **B** Wild type, *35S:Myc-ZTL*, *35S:HA-FKF1/fkf1*, *35S:HA-GI/gi-2*, *35S:PRR5*, *35S:ELF3-6H3F*, and *35S:3HA-CO #10*. **C** Comparative phenotypes of wild type, *phyA-211*, and *phyB-9* at 2 weeks after irradiation

Table 1 Phenotypic responses of Arabidopsis mutants and overexpression lines to 125 Gy of γ-rays

Genotypes	Growth retardation	Flowering time	H_2O_2 accumulation
Col-0	Moderate	Early	Moderate
ztl-4	Moderate	Unaltered	Moderate
fkf1	Moderate	Very early	Low
gi-2	Moderate	Very early	Low
phyA-211	Severe	n.a.	Moderate
phyB-9	Moderate	n.t.	n.t.
cry1 cry2	Moderate	Early	Low
cry1 cry2 fkf1-2	Moderate	Unaltered	Low
cca1-1 lhy-20	Moderate	Unaltered	High
35S:Myc-ZTL	Moderate	Unaltered	Moderate
35S:HA-FKF1/fkf1	Moderate	Unaltered	High
35S:HA-GI/gi-2	Moderate	Slightly early	High
35S:PRR5	Moderate	Unaltered	High
35S:ELF3-6H3F	Moderate	Early	Moderate
35S:3HA-CO #10	Moderate	Unaltered	Moderate

n.a. and n.t. denote not available and not tested, respectively

For all traits, the response of the wild type was defined as moderate, and variations were compared against this standard

to survive (Fig. 1A, B). Most plants including *fkf1*, *gi-2*, *cry1 cry2*, *cry1 cry2 fkf1*, *cca1-1 lhy-20* mutants, exposed to 125 Gy did not display noticeable morphological changes compared with untreated controls (Table 1). Strikingly, the far-red photoreceptor mutant *phyA-211* showed hypersensitivity to IR, exhibiting growth retardation and eventually death even at 125 Gy. In contrast, the red light photoreceptor mutant *phyB-9* displayed growth comparable to the wild type (Fig. 1C) (Table 1). These findings suggest that *phyA*, particularly the *phyA-211* allele, is critical for normal growth under IR stress.

Because anthocyanins accumulate in plants in response to various environmental stresses, including strong visible/UV light and low temperatures, we next measured anthocyanin content in wild-type, *phyA-211*, and *phyB-9* plants after 2 and 3 weeks of growth following IR treatment. Compared with wild-type plants, *phyA-211* mutants exhibited dramatically elevated anthocyanin accumulation at all doses of γ-rays, whereas *phyB-9* mutants showed no significant changes (Fig. S1A). Moreover, anthocyanin levels in *ztl-4* and *fkf1* exhibited no marked differences (Fig. S1B). Moderate anthocyanin accumulation can enhance plant stress tolerance; however, excessive accumulation may impair

growth (Li and Ahammed 2023). Thus, our results indicate that IR-induced anthocyanin overaccumulation in the *phyA-211* mutant likely contributes to reduced growth and survival in *Arabidopsis*.

125 Gy γ-ray Exposure Accelerates Flowering via *FT* Upregulation

We next examined the effects of IR exposure on flowering time in wild-type plants. Because plants failed to recover growth after exposure to 500 Gy or higher, we focused on the impact of 125 Gy and 250 Gy. At 250 Gy, most plants either failed to resume growth or exhibited abnormal development due to damage to the shoot apical meristem (SAM), rendering flowering time analysis impractical. By contrast, plants exposed to 125 Gy displayed markedly accelerated flowering (Fig. 2A).

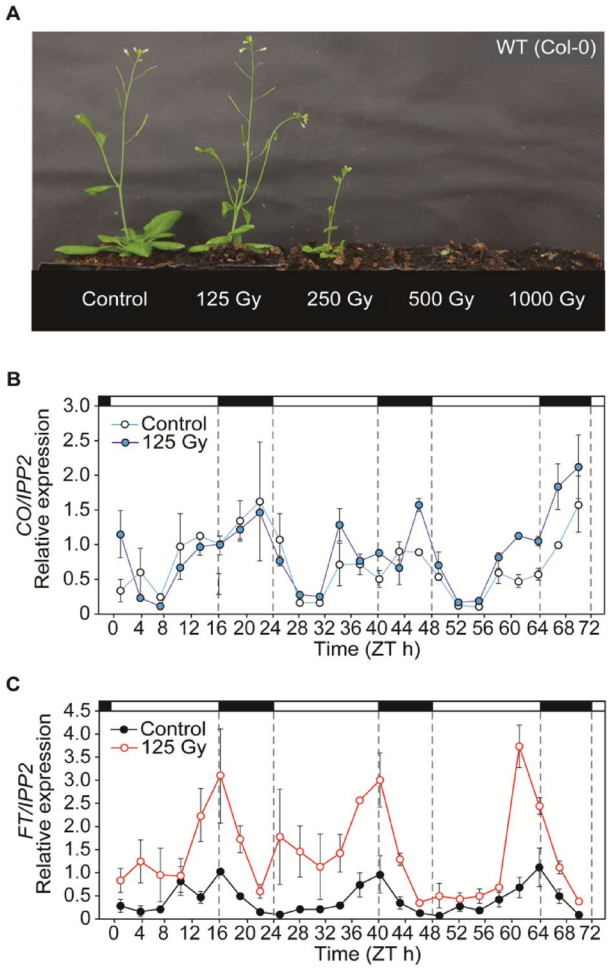


Fig. 2 Flowering time and expression analysis under IR. **A** Ten-day-old plants grown under long-day (LD) conditions were irradiated with 0, 125, 250, 500, or 1000 Gy of γ-rays, and flowering time was recorded at bolting. **B**, **C** Gene expression analysis of *CO* (**B**) and *FT* (**C**). Wild-type plants grown for 10 days under LDs were irradiated with 0–125 Gy and sampled at the indicated time points for three consecutive days. Transcript levels were measured by quantitative real-time PCR, and expression values were normalized to *IPP2* as an internal control

To investigate the basis of this early-flowering phenotype, we compared the expression of the conserved floral promoters, the *CO* and *FT* genes.. Wild-type plants were exposed to 0–125 Gy of γ-rays and grown under LDs. Plants were collected every 3 h starting from Zeitgeber time (ZT) 1 (i.e., hours after light onset within a day) on the day after irradiation for three consecutive days. Transcript analyses revealed that *CO* expression was not significantly altered on the first day after IR treatment but showed an upregulation trend during the evening and night on days 2 and 3 (Fig. 2B). In contrast, *FT* expression was strongly induced from the first day of irradiation throughout the day and remained elevated during all days, with a pronounced peak in the late afternoon under LDs (Fig. 2C). These results suggest that accelerated flowering in response to IR exposure is primarily attributable to enhanced transcription of *FT* rather than changes in *CO* expression.

Blue Light Photoreceptors Play Important Roles in IR Stress-induced Flowering

To identify key regulators of IR-induced *FT* expression, flowering time was analyzed using various mutants and overexpression lines of *FT* transcriptional regulators, including *ztl-4*, *fkf1*, *gi-2*, *cry1 cry2*, *cry1 cry2 fkf1-2*, *cca1-1 lhy-20*, and *35S:Myc-ZTL*, *35S:HA-FKF1/fkf1*, *35S:HA-GI/gi-2*, *35S:PRR5*, *35S:ELF3-6H3F*, and *35S:3HA-CO #10*. Under LDs, wild-type plants exhibited significantly accelerated flowering after 125 Gy γ-ray treatment. In contrast, the early-flowering *ztl-4* and *cca1-1 lhy*-20 mutants did not show changes in flowering time upon IR exposure (Fig. 3A) (Table 1). This suggests that their *FT*-repressing functions are involved in IR-induced flowering promotion. By contrast, the late-flowering *fkf1* and *gi-2* mutants displayed significantly enhanced flowering responses to IR compared with wild type (Fig. 3A) (Table 1). Considering that these mutants exhibit very low *FT* expression levels (Sawa et al. 2007), our data suggest that IR-induced flowering in these genotypes may be only partially dependent on *FT*, or that redundant factors may regulate *FT* induction. The late-flowering *cry1 cry2* double mutant also flowered earlier in response to IR, although less rapidly than *fkf1* or *gi-2* (Fig. 3A) (Table 1). Because FKF1 and CRY1/2 are both blue-light photoreceptors that stabilize CO protein and promote *FT* transcription (Liu et al. 2008; Song et al. 2012; Lee et al. 2017; Liu et al. 2018; Cho et al. 2024), we hypothesized that they act in the same pathway under IR stress. To test this possibility, we generated a *cry1 cry2 fkf1-2* triple mutant by a genetic cross between *cry1 cry2* and *fkf1-2* mutants. The *cry1 cry2 fkf1-2* mutant exhibited flowering

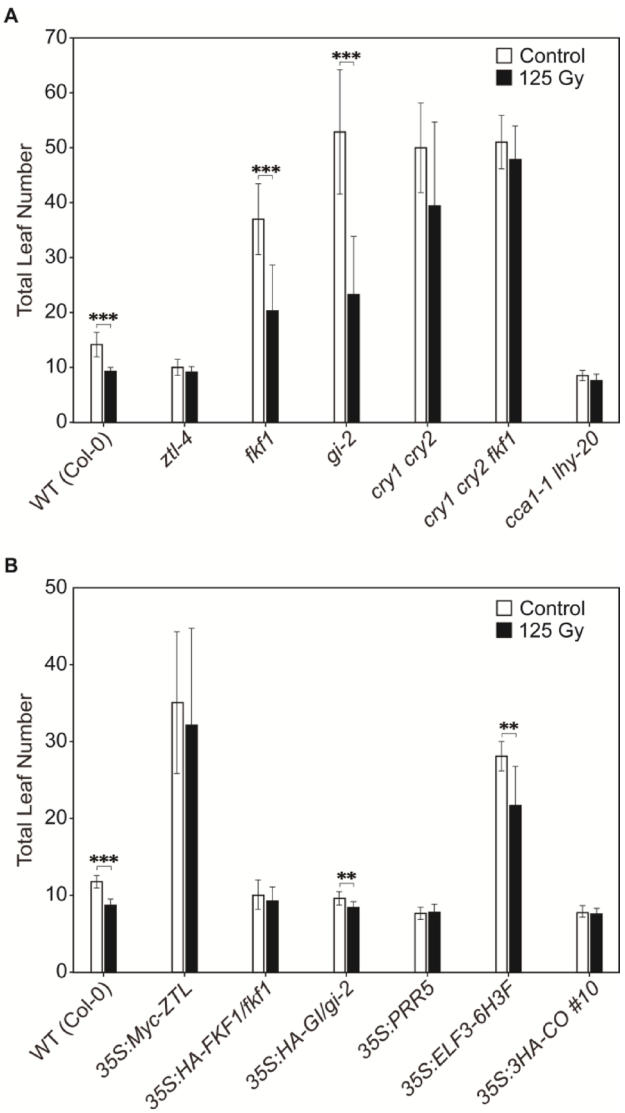


Fig. 3 Flowering time phenotypes under IR. Ten-day-old plants grown under long-day (LD) conditions were irradiated with 0–125 Gy of γ-rays, and flowering time was recorded at bolting. **A** Comparison of flowering time in wild type (Col-0), *ztl-4*, *fkf1*, *gi-2*, *cry1 cry2*, *cry1 cry2 fkf1-2*, and *cca1-1 lhy-20* plants. **B** Comparison of flowering time in wild type, *35S:Myc-ZTL*, *35S:HA-FKF1/fkf1*, *35S:HA-GI/gi-2*, *35S:PRR5*, *35S:ELF3-6H3F*, and *35S:3HA-CO #10* plants. At least 12 plants per line were analyzed. The bars indicate the mean ± *SEM*. ***$P<0.001$, **$P<0.01$; no significance at $P<0.05$ was observed

behavior similar to that of *cry1 cry2* under non-irradiated conditions. However, unlike the *cry1 cry2* mutant, the *cry1 cry2 fkf1-2* plants did not exhibit accelerated flowering after IR treatment (Fig. 3A) (Table 1).

Although *CO* transcript levels were not strongly altered, *FT* expression was markedly increased under IR exposure (Fig. 1A), suggesting that CO protein stability may be critical for IR-induced flowering. To test this, flowering time was analyzed in overexpression lines of *CO* and CO stability regulators. *CO* overexpression lines did not show altered flowering after 125 Gy γ-ray treatment (Fig. 3B) (Table 1). Similarly, the late-flowering *ZTL* overexpression line and the early-flowering *FKF1* and *PRR5* overexpression lines exhibited IR-insensitive flowering responses (Fig. 3B) (Table 1). By contrast, *GI* and E*LF3* overexpression lines showed a trend toward earlier flowering under IR stress (Fig. 3B) (Table 1). Together, these findings suggest that regulation of CO protein stability is important for IR-induced flowering.

ZTL destabilizes CO, whereas PRR5 stabilizes CO (Song et al. 2014; Hayama et al. 2017; Hwang et al. 2019). Additionally, ZTL targets PRR5 for degradation through its E3 ubiquitin ligase activity (Kim et al. 2007). Thus, the unaltered flowering responses of *CO*, *ZTL*, and *PRR5* overexpression lines to IR treatment may further support the importance of CO protein stabilization in mediating IR-induced flowering. Moreover, the IR-insensitive phenotypes of the *cry1 cry2 fkf1-2* mutant and the *FKF1* overexpression line highlight important roles of blue-light photoreceptors in controlling flowering responses under IR stress.

Changes in H_2O_2 Accumulation may Contribute to Flowering Time Under IR Stress

IR can directly affect plant growth and development through DNA damage or indirectly through the generation of reactive oxygen species (ROS) (Laanen et al. 2023). Increased ROS levels have also been associated with accelerated flowering patterns (Laanen et al. 2023). We therefore investigated whether the functions of flowering regulators are linked to IR-induced ROS production. Mutant and overexpression lines were exposed to γ-radiation after 10 days of growth, and changes in H_2O_2 accumulation were analyzed for three days thereafter by DAB staining. Although we initially expected higher IR doses to correlate with increased H_2O_2 accumulation, no clear relationship was observed in our experiments. Notably, late-flowering mutants such as *fkf1*, *cry1 cry2*, and *cry1 cry2 fkf1-2* accumulated lower levels of H_2O_2 compared with the wild type, regardless of IR dosage (Fig. 4A–C) (Table 1). In contrast, the early-flowering *cca1-1 lhy-20* mutant exhibited markedly elevated H_2O_2 accumulation (Fig. 4A–C) (Table 1). Similarly, early-flowering overexpression lines of *FKF1*, *GI*, and *PRR5* showed higher H_2O_2 accumulation than the wild type during the first two days after IR exposure (Fig. 4D–F) (Table 1). These results suggest that, in addition to *FT* transcriptional regulation, modulation of H_2O_2 accumulation may play an important role in IR stress-induced flowering, with blue-light photoreceptors and circadian regulators contributing significantly to this process. By contrast, the *phyA-211* mutant did not exhibit noticeable changes in H_2O_2 accumulation (Fig. 4A–C) (Table 1).

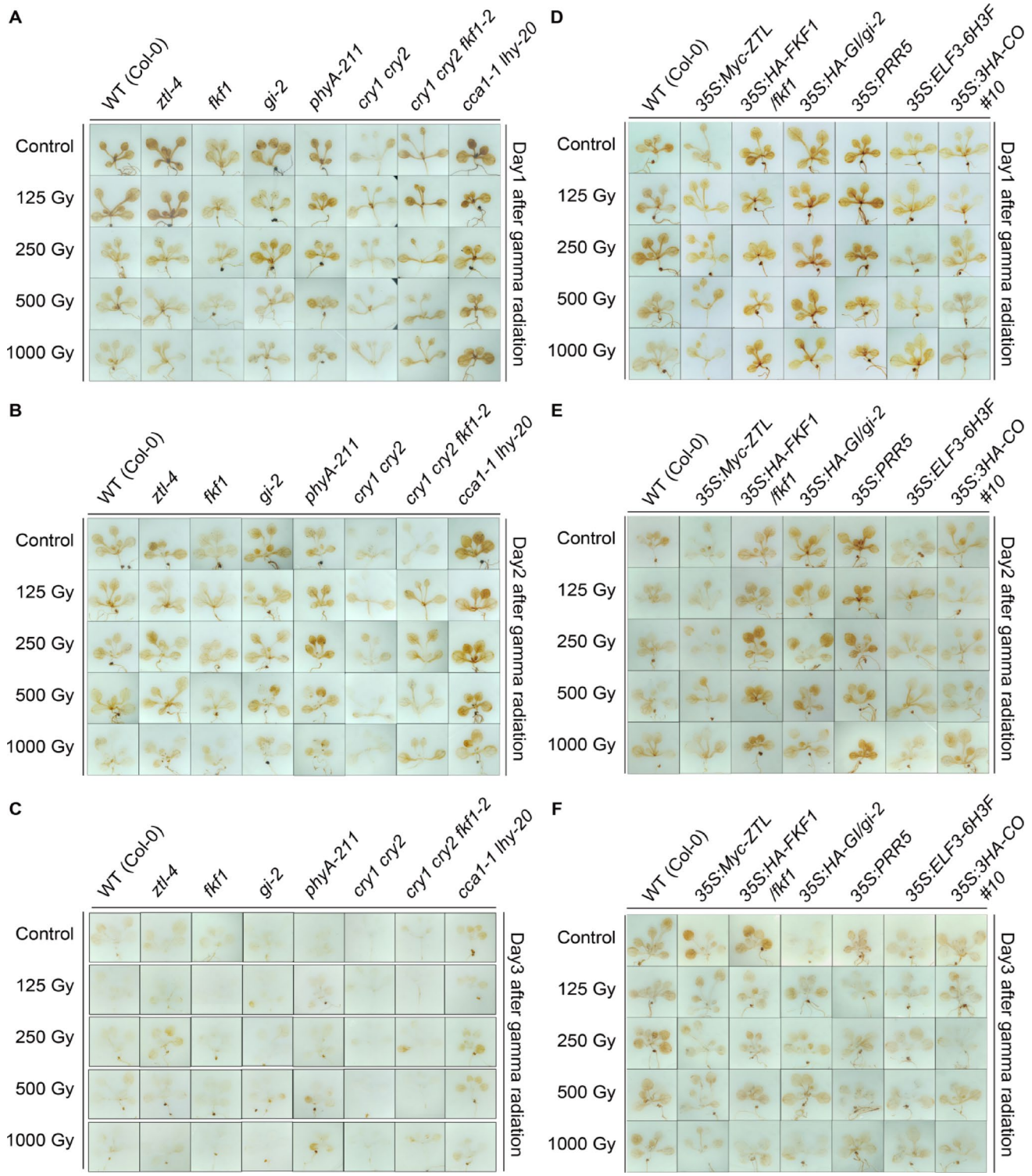


Fig. 4 Hydrogen peroxide (H_2O_2) accumulation under IR. Ten-day-old plants grown under long-day (LD) conditions were irradiated with 0, 125, 250, 500, or 1000 Gy of γ-rays, and H_2O_2 accumulation was monitored for 3 days by DAB staining. **A**–**C** Representative images of H_2O_2 accumulation in wild type (Col-0), *ztl-4*, *fkf1*, *gi-2*, *phyA-211*, *cry1 cry2*, *cry1 cry2 fkf1-2*, and *cca1-1 lhy-20* at 1 day (**A**), 2 days (**B**), and 3 days (**C**) after irradiation. **D**–**F** Representative images of H_2O_2 accumulation in wild type, *35S:Myc-ZTL*, *35S:HA-FKF1/fkf1*, *35S:HA-GI/gi-2*, *35S:PRR5*, *35S:ELF3-6H3F*, and *35S:3HA-CO #10* at 1 day (**D**), 2 days (**E**), and 3 days (**F**) after irradiation

IR-induced ROS accumulation has been reported to be associated with the transcriptional regulation of *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CO 1*), a downstream target of *FT* (Laanen et al. 2023). *CO* overexpression lines did not show altered H_2O_2 accumulation (Fig. 4D–F) (Table 1), implying that CO protein regulation and ROS production may function independently in IR-induced flowering. Taken together, these findings support the idea that both *FT*-dependent and *FT*-independent mechanisms contribute to flowering regulation under IR stress. Our study thus provides new insights into how plants adapt to extreme environments such as IR exposure. Future research should address the long-term effects of chronic low-dose IR on the roles of blue-light photoreceptors and circadian regulators in plant survival and flowering control.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12374-025-09491-7.

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Author Contributions S.W.C. performed the experiments. W.L. analyzed the data and designed the figures. S.S.L. and J.-I.K. contributed to the experimental design and reviewed the manuscript. Y.H.S. conceptualized the experiments, supervised the study, and wrote the manuscript. All authors have read and approved the contents of this paper.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

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