CRK2 modulates flowering in *Arabidopsis* together with GLYCINE-RICH RNA BINDING PROTEIN 7 (GRP7)

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Summary

Timely flowering is critical for the reproductive success of plants. Plants adjust flowering time through integration of multiple internal and environmental cues. A complex signalling system lies behind this integration, including multiple proteins like the GLYCINE-RICH RNA-BINDING PROTEIN 7 (GRP7), linked to the modulation of stress and flowering in Arabidopsis. Available data show the interaction between GRP7 and the receptor-like kinase CYSTEINE-RICH RECEPTOR-LIKE KINASE 2 (CRK2). Like GRP7, CRK2 plays multifaceted roles in the coordination of stress responses and development, but its role in development remains unclear. Phenotypic analyses of the *crk2* and *grp7-1* single mutants as well as the *crk2/grp7-1* double mutant supported the interaction between CRK2 and GRP7. Moreover, exogenous gibberellic acid did not recover the rosette phenotype of *crk2* mutants but as in sensitive plants, inhibited GA-biosynthesis transcripts. The pseudo insensitivity of *crk2* mutant was recovered in the *crk2/grp7-1* line pointing to a common role of CRK2 and GRP7 in the modulation of gibberellin signalling. Together, CRK2 and GRP7 integrate hormonal and spatial signals required for floral transition. These insights highlight a previously unrecognized layer of regulation in GA signalling and open new avenues to understand

how membrane-localized kinases and RNA-binding proteins intersect to control developmental timing.

Introduction

Timely flowering is crucial for plants, as it ensures production of viable seeds and thereby the next generation. Plants adjust flowering time through integration of multiple internal and environmental cues, including energy status, temperature, photoperiod, in order to optimize plant reproductive output in a changing environment (Andrés & Coupland, 2012; Wahl *et al.*, 2013; Song *et al.*, 2015; Yu *et al.*, 2015; Kazan & Lyons, 2016).

Arabidopsis thaliana, a facultative long day (LD) plant, has been widely used to dissect the molecular mechanisms underlying flowering time regulation. Photoperiod plays a major role in controlling flowering time in Arabidopsis, (Andrés & Coupland, 2012; Song *et al.*, 2015). In addition, flowering in Arabidopsis is fine-tuned by vernalization, gibberellin (GA) and the autonomous pathway (AP) (Andrés & Coupland, 2012; Bao *et al.*, 2020). The vernalization pathway responds to prolonged exposure to cold by suppressing expression of *FLOWERING LOCUS C* (*FLC*), a central repressor of flowering, thereby enabling floral induction (Andrés & Coupland, 2012). In parallel, GA and AP convey internal signals and adjust flowering time either by directly influencing the expression of flowering integrators and meristem identity genes (GA) or by modulating the expression of *FLC* (AP) (Andrés & Coupland, 2012; Xiao *et al.*, 2015; Bao *et al.*, 2020). Similar to vernalization, the AP acts through repression of *FLC* (Xiao *et al.*, 2015). Because both pathways repress *FLC*, the flowering delay seen in mutants with a defective AP can be reversed by exposing them to prolonged cold, which activates the vernalization pathway (Streitner *et al.*, 2008; Steffen *et al.*, 2019). AP components, including GLYCINE-RICH RNA-BINDING PROTEIN 7 (GRP7), modulate *FLC* but frequently also have roles in stress responses in plants (Nicaise *et al.*, 2013; Wang *et al.*, 2020). Intriguingly, GRP7 also participates in gibberellic acid (GA) signalling (Kim *et al.*, 2008; Löhr *et al.*, 2014; Wang *et al.*, 2020), which is tightly linked to stress and development (Colebrook *et al.*, 2014). GRP7 is phosphorylated and regulated by the receptor-like protein kinase (RLK) FERONIA (FER) (Wang *et al.*, 2020). Signals perceived by the RLK control GRP7 activity and, in turn, regulate the splicing-mediated maturation of numerous stress- and development-related transcripts (Wang *et al.*, 2020). GRP7 also interacts with other RLKs (Nicaise *et al.*, 2013) but the functional significance of those interactions remains to be clarified.

RLKs, such as the CYSTEINE-RICH RECEPTOR-LIKE KINASE 2 (CRK2), play multifaceted roles in the coordination of stress responses and development (Bourdais *et al.*, 2015). The *crk2* mutant combines altered sensitivity to biotic (Kimura *et al.*, 2020) and abiotic (Hunter *et al.*, 2019) stress with developmental phenotypes, including late flowering and small rosette size (Bourdais *et al.*, 2015). While the molecular role of CRK2 in controlling Arabidopsis development remains unclear, some of its known functions may point to possible mechanisms. CRK2 regulates apoplastic reactive oxygen species (ROS) production via phosphorylation of RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) during immune responses (Kimura *et al.*, 2020). ROS also contribute to shoot apical meristem (SAM) maintenance (Zeng *et al.*, 2017; Foyer & Noctor, 2020). Tomato knockout lines for *SlRBOH1/2* show accelerated flowering transition linked to ROS-mediated modulation of the transcription factor TERMINATING FLOWERING (TMF) (Huang *et al.*, 2021). CRK2 has also been linked to regulation of callose deposition (Hunter *et al.*, 2019). Callose has important roles in controlling plasmodesmal permeability and has been linked to regulating transport of mobile flowering signals like the flowering promotor FLOWERING LOCUS T (FT) to the SAM (Murata *et al.*, 2025). In addition, CRK2 has previously been associated with GA signaling. Exogenous gibberellic acid (GA_3) treatment reduces *CRK2* transcript abundance in the GA biosynthesis deficient *ga1-3* mutant (Bassel *et al.*, 2011) and DELLAs, repressors of GA responses, positively regulate *CRK2* transcript levels (Cao *et al.*, 2006). Moreover, the abundance of *CRK2* correlates with the GA biosynthesis-related transcripts *GIBBERELLIN 3-BETA-DIOXYGENASE 1* (*GA3ox1*) and *GIBBERELLIN 20-BETA-DIOXYGENASE 1* (*GA20ox1*) (Bassel *et al.*, 2011). Interestingly, GRP7 has been identified as an *in planta* interaction partner for CRK2 (Hunter *et al.*, 2019). Although no direct link is currently known between callose, ROS and GRP7, GRP7 is present in plasmodesmata-enriched fractions (Fernandez-Calvino *et al.*, 2011) and phloem exudates (Batailler *et al.*, 2012), suggesting a possible role in symplastic communication. These data also point towards a link between CRK2 and GRP7 in the regulation of flowering pathways.

Here we explored the involvement of CRK2 in the modulation of flowering time in Arabidopsis. Through analyses of *crk2*, *grp7-1* and the *crk2/grp7-1* double mutant, we show that CRK2 modulates flowering time in Arabidopsis through photoperiod-independent pathways, including the GA-mediated pathway. Based on our findings, we

propose a model in which CRK2 impacts flowering time regulation in Arabidopsis by acting as a negative regulator of GRP7, thus integrating signals perceived by the RLK in the modulation of flowering time.

Results and Discussion

CRK2 is involved in photoperiod-independent flowering regulation

To determine the flowering pathways regulated by CRK2 and whether CRK2 acts in concert with GRP7, we analysed the flowering phenotypes of *crk2*, *grp7-1*, the *crk2/grp7-1* double mutant, and complementation lines (*crk2/CRK2p::CRK2-YFP* #1-14 and #1-22; Kimura *et al.*, 2020) in comparison to Col-0. Under LD conditions, *crk2* exhibits a late flowering phenotype (Bourdais *et al.*, 2015). Surprisingly, *crk2* showed a more severe flowering delay in short day (SD) (Figure S1; Table S1) suggesting that CRK2 could be involved in photoperiod-independent flowering pathways. In SD-grown Arabidopsis, flowering transition relies on endogenous pathways, including the AP and GA pathways. Mutants in these pathways, including *grp7-1*, frequently show more severe delay in flowering under SD compared to LD (Macknight *et al.*, 2002; Ausín *et al.*, 2004; Streitner *et al.*, 2008; Steffen *et al.*, 2019), suggesting that CRK2 might act via similar mechanisms. In SD, *crk2* flowered later than *grp7-1* and *crk2/grp7-1*, which flowered later than Col-0 (Figure 1a, Table S2a). In addition, *crk2* showed fewer rosette leaves than Col-0 at the time of bolting (Figure 1b, Table S2b). This suggested internal factors, including age or carbohydrate levels, rather than external cues in the regulation of flowering time in *crk2*. The rosette size of *crk2* was consistently smaller compared to Col-0 while rosettes of *grp7-1* and *crk2/grp7-1* showed a similar size to Col-0 (Figure 1c). This partial rescue of the *crk2* developmental phenotypes by *grp7-1* suggested that GRP7 and CRK2 interact functionally or converge at common regulatory processes.

The identification of GRP7 as a component in CRK2-mediated flowering regulation functionally links CRK2 with the GA and AP pathways, which are both modulated by GRP7 (Kim *et al.*, 2008; Löhr *et al.*, 2014; Xiao *et al.*, 2015; Wang *et al.*, 2020). The AP and vernalization pathways converge at the repression of the major flowering repressor *FLC*. Therefore, the flowering delay of AP mutants is reversed by vernalization (Streitner *et al.*, 2008; Steffen *et al.*, 2019). Vernalization at 4ºC for 45 days rescued the flowering delay of *grp7-1* and *crk2/grp7-1*, but only partially for *crk2* (Figure 1d; Table S3). Interestingly, Col-0 and *crk2* showed similar *FLC* abundance in 3 and 4-week-old plants (Figure S2a, b; Table S4) and vernalization reduced *FLC*

abundance similarly in Col-0 and *crk2* (Figure S2b; Table S4b). By contrast, the *crk2/grp7-1* double mutant showed high *FLC* abundance similar to *grp7-1* (Figure S2a; Table S4a). Together with the similar flowering phenotypes of *grp7-1* and *crk2/grp7-1* (Figure 1; Table S2-3) this suggests that GRP7 modulates *FLC* independently of CRK2. CRK2 may regulate flowering through alternative GRP7-dependent pathways, e.g. GA, suggesting a partial functional overlap between both proteins.

CRK2 regulates GA signaling in Arabidopsis via GRP7

CRK2 and GRP7 have previously been linked to GA biosynthesis and signaling (Cao *et al.*, 2006; Kim *et al.*, 2008; Bassel *et al.*, 2011; Löhr *et al.*, 2014; Wang *et al.*, 2020) suggesting that CRK2 could potentially regulate GA signaling via GRP7. GA plays central roles in Arabidopsis development and growth, including flowering induction and cell elongation. Exogenous GA_3 treatment promotes flowering and increases rosette size and internode length, due to enhanced cell elongation (Ribeiro *et al.*, 2012; Löhr *et al.*, 2014). Treatment with GA_3 increased the rosette size of Col-0 but not in *crk2* compared to mock treatment (Figure 2a). By contrast, GA_3 treatment increased rosette size in *grp7-1* and *crk2/grp7-1*, similar to Col-0 (Figure 2a) suggesting an involvement of CRK2 and GRP7 in GA signaling.

GRP7 overexpression plants (GRP7-OX) are sensitive to exogenous GA_3 but have been shown to contain reduced levels of GA, and reduced transcript abundance of GA biosynthesis and response genes like *ENT-KAURENE SYNTHASE* (*GA2*) and *GIBBERELLIC ACID STIMULATED ARABIDOPSIS 9* (*GASA9*), respectively (Löhr *et al.*, 2014). This suggests that GRP7 acts as negative regulator of GA signaling. The *crk2* mutant exhibited reduced transcript abundance of *GASA9* compared to complementation lines following mock treatment (Figure 2b, Table S5a). Addition of GA_3 reduced *GASA9* abundance in Col-0 as previously described (Zhang & Wang, 2008), but not in *crk2* (Figure 2b; Table S5a) indicating altered transcriptional responses to GA in *crk2*. *GASA9* transcript abundance did not change in *grp7-1* following GA_3 treatment, similar to *crk2* (Figure 2b; Table S5a). However, *crk2* plants showed higher abundance of *GA3ox1* and *GA2*, which encode GA biosynthesis enzymes, compared to Col-0 under mock conditions (Figure 2c, Figure S3, Table S5b, Table S6). GA3ox1, involved in the final steps of bioactive GA synthesis, is subject to feedback repression by GA (Chiang *et al.*, 1995). Under mock treatment conditions, transcript abundance of *GA3ox1* was higher in *crk2* than in Col-0 (Figure 2c, Figure S3b, Table S5b, Table S6b).

Surprisingly, while GA_3 treatment did not affect *crk2* rosette size, transcript abundance of *GA3ox1* was reduced following GA_3 treatment in *crk2* similar to Col-0 (Figure 2c; Table S5b), suggesting that GA signaling in *crk2* is impaired downstream of perception. Transcript abundance of *GA3ox1* in *grp7-1* and *crk2/grp7-1* was similar to Col-0. Notably, the *crk2*/*grp7-1* double mutant also displayed restored rosette size and GA_3 responsiveness (Figure 2a-c; Table S5).

The dwarf and late flowering phenotype of *crk2* resembles GA insensitive mutants such as *gai*, which also display small rosette size and partially vernalization-reversible late flowering (Wilson *et al.*, 1992), similar to *crk2*. Similar to mutants like *gai*, treatment with paclobutrazol (PCL), a GA-biosynthesis inhibitor, show enhanced stability of DELLAs, resulting in reduced growth and late flowering (Serrano-Mislata *et al.*, 2025). Interestingly, a positive correlation between *DELLA* and *CRK2* abundance (Cao *et al.*, 2006) and reduced PCL sensitivity of *crk2* have been described (Bassel *et al.*, 2011). Thus, we speculate that CRK2 might function downstream of DELLAs and modulate GA responses partially through GRP7 (Fig. 2d). Available transcriptomic data suggest that GRP7 targets multiple *GASA* transcripts (Meyer *et al.*, 2017; Wang *et al.*, 2020). Therefore, it is tempting to speculate that CRK2 controls GRP7 and thereby the maturation of GA signaling transcripts.

Symplastic transport is essential for developmental regulators like FT (Murata *et al.*, 2025) and possibly for GA-dependent signaling components. For example, the mRNA of the DELLA protein *GAI*, has been shown to move through the phloem and induce dwarfism in distal tissues (Haywood *et al.*, 2005). CRK2 has been proposed to control callose deposition at plasmodesmata (Hunter *et al.*, 2019) and GRP7 has been identified in phloem exudates (Batailler *et al.*, 2012) and plasmodesmata enriched fractions (Fernandez-Calvino *et al.*, 2011). The flowering phenotype of *crk2* plants, along with its genetic interaction with *grp7-1*, suggest that CRK2 could influence mobility and processing of mobile transcripts. CRK2 might modulate GRP7 function to coordinate RNA processing and intercellular transport, thereby integrating hormonal, spatial, and post-transcriptional regulatory layers essential for developmental transitions such as flowering.

In summary, our results position CRK2 as a key modulator of GA signaling, acting partly through GRP7 to control development (Fig. 2d). It will be interesting in the future

to elucidate the molecular mechanism of how CRK2, together with GRP7, regulate GA responses and flowering time control.

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Competing interests

The authors declare no competing interests.

Author contribution

FC and MW conceived and designed the project. FC, AP, and JKW carried out experiments. FC analyzed the data. FC, JKW and MW wrote the article. All authors read and contributed to the final article.

Data availability

Data from this manuscript is available from the authors upon reasonable request.

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Figure legends

Figure 1. CRK2 and GRP7 modulate Arabidopsis flowering time under non inductive conditions. (a) Flowering time (in days) of *Arabidopsis thaliana* Col-0, *crk2*, *grp7-1*, *crk2/grp7-1* double mutant, and two complementation lines (*CRK2p::CRK2-YFP/crk2* #1-14 and #1-22) grown under short-day (SD) conditions. Statistical significance was assessed by Kruskal–Wallis followed by Wilcoxon post hoc test. Letters indicate statistically significant differences between genotypes ($p < 0.05$). **(b)** Number of rosette leaves at flowering for the same genotypes under SD conditions. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. Letters indicate statistically significant differences between genotypes ($p < 0.05$). **(c)** Representative rosettes of 75-day-old plants grown under SD conditions. **(d)** Ratio of flowering time (in days) for each genotype with and without vernalization treatment under SD conditions. Pairwise comparisons were performed within each genotype using Wilcoxon rank-sum test. P-values were adjusted for multiple testing using the Benjamini–Hochberg (BH) method (** $p<0.05$, *** $p<0.01$). **(a, b, d)** Data are presented as boxplots showing the median, interquartile range (IQR), and whiskers extending to 1.5×IQR. Individual data points are overlaid.

Figure 2. Effect of GA_3 treatment. (a) Representative rosettes of two-month-old plants grown under SD conditions and under weekly spraying with mock or GA_3 containing solutions. **(b)** Abundance of GA-responsive transcript *GASA9*. Pairwise comparisons were performed within each genotype using Wilcoxon rank-sum test. P-values were adjusted for multiple testing using the Benjamini–Hochberg (BH) method (* $p<0.1$). **(c)** Abundance of GA-biosynthesis transcript *GA3ox1* Pairwise comparisons were performed within each genotype using Wilcoxon rank-sum test. P-values were adjusted for multiple testing using the Benjamini–Hochberg (BH) method (* $p<0.1$, ** $p<0.05$). **(b, c)** Data are presented as boxplots showing the median, interquartile range (IQR), and whiskers extending to 1.5×IQR. Individual data points are overlaid. **(d)** Schematic model for the CRK2-GRP7-based modulation of flowering time in Arabidopsis. CRK2 acts as a flowering modulator in Arabidopsis. In this role, the RLK acts over the GA signaling pathway, partially via the inhibition of GRP7, a known modulator of GA signaling. CRK2 might also modulate GA signaling independently of GRP7 and flowering results suggest that the regulation of *FLC* by GRP7 does not involve CRK2.

Supplementary figure legends

Figure S1. The flowering delay of the *crk2* mutant is more severe under SD.

Figure S2. The *crk2* mutant does not show increased *FLC* expression.

Figure S3. Expression of the GA biosynthesis genes *GA2* and *GA3ox1* is altered in *crk2*.

Supplementary table legends

Table S1. Photoperiod experiment. Raw data and statistical analysis.

Table S2. Flowering time and rosette leaf number at flowering. Raw data and statistical analysis.

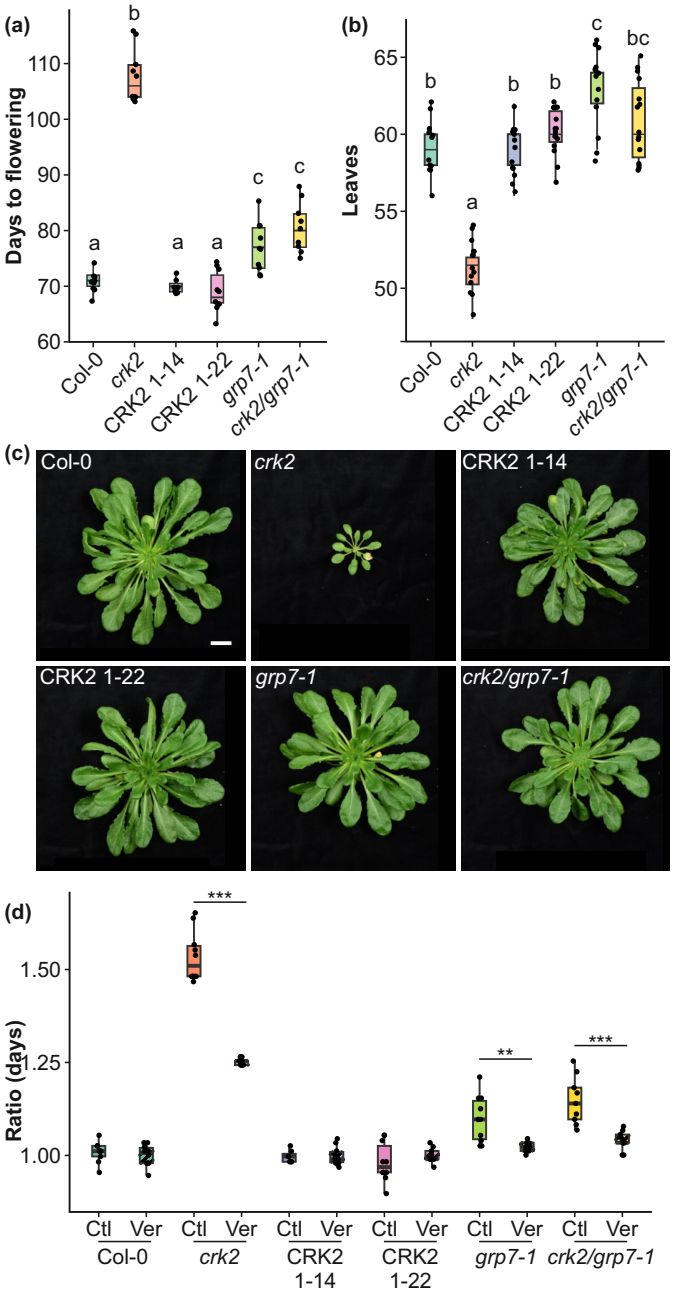
Table S3. Vernalization experiment. Normalized data and statistical analysis.

Table S4. Relative transcript abundance of *FLC*. Raw data and statistical analysis.

Table S5. Relative transcript abundance of *GASA9* & *GA3ox1*. Raw data and statistical analysis.

Table S6. Expression of GA biosynthesis-related genes. Raw data and statistical analysis.

Table S7. List of oligonucleotides used for gene expression analysis.


(a)
Days to flowering
110
100
90
80
70
60
a
b
a
a
c
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Col-0
crk2
CRK2 1-14
CRK2 1-22
grp7-1
crk2/grp7-1
(b)
Leaves
65
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b
a
b
b
c
bc
Col-0
crk2
CRK2 1-14
CRK2 1-22
grp7-1
crk2/grp7-1
(c)
Col-0
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CRK2 1-22
grp7-1
crk2/grp7-1
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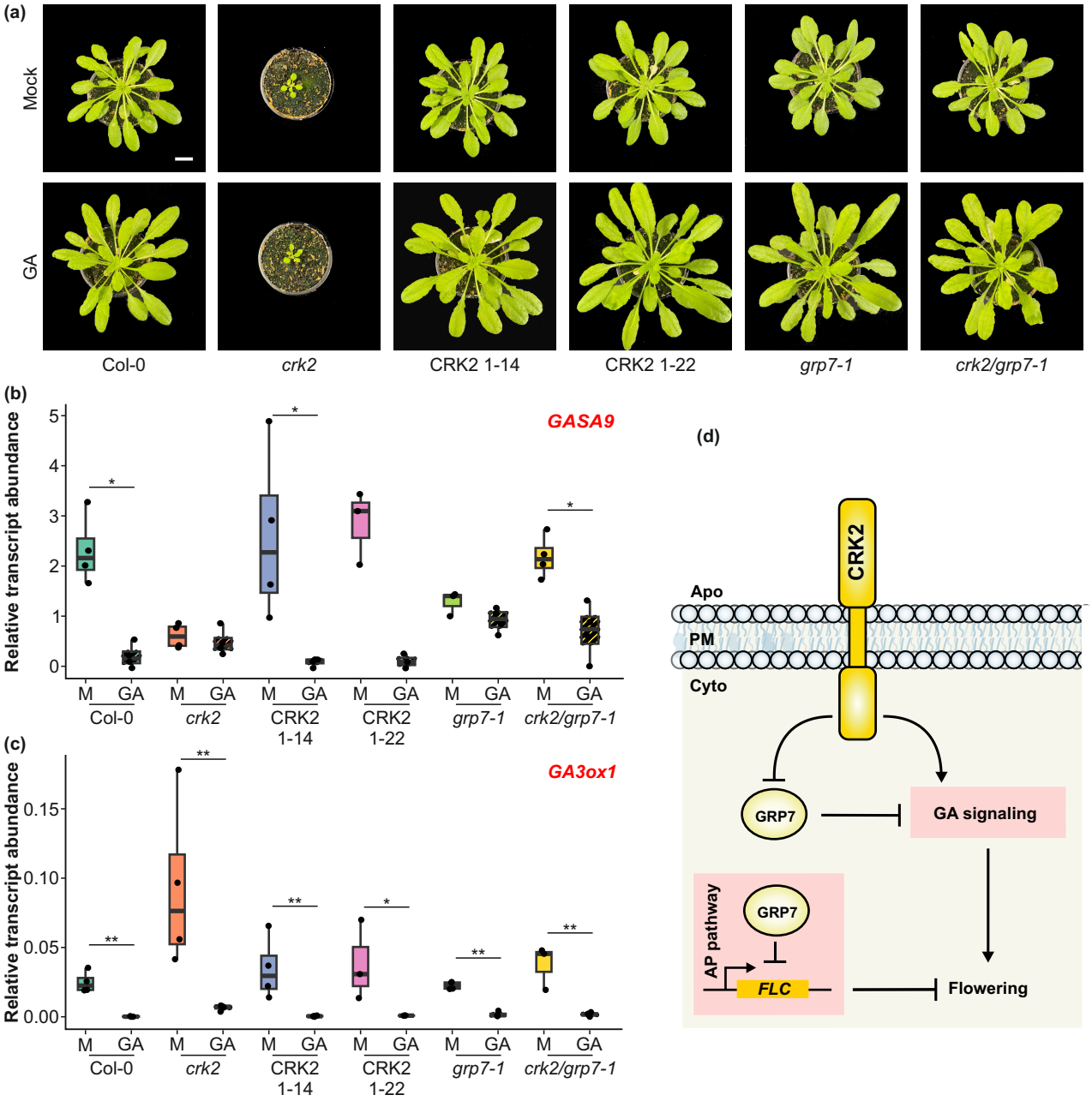
(a)
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crk2/grp7-1
(b)
GASA9
Relative transcript abundance
M GA
Col-0
crk2
CRK2 1-14
CRK2 1-22
grp7-1
crk2/grp7-1
(c)
GA3ox1
Relative transcript abundance
(d)
CRK2
Apo
PM
Cyto
GRP7
GA signaling
AP pathway
GRP7
FLC
Flowering