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# MOTHER-OF-FT-AND-TFL1 represses seed germination under far-red light by modulating phytohormone responses in *Arabidopsis thaliana*

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Seed germination in many plant species is triggered by sunlight, which is rich in the red (R) wavelength and repressed by under-the-canopy light rich in far red (FR). R:FR ratios are sensed by phytochromes to regulate levels of gibberellins (GAs) and abscisic acid (ABA), which induce and inhibit germination respectively. In this study we have discovered that, under FR light conditions, germination is repressed by MOTHER-OF-FT-AND-TFL1 (MFT) through the regulation of the ABA and GA signaling pathways. We also show that MFT gene expression is tightly regulated by light quality. Previous work has shown that under FR light conditions the transcription factor PHYTOCHROME-INTERACTING-FACTOR1 (PIF1) accumulates and promotes expression of SOMNUS (SOM) that, in turn, leads to increased ABA and decreased GA levels. PIF1 also promotes expression of genes encoding ABA-INSENSITIVE5 (ABI5) and DELLA growth-repressor proteins, which act in the ABA and GA signaling pathways, respectively. Here we show that MFT gene expression is promoted by FR light through the PIF1/SOM/ABI5/DELLA pathway and is repressed by R light via the transcription factor SPATULA (SPT). Consistent with this, we also show that SPT gene expression is repressed under FR light in a PIF1-dependent manner. Furthermore, transcriptomic analyses presented in this study indicate that MFT exerts its function by promoting expression of known ABA-induced genes and repressing cell wall expansion-related genes.

seed germination | far-red light | abscisic acid | gibberellin | PIF1

The timing of seed germination is critical for the survival of plants and the completion of their life cycle. Environmental conditions regulate accumulation and perception of the gibberellin (GA) and abscisic acid (ABA) phytohormones, which promote and repress seed germination, respectively. GA triggers degradation of the growth-repressing DELLA proteins (1, 2), whereas ABA acts through ABA-response transcription factors such as ABA-INSENSITIVE3 (ABI3), ABI4, and ABI5 (3–5). ABA and GA do not exert their effects independently of each other, but rather interact in a complex crosstalk (6–9). During development on the mother plant, seeds acquire an ABA-dependent dormant state (primary dormancy), which prevents germination even under favorable environmental conditions and contributes to seed dispersal (10). Once developed, seeds undergo an afterripening process resulting in a gradual loss of dormancy and increased ability to germinate when exposed to the right environmental conditions, such as the appropriate quality of light.

Sunlight is enriched in the red (R) wavelength, whereas under-the-canopy light (shade) is rich in far red (FR). *Arabidopsis thaliana* (*Arabidopsis*) has five phytochrome photoreceptors that perceive R and FR light and two of them, PHYA and PHYB, are essential in promoting seed germination (11, 12). Phytochromes are synthesized as inactive forms and become active in a light-quality-dependent manner. Excess of R and FR light activates and inactivates PHYB, respectively (13). In the dark, the active PHYB also reverts to its inactive form through a phosphorylation-dependent process (14). Unlike PHYB, PHYA is irreversibly activated by both R and FR

light and then subjected to proteasome-mediated degradation (13). Hence, FR light has opposite effects in PHYA and PHYB (i.e., while it activates PHYA, it deactivates PHYB). Also, the timing and length of FR light exposure affects PHYA and PHYB differently and, as a consequence, influences the germination response. Compared with PHYB, PHYA accumulates at high levels only after relatively long periods of seed imbibition and needs high levels of FR light to respond (8). Thus, under controlled experimental conditions, a pulse of FR light relatively early during seed imbibition (when PHYA is absent) is sufficient to deactivate PHYB, which leads to repression of germination; whereas a long FR light pulse later in imbibition still deactivates PHYB while activating PHYA to induce germination. Upon activation, both PHYA and PHYB are translocated into the nucleus to induce proteasome-directed degradation of PHYTOCHROME-INTERACTING-FACTORS (PIFs), which are a subfamily of basic helix–loop–helix (bHLH) transcription factors possessing an active phytochrome binding (APB) domain (15). In *Arabidopsis*, PIF1 (previously known as PIL5) plays a major role in the repression of seed germination (16, 17). PIF1 directly promotes expression of ABI5 and the DELLA-encoding GA-INSENSITIVE (*GAI*) and REPRESSOR-OF-GAI (*RGA*) genes (18). PIF1 also promotes expression of SOMNUS (*SOM*), which encodes a CCH-type zinc finger protein that regulates expression of genes leading to increased and decreased accumulation of ABA and GA, respectively (19).

SPATULA (SPT) is another bHLH transcription factor closely related to the PIFs, but it lacks the APB domain (20). SPT was originally described as a regulator of gynoecium development (21)

## Significance

Seeds sense environmental conditions, such as light quality, to regulate their germination. Light enriched in the far-red wavelength, such as under-the-canopy light (shade), increases abscisic acid (ABA) and decreases gibberellin (GA) levels, the phytohormones repressing and promoting germination, respectively. We have discovered that MFT functions as a key repressor of germination under far-red light conditions by modulating ABA and GA responses.

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The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in The National Center for Biotechnology Information (NCBI) RNA database (accession nos. SRR7175460–SRR7175468).

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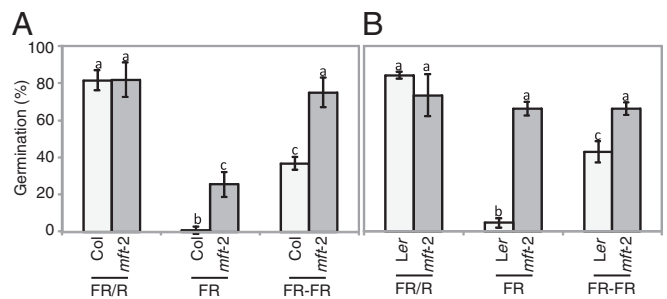
This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1806460115/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1806460115/-DCSupplemental).

and is also involved in regulating vegetative growth and seed dormancy (22–24). SPT regulation of seed dormancy involves the direct repression of *MOTHER-OF-FT-AND-TFL1* (*MFT*) (25). *MFT* is a member of the phosphatidylethanolamine-binding protein (PEBP) family, which is involved in diverse growth and differentiation signaling pathways in bacteria, animals, and plants. In *Arabidopsis* there are six PEBP proteins and extensive analyses of two of them, *FLOWERING-LOCUS-T* (*FT*) and *TERMINAL-FLLOWER1* (*TFL1*), have demonstrated that they antagonistically regulate flowering time and seed dormancy (26–29). In wheat it has been established that up-regulation of *MFT* expression by cold during seed development or by a mutation in its promoter leads to strong seed dormancy (30, 31). In *Arabidopsis* *MFT* is also a strong promoter of seed dormancy (25), but its expression is only weakly promoted by low temperature during seed development (32). However, *MFT* expression in the soil seed bank is seasonally regulated and correlates with low temperatures and high levels of cycling secondary dormancy (33). Furthermore, expression of *MFT* has also been shown to be promoted by ABA and DELLA proteins (34). Intriguingly, we and others have found that while freshly matured *MFT*-deficient seeds are less dormant than wild-type seeds, afterripened (nondormant) seeds are, counterintuitively, more sensitive to ABA treatments (25, 34). This raised the possibility that *MFT* has opposite roles in regulating seed dormancy and nondormant seed germination.

In the present study, we investigate the role of the *MFT* gene in regulating germination of afterripened seeds under R and FR light. We demonstrate that *MFT* is a key component of the PIF1-dependent pathway of repression of germination and acts by integrating ABA and GA signals. Furthermore, we show that expression of the *MFT* gene is regulated by light quality: While it is promoted by the PIF1/SOM/DELLA pathway under FR light, it is repressed by SPT under R light. Consistent with this, we also show that FR light represses *SPT* gene expression also in a PIF1-dependent manner. Our genetic, physiological and transcriptomic analyses reveal that *MFT* plays a major role in repressing seed germination by integrating the ABA and GA signaling pathways.

## Results and Discussion

***MFT* Represses Germination Under FR Light.** We reported previously that freshly matured *Arabidopsis* loss-of-function *mft-2* mutant seeds are less dormant than wild-type seeds, which led us to conclude that *MFT* promotes dormancy (25). However, ABA treatments of *mft-2* nondormant seeds indicated that *MFT* promotes germination of afterripened seeds (25, 34). This prompted us to investigate the role of *MFT* in regulating germination of nondormant seeds under shade light conditions, which repress germination by inducing accumulation of endogenous ABA. To do this, germination assays were performed on control and *mft-2* afterripened seeds (in the Col and *Ler* backgrounds) under different light treatments (*SI Appendix*, Fig. S1). Seeds were imbibed for 3 h under white light, then treated with: (i) two consecutive short (5 min) pulses of FR and R light (FR/R) to activate PHYB; (ii) a single FR light pulse (FR) to deactivate PHYB; and (iii) an initial short FR pulse followed [46 h after imbibition (hai)] by a long (120 min) FR light exposure (FR–FR) to activate PHYA while also deactivating PHYB. We found that under FR/R light conditions, wild-type and *mft-2* seeds germinate at similarly high levels (80%) (Fig. 1 *A* and *B*). Upon FR treatments, germination of wild-type seeds was, as expected, severely repressed (less than 5%), whereas *mft-2* seeds germinated at higher rates (30–70%) (Fig. 1 *A* and *B*). We used an *mft-2* complemented line (33) to confirm that this mutant phenotype is due to a mutation in the *MFT* gene (*SI Appendix*, Fig. S2). Under FR–FR conditions, germination rates of control seeds were, as expected, approximately intermediate (40%) to those of FR/R- and FR-treated seeds; and *mft-2* seeds germinated at higher rates (70%) than the controls (Fig. 1 *A* and *B*). Taken together

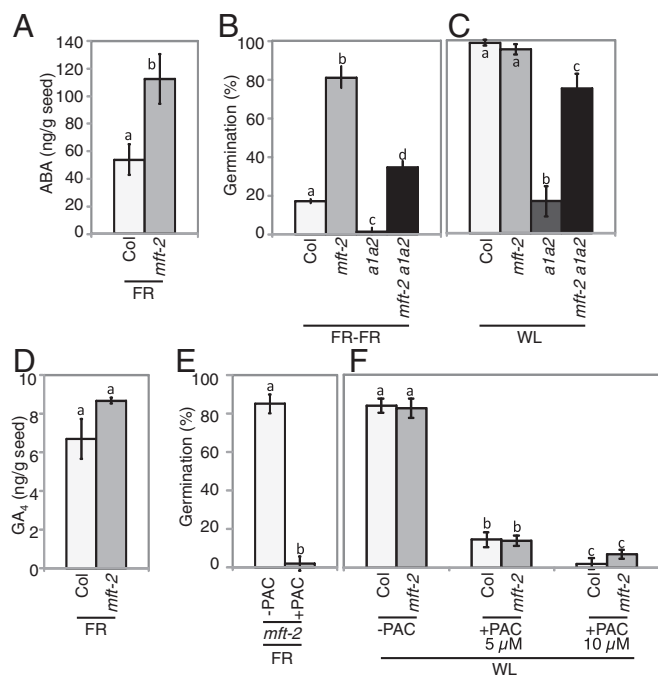


**Fig. 1.** Germination of *mft-2* seeds under different light quality treatments. Control and *mft-2* afterripened seeds [in Col (*A*) and *Ler* (*B*) backgrounds] upon FR/R, FR, and FR–FR light treatments as indicated in *SI Appendix*, Fig. S1. Data are means of four biological replicates and error bars represent SD. Different lowercase letters indicate statistically significant differences (*t* test,  $P < 0.01$ ).

these observations demonstrate that *MFT* is required to repress germination under FR light conditions.

***MFT* Promotes Hypocotyl Elongation in the Dark.** In addition to their role in seed germination, phytochromes and the PIF1-dependent pathway also control seedling hypocotyl elongation (35, 36). Thus, we investigated whether *MFT* also regulates seedling development and measured wild-type and *mft-2* hypocotyls of seedlings grown under dark conditions, which are known to trigger PHYB inactivation (14). We found that *mft-2* hypocotyls were significantly shorter than the wild-type controls (*SI Appendix*, Fig. S3) supporting the hypothesis that *MFT* promotes seedling hypocotyl elongation. These roles for *MFT* in repressing seed germination and promoting seedling hypocotyl elongation parallel the established roles of the phytochrome interacting factor PIF1 (16, 17, 37, 38) and further indicates that *MFT* operates in the PIF1 pathway.

***MFT* Is a Positive Regulator of ABA Signaling.** To investigate how *MFT* inhibits germination, we measured phytohormone levels in afterripened seeds under FR light conditions (24 hai). ABA levels were actually elevated in FR-treated *mft-2* seeds compared with the wild type (Fig. 2*A*), even though germination is higher in the mutant (Fig. 1 *A* and *B*). This suggests that the inhibitory effect of *MFT* on germination of FR-treated seeds acts downstream of ABA. To test this hypothesis, we analyzed triple *mft-2 cyp707a1-1 cyp707a2-1* mutant and control seeds. Both *CYP707A1* and *CYP707A2* are involved in ABA degradation, and double *cyp707a1-1 cyp707a2-1* (*ala2*) mutant seeds germinate poorly due to their elevated levels of endogenous ABA (39). Under FR–FR light conditions, we found that the almost zero percentage germination phenotype of the *ala2* double mutant is rescued by *mft-2* to levels that are intermediate between the wild type and the *mft-2* single mutant (Fig. 2*B*). We also performed germination assays of the afterripened seeds under white light, and observed that, whereas *ala2* seed germination was impaired, *mft-2 ala2* seed germination was almost as high as *mft-2* and wild-type control seeds (Fig. 3*C*). Considering that ABA induces *MFT* expression (34), these observations indicate that *MFT* is involved in the transduction of the ABA signal that results in germination inhibition. However, since the repression of germination in *ala2* seeds is not completely removed in the *mft-2* background, we conclude that not all of the ABA repression of germination is due to its effect on *MFT*. We and others have previously shown that *mft-2* seeds are hypersensitive to exogenous ABA treatment (25, 34). This contrasts with our present observation, which indicates *mft-2* to be hypo- rather than hypersensitive to elevated endogenous ABA in *ala2* seeds (Fig. 2 *B* and *C*). The hypersensitivity of *mft-2* to exogenous ABA also contrasts with studies analyzing sensitivity of *mft-2* seeds to exogenous ABA during dormancy cycling (40).



**Fig. 2.** Phytohormone and germination analyses of *mft-2* seeds. (A) ABA accumulation in FR-treated wild-type (Col) and *mft-2* seeds (24 hai). (B and C) Germination assays of Col, single *mft-2*, double *cyp707a1-1 cyp707a2-1* (*a1a2*), and triple *mft-2 a1a2* mutant nondormant seeds upon FR-FR (B) and white light (WL; C) conditions. (D) GA<sub>4</sub> (ng/g seed) accumulation in FR-treated wild-type (Col) and *mft-2* seeds (24 hai). (E and F) Germination assays of Col and *mft-2* seeds on control (-PAC) and paclobutrazol-supplemented plates under FR (+PAC, 10 μM; E) and WL light conditions (+PAC, 5 and 10 μM; F). Phytohormone and germination data are means of four biological replicates and error bars represent SD. Different lowercase letters indicate statistically significant differences (*t* test, *P* < 0.01).

We also measured GA accumulation in FR-treated seeds and observed no statistically significant difference between *mft-2* and control seeds (Fig. 2D). However, we found that, under FR light, germination of *mft-2* seeds treated with the GA biosynthesis inhibitor paclobutrazol (PAC) was strongly inhibited (Fig. 2E), which shows that GA is required for *mft-2* seeds to germinate. We also compared the response to PAC of wild-type and *mft-2* nondormant seeds under white light. We found that both sets of seeds were equally affected by PAC treatments (Fig. 2F), demonstrating that high *mft-2* germination is not due to increased GA sensitivity.

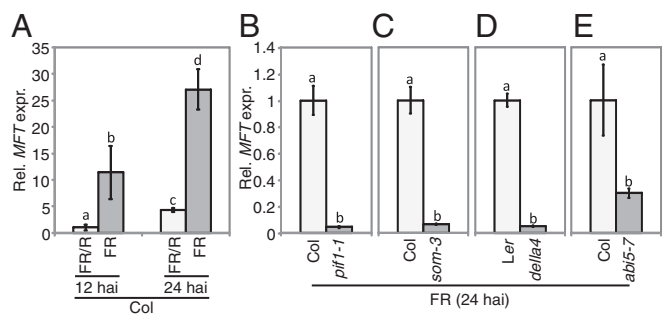
***MFT* Expression Is Promoted by FR Light in a PIF1 Pathway-Dependent Manner.** Publicly available transcriptomic data indicate that *MFT* gene expression is high during seed development when primary dormancy is set and, conversely, low in imbibed seeds when germination is triggered ([vseed.nottingham.ac.uk](http://vseed.nottingham.ac.uk)). This prompted us to investigate whether light quality plays a role in regulating *MFT* expression in imbibed seeds. To test this, we quantified *MFT* transcript accumulation in FR/R and FR light treated afterripened seeds 12 and 24 hai. We observed that, compared with FR/R, *MFT* transcript accumulation is strongly induced under FR light conditions (Fig. 3A). Moreover, we established that this FR-triggered increase of *MFT* expression is strongly compromised (over 20-fold) in *pif1-1* mutant seeds (Fig. 3B), which are impaired in the FR-dependent repression of germination (18). Since active PHYA represses PIF1 accumulation, we also analyzed *phyA-211* mutant seeds under FR-FR light conditions and found increased *MFT* transcript accumulation (SI Appendix, Fig. S4). This is consistent with our finding that *MFT* expression is positively regulated by PIF1 (Fig. 3B).

As a member of the bHLH family of transcription factors, PIF1 preferentially binds to G-box motifs (41). Since the *MFT* promoter harbors three G-box motifs (25), we investigated by chromatin immunoprecipitation (ChIP) followed by quantitative PCR (qPCR) whether PIF1 binds the *MFT* promoter in FR-treated seeds but we found no evidence of such an interaction (SI Appendix, Fig. S5). This indicates that the PIF1 involvement in promoting *MFT* expression under FR conditions is likely to be indirect. It is known that PIF1 does interact directly with the promoters of *SOM* and *ABI5* as well as the DELLA-encoding *RGA* and *GAI* genes (18, 19). It was previously shown in imbibed seeds under white light that *MFT* expression is promoted by ABA and repressed by GA in an *ABI5*- and DELLA-dependent manner, respectively (34). This prompted us to test whether this regulation also occurs under FR light conditions. We analyzed *som-3*, *abi5-7*, and quadruple *rgl1-1 rgl2-2 gai-6 rga-2* (hereafter referred to as *della4*) mutant seeds and found that *MFT* expression is decreased in all three mutant backgrounds (Fig. 3C–E), which indicates that *MFT* expression is promoted by these factors acting downstream of PIF1.

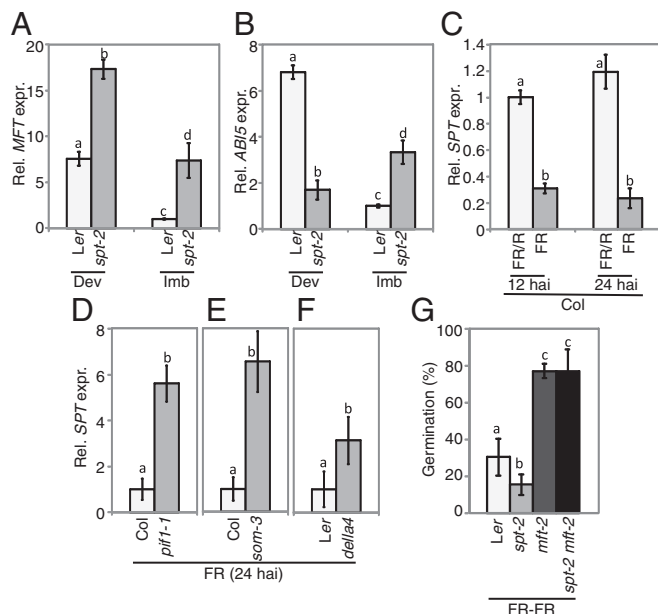
It is worth noting that the decrease of *MFT* transcript accumulation in *pif1-1*, *som-3*, and *della4* seeds is much more pronounced (15- to 20-fold) than its decrease in *abi5-7* seeds (3-fold) (Fig. 3B–E). This shows that, relative to PIF1/SOM/DELLA, *ABI5* is a weak promoter of *MFT* expression. Interestingly, while *pif1-1*, *som-3*, and multiple combinations of *della* mutant seeds germinate under FR light (17, 19, 42), *abi5* mutant seeds do not (8). This suggests that the relatively high levels of residual *MFT* transcript in *abi5* mutant seeds may be sufficient to repress germination under FR light conditions.

#### SPT Represses both *MFT* and *ABI5* Gene Expression in Imbibed Seeds.

We showed previously that freshly matured *spt-2* mutant seeds (in the *Ler* background) are extremely dormant, but their dormancy is lost over dry storage, and afterripened *spt-2* seeds germinate as much as *Ler* control seeds (22). In freshly matured dry seeds, SPT binds to the *MFT* and *ABI5* promoters to repress and promote their expression, respectively. However, at least in the case of SPT-*MFT*, this direct interaction is lost in imbibed seeds (25). We therefore reassessed the role of SPT in regulating these genes in developing and imbibed seeds. As previously reported in dry seeds, *MFT* transcript accumulation is increased in both developing and imbibed *spt-2* seeds (Fig. 4A), which indicates that SPT represses *MFT* expression in seeds irrespective of their developmental stage. Regarding *ABI5*, we found that its expression is decreased in developing *spt-2* seeds, which is consistent



**Fig. 3.** Characterization of *MFT* gene expression. (A) Relative *MFT* expression in FR/R and FR-treated wild-type (Col) seeds (12 and 24 hai). (B–E) Relative *MFT* expression in FR-treated *pif1-1*, *som-3*, *della4* (*rgl1-1*, *rgl2-2*, *gai-6*, and *rga-2*), and *abi5-7* mutant seeds and their respective Col/Ler controls (24 hai). Data are means of three biological replicates and error bars represent SD. Different lowercase letters indicate statistically significant differences (*t* test, *P* < 0.01).



**Fig. 4.** The role of SPT in light-quality-dependent germination. (A and B) Relative *MFT* and *ABI5* expression in developing (Dev) and stratified imbibed (Imb) control (*Ler*) and *spt-2* seeds. (C) Relative *SPT* expression in FR/R and FR-treated wild-type (*Col*) seeds (12 and 24 hai). (D–F) Relative *SPT* expression in FR-treated *pif1-1*, *som-3*, and *della4* (*rgl1-1*, *rgl2-2*, *gai-6*, and *rga-2*) mutant seeds and their respective *Col/Ler* controls (24 hai). (G) Germination assays of *Ler*, *spt-2*, *mft-2*, and *spt-2 mft-2* afterripened seeds under FR–FR light conditions. Error bars represent SD of at least three biological replicates. Different lowercase letters indicate statistically significant differences (*t* test,  $P < 0.05$ ).

with our previous finding for dry seeds. However, *ABI5* expression is increased in imbibed *spt-2* seeds (Fig. 4B). Thus, SPT promotes *ABI5* expression in developing seeds and represses it in imbibed seeds. Using ChIP–qPCR assays, we found no evidence for direct interaction of SPT with *MFT* or *ABI5* in imbibed seeds (*SI Appendix*, Fig. S5).

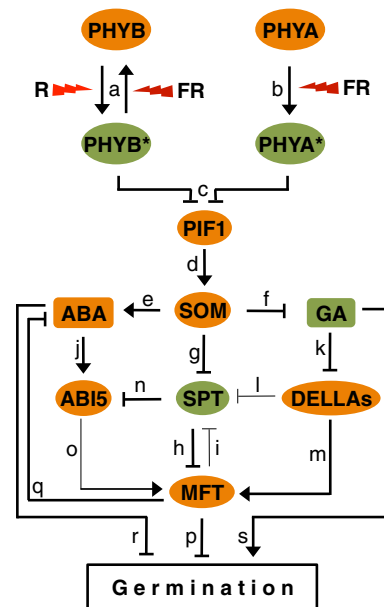
#### SPT Expression Is Repressed by FR Light in a PIF1 Pathway-Dependent Manner.

We next investigated whether *SPT* expression is regulated by light quality. We established that, compared with FR/R conditions, *SPT* transcript accumulation is inhibited by FR light in a PIF1 pathway-dependent manner (Fig. 4C and D). Consistent with this, we also established that PHYA promotes *SPT* expression (*SI Appendix*, Fig. S4). ChIP–qPCR assays established that repression of *SPT* by PIF1 is likely to be indirect (*SI Appendix*, Fig. S5). Furthermore, genetic analysis revealed that *SPT* gene expression is repressed by SOM and DELLA proteins, although the repressing effect of the latter appears to be weaker (Fig. 4E and F). Finally, we also assessed germination under FR–FR light conditions of *spt-2 mft-2* double mutant and *Ler* and parental control seeds. We found that *spt-2* seeds germinate at a lower rate than *Ler* control seeds and, importantly, *spt-2 mft-2* seeds germinate as much as the single *mft-2* mutant seeds (Fig. 4G), which is consistent with our previous observation in freshly harvested seeds and shows that MFT acts downstream of SPT in the light quality regulation of germination.

**MFT Regulates Expression of Growth-Related Genes.** It has been established that MFT localizes in the nucleus and interacts indirectly with DNA to regulate gene expression (34, 43). To gain insight into the MFT mode of action, we performed RNA sequencing-based transcriptomic analyses to identify genes regulated by MFT. We analyzed wild-type (*Col*) and *mft-2* afterripened seeds under FR/R and FR light conditions (24 hai). We made two sets of gene

expression profile comparisons from three transcriptomic datasets: (i) FR- vs. FR/R-treated *Col* seeds ( $Col_{FR}$  vs.  $Col_{FR/R}$ ) and (ii) FR-treated *Col* seeds vs. FR-treated *mft-2* seeds ( $Col_{FR}$  vs. *mft-2*<sub>FR</sub>). We reasoned that genes that are up-regulated by the MFT-mediated FR light response will show increased expression in  $Col_{FR}$  compared with  $Col_{FR/R}$  and *mft-2*<sub>FR</sub>. Similarly, genes that are down-regulated by MFT will have decreased expression in  $Col_{FR}$ . Using at least a twofold-difference threshold in each comparison, we found 175 MFT up-regulated genes and 74 MFT down-regulated genes (Datasets S1 and S2).

Among the MFT up-regulated group are *MFT* itself and genes known to be induced by ABA and involved in ABA signaling, such as *ABI2*, *PYL13/RCAR7*, and *GCR2/GPCR*, although the role of the latter in this pathway has been controversial (44–46). The gene most strongly up-regulated by MFT is *PLP1/PLAVIA*, which encodes a phospholipase known to promote hypocotyl elongation (47). Also among this group of MFT up-regulated genes are *CAT1*, which is known to act downstream of *ABI5* to regulate reactive oxygen species (ROS) homeostasis in germinating seeds (48), and *DOGL1*, *DOGL2*, and *DOGL3*, which are closely related to the strong dormancy promoter DELAY-OF-GERMINATION1 (*DOG1*) (49). Notably, another of the genes up-regulated by MFT, *NCED4/CCD4*, encodes a negative regulator of  $\beta$ -carotene, a biosynthetic precursor of ABA (50, 51). Thus, it is possible that the higher-than-normal ABA levels found in *mft-2* seeds is a consequence of the decreased *NCED4/CCD4*



**Fig. 5.** The role of MFT and SPT in the light-quality-dependent regulation of seed germination. PHYB, which is present at the onset of imbibition, is activated and deactivated by R and FR light, respectively (a). PHYA, which accumulates a few hours after imbibition, is activated by FR light (b). Active phytochromes (PHYA\* and PHYB\*) trigger PIF1 degradation (c). In the absence of PHYA\* and PHYB\*, PIF1 accumulates to promote *SOM* expression (d). *SOM* induces ABA and inhibits GA accumulation (e and f), as well as repressing *SPT* expression (g). *SPT* represses *MFT* expression (h) and MFT partially contributes to *SPT* repression under FR light (i). ABA acts, at least partially, through *ABI5* (j), whereas GA triggers degradation of the DELLA proteins (k). The growth-repressing DELLA proteins inhibit, albeit weakly, *SPT* expression (l) and promote *MFT* expression (m). *SPT* represses *ABI5* expression (n), and *ABI5* is a weak promoter of *MFT* expression (o). MFT inhibits seed germination (p) through regulation of growth-related genes (not depicted in the model). MFT also represses ABA accumulation (q). Both ABA and GA have MFT-independent effects on germination (r and s).

transcript abundance and a concomitant increase of  $\beta$ -carotene. MFT also promotes expression of genes involved in seed development and seed storage, as well as in lipid metabolism, including oxylipin biosynthesis. *AOS/CYP74A*, which is involved in an early step in the biosynthesis of the oxylipin 12-oxo-phytodienoic acid (OPDA) (52), is up-regulated by MFT. We reported previously that OPDA is a potent repressor of germination that interacts with MFT and ABA in a complex network of feedback interactions (53, 54).

*SPT* gene expression is 11-fold decreased by FR light and MFT partially contributes to this repression, highlighting the complex feedback interactions between these two key players. Many of the other MFT down-regulated genes are predicted to be involved in cell wall metabolism, response to GA, and root growth. The *DVL19/RTFL10* gene, belonging to the *DEVIL/ROTUNDIFOLIA* family, is the most strongly down-regulated by FR light and this appears to be completely dependent on MFT. Little is known about the function of this gene; however, over-expression of other members of this gene family results in elongated cells at the base of trichomes (55). Many genes involved in auxin signaling (for example *AUX1*, *HLS1/COP3*, *GH3.1/WES1*, *WOX11*, *SAUR77*, and *PAP1/LAA26*) are also repressed by MFT, as well as *CYP90A1*, involved in brassinosteroid biosynthesis. Both auxin and brassinosteroid phytohormones regulate a wide range of plant development and physiological processes and are recognized as positive growth regulators of root and shoot development (56). *GASA6*, *GASA14*, and *EXPA1* are also repressed by MFT and are known to be promoted by GA and repressed by ABA and DELLA proteins. It has been reported that *GASA6* and *GASA14* promote cell elongation and leaf expansion, as well as seed germination (57, 58). *GASA6* requires the cell wall loosening function of *EXPA1* (59) and it is expressed in the hypocotyl–radical transition zone of the embryonic axis (58). This is consistent with the reported tissue specificity of *MFT* gene promoter activity in ABA-treated seeds (34).

## Conclusion

The integration of the data presented in this study with current knowledge of FR light signaling allows us to propose a model (Fig. 5) in which MFT plays a key role in the integration of signals downstream of core signaling components and the antagonistically acting phytohormones ABA and GA. Previous reports have established that light-activated phytochromes lead to PIF1 degradation (17) and, when phytochromes are deactivated, PIF1 accumulates and induces *SOM* expression. *SOM*, in turn, promotes and represses ABA and GA accumulation, respectively (19). This then leads to repression of seed germination through a mechanism involving *ABI5* and *DELLA* proteins (2, 4). In the present work, we have shown that *SOM* also represses germination through the promotion of the germination inhibitor MFT. We show that this effect of *SOM* on MFT is most likely due to *SOM* repressing *SPT*, which is a negative regulator of *MFT* expression. Our data also demonstrate that the growth-repressing *DELLA* proteins inhibit and promote *SPT* and *MFT* expression, respectively, which is entirely consistent with the fact that *DELLA*s accumulate under light conditions that repress germination. Furthermore, MFT is also promoted, albeit weakly, by *ABI5*, which itself is repressed by *SPT*. Our data also highlight that, as well as acting downstream of ABA, MFT represses its accumulation. Thus, MFT counteracts the effect of *SOM* on ABA accumulation. Finally, while we show that MFT plays a key role in the integration of signaling downstream of ABA and GA, both phytohormones can still affect germination independently of MFT.

In summary, our findings establish that shade (FR light) promotes expression of *MFT*, which in turn represses germination by up- and down-regulating expression of genes involved in ABA signaling and cell wall expansion, respectively. Moreover, we show that this MFT-dependent mechanism of germination inhibition is

actively repressed by *SPT* under R light conditions that induce germination. Publicly available data indicate that *MFT* is strongly expressed in the endosperm during the late stages of seed development ([vseed.nottingham.ac.uk/](http://vseed.nottingham.ac.uk/)). It has been established that the endosperm plays a crucial role in promoting dormancy and repressing germination of nondormant seeds under FR light conditions (7, 8). Whether MFT plays a role in this endosperm-based pathway leading to repression of germination remains to be established.

## Experimental Procedures

**Growth Conditions and Biological Materials.** Plants were grown in a greenhouse supplemented with artificial light to give a photoperiod of 16 h light at a temperature of 20–22 °C. Seeds were harvested and afterripened for at least 8 wk before analyses. Original mutant and transgenic lines were described previously: *mft-2* (Col/Ler) and *gmft* (34); *cyp707a1-1 cyp707a2-1* (*a1a2*) (39); *pi1-1/pil5-1* (16); *som-3* (19) and *rgl1-1 rgl2-2 gai-6 rga-2* (*della4*) (2); *abi5-7* (60); *spt-2* (61); 35S:PIF1myc (18); and 35S:SPTmyc (25).

**Germination Assays.** Sterilized seeds were plated on water agar (0.9% wt/vol) and allowed to imbibe under dim light for 3 h and then LED irradiated with FR (4.5  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) and R (20  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) as indicated in Fig. 1A. After FR/R, FR, and FR–FR treatments, plates were wrapped in foil and kept at 20 °C. Germination was scored on the basis of radicle emergence of 50–100 seeds per replica. Paclobutrazol (Sigma-Aldrich) was included in the water agar medium where indicated.

**Phytohormone Analyses.** Phytohormones were extracted and quantified from four biological replicates of 100 mg of treated seeds according to the protocol described previously (53).

**Molecular Biology Techniques.** RNA extractions were performed as described previously (25). Standard protocols were used for RQ1 RNase-Free DNase treatments (Promega), cDNA synthesis (SuperScriptII, Invitrogen), and qPCRs (iTaQ Universal Syber Green, Bio-Rad). Expression of *UBQ11* was used for normalization, and gene expression was expressed as a fold change relative to the control sample. ChIP assays were performed as described previously (18) followed by qPCR and DNA amplification normalized to the amplification of *UBQ11* and expressed relative to the negative control (–Ab). Relative qPCR amplifications were calculated following the delta–delta Ct method ( $2^{-\Delta\Delta\text{Ct}}$ ). Primer sequences for the qPCRs are described in *SI Appendix, Table S1*.

**Transcriptomic Analyses.** RNA yield from three independent biological samples was quantified and quality checked using a Nanodrop Spectrophotometer and Agilent Bioanalyser RNA nanochip. A total of 500 ng of total RNA was used for library preparation using the NEBNext RNA Ultra Library preparation kit for Illumina in conjunction with the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England BioLabs, Inc.) and NEBNext single 6-bp indexing primers, according to the manufacturer's instructions. Libraries were pooled at equimolar ratios, and the pool was sent for 2  $\times$  150 base-paired-end sequencing on a HiSeq 3000 at the University of Leeds Next Generation Sequencing Facility. Ribosomal RNA was filtered with mapping to rRNA\_115\_tax\_silva\_v1.0 downloaded from SILVA database (<https://www.arb-silva.de/>). The remaining RNAseq reads were mapped to the cDNA reference sequence from TAIR10. BWA (62) was used with default parameters for mapping. SAMtools (63) was used for read quantification (*SI Appendix, Table S2*). The methods in the Bioconductor package EdgeR (64) were used to identify the differentially expressed genes from comparisons described in the main text. Only genes with at least a twofold change in each of the two comparisons, and a minimum of 5 reads per kilobase million (RPKM) in Col<sub>FR</sub> (for MFR promotion) or Col<sub>FR/R</sub> and *mft-2*<sub>FR</sub> (for MFT repression), were considered in the analyses. Raw RNAseq data have been deposited at the National Center for Biotechnology Information (accession nos. SRR7175460–SRR7175468).

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- Dill A, Jung HS, Sun TP (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. *Proc Natl Acad Sci USA* 98:14162–14167.
- Cao D, Hussain A, Cheng H, Peng J (2005) Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in Arabidopsis. *Planta* 223:105–113.
- Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The Arabidopsis abscisic acid response locus ABI4 encodes an APETALA 2 domain protein. *Plant Cell* 10:1043–1054.
- Finkelstein RR, Lynch TJ (2000) The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. *Plant Cell* 12:599–609.
- Clerkx EJ, Vries HB, Ruys GJ, Groot SP, Koornneef M (2003) Characterization of green seed, an enhancer of *abi-3* in Arabidopsis that affects seed longevity. *Plant Physiol* 132:1077–1084.
- Seo M, et al. (2006) Regulation of hormone metabolism in Arabidopsis seeds: Phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. *Plant J* 48:354–366.
- Lee KP, Piskurewicz U, Turecková V, Strnad M, Lopez-Molina L (2010) A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. *Proc Natl Acad Sci USA* 107:19108–19113.
- Lee KP, et al. (2012) Spatially and genetically distinct control of seed germination by phytochromes A and B. *Genes Dev* 26:1984–1996.
- Lim S, et al. (2013) ABA-insensitive3, ABA-insensitive5, and DELLAs interact to activate the expression of SOMNUS and other high-temperature-inducible genes in imbibed seeds in Arabidopsis. *Plant Cell* 25:4863–4878.
- Chahtane H, Kim W, Lopez-Molina L (2017) Primary seed dormancy: A temporally multilayered riddle waiting to be unlocked. *J Exp Bot* 68:857–869.
- Reed JW, Nagatani A, Elich TD, Fagan M, Chory J (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. *Plant Physiol* 104:1139–1149.
- Shinomura T, Nagatani A, Chory J, Furuya M (1994) The induction of seed germination in Arabidopsis thaliana is regulated principally by phytochrome B and secondarily by phytochrome A. *Plant Physiol* 104:363–371.
- Shinomura T, et al. (1996) Action spectra for phytochrome A- and B-specific photo-induction of seed germination in Arabidopsis thaliana. *Proc Natl Acad Sci USA* 93:8129–8133.
- Medzhradszky M, et al. (2013) Phosphorylation of phytochrome B inhibits light-induced signaling via accelerated dark reversion in Arabidopsis. *Plant Cell* 25:535–544.
- Shen Y, Khanna R, Carle CM, Quail PH (2007) Phytochrome induces rapid PIF5 phosphorylation and degradation in response to red-light activation. *Plant Physiol* 145:1043–1051.
- Oh E, et al. (2004) PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. *Plant Cell* 16:3045–3058.
- Oh E, et al. (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *Plant J* 47:124–139.
- Oh E, et al. (2007) PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. *Plant Cell* 19:1192–1208.
- Kim DH, et al. (2008) SOMNUS, a CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination downstream of PIL5. *Plant Cell* 20:1260–1277.
- Reymond MC, et al. (2012) A light-regulated genetic module was recruited to carpel development in Arabidopsis following a structural change to SPATULA. *Plant Cell* 24:2812–2825.
- Alvarez J, Smyth DR (1999) CRABS CLAW and SPATULA, two Arabidopsis genes that control carpel development in parallel with AGAMOUS. *Development* 126:2377–2386.
- Penfield S, et al. (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. *Curr Biol* 15:1998–2006.
- Ichihashi Y, Horiguchi G, Gleissberg S, Tsukaya H (2010) The bHLH transcription factor SPATULA controls final leaf size in Arabidopsis thaliana. *Plant Cell Physiol* 51:252–261.
- Josse EM, et al. (2011) A DELLA in disguise: SPATULA restrains the growth of the developing Arabidopsis seedling. *Plant Cell* 23:1337–1351.
- Vaistij FE, et al. (2013) Differential control of seed primary dormancy in Arabidopsis ecotypes by the transcription factor SPATULA. *Proc Natl Acad Sci USA* 110:10866–10871.
- Bradley D, Ratcliffe O, Vincent C, Carpenter R, Coen E (1997) Inflorescence commitment and architecture in Arabidopsis. *Science* 275:80–83.
- Kardailsky I, et al. (1999) Activation tagging of the floral inducer FT. *Science* 286:1962–1965.
- Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 286:1960–1962.
- Chen M, Penfield S (2018) Feedback regulation of COOLAIR expression controls seed dormancy and flowering time. *Science* 360:1014–1017.
- Nakamura S, et al. (2011) A wheat homolog of MOTHER OF FT AND TFL1 acts in the regulation of germination. *Plant Cell* 23:3215–3229.
- Chono M, et al. (2015) Molecular and genealogical analysis of grain dormancy in Japanese wheat varieties, with specific focus on MOTHER OF FT AND TFL1 on chromosome 3A. *Breed Sci* 65:103–109.
- Kendall SL, et al. (2011) Induction of dormancy in Arabidopsis summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. *Plant Cell* 23:2568–2580.
- Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. *Proc Natl Acad Sci USA* 108:20236–20241.
- Xi W, Liu C, Hou X, Yu H (2010) MOTHER OF FT AND TFL1 regulates seed germination through a negative feedback loop modulating ABA signaling in Arabidopsis. *Plant Cell* 22:1733–1748.
- Fankhauser C, Casal JJ (2004) Phenotypic characterization of a photomorphogenic mutant. *Plant J* 39:747–760.
- Yang C, Li L (2017) Hormonal regulation in shade avoidance. *Front Plant Sci* 8:1527.
- Shin J, et al. (2009) Phytochromes promote seedling light responses by inhibiting four negatively-acting phytochrome-interacting factors. *Proc Natl Acad Sci USA* 106:7660–7665.
- Soy J, Leivar P, Monte E (2014) PIF1 promotes phytochrome-regulated growth under photoperiodic conditions in Arabidopsis together with PIF3, PIF4, and PIF5. *J Exp Bot* 65:2925–2936.
- Okamoto M, et al. (2006) CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. *Plant Physiol* 141:97–107.
- Footitt S, Ölçer-Footitt H, Hambidge AJ, Finch-Savage WE (2017) A laboratory simulation of Arabidopsis seed dormancy cycling provides new insight into its regulation by clock genes and the dormancy-related genes DOG1, MFT, CIPK23 and PHYA. *Plant Cell Environ* 40:1474–1486.
- Oh E, et al. (2009) Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in Arabidopsis. *Plant Cell* 21:403–419.
- Piskurewicz U, Lopez-Molina L (2009) The GA-signaling repressor RGL3 represses testa rupture in response to changes in GA and ABA levels. *Plant Signal Behav* 4:63–65.
- Hou CJ, Yang CH (2016) Comparative analysis of the pteridophyte Adiantum MFT ortholog reveals the specificity of combined FT/MFT C and N terminal interaction with FD for the regulation of the downstream gene AP1. *Plant Mol Biol* 91:563–579.
- Finkelstein RR (1994) Maternal effects govern variable dominance of two abscisic acid response mutations in Arabidopsis thaliana. *Plant Physiol* 105:1203–1208.
- Fuchs S, Tischer SV, Wunschel C, Christmann A, Grill E (2014) Abscisic acid sensor RCAR7/PYL13, specific regulator of protein phosphatase coreceptors. *Proc Natl Acad Sci USA* 111:5741–5746.
- Klingler JP, Batelli G, Zhu JK (2010) ABA receptors: The START of a new paradigm in phytohormone signalling. *J Exp Bot* 61:3199–3210.
- Holk A, Rietz S, Zahn M, Quader H, Scherer GF (2002) Molecular identification of cytosolic, patatin-related phospholipases A from Arabidopsis with potential functions in plant signal transduction. *Plant Physiol* 130:90–101.
- Bi C, et al. (2017) Arabidopsis ABI5 plays a role in regulating ROS homeostasis by activating CATALASE 1 transcription in seed germination. *Plant Mol Biol* 94:197–213.
- Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in Arabidopsis. *Proc Natl Acad Sci USA* 103:17042–17047.
- Gonzalez-Jorge S, et al. (2013) Carotenoid cleavage dioxygenase4 is a negative regulator of  $\beta$ -carotene content in Arabidopsis seeds. *Plant Cell* 25:4812–4826.
- Dong T, Park Y, Hwang I (2015) Abscisic acid: Biosynthesis, inactivation, homeostasis and signalling. *Essays Biochem* 58:29–48.
- Park JH, et al. (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis. *Plant J* 31:1–12.
- Dave A, et al. (2011) 12-oxo-phytodienoic acid accumulation during seed development represses seed germination in Arabidopsis. *Plant Cell* 23:583–599.
- Dave A, Vaistij FE, Gilday AD, Penfield SD, Graham IA (2016) Regulation of Arabidopsis thaliana seed dormancy and germination by 12-oxo-phytodienoic acid. *J Exp Bot* 67:2277–2284.
- Valdivia ER, et al. (2012) DVL genes play a role in the coordination of socket cell recruitment and differentiation. *J Exp Bot* 63:1405–1412.
- Tian H, Lv B, Ding T, Bai M, Ding Z (2018) Auxin-BR interaction regulates plant growth and development. *Front Plant Sci* 8:2256.
- Sun S, et al. (2013) GASA14 regulates leaf expansion and abiotic stress resistance by modulating reactive oxygen species accumulation. *J Exp Bot* 64:1637–1647.
- Zhong C, et al. (2015) Gibberellin acid-stimulated Arabidopsis6 serves as an integrator of gibberellin, abscisic acid, and glucose signaling during seed germination in Arabidopsis. *Plant Physiol* 169:2288–2303.
- Li Y, et al. (2002) Plant expansins are a complex multigene family with an ancient evolutionary origin. *Plant Physiol* 128:854–864.
- Nambara E, et al. (2002) A screen for genes that function in abscisic acid signaling in Arabidopsis thaliana. *Genetics* 161:1247–1255.
- Heisler MG, Atkinson A, Bylstra YH, Walsh R, Smyth DR (2001) SPATULA, a gene that controls development of carpel margin tissues in Arabidopsis, encodes a bHLH protein. *Development* 128:1089–1098.
- Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760.
- Li H, et al.; 1000 Genome Project Data Processing Subgroup (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079.
- Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139–140.