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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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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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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

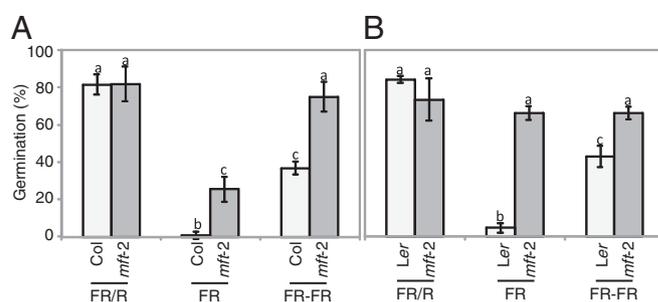


Fig. 1. Germination of *mft-2* seeds under different light quality treatments. Control and *mft-2* afterripened seeds [in Col (*A*) and *Ler* (*B*) backgrounds] upon FR/R, FR, and FR–FR light treatments as indicated in *SI Appendix*, Fig. S1. Data are means of four biological replicates and error bars 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).

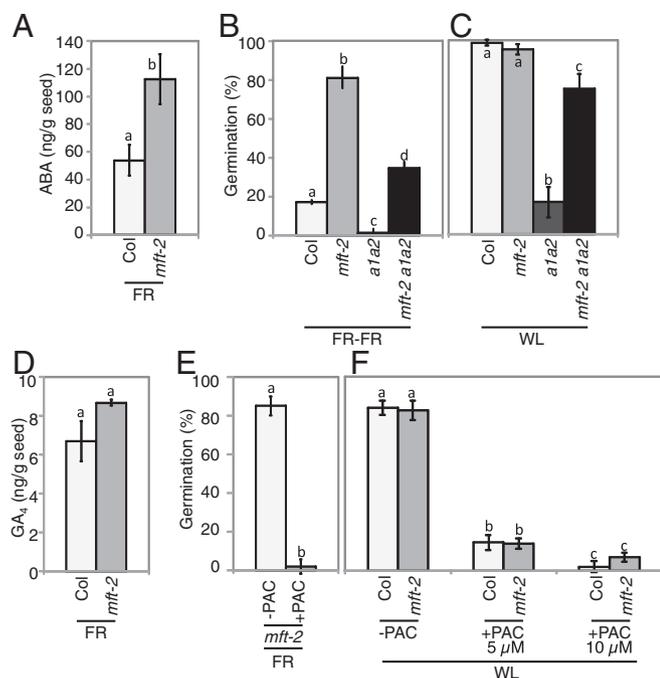


Fig. 2. Phytohormone and germination analyses of *mft-2* seeds. (A) ABA accumulation in FR-treated wild-type (Col) and *mft-2* seeds (24 hai). (B and C) Germination assays of Col, single *mft-2*, double *cyp707a1-1 cyp707a2-1* (*a1a2*), and triple *mft-2 a1a2* mutant nondormant seeds upon FR-FR (B) and white light (WL; C) conditions. (D) GA₄ 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). 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

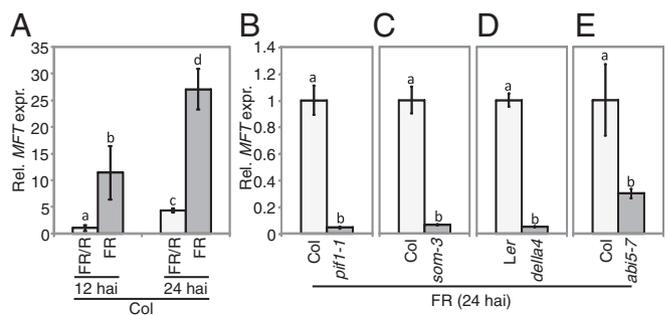


Fig. 3. Characterization of *MFT* gene expression. (A) Relative *MFT* expression in FR/R and FR-treated wild-type (Col) seeds (12 and 24 hai). (B–E) Relative *MFT* expression in FR-treated *pif1-1*, *som-3*, *della4* (*rgl1-1*, *rgl2-2*, *gai-6*, and *rga-2*), and *abi5-7* mutant seeds and their respective 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).

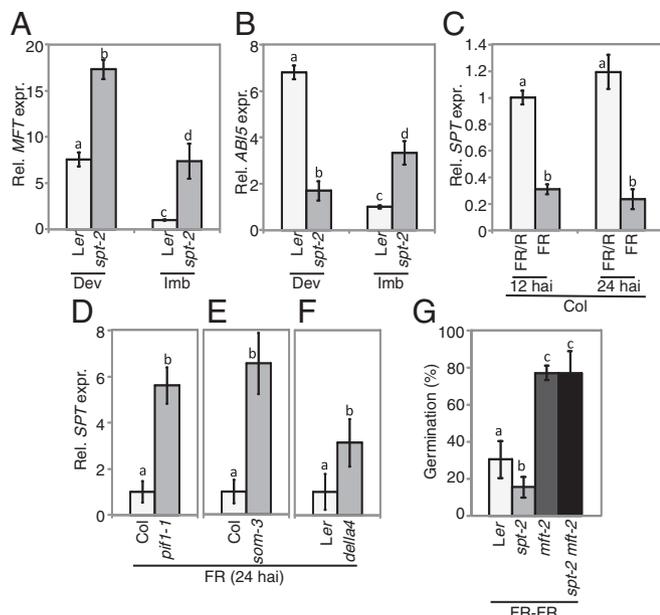


Fig. 4. The role of SPT in light-quality-dependent germination. (A and B) Relative *MFT* and *ABI5* expression in developing (Dev) and stratified imbibed (Imb) control (Ler) and *spt-2* seeds. (C) Relative *SPT* expression in FR/R and FR-treated wild-type (Col) seeds (12 and 24 hai). (D–F) Relative *SPT* 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*_{FR}). 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*_{FR}. 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 β -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*

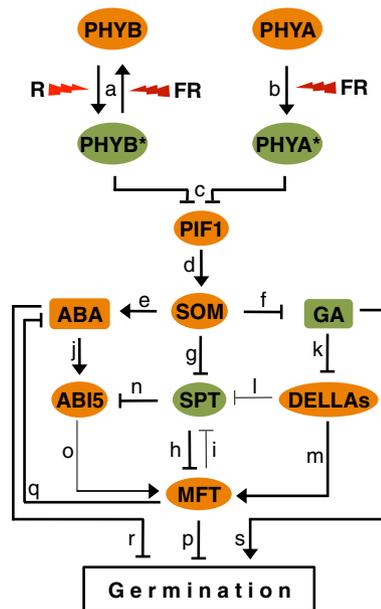


Fig. 5. The role of MFT and SPT in the light-quality-dependent regulation of seed germination. PHYB, which is present at the onset of imbibition, is activated and deactivated by R and FR light, respectively (a). PHYA, which accumulates a few hours after imbibition, is activated by FR light (b). Active 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 β -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/). 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_{FR} (for MFR promotion) or Col_{FR/R} and *mft-2*_{FR} (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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