cis-12-Oxo-phytodienoic acid represses Arabidopsis seed germination in shade conditions

Thiago Barros-Galvão, Anuja Dave, Adama Cole, David Harvey, Swen Langer, Tony R. Larson, Fabián E. Vaistij and Ian A. Graham*

Centre for Novel Agricultural Products, Department of Biology, University of York, York YO10 5DD, UK

* Correspondence: ian.graham@york.ac.uk

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Abstract

Light-dependent seed germination is induced by gibberellins (GA) and inhibited by abscisic acid (ABA). The widely accepted view of the GA/ABA ratio controlling germination does not, however, explain the fact that seeds deficient in ABA still germinate poorly under shade conditions that repress germination. In Arabidopsis, MOTHER-OF-FT-AND-TFL1 (MFT) acts as a key negative regulator of germination, modulating GA and ABA responses under shade conditions. Under full light the oxylipin cis-12-oxo-phytodienoic acid (OPDA), a precursor of the stress-related phytohormone jasmonic acid, interacts with ABA and MFT to repress germination. Here, we show that under shade conditions both OPDA and ABA repress germination to varying extents. We demonstrate that the level of shade-induced MFT expression influences the ability of OPDA and/or ABA to fully repress germination. We also found that MFT expression decreases with seed age and this again correlates with the response of seeds to OPDA and ABA. We conclude that OPDA plays an essential role alongside ABA in repressing germination in response to shade and the combined effect of these phytohormones is integrated to a significant extent through MFT.

Keywords: ABA, FR-light, MFT, OPDA, phytochrome, seed germination, shade.

Introduction

Timing of seed germination is one of the most important decision points in the life cycle of a higher plant. The environmental conditions under which a seed germinates are critical for survival, and consequently mechanisms that integrate environmental cues such as temperature and light quality have evolved to control the timing of germination in a number of species (Smith, 2000; Linkies et al., 2010; Kendall et al., 2011; Lee and Lopez-Molina, 2012). These cues regulate accumulation and perception of the phytohormones gibberellins (GA) and abscisic acid (ABA), which promote and repress seed germination, respectively (Seo et al., 2006; Kendall et al., 2011; Shu et al., 2016). ABA, acting through ABA response transcription factors such as ABA-INSENSITIVE3 (ABI3), ABI4, and ABI5 (Finkelstein et al., 1998; Finkelstein and Lynch, 2000; Clerkx et al., 2003), accumulates during seed development to induce a physiologically dormant state in which newly formed seeds do not germinate even under favourable environmental conditions (Graeber et al., 2012; Chahtane et al., 2017). Seeds gradually lose their dormancy through an after-ripening process after which they can germinate if environmental conditions are favourable (Smith, 2000; Jiao et al., 2007; Holdsworth et al., 2008).

In many plant species, including Arabidopsis, the probability of seedling establishment is generally greater if germination occurs under direct sunlight (white), which is rich in the red (R) wavelength, rather than in under-canopy light (shade), which is rich in far-red (FR) (Lee and Lopez-Molina, 2012).
In photoblastic seeds, phytochrome photoreceptors distinguish between these different light conditions on the basis of their R and FR light intensities and ratios (Shinomura, 1997; Smith, 2000; Quail, 2002; Jung et al., 2016). Excess R triggers GA accumulation and germination, whereas excess FR, typical of shade, triggers ABA accumulation and a block in germination (Oh et al., 2006; Seo et al., 2006; Piskurewicz et al., 2008). Arabidopsis has five phytochromes (phyA–E; Clack et al., 1994), with phyB being the main promoter of germination under sunlight, while phyA is responsible for germination in under-canopy light conditions (Shinomura et al., 1994). Both phyA and phyB are synthesized as inactive proteins and become active in a light-quality-dependent manner. However, while short pulses of R and FR light are sufficient to activate and deactivate phyB, respectively, longer exposures to R and FR activate phyA (Reed et al., 1994; Shinomura et al., 1994). Furthermore, compared with phyB, phyA accumulates at high levels only after relatively long periods of seed imbibition (Lee et al., 2012).

Upon activation, both phyA and phyB induce degradation of the transcription factor PHYTOCHROME INTERACTING FACTOR1 (PIF1, formerly known as PIL5) (Shen et al., 2005; Park et al., 2012). Upon phytochrome inactivation, PIF1 accumulates and regulates transcription of many genes, including SOMNUS (SOM), which encodes a CCCH-type zinc finger protein that is part of the phytochrome signal transduction pathway controlling genes involved in regulating ABA and GA levels ultimately leading to high ABA:GA ratios to repress germination (Oh et al., 2004, 2007; Kim et al., 2008; Park et al., 2011; Kim et al., 2016). We showed recently that PIF1 and SOM also promote MOTHER-OF-FT-AND-TFL1 (MFT) expression and that MFT plays a key role in repressing germination by modulating ABA and GA responses (Vaistij et al., 2018). Furthermore, PIF1 stimulates the expression of GA1 and RGA, which encode growth-repressing DELLA proteins (Oh et al., 2004, 2007; Piskurewicz et al., 2008; Piskurewicz and Lopez-Molina, 2009). GA promotes germination by targeting destruction of the DELLA proteins through the 26S proteasome. Under FR conditions, the DELLA proteins RGL2, GAI, and RGA repress germination by stimulating the expression of ABA biosynthetic genes, further increasing the ABA:GA ratio (Piskurewicz et al., 2008; Piskurewicz and Lopez-Molina, 2009; Lee et al., 2012).

The phytohormone jasmonic acid (JA) and its precursor cis-12-oxo-phytodienoic acid (OPDA) are oxilipins derived from linolenic acid (Wasternack and Song, 2017). The biologically active conjugated JA-isoleucine (JA-Ile) form is involved in responses to biotic and abiotic stress as well as in many other biological processes including seed germination (Linkies and Leubner-Metzger, 2012; Wasternack and Hause, 2013; Wasternack and Strnad, 2016; Singh et al., 2017). OPDA also exhibits signalling properties, some of which are shared with JA-Ile, but others are distinct (Dave et al., 2011; Goetz et al., 2012; Bosch et al., 2014; Guo et al., 2014; Savchenko and Dehesh, 2014). Previously we characterized the role of oxilipins in seed dormancy. We did this by analysing mutant seeds defective in (i) ALLENE OXIDE SYNTHASE (AOS), which encodes a cytochrome P450 oxidase enzyme involved in one of the final steps of OPDA biosynthesis inside plastids (Park et al., 2002); (ii) PXA1 (also known as CTS and PED3), which encodes an ABC-type transporter that imports OPDA into peroxisomes (Zolman et al., 2001; Footitt et al., 2002; Hayashi et al., 2002); and (iii) 12-OXOPHYTODIENOIC ACID REDUCTASE (OPR3), which is involved in the conversion of OPDA to JA in peroxisomes (Stintzi and Browse, 2000). It has been determined that seeds of the aos mutant, which is compromised in OPDA and JA/IA-Ile accumulation, are less dormant than wild-type seeds, whereas seeds of the pxa1-1 and opr3-1 single mutants, which overaccumulate OPDA but are deficient in JA/IA-Ile, are more dormant (Chehab et al., 2011; Dave et al., 2011, 2016). These observations led us to conclude that OPDA specifically acts as a dormancy-promoting factor.

In the present work we investigated the role of OPDA in the FR-triggered repression of germination of after-ripened seeds. We show that endogenous OPDA in seeds plays a key role alongside ABA to repress germination under shade conditions through an MFT-modulated process.

Material and methods

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 when plants stopped flowering and siliques started to dehisc. In all experiments, except for that involving extended after-ripening shown in Fig. S6, seeds were after-ripened for no longer than 8 weeks. All mutant lines used in this study were described previously: aos (Park et al., 2002); opr3-1 (Stintzi and Browse, 2000); opr3-3 (Chima et al., 2018); aba2-1 (León-Kloosterziel et al., 1996); mfi-2 (Xi et al., 2010); gfl1-1 gfl2-2 gai-6 nga-2 (della4) (Cao et al., 2005); and cyp20-3 (Park et al., 2013).

Germination assays

Seeds were vapour-phase sterilized by exposure to chlorine gas in a sealed glass container for at least 3 h, the gas having been produced by mixing 100 ml of bleach with 3 ml of concentrated HCl. Sterilized seeds were plated on water agar (0.9% w/v) and allowed to imbibed under low light at 20 °C. After FR/R, FR, and FR48 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 replication. In experiments where germination assays were conducted with ABA (Sigma-Aldrich), OPDA (Larodon), palbociclib (Sigma-Aldrich) or norflurazon (Sigma-Aldrich), the appropriate amounts of these compounds were included in the water agar media.

Phytohormone analyses

At least four biological replicates of 100 mg of seeds were imbibed and light-treated as depicted in Fig. 1A; all imbibed seeds were collected and treated as depicted in Fig. 1A; all imbibed seeds were collected and analysed on an ultraperformance liquid chromatography (UPLC)-MS system consisting of an Acquity UPLC I-Class system (Waters) coupled to a TSQ Endura triple quadrupole mass spectrometer (Thermo Scientific). Chromatographic separation of phytohormones was performed at 40 °C on a Waters Acquity C18 BEH column (50 mm ×2.1 mm ×1.7 µm particle size), using a binary gradient of mobile phases with A=water+0.1% (v/v) acetic acid and B=acetonitrile+0.1% (v/v) acetic acid. The gradient elution program was as follows: 0–0.61 min isocratic 100% B; 0.61–2.34 min to 100% B; 2.34–2.82 min isocratic 100% B; 2.82–2.83 min to 100% B; 2.83–3.30 min isocratic 100% B. Eluted compounds were ionized on the mass spectrometer using an electrospray (ESI) source.
OPDA represses seed germination under far-red light conditions

Gene expression analysis

RNA extractions were performed as described previously (Vaištij et al., 2013). Standard protocols were used for RQ1 RNase-free DNase treatments (Promega), cDNA synthesis (SuperScript®II, Invitrogen) and qPCRs (iTaq™ Universal Syber® Green, Bio-Rad). Transcript abundance of a stable endogenous control (UBQ11; see Supplementary Fig. S1 at JXB online) was used for normalization and gene expression was expressed as a fold change relative to the control sample. Primer sequences for the qPCRs are described in Supplementary Table S1.

Results and discussion

Differential expression of AOS and OPR3 does not result in OPDA accumulation in FR-treated seeds

In a recent RNAseq-based transcriptomic analysis, we observed that AOS expression is FR induced (Vaištij et al., 2018; Supplementary Fig. S1). This suggested that, as for ABA, OPDA biosynthesis is induced by FR light. In order to validate the transcriptomic data, we performed RT-qPCR to quantify transcript abundance of AOS and OPR3 in wild-type (Col) after-ripened seeds treated with two consecutive short pulses of FR and R light (FR/R) or a single FR pulse (Fig. 1A) in order to activate and deactivate phyB, respectively. Consistent with the RNAseq data, we found that AOS expression was approximately 4- to 5-fold higher in FR-treated seeds compared with FR/R controls at 12 and 24 h after imbibition (hai) (Fig. 1B). In contrast, the OPR3 transcript abundance was unaffected at 12 hai and was less than 2-fold lower in FR-treated seeds compared with the FR/R treatment at 24 hai (Fig. 1C). These results at the level of gene expression prompted us to assess OPDA levels in FR/R- and FR-treated wild-type seeds. We also measured ABA levels and analysed FR-treated mft-2 seeds. As previously reported, ABA levels were increased in FR-treated wild-type seeds compared with FR/R-treated seeds, and in mft-2 seeds compared with wild-type seeds under FR light conditions (See et al., 2006; Vaištij et al., 2018; Fig. 1D). Surprisingly, despite the differential AOS expression, we detected no significant changes in OPDA accumulation in wild-type and mft-2 seeds under FR light conditions compared with the respective controls (Fig. 1E). We also measured JA and JA-Ile levels in wild-type seeds upon FR/R and FR treatments (24 hai) but found no significant changes in their accumulation (see Supplementary Fig. S2). Thus, our findings indicate that total OPDA, JA, and JA-Ile accumulation in seeds is not regulated by light quality. However, we cannot rule out the possibility that light may affect oxylipin accumulation in a localized cell-specific manner, which would not be detected by the whole seed phytohormone extraction methodology we have available. Future work could explore detection methods that allow more localized mapping of phytohormones within seed tissues.

OPDA acts in addition to ABA to repress seed germination in the shade

Although we could not detect changes in OPDA levels in FR-treated seeds, we were curious as to whether oxylipins played a role in regulating germination under FR light conditions.

Fig. 1. Analyses of gene expression and accumulation of OPDA and ABA. (A) Scheme of the experimental design: after-ripened seeds were imbibed for 4 h under white light (WL) and then treated with (i) two consecutive 5 min FR and R pulses (FR/R); (ii) only one FR pulse (FR); or (iii) 48 h of continuous FR irradiation (FR48). Seeds were kept in the dark after light treatments. Samples were collected for analyses at 12 and 24 h after imbibition (hai) as shown. (B, C) Relative AOS and OPR3 expression. (D, E) ABA and OPDA levels in FR/R and FR-treated wild-type (Col) and FR-treated mft-2 seeds. Data are means of three and four biological replicates for gene expression and germination assays, respectively, and error bars represent standard deviation. Asterisks denote statistically significant difference compared with the respective controls as determined by Student’s t-test (P<0.05).

from 0.2 to 2.5 min, in negative ion mode (spray 2500 V; sheath N2 gas 60 units, auxiliary N2 gas 20 units, sweep N2 gas 2 units, vaporizer 400 °C, ion transfer tube 380°C). Precursor and product ions were filtered through Q1 and Q3, respectively, at a mass resolution of 1.2 Da and at a fixed dwell time of 35 ms per transition. The following precursor–product ion transitions were programmed using Thermo Xcalibur software in SRM mode: ABA 263.2–153.2; d6-ABA 269.2–159.2; JA 209.2–59.4; GA4 331.2–287.1; JA-Ileu 322.2–130.2; prostaglandin A1 317.2–273.2; OPDA 291.2–165.2. For ABA and GA4, product ion peak area ratios relative to their respective deuterated analogues added as internal standards were used to construct calibration curves and calculate concentrations. For all other compounds, prostaglandin A1 was used as the reference internal standard. All standards were obtained as described in Dave et al. (2011).
Hence, we analysed germination of after-ripened aos mutant seeds, which are deficient in OPDA, JA, and JA-Ile (Dave et al., 2011). We observed that, as expected, wild-type and aos seeds germinated at high rates upon FR/R treatment and at very low rates (routinely less than 10%) under FR conditions (Fig. 2A). Presumably, the well-documented ABA inhibition of germination in response to FR light causes inhibition of germination in these oxylipin mutants as well as in wild-type seeds. We then used the ABA biosynthesis inhibitor norflurazon to further investigate a possible interaction between OPDA and ABA. We found that blocking ABA biosynthesis in the wild-type background did not rescue the FR block on wild-type seed germination (Fig. 2A), which is consistent with a previous report by Lee et al. (2012). This implies that something else is blocking germination in response to FR treatment when ABA biosynthesis is impaired. Interestingly, we found that germination of FR-treated aos seeds in the presence of norflurazon germinated at high rates, in a dose-dependent manner, suggesting a role for oxylipins in regulating the FR response, at least when ABA biosynthesis is compromised (Fig. 2A). We also assessed germination of two OPR3 mutant alleles, opr3-1 and opr3-3. Seeds of both opr3-1 and opr3-3 accumulated OPDA and ABA at similar levels to those of wild-type but were both significantly impaired in JA and JA-Ile accumulation (see Supplementary Fig. S2). The opr3-1 and opr3-3 seeds did accumulate very low amounts of JA-Ile, possibly due to the activity of the recently reported OPR3-independent biosynthetic pathway (Chini et al., 2018; Wasternack and Hause, 2018). We found that, similar to wild-type, both opr3-1 and opr3-3 seeds germinated at high levels under FR/R conditions, and at extremely low levels upon FR-light treatments even in the presence of norflurazon (Fig. 2B). This indicates that OPDA rather than JA/JA-Ile acts to repress germination under FR light, as was found to be the case under white light conditions (Dave et al., 2011, 2016).

In order to further validate our observations, we also analysed aos seeds in the ABA biosynthesis-deficient aba2-1 mutant.

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**Fig. 2.** Germination assays of OPDA- and ABA-deficient seeds. (A) Wild-type (Col) and aos seeds on control and norflurazon (Norf; 50 and 100 µM)-supplemented plates under FR/R and FR light. (B) Col, opr3-1, and opr3-3 seeds on control and norflurazon (Norf; 50 and 100 µM)-supplemented plates under FR/R and FR light. (C) Col, aos, and aba2-1 single- and aos aba2-1 double-mutant seeds under FR/R, FR, and FR-FR treatments. (D) Germination of aos aba2-1 seeds treated with OPDA or ABA (1 and 10 µM) under FR light conditions. Germination was assessed 144 hai (seeds had been after-ripened for not longer than 8 weeks). Data are means of four biological replicates and error bars represent standard deviation. Asterisks denote statistically significant difference compared with the respective controls as determined by Student’s t-test (*P<0.05).*
background (Léon-Kloosterziel et al., 1996). We found that while aos and aba2-1 single mutant seeds did not germinate, aos aba2-1 double mutant seeds germinated at high rates under the normally repressing FR light conditions (Fig. 2C). These results support the view that blocking the biosynthesis of both OPDA and ABA is required to allow germination of after-ripened seeds under strong phyB-deactivating FR light conditions. Consistent with this, we determined that, while application of either OPDA or ABA repressed germination of FR-treated aos aba2-1 seeds (Fig. 2D), exogenous JA had no significant effect on germination of the double mutant (see Supplementary Fig. S3). Overall, these results demonstrate that the strong repression of germination imposed by the FR light treatment is alleviated when the accumulation of both OPDA and ABA is compromised.

PhyA-dependent germination of aos aba2-1 double mutant and control seeds was also assessed after 48 h of continuous FR light treatment followed by 4 d in the dark (FR48; Fig. 1A). Under these conditions phyA and phyB are activated and deactivated respectively. As expected, we observed that FR48-treated wild-type seeds germinated at higher rates than seeds given just a short FR pulse; aos and aba2-1 single mutants germinated at higher levels than the wild-type control seeds; and aos aba2-1 double mutant seeds germinated at even higher rates than the single mutants (Fig. 2C). There are similarities between the role of ABA in this study and the one described by Lee et al. (2012), which showed that attenuation of ABA-dependent responses is required to promote phyA-dependent germination. Overall, these results demonstrate that disruption of either ABA or OPDA biosynthesis results in increased phyA-dependent seed germination.

To gain insight into the interplay between OPDA, ABA, and GA in the control of germination in response to FR light, we analysed the GA requirement of the aos aba2-1 double mutant under FR light conditions by treating seeds with the GA-biosynthesis inhibitor paclobutrazol. We observed that under these conditions aos aba2-1 seeds failed to germinate (Fig. 3A). It is well established that FR conditions are associated with low GA/ABA ratios in seeds (See et al., 2006), and that DELLA factors play a critical role in stimulation of ABA biosynthesis under these conditions (Piskurewicz and Lopez-Molina, 2009). Thus, we also analysed rgl1-1 rgl2-2 gai-6 nga-2 quadruple mutant seeds (della4) and determined that, as is the case with exogenous ABA, exogenous OPDA represses the high levels of germination that these seeds exhibit under FR light (Fig. 3B). These results suggest that OPDA acts downstream of DELLA factors to repress germination under FR light conditions, which is similar to what has been previously reported for ABA (Piskurewicz and Lopez-Molina, 2009). However, it is also possible that OPDA and DELLA have parallel pathways that repress germination under FR conditions.

**OPDA and/or ABA repression of germination correlates with MFT expression**

Previously, we established that OPDA has no effect on repressing germination of mft-2 mutant seeds under white light (Dave et al., 2016); here we show that OPDA also failed to repress mft-2 germination under FR light conditions (Fig. 3C). This strengthens our view that OPDA acts upstream of MFT. In addition, we previously demonstrated that MFT expression is induced in a PIF1- and SOM-dependent manner under FR conditions (Vaistij et al., 2018). Here we show that the transcript levels of PIF1, SOM, and MFT were strongly increased upon FR-treatment compared with FR/R in wild-type seeds, and this increase is intermediate under FR48 conditions (Fig. 4A). Comparing germination rates of wild-type (Fig. 2C) with MFT expression under FR/R, FR, and FR48 (Fig. 4A) revealed a negative correlation. These observations led us to hypothesize that a deficiency in both ABA and OPDA is required to overcome the strong germination-inhibitory effects of FR conditions when MFT is highly expressed, whereas under phyA-dependent germination conditions (FR48), where MFT expression is reduced, the absence of either ABA or OPDA is sufficient to alleviate the block on germination. To test this hypothesis we assessed wild-type, aos, aba2-1, and aos aba2-1 seeds under FR48 light conditions and determined that MFT expression was reduced in the mutant backgrounds, with the strongest effect seen in aba2-1 and aos aba2-1 (Fig. 4B). Taken together, these observations led us to conclude that MFT integrates both ABA and OPDA signalling pathways in order to repress germination in the shade: the necessity for just one or both of ABA and OPDA for repression of germination depends on endogenous levels of MFT. It is worth noting however, that although MFT expression is at a similar low level in aba2-1 and aos aba2-1 (Fig. 4B), the germination rate of the double mutant seeds was higher than that of the single mutant seeds (Fig. 2C), suggesting that factors other than MFT also play a role.

**OPDA and/or ABA repression of germination correlates with seed age**

Lee et al. (2012) observed that blocking ABA biosynthesis by norflurazon treatment of wild-type seeds does not alleviate the repression of germination by FR light, which is in agreement with our observations (Fig. 2A). However, Seo et al. (2006)
reported that ABA biosynthesis-deficient mutant seeds germinate partially under FR conditions, which contrasts with our analyses of aba2-1 seeds (Fig. 2B). While the report of Seo et al. (2006) did not indicate the age of after-ripened seeds used in their germination assays, our study and that of Lee et al. (2012) were performed with seeds not older than 8 weeks from the time of maturation/collection. This led us to question whether seed age may influence the sensitivity to OPDA and ABA in terms of germination repression under shade conditions. In order to address this, seeds after-ripened for more than 9 months were treated with FR/R and FR light. Interestingly, germination rates of long-term after-ripened norflurazon-treated wild-type seeds and aos seeds (not treated with norflurazon) were 75% and 40%, respectively, under FR conditions (Fig. 5A, B). These germination rates are much higher than those found routinely for the same treatments of short-term (less than 8 weeks) after-ripened seeds (Fig. 2A). Noteworthy also is the fact that long-term after-ripened wild-type seeds are still very responsive to the germination-repressing effects of FR light. Taken together these results demonstrate that as seeds age there is a necessity for both ABA and OPDA to block germination under FR light, whereas in younger after-ripened seeds either one is sufficient. A possible explanation for this might be that aged seeds are less sensitive to dormancy-promoting factors than younger seeds (Holdsworth et al., 2008; Holman et al., 2009). We have previously shown that MFT is a strong promoter of seed dormancy (Vaijti et al., 2013). Therefore, we hypothesized that MFT may be involved in the age-dependent requirement of OPDA and/or ABA to repress germination. To test this we assessed MFT expression in young (less than 8 weeks) and old (more than 9 months) wild-type seeds treated with FR light and found that MFT expression is reduced in the older seeds (Fig. 5C). This parallels the negative correlation between MFT expression levels and OPDA and/or ABA requirements of young seeds under FR and FR48 conditions (Figs 2B, 4A). These observations further support our conclusion that MFT integrates both ABA and OPDA signalling pathways in order to repress germination and that both environmental conditions such as light quality and developmental factors such as seed age play an important role in regulating germination through MFT expression. As seeds age other changes may also occur, such as a decrease in phytohormone levels. While we have demonstrated an important role for MFT, we cannot rule out the possibility of other factors also having an effect on the sensitivity to OPDA and ABA under FR light conditions.

**CYP20-3 is involved in OPDA signalling in seeds**

The crosstalk between ABA and OPDA may influence their abundance as well as their associated signalling pathways. We established previously that both gene expression and protein accumulation of the ABI5 transcription factor are induced by OPDA (Dave et al., 2011, 2016). It has also been shown that the forever-dormant phenotype of the OPDA over-accumulating ped3-3 mutant is dependent on ABI5 (Kanai et al., 2010), and that ABI5 accumulation is induced by FR light (Piskurewicz and Lopez-Molina, 2009). However, despite this apparent involvement of ABI5 in signalling both ABA and OPDA, abi5 mutant seeds fail to germinate under FR light (Lee et al., 2012). This indicates that factors other than ABI5 are involved in signalling the ABA- and OPDA-triggered repression of germination under shade conditions. Interestingly, it has been shown that, in wounded leaves, CYCLOPHILIN20-3 (CYP20-3) acts as a plastid-localized receptor linking OPDA signalling to cellular redox homeostasis in the response to stress in Arabidopsis (Park et al., 2013). We tested whether CYP20-3 also plays a role in seed OPDA signalling under different light conditions. To do this we assessed germination of cyp20-3 knockout mutant seeds under FR and FR48 treatments, but
observed no significant germination increase, even in the presence of norflurazon (see Supplementary Fig. S4). However, we did find that cyp20-3 seeds were resistant to the germination-repressive effect of exogenously applied OPDA under white light conditions (Supplementary Fig. S4). These results indicate that CYP20-3 is involved in the mechanism by which exogenous OPDA inhibits seed germination, but that CYP20-3 is not required for transducing the OPDA effect under FR light conditions (although we cannot exclude that it may act redundantly with other signalling factors).

Conclusions

The integration of the data presented in this and our previous studies allows us to propose a model in which the germination repression effect of OPDA and ABA under shade conditions is, at least partially, modulated by MFT (Fig. 6). We have demonstrated that under FR light conditions that lead to phyB deactivation, accumulation of OPDA or ABA is sufficient to repress germination (i.e. the presence of either phytohormone is enough for the complete FR-driven repression of germination). In contrast, under FR48 light conditions, when the effect of phyB deactivation is partially compensated by phyA activation, both OPDA and ABA are required for the complete repression of germination. We show a correlation of this dependence on OPDA and/or ABA to repress germination with the levels of MFT expression: when MFT is highly expressed (FR light conditions) OPDA and ABA act redundantly whereas when MFT expression is low (FR48 light conditions) OPDA and ABA act non-redundantly. Moreover, we also show a correlation of the OPDA and/or ABA requirements of young and old seeds to repress germination under FR light conditions with MFT expression: compared with young seeds, old seeds express MFT at a lower level and require both OPDA and ABA to fully repress germination. It is still not obvious why two phytohormone-based repression pathways have evolved to control seed germination. One could argue that, because of the critical importance of germination in the plant life cycle, it has been advantageous to adopt a ‘belt and braces’ approach to its control. The deployment of two repres sor systems may also allow a greater flexibility or fine tuning of the different temporal, spatial, and physiological factors that could all be influencing when a seed germinates.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Relative AOS, OPR3, and UBQ11 gene expression.

Fig. S2. OPDA, JA, and JA-Ile accumulation in Col, opr3-1, and opr3-3.

Fig. S3. Effect of JA treatment on germination of aos aba2-1 and della quadruple mutant seeds.

Fig. S4. Analysis of cyp20-3 seed germination.

Table S1. Sequence of primers used in this study for RT-qPCR in gene expression analyses.

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