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1 ***cis*-12-oxo-phytodienoic acid represses *Arabidopsis thaliana* seed germination in shade light**  
2 **conditions**

3

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18

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22

23 **Highlight**

24 OPDA acts in addition to ABA to repress seed germination under far-red light conditions. The response  
25 to both these phytohormones is integrated by MFT, a negative regulator of germination.

26

27 **Abstract**

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

41

42 **Key words**

43 OPDA, ABA, MFT, phytochrome, FR-light, shade, seed germination

44

45 **Abbreviations**

46 ABA: abscisic acid  
47 FR: Far Red  
48 GA: gibberellins  
49 hai: hours after imbibition  
50 JA: jasmonic acid  
51 JA-Ile: jasmonic acid -isoleucine  
52 OPDA: cis-12-oxo-phytodienoic acid  
53 R: Red

54

## 55 **Introduction**

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

69

70 In many plant species, including *Arabidopsis thaliana*, the probability of seedling establishment is  
71 generally greater if germination occurs under direct sun (white) light, which is rich in the red (R) wave  
72 length; rather than under-the-canopy light (shade), which is rich in far-red (FR) (Lee and Lopez-Molina,  
73 2012). In photoblastic seeds, phytochrome photoreceptors distinguish between these different light  
74 conditions on the basis of their R and FR light intensities and ratios (Shinomura, 1997; Smith, 2000;  
75 Quail, 2002; Jung *et al.*, 2016). Excess R triggers GA accumulation and germination, whereas excess  
76 FR, typical of shade light, triggers ABA accumulation and a block in germination (Oh *et al.*, 2006; Seo  
77 *et al.*, 2006; Piskurewicz *et al.*, 2008). *Arabidopsis* has five phytochromes, (phyA-E); Clack *et al.*,  
78 1994), with phyB being the main promoter of germination under sun light; while phyA is responsible for  
79 germination under-the-canopy light (Shinomura *et al.*, 1994). Both phyA and phyB are synthesized as  
80 inactive proteins and become active in a light-quality dependent manner. However, while short pulses of  
81 R and FR light are sufficient to activate and deactivate phyB respectively; longer exposures to R and FR  
82 activate phyA (Reed *et al.*, 1994; Shinomura *et al.*, 1994). Furthermore, compared to phyB, phyA  
83 accumulates at high levels only after relatively long periods of seed imbibition (Lee *et al.*, 2012).

84

85

86 Upon activation, both phyA and phyB induce degradation of the transcription factor PHYTOCHROME  
87 INTERACTING FACTOR1 (PIF1, formerly known as PIL5) (Shen *et al.*, 2005; Park *et al.*, 2012).

88 Upon phytochrome inactivation, PIF1 accumulates and regulates transcription of many genes, including  
89 SOMNUS (SOM), which encodes a CCCH- type zinc finger protein that is part of the phytochrome  
90 signal transduction pathway controlling genes involved in regulating ABA and GA levels ultimately  
91 leading to high ABA:GA ratios to repress germination (Oh *et al.*, 2004; Oh *et al.*, 2007; Kim *et al.*,  
92 2008; Kim *et al.*, 2016; Park *et al.*, 2011). We showed recently that PIF1 and SOM also promote  
93 *MOTHER-OF-FT-AND-TFL1* (*MFT*) expression and that *MFT* plays a key role in repressing  
94 germination by modulating ABA and GA responses (Vaistij *et al.*, 2018). Furthermore, PIF1 stimulates  
95 the expression of *GAI* and *RGA*, which encode growth repressing DELLA proteins (Oh *et al.*, 2004; Oh  
96 *et al.*, 2007; Piskurewicz *et al.*, 2008; Piskurewicz *et al.*, 2009). GA promotes germination by targeting  
97 destruction of the DELLA proteins through the 26S-proteasome. Under FR conditions, the DELLA  
98 proteins RGL2, GAI, and RGA repress germination by stimulating the expression of ABA biosynthetic  
99 genes, further increasing the ABA:GA ratio (Piskurewicz *et al.*, 2008; Piskurewicz *et al.*, 2009; Lee *et*  
100 *al.*, 2012).

101

102 The phytohormone jasmonic acid (JA) and its precursor *cis*-12-oxo-phytodienoic acid (OPDA) are  
103 oxilipins derived from linolenic acid (Wasternack and Song, 2016). The biologically active conjugated  
104 JA-isoleucine (JA-Ile) form is involved in responses to biotic and abiotic stress as well as in many other  
105 biological processes including seed germination (Linkies and Leubner-Metzger, 2012; Wasternack and  
106 Hause, 2013; Wasternack and Strnad, 2015; Singh *et al.*, 2017). OPDA also exhibits signalling  
107 properties, some of which are shared with JA-Ile, but others are distinct (Goetz *et al.*, 2012; Guo *et al.*,  
108 2014; Savchenko and Dehesh, 2014; Bosh *et al.*, 2014; Dave *et al.*, 2011). Previously we characterised  
109 the role of oxilipins in seed dormancy. We did this by analyzing mutant seeds defective in: (i) *ALLENE*  
110 *OXIDE SYNTHASE* (*AOS*), which encodes a cytochrome P450 oxidase enzyme involved in one of the  
111 final steps of OPDA biosynthesis inside plastids (Park *et al.*, 2002); (ii) *PXA1* (also known as *CTS* and  
112 *PED3*), which encodes an ABC-type transporter that imports OPDA into peroxisomes (Zolman *et al.*,  
113 2001; Footitt *et al.*, 2002; Hayashi *et al.*, 2002); and (iii) *12-OXOPHYTODIENOIC ACID REDUCTASE*  
114 (*OPR3*), which is involved in the conversion of OPDA to JA in peroxisomes (Stintzi and Browse, 2000).  
115 It has been determined that seeds of the *aos* mutant, which is compromised in OPDA and JA/JA-Ile

116 accumulation, are less dormant than wild-type seeds; whereas seeds of the *pxa1-1* and *opr3-1* single  
117 mutants, which over-accumulate OPDA but are deficient in JA/JA-Ile, are more dormant (Chehab *et al.*,  
118 2011; Dave *et al.*, 2011; Dave *et al.*, 2016). These observations led us to conclude that OPDA  
119 specifically acts as a dormancy-promoting factor. In the present work we investigated the role of OPDA  
120 in the FR triggered repression of germination of after-ripened seeds. We show that endogenous OPDA  
121 in seeds plays a key role alongside ABA to repress germination under shade conditions through an MFT  
122 modulated process.

123

## 124 **Material and methods**

125 **Growth conditions and biological materials.** Plants were grown in a greenhouse supplemented with  
126 artificial light to give a photoperiod of 16 h light at a temperature of 20-22 °C. Seeds were harvested  
127 when plants stopped flowering and siliques started to dehisce. In all experiments, except for that  
128 involving extended after-ripening shown in Figure 6, seeds were after-ripened for no longer than 8  
129 weeks. All mutant lines used in this study were described previously: *aos* (Park *et al.*, 2002); *opr3-1*  
130 (Stintzi and Browse, 2000); *opr3-3* (Chini *et al.*, 2018); *aba2-1* (Leon-Kloosterziel *et al.*, 1996); *mft-2*  
131 (Xi *et al.*, 2010); *rgl1-1 rgl2-2 gai-6 rga-2 (della4)* (Cao *et al.*, 2005); *cyp20-3* (Park *et al.*, 2013).

132

133 **Germination assays.** Seeds were vapour-phase sterilized by exposure to chlorine gas in a sealed glass  
134 container to at least three hours, the gas having been produced by mixing 100 ml of bleach with 3 ml of  
135 concentrated HCl. Sterilized seeds were plated on water agar (0.9 % w/v) and allowed to imbibe under  
136 low light for 4 hours and then LED- irradiated with FR ( $4.5 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ ) and R ( $20 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) as  
137 indicated in Figure 1A. After FR/R, FR and FR48 treatments plates were wrapped in foil and kept at 20  
138 °C. Germination was scored on the basis of radical emergence of 50-100 seeds per replica. In  
139 experiments where germination assays were conducted with ABA (Sigma-Aldrich), OPDA (Larodan),  
140 paclobutrazol (Sigma-Aldrich) or norflurazon (Sigma-Aldrich) the appropriate amounts of these  
141 compounds were included in the water agar media.

142

143 **Phytohormone analyses.** At least four biological replicates of 100 mg of seeds were imbibed and light-  
144 treated as depicted in Fig. 1A, all imbibed seeds were collected and phytohormones extracted as  
145 described previously (Dave *et al.*, 2011). Extracts were resuspended in methanol and 2  $\mu\text{L}$  injected and  
146 analyzed on an ultraperformance liquid chromatography (UPLC)-MS system consisting of an Acquity

147 UPLC I-Class system (Waters) coupled to a TSQ Endura triple quadrupole mass spectrometer (Thermo  
148 Scientific). Chromatographic separation of phytohormones was performed at 40°C on a Waters Acquity  
149 C18 BEH column (50 mm x 2.1 mm x 1.7µm particle size), using a binary gradient of mobile phases  
150 with A = water + 0.1% (v/v) acetic acid and B = acetonitrile + 0.1% (v/v) acetic acid. The gradient  
151 elution program was as follows: 0 - 0.61 min isocratic 10% B; 0.61 - 2.34 min to 100% B; 2.34 - 2.82  
152 min isocratic 100% B; 2.82 - 2.83 min to 10% B; 2.83 - 3.30 min isocratic 10% B. Eluted compounds  
153 were ionized on the mass spectrometer using a heated electrospray (HESI) source from 0.2 - 2.5 min, in  
154 negative ion mode (spray 2500 V; sheath N2 gas 60 units, Aux N2 gas 20 units, sweep N2 gas 2 units,  
155 vaporizer 400 °C, ion transfer tube 380°C). Precursor and product ions were filtered through Q1 and Q3  
156 respectively at a mass resolution of 1.2 Da and at a fixed dwell time of 35 ms per transition. The  
157 following precursor - product ion transitions were programmed using Thermo Xcalibur software in SRM  
158 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 -  
159 130.2; prostaglandin A1 317.2 - 273.2; OPDA 291.2 - 165.2. For ABA and GA4, product ion peak area  
160 ratios relative to their respective deuterated analogs added as internal standards were used to construct  
161 calibration curves and calculate concentrations. For all other compounds, prostaglandin A1 was used as  
162 the reference internal standard. All standards were obtained as described in Dave et al (2011).

163

164 **Gene expression analysis.** RNA extractions were performed as described previously (Vaistij *et al.*,  
165 2013). Standard protocols were used for RQ1 RNase-Free DNase treatments (Promega), cDNA  
166 synthesis (SuperScript®II, Invitrogen) and qPCRs (iTaQ™ Universal Syber® Green, Bio-Rad).  
167 Transcript abundance of a stable endogenous control (*UBQ11*; see Sup. Fig. 1) was used for  
168 normalization and gene expression was expressed as a fold change relative to the control sample. Primer  
169 sequences for the qPCRs are described in Table S1.

170

## 171 **Results and discussion**

172 **Differential expression of *AOS* and *OPR3* does not result in OPDA accumulation in FR-treated**  
173 **seeds.** In a recent RNAseq-based transcriptomic analysis, we observed that *AOS* expression is FR  
174 induced (Vaistij *et al.*, 2018; Sup. Fig. 1). This suggested that, as for ABA, OPDA biosynthesis is  
175 induced by FR light. In order to validate the transcriptomic data, we performed RT-qPCR to quantify  
176 transcript abundance of *AOS* and *OPR3* in wild-type (Col) after-ripened seeds treated with two  
177 consecutive short pulses of FR and R light (FR/R) or a single FR pulse (Fig. 1A) in order to activate and

178 deactivate phyB, respectively. Consistent with the RNAseq data, we found that *AOS* expression was  
179 approximately 4 to 5-fold higher in FR-treated seeds compared to FR/R controls at 12 and 24 hours-  
180 after-imbibition (hai) (Fig. 1B). In contrast, the *OPR3* transcript abundance was unaffected at 12 hai and  
181 was less than 2-fold lower in FR-treated seeds compared to the FR/R treatment at 24 hai (Fig. 1C).  
182 These results at the level of gene expression prompted us to assess OPDA levels in FR/R and FR-treated  
183 wild-type seeds. We also measured ABA levels and analysed FR-treated *mft-2* seeds. As previously  
184 reported, ABA levels were increased in FR-treated wild-type seeds compared to FR/R-treated seeds, and  
185 in *mft-2* seeds compared to wild-type seeds under FR light conditions (Seo *et al.*, 2006; Vaistij *et al.*  
186 2018; Fig. 1D). Surprisingly, despite the differential *AOS* expression, we detected no significant changes  
187 in OPDA accumulation in wild-type and *mft-2* seeds under FR light conditions compared to the  
188 respective controls (Fig. 1E). We also measured JA and JA-Ile levels in wild-type seeds upon FR/R and  
189 FR treatments (24 hai) but found no significant changes in their accumulation (Sup. Fig. 2). Thus, our  
190 findings indicate that total OPDA, JA and JA-Ile accumulation in seeds is not regulated by light quality.  
191 However, we cannot rule out the possibility that light may affect oxylipin accumulation in a localised  
192 cell specific manner, which would not be detected by the whole seed phytohormone extraction  
193 methodology we have available. Future work could explore detection methods that allow more localised  
194 mapping of phytohormones within seed tissues.

195

196 **OPDA acts in addition to ABA to repress seed germination in the shade.** Although we could not  
197 detect changes in OPDA levels in FR-treated seeds we were curious as to whether oxylipins played a  
198 role in regulating germination under FR light conditions. Hence, we analysed germination of after-  
199 ripened *aos* mutant seeds, which are deficient in OPDA, JA and JA-Ile (Dave *et al.*, 2011). We observed  
200 that, as expected, wild-type and *aos* seeds germinate at high rates upon FR/R treatment and at very low  
201 rates (routinely less than 10 %) under FR conditions (Fig. 2A). Presumably, the well documented ABA  
202 inhibition of germination in response to FR light causes inhibition of germination in these oxylipin  
203 mutants as well as in wild-type seeds. We then used the ABA biosynthesis inhibitor norflurazon to  
204 further investigate a possible interaction between OPDA and ABA. We found that blocking ABA  
205 biosynthesis in the wild-type background does not rescue the FR block on wild-type seed germination  
206 (Fig. 2A), which is consistent with a previous report by Lee *et al.* (2012). This implies that something  
207 else is blocking germination in response to FR treatment when ABA biosynthesis is impaired.  
208 Interestingly, we found that germination of FR-treated *aos* seeds in the presence of norflurazon



209 germinated at high rates, in a dose dependent manner, suggesting a role for oxylipins in regulating the  
210 FR response, at least when ABA biosynthesis is compromised (Fig. 2A). We also assessed germination  
211 of two *OPR3* mutant alleles, *opr3-1* and *opr3-3*. Seeds of both *opr3-1* and *opr3-3* accumulate OPDA  
212 and ABA at similar levels as wild-type but are both significantly impaired in JA and JA-Ile  
213 accumulation (Sup. Fig. 2). The *opr3-1* and *opr3-3* seeds do accumulate very low amounts of JA-Ile,  
214 possibly due to the activity of the recently reported OPR3-independent biosynthetic pathway (Chini *et*  
215 *al.*, 2018; Wasternack and Hause; 2018). We found that, similar to wild-type, both *opr3-1* and *opr3-3*  
216 seeds germinated at high levels under FR/R conditions; and at extremely low levels upon FR-light  
217 treatments even in the presence of norflurazon (Fig. 2B). This indicates that OPDA rather than JA/JA-Ile  
218 acts to repress germination under FR light, as was found to be the case under white light conditions  
219 (Dave *et al.*, 2011; Dave *et al.*, 2016).

220

221 In order to further validate our observations, we also analysed *aos* seeds in the ABA biosynthesis  
222 deficient *aba2-1* mutant background (Leon-Kloosterziel *et al.*, 1994). We found that while *aos* and  
223 *aba2-1* single mutant seeds do not germinate, *aos aba2-1* double mutant seeds germinate at high rates  
224 under the normally repressing FR light conditions (Fig. 2C). These results support the view that blocking  
225 the biosynthesis of both OPDA and ABA is required to allow germination of after-ripened seeds under  
226 strong phyB-deactivating FR light conditions. Consistent with this, we determined that while application  
227 of either OPDA or ABA represses germination of FR-treated *aos aba2-1* seeds (Fig. 2D), exogenous JA  
228 has no significant effect on germination of the double mutant (Sup. Fig. 3). Overall, these results  
229 demonstrate that the strong repression of germination imposed by the FR light treatment is alleviated  
230 when the accumulation of both OPDA and ABA is compromised.

231

232 PhyA-dependent germination of *aos aba2-1* double mutant and control seeds was also assessed after 48  
233 h of continuous FR light treatment followed by four days in the dark (FR48; Fig. 1A). Under these  
234 conditions phyA and phyB are activated and deactivated respectively. As expected, we observed that  
235 FR48-treated wild-type seeds germinated at higher rates than seeds given just a short FR pulse; *aos* and  
236 *aba2-1* single mutants germinate at higher levels than the wild-type control seeds; and *aos aba2-1*  
237 double mutant seeds germinate at even higher rates than the single mutants (Fig. 2C). There are  
238 similarities between the role of ABA in this study and the one described by Lee *et al.* (2012), which  
239 showed that attenuation of ABA-dependent responses is required to promote phyA-dependent

240 germination. Overall, these results demonstrate that disruption of either ABA or OPDA biosynthesis  
241 results in increased phyA-dependent seed germination.

242

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

255

256 **OPDA and/or ABA repression of germination correlates with *MFT* expression.** Previously, we  
257 established that OPDA has no effect on repressing germination of *mft-2* mutant seeds under white light  
258 (Dave *et al.*, 2016); here we show that OPDA also fails to repress *mft-2* germination under FR light  
259 conditions (Fig. 3C). This strengthens our view that OPDA acts upstream of MFT. In addition, we  
260 previously demonstrated that *MFT* expression is induced in a PIF1- and SOM-dependent manner under  
261 FR conditions (Vaistij *et al.*, 2018). Here we show that the transcript levels of *PIF1*, *SOM* and *MFT* are  
262 strongly increased upon FR-treatment compared to FR/R in wild-type seeds, and this increase is  
263 intermediate under FR48 conditions (Fig. 4A). Comparing germination rates of wild-type (Fig. 2C) with  
264 *MFT* expression under FR/R, FR and FR48 (Fig. 4A) reveals a negative correlation. These observations  
265 led us to hypothesise that a deficiency in both ABA and OPDA is required to overcome the strong  
266 germination inhibitory effects of FR conditions when *MFT* is highly expressed; whereas under phyA-  
267 dependent germination conditions (FR48), where *MFT* expression is reduced, the absence of either ABA  
268 or OPDA is sufficient to alleviate the block on germination. To test this hypothesis we assessed wild-  
269 type, *aos*, *aba2-1* and *aos aba2-1* seeds under FR48 light conditions and determined that *MFT*  
270 expression is reduced in the mutant backgrounds, with the strongest effect seen in *aba2-1* and *aos aba2-*

271 *1* (Fig. 4B). Taken together, these observations lead us to conclude that MFT integrates both ABA and  
272 OPDA signalling pathways in order to repress germination in the shade: The necessity for just one or  
273 both of ABA and OPDA for repression of germination depends on endogenous levels of MFT. It is  
274 worth noting however, that although *MFT* expression is at a similar low level in *aba2-1* and *aos aba2-1*  
275 (Fig. 4B), the germination rate of the double mutant seeds is higher than the single mutant seeds (Fig.  
276 2C) suggesting that factors other than MFT also play a role.

277

278 **OPDA and/or ABA repression of germination correlates with seed age.** Lee *et al.* (2012) observed  
279 that blocking ABA biosynthesis by norflurazon treatment of wild-type seeds does not alleviate the  
280 repression of germination by FR light, which is in agreement with our observations (Fig. 2A). However,  
281 Seo *et al.* (2006) reported that ABA biosynthesis deficient mutant seeds germinate partially under FR  
282 conditions, which contrasts with our analyses of *aba2-1* seeds (Fig. 2B). While the report of Seo *et al.*  
283 (2006) did not indicate the age of after-ripened seeds used in their germination assays, our study and that  
284 of Lee *et al.* (2012) were performed with seeds not older than 8 weeks from the time of  
285 maturation/collection. This led us to question whether seed age may influence the sensitivity to OPDA  
286 and ABA in terms of germination repression under shade light conditions. In order to address this, seeds  
287 after-ripened for more than nine months were treated with FR/R and FR light. Interestingly, germination  
288 rates of long-term after-ripened norflurazon-treated wild-type seeds and *aos* seeds (not treated with  
289 norflurazon) were 75 % and 40 %, respectively, under FR conditions (Fig. 5A and 5B). These  
290 germination rates are much higher than those found routinely for the same treatments of short-term (less  
291 than 8 weeks) after-ripened seeds (Fig. 2A). Noteworthy also is the fact that long-term after-ripened wild  
292 type seeds are still very responsive to the germination repressing effects of FR light. Taken together  
293 these results demonstrate that as seeds age there is a necessity for both ABA and OPDA to block  
294 germination under FR light whereas in younger after-ripened seeds either one is sufficient. A possible  
295 explanation for this might be that aged seeds are less sensitive to dormancy-promoting factors than  
296 younger seeds (Holdsworth *et al.*, 2008; Holman *et al.*, 2009). We have previously shown that MFT is a  
297 strong promoter of seed dormancy (Vaistij *et al.*, 2013). Therefore, we hypothesized that MFT may be  
298 involved in the age-dependent requirement of OPDA and/or ABA to repress germination. To test this we  
299 assessed *MFT* expression in young (less than 8 weeks) and old (more than 9 months) wild-type seeds  
300 treated with FR light and found that *MFT* expression is reduced in the older seeds (Fig. 5C). This  
301 parallels the negative correlation between *MFT* expression levels and OPDA and/or ABA requirements

302 of young seeds under FR and FR48 conditions (Fig. 2B and 4A). These observations further support our  
303 conclusion that MFT integrates both ABA and OPDA signalling pathways in order to repress  
304 germination and that both environmental conditions such as light quality or developmental factors such  
305 as seed age playing an important role in regulating germination through MFT expression. As seeds age  
306 other changes may also occur, such as decrease in phytohormone levels. While we have demonstrated an  
307 important role for MFT, we cannot rule out the possibility of other factors also having an effect on the  
308 sensitivity to OPDA and ABA under FR light conditions.

309

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

329

### 330 **Conclusions**

331 The integration of the data presented in this and our previous studies allows us to propose a model in  
332 which the germination repression effect of OPDA and ABA under shade light conditions is, at least

333 partially, modulated by *MFT* (Fig. 6). We have demonstrated that under FR light conditions that lead to  
334 *phyB* deactivation, accumulation of OPDA or ABA are sufficient to repress germination (*i.e.* the  
335 presence of either phytohormone is enough for the complete FR-driven repression of germination). In  
336 contrast, under FR48 light conditions, when the effect of *phyB* deactivation is partially compensated by  
337 *phyA* activation, both OPDA and ABA are required for the complete repression of germination. We  
338 show a correlation of this dependence on OPDA and/or ABA to repress germination with the levels of  
339 *MFT* expression: When *MFT* is highly expressed (FR light conditions) OPDA and ABA act redundantly  
340 whereas when *MFT* is lowly expressed (FR48 light conditions) OPDA and ABA act non-redundantly.  
341 Moreover, we also show a correlation of the OPDA and/or ABA requirements of young and old seeds to  
342 repress germination under FR light conditions with *MFT* expression: Compared to young seeds, old  
343 seeds express *MFT* at a lower level and require both OPDA and ABA to fully repress germination. It is  
344 still not obvious why two phytohormone-based repression pathways have evolved to control seed  
345 germination. One could argue that, because of the critical importance of germination in the plant life  
346 cycle, it has been advantageous to adopt a ‘belt and braces’ approach to its control. The deployment of  
347 two repressor systems may also allow a greater flexibility or fine tuning of the different temporal, spatial  
348 and physiological factors that could all be influencing when a seed germinates.

349

#### 350 **Supplementary data**

351 Figure S1. Relative *AOS*, *OPR3* and *UBQ11* gene expression.

352 Figure S2. OPDA, JA and JA-Ile accumulation in Col, *opr3-1* and *opr3-3*.

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

354 Figure S4. Analysis of *cyp20-3* seed germination.

355 Table S1. Sequence of primers used in this study

356

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366

## 367 **References**

368 **Bosch M, Wright LP, Gershenzon J, Wasternack C, Hause B, Schaller A, Stintzi A.** 2014. Jasmonic  
369 acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced  
370 herbivore defenses in tomato. *Plant Physiology* **166**, 396-410.

371 **Cao DN, Hussain A, Cheng H, Peng JR.** 2005. Loss of function of four DELLA genes leads to light-  
372 and gibberellin-independent seed germination in Arabidopsis. *Planta* **223**, 105-113.

373 **Chahtane H, Kim W, Lopez-Molina L.** 2017. Primary seed dormancy: a temporally multilayered  
374 riddle waiting to be unlocked. *Journal of Experimental Botany* **68**, 857-869.

375 **Chehab EW, Kim S, Savchenko T, Kliebenstein D, Dehesh K, Braam J.** 2011. Intronic T-DNA  
376 insertion renders Arabidopsis *opr3* a conditional jasmonic acid-producing mutant. *Plant Physiology* **156**,  
377 770-778.

378 **Chini A, Monte I, Zamarreño AM, et al.** 2018. An OPR3-independent pathway uses 4,5-  
379 didehydrojasmonate for jasmonate synthesis. *Nature Chemistry Biology* **14**, 171-178.

380 **Clack T, Mathews S, Sharrock RA.** 1994. The phytochrome apoprotein family in Arabidopsis is  
381 encoded by five genes: the sequences and expression of PHYD and PHYE. *Plant Molecular Biology* **25**,  
382 413-427.

383 **Clerkx EJ, Vries HB, Ruys GJ, Groot SP, Koornneef M** (2003) Characterization of green seed, an  
384 enhancer of *abi3-1* in Arabidopsis that affects seed longevity. *Plant Physiology* **132**:1077–1084.

385 **Dave A, Hernández ML, He Z, Andriotis VM, Vaistij FE, Larson TR, Graham IA.** 2011. 12-oxo-  
386 phytodienoic acid accumulation during seed development represses seed germination in Arabidopsis.  
387 *The Plant Cell* **23**, 583-599.

388 **Dave A, Vaistij FE, Gilday AD, Penfield SD, Graham IA.** 2016. Regulation of Arabidopsis thaliana  
389 seed dormancy and germination by 12-oxo-phytodienoic acid. *Journal of Experimental Botany* **67**, 2277-  
390 2284.

391 **Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM.** 1998. The Arabidopsis abscisic acid  
392 response locus *ABI4* encodes an APETALA 2 domain protein. *Plant Cell* **10**: 1043–1054.

393 **Finkelstein RR, Lynch TJ.** 2000. The Arabidopsis abscisic acid response gene *ABI5* encodes a basic  
394 leucine zipper transcription factor. *Plant Cell* **12**:599–609.

395 **Footitt S, Slocombe SP, Lerner V, Kurup S, Wu Y, Larson T, Graham I, Baker A, Holdsworth M.**  
396 2002. Control of germination and lipid mobilization by COMATOSE, the Arabidopsis homologue of  
397 human ALDP. *EMBO Journal* **21**, 2912-2922.

398 **Goetz S, Hellwege A, Stenzel I, et al.** 2012. Role of cis-12-oxo-phytodienoic acid in tomato embryo  
399 development. *Plant Physiology* **158**, 1715-1727.

400 **Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJJ.** 2012. Molecular  
401 mechanisms of seed dormancy. *Plant, Cell and Environment* **35**, 1769-1786.

402 **Guo HM, Li HC, Zhou SR, Xue HW, Miao XX.** 2014. *cis*-12-oxo-phytodienoic acid stimulates rice  
403 defense response to a piercing-sucking insect. *Mol Plant* **7**, 1683-1692.

404 **Hayashi M, Nito K, Takei-Hoshi R, Yagi M, Kondo M, Suenaga A, Yamaya T, Nishimura M.**  
405 2002. Ped3p is a peroxisomal ATP-binding cassette transporter that might supply substrates for fatty  
406 acid beta-oxidation. *Plant and Cell Physiology* **43**, 1-11.

407 **Holdsworth MJ, Bentsink L, Soppe WJ.** 2008. Molecular networks regulating Arabidopsis seed  
408 maturation, after-ripening, dormancy and germination. *New Phytologist* **179**, 33-54.

409 **Holman TJ, Jones PD, Russell L, et al.** 2009. The N-end rule pathway promotes seed germination and  
410 establishment through removal of ABA sensitivity in Arabidopsis. *Proceedings of the National Academy*  
411 *of Sciences of the USA* **106**, 4549-4554.

412 **Jiao Y, Lau OS, Deng XW.** 2007. Light-regulated transcriptional networks in higher plants. *Nature*  
413 *Reviews Genetics* **8**, 217-230.

414 **Jung, JH, Domijan M, Klose C, Biswas S, Ezer D, Gao M, Khattak AK, Box MS, Charoensawan**  
415 **V, Cortijo S, Kumar M, Grant A, Locke JC, Schäfer E, Jaeger KE, Wigge PA.** 2016. Phytochromes  
416 function as thermosensors in Arabidopsis. *Science* **354**, 886-889.

417 **Kanai M, Nishimura M, Hayashi M.** 2010. A peroxisomal ABC transporter promotes seed  
418 germination by inducing pectin degradation under the control of ABI5. *The Plant Journal* **62**, 936-947.

419 **Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S.** 2011. Induction of  
420 dormancy in Arabidopsis summer annuals requires parallel regulation of DOG1 and hormone  
421 metabolism by low temperature and CBF transcription factors. *The Plant Cell* **23**, 2568-2580.

422 **Kim DH, Yamaguchi S, Lim S, Oh E, Park J, Hanada A, Kamiya Y, Choi G.** 2008. SOMNUS, a  
423 CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination  
424 downstream of PIL5. *The Plant Cell* **20**, 1260-1277.

425 **Kim J, Kang H, Park J, Kim W, Yoo J, Lee N, Kim J, Yoon TY, Choi G.** 2016. PIF1-interacting  
426 transcription factors and their binding sequence elements determine the in vivo targeting sites of PIF1.  
427 *The Plant Cell* **28**, 1388-1405.

428 **Lee KP, Lopez-Molina L.** 2012. Control of seed germination in the shade. *Cell Cycle* **11**, 4489-4490.

429 **Lee KP, Piskurewicz U, Turečková V, Carat S, Chappuis R, Strnad M, Fankhauser C, Lopez-**  
430 **Molina L.** 2012. Spatially and genetically distinct control of seed germination by phytochromes A and  
431 B. *Genes & Development* **26**, 1984-1996.

432 **Léon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH, Zeevaart**  
433 **JA, Koornneef M.** 1994. Isolation and characterization of abscisic acid-deficient *Arabidopsis* mutants at  
434 two new loci. *Plant Journal* **10**, 655-661.

435 **Linkies A, Graeber K, Knight C, Leubner-Metzger G.** 2010. The evolution of seeds. *New*  
436 *Phytologist* **186**, 817-831.

437 **Linkies A, Leubner-Metzger G.** 2012. Beyond gibberellins and abscisic acid: how ethylene and  
438 jasmonates control seed germination. *The Plant Cell Rep* **31**, 253-270.

439 **Oh E, Kim J, Park E, Kim JI, Kang C, Choi G.** 2004. PIL5, a phytochrome-interacting basic helix-  
440 loop-helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. *The Plant*  
441 *Cell* **16**, 3045-3058.

442 **Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G.** 2006. Light activates the degradation of  
443 PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. *Plant Journal* **47**, 124-139.

444 **Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, Lee HS, Sun TP, Kamiya Y, Choi G.** 2007.  
445 PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly  
446 to the GAI and RGA promoters in *Arabidopsis* seeds. *The Plant Cell* **19**, 1192-1208.

447 **Park E, Park J, Kim J, Nagatani A, Lagarias JC, Choi G.** 2012. Phytochrome B inhibits binding of  
448 phytochrome-interacting factors to their target promoters. *The Plant Journal* **72**, 537-546.

449 **Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R.** 2002. A knock-out  
450 mutation in allene oxide synthase results in male sterility and defective wound signal transduction in  
451 *Arabidopsis* due to a block in jasmonic acid biosynthesis. *Plant Journal* **31**, 1-12.

452 **Park J, Lee N, Kim W, Lim S, Choi G.** 2011. ABI3 and PIL5 collaboratively activate the expression  
453 of SOMNUS by directly binding to its promoter in imbibed *Arabidopsis* seeds. *Plant Cell* **23**, 1404-  
454 1415.



455 **Park SW, Li W, Viehhauser A, et al.** 2013. Cyclophilin 20-3 relays a 12-oxo-phytodienoic acid signal  
456 during stress responsive regulation of cellular redox homeostasis. Proceedings of the National Academy  
457 of Sciences of the USA **110**, 9559-9564.

458 **Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L.** 2008. The  
459 gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic  
460 acid synthesis and ABI5 activity. The Plant Cell **20**, 2729-2745.

461 **Piskurewicz U, Lopez-Molina L.** 2009. The GA-signaling repressor RGL3 represses testa rupture in  
462 response to changes in GA and ABA levels. Plant Signal Behavior **4**, 63-65.

463 **Quail PH.** 2002. Phytochrome photosensory signalling networks. Nature Reviews Molecular Cell  
464 Biology **3**, 85-93.

465 **Reed JW, Nagatani A, Elich TD, Fagan M, Chory J.** 1994. Phytochrome A and phytochrome B have  
466 overlapping but distinct functions in Arabidopsis development. Plant Physiol **104**, 1139-1149.

467 **Savchenko T, Dehesh K.** 2014. Drought stress modulates oxylipin signature by eliciting 12-OPDA as a  
468 potent regulator of stomatal aperture. Plant Signal Behavior **9**, e28304.

469 **Seo M, Hanada A, Kuwahara A, et al.** 2006. Regulation of hormone metabolism in Arabidopsis seeds:  
470 phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin  
471 metabolism. The Plant Journal **48**, 354-366.

472 **Singh P, Dave A, Vaistij FE, Worrall D, Holroyd GH, Wells JG, Kaminski F, Graham IA, Roberts**  
473 **MR.** 2017. Jasmonic acid-dependent regulation of seed dormancy following maternal herbivory in  
474 Arabidopsis. New Phytologist **214**, 1702-1711.

475 **Smith H.** 2000. Phytochromes and light signal perception by plants - an emerging synthesis. Nature **407**,  
476 585-591.

477 **Shen H, Moon J, Huq E.** 2005. PIF1 is regulated by light-mediated degradation through the ubiquitin-  
478 26S proteasome pathway to optimize seedling photomorphogenesis in Arabidopsis. The Plant Journal  
479 **44**, 1023-1035.

480 **Shinomura T, Nagatani A, Chory J, Furuya M.** 1994. The induction of seed germination in  
481 *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A.  
482 Plant Physiology **104**, 363-371.

483 **Shinomura T.** 1997. Phytochrome regulation of seed germination. Journal of Plant Research **110**, 151-  
484 161.

485 **Shu K, Chen Q, Wu Y, et al.** 2016. ABI4 mediates antagonistic effects of abscisic acid and gibberellins  
486 at transcript and protein levels. *Plant Journal* **85**, 348-361.

487 **Stintzi A, Browse J.** 2000. The Arabidopsis male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic  
488 acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Sciences of the*  
489 *USA* **97**, 10625-10630.

490 **Vaistij FE, Barros-Galvão T, Cole A, Gilday AD, He Z, Li Y, Harvey D, Larson T, Graham IA.**  
491 2018. MOTHER-OF-FT-AND-TFL1 is a key repressor of seed germination under far-red light in  
492 *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* **115**, 8442-8447.

493 **Vaistij FE, Gan Y, Penfield S, Gilday AD, Dave A, He Z, Josse EM, Choi G, Halliday KJ, Graham**  
494 **IA.** 2013. Differential control of seed primary dormancy in Arabidopsis ecotypes by the transcription  
495 factor SPATULA. *Proceedings of the National Academy of Sciences of the USA* **110**, 10866-10871.

496 **Wasternack C, Hause B.** 2018. A bypass in jasmonate biosynthesis: the OPR3-independent formation.  
497 *Trends in Plant Sciences* **23**, 276-279.

498 **Wasternack C, Hause B.** 2013. Jasmonates: biosynthesis, perception, signal transduction and action in  
499 plant stress response, growth and development. *Annals of Botany* **111**, 1021-1158.

500 **Wasternack C, Song S.** 2016. Jasmonates: biosynthesis, metabolism, and signaling by proteins  
501 activating and repressing transcription. *Journal of Experimental Botany* **68**, 1303-1321.

502 **Wasternack C, Strnad M.** 2015. Jasmonate signaling in plant stress responses and development -  
503 active and inactive compounds. *N Biotechnol* **25**, 604-613.

504 **Xi W, Liu C, Hou X, Yu H.** 2010. MOTHER OF FT AND TFL1 regulates seed germination through a  
505 negative feedback loop modulating ABA signaling in Arabidopsis. *The Plant Cell* **22**, 1733-1748.

506 **Zolman BK, Silva ID, Bartel B.** 2001. The Arabidopsis *pxa1* mutant is defective in an ATP-binding  
507 cassette transporter-like protein required for peroxisomal fatty acid b-oxidation. *Plant Physiology* **127**,  
508 1266-1278.

509

## 510 **Figure Legends**

511 **Fig. 1. Analyses of gene expression and accumulation of OPDA and ABA.** (A) Scheme of the  
512 experimental design: after-ripened seeds were imbibed for 4 hours under white-light (WL) and then  
513 treated with (i) two consecutive 5 minutes FR and R pulses (FR/R); (ii) only one FR pulse (FR); or (iii)  
514 48 hours of continuous FR irradiation (FR48). Seeds were kept in the dark after light treatments.  
515 Samples were collected for analyses at 12 and 24 hours-after-imbibition (hai) as stated in the figure. (B-

516 C) Relative *AOS* and *OPR3* expression. (D-E) ABA and OPDA levels in FR/R and FR-treated wild-type  
517 (Col) and FR-treated *mft-2* seeds. Data are means of three and four biological replicates for gene  
518 expression and germination assays, respectively, and error bars represent standard deviation. Asterisks  
519 (\*) denote statistical significant difference compared to the respective controls as determined by  
520 Student's t-test ( $P < 0.05$ ).

521

522 **Fig. 2. Germination assays of OPDA and ABA deficient seeds.** (A) Wild-type (Col) and *aos* seeds on  
523 control and norflurazon (Norf; 50 and 100  $\mu\text{M}$ ) supplemented plates under FR/R and FR light. (B) Col,  
524 *opr3-1* and *opr3-3* seeds on control and norflurazon (Norf; 50 and 100  $\mu\text{M}$ ) supplemented plates under  
525 FR/R and FR light. (C) Col, *aos* and *aba2-1* single, and *aos aba2-1* double mutant seeds under FR/R, FR  
526 and FR-FR treatments. (D) Germination of *aos aba2-1* seeds treated with OPDA or ABA (1 and 10  $\mu\text{M}$ )  
527 under FR light conditions. Germination was assessed 144 hai (seeds had been after-ripened for not  
528 longer than 8 weeks). Data are means of four biological replicates and error bars represent standard  
529 deviation. Asterisks (\*) denote statistical significant difference compared to the respective controls as  
530 determined by Student's t-test ( $P < 0.05$ ).

531

532 **Fig. 3. Effect of GA, OPDA or ABA on germination of mutant seeds under FR light conditions.** (A)  
533 *aos aba2-1* double mutant seeds on control and Paclobutrazol (PAC; 5  $\mu\text{M}$ ) supplemented plates. (B)  
534 *rgl1-1 rgl2-2 gai-6 rga-2* quadruple (*della4*) mutant seeds on control, OPDA and ABA (1 and 5  $\mu\text{M}$ )  
535 supplemented plates. (C) *mft-2* mutant seeds on control and OPDA (10  $\mu\text{M}$ ) supplemented plates.  
536 Germination was assessed 144 hai. Data are means of four biological replicates and error bars represent  
537 standard deviation. Asterisks (\*) denote statistical significant difference compared to the respective  
538 controls as determined by Student's t-test ( $P < 0.05$ ).

539

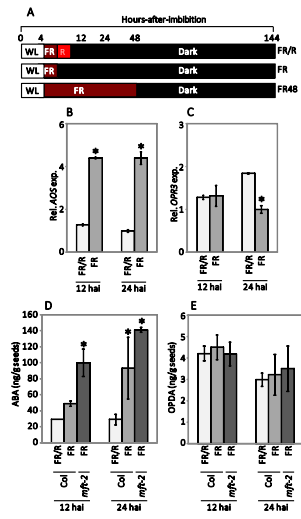
540 **Fig. 4. *PIF1*, *SOM* and *MFT* gene expression.** (A) Relative gene expression in after-ripened wild-type  
541 (Col) seeds under FR/R, FR and FR48 light treatments (48 hai). (B) Relative MFT expression in after-  
542 ripened Col, *aos*, *aba2-1* and *aos aba2-1* seeds under FR48 light treatment (48 hai). Data presented are  
543 the means of three biological replicates and error bars represent standard deviation. Asterisks (\*) denote  
544 statistical significant difference compared to the respective controls as determined by Student's t-test ( $P$   
545  $< 0.05$ ).

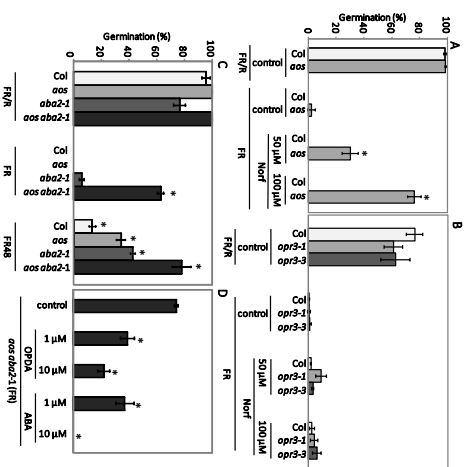
546

547 **Fig. 5. Analyses of long-term after-ripened seeds.** (A) Germination (144 hai) of wild-type (Col) seeds  
548 upon FR/R and FR treatments on control and norflurazon (Norf; 100  $\mu$ M) supplemented plates. (B)  
549 Germination (144 hai) of Col and *aos* seeds upon FR/R and FR treatments. All seeds were after-ripened  
550 for at least nine months before conducting germination assays. (C) Relative *MFT* expression in young  
551 and old (8-weeks and 9-months after-ripened, respectively) FR-treated seeds. Data are means of four  
552 (for germination) and three (for gene expression) biological replicates and error bars represent standard  
553 deviation. Asterisks (\*) denote statistical significant difference compared to the respective controls as  
554 determined by Student's t-test ( $P < 0.05$ ).

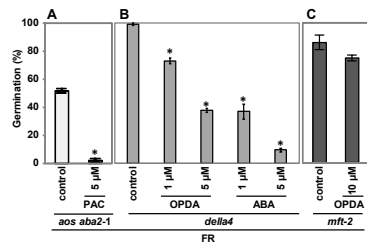
555  
556 **Fig. 6. Model of interaction between OPDA, ABA and MFT to repress germination.** Treatment with  
557 FR light early after seed imbibition deactivates phyB but has no effect on phyA as it has not yet  
558 accumulated. Under these conditions endogenous OPDA and ABA fully repress seed germination and  
559 promote expression of the germination repressor MFT (A). In the absence of either OPDA and ABA, the  
560 action of the remaining phytohormone and the reduced level of MFT is sufficient to fully repress  
561 germination (B and C). When both phytohormones are absent, the low level of MFT expression is not  
562 sufficient to repress germination (D). This model also explains the partial germination of OPDA or ABA  
563 deficient seeds following a FR48 treatment, which activates phyA and deactivates phyB leading to a  
564 reduction of *MFT* expression (compare Figs 2 and 4). Similarly, the model explains the partial  
565 germination in response to FR treatment of old seeds deficient in ABA or OPDA.

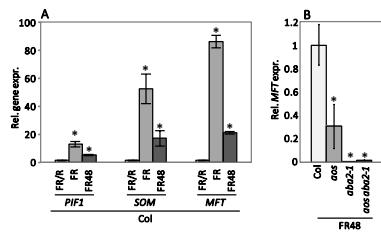
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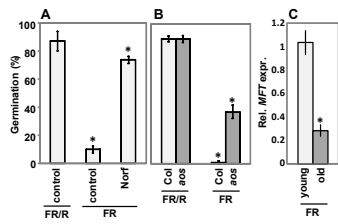


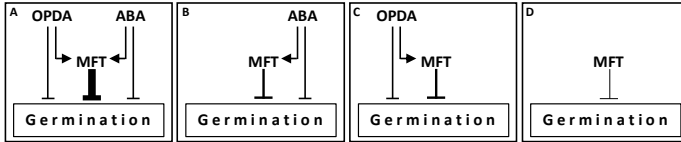
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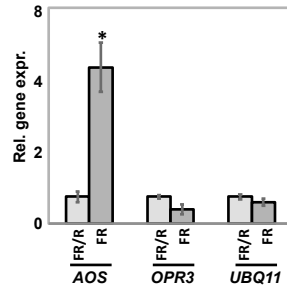






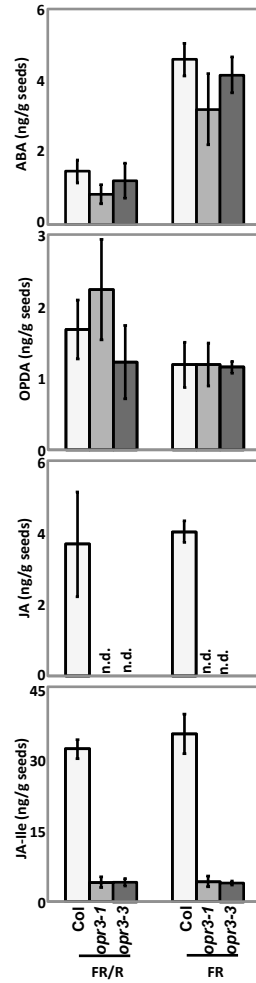




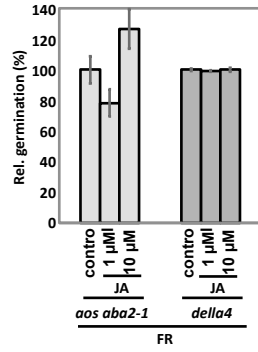


**Sup. Fig. 1 Relative AOS, OPR3 and UBQ11 gene expression.** Data extracted from an RNAseq-based transcriptomic analysis performed previously (Vaistij *et al.*, 2016) of FR/R- and FR-treated Col seeds (24 hai). Data presented are the means of three biological replicates and error bars represent standard deviation. Asterisks (\*) denote statistical significant difference compared to the respective controls as determined by Student's t-test ( $P < 0.05$ ).

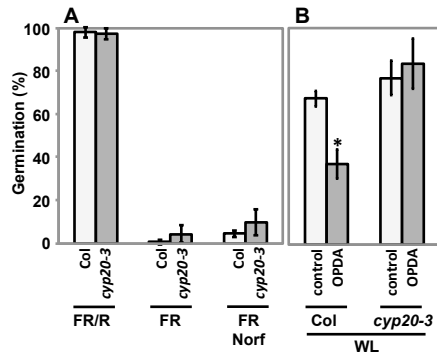
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584



**Sup. Fig. 2** OPDA, JA and JA-Ile accumulation in Col, *opr3-1* and *opr3-3* seeds. Seeds were FR/R and FR treated and material collected 24 hai. Data presented are the means of four biological replicates and error bars represent standard deviation. (n.d.: not detected.)



**Sup. Fig. 3 Effect of JA-treatment on germination of *aos aba2-1* double and *della* quadruple mutant seeds. A-B** Relative germination (144 hai; FR-treated) of *aos aba2-1* double (A) and *rgl1-1 rgl2-2 gai-6 rga-2* quadruple (*della4*; B) mutant seeds on control and JA (1 and 10 μM) supplemented plates. Data presented are the means of four biological replicates and error bars represent standard deviation.



**Sup. Fig. 4 Analysis of *cyp20-3* seed germination.** **A** Germination (144 hai) of Col and *cyp20-3* seeds treated with FR/R, FR and FR supplemented with 100 μM norflurazon (Norf). **B** Germination (144 hai) of Col and *cyp20-3* seeds under white light (WL) on control and OPDA (10 μM) supplemented plates. Data presented are the means of four biological replicates and error bars represent standard deviation. Asterisks (\*) denote statistical significant difference compared to the respective controls as determined by Student's t-test ( $P < 0.05$ ).

587  
588  
589

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

Oligo name	Forward	Reverse
AOS	AAGTCAAAGCCGGTCAAAT	CTTACCGGCGCATTGTTTAT
OPR3	TGGACGCAACTGATTCTGAC	CTCATCACTCCCTTGCCTTC
PIF1	TGTCAATGGGATGTGGAATGA	CATCGCCATATGAGGCATGTA
SOM	TCCGGATGTTCTGAATTCAAGAT	GCAAAAGGACAATCAGTCCAATC
MFT	ATCACTAACGGCTGCGAGAT	CGGGAATATCCACGACAATC
UBQ11	TTCATTTGGTCTTGCGTCTG	GAAGATGAGACGCTGCTGGT

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