

This is a repository copy of *Regulation of Arabidopsis thaliana seed dormancy and germination by 12-oxo-phytodienoic acid.*

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/95707/>

Version: Published Version

Article:

Dave, Anuja Rajhuns, Vaistij, Fabian Emmanuel, Gilday, Alison D. et al. (2 more authors) (2016) Regulation of Arabidopsis thaliana seed dormancy and germination by 12-oxo-phytodienoic acid. Journal of Experimental Botany. pp. 2277-2284. ISSN 0022-0957

<https://doi.org/10.1093/jxb/erw028>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



RESEARCH PAPER

Regulation of *Arabidopsis thaliana* seed dormancy and germination by 12-oxo-phytodienoic acid

Anuja Dave^{1,*}, Fabián E. Vaistij¹, Alison D. Gilday¹, Steven D. Penfield² and Ian A. Graham^{1,†}

¹ Centre for Novel Agricultural Products, Department of Biology, University of York, York YO10 5DD, United Kingdom

² Department of Crop Genetics, John Innes Centre, Norwich NR4 7UH, United Kingdom

* Present address: Department of Crop Genetics, John Innes Centre, Norwich, NR4 7UH, United Kingdom

† To whom correspondence should be addressed. E-mail: ian.graham@york.ac.uk

Received 27 November 2015; Revised 12 January 2016; Accepted 14 January 2016

Editor: Christine Raines, University of Essex

Abstract

We previously demonstrated that the oxylipin 12-oxo-phytodienoic acid (OPDA) acts along with abscisic acid to regulate seed germination in *Arabidopsis thaliana*, but the mechanistic details of this synergistic interaction remain to be elucidated. Here, we show that OPDA acts through the germination inhibition effects of abscisic acid, the abscisic acid-sensing ABI5 protein, and the gibberellin-sensing RGL2 DELLA protein. We further demonstrate that OPDA also acts through another dormancy-promoting factor, MOTHER-OF-FT-AND-TFL1 (MFT). Both abscisic acid and MFT positively feed back into the OPDA pathway by promoting its accumulation. These results confirm the central role of OPDA in regulating seed dormancy and germination in *A. thaliana* and underline the complexity of interactions between OPDA and other dormancy-promoting factors such as abscisic acid, RGL2, and MFT.

Key words: Abscisic acid, dormancy, germination, MFT, OPDA, RGL2.

Introduction

Seed dormancy prevents germination until conditions are favourable for plant growth. In *Arabidopsis thaliana* (*Arabidopsis*), dormancy can be released by exposing imbibed seeds to low temperatures (i.e. stratification) or by an extended period of dry seed storage (i.e. after-ripening). Plant hormones are chemically diverse compounds, which occur in plants at low concentrations and regulate plant growth, development, and responses to external stimuli (Davies, 2010). Among these, the two hormones that are of the utmost importance in seed dormancy and germination regulation are abscisic acid (ABA) and gibberellic acid (GA). ABA plays a key role in induction of primary dormancy during *Arabidopsis* seed development and acts as a germination repressor, while GA promotes germination and the alleviation

of dormancy (Finkelstein *et al.*, 2008; Graeber *et al.*, 2012; Holdsworth *et al.*, 2008). Both biosynthesis and catabolism determine the final amount of ABA and GA in the seed, and the interactions between these two antagonistic hormones, at the metabolism and signalling levels, are crucial in the regulation of germination.

The transcription factors ABI3, ABI4, and ABI5 are important components of the ABA signal transduction pathway involved in germination regulation (Clerkx *et al.*, 2003; Finkelstein, 1994; Finkelstein and Lynch, 2000; Finkelstein *et al.*, 1998; Lopez-Molina and Chua, 2000; Lopez-Molina *et al.*, 2002; Xu *et al.*, 2014). ABI5 is a basic leucine-zipper (bZIP) transcription factor (Finkelstein and Lynch, 2000; Lopez-Molina and Chua,

2000); in *Arabidopsis* seeds it is expressed in the embryo and micropylar endosperm (Penfield *et al.*, 2006a). ABA causes an increase in *ABI5* transcript levels and protein abundance, and also activates *ABI5* via phosphorylation (Lopez-Molina *et al.*, 2001; Piskurewicz *et al.*, 2008). Integral to GA signal transduction are the growth-repressing DELLA proteins. GA binds to the receptor GA INSENSITIVE DWARF1 (*GID1*) and enhances *GID1*-DELLA interaction, leading to DELLA degradation by the ubiquitin-proteasome pathway (Murase *et al.*, 2008; Sun, 2010). DELLA proteins repress germination, and of the five *DELLA* genes in *Arabidopsis*, *RGA-LIKE2* (*RGL2*) is the major DELLA involved in seed germination (Cao *et al.*, 2005; Lee *et al.*, 2002; Penfield *et al.*, 2006b; Piskurewicz and Lopez-Molina, 2009; Piskurewicz *et al.*, 2008; Tyler *et al.*, 2004). *RGL2* accumulation can also lead to an increase in ABA levels via regulation of the RING-zinc finger protein-encoding gene *XERICO*, which has been shown to promote accumulation of ABA in an as yet unknown manner (Ko *et al.*, 2006; Piskurewicz *et al.*, 2008; Zentella *et al.*, 2007).

FLOWERING-LOCUS-T (*FT*), TERMINAL-FLOWER1 (*TFL1*), and MOTHER-OF-FT-AND-TFL1 (*MFT*) belong to the phosphatidyl ethanolamine-binding protein family of proteins in *Arabidopsis*. While *FT* and *TFL1* are involved in regulation of flowering time control, *MFT* has been shown to be a regulator of germination and dormancy in different species (Li *et al.*, 2014; Nakamura *et al.*, 2011; Vaistij *et al.*, 2013; Xi and Yu, 2010; Xi *et al.*, 2010). Data reported by Vaistij *et al.* (2013) and Nakamura *et al.* (2011) indicate that *MFT* promotes dormancy, and this is consistent with the fact that *MFT* expression is promoted by *RGL2* and *ABI5* in *Arabidopsis* (Xi *et al.*, 2010). However, *MFT* has also been found to negatively regulate ABA signalling (Xi *et al.*, 2010).

Jasmonic acid (*JA*) and its precursor 12-oxo-phytodienoic acid (*OPDA*) are oxylipins derived from α -linolenic acid (Wasternack and Hause, 2013). *JA* regulates biotic and abiotic stress responses, as well as plant growth and developmental processes (Balbi and Devoto, 2008; Santino *et al.*, 2013; Wasternack, 2014; Wasternack *et al.*, 2013). *OPDA* has also been shown to possess distinct, *JA*-independent, signalling properties in different species, and its involvement has been reported in biotic and abiotic stress responses, embryo development, and seed germination (Bosch *et al.*, 2014; Dave and Graham, 2012; Dave *et al.*, 2011; Goetz *et al.*, 2012; Guo *et al.*, 2014; Park *et al.*, 2013; Ribot *et al.*, 2008; Satoh *et al.*, 2014; Savchenko and Dehesh, 2014; Savchenko *et al.*, 2014; Taki *et al.*, 2005). A role for *OPDA* in repressing germination in *Arabidopsis* was proposed based on analysis of *comatose* (*cts*) seeds disrupted in the activity of the ATP binding cassette (*ABC*) transporter *CTS*, also known as *PXA1* or *PED3* (Footitt *et al.*, 2002; Hayashi *et al.*, 2002; Russell *et al.*, 2000; Zolman *et al.*, 2001). *CTS* is involved in the transport of *OPDA* into the peroxisome, where *JA* biosynthesis occurs. *cts-2/pxal-1* seeds accumulate high levels of *OPDA*, leading to the low germination rates observed for this mutant (Dave *et al.*, 2011). Moreover, exogenous *OPDA* leads to an increase

in *ABI5* protein abundance and inhibition of the germination of wild-type (*WT*) seeds (Dave *et al.*, 2011).

Here, we show that ABA, *ABI5*, *RGL2*, and *MFT* are key components of the *OPDA* pathway that represses germination. We also show that there is a complex network of feedback interactions by which these components downstream of *OPDA* affect *OPDA* levels in seeds.

Materials and methods

Plant and growth conditions

Arabidopsis thaliana *WT* and mutant plants used were in the Columbia-0 (*Col*), Landsberg *erecta* (*Ler*) and Wassilewskija (*Ws*) ecotypes as stated. The mutants used in this study have been described previously: *pxal-1* (Zolman *et al.*, 2001), *cts-2* (Footitt *et al.*, 2002), *aos* (Park *et al.*, 2002), *opr3-1* (Stintzi and Browse, 2000), *rgl2-1* (Lee *et al.*, 2002), *abal-1* (Koorneef *et al.*, 1982), *mft-2* (Xi *et al.*, 2010), and *gal-3* (Sun and Kamiya, 1994). Plants were grown in controlled environment growth cabinets at 22 °C with 16h white light at 80–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity. Seeds were harvested from brown siliques that had begun to dehisce, and were size-sieved using a 250 μm mesh sieve. Experiments performed with freshly harvested seeds were conducted within 24h of harvest. For the experiments with after-ripened seeds, freshly harvested seeds were dry stored for 6–8 weeks. For stratification, seeds were imbibed on water agar plates and stratified in the dark at 4 °C for 3 days before being placed in growth cabinets.

Seed germination assays

Germination assays were performed on 0.9% (w/v) water agar plates, with 50–100 sterilized seeds, and plates were placed in a controlled environment growth cabinet with continuous light (150 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at 20 °C. In experiments where germination assays were conducted with *OPDA* (Larodan), *ABA* (Sigma), *Norflurazon* (Sigma), and *Paclbutrazol* (Sigma), the appropriate amounts of these compounds were included in the water agar medium. Germination was scored as radicle emergence after 7 days of incubation.

Hormone and oxylipin analysis

ABA, *GA*, *OPDA*, and *JA*-isoleucine (*JA*-Ile) conjugate were extracted and quantified from dry seed (80–100mg dry weight) and imbibed seed (300–400mg fresh weight) according to the protocol described for *GA* and *ABA* quantification (Dave *et al.*, 2011), with d_2 -*GA4*, d_6 -*ABA* and Prostaglandin *A1* included as internal standards for *GA4*, *ABA*, and *OPDA*/*JA*-Ile, respectively.

Gene expression analysis

RNA extractions from developing seeds and imbibed seeds, cDNA synthesis, and quantitative PCR analysis were performed according to methods described previously (Penfield *et al.*, 2005; Vaistij *et al.*, 2013). cDNA amplifications were normalized to *ACTIN2* and relative gene expression was calculated as a ratio between the value of each sample and their respective controls. The primers used are described in Supplementary Table 1 at *JXB* online.

Statistical analysis

In all Figures the data represent the mean of three biological replicates \pm standard deviation. Differences between controls and samples stated in the text are statistically significant as determined by Student's *t*-test ($P \leq 0.05$).

Results and discussion

Seed dormancy is affected in OPDA biosynthetic and catabolic mutants

We demonstrated previously that *Arabidopsis pxa1-1/cts-2* mutant seeds are highly dormant due to an over-accumulation of OPDA (Dave *et al.*, 2011). We also showed that seeds of the *aos* mutant (disrupted in an early step in the oxylipin pathway prior to OPDA; Park *et al.*, 2002) and of the *opr3-1* mutant (unable to convert OPDA into JA; Stintzi and Browse, 2000) have lower and higher levels of OPDA, respectively, than WT seeds (Dave *et al.*, 2011). Based on these observations, we hypothesized that seeds of these mutants should also have altered dormancy levels correlating with their OPDA levels in seeds. To test this hypothesis, germination levels of freshly harvested seeds were determined. We found that *aos* seeds, which are deficient in OPDA accumulation, indeed germinated faster and at a higher frequency than WT (Col) seeds (Fig. 1). In contrast, *opr3-1* seeds, which contain more OPDA, germinated at a lower frequency than their respective WT (Ws) seeds (Fig. 1). These observations further confirm that levels of dormancy positively correlate with OPDA accumulation in freshly harvested seeds.

OPDA acts through the ABA pathway

We showed in our previous work that exogenously applied OPDA and ABA synergistically inhibit germination of after-ripened seeds (Dave *et al.*, 2011). Here, we extended our analyses by performing germination assays using seeds of the ABA-biosynthesis mutant *aba1-1* and the GA-signalling mutant *rgl2-1*. As shown for Col and Ws (Dave *et al.*, 2011), germination of after-ripened *Ler* seeds was inhibited by exogenously applied OPDA (Fig. 2A). However, OPDA was almost ineffective when germination assays were performed with *aba1-1* and *rgl2-1* seeds (Fig. 2A). The ABA-dependent effect of OPDA was also observed when WT (Col) seeds were

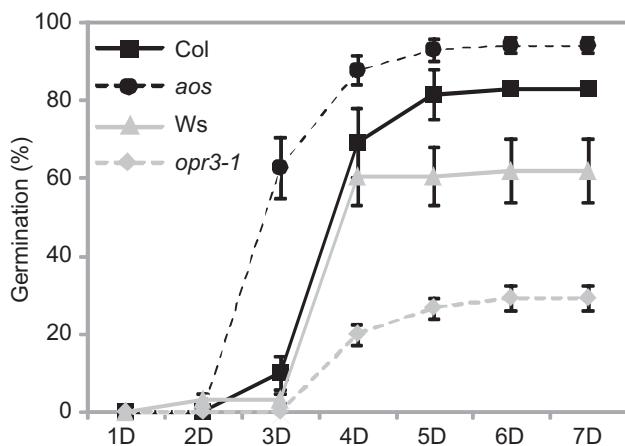


Fig. 1. Seeds affected in OPDA metabolism have altered dormancy. Germination profiles of WT Col and Ws, *aos* (in Col background), and *opr3-1* (in Ws background) freshly harvested, unstratified seeds from 1 to 7 days after imbibition (1D–7D) on water agar plates under constant light. In this and all other Figures, error bars represent the standard deviation of three independent measurements.

treated jointly with OPDA and the indirect ABA biosynthesis inhibitor Norflurazon, to mimic the *aba1-1* mutation (Supplementary Fig. S1). We also measured GA and ABA accumulation in OPDA-treated seeds and found that while GA levels were unchanged (Fig. 2B), ABA accumulation

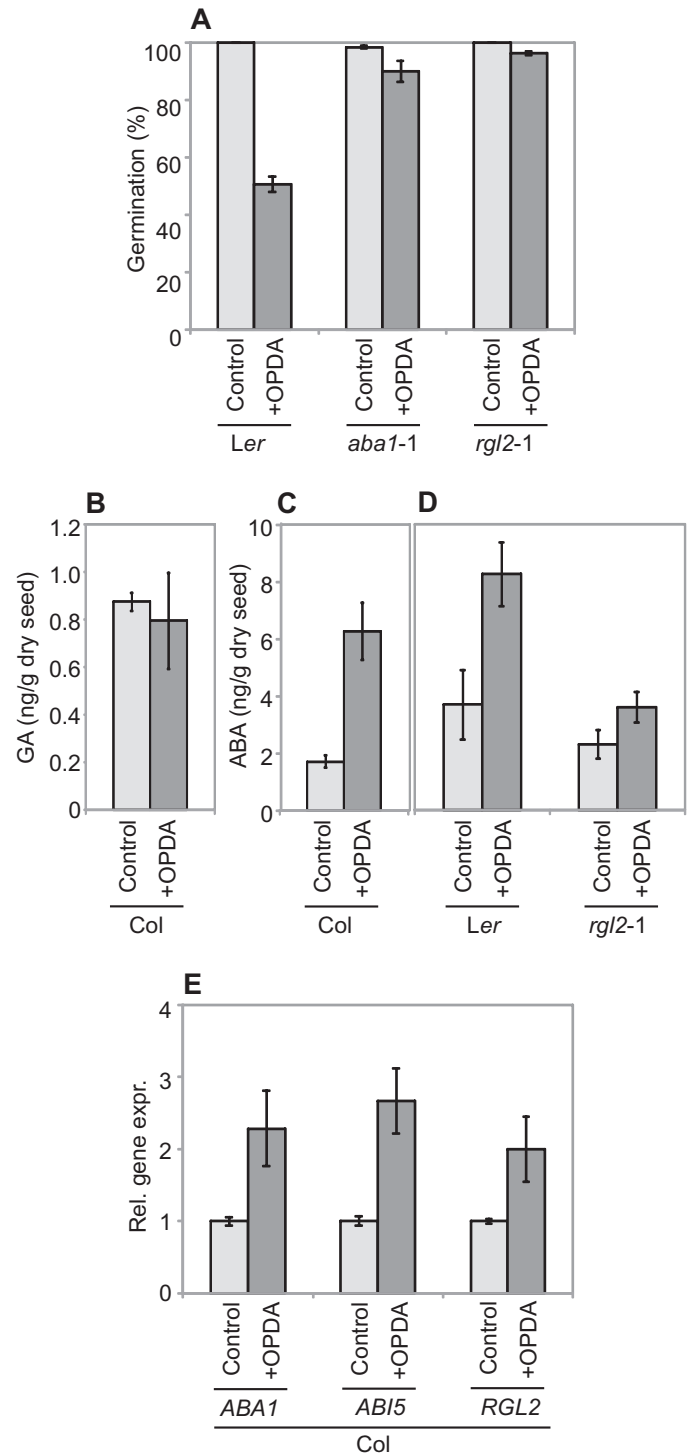


Fig. 2. The ABA biosynthetic pathway is required for OPDA-driven inhibition of germination. (A) Germination profiles [7 days after imbibition (DAI)] of after-ripened WT (*Ler*), *aba1-1* and *rgl2-1* seeds on water agar (Control) plates and water agar supplemented with 10 μ M OPDA (+OPDA). (B–D) GA (GA4) and ABA levels in after-ripened WT (Col and *Ler*) and *rgl2-1* seeds on Control or +OPDA plates measured 2 DAI. (E) Relative gene expression of *ABA1*, *ABI5*, and *RGL2* in after-ripened seeds.

was increased in both Col and *Ler* WT seeds (Fig. 2C, D). However, the ABA increase was strongly reduced in *rgl2-1* mutant seeds (Fig. 2D). This is in accordance with previous observations showing that RGL2 indirectly promotes ABA biosynthesis (Lee et al., 2010). Taken together, these observations demonstrate that exogenously applied OPDA triggers ABA accumulation.

We then investigated whether elevated levels of endogenous OPDA in seeds also correlated with increased amounts of ABA. To do this, we quantified ABA in OPDA over-accumulating *cts-2* seeds (Dave et al., 2011) and found that, as with OPDA-treated seeds, their ABA levels were increased (Supplementary Fig. S2).

In water-stressed Arabidopsis roots, induction of the JA-Ile conjugate, but not OPDA, is required for ABA accumulation in an *NCED3*-dependent manner (De Ollas et al., 2015). *NCED3* is a key gene regulating ABA biosynthesis under stress conditions (Nambara and Marion-Poll, 2005). One possible explanation for the germination-inhibiting effect of OPDA is that OPR3 converts OPDA into JA/JA-Ile, which in turn promotes ABA biosynthesis to inhibit germination. However, *opr3-1* seeds, which over-accumulate OPDA but not JA/JA-Ile (Dave et al., 2011), are more dormant than WT seeds (Fig. 1). Additionally, exogenously applied OPDA

represses germination of *opr3-1* seeds as well as WT control seeds (Supplementary Fig. S3). Hence, in the germination inhibition response, it is OPDA rather than JA/JA-Ile that leads to the increase in ABA to inhibit germination. Thus, from our own work and that of others, it seems that both JA/JA-Ile and OPDA are able to affect ABA biosynthesis, but which compound does so depends on the tissue, stress stimuli, and developmental stage. Drought also induces OPDA and ABA accumulation, and these two compounds cooperatively regulate stomatal aperture in a JA-independent manner; however, in this case the response initiated by OPDA is unlikely to be via its effect on ABA biosynthesis (Savchenko et al., 2014). Thus, a mode of action for OPDA whereby it acts along with ABA, but not through a direct effect on ABA biosynthesis, is also possible.

To gain more insight into the mechanism of OPDA-driven ABA accumulation in seeds, we analysed the expression of *ABAI* and *RGL2*. We found that exogenously applied OPDA promotes the expression of both genes (Fig. 2E). Thus, OPDA has the ability to regulate the expression of genes involved in ABA production, but it remains unknown how OPDA operates to achieve this. Work from other laboratories has shown that expression of the ABA-signalling gene *ABI5* is up-regulated by endogenous and exogenous ABA (Lopez-Molina

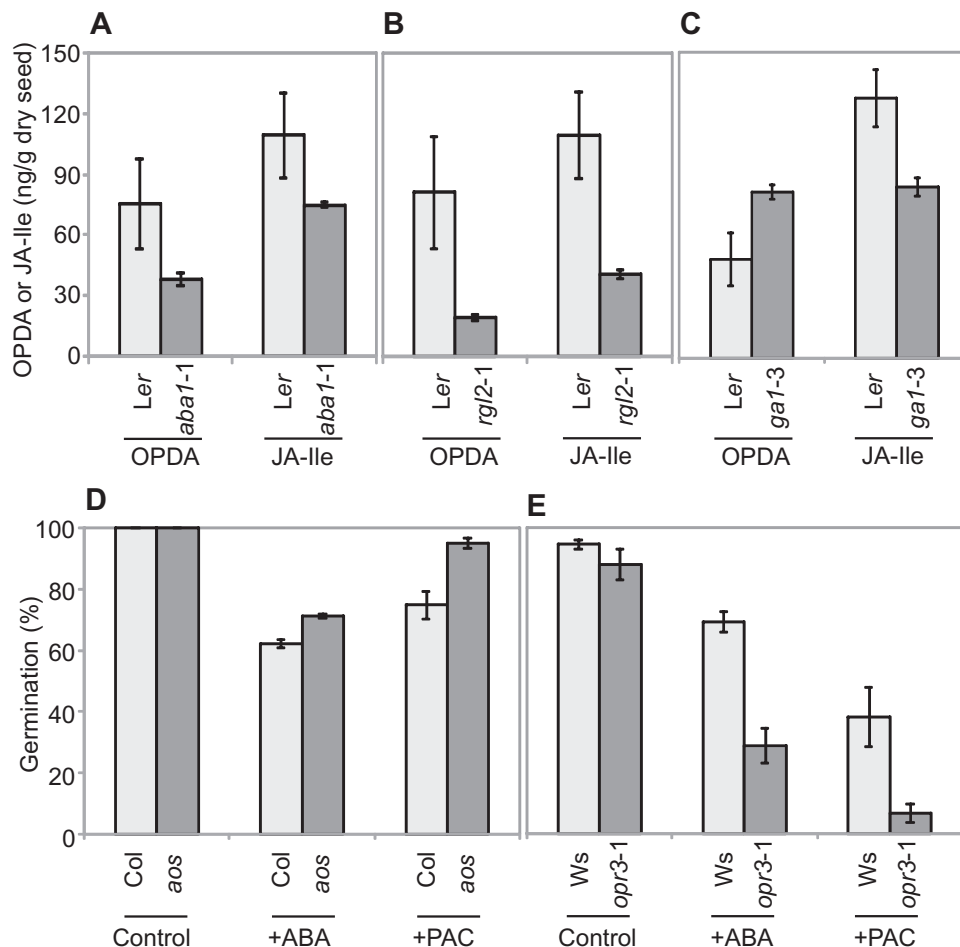


Fig. 3. ABA affects the oxylipin pathway. (A–C) OPDA and JA-Ile levels in WT (*Ler*), *aba1-1*, *rgl2-1*, and *ga1-3* freshly harvested dry seeds. (D–E) Germination profiles (7 DA) of WT (*Col*), *aos*, and *opr3-1* freshly harvested, stratified (3 days in the dark at 4 °C), seeds on water agar (Control) plates and water agar supplemented with 2 μ M ABA (+ABA) or 1 μ M Paclobutrazol (+PAC).

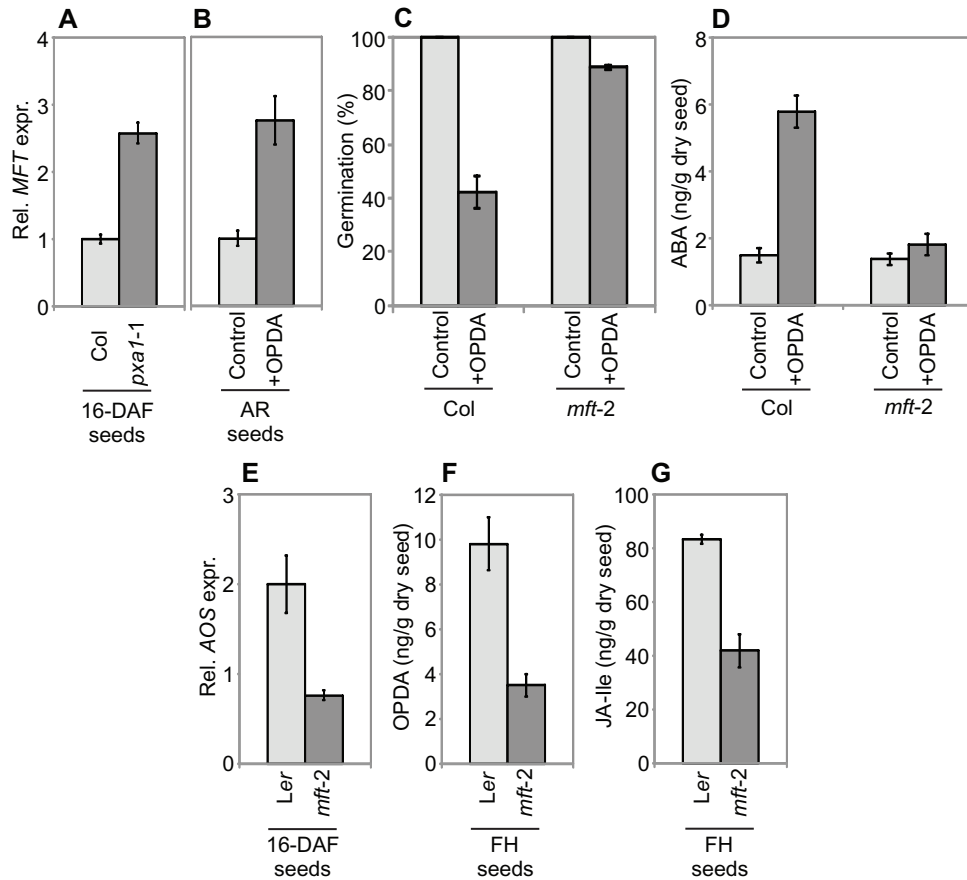


Fig. 4. Interactions between OPDA and MFT. (A, B) Relative *MFT* expression in WT (Col) and *pxa1-1* developing seeds 16 days after flowering (16-DAF) (A), and in after-ripened (AR) WT (Col) seeds on water agar (Control) plates and water agar supplemented with 10 μ M of OPDA (+OPDA) (panel B). (C) Germination profiles (7 DAI) of WT (Col) and *mft-2* after-ripened seeds on Control and +OPDA plates. (D) ABA levels in WT (Col) and *mft-2* after-ripened seeds imbibed on Control and +OPDA plates measured 2 DAI. (E) Relative AOS expression in 16-DAF WT (Col) and *mft-2* seeds. (F, G) OPDA and JA-Ile levels in WT (*Ler*) and *mft-2* freshly harvested (FH) dry seeds.

et al., 2001; Okamoto *et al.*, 2010; Piskurewicz *et al.*, 2008). This prompted us to assess whether exogenously applied OPDA has a similar effect on *ABI5* expression, and we found that it does (Fig. 2E). Hence, OPDA not only triggers ABA accumulation but can also increase ABA sensitivity. Furthermore, the importance of *ABI5* in the OPDA-imposed dormancy pathway has been demonstrated *in planta* by studying *ped3* mutant seeds, which are deficient in the same ABC transporter as *cts-2/pxa1-1*. The *ped3* seeds are highly dormant, have increased *ABI5* expression, and are rescued by the *abi5* mutation (Kanai *et al.*, 2010). However, the regulation of *ABI5* by OPDA does not occur exclusively at the level of gene expression, since OPDA also increases *ABI5* protein stability (Dave *et al.*, 2011). Taken together, these observations show that OPDA promotes ABA biosynthesis by increasing *ABA1* and *RGL2* expression, and ABA sensitivity by increasing *ABI5* gene expression and *ABI5* protein stability.

ABA regulates OPDA accumulation

It has been reported that Arabidopsis seedlings infected with the fungus *Phytophthora irregulare* accumulate JA and OPDA in an ABA-biosynthesis-dependent manner (Adie *et al.*, 2007). This led us to investigate a possible feedback of ABA and

GA into oxylipin biosynthesis in seeds. Thus, OPDA and JA-Ile levels were measured in freshly harvested dry seeds of ABA/GA metabolism and signalling mutants. We found that *aba1-1* and *rgl2-1* seeds accumulate less OPDA and JA-Ile than WT (*Ler*) controls (Fig. 3A, B). However, GA-deficient *gal-3* mutant seeds, which also accumulated lower levels of JA-Ile, contained a small but significant increase in OPDA levels (Fig. 3C). We also assessed whether the germination-inhibiting effects of ABA and Paclobutrazol (a GA-biosynthesis inhibitor) were dependent on OPDA by analysing *aos* and *opr3-1* mutants. We found a small but significant reduction in the effect of ABA and Paclobutrazol on after-ripened *aos* mutant seeds, which lack OPDA (Fig. 3D). Consistent with this result, *opr3-1* seeds, which accumulate OPDA, were found to be hypersensitive to ABA and Paclobutrazol (Fig. 3E).

Cross-talk between oxylipin (specifically JA) signalling and GA signalling has been reported previously in non-seed systems (Hou *et al.*, 2010; Navarro *et al.*, 2008; Qi *et al.*, 2014; Wild *et al.*, 2012; Yang *et al.*, 2012), but interaction between OPDA and GA at the level of biosynthesis has not been shown before. It is possible that the GA and OPDA interactions are indirect via ABA, since GA triggers degradation of *RGL2*, which in turn indirectly promotes accumulation of

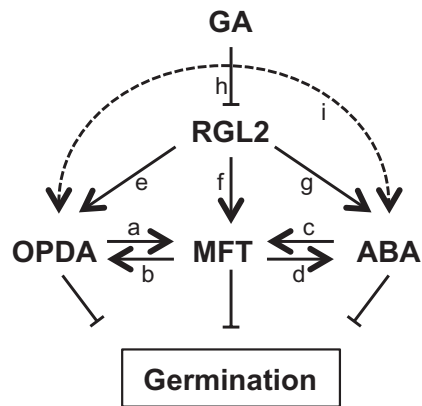


Fig. 5. Model of interactions between the germination repressors OPDA, MFT, and ABA. OPDA promotes *MFT* gene expression (a; Fig. 4A, 4B) and MFT protein is required for OPDA accumulation (b; Fig. 4F). ABA also promotes *MFT* expression (c; Xi et al., 2010) and MFT is required for OPDA-driven ABA accumulation (d; Fig. 4D). RGL2 is necessary for OPDA accumulation (e; Fig. 3B), *MFT* expression (f; Xi et al., 2010), and OPDA-driven ABA accumulation (g; Fig. 2D). GA triggers RGL2 protein degradation (h; Murase et al., 2008). The reciprocal positive feedback between OPDA and ABA (Figs 2C and 3A) may happen through MFT (a and d; c and b) or another indirect pathway (i). OPDA and MFT may *per se* have ABA-independent germination inhibitory effects. For simplicity, the involvement of ABI5 has not been depicted in this model.

ABA and oxylipins (Figs 2E and 3B). Taken together, these observations demonstrate that the germination-inhibitory effects of ABA and Paclobutrazol are (at least partially) augmented by OPDA, and that there is a feedback from ABA and GA to regulate OPDA accumulation.

OPDA and MFT interact reciprocally to inhibit germination

We reported previously that MFT plays a major role in promoting dormancy in Arabidopsis seeds (Vaistij et al., 2013). This prompted us to assess whether the OPDA and MFT pathways are linked. Analysis of a transcriptomic dataset derived from *pxa1-1* developing seeds (Dave et al., 2011) revealed that *MFT* expression is higher in the mutant compared with WT. This observation was validated in independent samples of developing *pxa1-1* seeds (Fig. 4A). Furthermore, MFT expression was also found to be elevated in OPDA-treated after-ripened WT seeds (Fig. 4B). Thus, high endogenous or exogenous OPDA levels induce expression of the dormancy-promoting *MFT* gene. Interestingly, we also found that *mft-2* after-ripened seeds are almost insensitive to OPDA (Fig. 4C), which shows that MFT is required for OPDA-induced inhibition of germination. In order to gain more insight into the interactions between OPDA and MFT, we assessed ABA levels in OPDA-treated *mft-2* after-ripened seeds, and found that they were lower than the levels observed in WT (Col) controls (Fig. 4D). This suggests that in order to trigger ABA biosynthesis, in addition to RGL2 (Fig. 2D), OPDA also requires MFT. We also investigated possible MFT-to-OPDA feedback interactions. To do this, we assessed *AOS* expression and found that it is down-regulated in *mft-2* developing seeds (Fig. 4E). Whether MFT

regulates *AOS* expression directly or indirectly remains to be determined but, consistent with *AOS* expression levels, both OPDA and JA-Ile levels were found to be lower in *mft-2* freshly harvested dry seeds than levels in WT seeds (Fig. 4F, G).

Our results indicate that at least one role of the phosphatidyl ethanolamine-binding protein MFT and the DELLA protein RGL2 in the OPDA response is to elevate ABA levels. DELLA proteins are known to promote ABA accumulation by inducing *XERICICO* expression, and *XERICICO* protein promotes ABA accumulation by an unknown mechanism (Ko et al., 2006; Piskurewicz et al., 2008; Zentella et al., 2007). MFT is also capable of influencing ABA biosynthesis by promoting *ABA1* expression (Li et al., 2014). Previous work has shown that MFT also influences ABA sensing, since it negatively regulates *ABI5* and, in turn, *MFT* gene expression is promoted by *ABI5* and RGL2 (Xi et al., 2010). Our data demonstrate that in terms of germination, OPDA acts through MFT to promote both biosynthesis of and sensitivity towards ABA. Furthermore, RGL2 and MFT positively feed back on this regulation by promoting OPDA accumulation. However, we cannot exclude the possibility that OPDA and ABA also interact independently of MFT, or that OPDA directly inhibits germination independently of both ABA and MFT (Fig. 5).

In conclusion, cross-talk between different hormone pathways is known to occur in order to regulate physiological processes at various developmental stages and in response to external stimuli. In this work we have confirmed the role of OPDA as a potent dormancy-promoting/germination-inhibiting factor in Arabidopsis. We established that it acts in conjunction (but possibly not exclusively) with ABA, ABI5, RGL2, and MFT via a complex network of positive and negative feedback interactions. Further research is required to understand how OPDA is regulated by external stimuli and what other physiological processes can be influenced by OPDA in Arabidopsis.

Supplementary data

- Figure S1. OPDA requires ABA to inhibit germination.
- Figure S2. OPDA and ABA levels are increased in *cts-2* seeds.
- Figure S3. *opr3-1* seeds are sensitive to OPDA.
- Table S1. Sequence of primers used for gene expression analyses.

Acknowledgements

This work was supported by the Biotechnology and Biological Sciences Research Council (grant number BB/J00216X/1). We thank Dr Tony Larson for his assistance with measurement of phytohormones and oxylipins, the departmental horticultural service for plant care, and Thiago Barros-Galvão and Judith Mitchell for comments on and proofreading of the manuscript, respectively.

References

- Adie BA, Pérez-Pérez J, Pérez-Pérez MM, Godoy M, Sánchez-Serrano JJ, Schmelz EA, Solano R. 2007. ABA is an essential signal for

- plant resistance to pathogens affecting JA biosynthesis and the activation of defences in *Arabidopsis*. *The Plant Cell* **19**, 1665–1681.
- Balbi V, Devoto A.** 2008. Jasmonate signalling network in *Arabidopsis thaliana*: crucial regulatory nodes and new physiological scenarios. *New Phytologist* **177**, 301–318.
- Bosch M, Wright LP, Gershenzon J, Wasternack C, Hause B, Schaller A, Stintzi A.** 2014. Jasmonic acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. *Plant Physiology* **166**, 396–410.
- Cao D, Hussain A, Cheng H, Peng J.** 2005. Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in *Arabidopsis*. *Planta* **223**, 105–113.
- Clerkx EJ, Vries HB, Ruys GJ, Groot SP, Koornneef M.** 2003. Characterization of *green seed*, an enhancer of *abi3-1* in *Arabidopsis* that affects seed longevity. *Plant Physiology* **132**, 1077–1084.
- Dave A, Graham IA.** 2012. Oxylin signalling: a distinct role for the jasmonic acid precursor 12-oxo-phytodienoic acid (OPDA). *Frontiers in Plant Science* **3**, 42.
- Dave A, Hernández ML, He Z, Andriotis VME, Vaistij FE, Larson TR, Graham IA.** 2011. 12-Oxo-phytodienoic acid accumulation during seed development represses seed germination in *Arabidopsis*. *The Plant Cell* **23**, 583–599.
- Davies PJ.** 2010. The plant hormones: their nature, occurrence, and function. In PJ Davies, ed., *Plant hormones: biosynthesis, signal transduction, action!* Dordrecht: Springer, 1–15.
- De Ollas C, Arbona V, Gómez-Cadenas A.** 2015. Jasmonoyl isoleucine accumulation is needed for abscisic acid build-up in roots of *Arabidopsis* under water stress conditions. *Plant Cell & Environment* **38**, 2157–2170.
- Finkelstein RR.** 1994. Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. *The Plant Journal* **5**, 765–771.
- Finkelstein RR, Ariizumi T, Steber C.** 2008. Molecular aspects of seed dormancy. *Annual Reviews of Plant Biology* **59**, 387–415.
- Finkelstein RR, Lynch TJ.** 2000. The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *The Plant Cell* **12**, 387–415.
- Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM.** 1998. The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA2 domain protein. *The Plant Cell* **10**, 1043–1054.
- Footitt S, Slocombe SP, Larner V, Kurup S, Wu Y, Larson T, Graham IA, Baker A, Holdsworth M.** 2002. Control of germination and lipid mobilization by *COMATOSE*, the *Arabidopsis* homologue of human ALDP. *The EMBO Journal* **21**, 2912–2922.
- Goetz S, Hellwege A, Stenzel I, et al.** 2012. Role of cis-12-oxo-phytodienoic acid in tomato embryo development. *Plant Physiology* **158**, 1715–1727.
- Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ.** 2012. Molecular mechanisms of seed dormancy. *Plant Cell & Environment* **35**, 1769–1786.
- Guo HM, Li HC, Zhou SR, Xue HW, Miao XX.** 2014. Cis-12-oxo-phytodienoic acid stimulates rice defense response to a piercing-sucking insect. *Molecular Plant* **7**, 1683–1692.
- Hayashi M, Nito K, Takei-Hoshi R, Yagi M, Kondo M, Suenaga A, Yamaya T, Nishimura M.** 2002. Ped3p is a peroxisomal ATP-binding cassette transporter that might supply substrates for fatty acid beta-oxidation. *Plant and Cell Physiology* **43**, 1–11.
- Holdsworth MJ, Bentsink L, Soppe WJ.** 2008. Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. *New Phytologist* **179**, 33–54.
- Hou X, Lee LY, Xia K, Yan Y, Yu H.** 2010. DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Developmental Cell* **19**, 884–894.
- Kanai M, Nishimura M, Hayashi M.** 2010. A peroxisomal ABC transporter promotes seed germination by inducing pectin degradation under the control of *ABI5*. *The Plant Journal* **62**, 936–947.
- Ko JH, Yang SH, Han KH.** 2006. Upregulation of an *Arabidopsis* RING-H2 gene, *XERICO*, confers drought tolerance through increased abscisic acid biosynthesis. *The Plant Journal* **47**, 343–355.
- Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM.** 1982. The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.). *Theoretical and Applied Genetics* **61**, 385–393.
- Lee KP, Piskurewicz U, Turecková V, Strnad M, Lopez-Molina L.** 2010. A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in *Arabidopsis* dormant seeds. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 19108–19113.
- Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng J.** 2002. Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a *GAI/RGA*-like gene whose expression is up-regulated following imbibition. *Genes & Development* **16**, 646–658.
- Li Q, Fan C, Zhang X, Wang X, Wu F, Hu R, Fu Y.** 2014. Identification of a soybean *MOTHER OF FT AND TFL1* homolog involved in regulation of seed germination. *PLoS ONE* **9**, e99642.
- Lopez-Molina L, Chua NH.** 2000. A null mutation in a bZIP factor confers ABA-insensitivity in *Arabidopsis thaliana*. *Plant Cell & Environment* **41**, 541–547.
- Lopez-Molina L, Mongrand S, Chua NH.** 2001. A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 4782–4787.
- Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH.** 2002. ABI5 acts downstream of ABI3 to execute an ABA dependent growth arrest during germination. *The Plant Journal* **32**, 317–328.
- Murase K, Hirano Y, Sun TP, Hakoshima T.** 2008. Gibberellin-induced DELLA recognition by the gibberellin receptor *GID1*. *Nature* **456**, 459–463.
- Nakamura S, Abe F, Kawahigashi H, et al.** 2011. A wheat homolog of *MOTHER OF FT AND TFL1* acts in the regulation of germination. *The Plant Cell* **23**, 3215–3229.
- Nambara E, Marion-Poll A.** 2005. Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56**, 165–185.
- Navarro L, Bari R, Achard P, Lisón P, Nemri A, Harberd NP, Jones JD.** 2008. DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signalling. *Current Biology* **18**, 650–655.
- Okamoto M, Tatematsu K, Matsui A, et al.** 2010. Genome-wide analysis of endogenous abscisic acid-mediated transcription in dry and imbibed seeds of *Arabidopsis* using tiling arrays. *The Plant Journal* **62**, 39–51.
- Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R.** 2002. A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. *The Plant Journal* **31**, 1–12.
- Park SW, Li W, Viehhauser A, et al.** 2013. Cyclophilin 20–3 relays a 12-oxo-phytodienoic acid signal during stress responsive regulation of cellular redox homeostasis. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 9559–9564.
- Penfield S, Gilday AD, Halliday KJ, Graham IA.** 2006b. DELLA-mediated cotyledon expansion breaks coat-imposed seed dormancy. *Current Biology* **16**, 2366–2370.
- Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ, Graham IA.** 2005. Cold and light control seed germination through the bHLH transcription factor *SPATULA*. *Current Biology* **15**, 1998–2006.
- Penfield S, Li Y, Gilday AD, Graham S, Graham IA.** 2006a. *Arabidopsis* ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. *The Plant Cell* **18**, 1887–1899.
- Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L.** 2008. The gibberellin acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5 activity. *The Plant Cell* **20**, 2729–2745.
- Piskurewicz U, Lopez-Molina L.** 2009. The GA-signaling repressor RGL3 represses testa rupture in response to changes in GA and ABA levels. *Plant Signaling & Behavior* **4**, 63–65.
- Qi T, Huang H, Wu D, Yan J, Qi Y, Song S, Xie D.** 2014. *Arabidopsis* DELLA and JAZ proteins bind the WD-repeat/bHLH/MYB complex to modulate gibberellin and jasmonate signaling synergy. *The Plant Cell* **26**, 1118–1133.

- Ribot C, Zimmerli C, Farmer EE, Reymond P, Poirier Y.** 2008. Induction of the *Arabidopsis* PHO1;H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. *Plant Physiology* **147**, 696–706.
- Russell L, Larner V, Kurup S, Bougourd S, Holdsworth M.** 2000. The *Arabidopsis* COMATOSE locus regulates germination potential. *Development* **127**, 3759–3767.
- Santino A, Taurino M, De Domenico S, Bonsegna S, Poltronieri P, Pastor V, Flors V.** 2013. Jasmonate signaling in plant development and defense response to multiple (a)biotic stresses. *Plant Cell Reports* **32**, 1085–1098.
- Sato M, Tokaji Y, Nagano AJ, Hara-Nishimura I, Hayashi M, Nishimura M, Ohta H, Masuda S.** 2014. *Arabidopsis* mutants affecting oxylipin signaling in photo-oxidative stress responses. *Plant Physiology and Biochemistry* **81**, 90–95.
- Savchenko T, Dehesh K.** 2014. Drought stress modulates oxylipin signature by eliciting 12-OPDA as a potent regulator of stomatal aperture. *Plant Signaling & Behavior* **9**, e28304.
- Savchenko T, Kolla VA, Wang CQ, Nasafi Z, Hicks DR, Phadungchob B, Chehab WE, Brandizzi F, Froehlich J, Dehesh K.** 2014. Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought. *Plant Physiology* **164**, 1151–1160.
- Stintzi A, Browse J.** 2000. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 10625–10630.
- Sun TB, Kamiya Y.** 1994. The *Arabidopsis* GA1 locus encodes the cyclase ent-kaurene synthetase A of gibberellin biosynthesis. *The Plant Cell* **6**, 1509–1518.
- Sun TP.** 2010. Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. *Plant Physiology* **154**, 567–570.
- Taki N, Sasaki-Sekimoto Y, Obayashi T, et al.** 2005. 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. *Plant Physiology* **139**, 1268–1283.
- Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP.** 2004. DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. *Plant Physiology* **135**, 1008–1019.
- Vaistij FE, Gan Y, Penfield S, Gilday AD, Dave A, He Z, Josse EM, Choi G, Halliday KJ, Graham IA.** 2013. Differential control of seed primary dormancy in *Arabidopsis* ecotypes by the transcription factor SPATULA. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 10866–10871.
- Wasternack C.** 2014. Action of jasmonates in plant stress responses and development-applied aspects. *Biotechnology Advances* **32**, 31–39.
- Wasternack C, Forner S, Strnad M, Hause B.** 2013. Jasmonates in flower and seed development. *Biochimie* **95**, 79–85.
- Wasternack C, Hause B.** 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. an update to the 2007 review in *Annals of Botany*. *Annals of Botany* **111**, 1021–1058.
- Wild M, Davière JM, Cheminant S, Regnault T, Baumberger N, Heintz D, Baltz R, Genschik P, Achard P.** 2012. The *Arabidopsis* DELLA *FGA-LIKE3* is a direct target of MYC2 and modulates jasmonate signaling responses. *The Plant Cell* **24**, 3307–3319.
- Xi W, Liu C, Hou X, Yu H.** 2010. *MOTHER OF FT AND TFL1* regulates seed germination through a negative feedback loop modulating ABA signaling in *Arabidopsis*. *The Plant Cell* **22**, 1733–1748.
- Xi W, Yu H.** 2010. *MOTHER OF FT AND TFL1* regulates seed germination and fertility relevant to the brassinosteroid signaling pathway. *Plant Signaling & Behavior* **5**, 1315–1317.
- Xu D, Li J, Gangappa SN, Hettiarachchi C, Lin F, Andersson MX, Jiang Y, Deng XW, Holm M.** 2014. Convergence of Light and ABA signaling on the *ABI5* promoter. *PLoS Genetics* **10**, e1004197.
- Yang DL, Yao J, Mei CS, et al.** 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America* **109**, E1192–E1200.
- Zentella R, Zhang ZL, Park M, et al.** 2007. Global analysis of DELLA direct targets in early gibberellin signaling in *Arabidopsis*. *The Plant Cell* **19**, 3037–3057.
- Zolman BK, Silva ID, Bartel B.** 2001. The *Arabidopsis* *pxa1* mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid β -oxidation. *Plant Physiology* **127**, 1266–1278.