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Short title: BRM controls seed biology through *asDOG1*

The authors responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors

(https://academic.oup.com/plphys/pages/General-Instructions) are Szymon Swiezewski and Ruslan Yatusevich.

Abstract

The SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeling complex is involved in various aspects of plant development and stress responses. Here, we investigated the role of BRM (BRAHMA), a core catalytic subunit of the SWI/SNF complex, in *Arabidopsis thaliana* seed biology. *brm-3* seeds exhibited enlarged size, reduced yield, increased longevity, and enhanced secondary dormancy, but did not show changes in primary dormancy or salt tolerance. Some of these phenotypes depended on the expression of *DOG1*, a key regulator of seed dormancy, as they were restored in the *brm-3 dog1-4* double mutant. Transcriptomic and metabolomic analyses revealed that BRM and DOG1 synergistically modulate the expression of numerous genes. Some of the changes observed in the *brm-3* mutant, including increased glutathione levels, depended on a functional *DOG1*. We demonstrated that the BRM-containing chromatin remodeling complex directly controls secondary dormancy through *DOG1* by binding and remodeling its 3' region, where the promoter of the long non-coding RNA *asDOG1* is located. Our results suggest that BRM and DOG1 cooperate to control seed physiological properties and that BRM regulates *DOG1* expression through *asDOG1*. This study reveals chromatin remodeling at the *DOG1* locus as a molecular mechanism controlling the interplay between seed viability and dormancy.

Introduction

Seeds encapsulate plant embryos in a state of suspended development, poised to resume life cycle upon encountering favorable environmental conditions. Many plant species, including *Arabidopsis thaliana,* produce seeds that can be stored in a dry stage for an extended time. This ability is known as seed longevity. In addition, seeds can also postpone germination despite optimal conditions, in a process known as dormancy that helps to adjust germination capability to changes in the environment. Dormancy established during seed maturation is known as primary dormancy, and is defined as a state in which freshly harvested seeds cannot germinate even under favorable conditions. Primary dormancy can be relieved by different means including after ripening – dry storage, or stratification – exposure to cold in the imbibed state. In contrast, dormancy developed by a non-dormant, imbibed seed in response to an unfavorable germination condition is known as secondary dormancy (Cadman *et al.*, 2006; Finch-Savage & Leubner-Metzger, 2006; Baskin & Baskin, 2014).

DELAY OF GERMINATION1 gene (*DOG1*), the main regulator of seed dormancy, has been identified by population analysis (Bentsink *et al.*, 2006) and further characterized in numerous molecular studies (Carrillo-Barral *et al.*, 2020). Primary dormancy and longevity are both acquired during seed development and are strictly regulated by numerous external and internal factors (Holdsworth *et al.*, 2008; Sano *et al.*, 2016). Interestingly, in Arabidopsis a trade-off between seed longevity and dormancy was described, as deep dormancy was associated with low longevity, suggesting that longevity and dormancy are genetically negatively correlated. On the contrary, *DOG1* has been shown to act as a positive regulator of both dormancy and longevity as *dog1* mutants show low primary and secondary dormancy as well as low longevity (Nguyen *et al.*, 2012; Dekkers *et al.*, 2013; Footitt *et al.*, 2015). As a central regulator of seed biology, *DOG1* expression is extensively regulated (Tognacca & Botto, 2021). Known regulators include two long non-protein coding RNA (lncRNA): one is the *PUPPIES* that activates *DOG1* expression and is transcribed from the *DOG1* promotor (Montez *et al.*, 2023) and a second is *asDOG1* that is transcribed from within the *DOG1* intron 2 in antisense orientation and suppresses *DOG1* expression (Fedak *et al.*, 2016).

Secondary dormancy modulation underlies the dormancy cycling phenomena described for seeds forming soil seed banks (Footitt *et al.*, 2015, 2017). Analysis of histone posttranslational modifications at the *DOG1* gene during dormancy cycling has led to a model where chromatin remodeling at the *DOG1* locus underpins this process (Footitt *et al.*, 2015). Compared to primary

dormancy, the mechanisms of secondary dormancy establishment in various plant species including *Arabidopsis thaliana* are mostly uncharted. Only few regulators have been identified so far and most of them influence both primary and secondary dormancy (Skubacz & Daszkowska-Golec, 2017; Buijs, 2020). Both types of dormancy are intricately modulated by environmental cues, such as variations in light quality, moisture levels, and transient cold exposure. They are also dependent on internal hormones, namely gibberellic acid (GA) and abscisic acid (ABA, Hauvermale *et al.*, 2015; Sano & Marion-Poll, 2021). ABA plays a pivotal role in initiating and sustaining dormancy, while GA acts as the trigger that breaks dormancy and promotes the germination process (Iwasaki *et al.*, 2022).

SWI/SNF (SWItch/Sucrose Non-Fermentable) is a highly conserved chromatin-remodeling complex that uses ATP to remodel chromatin. SWI/SNF complexes have been implicated in the control of multiple developmental processes and in orchestrating responses to environmental stimuli in yeast, plants, and animals (Ojolo *et al.*, 2018; Hernández-García *et al.*, 2022; Bieluszewski *et al.*, 2023). In Arabidopsis, several homologous subunits of the SWI/SNF complex have been described and many of these subunits are encoded by gene families, including SNF2-type ATPases: SPLAYED (SYD), BRAHMA (BRM), CHR12/MINU1 and CHR23/MINU2 (Shang & He, 2022; Guo *et al.*, 2022a). These subunits create the basis for plant SWI/SNF complex taxonomy dividing them into BRM-associated (BAS), SYD-associated (SAS) and MINU-associated (MAS) (Guo *et al.*, 2022b; Fu *et al.*, 2023). The BRM-containing complex (BAS) is probably the best-studied chromatin remodeling complex in plants. BRM ATPase contains multiple protein domains, including a bromodomain that binds acetylated histones and is thought to facilitate the complex recruitment to targeted DNA. In addition to BRM, the BAS complex contains other bromodomain-containing proteins - BRDs (BRD1/2/13) (Jarończyk *et al.*, 2021). Studies of Arabidopsis mutants of BAS complex subunits have shown that the BRM containing BAS-SWI/SNF complex is involved in multiple environmental responses and developmental transitions including seed maturation, embryogenesis, cotyledon separation, leaf development, root stem cell maintenance, floral patterning or flowering (Yu *et al.*, 2021; Shang & He, 2022; Guo *et al.*, 2022b; Bieluszewski *et al.*, 2023). Consistent with these findings, Arabidopsis *brm* knockout mutants (*brm-1)* display severe phenotypes including dwarfism, leaf curling, and sterility (Farrona *et al.*, 2004). Conversely, the *brm-3* mutant, which lacks the bromodomain in the BRM protein, is not sterile and exhibits mild phenotypic abnormalities, including seed coat defects (Hurtado *et al.*, 2006; Farrona *et al.*, 2007). Similar analysis of single and multiple mutants of the *BRD1/2/13* genes revealed their redundant role in regulating

vegetative development, flowering, as well as the responses to GA and ABA hormones (Jarończyk *et al.*, 2021; Stachula *et al.*, 2023). Despite the extensive work describing SWI/SNF and BRM's role in seeds development and germination (Han *et al.*, 2012; Ding *et al.*, 2022), the role of the BAS complex in seed biology remains poorly understood.

We and others showed before that apart from promoter regions, chromatin remodelers exhibit extensive binding at the 3'ends of genes (Brzezinka *et al.*, 2016; Archacki *et al.*, 2016; Jégu *et al.*, 2017). This led us to hypothesize that the BAS-containing SWI/SNF complex may regulate antisense transcription to indirectly control the sense gene expression (Archacki *et al.*, 2016). A reporter-effector study in young Arabidopsis seedlings identified *DOG1* as one of the genes displaying BRM binding at the 3'end (Archacki *et al.*, 2016). Here, we asked whether the link between BRM and *DOG1* may be important in seeds where the role of DOG1 and its regulation through *asDOG1* is well established (Fedak *et al.*, 2016). Here we show that BRM is implicated in multiple aspects of seed biology including seed size, seed longevity and seed dormancy. We demonstrate that some of the affected seed properties, including dormancy, are *DOG1* dependent and that the BAS complex controls secondary dormancy but not primary seed dormancy through *asDOG1* antisense transcription.

Results

BRM-mediated gene expression and metabolite composition in mature Arabidopsis seeds

To investigate the role of BRM in Arabidopsis seed biology in the context of DOG1, we created *brm-3dog1-4* double mutant and compared it with the wild type and single mutants in downstream analyses. *BRM* knockout allele *brm-1* is sterile, we therefore used the T-DNA insertion line *brm-3* (Tang *et al.*, 2008) and the double mutant *brm-3dog1-4*. 3'RNA-seq data analysis identified 77, 211 and 911 transcripts with decreased transcript levels in *brm-3, dog1-4* and *brm-3dog1-4*, respectively, and 167, 385 and 1391 transcripts with increased expression (absolute fold change > 1, FDR < 0.05) (Fig. 1A). As expected, comparison of differentially expressed genes in *brm-3* and *dog1-4* single mutants showed strong overlap with genes misregulated in double *brm-3dog1-4* mutant (Fig. 1B, Supplementary Figure S1C). Moreover, genes misregulated in *brm-3* and *dog1-4* also showed a substantial overlap (Fig. 1B). Interestingly, *dog1-4*, *brm-3* and double *brm-3dog1-4* mutants showed a substantial number of genes misregulated in the same directions (up and/or down), suggesting that DOG1 and BRM act synergistically in controlling gene expression in seeds (Fig. 1B). Self-clustering of expression profiles among genes misregulated in *brm-3* identified nine groups of genes (Fig.1C). Three

groups showed opposite changes in *brm-3* and *dog1-4* and at least partial suppression of the *brm* mutation-caused defects in the double *brm-3dog1-4* mutant (Fig.1C). Based on this, we considered the BRM effect on those genes as *DOG1*-gene dependent (Fig. 1C, D).

Transcriptomic analysis showed that both BRM and DOG1 are important players in seed biology and gene expression regulation. Our data revealed that in seeds, BRM and DOG1 control expression of a large number of genes synergistically. There is, however, a substantial subset (55 out of 244) of genes that are regulated by BRM in DOG1 dependent manner (Fig. 1C, 1D).

Gene Ontology (GO) analysis identified 20 and 30 significantly enriched GO terms among genes that were down- and up-regulated in the *brm-3* mutant, respectively. Among those, many GO terms represented molecular functions involved in response to oxidative stress, with some related to glutathione metabolism, binding and transferase activity, which are essential for the control of reactive oxygen species (ROS) (Supplementary Table 1). Single *dog1-4* mutant and *brm-3dog1-4* double mutant showed similar GO term profiles. Those included response to multiple factors, like stimulus or ABA for down-regulated genes (Supplementary Fig. S2A), as well as GO terms related to translation – for up-regulated genes (Supplementary Table 1).

Next, we analyzed the metabolic profiles of *brm-3, dog1-4* and *brm-3dog1-4* double mutant mature seeds using a non-targeted comparative metabolomics approach based on high-resolution mass spectrometry. Molecular features detected in each single mutant and in the double mutant *brm-3dog1-4* were compared to Col-0 wild type (WT). This identified a total of 410, 112 and 418 differentially abundant molecular features (metabolites) in *brm-3*/WT, *dog1-4*/WT and *brm-3dog1-4*/WT comparisons, respectively (Wilcoxon rank sum test, fold change≥2; p-value<0.05, Supplementary Fig. S1). Among them, 223 were more abundant and 187 were less abundant in *brm-3* in comparison to Col-0 WT. In *dog1-4* mutant, 30 were more and 82 less abundant when compared to WT. Finally, in the double mutant, 254 metabolites were more, and 164 were less abundant when compared to Col-0 WT, indicating a significant metabolic remodeling in *brm-3* and *brm-3dog1-4* mutants. Assigned masses allowed us to identify a putative molecular formula for 170 out of 410 differentially expressed metabolites for *brm-3*/WT, 37 out of 112 for *dog1-4/WT* and 143 out of 418 compounds for double mutant (Supplementary Table 1). To visualize the metabolic changes between mutants and the Col-0 WT, we used a chemical enrichment analysis named ChemRICH (Chemical Similarity Enrichment Analysis for Metabolites) according to Barupal and Fiehn, 2017, which provides differentially enriched clusters of metabolites families. Such clusters were identified for each mutant separately (Fig. 1E and

Supplementary Table 1). For *brm-3*, we detected 26 differentially abundant metabolic families (p-value<0.05), with a decrease in compounds such as phosphatidylcholine, phosphatidylethanolamine, aldehydes, flavonoids and glucosinolates and an enrichment of indoles, polyunsaturated alkamides, phenols, unsaturated fatty acids and amides (Fig. 1E). Interestingly, a 1.6 FC (p-value=0.0012) enrichment in glutathione was found in *brm-3* mutant compared to Col-0 WT (Fig. 1F, Supplementary Table 1). Similarly, in the seeds of the *dog1-4* mutant metabolomic analysis revealed decrease in flavonoids and peptides levels and an enrichment in phenols and glucosides (Fig. 1E). Interestingly, in *dog1-4* mutant we found a -2.08 FC decrease (p-value=0.0059) in glutathione enrichment compared to Col-0 (Fig. 1E, 1F). In the double mutant *brm-3dog1-4,* we found a broad variety of metabolic families up or downregulated compared to Col-0 WT. We noticed an enrichment in phospholipids ethers, phenols, saturated and unsaturated lysophosphatidylcholine, lactones, saturated fatty acids and indoles; and lower levels of phosphatidylcholine, phosphatydylethanolamine, glucosinolates, flavonoids and glucosides (Supplementary Table 1). Interestingly, as observed for the single mutants, the double mutant glutathione was downregulated compared to the wild type (Fig. 1E, 1F, Supplementary Table 1), with a FC of –1.85 (p-value=0.00058). We conclude that similarly to RNA-seq analysis, metabolomic analysis shows that *dog1* enhances metabolic changes in the *brm-3* background as many changes are only visible or more pronounced in the double *brm-3dog1-4* mutant. In addition to untargeted analysis of metabolites, a targeted analysis of soluble sugars and hormones was performed in the mature seeds of the mutants. This analysis showed that ABA levels were slightly reduced in *brm-3* and *dog1-4* mutant seeds while double mutant seeds showed intermediate levels of ABA, with no significant difference to either Col-0 or single mutants (Supplementary Fig. S3A). The small effect on ABA suggests that phenotypic defects observed in *brm-3* and double mutant are probably not driven through ABA. Similarly, analysis of GA levels in dry seeds showed no significant difference for *brm-3* seeds and higher but not significant levels for *dog1-4* and double mutant seeds (Supplementary Fig. S3B). Given the published role of sugars in seed maturation, we also analyzed levels of sucrose, raffinose and stachyose (Li *et al.*, 2017; Salvi *et al.*, 2022). Seeds of *brm-3* and *brm-3dog1-4* contained significantly higher levels of raffinose and lower levels of sucrose, resulting in increased RFO/sucrose ratio (Supplementary Fig. S4A, 4B, 4C). Likewise, stachyose levels were also lower in seeds of the *brm-3* single and *brm-3dog1-4* double mutant (Supplementary Fig. S4D). In contrast, seeds of *dog1-4* mutant did not show changes in sugar levels. These results suggest that BRM is implicated in sugar level control in seeds but independently of DOG1 (Supplementary Fig. S4).

In summary, our metabolomic analysis revealed that *brm-3* and *dog1-4* share some common differentially enriched compounds compared to the wild type, while much more diverse families of metabolites are differentially enriched in the double mutant, confirming the synergistic action of BRM and DOG1 in seeds biology (Supplementary Fig. S1, Supplementary Table 1). Interestingly, our data suggest the glutathione level is controlled by BRM in a DOG1 dependent manner, as it was increased in *brm-3* mutant while in *dog1-4* and *brm-3dog1-4* double mutant glutathione was less abundant compared to Col-0 WT seeds (Fig. 1F, 1E, Supplementary Fig. S5).

BRM regulates seed quality and physiology

To assess the BRM and DOG1 effect on seed quality, we first checked the seed size in single and double mutants (Fig. 2A, B and Supplementary Fig. S6A). The *brm-3* mutant seeds were 20% bigger compared to the wild type and the *dog1-4* mutant, with an average size of 0.27, 0.21 and 0.23 mm^2 for *brm-3*, *dog1-4* and the wild type, respectively (Fig. 2B, Supplementary Fig. S6A). The *brm-3* mutant seeds were also 25% heavier (p-value < 0.00095) compared to the wild type. In addition, even though *brm-3dog1-4* seeds were not significantly larger than Col-0 WT seeds, double mutant seeds were significantly heavier than Col-0 seeds (Supplementary Fig. S6D). In addition, germination rate of *brm3* and *brm3dog1-4* double mutants in the presence of ABA was similar to Col-0 WT seeds (Supplementary Fig. S6C). As previously reported for *brm-3* (Farrona et al., 2007), *brm-3* and *brm-3dog1-4* mutants had significantly reduced seed yield while *dog1-4* mutant did not show a difference compared to the wild type (Fig. 2C). Our data suggest that seed quality measured by seed size, weight but not yield is affected by BRM in DOG1 dependent manner.

Glutathione is one of the main antioxidants in seeds and its level decreases during seed ageing (Ranganathan & Groot, 2023). Our metabolomic analysis showed changes in glutathione levels prompting us to analyze the role of BRM in longevity. Seeds of *brm-3* mutant showed increased longevity compared to the wild type (Supplementary Fig. S6B), as seen in the analysis of the time required for 50% loss of viability (P_{50}, Fig. 2D). As published by Dekkers et al. 2016, we observed that the P_{50} of the *dog1-4* mutant was lower compared to the wild type, demonstrating a role of DOG1 in enhancing longevity. Interestingly, the P_{50} of the double mutant *brm-3dog1-4* was only slightly higher than for the single *dog1-4* mutant. In addition, we performed a germination analysis of 4-year-old naturally aged seeds of the tested mutants. This analysis revealed a germination phenotype similar to that observed in artificially aged seeds: the *brm3*

mutant showed significantly higher germination rates compared to Col-0 WT seeds, but this effect was suppressed in the *brm3dog1-4* double mutant (Supplementary Fig. S6E). This suggests that BRM role in seed longevity is partially DOG1 dependent.

As we found BRM affects seed longevity, we were further interested in its effect on seed's vigor. We analyzed salt sensitivity during germination of after-ripened seeds (Fig. 2E). After-ripened seeds of *brm-3* showed no difference in germination in presence of 100mM NaCl compared to Col-0. In contrast to what has been observed for freshly harvested stratified seeds, the *dog1-4* mutant showed reduced germination when compared to Col-0 WT (Montez *et al.*, 2023). The *brm-3dog1-4* double mutant behaved like the *dog1-4* single mutant suggesting that BRM is not involved in salt-mediated germination delay (Fig. 2E). Given the role of DOG1 in dormancy, we performed primary and secondary dormancy tests on the mutants. Primary dormancy was tested by germination of freshly harvested seeds and secondary dormancy was analyzed on seeds pre-treated with high temperature in the darkness (Footitt *et al.*, 2015; Krzyszton *et al.*, 2022). In agreement with published results (Krzyszton *et al.*, 2022), we observed that the *DOG1* gene is required for primary as well as secondary dormancy, as the *dog1-4* mutant showed nearly 100% germination of both freshly harvested seeds and seeds induced into secondary dormancy. In contrast, the *brm-3* mutant displayed a stronger secondary but unaffected primary dormancy when compared to Col-0 WT seeds (Fig 2F, G), suggesting a specific function of BRM in secondary dormancy regulation. Likewise for *brm-3*, stronger secondary seed dormancy was also observed in *brm-5*, *3xbrd* and *swp73a*, other *SWI/SNF* subunit mutants (Supplementary Fig. S7, S8A).

In summary, we showed that BRM is an important regulator of seed biology required for many aspects of seed development and environmental sensing. Our data reveals a genetic requirement of the functional *DOG1* gene for BRM-mediated control of seed longevity and secondary dormancy. The lack of primary seed dormancy defects and stronger secondary seed dormancy observed in *brm-3* and *brm-5* mutants is surprising, as primary and secondary dormancy levels are usually correlated in Arabidopsis mutants (Buijs , 2020; Sajeev *et al.*, 2024).

BRM binds to the *DOG1* 3' region and regulates *asDOG1* antisense expression

Our genetic analyses suggested that BRM-mediated regulation of secondary dormancy requires the *DOG1* gene. Previously, it has been shown that the *DOG1* gene 3' end is bound by BRM and BRD in seedlings (Yu *et al.*, 2020, Yu *et al*., 2021, Archacki *et al.*, 2016, Supplementary Fig.

S9A, S9B). To confirm BRM binding at the *DOG1* locus in seeds, we performed ChIP-qPCR experiment using a transgenic line expressing BRM-GFP under a native promoter in the background of *brm-1* null mutant (Jarończyk *et al.*, 2021). We observed BRM binding mostly within exon 2 and exon 3 of the *DOG1* gene, matching the location of previously described by us antisense lncRNA *DOG1* promoter (Fig. 3A). This suggests that BRM could control the *DOG1* gene expression in seeds via as*DOG1* (Fedak *et al.*, 2016).

In non-dormant dry seeds, BRM was bound at exon 2 and exon 3 regions (*asDOG1* promoter), similar to genome-wide ChIP data from Arabidopsis seedlings (Fig. 3B, Supplementary Fig. S9). During SD induction, BRM was predominantly bound at exon 3 (Fig. 3B). This suggests that secondary dormancy induction may lead to changes in the way BRM controls *DOG1* sense and antisense expression. This is in agreement with our phenotypic analysis (Fig. 2G, Supplementary Fig. S7) that demonstrated that *brm-3* seeds show an enhanced propensity to enter secondary dormancy while seeds collected from *brm-3dog1-4* double mutant are unable to enter dormancy.

Next, we performed RT-qPCR analysis of *DOG1* gene expression in the Col-0 WT and selected mutants during secondary dormancy induction. Upon imbibition at 4 hours, we observed a strong *DOG1* mRNA reduction followed by a gradual rebuild of *DOG1* mRNA levels during secondary dormancy induction (Fig. 3C). This is in agreement with previously published by us and others' results (Buijs G. 2020; Krzyszton et al. 2022; Sajeev et al. 2024). Interestingly, we observed a much stronger increase in *DOG1* mRNA levels in *brm-3* mutant (Fig 3C). To test if the observed BRM role in *DOG1* expression regulation during secondary dormancy induction requires activity of the whole BAS complex, we used a single *swp73a* and a triple *BRD1/2/13* (*3xbrd*) mutant that both are components of BAS SWI/SNF complex (Guo *et al.*, 2022; Fu *et al.*, 2023). We observed hyper activation of *DOG1* expression in *3xbrd* and *swp73a* mutant seeds (Fig. 3C, Supplementary Fig. S8B) that was very similar to the one observed in *brm-3*. We also note a stronger dormancy phenotype during secondary dormancy induction for *3xbrd* and *swp73a* mutants (Supplementary Fig. S7, S8A). The observed upregulation of *DOG1* expression in the *brm-3, swp73a* and *3xbrd* mutants suggests a previously unrecognized role for BRM and the SWI/SNF BAS-complex in the suppression of *DOG1* expression during secondary dormancy establishment. Gene expression analysis showed increase in the mRNA levels of the *BRM* and *SWP73A* genes but not for *BRD1*, suggesting that the main SWI/SNF subunits are co-induced with *DOG1* gene during secondary dormancy establishment. However, BRM expression was not affected in *dog1-4* mutant compared to Col-0 WT seeds during induction (Supplementary Fig.

S10A). Thus, our genetic and RT-qPCR analyses suggested that BRM function upstream of the DOG1 gene.

In parallel to sense transcript expression during secondary dormancy induction, we analyzed *asDOG1* transcript levels. Similarly, to sense transcript expression, we observed a gradual accumulation of antisense transcript during dormancy induction (Fig. 3D). All *brm-3, swp73a* and 3x*brd* mutants showed a clear reduction in the levels of *asDOG1* expression when compared to the Col-0 seeds at later stages of dormancy induction (Fig. 3D, Supplementary Fig. S9C). Together with BRM binding at *DOG1* 3'end this suggests that BRM directly regulates *asDOG1* transcription. To test this possibility, we used the p_{AS}*DOG1*promoter-driven IRES-LUC reporter line and crossed it with the *brm-3* mutant. RT-qPCR using LUC primers showed significant downregulation of *LUC* transcript in *brm-3*, suggesting that BRM directly regulates *asDOG1* promoter activity (Fig. 3F).

BRM regulation of *DOG1* gene expression requires *asDOG1*

Observed by us direct regulation of *DOG1* antisense expression by BRM and the lack of BRM binding to canonical *DOG1* promoter suggested a model where BRM regulates *DOG1* sense expression through *DOG1* antisense. To test this model, we first asked if BRM can regulate *DOG1* expression in the absence of as*DOG1*. We used a previously published (Fedak *et al.*, 2016) transgenic truncated *DOG1* gene ($_{pDOG1}$*shDOG1::LUC*) with an antisense promoter deleted and crossed it to a *brm-3* mutant (Fig. 3E). Importantly, no significant differences were observed between Col-0 and *brm-3* suggesting that BRM is unable to regulate the *DOG1* gene expression when the *DOG1* 3' region is removed. Surprisingly, p_{DOG1}*shDOG1::LUC* did not show the induction of expression that we observed for endogenous sense mRNA.

Therefore, the inability of BRM to regulate *DOG1* gene with antisense deleted (p_{sense}*DOG1-LUC*), together with the fact that in *brm-3* mutant p_{AS}*DOG1* activity is suppressed, suggest that BRM acts through antisense. The fact that p_{sense}*DOG1-LUC* transgene did not recapitulate endogenous *DOG1* sense expression inductions suggest that some of the elements required for *DOG1* induction upon secondary dormancy induction are located in regions deleted in the construct.

To test this possibility, we took advantage of a set of TATA mutations shown by us previously to greatly reduce *asDOG1* transcript expression (Yatusevich *et al.*, 2017). We engineered these mutations in an antisense promotor of the *DOG1* genomic reporter construct creating *LUC-*

DOG1-deltaTATA transgenic lines. RT-qPCR analysis using primers that amplify only transgene-originating *DOG1* mRNA showed that removal of antisense transcription indeed resulted in strong upregulation of *DOG1* sense transcript at early stages of dormancy induction (Fig. 3G, Supplementary Fig. S11A, S11B). Together the transgenes analysis shows that BRM requires *asDOG1* to control *DOG1* expression during secondary dormancy induction and that *asDOG1* acts as negative regulator of *DOG1* expression during secondary dormancy establishment. Similar to *brm-3* and *brm-5* mutants, transgenic *LUC-DOG1* lines carrying dTATA mutations showed stronger secondary seed dormancy compared to WT *LUC-DOG1* lines, confirming *asDOG1* acts as a negative regulator of *DOG1* expression during secondary dormancy (Supplementary Fig. S11C).

BRM is part of the SWI/SNF complex and our data show that both BRM and another SWI/SNF complex subunits - BRDs are implicated in seed secondary dormancy control through *asDOG1* antisense transcript promoter regulation. SWI/SNF is a chromatin remodeling complex that utilizes ATP to remodel chromatin at target loci (Mashtalir *et al.*, 2018). To test if BRM-mediated regulation of *DOG1* sense expression through *asDOG1* is accompanied by DNA accessibility changes, we performed FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) during secondary dormancy induction (Omidbakhshfard *et al.*, 2014). On the third day of secondary dormancy induction, *brm-3* showed a marked increase in DNA accessibility using FAIRE at the end of exon 2 compared to dry seeds (Fig. 4A, 4B). This increase was even more pronounced in the last intron and exon 3 of the *DOG1* region on the 5th day of induction, centering around the *DOG1* antisense promoter region (Supplementary Fig. S8D). This region colocalized with the BRM binding site identified in ChIP experiments (Fig. 3A, B; Fig. 4A, B; Supplementary Fig. S8C, S8D). In agreement with BRM involvement in *DOG1* regulation during secondary dormancy, the FAIRE experiment in dry seeds failed to detect a localized increase at *DOG1* 3' end in *brm-3* compared to Col-0 WT dry seeds (Fig. 4A). Thus, this suggests that during secondary dormancy induction, BRM is directly bound at *DOG1* 3' end and locally remodels chromatin presumably to regulate *asDOG1* expression. This is consistent with the *asDOG1* requirement for BRM's ability to suppress *DOG1* gene expression during secondary dormancy induction.

BRM affects *DOG1* splicing and polyadenylation

To gain a deeper understanding of *DOG1* regulation by BRM, we analyzed the misregulation of *DOG1* splicing and polyadenylation in *bmr-3* seeds during the induction of secondary dormancy.

Our RT-qPCR analysis examined early time points (2- and 4-days post-induction) for splicing and up to 14 days post-induction for polyadenylation. We detected increased levels of the short proximally polyadenylated *DOG1* mRNA (*shDOG1*), but not the long form (*lgDOG1*), during secondary dormancy induction in *brm-3* (Fig. 4C, 4D). We also found a significant increase in *β* (beta) and *γ* (gamma) *DOG1* mRNA splicing forms in *brm3* mutants compared to Col-0 seeds (Fig. 4E-H). Increased *β*, *γ* and *shDOG1* were not only observed in *brm-3* but also in *3xbrd* mutant, suggesting that they are a result of BRM activity linked to SWI/SNF complex. The increase in *β*, *γ* and *shDOG1* mRNA is consistent with the observed stronger *brm-3* mutant secondary seed dormancy phenotype as *shDOG1* has been reported to be the predominant *DOG1* isoform that can complement the *DOG1* mutant phenotype and *β* and *γ* mRNA isoforms lead to production of the same protein as encoded by *shDOG1*. Interestingly, in the *ntr1* mutant, known as a spliceosome disassembly factor (Dolata J *et al.*, 2015), we observed a significant reduction in *αDOG1* mRNA splicing forms, while other *β*, *γ* and δ splicing forms showed similar kinetics to one observed in Col-0 WT (Supplementary Fig. S15). The differential effect of *brm-3* and *ntr1* on *DOG1* splicing suggest that BRM does not control *DOG1* splicing through NTR1.

DISCUSSION

BRM containing SWI/SNF complex controls seed physiological quality

We show that *brm-3* mutant exhibit multiple seed-related phenotypes, including enlarged seed size, reduced seed yield, increased seed longevity and enhanced secondary dormancy induction but no change in primary dormancy or germination in the presence of salt (Fig. 2, Supplementary Fig. S14). Given the central role of the *DOG1* gene in seed biology as well as BRM binding to *DOG1* locus, we tested the interplay of *BRM* and *DOG1* genes in seeds. We found that some of the *brm-3* mutant phenotypes are genetically dependent on *DOG1* gene as a double mutant of *brm-3dog1-4* shows a reversal of the longevity and secondary dormancy phenotypes (Fig. 2). Transcriptomic analysis in dry seeds showed that around 20% of genes misregulated in *brm-3* are *DOG1* gene-dependent as *dog1-4* mutation can partially or fully suppress the *brm* mutation effect on their expression in the *brm-3dog1-4* double mutant (Fig. 1D). In addition, we observed a pronounced misregulation of gene expression in the double *brm-3dog1-4* double mutant (Fig. 1A) which suggests a synergistic function in the case of the majority of affected genes.

Longevity and metabolite accumulation

GO terms analysis among differentially regulated genes suggested changes in genes involved in the biosynthesis of metabolites known to be important in seed biology (Supplementary Table 1) that were mostly consistent with changes observed in seed metabolome analysis (Supplementary Fig. S1A, 1B). RNA-seq data showed that genes related to glutathione metabolism were enriched among genes downregulated in *brm-3dog1-4* double and *dog1-4* single mutant and upregulated in single *brm-3* mutant (Supplementary Fig. S2, S5). This is consistent with our metabolomic analysis that showed an increase in *brm-3* and lower levels of glutathione in *dog1-4* and *brm-3dog1-4* mutants (Fig. 1F, Supplementary Fig. S5). Given the published link between glutathione level, dormancy and longevity in Arabidopsis (Cairns *et al.*, 2006; Nguyen *et al.*, 2015; Koramutla *et al.*, 2021), we note that the observed changes in glutathione level are probably responsible for the observed *DOG1* gene-dependent partial increase in longevity of *brm-3* mutant. This observation is corroborated by the suggested previously positive role of *DOG1* in longevity based on analysis of natural variation and *DOG1* NIL line analysis (Nguyen *et al.*, 2012).

Changes in soluble sugar contents have been suggested to be involved in germination and longevity regulation (He *et al.*, 2016). In legume species, a correlation between lower seed storability and a lower ratio between RFO and sucrose has been reported (Pereira Lima et al., 2017). While in Arabidopsis, this correlation remains unclear, as an increase in the RFO/sucrose ratio was not found to be correlated with seed vigor (Bentsink *et al.*, 2000; Li *et al.*, 2017). We found a significant decrease in RFO/sucrose ratio in both single *dog1-4*, *brm-3* and double mutants (Supplementary Fig. S4B). However, there is no significant difference in this ratio between *dog1-4* and *brm-3* mutants, despite the difference in seed longevity between them. Thus, our data suggest that in Arabidopsis, there might be no direct link between RFO and seed longevity. However, it is interesting to further investigate galactinol contents as it has been linked to seed biology and has not been measured by us (De Souza Vidigal *et al.*, 2016).

In addition, in dry *brm-3* mutant seeds, we observed significant alterations in various classes of tryptophan-derived metabolites, including auxin, camalexin, and indole-glucosinolates. The deregulation of genes involved in the tryptophan-derived metabolite pathways (such as *MYB34*, *MYB51*, MYB122, *NITs*, *ARFs*, *CYP79B3* etc.) in the *brm* mutant may contribute to the observed auxin-related phenotypes and reduced seed yield of *brm-3* (Supplementary Fig. S2B). While camalexin and indole-glucosinolates are recognized as plant defensive secondary compounds against pathogens and herbivores (Stotz *et al.*, 2011; Nguyen *et al.*, 2020), their specific biological roles in seeds dormancy remain to be fully elucidated.

The role of BRM in glutathione accumulation in seed is *DOG1*-dependent

Glutathione is an important player in redox signaling and is involved in protection against excessive oxidation in multiple plant tissues (Mhamdi *et al.*, 2010). Accumulation of oxidative damage during dry seed storage is probably the most important factor behind deterioration of seed quality and eventually loss of viability determining seed longevity (Kumar *et al.*, 2015).Our transcriptomic analysis showed that genes related to glutathione metabolism were misregulated in *brm*-3 mutant in the opposite direction to changes observed in *dog1-4* mutant, including *GPX1* and *GPX6* that are responsible for glutathione biosynthesis. We also observed multiple other misregulated genes in different pathways related to glutathione synthesis, degradation and recycling. Genes coding for GLUTATHIONE S-TRANSFERASEs: GSTU9, GSTU10, GSTU11, GSTU12 and GSTU19 were significantly up-regulated (FDR < 0.05, FC > 2) in mature seeds of *brm-3* mutant compared to Col-0 WT seeds (Fig. 1F, Supplementary Fig. S2, S5). No consistent change of the transcript levels of these genes were observed in *dog1-4* seeds.

In agreement with the observed deregulation of glutathione-related transcripts, we observed a higher level of glutathione in the *brm-3* mutant, and depletion in *dog1-4* and double mutant compared to Col-0. Those results are concordant with the reduced longevity in both *dog1-4* and double mutant, and the increased longevity of the *brm-3* mutant.

ABA and GA hormonal signaling in *brm-3* mutant seeds during SD

Our analysis of hormone levels in dry seeds revealed relatively minor differences in the single mutants *brm-3* and *dog1-4* regarding GA and auxin content (Supplementary Fig. S3B, S3C). Interestingly, we observed a significantly elevated auxin level in the *brm-3 dog1-4* double mutant (Supplementary Fig. S2C), correlating with increased expression of auxin pathway genes (Supplementary Fig. S7B). A slight reduction in ABA levels was detected in *dog1-4* and slightly in the double mutant (Supplementary Fig. S3A). Members of the SWITCH2 (SWI2)/SNF2 chromatin-remodeling complexes play a role in seed germination under ABA treatment. BRAHMA (BRM) directly suppresses the expression of ABI5 and, consequently, the *brm-3* mutant exhibits increased ABA sensitivity during seed germination (Han *et al.*, 2012). The role of ABA and its signaling pathway in seed biology has been extensively studied, including its role in secondary seed dormancy establishment (Auge et al., 2015; Ibarra et al., 2016).

In agreement with published results, we have previously reported that quadruple *nced2469* mutant failed to enter into secondary dormancy (Lefebvre *et al.*, 2006; Krzyszton *et al.*, 2022). Here, we show that *nced2469* shows no defect in *DOG1* expression during secondary dormancy

induction when compared to Col-0 seeds (Supplementary Fig.S10B). Also, analysis of the expression of genes related to ABA biosynthesis and catabolism (*NCED4/5* and *CYP707A2*) showed only minor fluctuations during secondary dormancy induction in Col-0 seeds and no major differences when compared to *brm-3* or *dog1-4* mutants (Supplementary Fig. S12A, S13A). In contrast, we observed a strong induction of *RGL1*, *RGL2* and *GAI* genes - known negative regulators of the GA pathway, during secondary dormancy induction. Interestingly *RGL1* and *RGL2* but not *GAI* showed strong upregulation in *brm-3* mutant when compared to WT seeds during dormancy induction (Supplementary Fig. S12B). In contrast, in *dog1-4* mutant only *RGL1* and *GAI* genes were induced (Supplementary Fig. S13B). While we did not analyze the levels of gibberellins during secondary dormancy induction, this may suggest a role of GA catabolism genes rather than ABA in enhanced secondary dormancy induction in *brm-3* (Ibarra *et al.*, 2016; Chang *et al.*, 2018) and required functional *DOG1*.

BRM controls secondary seed dormancy through *DOG1* antisense

In agreement with the genetic interplay between *BRM* and *DOG1* genes, we detected direct BRM binding to exon 2 and exon 3 of the *DOG1* gene in dry seeds. Interestingly, during secondary dormancy induction, BRM binding increased towards the 3' end of the *DOG1* locus (Fig. 3A, B). Together with the observed deregulation of the *DOG1* gene expression and changes in chromatin accessibility, this suggests that BRM controls secondary dormancy directly through *DOG1*. Primary and secondary dormancy are intrinsically linked and multiple factors including DOG1, AFP2, ABI5 and ABI3 have been shown to affect both primary and secondary dormancy (Han *et al.*, 2012; Ibarra *et al.*, 2016; Chang *et al.*, 2018). Here, we show that BRM is specifically implicated in secondary but not primary dormancy control. To the best of our knowledge, this is the first example of where a factor is required only for secondary but not primary seed dormancy.

DOG1 is a known positive regulator of dormancy (Bentsink *et al.*, 2006). Here we show that during secondary dormancy induction, sense *DOG1* transcript is induced (Fig. 3C). This is in agreement with published by us and others requirement of functional *DOG1* gene for secondary dormancy establishment (Ibarra *et al.*, 2016; Krzyszton *et al.*, 2022; Sajeev *et al.*, 2024). Our data show that in *brm-3* seeds *DOG1* transcript is upregulated while *asDOG1* is downregulated during secondary dormancy induction, when compared to Col-0 (Fig. 3C, 3D). We also observe BRM binding to *DOG1* 3'end region, and that BRM regulates *asDOG1* but not *DOG1* sense promoter activity during secondary dormancy establishment (Fig. 3). Supporting a direct role of BRM in control of *asDOG1* we observed increased DNA accessibility at *asDOG1* promoter region of the

DOG1 locus in *brm-3* mutant during secondary dormancy establishment (Fig. 4A). Interestingly, BRM binds to the 3' end region of selected differentially expressed genes (DEGs) involved in ABA and GA pathways (such as *RGL3*, *NCED4*, *CYP707A1* etc.) (Supplementary Table 2).

Previous research has shown that *asDOG1* is a negative regulator of *DOG1* expression, as its deletion results in high *DOG1* expression (Fedak *et al.*, 2016, Yatusevich et al., 2017). BRM appears to positively control antisense, thus also negatively regulating *DOG1* gene expression (Fig. 4, 5). The mechanism of *DOG1* silencing by its antisense, aka *1GOD*, is not yet fully understood. We hypothesize that during secondary dormancy establishment, BRM indirectly limits *DOG1* induction by enhancing *asDOG1* expression (Fig. 3D). In agreement, we show that mutation of TATA boxes located in the antisense promoter region resulted in much stronger *DOG1* upregulation compared to not mutated *DOG1* transgene and, in agreement, enhanced secondary dormancy phenotype in seeds. Our model suggests that observed by us in Col-0 upregulation of *asDOG1* during secondary dormancy induction serves to limit *DOG1* induction attenuating dormancy strength. In contrast to BRM function in secondary dormancy, both RNA-seq and RT-qPCR showed no major differences in *DOG1* sense and antisense transcripts levels between Col-0 and *brm-3* mutant in dry seeds. This agrees with the observed lack of primary dormancy defects in *brm-3* as well as in *3xbrd and swp73a* mutants (Fig. 2F, Supplementary Fig. S14).

Notably, the introduction of triple *3xbrd* mutation into the *brm-1* knockout mutant background did not enhance the *brm-1* phenotype, confirming the conclusion that BRD subunits operate within the same complex as BRM (Stachula *et al.*, 2023). Also, here we show that *3xbrd* and *swp73a* display similar *DOG1* expression changes to *brm-3* mutant consistent with BRM operating as part of BAS SWI/SNF complex in controlling *DOG1* expression (Fig. 3, Supplementary Fig. S9).

Thus, we propose a model where BRM-containing the SWI/SNF complex binds to the *DOG1* 3'end region and in response to secondary dormancy-inducing conditions remodels nucleosomes which activate *asDOG1* antisense promoter (Fig 5). This leads to *asDOG1* transcript expression that limits the activation of *DOG1* and subsequently to a strong dormancy establishment. Surprisingly, analysis of p_{sense}*DOG1-LUC* transgene that lacks a function *DOG1* antisense transcript in seeds showed that *DOG1* 5'region is insufficient to support *DOG1* expression upregulation in response to secondary dormancy induction. This suggests that in addition to *DOG1* antisense that limits the full activation of *DOG1* expression, the *DOG1* 3'end region

contains unknown positive regulators responsible for secondary dormancy-mediated *DOG1* expression induction.

Analysis of *DOG1* splicing and polyadenylation during secondary seed dormancy induction in Col-0 WT, *brm3* showed increased levels of β and γ alternatively spliced mRNA as well as increased levels of *shDOG1* resulting from selection of proximal termination site (Fig. 4F, 4G). Whereas in the *ntr1* mutant, we observed predominantly changes in α and not significantly in β *DOG1* mRNA splicing forms compared to Col-0 WT seeds (Supplementary Fig. S15). Given that our work implicated NTR1 in splicing control through Pol II speed on *DOG1* we speculate that defects observed in *brm-3* are unlikely to results from NTR1 dependent splicing defects or direct Pol II speed regulation. One possibility is that changes in *DOG1* splicing and termination site selection observed in *brm-3* and *3xbrd* result from defects in antisense expression in these mutants. The proximity of *DOG1* alternative splice sites - proximal termination site and antisense promoter make deletional confirmation of this hypothesis difficult, if possible. We, however, showed that BRM regulates the *DOG1* antisense promoter in seeds in the absence of sense promoter driving *DOG1* alternative splicing or proximal termination site selection.

In summary, our work explores the functions of the BRM-containing SWI/SNF complex in seed biology. We observe that BRM is required for multiple aspects of seed physiology as underpinned by metabolomic and transcriptomic analysis. We show that BRM controls some of the seed-related phenotypes including longevity and secondary dormancy regulation through the *DOG1* gene. Our analysis demonstrates that in response to environmental signals triggering secondary dormancy induction BRM containing SWI/SNF complex controls *DOG1* expression through *DOG1* antisense.

Materials and Methods

Plant Material and Growth Conditions

Arabidopsis thaliana plants were grown in pots with mixed coconut and normal soil in a greenhouse with a long-day photoperiod (16-h light/8-h dark) at 22°C/18°C. After harvest, seeds were stored in paper bags at room temperature. Ecotype Col-0 plants were used as a wild type (WT). The *brm-3* (SALK_088462) and *dog1- 4* (SM_3_20886) mutants were obtained from the Nottingham Arabidopsis Stock Centre (NASC) and are described in (Farrona et al., 2007; Fedak et al., 2016). Transgenic reporter lines: genomic *LUC-DOG1dTATA*, *asDOG1dTATA-LUC*,

senseDOG1-LUC, *asDOG1-LUC*, and g*enomicLUC-DOG1* were generated and characterized previously (Fedak et al., 2016; Yatusevich et al., 2017). The *brm-5*, *swp73a* (SM_3_30546), *3xbrd, ntr-1, 4xnced* and *brm-1*/*BRM-GFP* lines were described previously (Tang et al., 2008; Dolata *et al.*, 2015; Sacharowski *et al.*, 2015; Jarończyk *et al.*, 2021; Krzyszton et al., 2022). The double mutants: *brm-3dog1-4*; *brm-3p$_{sense}$DOG1-LUC*; *brm-3p$_{AS}$DOG1-LUC*; *brm-3genomic LUC-DOG1dTATA*; *brm-3p$_{AS}$DOG1dTATA-LUC* were generated by crossings and homozygous plants were identified using *brm-3* T-DNA insertion primers described in Jarończyk et al., 2021.

For salt stress, sterilized after-ripened seeds were sown on agar plates supplemented with 100mM NaCl. After sowing, plates were taped, wrapped with silver foil, and kept for stratification for 3 days at 4°C. Then plates were unwrapped from the silver foil and transferred to the growth chamber under long-day conditions (16h light/8h dark) to check the germination rate (Montez *et al.*, 2023).

Seed Longevity Measurement

To perform artificial ageing, mature post-harvested *Arabidopsis* seeds were stored in the darkness at 35ºC in hermetically closed containers with saturated NaCl (75% of relative humidity). 50 seeds per each biological replica were imbibed on blue paper (Anchor) plates after different storage times (0, 7, 14, 21, 28, 35, 42, 49, 56 days) and placed in a phytotron at 22ºC/long day photoperiod. The final germination percentage was counted after 10 days. P50 was determined as the time after which seeds lost 50% of their germination capacity (Zinsmeister *et al.*, 2020).

Seed primary dormancy assay

Freshly harvested seeds were sown on plates with water-soaked blue paper (Anchor) and sealed with tape. Plates with the seeds were put in the growth chamber under long-day conditions (16 hours light/8 hours dark) at 22°C/18°C. The germination rate was scored every day until 100% germination was observed. Control plates were initially stratified for 3 days at 4°C to break seed dormancy and to ensure that the seeds were not dead.

Seed secondary dormancy assay

Seeds stored for at least 3 months that showed full loss of primary dormancy were used for secondary dormancy induction. Seeds were sown on water-soaked blue paper plates, sealed and kept in the dark. Plates were incubated at 30ºC for 4 hours, 1, 3, 5, 7, 10 or 14 days. After high incubation, the plates were transferred to the growth chamber at 22ºC under long-day conditions.

599 Seed germination was assayed after 4 and 7 days. The control plates were placed in a phytotron
600 immediately after sowing the seeds, without a dormancy induction.

601 **RNA extraction, cDNA synthesis and RT–qPCR analysis**

602 RNA extraction from seeds was performed using the phenol-chloroform protocol. The frozen
603 seeds were ground to a powder using an electric drill and then mixed with 600 µl of RNA
604 extraction buffer (100 mM Tris pH 8.5, 5 mM EDTA pH 8.0, 100 mM NaCl, 0.5% SDS, 1% β-
605 mercaptoethanol). Afterwards, samples were centrifuged for 5 min at 14,000 g at 4°C. The
606 supernatant was transferred to new tubes and 250 µl of chloroform was added and samples were
607 shaken at room temperature (RT) for 15 min. Then 250 µl of phenol was added and samples were
608 shaken for a further 15 min and centrifuged for 10 min at 14,000 g at 4°C. Then, 550 µl of the
609 aqueous layer was transferred to new tubes and mixed with 550 µl of phenol–chloroform–
610 isoamyl alcohol 25:24:1. Samples were shaken for 10 min at RT and centrifuged for 10 min at
611 14,000 g at 4°C. Then, 500 µl of supernatant after transferring to new tubes was mixed with 50 µl
612 of 3 M sodium acetate and 400 µl of pure ice-cold isopropanol and incubated for 15 min at RT.
613 After the incubation, samples were centrifuged for 30 min at 14,000 g at 4°C. Finally, the RNA
614 pellet was washed in 1 ml of 80% ethanol, dried and resuspended in Milli-Q water. DNase
615 treatment of RNA samples was performed using a TURBO DNA-free^{TM} Kit (ThermoFisher
616 Scientific), according to the manufacturer's protocol. DNase treatment effectiveness was checked
617 by PCR with pp2A primers. Reverse transcription of RNA was performed using a RevertAid
618 First Strand cDNA Synthesis (ThermoFisher Scientific) or RevM First Strand cDNA Synthesis
619 (KleverLab) kits according to the manufacturer's protocol. Two types of cDNA synthesis were
620 performed: using 1,000 ng of RNA and oligo(dT) primers for *DOG1* analysis and gene-specific
621 synthesis for as*DOG1* analysis using 2,500 ng of RNA and primers with overhangs as described
622 (Fedak *et al.*, 2016). qPCR was performed with SYBR Green mix and specific primers for PCR
623 amplification and with using LightCycler 480 real-time system (Roche). The sequences of all
624 primers are published previously (Cyrek *et al.*, 2016; Fedak *et al.*, 2016) and provided in the
625 Supplementary Table 2. RT–qPCR results were normalized to the expression level of the *UBC21*
626 (AT5G25760) gene.

627 **RNA-sequencing and data analysis**

628 3'RNA-seq and data analysis were performed as described previously using 500 ng of total RNA
629 as starting material (Krzyszton *et al.*, 2022).

Chromatin Immunoprecipitation (ChIP)

Chromatin was isolated from dry, non-dormant mature seeds and after 1, 3 and 5 days of secondary dormancy induction for WT and *brm-1/BRM-GFP* lines (Jarończyk *et al.*, 2021). ChIP was performed as described previously (Kowalczyk, 2017) with some modifications. The 60mg of frozen seeds were ground to a powder using a pestle and mortar and suspended in 10ml of MC buffer (0.1M sucrose; 10mM sodium phosphate pH 7; 50mM NaCl). Then 37% formaldehyde was added to the final concentration of 1% and samples were mixed softly on a rotating wheel for 10 min at 4°C. After mixing, 625µl of 2M glycine was added and samples were rotated for another 10 min. Then the samples were filtered through a double layer of Miracloth Quick Filtration Material, and centrifuged for 10 min at 1500xg at 4°C. After centrifugation, samples were resuspended in 5ml of Honda buffer (0.44M sucrose; 1.25% Ficoll; 2.5% Dextran T40; 20mM HEPES-KOH pH 7.4; 0.5M EDTA; 0.5% Triton X-100; 10mM β-Mercaptoethanol and freshly added 0.0005M PMSF and 1×Complete EDTA-free protease inhibitor) and spun for 10min at 1800xg at 4°C. The nuclear pellet was resuspended in 500µl ChIP Lysis/sonication buffer (50mM HEPES-KOH pH 7.4; 150mM NaCl; 1mM EDTA; 1% Triton X- 100; 0.8% SDS; 10mM β-Mercaptoethanol and freshly added 0.0005M PMSF and 1×Complete EDTA-free protease inhibitor) and sonicated twice 30sec-ON/30s-OFF for 25min using Bioruptor Sonication System (Diagenode). 1/10 of each sample was saved as input control and 20µl for sonication control. Sonication efficiency was verified by running de-crosslinked samples on 1% agarose gel. GFP-Trap® Agarose beads were prepared according to the manufacturer's protocol (Chromotek). The lysates of sonicated samples were added to equilibrated beads and placed on a rotating wheel in a cold room for 2-4 hours. After incubation, beads were centrifuged for 2500 x g for 5min at 4°C and washed twice for 5min with 1ml of low salt wash buffer (150mM NaCl; 1% Triton X-100; 2mM EDTA; 20mM Tris pH 8.0; 0.1% SDS). Then beads were washed for 5min with 1ml of high salt wash buffer (500mM NaCl; 1% Triton X-100; 2mM EDTA; 20mM Tris pH 8.0; 0.1% SDS) and centrifuged for 2500 x g for 5min at 4°C. Then 500 µl of phenol:chloroform:isoamyl alcohol mixture (25:24:1; pH 8.0) were added and the samples were shaken for 10 min at 22°C. After centrifugation for 10 min at 14,000 g the upper aqueous layers were collected and 0,1 volume of 3M sodium acetate (pH 5.2), 1 µl of glycogen (ThermoFisher Scientific), and 1 ml 96% ethanol were added. The mixed samples were held at -80°C for >1 hour and then centrifuged for 30 min at 14,000 g at room temperature. The pellets were washed with cold 70% ethanol, air-dried and suspended in water. For the quantification of DNA fragments, samples were tested by qPCR. The sequences of all primers are given in the Supplementary Table 2 or published by (Dolata *et al.*, 2015).

Formaldehyde-assisted isolation of regulatory DNA elements (FAIRE)

The FAIRE method was performed according to the published protocol (Omidbakhshfard *et al.*, 2014) with minor modifications. For nuclei isolation, 100 mg of the dry and secondary dormancy-induced seeds of *Col-0* and *brm-3* were used. Chromatin was sheared by sonication and the sonication efficiency was checked by electrophoresis in agarose gel as previously described for the ChIP protocol. To separate NDR (nucleosome-depleted regions), the sheared chromatin DNA was extracted by PCI (phenol:chloroform:isopropanol) method. The enrichment was assessed by qPCR using primers indicated in Supplementary Table 2. Calculations were performed using the ΔΔCt method with normalization of a crosslinked sample (FAIRE) to non-crosslinked sample (UN-FAIRE) as described in Omidbakhshfard et al., 2014 and then to *PP2A* – AT1G13320 (Supplementary Table 2) as an internal control.

METABOLOME ANALYSIS

Sample preparation

Mature post-harvested seeds (50 mg) in biological triplicates were ground with metal beads for 2x 90s on a Tissue Lyser (Qiagen) at 30 Hz in 1.5 ml of cold (-20°C) methanol spiked with an internal standard of deuterium labelled abscisic acid ($^2H_6$ ABA, 0.2µgg/ml). Samples were shaken for 10 minutes at RT, and centrifuged for 5 minutes at 13,000 rpm, RT. The supernatant was transferred to a glass vial and the extract was dried with a SpeedVac concentrator (Savant SPD121P, ThermoFisher Scientific) at RT. The pellets were extracted twice with 1.5 ml methanol, shaken, centrifuged and collected in the same glass vial to be evaporated. After this, dry samples were solubilized in 100 µl of methanol.

Non-targeted metabolites analysis

Extracts were analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) using an UltiMate 3000 UHPLC system (ThermoFisher Scientific) coupled to the ImpactII (Bruker) high-resolution Quadrupole Time-of-Flight (QTOF) mass spectrometry according to Villette et al. (2018) and Graindorge et al. (2022). Chromatographic separation was performed on an Acquity UPLC ® HSS T3 C18 column (2.1 × 100 mm, 1.8 µm, Waters) coupled to an Acquity UPLC HSS T3 C18 pre-column (2.1 × 5 mm, 1.8 µm, Waters) using a gradient of solvents A (H_20, 0.1% formic acid) and B (methanol, 0.1% formic acid). Chromatography was carried out at 35°C, at a flux of 0.3 ml min^{-1}, starting with 5% B for 2 min, reaching 100% B at 10 min, holding 100% B for 3 min and coming back to 5% B in 2 min (run time 15 min).

Samples were kept at 4 ◦C, 5 µL were injected in full loop mode with a washing step after sample injection with 150µL of wash solution (H2O/MeOH, 90/10, v/v). The spectrometer was equipped with an electrospray ionization (ESI) source and operated in positive and negative ion modes on a mass range from 20 to 1000 Da with a spectra rate of 8 Hz in Auto MS/MS fragmentation mode. The end plate offset was set at 500 V, the capillary voltage set at 2.5 kV, the nebulizer at 29 psi, the dry gas at 8 l min^{-1}, and the dry temperature of 200°C. The transfer time was set at 20–70 µs (positive mode) and 40.8–143 µs (negative mode) and the MS/MS collision energy was at 80–120% with a timing of 50–50% for both parameters. The MS/MS cycle time was set to 3 s, the absolute threshold to 816 cts, and active exclusion was used with an exclusion threshold at 3 spectra, release after 1 min, and the precursor ion was reconsidered if the ratio current intensity/previous intensity was higher than 5. A calibration segment was included at the beginning of the runs allowing the injection of a calibration solution from 0.05 to 0.25 min. The calibration solution used was a fresh mix of 50 mL isopropanol/water (50/50, v/v), 500 µL NaOH 1M, 75 µL acetic acid, and 25 µL formic acid. The spectrometer was calibrated on the [M+H]+/[M−H]−form of reference ions (57 masses from m/z 22.9892 to m/z 990.9196 in positive mode; 49 masses from m/z 44.9971 to m/z 996.8221 in negative mode) in high-precision calibration (HPC) mode with a standard deviation below 1 ppm before the injections for each polarity mode, and re-calibration of each raw data was performed after injection using the calibration segment. Molecular features were processed with MetaboScape version 4.0 (Bruker, Bremen, Germany). Molecular features were considered and grouped into buckets containing one or several adducts and isotopes from the detected ions with their retention time and MS/MS information when available. The parameters used for bucketing are a minimum intensity threshold of 10,000 (positive mode) or 1,000 (negative mode), a minimum peak length of 3 spectra, a signal-to-noise ratio (S/N) of 3, and a correlation coefficient threshold set at 0.8. The [M+H]+, [M+Na]+, [M+K]+, and [M+NH4]+ ions (positive mode); [M-H]- and [M+Cl]− ion (negative mode) were authorized as possible primary and seed ions. Replicate samples were grouped and only the features found in 80% of the samples of one group were extracted from the raw data. The obtained lists of features from positive and negative ion modes were merged. The parameters used for metabolite annotation were as follows. The maximum allowed variation on the mass (Δm/z) was set to 3 ppm, and the maximum mSigma value (assessing the good fitting of isotopic patterns) was set to 30. The merged list of features was annotated using SmartFormula to generate a raw formula based on the exact mass of the primary ions and the isotopic pattern. Analyte lists were derived from KNApSAcK (http://www.knapsackfamily.com/KNApSAcK_Family/), PlantCyc (https://plantcyc.org/),

FooDB (http://foodb.ca), LipidMaps (https://www.lipidmaps.org/), and SwissLipids (https://www.swisslipids.org/) to obtain a level 3 annotation according to Schymanski classification (tentative candidates based on exact mass and isotopic profile) (Schymanski *et al.*, 2014). Spectral libraries (Bruker MetaboBASE Personal Library 3.0, MoNA_LCMSMS_spectra, MSDIAL_LipidBDs-VS34) were searched to obtain level 2 annotations (probable structure based on library spectrum match (MS2 data) according to Schymanski et al (2014). PubChem IDs, SMILES and InChiKeys were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/idexchange/idexchange.cgi) for chemical enrichment studies using ChemRICH tool (Barupal & Fiehn, 2017).

Sugar determination

Soluble sugar contents were assessed by HPLC (Dionex) according to Rosnoblet et al. 2007. Analysis was performed on four replicates of 15 mg of mature Arabidopsis seeds. In brief, Seed were ground in a mortar in the presence of 1 ml 80% methanol containing melizitose as the internal sugar standard. After heating at 76°C for 15 min, the liquid was evaporated under vacuum. The residue was dissolved in 1 ml distilled water and centrifuged for 1 min at 13 000 g. Sugars were analyzed by HPLC on a Carbopac PA-1 column (Dionex Corp.) (Rosnoblet *et al.*, 2007).

Targeted hormone analysis

Auxin, ABA and GA were analyzed by ultra-high-performance liquid chromatography (UHPLC) on an UltiMate 3000 UHPLC system (ThermoFisher Scientific)coupled to EvoQ Elite (Bruker) mass spectrometer equipped with an electrospray ionization (ESI) source in MS/MS mode as described in (Zumsteg *et al.*, 2023).

Statistical analyses

Statistical tests were done using a Student-Newman-Keuls test or a two-tailed t-test, implemented in Microsoft Office Excel.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers listed in Supplementary Tables 1 and 2.

Supplementary Data

Supplementary Figure S1. Untargeted metabolite analysis of mature seeds of *brm3*, *dog1-4* and *brm3 dog1-4*.

Supplementary Figure S2. Heatmap showing significant and differential transcript levels of genes of selected pathways.

Supplementary Figure S3. HPLC quantification of hormones in dry mature seeds of mutants compared to Col-0 WT.

Supplementary Figure S4. UHPLC quantification of total soluble sugars in dry mature seeds of mutants compared to Col-0 WT.

Supplementary Figure S5. Glutathione biosynthesis pathway regulation by BRM is *DOG1* gene-dependent.

Supplementary Figure S5. Seed physiology is affected in brm3 mutants compared to wild type.

Supplementary Figure S6. Secondary seed dormancy phenotype of the selected mutants.

Supplementary Figure S7. Inactivation of SWP73A - BAS SWI/SNF specific subunit - results in stronger dormancy, higher *DOG1* expression and decreased expression of *asDOG1*.

Supplementary Figure S8. ChIP-qPCR on BRM binding to *DOG1* locus.

Supplementary Figure S10. RT-qPCR expression analysis of the *DOG1* mRNA level.

Supplementary Figure S11. RT-qPCR analysis of selected ABA and GA marker genes during secondary dormancy induction.

Supplementary Figure S12. RT-qPCR analysis of selected ABA and GA marker genes in *dog1-4* mutant.

Supplementary Figure S13. RT-qPCR expression analysis of the native *DOG1* vs *LUC::DOG1* transgene with *dTATA* mutations in antisense region of independent transgenic lines.

Supplementary Figure S14. Primary seed dormancy phenotype of *3xbrd, brm-3, dog1-4* and double *brm-3dog1-4* mutants in comparison to Col-0 WT seeds.

Supplementary Figure S15. *DOG1* gene scheme and splicing analysis.

Supplementary Table S1. GO terms and ChemRICH metabolite analyses.

Supplementary Table S2. List of all primers used in this study.

Funding

This work was funded by a National Science Centre, Poland OPUS16 grant (UMO-2018/31/B/NZ3/03363), SONATA BIS UMO-2018/30/E/NZ1/00354. SPS was supported by a NCN Preludium grant UMO-2015/17/N/NZ2/01919. JZ funded by European Union's Horizon H2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101038075 and from EMBO scientific exchange grant No 9107.

Acknowledgments

The authors thank Dimitri Heintz (metabolomics platform) and Jérôme Mutterer (Imaging platform) from IBMP institute, for their help and support. The authors declare that they have no conflict of interest.

Author Contributions

RY and SS conceived and designed the research project. RY, MW, JZ, MK, SPS, RA,CV, JZ, BC, PM and MN performed experiments. RY, MK, JZ, CV, JZ, HS, JB and SS analyzed data. RY and SS wrote the paper with contribution of all authors.

Data availability

The 3'RNA-seq data generated for this study have been deposited at the Gene Expression Omnibus (GEO) under the accession code GSE251921.

Figure Legends

Figure 1. **Seeds transcriptome and metabolome of *brm* and *dog1* single and double mutants.** **A**. Identification of differentially expressed genes in mature, dry *brm-3*, *dog1-4* and *brm-3dog1-4* seeds compared to Col-0 Wild Type (3'RNA-seq, differential analysis was performed using DESeq2 – genes with FDR < 0.05 and absolute fold change > 1 were considered as differentially expressed). **B**. Analysis of overlap between genes whose expression was affected in analyzed mutants (extended graph version shown in Supplementary Figure S1C). **C**. Self-clustering of expression profiles for genes differentially expressed in *brm-3,* identifies the *DOG1*-dependent genes among ones affected in *brm* mutant seeds. Number of DEGs is indicated on panels. **D**. Heatmap of genes' expression for genes misregulated in *brm-3* mutant across mutants used.

817 Genes marked as *DOG1*-gene dependent show suppression of the *brm* effect in the double *brm-*
818 *3dog1-4* mutant. Second column colors correspond to specific color of the cluster. **E**. Chemical
819 enrichment analysis of *brm* and *dog* single and double mutants. Colored circles represent clusters
820 of metabolites from given chemical families (red, increased cluster; blue, decreased cluster;
821 purple, increased and decreased metabolites in a cluster). The number of metabolites as indicated
822 as circle size. **F.** The graph represents the fold change of glutathione levels compared to Col-0 in
823 the seeds of mutants indicated values were ranked into groups as indicated by the respective
824 letter using a Student-Newman-Keuls test, n=?.

825
826 Figure 2. **The *brm-3* mutant seeds showed multiple morphological and physiological defects.**
827 **A**. Scanning electron visualisation of seed from Col-0 WT, *brm-3*, *dog1-4* and *brm-3dog1-4*
828 mutants. Bar corresponds to 200 nm. **B**. Seeds size analysed using Boxed robot, **C.** Seed yield
829 analysed based on total number of seeds produced by mature plants. **D.** Seed longevity analysed
830 using artificial aging. **E.** Germination in presence and absence of 100mM NaCl. **F.** Primary seed
831 dormancy analysed with freshly harvested seeds. **G** Secondary dormancy analysis for seed of
832 *brm-3*, *dog1-4* and *brm-3dog1-4* mutants. Asterisks indicate significant differences compared to
833 Col-0 dry seeds. Statistical analysis applies to all figure panels; t-test, *, P<0.05, **, P<0.01 and
834 ***, P<0.0001; n=4, one biological replicate is a mixture of independent 5 plants; error bars
835 represent standard deviation.
836
837 Figure 3. **BRM directly regulates *DOG1* antisense transcription to control seed secondary**
838 **dormancy. A.** BRM ChIP-qPCR in dry seeds and **B.** seeds subjected to 3 days of secondary
839 dormancy induction. Col-0 and BRM-GFP *brm-1* seeds were analysed using GFP antibodies. x-
840 axis shows beginning of amplicon relative to TSS, TSS=0. Percent of input normalized to PP2A
841 gene region. **C.** RT-qPCR analysis of *DOG1* sense and **D.** antisense transcripts in Col-0, *brm-3*
842 and *3xbrd* mutants during secondary dormancy induction; **E.** RT-qPCR analysis of reporter lines
843 activity during secondary dormancy induction for p_{sense}*DOG1-LUC* and **F.** p_{AS}*DOG1-LUC* lines
844 in Col-0 and *brm-3* background. **G.** RT-qPCR for endogenous and *LUC-DOG1 deltaTATA* line
845 activity during secondary dormancy induction shows that inactivation of *asDOG1* results in
846 stronger induction of *DOG1* during secondary dormancy induction. H. BRM, SWP73A and
847 BRD1 genes expression analysis during SD induction in Arabidopsis seeds. RT-qPCR analysis
848 in C-H is normalized using *UBC21* gene. Statistical analysis applies to all figure panels, t-test, *,
849 P<0.05, **, P<0.01 and ***, P<0.0001; n=4, one biological replicate is a mixture of independent
850 5 plants; error bars represent standard deviation.

Figure 4. **The *brm3* mutant shows enhanced chromatin accessibility at *DOG1* 3'end during secondary dormancy induction. A**. FAIRE in Col-0 and *brm-3* seeds on the 3rd day and **B.** 5th day of secondary dormancy induction. Chromatin accessibility at *DOG1* shown as % recovery to non-crosslinked samples (UNFAIRE) and relative to PP2A. The x-axis shows beginning of amplicon relative to TSS, TSS=0. **C-F**. RT-qPCR analysis of *α-, β-, γ-, δ-DOG1* mRNA splicing forms during secondary seed dormancy induction. Transcript level of short (**G**) and long (**H**) polyadenylated *DOG1* mRNA forms The x-axis shows time/days of secondary dormancy induction. Statistical analysis applies to all figure panels; t-test, *, $P<0.05$, **, $P<0.01$ and ***, $P<0.0001$; n=4, one biological replicate is a mixture of independent 5 plants; error bars represent standard deviation.

Figure 5. **Model of the BRAHMA-associated SWI/SNF complex control of Arabidopsis seeds quality and physiology.** The BRAHMA-associated SWI/SNF complex controls seed yield, seed size and plant hormonal crosstalk to a large extend independently of DOG1. The BRAHMA controls longevity and secondary dormancy by controlling *DOG1* expression through *DOG1* antisense (in red color; dark grey arrows). The BRAHMA also either directly or through *DOG1* antisense negatively controls *DOG1* gene expression (grey arrow) by affecting its alternative splicing and alternative polyadenylation.

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Figure 1

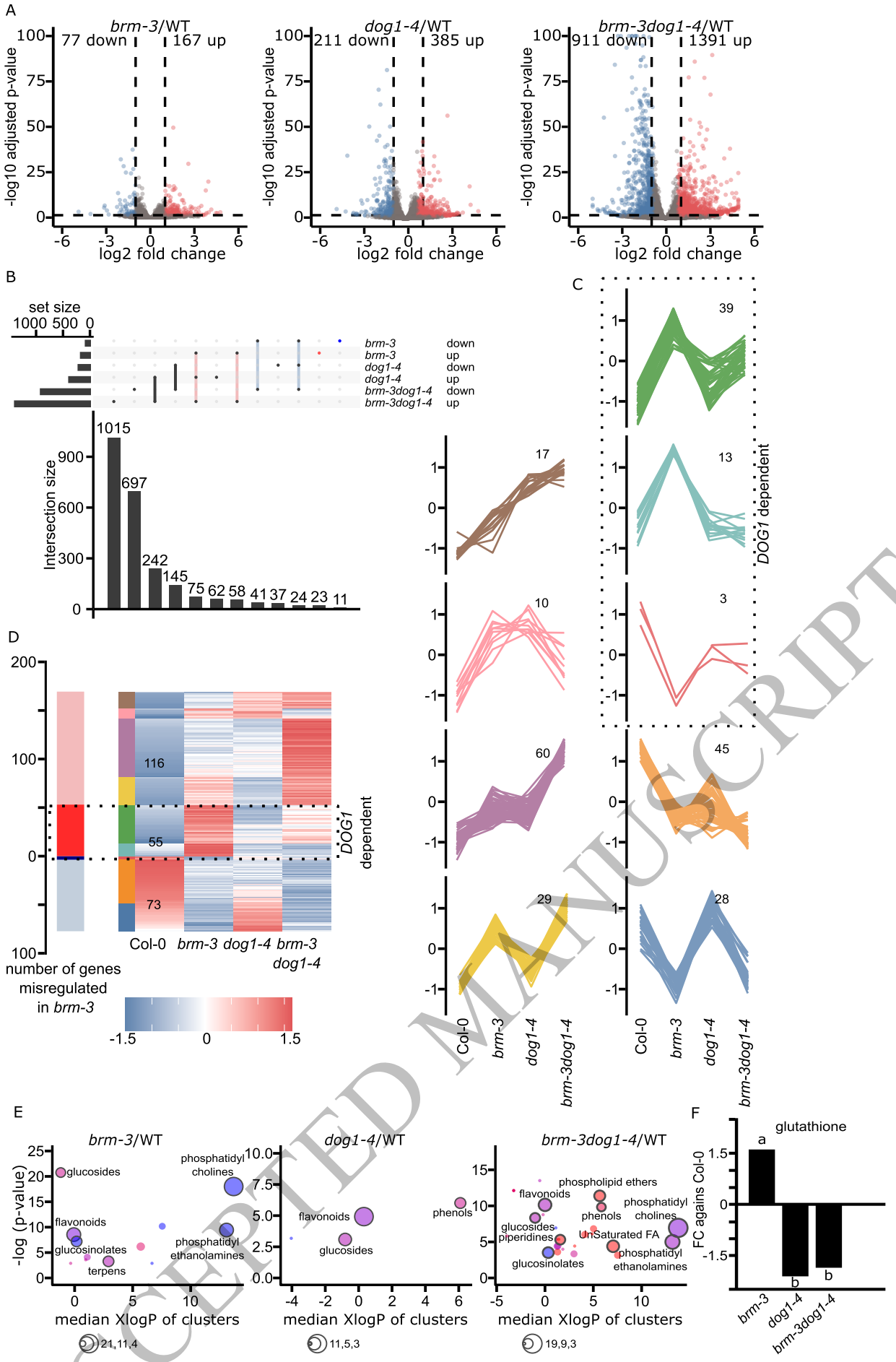

Figure 1. **Seeds transcriptome and metabolome of *brm* and *dog1* single and double mutants.** **A**. Identification of differentially expressed genes in mature, dry *brm-3*, *dog1-4* and *brm-3dog1-4* seeds compared to Col-0 Wild Type (3'RNA-seq, differential analysis was performed DESeq2 – genes with FDR < 0.05 and absolute fold change > 1 were considered as differentially expressed). **B**. Analysis of overlap between genes whose expression was affected in analyzed mutants (extended graph version shown in Supplementary Figure S1, the x-axis is set). **C**. Self-clustering of expression profiles for genes differentially expressed in *brm-3,* identifies the *DOG1*-dependent genes among ones affected in *brm* mutant seeds. Number of DEGs is indicated on panels. the y-axis is scaled expression. **D**. Heatmap of genes' expression for genes misregulated in *brm-3* mutant across mutants used. Genes marked as *DOG1*-gene dependent show suppression of the *brm* effect in the double *brm-3dog1-4* mutant. Second column colors correspond to specific color of the cluster. **E**. Chemical enrichment analysis of *brm* and *dog* single and double mutants. Colored circles represent clusters of metabolites from given chemical families (red, increased cluster; blue, decreased cluster; purple, increased and decreased metabolites in a cluster). The number of metabolites as indicated as circle size. **F.** The graph represents the fold change of glutathione levels compared to Col-0 in the seeds of mutants indicated values were ranked into groups as indicated by the respective letter using a Student-Newman-Keuls test, n=4.

Figure 2

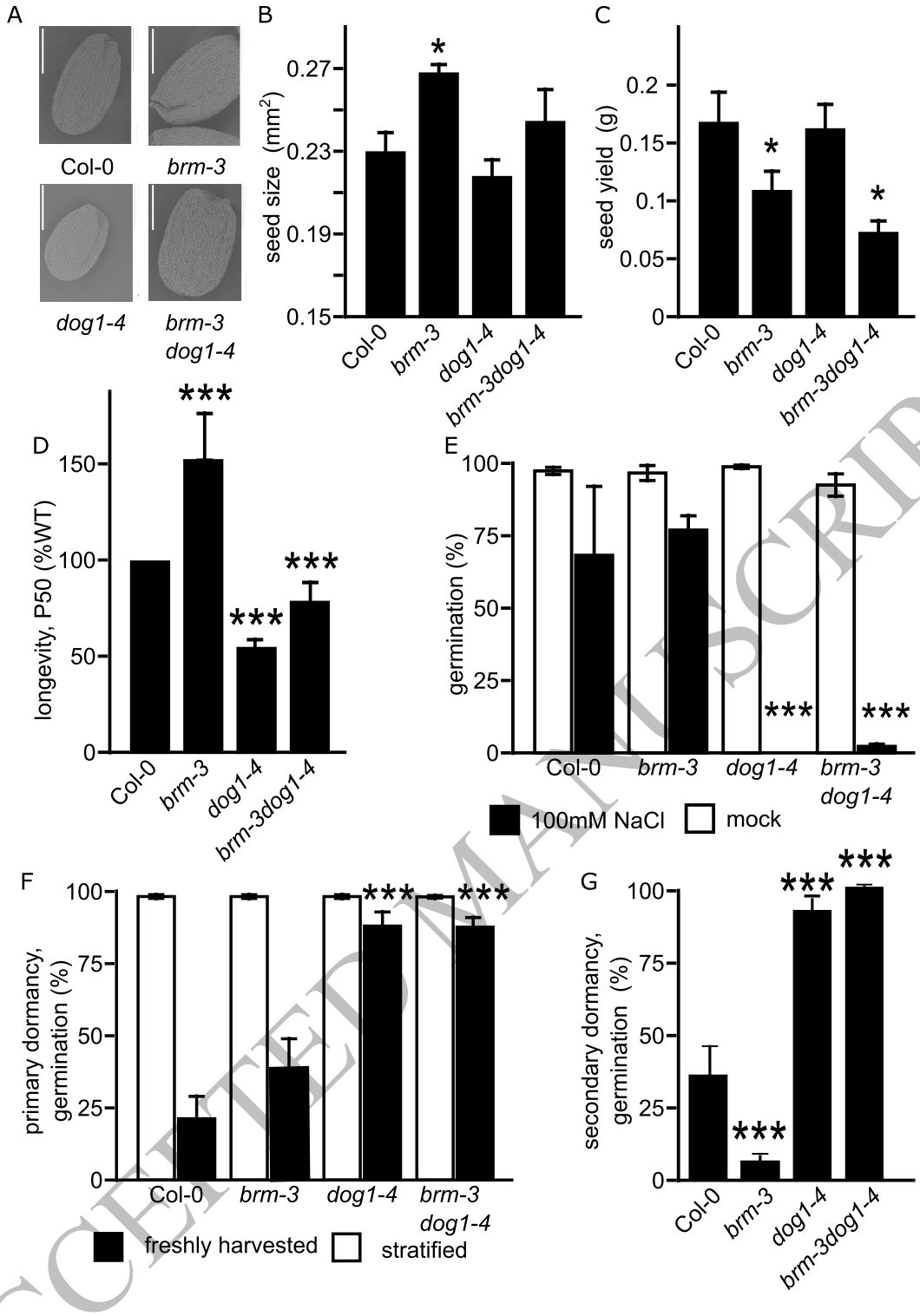

Figure 2. **The *brm-3* mutant seeds showed multiple morphological and physiological defects.** **A**. Scanning electron visualization of seed from Col-0 WT, *brm-3*, *dog1-4* and *brm-3dog1-4* mutants. Bar corresponds to 200 nm. **B**. Seeds size analyzed using Boxed robot, **C.** Seed yield analyzed based on total number of seeds produced by mature plants . **D.** Seed longevity analyzed using artificial aging. **E.** Germination in presence and absence of 100mM NaCl . **F.** Primary seed dormancy analyzed with freshly harvested seeds . **G.** Secondary dormancy analysis for seed of *brm-3*, *dog1-4* and *brm-3dog1-4* mutants. Asterisks indicate significant differences compared to Col-0 dry seeds. Statistical analysis applies to all figure panels; t-test, *, P<0.05, **, P<0.01 and ***, P<0.0001; n=4, one biological replicate is a mixture of independent 5 plants; error bars represent standard deviation (±SD).

Figure 3

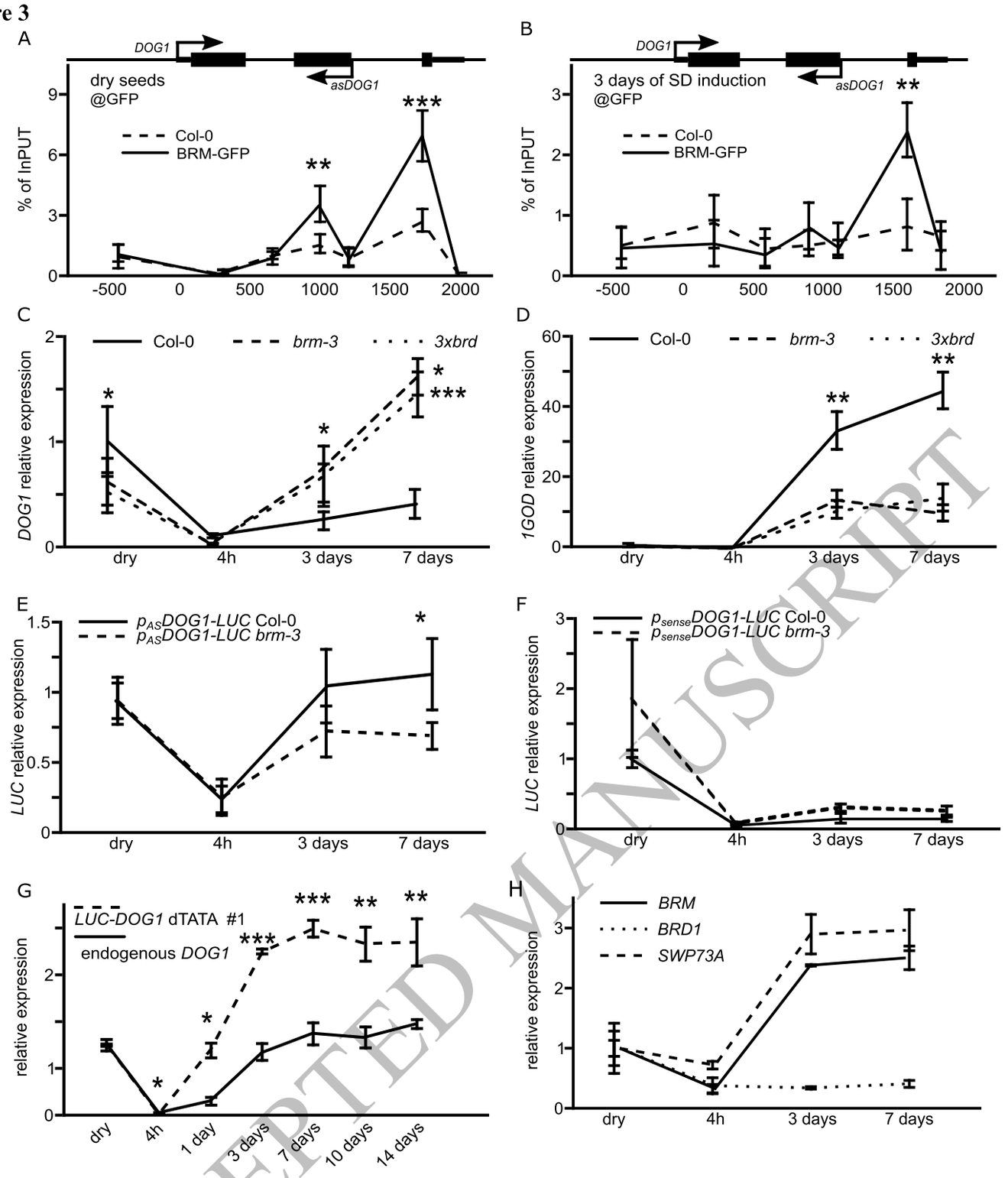

Figure 3. **BRM directly regulates *DOG1* antisense transcription to control seed secondary dormancy. A.** BRM ChIP-qPCR in dry seeds and **B.** seeds subjected to 3 days of secondary dormancy induction. Col-0 and BRM-GFP *brm-1* seeds were analyzed using GFP antibodies. x-axis shows beginning of amplicon relative to TSS, TSS=0. Percent of input normalized to PP2A gene region. **C.** RT-qPCR analysis of *DOG1* sense and **D.** antisense transcripts in Col-0, *brm-3* and *3xbrd* mutants during secondary dormancy induction; **E.** RT-qPCR analysis of reporter lines activity during secondary dormancy induction for p_{sense}*DOG1-LUC* and **F.** p_{AS}*DOG1-LUC* lines in Col-0 and *brm-3* background. **G.** RT-qPCR for endogenous and *LUC-DOG1 deltaTATA* line activity during secondary dormancy induction shows that inactivation of *asDOG1* results in stronger induction of *DOG1* during secondary dormancy induction. H. BRM, SWP73A and BRD1 genes expression analysis during SD induction in Arabidopsis seeds. RT-qPCR analysis in C-H is normalized using *UBC21* gene and the x-axis shows time/days of secondary dormancy induction (h-hours; d-days). Statistical analysis applies to all figure panels, t-test, *, P<0.05, **, P<0.01 and ***, P<0.0001; n=4, one biological replicate is a mixture of independent 5 plants; error bars represent standard deviation (±SD).

Figure 4

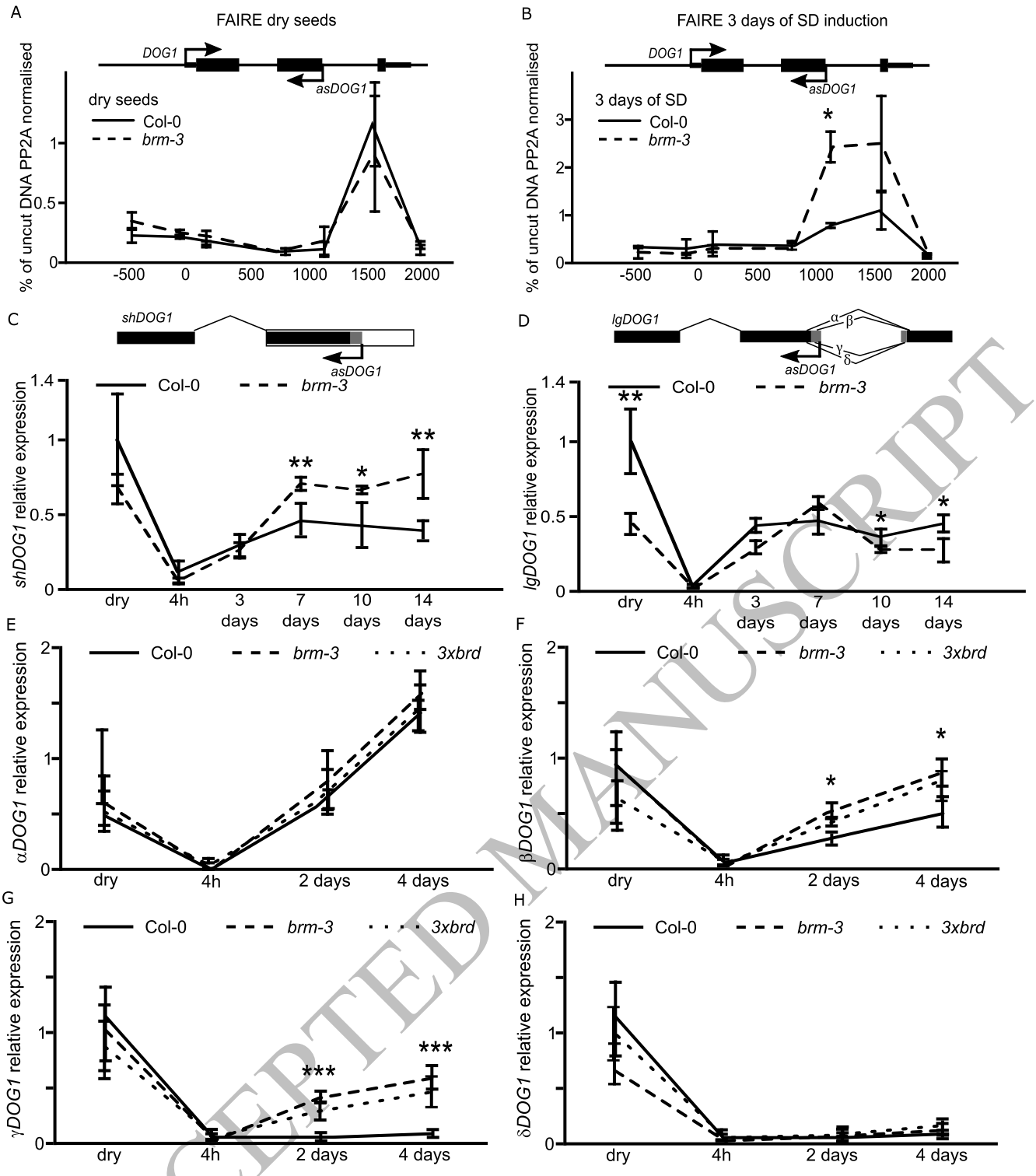

Figure 4. **The *brm3* mutant shows enhanced chromatin accessibility at *DOG1* 3'end during secondary dormancy induction.** **A**. FAIRE in Col-0 and *brm-3* seeds on the 3 rd day and **B.** 5th day of secondary dormancy induction. Chromatin accessibility at *DOG1* shown as % recovery to non-crosslinked samples (UNFAIRE) and relative to PP2A. The x-axis shows beginning of amplicon relative to TSS, TSS=0. **C-F**. RT-qPCR analysis of *α-, β-, γ-, δ-DOG1* mRNA splicing forms during secondary seed dormancy induction. Transcript l evel of short (**G**) and long (**H**) polyadenylated *DOG1* mRNA forms The x-axis shows time/days of secondary dormancy induction (h-hours; d-days). Statistical analysis applies to all figure panels; t-test, *, P<0.05, **, P<0.01 and ***, P<0.0001; n=4, one biological replicate is a mixture of independent 5 plants; error bars represent standard deviation (±SD).

Figure 5

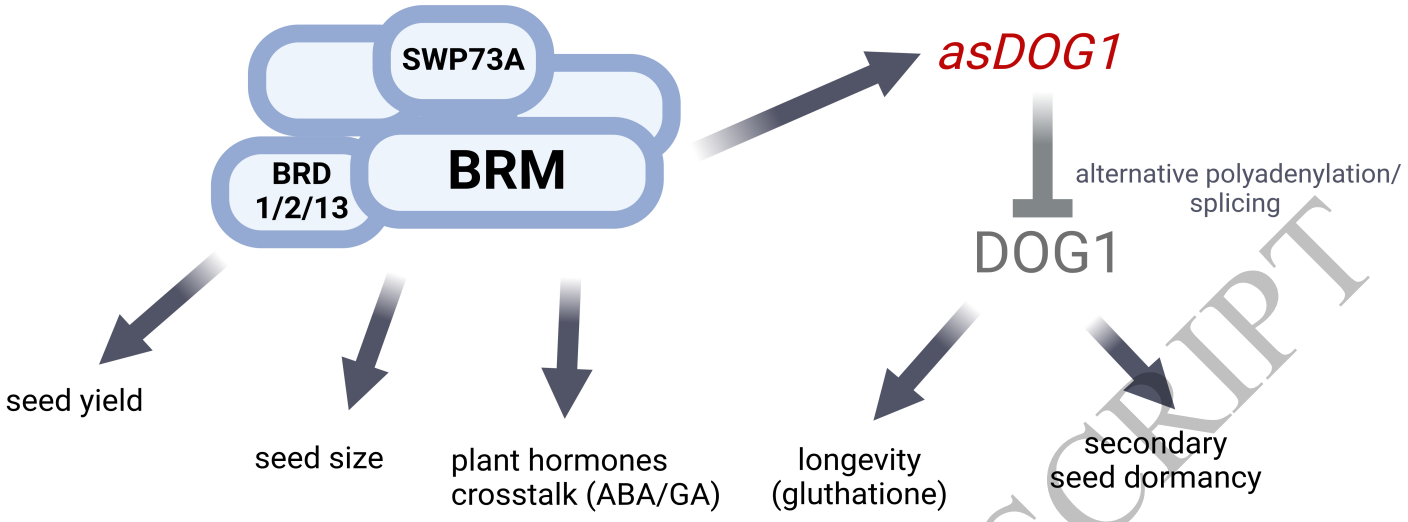

Figure 5. **Model of the BRAHMA-associated SWI/SNF complex control of Arabidopsis seeds quality and physiology.** The BRAHMA-associated SWI/SNF complex controls seed yield, seed size and plant hormonal crosstalk to a large extend independently of DOG1. The BRAHMA controls longevity and secondary dormancy by controlling *DOG1* expression through *DOG1* antisense (in red color; dark grey arrows). The BRAHMA also either directly or through *DOG1* antisense negatively controls *DOG1* gene expression (grey arrow) by affecting its alternative splicing and alternative polyadenylation.

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