

Research Paper

Cite this article: Barros-Galvão T, Chen X, Stefanato F and Penfield S (2026) Distinct roles of VEL family proteins in Arabidopsis seed development reveal VEL1 and VRN5 as regulators of seed abortion. *Seed Science Research* 1–8. <https://doi.org/10.1017/S0960258526100130>

Received: 27 January 2026

Revised: 18 March 2026

Accepted: 20 March 2026

Keywords:

endosperm; polymerase repressive complex 2 (PRC2); seed abortion; seed dormancy; triploid block; VERNALIZATION INSENSITIVE 3-LIKE

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Distinct roles of VEL family proteins in Arabidopsis seed development reveal VEL1 and VRN5 as regulators of seed abortion

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Abstract

During seed development, plants detect the paternal genome dose through gene expression from the paternally derived genome in the endosperm. To achieve this, the homologous genes in the maternally derived genome are silenced by polycomb repressive complex 2 (PRC2), and loss of PRC2 function or excess paternal genome dose both lead to seed abortion through the so-called 'triploid block'. PRC2 requires the activity of accessory proteins of the VERNALIZATION INSENSITIVE 3-LIKE (VEL) family, of which the atypical VEL3 protein is required for endosperm development. Here, we characterize the function of the remaining family members, using CRISPR-Cas9 to generate multiple mutants in Arabidopsis. Loss-of-function analyses establish VEL3 as the sole regulator of primary seed dormancy depth, whereas VEL1 and VRN5 act redundantly alongside VEL3 to prevent seed abortion but without affecting dormancy. VEL2 and VIN3 exhibited no detectable influence on seed abortion or dormancy. Comparative RNA-seq of mature endosperm revealed that both *vel3-1* and *vel1-2 vrn5-9* mutants upregulate programmed cell-death genes, whereas *vel3-1* uniquely deregulates chromatin remodellers and metabolic repressors, consistent with heterochromatin relaxation at germination loci and elevated metabolic activity. Our results establish that the role of VEL3 in seed dormancy is independent of PRC2, as they suggest the involvement of novel members of central cell and endosperm PRC2 complexes in regulating the maternally derived genome in the endosperm.

Introduction

After double fertilization, coenocytic proliferation and subsequent cellularization of the triploid endosperm are necessary for normal growth and development of the embryo through to maturity (Yadegari and Drews, 2004; Lafon-Placette and Köhler 2014). Epigenetic repression by polycomb repressive complex 2 (PRC2) is indispensable for heterochromatin formation in the central cell of the female gametophyte and for normal gene expression from the maternally derived genome in the endosperm during seed development (Grossniklaus and Paro, 2014). In *Arabidopsis thaliana*, the FERTILIZATION-INDEPENDENT SEED-PRC2 (FIS-PRC2) variant, composed of MEDEA, FERTILIZATION-INDEPENDENT SEED 2, FERTILIZATION-INDEPENDENT ENDOSPERM and MULTICOPY SUPPRESSOR OF IRA1, catalyses H3K27 trimethylation at key loci to drive endosperm cellularization and embryo viability, and maternal loss of any of these components induces seed abortion (Grossniklaus et al., 1998; Luo et al., 1999; Ohad et al., 1999; Köhler et al., 2003).

Beyond serving as a nutritive tissue, the endosperm exerts a pivotal regulatory influence on seed dormancy. The endosperm synthesizes hormones, including abscisic acid (ABA) and gibberellins, to either enforce or alleviate dormancy in response to environmental stimuli (Lee et al., 2012; Piskurewicz et al., 2023). This is influenced by the epigenetic state of the endosperm, with disruption of repressive chromatin leading to production of non-dormant seeds under conditions that normally cause high dormancy levels in Col-0 (Iwasaki et al., 2019; Sato et al., 2021; Chen et al., 2023).

PRC2 complexes interact with accessory factors that are necessary for stable chromatin association (Holoch and Margueron, 2017). The VERNALIZATION INSENSITIVE 3-LIKE (VEL) family of PRC2 accessory proteins includes VIN3, VRN5, VEL1 and VEL2, which harbour plant homeodomain (PHD) and fibronectin type III domains that mediate their interaction with PRC2 and chromatin (De Lucia et al., 2008; Gao and He, 2024). VEL1, VIN3 and VRN5 recruit PRC2 to FLOWERING LOCUS C (FLC) during vernalization, via polymerization and phase separation driven by the VEL domain (Schulten et al., 2025). VEL2 and VEL3 are both

expressed in gametophytes and seeds, and *VEL3* encodes an atypical truncated VEL protein that lacks the PHD present in other VEL proteins. We recently showed that *VEL3* is required for H3K27me3 deposition in the endosperm and regulates dormancy via association with histone deacetylase (HDAC) complexes (Chen et al., 2023). It remains unclear if this dormancy regulation also requires FIS-PRC2 function. *VEL1* and *VRN5* also interact with HDACs (De Lucia et al., 2008) and are necessary for H3K27me3 deposition at *FLC* during vernalization, but it is unclear whether they also play a role in H3K27me3 deposition in heterochromatin in the maternally derived genome of the endosperm.

Here, we use CRISPR-Cas9 gene editing of the VEL family to overcome any redundancy in function and assess their contribution to seed development and primary seed dormancy. Our results show that *VEL2* does not contribute to seed dormancy or abortion. Only the loss of *VEL3* affects seed dormancy, while *VEL1* and *VRN5* are necessary alongside *VEL3* to prevent seed abortion that may be associated with hyperactivation of the maternally derived genome in the endosperm. These findings demonstrate that the two effects are genetically separable.

Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana mutants used in this study have been previously described: *vel3-1* (SALK_052041; Chen et al., 2023), *vel3-1 gVEL3-GFP (VEL3-GFP)* (Chen et al., 2023), *vil2-1 (vel1-1)* (SAIL_323_A03; Kim and Sung, 2010) and *vrn5-8* (SALK_136506; Greb et al., 2007). Three transgenic lines expressing 35S::GFP-*VEL2* were generated via *Agrobacterium tumefaciens*-mediated floral dipping. The *VEL2* coding sequence was PCR-amplified (forward: ttGGTCTCtTTCGATGGCTTCTTTT CATAAAGGAGCTGC; reverse: ttGGTCTCtAAGCTCAATCC TGCAGCAGAATCAAAGACAC) and subsequently assembled with eGFP using Golden Gate cloning (see Supplementary Table 1 for details of plasmids). The resulting constructs were introduced into Col-0 plants. Other mutant lines used here were generated by CRISPR-Cas9: *vel2-1*, *vel2-2*, *vel2-3*, *vel2-4* *vel3-1*, *vel1-2* *vrn5-9*, *vel2-5* *vin3-4* *vrn5-10 (vel^I)* and *vel1-3* *vel2-5* *vin3-5* *vrn5-10 (vel^Q)*. The *vel2-4* *vel3-1* double mutant was created by CRISPR-Cas9 knock out of *VEL2* in the *vel3-1* background. *Arabidopsis* plants were directly grown in long days (16 h light and 8 h darkness at 22°C) until bolting. In the vernalization experiment, 2-week-old seedlings were subjected to a 4-week period at 4°C, followed by a transfer to 22°C until bolting occurred. After bolting, plants were either maintained at 22°C or transferred to 15°C for seed set. All mutants and wild type (WT) were grown side-by-side for each experiment.

Seed germination

For dormancy assays, freshly matured seeds were sown onto 0.9% water agar plates and placed in long days (16 h light and 8 h darkness) at 22°C. Stratification was performed at 4°C in the dark for 3 days as indicated. For ABA sensitivity, seeds were sown onto 0.9% water agar plates with or without exogenous ABA (1, 5 or 10 µM), stratified and then placed in long days (16 h light and 8 h darkness) at 22°C. Seed batches from individual mother plants were used as biological replicates; at least 6 biological replicates of ~50 seeds were used for each experiment. Seed germination was scored by radicle emergence at 7 days after sowing.

Microscopy

Arabidopsis thaliana 35S::*VEL2* seeds carrying GFP reporter constructs were germinated and seedlings were grown under long-day conditions (16 h light/8 h dark, 22°C). Hypocotyls from 5- to 7-day-old seedlings were mounted in phosphate-buffered saline (pH 7.4) on glass slides. Fluorescence imaging was performed with a Leica SP8X confocal microscope using a white light laser, with GFP excited at 488 nm and emission collected between 500 and 550 nm. All samples were imaged under identical acquisition settings to ensure comparability across biological replicates. Images were processed in Leica LAS X software, applying only linear adjustments to brightness and contrast without altering raw fluorescence signals. Representative images were selected from at least three independent experiments.

VEL gene family editing by CRISPR-Cas9

CRISPR-Cas9 gene editing was performed as described in Lawrenson et al. (2015). In short, target sequences for *VEL1*, *VEL2*, *VIN3* and *VRN5* were selected for guide RNAs (gRNAs) using a web-based bioinformatics tool (CHOPCHOP – <https://chopchop.cbu.uib.no/>), with four guides designed for each gene. Separately, we performed CRISPR-Cas9 targeting the beginning of the first exon of the *VEL2* gene (see Supplementary Table 1 for details of gRNAs). Plasmids were constructed (see Supplementary Table 1 for details of plasmids and gRNAs) using the Golden Gate system following a previously described assembly strategy (Lawrenson et al. 2015). We used the Discosoma Red (DsRED) fluorescent protein marker to screen for putative transgene-free CRISPR-Cas9 edited plants of *Arabidopsis* (Aliaga-Franco et al., 2019). Plants were transformed using *Agrobacterium*-mediated floral dip with *Agrobacterium* resuspended in 5% sucrose and 0.02% (v/v) Silwet L-77. Genotyping of edited plants was carried out by PCR using the following primers (*VEL1*-forward: AGGTCTTGCAGGCATGGAGC; *VEL1*-reverse: AGAGGACC CACATCAGCTTCAAG; *VEL2.vel2-1*-forward: TGATCTGAAA TGAAGTTTGG; *VEL2.vel2-1* reverse: TAGCTTTAGCTAATGG AGTC; *VEL2.others* forward: GTTCTGCGGTTGTAGTTGAG GT; *VEL2.others*-reverse: GCAGGTTAACCAGAGACTAGGAT CT; *VIN3*-forward: GCCTGAAGAAGCTTCGGAGC; *VIN3*-reverse: GAGACCACTTTGTCCAGAGCTTCC; *VRN5*-forward: CCTCCGTAATCTCAATCACC GAAC; *VRN5*-reverse: GGC TGACAACTCCCCTGCC). A summary of the construct design, gRNA positioning and resulting alleles is given in Fig. 1.

RNA-seq and qPCR

RNA-seq was performed as described in Chen et al. (2023). In short, RNA was extracted from mature endosperm by first imbibing 0.05 g of seeds for 1 h. Following Iwasaki and Lopez-Molina (2021), endosperm-enriched tissues were then isolated using a glass slide to squeeze the seeds and centrifuged twice at maximum speed (16,000 rpm) with 40% sucrose. After washing three times with water, the samples were stored at -80°C. RNA extraction was performed using the borate method (Penfield et al., 2005). The RNA pellet was dissolved in water and further purified using a Qiagen RNeasy Plant Mini Kit, including on-column DNase I digestion. RNA sequencing was performed by Novogene using a HiSeq 4000 system, generating 150 bp paired-end sequences with a minimum of 25 million reads per sample. Three biological replicates were prepared. Raw RNA-seq reads were trimmed

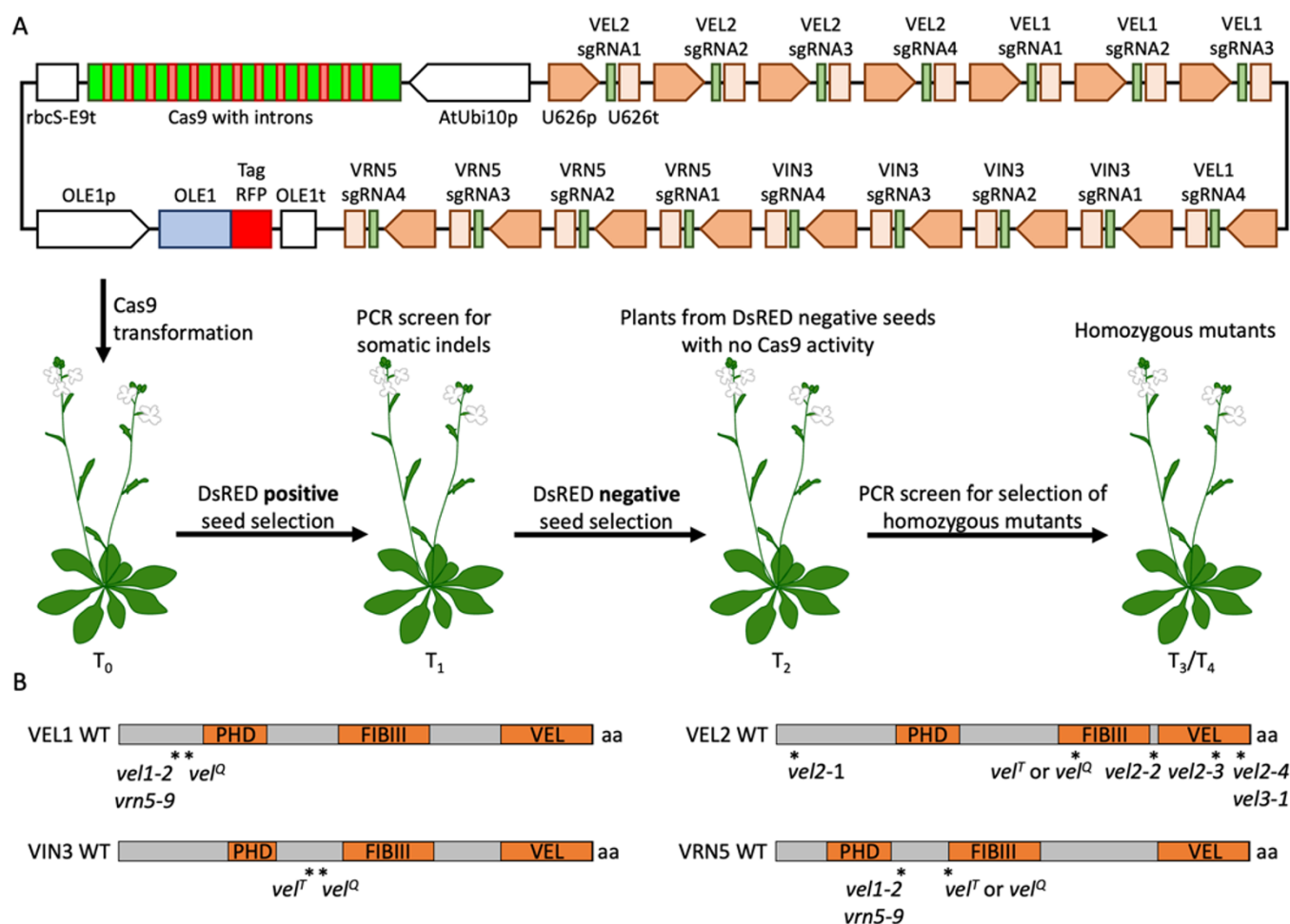


Figure 1. Gene editing strategy for targeted chromosomal indels in VEL family genes in Arabidopsis. (A) Schematic representation of the final CRISPR construct. In brief, DNA oligonucleotides encoding the sequences of 16 single-guide RNAs (sgRNAs) were used to generate double-stranded DNA fragments, which were subsequently integrated into the final construct with an intron-optimized *Cas9* and DsRED tagged selection via Golden Gate assembly, mediated by Type IIS restriction enzymes *BsaI*/*BpiI*. This enzymatic system facilitates the efficient assembly of large and complex constructs from standardized constitutive parts in a minimal number of reactions. Arabidopsis plants were transformed via floral dipping method with *Agrobacterium tumefaciens* carrying the final construct. Fluorescent DsRED-positive seeds were germinated, and plants were screened for somatic indels. Selected plants with indels produced DsRED-negative T1 seeds, which were further selected. T2 plants were grown, and homozygous mutants were screened through T4. (B) Sequence diagram of the VEL proteins (VEL1, VEL2, VIN3 and VRN5). Main domains of the VEL proteins (plant homeodomain, PHD; fibronectin type III, FIBIII and VERNALIZATION-INSENSITIVE3-like, VEL) are shown in orange boxes. Asterisks indicate the location of the first premature stop codon resulting from the frameshift mutation of each allele. *vel^T* refers to *vel2-5 vin3-4 vrn5-10* triple mutant. *vel^Q* refers to *vel1-3 vel2-5 vin3-5 vrn5-10* quadruple mutant.

using Cutadapt (Martin, 2011) and mapped to the *A. thaliana* TAIR10 genome with STAR (Dobin et al., 2013). Read counts for each gene were obtained using featureCounts (Liao et al., 2014), and only sense reads were analysed further. Differentially expressed genes (DEGs) were identified with edgeR (Robinson et al., 2010), using FDR (False Discovery Rate) < 0.05 and a fold-change ≥ 2. For qPCR, three seedlings were pooled as one biological replicate, and RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, 74904). cDNA samples were prepared using the Quantitect reverse transcription kit (Qiagen, 205311). qPCR was then performed on the *VEL2* gene (forward: TCCAAGACGAAG AAGTGGCAGC; reverse: CACTCTTGACCTGACCTGAGCAC) with the SYBR Green master mix (Bio-Rad, 1725271). *ACT11* (forward: GGAACAGTGTGACTCACACCATC; reverse: AAGCTG TTCTTTCCCTCTACGC) and *PP2A* (forward: TAACGTGGCCA AAATGATGC; reverse: GTTCTCCACAACCGCTTGGT) were used as the reference genes.

Results

Previously, we established a role for VEL3 in seed dormancy. In this paper, this function is being re-examined while also investigating the roles of other members of the VEL family. Although VEL2 is highly expressed in seeds (Klepikova et al., 2016), the role of VEL2 remained uncharacterized because we could not isolate *vel2* T-DNA mutants for analysis (Chen et al., 2023). To elucidate VEL2 function, we first generated Col-0 lines expressing GFP-tagged *VEL2* under the cauliflower mosaic virus 35S promoter (Supplementary Figure 1). To induce seed dormancy, seeds from the respective lines were matured at 16°C, and germination was evaluated in the absence of stratification. Notably, the three transgenic 35S:*GFP-VEL2* lines did not exhibit statistically significant differences in dormancy levels relative to the Col-0 control (Fig. 2A). Subsequently, seeds were matured at 22°C to generate non-dormant populations for ABA sensitivity assays. Under these

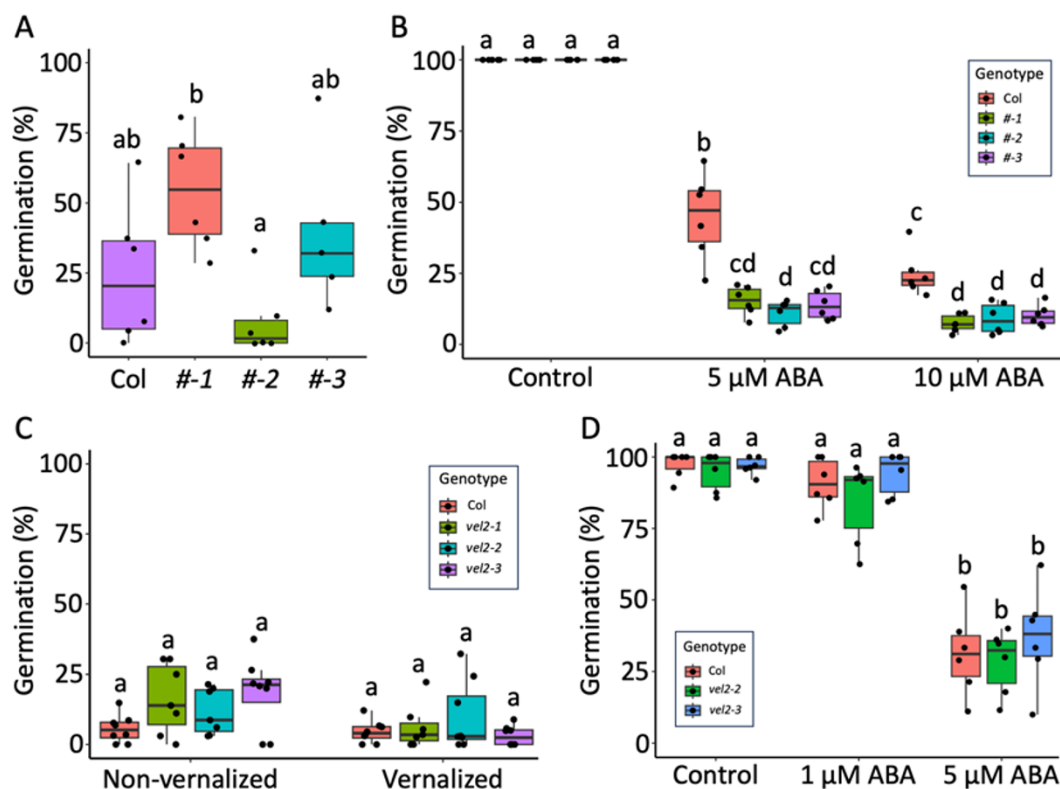


Figure 2. VEL2 does not play a role in seed dormancy. (A) Germination of Col-0, 35S::GFP-VEL2-1 (#-1), 35S::GFP-VEL2-2 (#-2) and 35S::GFP-VEL2-3 (#-3) seeds set at 16°C. (B) Germination of Col-0, OE-1, OE-2 and OE-3 seeds set at 22°C in the presence of exogenous ABA (5 or 10 µM). (C) Germination of Col, *vel2-1*, *vel2-2* and *vel2-3* seeds set at 15°C. Non-vernalized seedlings were grown continuously at 22°C until bolting, while vernalized seedlings had a 4°C treatment for 4 weeks before going back to 22°C until bolting. (D) Germination of Col-0, *vel2-2* and *vel2-3* seeds to 22°C in the presence of exogenous ABA (1 or 5 µM). Significant differences are shown using ANOVA with Tukey's post hoc test ($P < 0.05$; $n = 6-8$).

conditions, the 35S::GFP-VEL2 lines displayed slightly increased sensitivity to ABA compared to Col-0 (Fig. 2B) but were otherwise phenotypically normal.

To obtain various knockout mutants of VEL2, we used a CRISPR-Cas9 approach. We designed six RNA guides to target different portions of the VEL2 gene and obtained three independent *vel2* alleles with frameshift mutations predicted to cause premature termination of translation and the production of truncated VEL2 proteins (Fig. 1). To induce dormancy, seeds from these *vel2* single mutant lines were matured at 15°C, and germination was assessed in freshly harvested seeds. Dormancy of *vel2* mutants remained indistinguishable from WT (Fig. 2C). Because VEL2 expression is induced by vernalization (Kim and Sung, 2013), we vernalized Col-0 and *vel2* seedlings for 4 weeks at 4°C to test whether maternal vernalization had any impact on subsequent seed dormancy in *vel2* mutants. Again, no significant differences were found between genotypes (Fig. 2C). Next, we set seeds at 22°C to produce non-dormant seed lots and assess ABA sensitivity. Neither 1 µM nor 5 µM of exogenous ABA revealed any differences in seed germination between WT and *vel2* mutants (Fig. 2D). Together, these results indicate that VEL2 either does not function in processes affecting seed dormancy or that VEL2 functions redundantly with other VEL family members in the endosperm.

To test whether other VEL family members contribute redundantly to endosperm development or seed dormancy, we next targeted VEL1, VEL2, VIN3 and VRN5 together using CRISPR-Cas9

with the aim of generating lines with loss-of-function mutants for different combinations of VEL genes. For each gene, we designed four gRNAs, assembled into a single construct and obtained double, triple and quadruple mutants (Fig. 1). We matured seeds at 15°C from the following previously characterized mutants: *vel1-1*, *vel2-1*, *vel3-1* and *vrn5-8*, then from newly isolated *vel1-2 vrn5-9* and *vel2-4 vel3-1* double mutant lines; *vel2-5 vin3-4 vrn5-10* triple mutant line and *vel1-3 vel2-5 vin3-5 vrn5-10* quadruple mutant line. Strikingly, only seeds with the *vel3-1* background displayed an altered dormancy phenotype (Fig. 3A, B), suggesting that if these mutations cause loss of gene function as predicted, then VEL3 alone has a role in seed dormancy.

We previously found that *vel3-1* mutants exhibit high-frequency seed abortion accompanied by ectopic gene expression in endosperm closely resembling that of maternal PRC2 mutants and activation of the so-called 'triploid block' (Chen et al., 2023). After testing single and multiple mutant lines, we found that *vel1-2 vrn5-9* double mutant and *vel1-3 vel2-5 vin3-5 vrn5-10* quadruple mutant also showed high frequency seed abortion (Fig. 3C). The normal seed development of *vel1* and *vrn5* single mutants strongly suggested that VEL1 and VRN5 function redundantly alongside VEL3 to facilitate PRC2 activity in the central cell and/or endosperm. Of interest here is that seed abortion increases without compromising the establishment of dormancy in the *vel1-2 vrn5-9* double and *vel1-3 vel2-5 vin3-5 vrn5-10* quadruple mutants, whereas the *vel3-1* mutant disrupts both phenotypes, suggesting that VEL3 function in seed dormancy

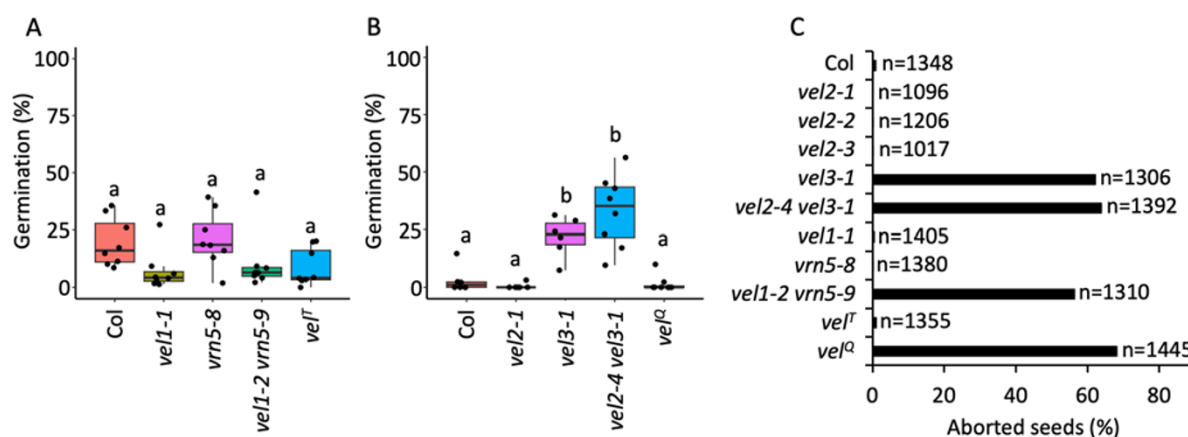


Figure 3. While VEL1, VEL2, VIN3 and VRN5 do not affect seed dormancy, VEL1 and VRN5 are essential to prevent seed abortion. (A) Germination of Col, *vel1-1*, *vrn5-8*, *vel1-2 vrn5-9* double mutant and *vel2-5 vin3-4 vrn5-10* triple (*vel1^l*) mutant seeds set at 15°C. (B) Germination of Col, *vel2-1*, *vel3-1*, *vel2-4 vel3-1* double mutant and *vel1-3 vel2-5 vin3-5 vrn5-10* quadruple (*vel1^l*) mutant seeds set at 15°C. Significant differences are shown using ANOVA with Tukey's post hoc test ($P < 0.05$; $n = 6-8$). (C) Seed abortion frequency (in percentage) in Col, *vel2-1*, *vel2-2*, *vel2-3*, *vel3-1*, *vel2-4 vel3-1*, *vel1-1*, *vrn5-8*, *vel1-2 vrn5-9*, *vel2-5 vin3-4 vrn5-10* (*vel1^l*) and *vel1-3 vel2-5 vin3-5 vrn5-10* (*vel1^l*). The total number of seeds analysed for each genotype is shown.

is independent of triploid block activation and possibly PRC2 activity.

We then aimed to isolate gene expression changes in *vel3-1* specifically associated with alteration of seed dormancy by comparing the *vel3-1* endosperm transcriptome with that of *vel1 vrn5*, performing RNA sequencing on mature endosperm from *vel1-2 vrn5-9* double mutants and comparing the resulting transcriptome to our previously published *vel3-1* dataset (Chen et al., 2023). Comparison between *vel1-2 vrn5-9* and *vel3-1* revealed 66 shared DEGs presumably associated with triploid block activation or another PRC2 function, including *NAC046* (NAC domain-containing protein 46), *WRKY75* (WRKY DNA-binding protein 75) and *ORESARA1* (ORE1) (Fig. 4A, B; Table S1). These genes were upregulated in both genotypes and have established roles in plant senescence (Balazadeh et al., 2010; Guo et al., 2017; Huysmans et al., 2018), with *NAC046* and *ORE1* specifically implicated in endosperm elimination via programmed cell death (Doll et al., 2023).

We next analysed DEGs unique to *vel3-1*, splitting them into upregulated and downregulated subsets. We identified 115 genes upregulated in *vel3-1* but not *vel1-2 vrn5-9* (Fig. 4B; Table S1), and Gene Ontology (GO) analysis highlighted stress-related categories such as salt stress, hypoxia, heat and hydrogen peroxide response (Fig. 4D). In our experience, these gene functions tend to be enriched in seed transcriptomes in any experimental situation, and we did not identify any strong candidate genes for *vel3*-specific dormancy regulators. Interestingly, 95% of DEGs in *vel1-2 vrn5-9* were upregulated relative to Col-0, whereas DEGs in *vel3-1* displayed an approximately equal number between induction and repression (Fig. 4B, C; Supplementary Table 1). When comparing downregulated genes between *vel1-2 vrn5-9* and *vel3-1*, we found 87 genes specifically repressed in *vel3-1* (Fig. 4C; Table S1). GO analysis revealed genes functioning at multiple levels of gene expression, including chromatin accessibility (chromatin remodelling), transcription initiation (DNA-templated transcription), RNA maturation (splicing) and organelle protein targeting (chloroplast import) (Fig. 4D), but no clear dormancy regulators were identified.

Discussion

In this study, we dissect the distinct roles of the VEL family members in seeds (Fig. 5A), uncovering VEL3 as the main regulator of seed dormancy and abortion processes, while VEL1 and VRN5 assume more specialized with redundant functions in controlling seed abortion. Genetic analyses confirm that only VEL3 plays a role in the depth of primary dormancy (Fig. 3B). Although VEL2 is highly expressed in the endosperm of developing seeds (Belmonte et al., 2013), neither *vel2* single mutants nor higher-order combinations lacking VEL1, VEL2, VIN3 or VRN5 affect dormancy (Fig. 2C Fig. 3A, B), thereby underscoring the unique, specific and non-redundant role of VEL3.

Strikingly, loss of VEL1 and VRN5 causes extensive seed abortion without altering dormancy (Fig. 2A, C). Like VIN3, these VEL proteins are known to fine-tune deposition of H3K27me3 together with PRC2 (VRN-PRC2), most notably at *FLC* during vernalization (De Lucia et al., 2008). We have recently shown that VEL3 similarly guides PRC2 to silence maternally derived PEGs (Paternal Expressed Genes) in the endosperm (Chen et al., 2023). Studies of triploid-block and hybridization barriers demonstrate that parental-genome dosage imbalances involve PRC2-mediated H3K27me3 to control seed abortion. Indeed, the FIS-PRC2 complex is dedicated to reproductive development, controlling female gametophyte differentiation, endosperm cellularization and seed maturation (Grossniklaus and Paro, 2014; Hugues et al., 2020). These findings implicate a role for VRN-PRC2 in endosperm cellularization and control of seed abortion but suggest that this process is unrelated to dormancy phenotypes of *vel3* mutants.

Comparative transcriptome profiling of *vel1-2 vrn5-9* and *vel3-1* matured endosperms reveals a shared deregulation of programmed cell-death genes that drive endosperm elimination (Fig. 4). Together, these findings suggest that VEL1, VRN5 and VEL3 act in parallel pathways with partially overlapping but also non-identical targets, and that PRC2 functional disruption does not necessarily result in a seed dormancy phenotype (Fig. 5B). However, we have previously found that upregulation of *ORE1* partially accounts for the low dormancy of *vel3-1* (Chen et al., 2023). Thus, we cannot rule out that differences in the timing of *ORE1*

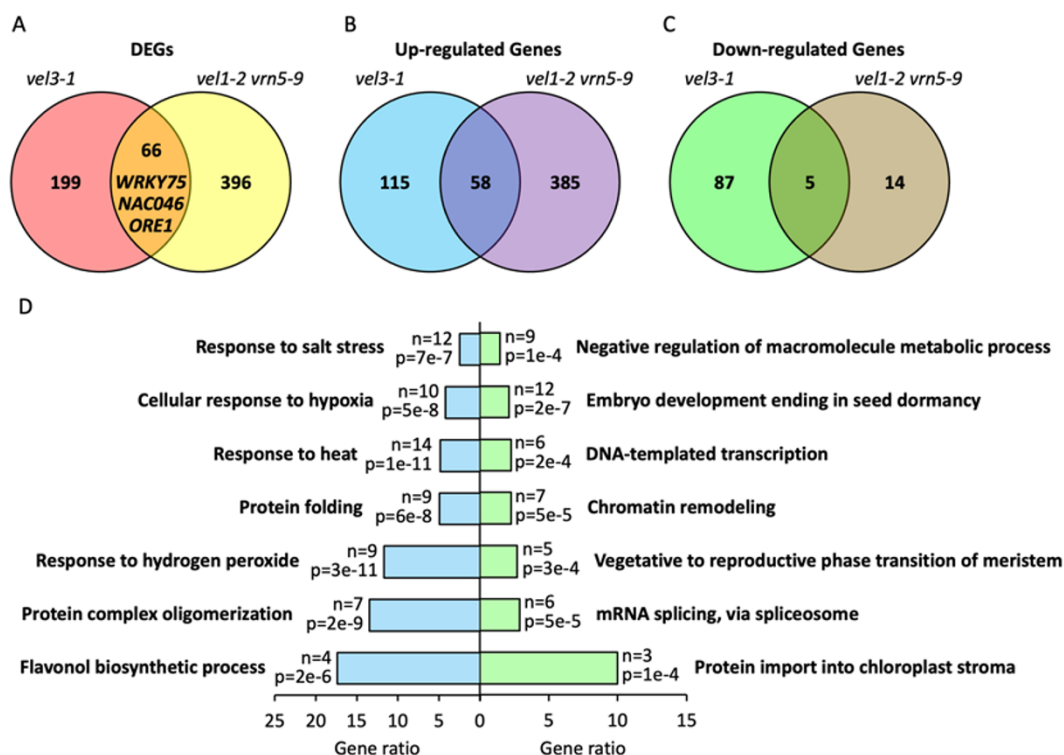


Figure 4. VEL1 and VRN5 are involved in the repression of senescence and programmed cell death-associated gene expression in the endosperm. (A) Comparison of differentially expressed genes (DEGs) in *vel3-1* and *vel1-2 vrn5-9* in the mature endosperm, genes in bold (*NAC046* and *ORE1*) regulate the pace of endosperm elimination by programmed cell death, after Doll *et al.* (2023). Comparison of up- (B) and downregulated (C) genes in *vel3-1* and *vel1-2 vrn5-9* in the mature endosperm. (D) GO-term analysis of up- (left, in blue) and downregulated (right, in green) genes between *vel3-1* and *vel1-2 vrn5-9* mature endosperms, showing gene function category, *p*-values and number of genes in each category.

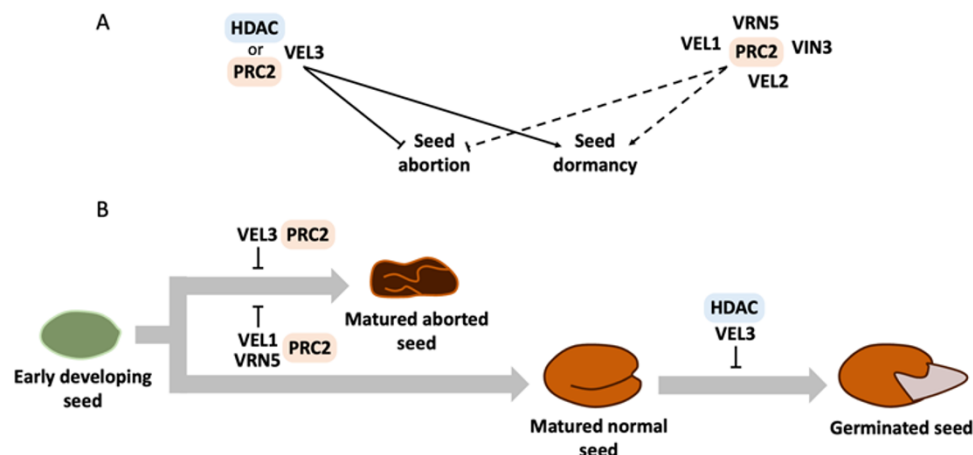


Figure 5. Hypotheses, experimental findings and proposed regulatory model. (A) VEL3, in association with HDAC and possibly PRC2, promotes seed dormancy and suppresses seed abortion in Arabidopsis (Chen *et al.*, 2023). Based on this, we hypothesized that other VEL family members (VEL1, VEL2, VIN3 and VRN5) may exert similar functions through PRC2. Solid arrows indicate previously established regulatory effects, while dotted arrows represent putative control. (B) Experimental results revealed that VEL2 and VIN3 had no detectable effect on seed abortion or dormancy. In contrast, knockout mutations in *VEL1* together with *VRN5* suppressed seed abortion at levels comparable to *vel3-1*, suggesting the involvement of a VEL-PRC2 complex in abortion control. Only VEL3, however, contributed to promoting seed dormancy, consistent with a role for the VEL3-HDAC complex in dormancy established in Chen *et al.* (2023). Grey thick arrows depict the timeline from early seed development through maturation to germination.

activation, effects on seed survival to maturity (which is necessary for dormancy testing) or other subtle differences account for the dormancy differences between *vel3-1* and *vel1 vrn5* double mutants.

Together with prior evidence for a partially redundant VEL1 and VRN5 mode of action in controlling *FLC* (De Lucia *et al.*, 2008), our results support a hypothesis in which VEL1 and

VRN5 proteins recruit or stabilize PRC2 at specific endosperm loci (Fig. 5B). This functional separation underscores a modular chromatin framework in seed development, where distinct components separate seed viability from dormancy programmes.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0960258526100130>.

Acknowledgements. We thank Caroline Dean for providing the *vill-2* (*vell-1*) and *vrn5-8* mutants.

Author contributions. TB-G and SP conceived the study; TB-G, XC and FS performed experiments; TB-G and SP analysed the data; TB-G and SP wrote the manuscript.

Funding statement. This work was supported by Biotechnology and Biological Sciences Research Council Grants BB/T003030/1 to SP.

Competing interests. The authors declare no competing interests.

Data availability. RNA-seq data have been deposited at European Nucleotide Archive under accession PRJEB100848.

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