Plant Direct

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RESEARCH ARTICLE OPEN ACCESS

The Arabidopsis PM19L1 Protein Functions as a Regulator of Germination Under Osmotic Stress

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Received: 4 November 2024 | **Revised:** 13 February 2025 | **Accepted:** 2 March 2025

Funding: This work was funded in part by a studentship granted by the Saudi Arabian Government to Omar Alsaif.

Keywords: abscisic acid | dormancy | germination | MAP kinase pathway | osmosensor | osmotic stress | salt stress | water stress

ABSTRACT

How plants perceive and respond to water availability, especially during the critical stages of seed formation and germination, is key to their survival. During development, ripening, and germination, seeds undergo large changes in water content, down to around 10% during maturation and up to 90% again within 24 h of germination. However, the mechanisms by which plants perceive and respond to their osmotic environment remain largely unknown.

The results presented here indicate that the osmotic environment of the seed is perceived by the PM19L1 protein. We find the Arabidopsis plasma membrane protein PM19L1 is evolutionarily conserved in all land plants, is highly expressed in seeds and seedlings, and regulates germination under osmotic stress, as shown by the reduced germination of the *pm19l1* mutant under salt and osmotic stress. The PM19L1 protein structurally resembles the yeast osmosensor Sho1, and expression of *PM19L1* in yeast will complement the osmosensitive *sho1* mutant, thus PM19L1 can function as an osmosensor.

In contrast to the Sho1-mediated mechanisms for osmotic tolerance in yeast, PM19L1 does not control osmolyte levels in plants, but is a regulator of genes governing abscisic acid and gibberellin synthesis, and of transcription factors that mediate the abscisic acid response. In the *pm19l1* mutant, expression of genes for *ABI3, LEC1,* and *FUS3,* which promote the late maturation of the seed, is downregulated, whereas expression of the *ABI4* and *ABI5* transcription factors, which confer abscisic acid-dependent inhibition of germination, is upregulated. The role of PM19L1 as an osmosensor in the plant was verified by ectopic expression of *PM19L1* which conferred the ability of vegetative plants to respond to imposed osmotic stress by enhanced expression of *ABI3, LEC1,* and *FUS3*. This suggests a function for PM19L1 as a factor that integrates information on the osmotic environment to modulate the developmental fate of the seed during development and germination. Analysis of endogenous hormone levels and phenotypes of digenic mutants, for example *pm19l1/abi3* and *pm19l1/abi4,* will help confirm and refine this model.

In a further parallel to ShoI osmosensing in yeast, intracellular signaling downstream of PM19L1 in the plant likely involves a MAP kinase signal transduction pathway, as shown by split ubiquitin analysis for protein–protein interactions, and by pull-down assays from plant extracts. The MAP kinase proteins AtMKK2 and AtMKK3 specifically bind to PM19L1, and the *atmkk2,* and *atmkk3* mutants

Abbreviations: abscisic acid, (ABA); beta-glucuronidase, (GUS); gibberellic acid, (GA); green fluorescent protein, (GFP); mitogen activated protein kinase, (MAP kinase); MAP kinase, (MKK); Murashige and Skoog, (MS); polyethylene glycol, (PEG); synthetic defined, (SD); synthetic dextrose arginine phosphate, (SDAP).

have strikingly similar germination and gene expression phenotypes to *pm19l1*; however, corroboration of the role of these proteins in the signaling pathway will require further analysis of knockout and gain of function MKK mutants in the *pm19l1* background. These results have implications for the study of dormancy, drought, and salinity tolerance in land plants including crops, and may also provide an insight into evolutionary adaptation of plants to a terrestrial environment.

1 | Introduction

The AWPM-19 (ABA-induced wheat plasma membrane polypeptide) protein was first identified in the plasma membrane fraction of abscisic acid (ABA) treated wheat cells (Koike et al. 1997), and subsequently in dormant embryos of barley (Ranford, Bryce, and Morris 2002). AWPM-19 homologues have been associated with preharvest sprouting, seed primary and secondary dormancy, seed aging and drought tolerance in a number of different species (Barrero et al. 2015; Chen et al. 2015; Yao et al. 2018; Barrero et al. 2019; Bai et al. 2023), and expression of AWPM-19-like genes is enhanced in rice and Arabidopsis by osmotic stress, drought, salt, ABA, and cold (Chen et al. 2015; Yao et al. 2018; Barrero et al. 2019). In the model plant *Arabidopsis thaliana*, AWPM-19 proteins are encoded for by a 4-member gene family (*PM19L1, PM19L2, PM19L3, and PM19L4*) (Barrero et al. 2019), but the function of these highly conserved genes has not been fully elucidated.

Embryo development, seed maturation, germination, and seedling development are physiological processes that are controlled by environmental parameters such as drought and osmotic stress, although the detailed mechanisms of perception for these stresses have remained elusive (Haswell and Verslues 2015; Nongpiur, Singla-Pareek, and Pareek 2020). It is known that the plant hormone ABA, through the agency of conserved transcription factors such as ABI3 (ABA Insensitive 3) (Koornneef, Reuling, and Karssen 1984), ABI4 (ABA Insensitive 4), and ABI5 (ABA Insensitive 5) (Finkelstein 1994), is an essential component regulating the developing and germinating seed's response to osmotic stresses.

ABI3 together with FUS3 (Fusca 3), LEC1 (Leafy Cotyledon 1), and LEC2 (Leafy Cotyledon 2), form the LAFL group of transcription factors which govern seed maturation and desiccation tolerance, and regulation of the phase transition between embryo maturation and seedling development (Parcy et al. 1997; Gazzarrini and Song 2024), whereas ABI4 and ABI5 primarily help control post germination arrest of seedling growth through enhanced sensitivity to ABA under stress conditions and also enhanced biosynthesis of ABA (Lopez-Molina et al. 2002; Lopez-Molina, Mongrand, and Chua 2001; Shu et al. 2013; Chahtane, Kim, and Lopez-Molina 2017), as well as having a role in seed maturation together with ABI3 (reviewed, Finkelstein, Gampala, and Rock 2002; Ali et al. 2022).

In this work we show that the AWPM-19 protein encoded by *PM19L1* (AT1G04560), in addition to its previously reported role in dormancy (Barrero et al. 2019; Bai et al. 2023), functions as an osmosensor and can regulate germination under osmotic stress, likely through signal transduction via a mitogen activated protein (MAP) kinase pathway, resulting in modulation of *ABI3, FUS3, LEC1*, *ABI4,* and *ABI5* transcription factor gene expression and consequently the seed response to the osmotic environment.

2 | Materials and Methods

2.1 | Plant Material and Growth Conditions

The plants used in this study were the wild type (accession Columbia, Col-0), the homozygous T-DNA insertion mutants pm19l1 (line SALK_075435) in the Col-0 background, and transgenic lines bearing the constructs *pCambia1302, pCaMV35S:PM19L1-GFP* and *pPM19L1:PM19L1* in both wild type and *pm19l1*mutant backgrounds, and *pPM19L1:UidA* in the wild type background. In addition, T-DNA insertion mutants *atmkk1,* (Salk line 027645) (Qiu et al. 2008), *atmkk2* (SAIL_511H01) (Qiu et al. 2008), and *atmkk3* (SALK_051970) (Takahashi et al. 2007) were studied. Plants were grown in a growth chamber with 8/16 h light/dark cycle, with 200 μE m-2 s-1 white light, 22/20°C light/dark temperature, and 70% relative humidity in a mixture of John Innes No. 2 (80%), and vermiculite (20%). Unless otherwise indicated, seeds were afterripened at room temperature for at least 2 weeks.

2.2 | Phylogenetic Tree Construction

An existing alignment from Yao et al. (2018) was used and additional sequences added. The Arabidopsis PM19L1 protein sequence was used for BLAST searches on proteomes available on Phytozome and other sources (see Tables S1, S2). No E value cut off was used and all possible sequences were investigated. New protein sequences were added to the existing alignment using mafft-add (MAFFT version 7). The alignment was trimmed to include only regions of the predicted transmembrane domains as illustrated in Figure 1B. This trimmed alignment was used to construct the phylogenetic tree using PhyML 3.0. The LG protein substitution model, nearest neighbor interchange for tree searching and approximate likelihood ratio (SH-like) branch support settings were used.

2.3 | Binary Plasmid Construction and Plant Transformation

To generate the *pCaMV35S:PM19L1-GFP* lines, the full-length ORF of *PM19L1* (AT1G04560) from cDNA clone *PAP111* (Genbank Z29867) (Cooke et al. 1996) was subjected to site-directed mutagenesis using primers PM19-SDM1 and PM19-SDM2 (Table S5) to remove an *Nco*I site from the coding region, and the coding region amplified with primers PM19-NcoI (F) and PM19-Spe1 (R) to introduce an *Nco*I site at the encoded N-terminal and an *Spe*I site at the C terminal, cloned into the pCambia1302 vector over *Nco*I and *Spe*I and checked by sequencing to yield the *pCaMV35S:PM19L1-GFP* construct. The construct was transferred into *Agrobacterium tumefaciens* strain EHA105, as was pCambia 1302 as a control, and

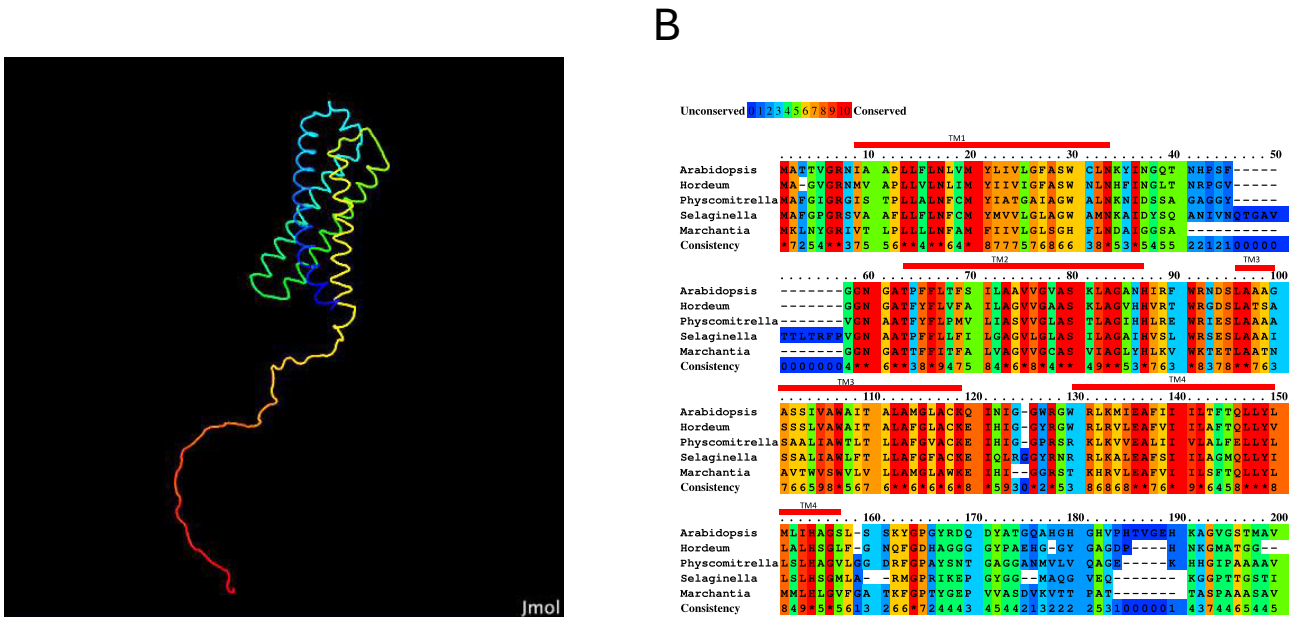


FIGURE 1 | PM19L1 has four transmembrane domains and is highly conserved through terrestrial plant evolution. (A) Predicted secondary structure of PM19L1 protein carried out with AlphaFold (Jumper et al. 2021; Varadi et al. 2022) and visualized with JMol (Jmol:an open-source Java viewer for chemical structures in 3D. http://www.jmol.org) The N terminal is colored blue, the C terminal red. (B) Alignment analysis of PM19L1 proteins from representative members of land plants, including bryophytes, vascular plants, and seed plants. Red bars indicate position of predicted transmembrane domains. Uniprot sequences *Arabidopsis thaliana* 023029 (the encoded protein from *PM19L1*), *Hordeum vulgare* A0A287T362, *Physcomitrella patens* A9SMF8, *Selaginella moellendorffii* D8S1W8, and *Marchantia polymorpha* A0A2R6WWV5 were aligned using Praline multiple sequence alignment (http://ibi.vu.nl/programs/pralinewww/).

used to produce transgenic plants by the floral dip method (Clough and Bent 1998).

To generate the *pPM19L1:PM19L1* complementation lines, a 2894 bp fragment was amplified from wild type Arabidopsis genomic DNA using primers PM19-3 (F) and PM19-4A (R) using Phusion proofreading enzyme. The fragment was digested with *Eco*RI and ligated into binary vector pBinHyg (Qiu et al. 2008) previously digested with *Eco*RI and *Sma*I, which replaces the CaMV35S promoter in the vector. This clone contained 1970 bp of sequence upstream to the *PM19L1* translational start site, the *PM19L1* gene including introns, and 165 bp of genomic sequence upstream to the *PM19L1* translational stop site.

To generate the *pPM19L1:UidA* construct, a 2282 bp fragment was amplified from wild type Arabidopsis genomic DNA using primers PM19-3 (F) and PM19-SDM2(R) and Phusion proofreading enzyme, and cloned into pGEM T-Easy. This clone contained 1970 bp of sequence upstream to the *PM19L1* translational start site, and a further 312 bp of coding sequence. A 1257 bp *Eco*RI-*Bgl*II genomic fragment (*Bgl*II is in the 5′ untranslated region of *PM19L1*) was excised and cloned into the binary vector pPR97 in front of the *UidA* gene (Szabados et al. 1995).

2.4 | Germination Assays and Seedling Growth

Seeds were sown on 0.8% agar media with half-strength Murashige and Skoog (MS) medium (0.5×MS salts, 10 mM MES pH 5.6) and additives as indicated in Figures 3, 4, and 8. Plates were placed in a growth chamber as before. Germination was scored for by observing radicle protrusion, 100 seeds per assay. For stress treatments of seedlings, plants were grown for 14 days on half-strength MS medium plates. Seedlings were then sprayed directly with either a 15% w/v solution of polyethylene glycol (PEG) 8000, 25 μM ABA, 200 mM NaCl, 200 mM KCl, or distilled water and incubated for 24 h. For precocious germination assays, developing siliques at 3 weeks post flowering (long-green stage) were collected, slightly opened at the replum-valve margin and plated on half-strength MS medium plates. For studies on osmotic stress in vegetative plants, 14-day old seedlings were transferred to fresh plates for an additional 7 days of growth. Following this, 2 mL of either 15% w/v PEG 8000, or distilled water was added to the plates and incubated for 24 h before samples were taken.

2.5 | Physiological Measurements

In all cases, triplicate extracts were prepared and measured. Measurement of free proline content was carried out according to Bates, Waldren, and Teare (1973). Soluble sugars were measured according to Yemm and Willis (1954). Potassium and sodium ion measurements were carried out on aqueous plant extracts using Laqua twin ion-specific electrodes (Horiba Scientific). Dormant and active axillary buds were prepared using a protocol described in Seale, Bennett, and Leyser (2017).

2.6 | Statistical Analysis

Germination data was analyzed for standard deviation based on a binomial distribution. Statistically significant differences were calculated using Fisher's exact test followed by a

Bonferroni-Šídák post-hoc analysis. Physiological data was analyzed for statistical significance by two-sided ANOVA and post-hoc Tukey's test for multiple comparisons. GraphPad Prism version 10 was used for statistical calculations.

2.7 | Histochemical Staining for Beta-Glucuronidase (GUS) Assay and GFP Imaging

Histochemical GUS staining was carried out on seeds and seedlings of pPM19L1: UidA lines, grown on agar plate media as described by Jefferson (1987). Images of GUS-stained seedlings were recorded with a Leica DFC320 microscope. Imaging of GFP-expressing stable Arabidopsis lines was performed using an inverted ZEISS LSM880 equipped with a C-Apochromat 40x/1.2 W Korr W27 water immersion objective. Prior to imaging, the seedlings were incubated in 10 μM FM4-64 for 10 min and mounted in water. GFP as well as FM4-64 were excited at 488 nm and GFP was detected at 510–550 nm while FM4-64 was detected at 660–740 nm. To avoid cross talk, the two fluorophores were acquired separately.

2.8 | RNA Extraction and mRNA Expression

Total RNA extraction and cDNA synthesis were performed as described by Alexander, Wendelboe-Nelson, and Morris (2019). Northern blotting was carried out at described by Alzwiy and Morris (2007). RT-qPCR was performed with a StepOnePlus Real-Time PCR System (Applied Biosystems). Primers are listed in Table S5. Standard cycling conditions were 10 min at 95°C followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C; the melting curve profiles were then determined. Reactions were performed in three technical replicas per biological replica and in three biological replicas per line and treatment. Expression values were normalized to Arabidopsis *Actin-2* (At3g18780) and *Ubiquitin-10* (At4g05320); gene expression values were calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak 2008).

2.9 | Yeast Strains and Plasmids

All strains and plasmids used are described in Tables S3 and S4. The ORF from *PM19L1* cDNA clone *PAP111* (Cooke et al. 1996) was amplified with primers PM19-3A and PM19-Xba1 to yield a full-length ORF which was cloned into the *Eco*RV site of pBluescript. *PM19L1* was re-excised with *Bam*HI and *Xba*I and cloned into pYES2 to yield pPMRA1, used to test for complementation of *trk1/trk2* (Bertl et al. 2003) and of *ena1* (Haro, Garciadeblas, and Rodríguez-Navarro 1991). To test for complementation of the YSH642 strain (*gdp1/gdp2*) (Pettersson et al. 2006), auxotrophic selection over leucine was required, therefore the LEU2 expression cassette from pGAD424 was cloned into the *Apa*I and *Nhe*I sites in the URA3 gene of pYES2 to give pPMRA2, and into pYES2-PM19 to give pPMRA3.

For complementation of the *sho1* mutant (Maeda, Takekawa, and Saito 1995), auxotrophic selection over histidine was required; the HIS3 expression cassette from pFA6a-HIS3MX6 was cloned into the *Apa*I and *Nhe*I sites in the URA3 gene of pYES2 to give pPMRA4, and into pYES2-PM19 to give pPMRA5. A shortened version of *PM19L1*, which removed the non-conserved C-terminal was created by use of primers PM19-HindIII and Short PM19- XbaI to yield an amplicon encoding a protein of 149 amino acids (PM19L1Δ) that was cloned into pPMRA4 to give pPMRA6.

To express Arabidopsis MAP kinase (*MKK)* genes together with *PM19L1* in the *sho1* mutant, selection over kanamycin was employed; the KAN expression cassette from pFA6a-KANMX6 was cloned into the *Apa*I and *Nhe*I sites in the URA3 gene of pYES2 to give pPMRA7. Subsequently the open reading frames of *AtMKK1* (At4g26070), *AtMKK2* (At4g29810), and *AtMKK3* (At5g40440) were cloned into pPMRA7 to give pPMRA8, pPMRA9, and pPMRA10 respectively. Site directed mutagenesis was used to change specific amino acids in PM19L1 and these modified versions were introduced into pPMRA4 to give pPMRA11 (79W-G), pPMRA12 (80R-G), and pPMRA13 (82D-G). The Arabidopsis tetraspanin 3 (At3g45600) ORF was amplified using primers TET3-F and TET3-R and cloned into pPMRA4 to give pPMRA14.

2.10 | Yeast Growth Conditions

Yeast cells were grown using standard microbial techniques and media; media designations are as follows: YPAD is 1% Yeast Extract, 2% Peptone, and 2% glucose plus Adenine medium; SDAP is synthetic dextrose arginine phosphate (Rodríguez-Navarro and Ramos 1984). For YP-Gal, glucose was replaced with 2% galactose. SD is Synthetic Defined dropout (SD-dropout) medium. Recombinant plasmid DNA constructs were introduced using the standard lithium acetate method (Gietz and Schiestl 2007).

2.11 | Functional Complementation of Yeast Mutants

For complementation analysis all transformed yeast cells were grown in selective media and collected at a density of OD 0.4–0.6. Subsequent fivefold dilutions were made and 5 μL were spotted onto minimal medium plates containing galactose as a carbon source and the indicated osmotica. Potassium and sodium-dependent phenotypes were analyzed using the PLY240 and L5709 strains (Table S3) as described in Bertl et al. (2003) and Haro, Garciadeblas, and Rodríguez-Navarro (1991) respectively. For potassium importer assays, wild type (JRY379) and *trk1/2* (PLY240) strains transformed with pYES2 or pPMRA1 were grown on SDAP agar plates (pH 5.8), supplemented with 20 mM KCl. For sodium assays wild type (W303-1A) and *ena1* (L5709) strains were transformed with pYES2 or pPMRA1 and grown on SD-ura plates supplemented with of NaCl at 500 mM. Aquaglyceroporin function was tested by transforming the wild type (W303-1A) strain or YSH642 (Pettersson et al. 2006) (Table S3) with pPMRA2 or pPMRA3 and growing them on SD-L plates supplemented with KCl or sorbitol. The *sho1*Δ, *ssk2*Δ, and *ssk22*Δ triple mutant (TM310) (Maeda, Takekawa, and Saito 1995) was transformed with the pPMRA4 control vector, pPMRA5 and pPMRA6, or mutant variants pPMRA11-13 (Table S4). Osmosensitivity of yeast strains was tested by growth

on SD-H (Gal) plates with and without different concentrations of NaCl or sorbitol. The *sho1*Δ, *ssk2*Δ, and ssk22Δ triple mutant containing pPMRA5 or pPMRA6 was then re-transformed with Arabidopsis *AtMKK1*, *AtMKK2* or *AtMKK3* in the yeast vector pPMRA7 containing a kanamycin resistance marker (plasmids pPMRA8-10). These strains were tested by growth on SD-H (Gal) /G418 plates, with and without different concentrations of sorbitol as described in the figure legend (Figure 5).

2.12 | Yeast Two Hybrid Screens

Protein–protein interaction analysis was carried out by utilizing the split-ubiquitin yeast two-hybrid system (Snider et al. 2010). Yeast strain NMY51 was first transformed with the pAMBV bait plasmid containing one of six different PM19L1 variants (pPMRA15-20, Table S4). Auto-activation was determined by co-expressing each bait with NubI "positive" and NubG "negative" control prey plasmids. All constructs proved suitable for screening. Prey vector pPR3C containing one of the full-length *MKK1*, *MKK2,* and *MKK3* sequences (pPMRA21-23, Table S4) was then co-transformed with strains containing the bait plasmids. Transformants were selected for the presence of both bait and prey plasmids during 3 days of growth at 30°C on SD-trp-leu medium. Positive colonies were transferred to liquid SD-trp-leu medium and grown overnight to an OD of 1.0. Five microliters of different dilutions (1:10, 1:100, 1:1000) were spotted onto transformation selection medium (SD-trp-leu, to ensure that spotted cells are carrying the appropriate plasmids) and onto interaction selection medium (SD-trp-leu-his-ade media and SD-trp-leu-Xgal, to select specifically for cells containing interacting bait–prey pairs).

2.13 | Pulldown Assays

Recombinant proteins were produced as maltose binding protein fusions. MBP-MKK1 has been described in (Huang et al. 2000). MBP-MKK2 and MBP-MKK3 were produced in a similar fashion by PCR amplifying and cloning the open reading frame of each gene (primers MKK2-EcoRI_F, MKK2-SalI_R, MKK3-XbaI_F, MKK3-PstI_R) into the pMAL-C2 vector and fusion proteins were expressed and purified using amylose-affinity chromatography as described by the manufacturer (New England Biolabs). The recombinant proteins were then coupled to CNBr-activated Sepharose (Sigma) at a ratio of 2.5 mg protein to 1 mL Sepharose slurry as described by the manufacturers.

Crude protein extracts from 6-day old transgenic lines expressing the constructs *pCaMV35S:PM19L1*-GFP and *pCaMV35S:* GFP were prepared as described in Kadota, Macho, and Zipfel (2016). For pull-down assays, equal amounts of protein extracts were incubated with 100 µL MKK column matrices for 1 h at room temperature followed by five washes with 1 mL wash buffer (50 mM Tris pH 7.5, 50 mM NaF, 500 mM NaCl). Proteins were eluted from the matrix by resuspension in denaturing SDS-PAGE sample buffer. Total soluble proteins were then separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were blocked with PBS containing 4% w/v skimmed milk powder followed by incubation with 1:1000 dilution of mouse anti-GFP antibody (GF28R, Invitrogen) in blocking buffer followed by incubation with 1:5000 dilution of goat anti-mouse IgG secondary antibody, HRP (A4416, Sigma-Aldrich) and detected by chemiluminescence.

3 | Results

The structure of the deduced PM19L1 protein sequence shows four transmembrane domains and a variable C-terminal tail (Figure 1A). The amino acid sequences in the transmembrane domains and in the intracellular loop between helices two and three are highly conserved in all land plants examined, showing the evolutionary antiquity (approximately 470 million years) of this protein (Figure 1B). Homologues were not found in any other organisms including algae (for example the Zygnematophyceae), the closest living relative of land plants (Cheng et al. 2019). This also suggests that the transmembrane domains are not simply structural but have a conserved function. In contrast, the extracellular loops and C-terminus are variable in length and not conserved. We confirmed the plasma membrane localisation of PM19L1 (Koike et al. 1997) by confocal imaging of cells from transgenic Arabidopsis seedlings expressing free GFP or a PM19L1-GFP fusion driven by the CaMV 35S promoter. PM19L1-GFP exhibited colocalization with FM4-64 at the plasma membrane (Figure 2A).

Transgenic Arabidopsis plants bearing the *PM19L1* promoter sequence fused to the UidA marker gene showed high levels of GUS activity in seeds, germinating seedling, but not in vegetative tissues, other than in root tips (Figure 2B). Transcripts of *PM19L1* mRNA were highest during seed development and early germination but were also observed in all other tissues at lower levels. Expression of the three other gene family members was lower than for *PM19L1* in seeds but was seen in all tissues including stems and roots (Figure 3A). *PM19L1* expression fell markedly during germination and early seedling growth (Figure 3B). PEG, salts and ABA treatments all upregulated *PM19L1* expression in 14-day old seedlings, but little effect of these treatments was seen for the other gene family members (Figure 3C).

Phylogenetic analysis for AWPM-19 proteins in representatives of all land plant families shows that the Arabidopsis PM19L1 protein clusters with monocot and eudicot AWPM-19 representatives, and most of the AWPM-19 representatives from the bryophytes. In contrast, PM19L2-4 are found in a separate clade and are more divergent from the bryophyte sequences (Figure S1, Table S1,S2), indicating that PM19L1 may be ancestral to PM19L2-4.

Given the predominantly seed and seedling-specific expression pattern of *PM19L1*, and the induction of *PM19L1* gene expression by osmotica and salts, we suspected that PM19L1 may play a role in seed development and germination. We analyzed a T-DNA insertion mutant for *PM19L1*, (which did not express detectable mRNA for *PM19L1,* Figure S2), for germination-specific traits. The *pm19l1* mutant was found to be more sensitive than the wild type to the presence of salt or sorbitol in the germination medium (Figure 4A). The decreased germination on NaCl or sorbitol was rescued by complementing the mutant line either with a constitutive promoter driving expression of a *PM19L1-GFP*

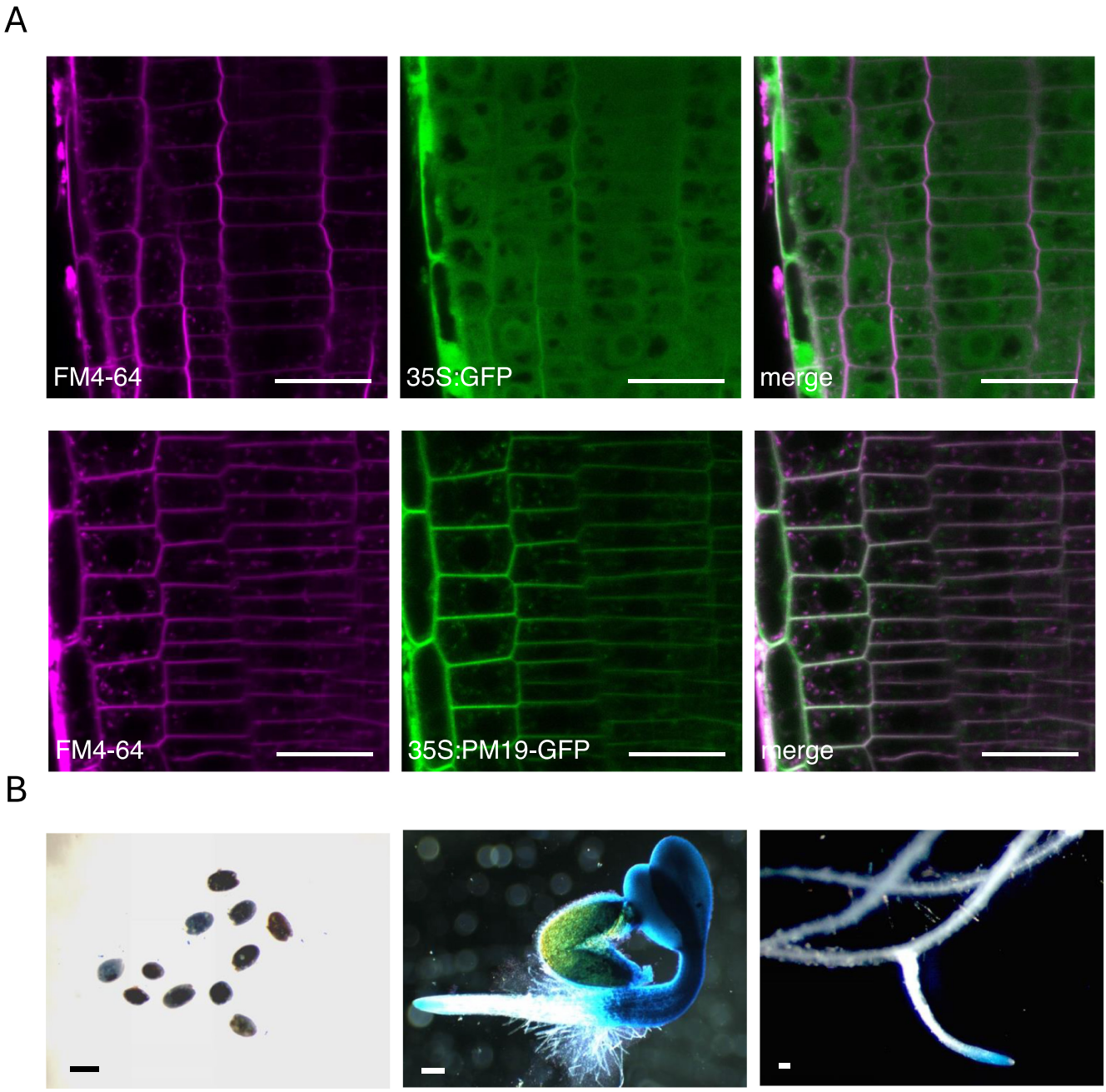


FIGURE 2 | PM19L1 is located in the plasma membrane and expressed in seeds and seedlings. (A) Intracellular localisation of free GFP (top row) and PM19L1-GFP (bottom row) (scale bar 20 μm) in transgenic Arabidopsis seedling roots as visualized by confocal microscopy. Cells were counterstained with FM4-64 (magenta) to visualize the plasma membrane. (B) Tissue-specific localisation of *PM19L1* gene expression as visualized by staining seedlings bearing the *pPM19L1:UidA* construct for GUS activity, showing from left to right high levels of expression in seeds (scale bar 250 μm), germinating seedlings and in lateral root tips of 14-day old seedlings (scale bars 100 μm).

fusion (*pCaMV 35S:PM19L1-GFP*), or by the native *PM19L1* promoter and gene (*pPM19L1:PM19L1*) (Figure 4B). The expression pattern and cell membrane location of *PM19L1*, together with the germination phenotype of the *pm19l1* mutant suggests a potential protein function associated with the seed response to osmotic stress.

We explored possible role of the PM19L1 protein by genetic complementation of different yeast mutants with phenotypes associated with osmotic stress. Sho1 is an osmosensor that permits growth of yeast on media of high osmolarity by stimulating the Hog1 signaling pathway controlling the accumulation of the osmolyte glycerol (Maeda, Takekawa, and Saito 1995; Saito and Posas 2012; Tatebayashi et al. 2020). A number of constructs were prepared to explore the ability of *PM19L1* to complement *shoI* and to interact with potential downstream signaling partners (Figure 5A). The *sho1* mutant was fully complemented when *PM19L1* was expressed, permitting growth on up to 500 mM NaCl or sorbitol. The deletion of the sequence encoding the variable C-terminus of PM19L1 had no impact on the ability of PM19L1 to complement sho1 (Figure 5B). Sho1, like PM19L1, is a four transmembrane domain plasma membrane

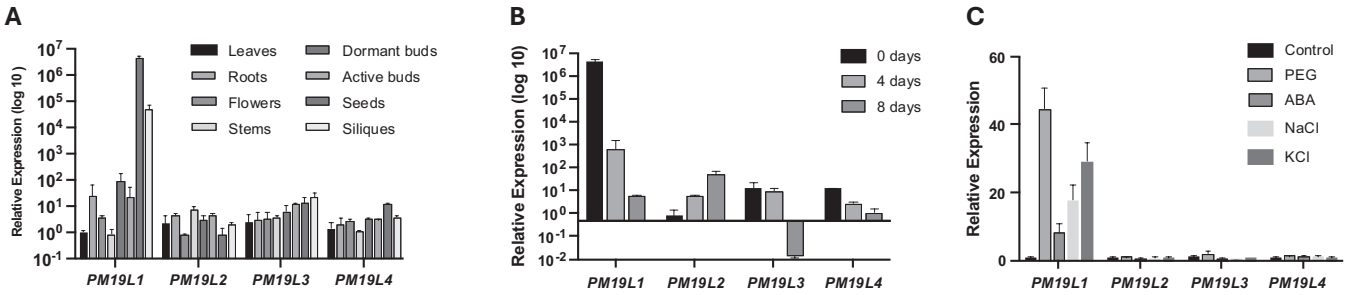


FIGURE 3 | *Q-PCR analysis of the PM19L1-4 genes.* Q-PCR analysis of expression patterns for Arabidopsis *PM19L* gene family members *PM19L1* (AT1G04560), *PM19L2* (AT5G46530), *PM19L3* (AT1G29520), and *PM19L4* (AT5G18970) in (A) different organs, (B) during germination (bars show days of germination), and (C) in 14-day old seedlings in response to osmotic stresses (15% PEG 8000, 200 mM NaCl, 200 mM KCl), and 25 μM ABA. Bars show standard deviation, three biological replicates.

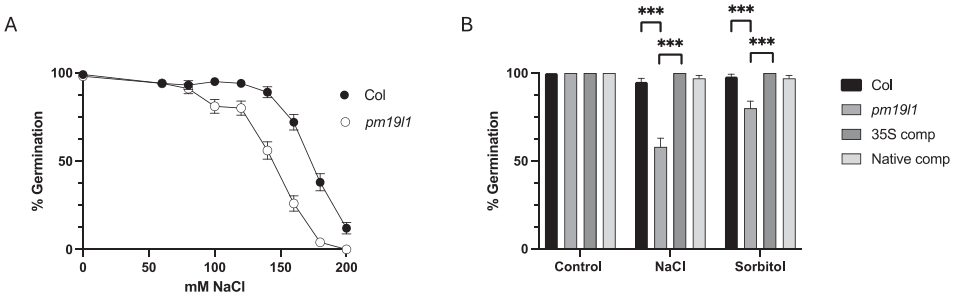


FIGURE 4 | The *pm19l1* mutant is sensitive to salt and osmotic stress during germination. (A) germination of 100 Columbia wild type and *pm19l1* mutant seeds on media with varying concentrations of NaCl. (B) Germination of 100 Columbia wild type, *pm19l1*and lines complemented with *pCaMV 35S:PM19L1-GFP* or the native promoter (*pPM19L1:PM19L1*) on control media and media containing 100 mM NaCl or 200 mM sorbitol. Bars show standard deviation (based on binomial distribution, 100 seeds). Statistically significant differences, using Fisher's exact test followed by a Bonferroni-Šídák post-hoc analysis are indicated with *** showing $p \leq 0.001$.

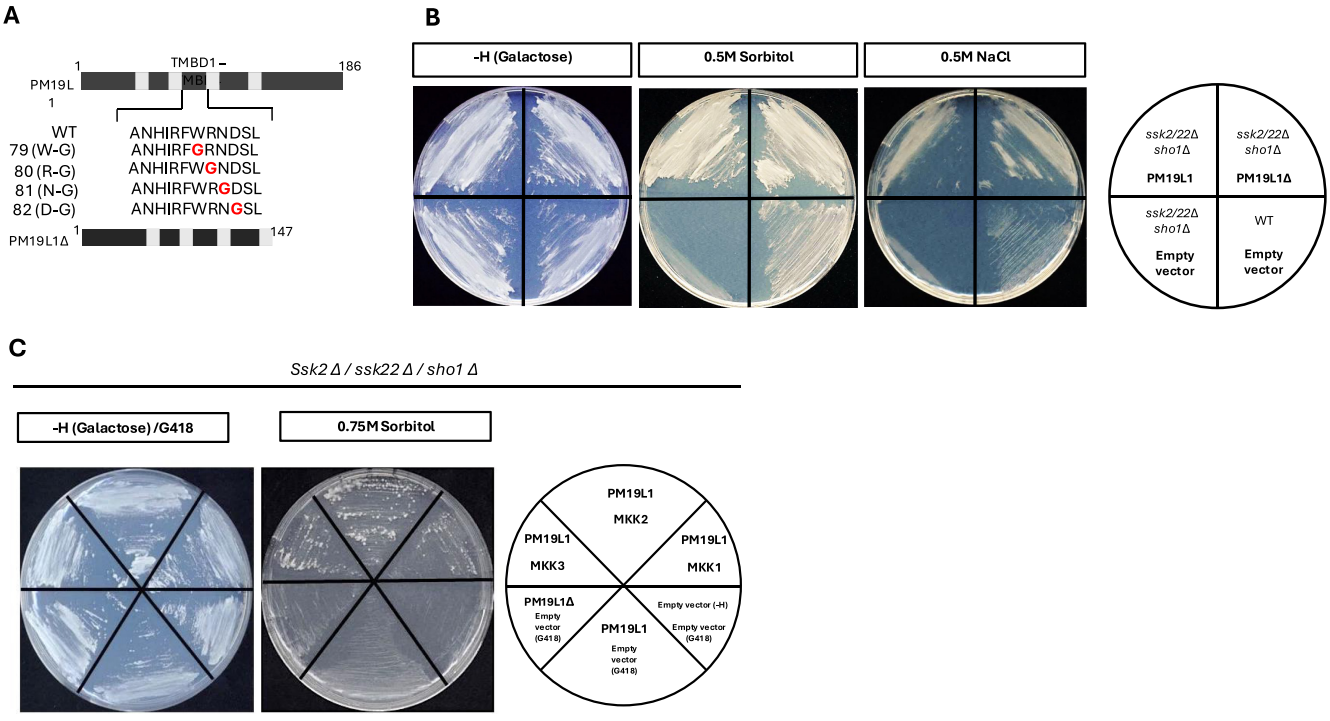


FIGURE 5 | Complementation of the *sho1* mutant in yeast by expression of *PM19L1* (A) shows the constructs, expression of full-length *PM19L1* (plasmid pPMRA5), site directed mutagenesis of conserved amino acids in the intracellular loop, and truncated *PM19L1* with the C terminus removed (*PM19L1Δ*) (plasmid pPMRA6). (B) growth of wild type, *skk1/skk2/sho1* mutant, *skk1/skk2/sho1* transformed with *PM19L1*, and with *PM19L1Δ* on basal media, 0.5 M sorbitol, and 0.5 M NaCl. (C) co-expression of Arabidopsis MAP kinase (*AtMKK1*, *AtMKK2,* and *AtMKK3*) enhances the ability of *PM19L1* to complement *sho1*, resulting in growth on up to 0.75 M sorbitol.

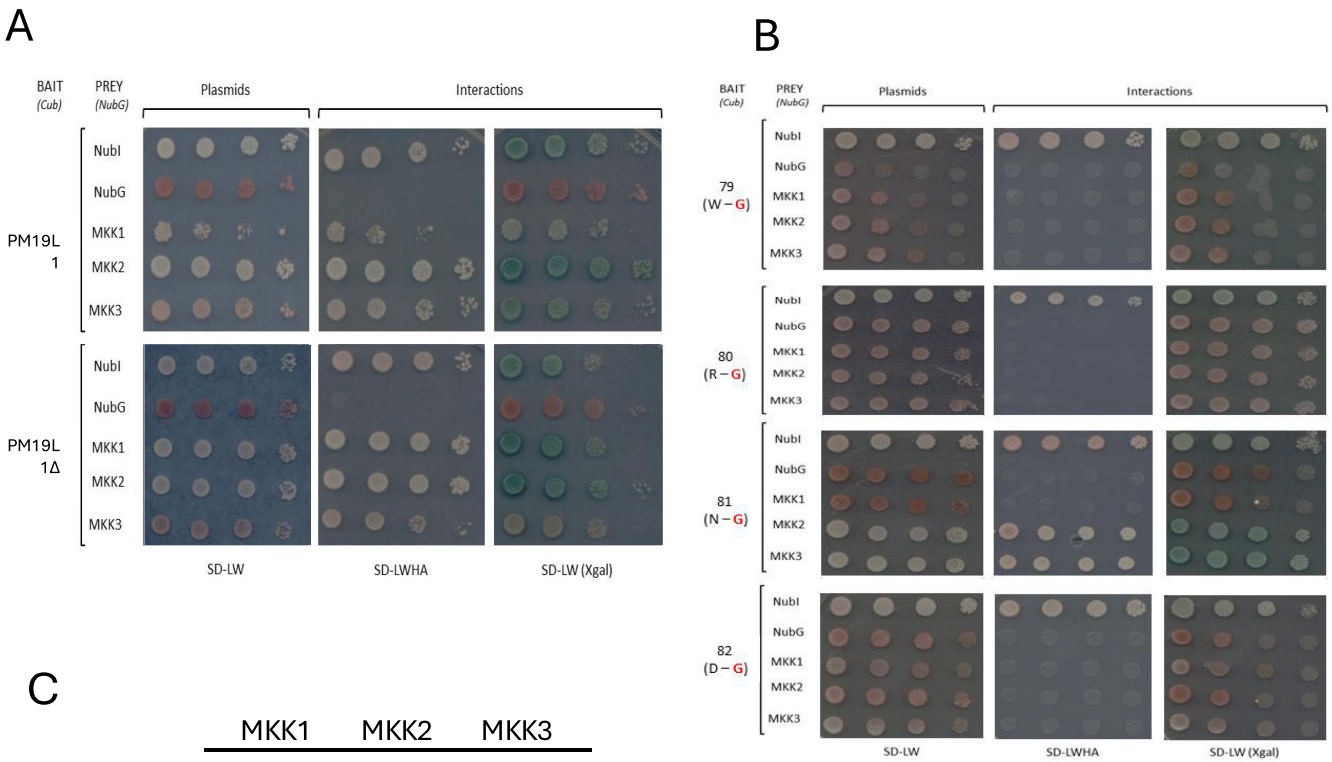
protein (Figure S3) with an extended C terminal tail but has no protein sequence homology with the AWPM-19 proteins. The ability of an unrelated Arabidopsis four transmembrane domain plasma membrane protein, tetraspanin 3, was tested for its ability to complement *sho1*, but it failed to do so (Figure S4), thus the PM19L1 protein is specific in its ability to function as an osmosensor and suggests a role in the control of seed physiology through sensing of water status. Other potential mechanisms for the role of PM19L1 were also explored using yeast mutants. No complementation was seen for the potassium influx transporter mutants *trk1* and *trk2* (Bertl et al. 2003) (Figure S5) nor the sodium efflux mutant *ena1* (Haro, Garciadeblas, and Rodríguez-Navarro 1991) (Figure S6). The yeast glycerol biosynthetic mutant *gdp1/gdp2* (Pettersson et al. 2006) was also not complemented, indicating that PM19L1 does not have an aquaporin-like function (Figure S7).

The yeast osmosensing pathway initiates from two osmosensors, the histidine kinase Sln1 and Sho1. Downstream from both osmosensors is a kinase signal transduction pathway that is activated under conditions of high osmolarity, with Sln1 activating Ssk2 and Ssk22 (two redundant MAP kinase kinases, MKKK), and the MKKK Ste11 being activated by Sho1. Both Ssk2/Ssk22 and Ste11 will in turn activate the MAP kinase (MKK) Pbs2 (which physically interacts with Sho1), and then Pbs2 activates the Map kinase Hog1, resulting in the accumulation of glycerol and the expression of aquaporins to permit water ingress (O'Rourke, Herskowitz, and O'Shea 2002; Tatebayashi et al. 2015). The *pbs2* mutant is compromised by osmotic stress, however, growth of *pbs2* on media of high osmolarity is restored by expression of Arabidopsis AtMKK2 (Ichimura et al. 1998). If MAP kinase signaling is also involved in osmosensing downstream of PM19L1, then co-expression of Arabidopsis MKK genes together with PM19L1 in the yeast *sho1* mutant background might be expected to further enhance growth under stress. This was found to be the case for *AtMKK1*, *AtMKK2,* and *AtMKK3*. These Arabidopsis MKK genes have all reported to be associated with germination stress, with knockout mutants more sensitive to salt, although we have not been able to confirm this for AtMKK1 (Figure S8) (Conroy et al. 2013; Hwa and Yang 2008; Teige et al. 2004). When introduced into the *sho1* mutant, co-expression of the Arabidopsis MKKs permitted growth on up to 0.75 M sorbitol, whereas PM19L1 on its own only permitted growth on up to 0.5 M sorbitol (Figure 5C), indicating that Arabidopsis MKK proteins may be involved with signaling from PM19L1. This association was corroborated by a split ubiquitin analysis in which PM19L1 (and C-terminally truncated PM19L1) was found to interact with AtMKK1 (weak interaction), AtMKK2 and AtMKK3 (which both showed a stronger interaction) (Figure 6A). MKKs are cytosolic proteins and thus might be expected to interact with the intracellular domains of PM19L1. To test this, site-directed mutagenesis of conserved amino acids within the predicted intracellular loop between transmembrane domains 2 and 3 (79 W-G; 80 R-G, 82 D-G) was carried out; it was found that these changes abolished any interaction. However, when a non-conserved amino acid (81 N-G) in the same loop was changed, this was not the case (apart for AtMKK1) (Figure 6B,C). Complementation of *sho1* by *PM19L1* expression was abolished by mutagenesis of the conserved amino acids, suggesting that the yeast MKK PBS2 also binds PM19L1 at these sites (Figure S9). The interaction of PM19L1 with Arabidopsis MKK proteins was confirmed by pull-down assays with immobilized recombinant MBP-MKK1, MBP-MKK2, and MBP-MKK3 proteins incubated with extracts from plants expressing free GFP or PM19L1-GFP. MBP-MKK2 and MBP-MKK3, but not MBP-MKK1, specifically interacted with PM19L1-GFP in this assay (Figure 7), which accords with the weak MKK1-PM19L1 interaction seen in the split ubiquitin assay.

The physiology of the *pm19l1* mutant was further investigated to gain an insight into the mechanism of action of this osmosensor in regulating germination. Levels of sodium and potassium ions in seedlings of the *pm19l1* mutant did not differ from the wild type when grown on 60 mM sodium or potassium salts (Figure S10), further evidence against the possibility that the *PM19L1* gene encodes a potassium or sodium transporter. Osmolyte accumulation (proline and total soluble sugars) in salt-stressed seedlings were no different in wild type and *pm19l1* seedlings (Figure S11A, B), making it unlikely that PM19L1 plays a role in adaptation to osmotic stress by means of osmotic accommodation.

Experiments using inhibitors of ABA and GA synthesis point towards an involvement of ABA in the *pm19l1* phenotype. Inclusion of 150 μM sodium tungstate, an inhibitor of ABA biosynthesis (Lee and Milborrow 1997), partially rescued *pm19l1* germination inhibition by 150 mM NaCl (Figure 8A), whereas inclusion of GA in the germination medium enhanced germination of *pm19l1* on 100 mM NaCl (Figure 8B). The biosynthesis of gibberellin can be inhibited by paclobutrazol (Dalziel and Lawrence 1984), and inclusion of 50 or 100 μM paclobutrazol in the germination medium inhibited germination, with a greater impact on *pm19l1*, implying that the *pm19l1* mutant is more dependent on GA synthesis for germination than the wild type (Figure 8C). Expression of genes for ABA and gibberellin metabolism were also investigated (Figure 9A). NCED6 is a 9-cis-epoxycarotenoid dioxygenase involved in the early steps of ABA biosynthesis (Lefebvre et al. 2006). *NCED6* transcripts were elevated relative to wild type in *pm19l1*, *atmkk2,* and *atmkk3* seeds, indicative of enhanced ABA synthesis. CYP707A1 is an ABA hydrolase (Okamoto et al. 2006), and transcripts for the corresponding gene were reduced in *pm19l1* and *atmkk3*. GA20OX1 catalyzes conversion of GA12 to GA9 (Phillips et al. 1995), and transcripts for this gene were downregulated in *pm19l1* and *atmkk3*. These results suggest that the relative levels of synthesis of ABA and GA are in part determinants of the *pm19l1* germination phenotype.

Transcript levels of the ABA-associated transcription factors that regulate phase transition, desiccation tolerance and germination, *ABI3*, *FUS3*, *LEC1*, *ABI4,* and *ABI5* were measured in after-ripened dry seeds of *pm19l1*, and in *atmkk2* and *atmkk3* mutant seeds. Relative to levels in wild type seeds, for all the mutant lines the LAFL transcription factor genes *ABI3, FUS3,* and *LEC1* levels were reduced between 10 and 100-fold, and both *ABI4* and *ABI5* transcripts were enhanced some 5 to 10-fold (Figure 9B), indicative of a physiological shift in these seeds away from the maturation phase governed by *ABI3, FUS3,* and *LEC1* towards preparation for germination, but also with enhanced ABA synthesis mediated by *ABI4* (Shu



C

	MKK1	MKK2	MKK3
PM19L1	+	++	++
79 (W-G)	-	-	-
80 (R-G)	-	-	-
81 (N-G)	-	++	++
82 (D-G)	-	-	-
PM19L1Δ	++	++	+

FIGURE 6 | Interaction of PM19L1 with Arabidopsis MKK proteins. (A) Split ubiquitin assays showing interactions between PM19L1 or PM19L1*Δ* with Arabidopsis MKK proteins AtMKK1, AtMKK2, and AtMKK3. NubI is a positive control, NubG a negative control, SD-LW medium selects for presence of bait and prey proteins, SD-LWHA shows growth on media minus histidine and adenine, and SD-LW (Xgal) shows activity of LacZ reporter gene (blue color). (B) Split ubiquitin assays showing interactions between PM19L1 or PM19L1*Δ* with Arabidopsis MKK proteins after site-directed mutagenesis to glycine of conserved (79 W-G, 80 R-G, and 82 D-G) or non-conserved (81 D-G) amino acids in the intracellular loop of PM19L1. (C) is a summary of the split ubiquitin analysis of PM19L1 interactions with Arabidopsis MKK proteins.

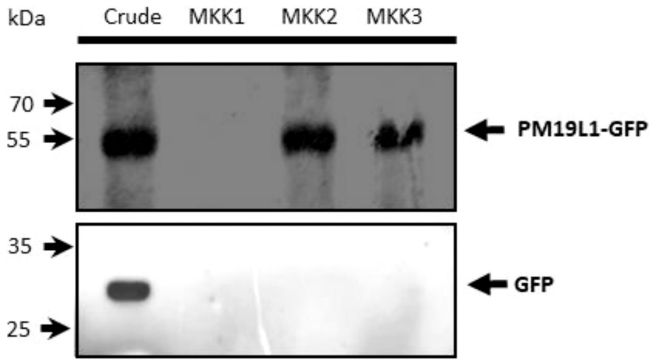


FIGURE 7 | Pulldown assay showing interaction between MKK proteins and PM19L1. Immobilized MBP-MKK was incubated with extracts from plants expressing PM19L1-GFP or free GFP. Bound proteins and crude extract were fractionated by SDS-PAGE followed by immunoassay for GFP. MBP-MKK2 and MBP-MKK3 bind to PM19L1-GFP but not to free GFP. Top panel, PM19L1-GFP, lower panel, free GFP.

et al. 2013) and greater sensitivity of *pm19l1* to ABA and osmotic stress through ABI4 and ABI5 (Finkelstein et al. 2011; Lopez-Molina, Mongrand, and Chua 2001; Lopez-Molina et al. 2002).

To further explore the function of PM19L as an osmosensor, wild type plants and plant ectopically expressing a *PM19L1-GFP* fusion (previously shown to complement the *pm19l1* mutant, Figure 4B), were grown on agar plates and three-week old plants, which normally do not express high levels of *PM19L1*, were subjected to 24 h osmotic stress by adding a 15% PEG solution to the plates followed by Q-PCR analysis. Expression levels of *ABI3*, *FUS3,* and *LEC1* were significantly enhanced in the stressed *PM19L1-GFP* plants, whereas no change was seen for *ABI4* or *ABI5* (Figure 10). This is further evidence that PM19L1 functions as an osmosensor in the plant, controlling the gene expression of key transcription factors that help the plant adjust to osmotic stresses.

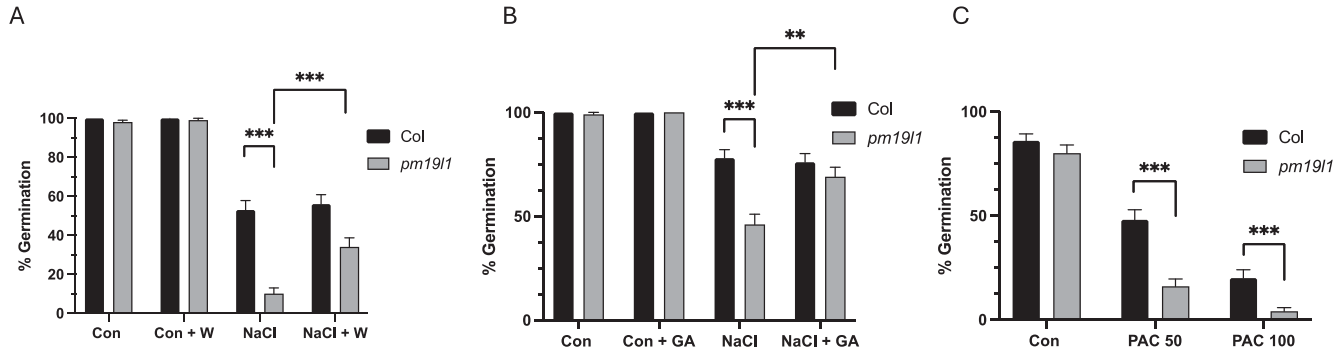


FIGURE 8 | Role of ABA and GA in salt sensitivity during germination. (A) After-ripened seeds were germinated for 7 days in media containing 150 mM NaCl with and without 150 μM of the ABA synthesis inhibitor sodium tungstate (W); seeds of *pm19l1* are partially rescued from salt inhibition by growth on tungstate. (B) Seeds were germinated in media containing 100 mM NaCl with and without 10 μM gibberellin (GA); seeds of *pm19l1* had enhanced germination in the presence of exogenous gibberellin. (C) Seeds were germinated in the presence of different concentrations (50 or 100 μM) of the GA synthesis inhibitor paclobutrazol (PAC); *pm19l1* seeds were more strongly inhibited from germination by PAC than the wild type. Bars show standard deviation (based on binomial distribution, 100 seeds). Statistically significant differences, using Fisher's exact test followed by a Bonferroni-Šídák post-hoc analysis, are indicated with *** showing $p \leq 0.001$ and ** showing $p \leq 0.01$.

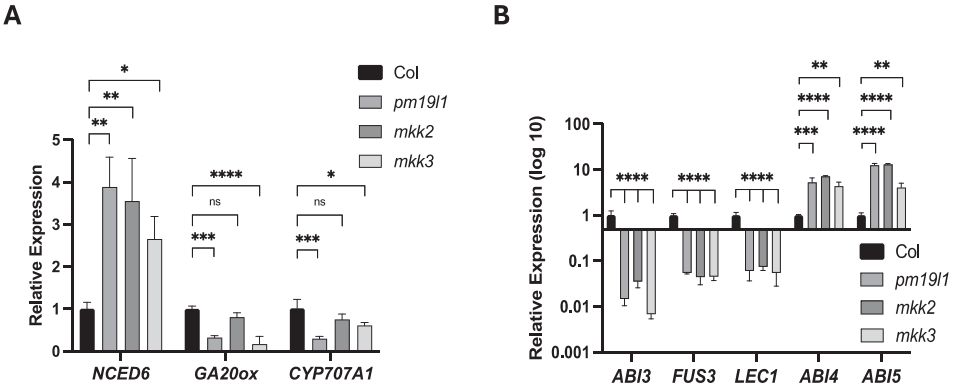


FIGURE 9 | Expression analysis of hormone metabolism and ABA associated transcription factor genes in seeds. (A) Q-PCR analysis of wild type, *pm19l1*, *atmkk2,* and *atmkk3* seeds for the ABA synthesis gene *NCED6*, the GA synthesis gene *GA20ox1* and the ABA catabolism gene *CYP707A1*. (B) Q-PCR analysis of wild type (Col), *pm19l1*, *atmkk2,* and *atmkk3* seeds for the transcription factor genes *ABI3*, *FUS3*, *LEC1*, *ABI4,* and *ABI5*. Both *atmkk2* and *atmkk3* resemble *pm19l1*, with reduced *ABI3, FUS3, LEC1* expression and enhanced *ABI4* and *ABI5* levels. Statistically significant differences relative to the wild type, using Dunnett's test at $a = 0.05$, are indicated with an asterisk, **** showing $p \leq 0.0001$, *** showing $p \leq 0.001$, ** showing $p \leq 0.01$, * showing $p \leq 0.05$, ns = not significant.

PM19L1 has previously been described as a negative regulator of dormancy in Arabidopsis (Barrero et al. 2019). To confirm and explore this further, green siliques at 3 weeks post fertilization were placed on agar plates at 20°C. Immature wild type seeds germinated after 7 days, whereas germination was reduced for the immature *pm19l1* seeds (Figure S12). To study primary dormancy in mature seeds, Columbia wild type and *pm19l1* plants were grown at 20°C or at 14°C to induce enhanced primary dormancy (Kendall et al. 2011). Freshly harvested seeds were put to germinate at 20°C. When grown at 14°C, *pm19l1* seeds showed greater dormancy than the wild type. Both wild type and mutant responded to 2 days stratification at 4°C with enhanced germination (Figure S12). The expression of *ABI3* was downregulated in *pm19l1* grown at both temperatures and also in dormant seeds of the wild type, whereas *ABI4* and *ABI5* were found to be upregulated 5–10 fold in *pm19l1* and in dormant wild type seeds (Figure S12). As *pm19l1* seeds express higher levels of the dormancy-inducing transcription factor genes *ABI4* and *ABI5,* even when grown at 20°C, and only minor increase in these transcripts when grown at lower temperatures, this may not be the reason for the enhanced dormancy of *pm19l1* seeds grown at 14°C, and other, as yet unidentified, factors may be involved.

4 | Discussion

The work presented here shows that in addition to its previously reported effects on dormancy (Barrero et al. 2019; Bai et al. 2023), the conserved Arabidopsis PM19L1 protein is an osmosensor that regulates germination under osmotic stress through modulation of the ABA-associated transcription factors ABI3, FUS3, LEC1, ABI4, and ABI5. Several lines of evidence independently point towards PM19L1 being an osmosensor. Firstly, the phenotype of the *pm19l1* mutant, as loss of function of *PM19L1* results in enhanced germination sensitivity to salt and osmotic stress. Secondly, *PM19L1* complements the yeast *sho1* osmosensor mutant, whereas a control gene (tetraspanin)

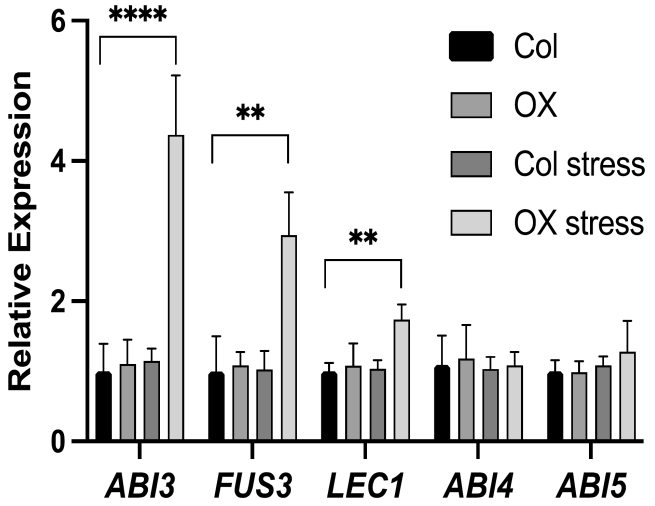


FIGURE 10 | Expression analysis of ABA associated transcription factor genes in stressed plants. Q-PCR analysis of transcription factor genes *ABI3*, *FUS3*, *LEC1*, *ABI4,* and *ABI5* in 21-day old wild type (Col) or plants ectopically overexpressing *PM19L1-GFP* (OX) with or without 24 h osmotic stress (15% w/v PEG 8000). Bars show standard deviation, three biological replicates. Statistically significant differences relative to the wild type, using Dunnett's test at $\alpha = 0.05$, are indicated with an asterisk, **** showing $p \leq 0.0001$, *** showing $p \leq 0.001$, ** showing $p \leq 0.01$, * showing $p \leq 0.05$, ns = not significant.

encoding a topologically similar protein does not. Thirdly, over-expression of *PM19L1* in Arabidopsis confers the ability of vegetative plants to react to osmotic stress by upregulating expression of the phase transition-regulating transcription factors *ABI3, FUS3,* and *LEC1*.

Complementation of yeast osmosensor mutants has been previously used to identify other potential plant osmosensors. The *sln1* mutant (defining the other branch of the yeast Hog1 pathway) is complemented by the Arabidopsis histidine kinase *AHK1* (Tran et al. 2007; Urao et al. 1999), and *ahk1* mutants are sensitive to osmolytes during germination (Wohlbach, Quirino, and Sussman 2008). However, the role of AHK1 in the mature plant has been contested as it is additionally associated with regulation of stomatal density (Kumar, Jane, and Verslues 2013). Two other candidates for plant osmosensors have recently been reported. The millet DROOPY LEAF 1 (DPY1) protein is a receptor-like kinase thought to mediate drought stress in vegetative tissue (Zhao et al. 2023). In Arabidopsis, the DECAPPING 5 (DCP5) protein is thought to be an intramolecular crowding sensor that responds to osmotic stress (Wang et al. 2024).

The function of PM19L1 in regulating seed germination is likely channeled through MKK protein signaling as demonstrated by a number of independent lines of evidence; enhancement of the yeast *shoI* complementation through co-expression of Arabidopsis MKK genes, split ubiquitin assays in yeast showing a physical interaction between PM19L1 and both AtMKK2 and AtMKK3, abolition of PM19L1-MKK interactions through site directed mutagenesis of the putative target site, and by pulldown assays with recombinant proteins and plant protein extracts. In addition, knockout lines of *atmkk2* and *atmkk3* also showed similar germination phenotypes and gene expression patterns to *pm19l1*. This accords with the observations that plants overexpressing constitutively active forms of AtMKK2 and AtMKK3 showed enhanced salt tolerance (Teige et al. 2004; Hwa and Yang 2008). AtMKK3 activation has also recently been shown to be involved in promotion of ABA catabolism and GA synthesis during afterripening of seeds (Otani et al. 2024). These observations are consistent with the concept that the AtMKK signaling proteins form part of the same osmotic stress signaling pathway together with PM19L1.However, full corroboration of the role of these proteins in the osmosensing pathway will require further analysis, for example phenotypic analysis of knockout and gain of function AtMKK mutants in the *pm19l1* background.

The plant hormone ABA is implicated in the control of seed development, dormancy, and germination. Analysis of the *pm19l1* mutant shows that PM19L1 modulates expression of ABA signaling genes. Levels of *ABI4* and *ABI5* expression are enhanced in *pm19l1* knockout seeds. This suggests a possible mechanism for the increased osmotic sensitivity of the *pm19l1* mutant. ABI4 controls seed development and germination by enhancing sensitivity to ABA (Finkelstein, Gampala, and Rock 2002; Finkelstein et al. 2011; Zhao et al. 2020) and is a positive regulator of ABA biosynthetic genes and a negative regulator of genes involved in ABA inactivation and GA biosynthesis (Shu et al. 2013; Shu et al. 2016). This accords with our findings that for *pm19l1*, *NCED6* expression is upregulated, and *CYP707A1* expression downregulated, expression of *GA20ox1* is reduced, that paclobutrazol has a greater impact on germination of *pm19l1* than of the wild type, and that exogenous GA will enhance germination of *pm19l1* on salt. A further mechanism by which ABI4 may act as an attenuator of germination is because ABI4 promotes ROS production through the expression of RbohD and hence represses germination (Luo et al. 2021).

ABI5 expression is promoted by ABA, and also by ABI4; ABI5 enhances sensitivity to ABA, leading to inhibition of germination and to seedling growth arrest under stress conditions, whereas *abi5* mutants are ABA and salt insensitive (Lopez-Molina, Mongrand, and Chua 2001; Skubacz, Daszkowska-Golec, and Szarejko 2016).

Taken together, these results suggest a pathway in the wild type in which the osmotic environment is perceived through PM19L1 and under favorable conditions for germination, signal transduction through AtMKK2 and AtMKK3 modulates *ABI4* and *ABI5* expression, resulting in a reduction of ABA sensitivity, and an increase in GA levels in the seed, hence promoting germination. In the *pm19l-1* mutant, a reduction in feedback on the osmotic status of the seed results in reduced germination, particularly under unfavorable conditions, as *ABI4* and *ABI5* expression is not repressed. An analysis of endogenous ABA and GA levels in *pm19l1* seeds and seedlings under osmotic stress conditions would help to refine this model. Given that other members of the AWPM-19 protein family, PM19L2, PM19L3, and PM19L4 are also expressed in seeds, there may be some genetic redundancy for osmosensing during germination.

In contrast to *ABI4* and *ABI5*, levels of the LAFL genes *ABI3, FUS3,* and *LEC1* are markedly reduced in mature *pm19l1* seeds. As well as contributing to the ABA signaling pathway, ABI3 (together with FUS3, LEC1, and LEC2), is a transcription factor that controls the transition between embryo development and seed

maturation (Parcy et al. 1997; Jia, Suzuki, and McCarty 2014; Gazzarrini and Song 2024). Severe *abi3* mutants are characterized by a failure to transition to the mature phase of seed development, remaining green and desiccation intolerant, and with reduced dormancy (Nambara, McCourt, and Naito 1995). This extreme phenotype was not observed in *pm19l1* presumably since low levels of *ABI3* transcript are still present, but the developmental phase transition controlled by the LAFL genes may be in part regulated by the osmotic environment of the desiccating seed through PM19L1.

The wheat PM19L1 homologues *PM19-A1* and *PM19-A2* have been identified as QTL candidate genes for preharvest sprouting and dormancy (locus Phs1 on chromosome 4A, (Barrero et al. 2015)), high levels of *PM19-A1* expression and deletions in the promoter region of *PM19-A2* are found in dormant wheat accessions. As observed by Barrero et al. (2019), the enhanced expression of *PM19-A1* resulting in dormancy is contrary to the finding that Arabidopsis *pm19l1* knockouts have enhanced dormancy, however, it should be noted that the two proteins differ by 40 non-conserved amino acids over a length of 186 amino acids for PM19L1, thus it is possible there are differences in the downstream mechanisms of action.

Other dormancy QTL genes in both wheat and barley have been identified as being homologues of *AtMKK3*, the wheat *MKK3*, also mapping to QTL Phs1 (Nakamura et al. 2016; Torada et al. 2016). This indicates that in cereals, PM19-A1/PM19-A2 and MKK3 may both contribute to regulation of germination and preharvest sprouting. The rice PM19L1 homologue OSPM has previously been noted to facilitate ABA influx into yeast (Yao et al. 2018), which may also be a potential mechanism that facilitates control of germination and dormancy.

Although there are strong parallels between the PM19L1 pathway in plants and the Sho1 pathway in yeast, the responses to osmotic stress signaling are very different. Sho1 signaling in response to stress results in the synthesis of osmolytes such as glycerol, GFP whereas PM19L1 signaling results in changes in seed developmental physiology. The mode of action of PM19L1 also differs from that of the DPY1 and DCP5 osmosensors as these enhance production of osmoprotectants (DPY1) and repression of growth-promoting genes/upregulation of genes involved in osmo-adjustment such as ion transporters (DCP5). Lastly, the evolutionary ancient roots of PM19L1, and the absence of this gene family from the aquatic ancestors of land plants, the algae, may indicate an important adaptation to terrestrial life for plants in regulating the plant response to osmotic stress.

Author Contributions

RA carried out yeast complementation, yeast split ubiquitin assays and Q-PCR work, and wrote the manuscript. PCP carried out mutant phenotype analysis and produced plant transformation constructs, OA carried out mutant phenotype analysis and plant transformations, NM carried out mutant phenotype analysis, VS carried out GFP imaging, YS carried out gene expression studies and helped with protein–protein interaction work, MS carried out gene expression studies and protein phylogeny, PCM conceived the project, designed experiments, carried out plant transformations and mutant phenotype analysis and wrote the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are available in the main text or the supplementary materials. Materials are available on request to the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.