Evolutionary Comparison of the Developmental/Physiological Phenotype and the Molecular Behavior of SPIRRIG Between Arabidopsis thaliana and Arabis alpina

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Beige and Chediak Higashi (BEACH) domain proteins mediate membrane-dependent processes in eukaryotic cells. The plant BEACH domain protein SPIRRIG in A. thaliana (AtSPI) was shown to display a similar molecular behavior as its yeast and animal homologs, along with a range of cell morphological defects. In addition, AtSPI was shown to interact with the P-body component DCP1, to differentially effect RNA levels and to be involved in the regulation of RNA stability in the context of salt stress responses. To determine, whether the dual function of SPI in apparently unrelated molecular pathways and traits is evolutionary conserved, we analyzed three Aaspi alleles in Arabis alpina. We show that the molecular behavior of the SPI protein and the role in cell morphogenesis and salt stress response are similar in the two species, though we observed distinct deviations in the phenotypic spectrum.

Keywords: morphogenesis, membrane trafficking, Arabis alpina, BEACH domain protein, SPIRRIG, salt response

INTRODUCTION

The mechanisms of cell growth and morphogenesis are in the focus of plant science for decades. Due to fully sequenced model organisms like Arabidopsis thaliana and excellent model systems for cell differentiation like root hairs and trichomes (Mathur and Hülskamp, 2002; Bögre et al., 2008), genetic and environmental factors regulating growth, size and shape of plant organs are better understood nowadays. Morphogenesis is thought to be controlled predominantly by endoreduplication and thereby cell size control, cytoskeletal dynamics, vesicle transport and small GTPase signaling (Guimil and Dunand, 2007).

A group of mutants with distorted cell shapes was identified in EMS screens in Arabidopsis thaliana (Koornneef et al., 1983, 1987; Hülskamp et al., 1994), which show altered trichome morphology, irregular shapes of leaf pavement cells, epidermal hypocotyl cells and epidermal root cells. The corresponding distorted genes were found to encode proteins of the actin regulating ARP2/3 or SCAR/WAVE complex (Mathur et al., 2003; Schwab et al., 2003; Basu et al., 2005), with one exception: the BEACH domain protein SPIRRIG (Saedler et al., 2009).
BEACH domain proteins are named after their corresponding mutations in mice and humans, the beige and Chediak Higashi (BEACH) syndromes (De Lozanne, 2003). They are known facilitators of membrane dynamics (De Lozanne, 2003) and regulators of endosomal sorting processes (Cullinane et al., 2013). In agreement with these essential functions, the BEACH domain protein family is well conserved in mammals, plants and yeast, often exhibiting several members within one species (Saedler et al., 2009).

In Arabidopsis, only the BEACH domain protein SPIRRIG has been characterized so far (Saedler et al., 2009). In the N-terminus, SPI carries tandemly repeated Armadillo domains, each approximately 42 amino acids long. Armadillo domains share a conserved three-dimensional structure of three α helices, which fold together and interact to form a surface for protein-protein interactions (Coates, 2003). The Armadillo repeats are followed by a Concanavalin A (ConA)-like lectin domain, which is thought to be involved in oligosaccharide binding and to mediate membrane fusion events (Burgess et al., 2009). In the C-terminus, SPI exhibits a Pleckstrin-Homology (PH) domain, followed by the name giving BEACH domain. PH domains were found to interact with BEACH domains to form a large groove, possibly serving as a ligand-binding site (Jogl et al., 2002). The BEACH domain of SPI is followed by several WD40 repeats, which can mediate protein-protein interactions.

The spirrig mutants in *A. thaliana* share all cell morphogenesis defects observed in other *distorted* mutants including lower complexity of epidermal pavement cells, weakly *distorted* and curled trichomes, disconnected and out-curved hypocotyl cells in dark grown seedlings as well as defects in vacuolar integrity (Saedler et al., 2009). AtSPI seems to act in two very different pathways. First, it was shown that AtSPI physically interacts with the ATPase Suppressor of K+-Transport Growth Defect 1 (AtSKD1) and LYST Interacting Protein 5 (AtLIP5; Steffens et al., 2017) suggesting that it functions in the context of the endosomal sorting complex required for transport (ESCRT) machinery. Second, AtSPI localizes to and facilitates the formation of processing bodies (P-bodies). In addition, Atspi mutations lead to pleiotropic transcriptional changes and the stabilization of several mRNAs and their recruitment to P-bodies under salt stress conditions. Also this pathway was suggested to be evolutionarily conserved because the AtSPI protein interacts with DECAPPING PROTEIN 1 (DCP1) from other species (Steffens et al., 2015).

The unusual finding that the *Arabidopsis thaliana* SPI gene has a dual role in unrelated pathways and traits raises the question, whether this is specific to one species or evolutionary conserved. We therefore studied this question in another distantly related Brassicaceae species that enables us to use genetic approaches and to unambiguously identify the true SPI ortholog by sequence similarity and synteny. We compared the developmental/physiological and molecular function between *A. thaliana* and *Arabis alpina*. *A. alpina* is suitable for laboratory use, since it is diploid, self-fertile, has a small and mostly sequenced genome and can be transformed with *Agrobacterium tumefaciens* (Wang et al., 2009). *A. alpina* was chosen for this work, since it is sufficiently closely related to *A. thaliana* to clearly identify orthologous genes, but also distant enough (26–40 million years; Koch et al., 2006; Beilstein et al., 2010) to
were confirmed by sequencing of the spi-3

MATERIALS AND METHODS

Plant Material, Growth Conditions, and Stress Treatments

Arabidopsis thaliana and A. alpina are highly conserved, especially in the C-terminus. The sequence identity reaches 94% for the whole protein and 97% for the PH-BEACH-WD40 (PBW) domain. Would Aaspi mutants show the same phenotypic spectrum as Atspi mutants? Would the molecular behavior be comparable? Using three Aaspi mutants identified in an exhaustive EMS screen for trichome mutants in A. alpina (Chopra et al., 2019) we reveal a similar molecular behavior and phenotypes, though the phenotypes show distinct differences.

expect phenotypic and molecular variations. The SPI proteins of A. thaliana and A. alpina are highly conserved, especially in the C-terminus. The sequence identity reaches 94% for the whole protein and 97% for the PH-BEACH-WD40 (PBW) domain. Would Aaspi mutants show the same phenotypic spectrum as Atspi mutants? Would the molecular behavior be comparable? Using three Aaspi mutants identified in an exhaustive EMS screen for trichome mutants in A. alpina (Chopra et al., 2019) we reveal a similar molecular behavior and phenotypes, though the phenotypes show distinct differences.

MATERIALS AND METHODS

Plant Material, Growth Conditions, and Stress Treatments

Arabidopsis thaliana EMS spirrig mutants spi-1, spi-2, and spi-3 were confirmed by sequencing of the SPIRRIG
FIGURE 4 | Intracellular localization of CFP-AaESCRT components and YFP-AaSPI-PBW. Transiently expressed CFP tagged AaESCRT proteins and YFP-AaSPI-PBW in Arabis alpina epidermal pavement cells. Arrow heads indicate dot-like structures. Scales bars: 25 µm.

(Aa_G228370) transcripts obtained from PCR (N-terminus: 5′-CGTGTTTAGAGAGGAAG-3′, 5′-TATGAACAACAGCAGGAAGTGTA-3′; Internal fragment: 5′-GCAACAGAACAAGACATGATT-3′, 5′-CATCGTTCAAGCTTTTGTC-3′; C-terminus: 5′-AA TCCAAAGGTTCTGAAG-3′, 5′-ACAGATGGGAGCTATACATAT-3′; GATC/Eurofins, Ebersberg). Seeds were grown on soil, or surface-sterilized and grown on full MS medium (Murashige and Skoog, 1962). The seeds were stratified for 5 days. Subsequently, plants were grown under long day conditions at 21 ± 1°C and 100 ± 20 µmol/m²s light intensity. To analyze plant
growth in the dark, seeds were removed from light conditions after 4 h, wrapped light-tight and vertically grown for 7 days. For germination assays, seeds were grown on MS plates and MS plates supplemented with 125 mM NaCl. Over the course of 20 days, seeds were checked for breakthrough of either root or cotyledons daily under a stereo microscope. For primary root growth assays, seeds were planted on MS plates, grown vertically, and transferred to MS plates and MS plates supplemented with different concentrations of NaCl after 7 days. Growth was then documented for 4 or 8 days. Seedling growth under transpiring conditions was carried out on soil under standard conditions, plants were watered by dipping every 3–4 days with water until day 20, followed by water or water supplemented with 125 mM NaCl until day 30. For transcript measurements, two to three 5–7 day old seedlings were transferred from plates to liquid 1/2 MS or liquid 1/2 MS with 125 mM NaCl for 4 h under constant shaking and subsequently frozen with liquid nitrogen. For short term salt stress of leaves, samples were transferred to 1/2 MS with 125 mM NaCl for 60–80 min.

Fluorescein Diacetate (FDA) Staining

Fluorescein diacetate was used as a negative stain to identify vacuole patterns in root hairs. Plants were treated with a 0.05% w/v solution of FDA (Sigma) in ddH2O for 15–20 min and analyzed under the fluorescence microscope.

Agarose Imprints

Agarose imprints were used to visualize the cell shapes of epidermal pavement cells. A 3% w/v agarose solution was applied to young cotyledons, peeled off after hardening and analyzed under the light microscope.

Microscopical Methods

Stereo microscopy was carried out with the Leica MZ 16F stereo microscope and the LAS AF software. Light and fluorescence microscopy was carried out with the Leica DMRB microscope equipped with a Sony Alpha 6000 camera or with the Leica DMRE microscope equipped with the Leica DFC 7000 T camera using the LAS X software. Confocal laser scanning microscopy was carried out with the Leica DM5500 and DM6000 CS Microscopes and documented with the TCS-SPE and TCS-SP8 imaging systems, respectively (Leica Microsystems, Heidelberg, Germany).

Transcript Analysis

Total RNA was isolated (TRI Reagent, Ambion by Life Technologies) and treated with DNasel (Thermo Fisher Scientific). Integrity of RNA was confirmed on a bleach gel (Aranda et al., 2012) and the concentration was quantified spectrophotometrically. One microgram of total RNA was reverse-transcribed (SuperScriptIII, Invitrogen).

qPCRs were carried out in a QuantStudio 5 System (ABI/Life Technologies) equipped with a 96 well block. The qPCRs were performed using plates (96 well, 0.2 ml) and cover foil (Opti-Seal Optical Disposable Adhesive, BIOplastics) and SYBR Green reagent (Thermo Fisher Scientific). All results represent the average of three biological and three technical replicates. Analysis was carried out with the QuantStudio TM Design and Analysis Software version 1.4.1 and Excel 2007. Outliers were removed using a two-sided Grubbs test at a significance level of 0.05.

Sequence Analysis, Plant Measurements, Statistical Analysis

Sequences were taken from the Genomic resources for Arabis alpina website1. In silico sequence analysis was carried out with CLC DNA Workbench version 5.6.1. Conserved domains were determined using CD-Search (Marchler-Bauer and Bryant, 2004) and/or PROSITE (Hulo et al., 2007). Phylogenetic trees were created using the Neighbor Joining method with the Unipro UGENE software (version 1.16.0) and subsequently modified for clarity using Adobe Illustrator CS4 version 14.0.0.

All plant measurements were carried out using ImageJ (Fabrice Cordelieres, Institut Curie, Orsay, January 2021 | Volume 11 | Article 596065

1www.arabis-alpina.org
FIGURE 5 | BiFC interactions of AaSPI-PBW with ESCRT components AaSKD1, AaLIP5, AaVPS60.1, and AaVPS60.2. Left—BiFC, middle—transformation control, right—overlay of BiFC and transformation control. Arrow heads indicate the sites of interaction. Scales bars: 25 µm.

France). Data was subsequently analyzed with Microsoft Excel 2007. Cell complexity was calculated using the formula complexity = \( \frac{\text{perimeter}^2}{4\pi \times \text{area}} \).

Statistical analysis was carried out with OriginPro 8.5 0G SR0, Microsoft Excel 2007 or R Studio. Significance levels were tested as indicated in the results section.

**Plasmids**

The coding sequences of AaSPI-PBW (Aa_G228370), AaDCP1 (Aa_G368370), AaSKD1 (Aa_G151760), AaLIP5 (Aa_G86640), AaVPS60.1 (Aa_G137100), AaVPS60.2 (Aa_G304080) were amplified from cDNA of pep1 (Supplementary Table S1). Gateway donor or destination vectors containing...
AtSPI-PBW (AT1G03060), AtDCP1 (AT1G08370), AtDCP2 (AT5G13570), AtDCP5 (AT1G26110), AtSKD1 (AT2G27600), AtLIP5 (AT4G26750), AtVPS60.1 (AT3G10640), AtVPS60.2 (AT5G04850), AtTTG1 (AT5G24520), AtGL3 (AT5G41315), and AtMYC1 (AT4G00480) have been described before (Zimmermann et al., 2004; Shahriari et al., 2010; Zhao et al., 2012; Steffens et al., 2014, 2015; Tian et al., 2015). Gateway vectors pENSG:YFP/CFP, pEXSG:YFP/CFP (Feys et al., 2005) and pAMARENA (M. Jakoby, NCBI:txid905036) were used for localization, pSYN/pSYC (Jakoby et al., 2006) and pCL112/113

FIGURE 6 | BIFC interactions of AaSPI-PBW with ESCRT components AtSKD1, AtLIP5, AtVPS60.1, and AtVPS60.2. Left—BIFC, middle—transformation control, right—overlay of BIFC and transformation control. Arrow heads indicate sites of interaction. Scale bars: 25 µm.
TABLE 5 | Yeast two-hybrid interaction between AaSPI-PBW and P-body components.

| GAL4BD       | Arabis alpina | Arabidopsis thaliana |
|--------------|---------------|----------------------|
| GAL4-AD      | DCP1          | DCP2                 |
| AaSPI-PBW    | +             | −                    |

Interaction between AaSPI-PBW, N-terminally fused to the GAL4-AD, and P-body components from Arabis alpina and Arabidopsis thaliana, N-terminally fused to the GAL4-BD, was tested on selective dropout medium lacking Leu, Trp, and His, supplemented with increasing concentrations of 3-aminotriazole (3AT). GFP, N-terminally fused to the GAL4-AD, was included as a negative control. The number of plus letters indicates the strength of the interaction [no growth on selective media (−); growth on selective media with < 15 mM 3AT above control (+)].

(provided by J. F. Uhrig) were used for BiFC, pACT/pAS (Clonetech) were used for yeast two-hybrid analyses.

Protein–Protein Interaction Assays and Co-localization

Yeast two-hybrid assays were carried out as described previously (Gietz et al., 1995). The selection on synthetic dropout interaction media lacking leucine (-L), tryptophan (-W), and histidine (-H), was performed with 3-aminotriazole (3AT) concentrations up to 30 mM. Bimolecular Fluorescence Complementation (BiFC) analysis was done in Arabidopsis leaves by biolistic transformation as described before (Mathur et al., 2003). Comparability of BiFC samples was guaranteed by fixed laser intensities, gain and line accumulation. The raw data was enhanced for clarity using Adobe Photoshop CS4 version 14.0.0 and ImageJ (Fabrice Cordelieres, Institut Curie, Orsay, France). Co-localization of AaSPI-PBW with ESCRT and P-body components was carried out in leaves of 3 week old Arabis seedlings by biolistic transformation (Mathur et al., 2003).

RESULTS

Arabis alpina spi Mutants and SPI Expression

Phylogenetic analysis has shown that BEACH domain proteins can be clustered into four groups (A–D), each containing plant as well as animal genes (Saedler et al., 2009). The Arabidopsis SPI gene belongs to group A, which includes animal genes that were described to be involved in membrane trafficking (De Lozanne, 2003). In our phylogenetic analysis, we found the SPI gene well conserved in all plant species analyzed (Supplementary Figure S1). In Arabis alpina, the orthologous SPI gene was identified by sequence homology and the synteny of homologous genes on the chromosome (Chopra et al., 2019). Three spi mutants were identified in an EMS mutagenesis screen for trichome phenotypes (Chopra et al., 2019) in the perpetual flowering 1 (pep1) mutant, in the pajares (paj) ecotype background (Wang et al., 2009). This line harbors a mutation in the floral repressor PEPI, which causes the plants to flower...
Arabidopsis thaliana Mutants Display Trichome-, Root-, Aaspi is the cause for the mutant phenotype.

nonsense mediated decay and that the truncation of the protein the aberrant transcripts are not recognized and degraded by wild-type reference for AaSPI mutant alleles are similar to that in pep1, to other tissues of the plant. The expression levels in the three seedlings, roots, mature leaves, hypocotyls and flowers (et al., 2009). The expression is lower in the hypocotyl, compared to cotyledons. We show that Aaspi mutant epidermal cells are less lobed than pep1 cells (Figure 2). To quantify the difference in the lobing of cells we determined the mean complexity as done before (Dewitte and Murray, 2003) and found a significant reduction in all three Aaspi alleles (Table 1).

**Hypocotyl Growth Is Not Reduced in Aaspi Mutants**

In Arabidopsis, young spi seedlings show an almost twofold reduction of the hypocotyl length (Saedler et al., 2009). Our analysis of Aaspi seedlings revealed no decrease of hypocotyl length as compared to pep1 (Table 2). If at all, hypocotyls of Aaspi seedlings were longer as observed in Aaspi-2 and Aaspi-3. We also never observed individual hypocotyl cells tearing out of the epidermal cell surface when grown in the dark. These observations correlate with our finding that AaSPI expression is relatively lower in the hypocotyl as compared to Arabidopsis, suggesting that AaSPI is less important for hypocotyl growth.

**Aaspi Is Not Important for Vacuolar Integrity in Arabis Root Hairs**

In Arabidopsis root hairs vacuoles were found to be fragmented, giving the initial hint toward a function of AtSPI in membrane trafficking/organization similar as reported for BEACH domain proteins in other species (Saedler et al., 2009). Toward this end we analyzed Fluorescein Diacetate (FDA) stained root hairs of pep1 and Aaspi mutants. Our analysis revealed a low frequency of root hairs with fragmented vacuoles in the range of 5% in both pep1 and all Aaspi mutants. This indicates that Aaspi mutants are not affected in vacuolar integrity of root hairs (Figure 3 and Table 3). This finding raised the question, whether the AaSPI protein interacts with ESCRT proteins, similar as reported for AtSPI (Steffens et al., 2017). In a first

### Aaspi Mutants Display Trichome-, Root-, and Epidermal Pavement Cell Phenotypes

The three Aaspi alleles had been identified by their weak distorted trichome phenotype (Chopra et al., 2019; Figure 2). In Arabidopsis, it was shown that spi mutant trichomes exhibit a reduced stalk length and width and a reduced average branch length (Saedler et al., 2009). In addition, we found that Arabidopsis Atspi mutants have a decreased number of branches (Supplementary Table S2). To compare the phenotypic spectrum of the Aaspi mutants with that in Atspi mutants, we studied the trichome phenotype in more detail. We found that almost all branches were curled or distorted in Aaspi mutants (Figure 2). Stalk length was significantly reduced in all three Aaspi alleles. Also, trichome branching was clearly reduced in the three Aaspi mutants (Figure 2). Stalk width and branch length varied in the three alleles such that some of the Aaspi alleles showed a difference to pep1 and others not (Table 1). We therefore considered these two aspects of the distorted phenotype as not affected by the Aaspi mutants.

Arabidopsis spi mutants are characterized by morphological changes in other cell types as well. In Atspi mutants root hairs are shorter as compared to wild-type (Saedler et al., 2009). We analyzed Aaspi mutant root hairs about 1 mm below the border between hypocotyl and the root of 7 days old seedlings. We found that Aaspi mutants also have significantly shorter root hairs than pep1 (Figure 2 and Table 1).

In Arabidopsis, Atspi epidermal pavement cells show less lobing and, as a result, less complexity than wild-type cells (Saedler et al., 2009). To assess whether this phenotype is also found in Aaspi mutants, we inspected the pavement cells on cotyledons. We show that Aaspi mutant epidermal cells are less lobed than pep1 cells (Figure 2). To quantify the difference in the lobing of cells we determined the mean complexity as done before (Dewitte and Murray, 2003) and found a significant reduction in all three Aaspi alleles (Table 1).
step, we studied the intercellular localization by transiently transforming Arabis alpina epidermal cells with YFP tagged proteins. In these experiments, YFP-AaSPI localized to the cytoplasm (Figure 4). Also, the Arabis ESCRT components CFP-AaSKD1 and CFP-AaLIP5 were found in the cytoplasm. CFP-AaVPS60.1 and CFP-AaVPS60.2 were found in the cytoplasm and in dot-like structures (Figure 4). In a second step, we studied the interaction of the AaSPI-PBW fragment with the four ESCRT proteins from A. alpina. In addition, we tested the interaction of AaSPI-PBW with ESCRT proteins from A. thaliana to assess the interspecies interactions (Table 4). In these experiments we found interactions between AaSPI-PBW and all Arabidopsis and Arabis ESCRT proteins, similar as reported for Arabidopsis SPI-PBW. To study the interaction behavior in Arabis alpina cells, we performed Bioluminescence Complementation (BiFC) experiments. BiFC interaction was found between AaSPI and all ESCRT proteins from Arabis and Arabidopsis (Figures 5, 6). Positive and negative BiFC controls are shown in Supplementary Figures S5–S7.

It would be desirable to further clarify the interaction behavior of AaSPI, however, also pull-down assays failed in our hands. Assays with bacterially expressed proteins did not work since we could not recover sufficient amounts of soluble proteins.

Interaction Between AaSPI and the P-Body Component DCP1

The PBW domain of AtSPI had been shown to interact with DCP1 homologs from mammals and yeast (Steffens et al., 2015). To determine, whether this behavior is conserved in A. alpina, we studied the interaction of AaSPI with DCP1 in yeast two-hybrid and BiFC assays. We found interactions with the AaDCP1 and AtDCP1 proteins (Table 5 and Figure 7).

In Arabidopsis, SPI-PBW co-localization to DCP1 is triggered by salt stress, suggesting that this stress treatment causes a re-localization of the AtSPI-PBW protein (Steffens et al., 2015).
In a first step we studied the co-localization of AaSPI-PBW and AaDCP1 under normal conditions. AaSPI-PBW alone was evenly distributed in the cytoplasm (Figure 8). When co-expressed with AaDCP1, AaSPI-PBW appeared in the cytoplasm and additionally in AaDCP1-marked P-bodies in the majority of cells (Figure 8). Thus, AaDCP1-PBW is recruited to P-bodies under normal conditions in these experiments. Upon salt stress (up to 300 mM of NaCl) the majority of leaf epidermal cells did not show a response to the treatment. However, we observed an increased number of small P-bodies and additional recruitment of AaSPI-PBW to AaDCP1-marked P-bodies in very few cells after 40 min of salt treatment (Supplementary Figure S3).

The Salt Stress Phenotype of Arabis spi Alleles

We analyzed the salt response of Aaspi mutants, as SPI was shown to be essential for the salt stress tolerance of Arabidopsis (Steffens et al., 2015). We tested effects of salt on germination, primary root growth and seedling growth, representing different tissues and stages of development.

Germination and primary root growth were significantly inhibited in Aaspi-1 and Aaspi-3 (Figures 9A,C). Strikingly, Aaspi-2 did not show an altered salt response compared to pep1. At a later stage of development and under transpiring conditions, salt hypersensitivity is only visible for Aaspi-1 (Figure 9B). The Aaspi-3 allele is indistinguishable from pep1 at this stage of development, while Aaspi-2 is hyposensitive to salt.

On the transcript level, we identified four genes which are differentially regulated under salt stress in Aaspi mutants (Figure 10). These genes were chosen following an RNAseq analysis in A. thaliana (Steffens et al., 2015). Two of which showed a similar salt response of all Aaspi alleles (Aa_G78660/AT3G07250 and Aa_NA/AT1G71520), while the other two reflected the phenotypic differences between the alleles (Aa_G499040/AT5G59310 and Aa_NA/AT3G44860). AT3G07250 is the nuclear transport factor family protein AtNTF2. This protein has a function in RNA binding and is likely to function as a ribonucleoprotein complex (Lamesch et al., 2012). AaNTF2 mRNA levels are strongly increased under salt stress, with a significantly higher upregulation in all three Aaspi mutants, compared to pep1 (Figure 10). AT5G59310 encodes the lipid transfer protein 4 (AtLTP4). Proteins of
this family can bind fatty acids and acyl-CoA esters. They can transfer phospholipids and function in response to abscisic acid, salt stress and drought (Lamesch et al., 2012). For this gene, Aaspi-2 showed a significantly higher upregulation in salt conditions compared to pep1 and the other two Aaspi alleles (Figure 10), possibly explaining the hyposensitivity to salt in later developmental stages. AT3G44860 encodes FARNESOIC ACID CARBOXYL-O-METHYLTRANSFERASE (AtFAMT). The role of farnesoin acid in plants has not been revealed so far, however, the transcript level of FAMT is increased upon various stresses, indicating that it might have general functions in stress responses (Yang et al., 2006). The qPCR results showed that the increase of transcript was significantly less in Aaspi-1 compared to pep1 and the other two spi alleles (Figure 10), which could explain the strong hyposensitivity of this allele.

DISCUSSION

Our phenotypic analysis of Arabis spi mutants revealed that they share most of the morphological phenotypes with Arabidopsis spi mutants. We also found a similar localization and protein-protein interaction behavior of the AtSPI and AaSPI proteins with ESCRT proteins. Taking these observations together, it is conceivable that the regulation of cell morphogenesis and a molecular function in the endosomal pathways is conserved in the two species. In support of this, also spi mutants of the moss Marchantia polymorpha exhibit short rhizoids reminiscent to the root hair phenotype of Aaspi and Atspi (Honkanen et al., 2016).

Interestingly, we also found that Arabis spi alleles show a different response to salt stress than pep1, suggesting that the dual function of SPI in morphogenetic processes and physiological processes is conserved between the two species. The molecular function of AaSPI in salt stress responses and mRNA regulation remains unclear. We found a physical interaction of AaSPI with AtDCP1 and AaDCP1. However, we cannot decide, whether the salt-dependent localization of cytoplasmic SPI protein to P-bodies as observed in Arabidopsis (Steffens et al., 2015) takes place in Arabis as co-expression of DCP1 and SPI is sufficient to trigger a recruitment of SPI to P-bodies. We also found transcriptional changes of several salt-stress response genes when comparing Aaspi alleles and pep1. Whether RNA stability is changed in Aaspi mutants was not tested in this study.

It is also not clear, whether AaSPI plays a role in salt stress responses. A salt stress phenotype is seen in Aaspi-1 and Aaspi-3 but not in Aaspi-2. One way to interpret this is to conclude that AaSPI is not involved in salt stress response because not all alleles show the phenotype. In this scenario, background mutations in Aaspi-1 and Aaspi-3 would cause the salt stress phenotype. Alternatively, it is possible that the different behavior of the three alleles is due to the type of mutations that they carry. While in Aaspi-1 and Aaspi-3 alleles premature stop codons lead to a loss of the PH-BEACH domain, the stop codon in Aaspi-2 leads to a truncation of only two of the four WD40 repeats in the C-terminus. Thus, it is possible that the protein region between the stop codons is relevant for the salt stress response. In this scenario it would be possible to assign protein domains that would be responsible for salt stress responses but not for cell morphogenesis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LS, MJ, and MH designed this study and wrote the manuscript. LS performed the research and analyzed the data. MH directed the project. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.596065/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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