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Original article

Comparative Expression Profiles of *SUVH7* in Sexual and Apomict *Boechera* spp. Display Differential Expression

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Abstract

Genomic imprinting is parent-of-origin specific gene expression in embryo nourishing tissues endosperm and placenta in flowering plants and mammals, respectively. Seeds are formed with double fertilization in flowering plants and the endosperm has a 3n chromosome set with the contribution of 2 maternal and 1 paternal genome. Any deviation from this ratio (2m+1p) results in seed abortion in many species, however, apomict species modify their gametogenesis or fertilization to survive. *Boechera divaricarpa* is a diploid apomict plant species that can produce seeds with a 4m:1p parental genome ratio in endosperm and produce viable seeds. *SUVH7*, on the other hand, is a histone methyltransferase that has a catalytic SET domain responsible for epigenetic control of gene expression. In this study, we characterized the structures of the *SUVH7* gene and compared the mRNA levels of *SUVH7* in diploid apomict and sexual *Boechera* spp. in unopened immature buds and manually pollinated siliques representing the -pre and -post pollination stages, respectively. The expression level of *SUVH7* in apomict *B. divaricarpa* has reached the max level 48 hours later following pollination, while in sexual *B. stricta* its expression level has dramatically decreased. Therefore, our study suggests the importance of epigenetic reprogramming in apomicts during seed development since chromatin marks via *SUVH7* are commonly associated with the activation of transcription in plants.

Keywords: *SUVH7*, *Boechera divaricarpa, Boechera stricta,* Apomixis, Seed development, qPCR.

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INTRODUCTION

Genomic imprinting is an epigenetic phenomenon that defines the nature of parent-of-origin-specific gene expression in both flowering plants and mammals. Genomic imprinting occurs in specialized tissues such as the endosperm and placenta to nourish the embryo. In angiosperms, genomic imprinting regulates the developmentally important genes through epigenetic modifications (Gehring et al., 2006). These epigenetic modifications were reported as either methylation of genomic regions by DNA methyltransferases or histones by Polycomb Repressive Complex 2 (PRC2) (Hsieh et al., 2011). Although the modification of DNA and histone proteins may have various effects on gene expression, generally DNA methylation is correlated with gene silencing (Ingelbrecht et al., 1994; Mette et al., 2000; Paszkowski and Whitham, 2001; Rodriguez-Negrete et al., 2013). Allele-specific methylation in plants is constituted during gametogenesis and results in highly methylated sperm cells and globally demethylated female central cells (Gehring et al., 2009; Hsieh et al., 2009; Huh and Rim, 2013; Park et al., 2016).

The gametogenesis in angiosperms requires reductional division of meiosis and following mitosis to produce haploid (n) gametophytes and, a double fertilization process generates a diploid embryo (2n). The female gametophyte originated from the megaspore mother cell that underwent meiosis, producing an egg cell and two central cells while the male gametophyte originated from the pollen mother cell, generates two sperm and vegetative cells. To develop a viable seed, double fertilization occurs, one sperm fertilizes the egg cell to produce an embryo, and the second sperm cell fuse with two central cells. Therefore, this plant-specific reproduction method produces a seed with a 2n embryo (1 maternal: 1 paternal genome) and 3n endosperm (2m:1p). However, the genome balance between parents in a functional endosperm is crucial since when disrupted it will lead to seed abortions (Scott et. al., 1998).

The fertilization of the polar nucleus creates a methylation imbalance between male and female genomes in endosperm and leads to genomic imprinting (Wollman and Berger, 2012). Therefore, in the endosperm, Paternally Expressed Imprinted Genes (PEGs) describes the expression of paternal allele only, while Maternally Expressed Genes (MEGs) describe the expression of allele originated from mother (Haig and Westoby, 1989). However, apomixis, an asexual reproduction mechanism through seeds, produce embryos that are clones of the mother plant without fertilization (Asker and Jerling, 1992). This reproduction mechanism is observed in more than 400 plant species (Carman, 1997) and suggests a revolutionary method to stabilize hybrid seed genotypes (Hanna and Bashaw, 1987). In apomict *B. divaricarpa*, the asexual embryo occurs spontaneously from unreduced egg cells (2n chromosome number) without fertilization (2 maternal:0 paternal). However, the asexual embryo is generally supported by a sexual endosperm in most apomicts, including *B. divaricarpa*. Therefore, the contribution of parental genomes (maternal + paternal) ratio to endosperm is different in apomicts, although, in sexual ones, the 2m+1p ratio in the endosperm is crucial for the development of an embryo.

In many apomicts, male meiosis yields reduced sperms cells (Spillane et al., 2001; Grimanelli et al., 2003). During the seed development of these species, the two unreduced polar nuclei (2m+2m), fertilize with a sperm produce a pentaploid endosperm with a 4m:1p ratio. Since any deviations from a 2m:1p ratio are lethal, apomict species modified the pathways of female gametogenesis or fertilization to overcome this "imprinting barrier" problem (Grossniklaus et al., 2001; Vinkenoog et al. 2003). Similarly, apomict *B. divaricarpa* is capable of the production of healthy seeds despite the genomic imbalance in the endosperm (4m+1p), by unknown mechanisms. Presumably, there have been some changes in genetic imprinting systems during the evolutionary development of the species (Spielman et al., 2003).

Apomictic plants could avoid the seed abortion because of unbalanced genome ratios in endosperm by various mechanisms; being insensitive to altered ratios of parental genomes, modifying the double fertilization process to constitute a genetically balanced endosperm, reducing the role of imprinting in endosperm development, producing four nuclei instead of eight and as a result, having only one unreduced polar nucleus in the embryo sac, or degenerating one of the central cells or fertilizing each with one sperm cell (Grossniklaus et al., 2001; Vinkenoog and Scott, 2001; Spielman et al., 2003). This suggests that the requirement of imprinted-mediated parental genome dosage control in apomict plants is less than usual (Spielman et al., 2003).

Over the last decades, whole-genome transcriptome analyses have revealed numbers of PEGs in *A. thaliana* and other plant species (Gehring et al., 2009; Gehring et al., 2011; Hsieh et al., 2011; Luo et al., 2011; Zhang et al., 2011; Kradolfer et al., 2013, Jeong et al., 2015; Lafon-Placette et al., 2018; Tuteja et al., 2019) including a histone-lysine N-methyltransferase (H3K9) encoding *SUVH7* gene in *Arabidopsis thaliana* (Baumbusch et al., 2001; Hsieh et al., 2011). SUVH7 protein has a catalytic SET domain which is responsible for epigenetic control of gene expression (Baumbusch et al., 2001). Although it is predicted that SUVH7 catalyzes mono- or di-methylation of H3K9 (Satish et al., 2018). SUVH7 has already been shown to play a role in constituting postzygotic hybridization barriers established by H3K9me2 (Wolff et al., 2015). Besides, a recent study revealed that SUVH7 protein has an anti-silencing effect on *ROS1* (a DNA demethylase) gene by recognizing DNA methylation in methylation monitoring sequences in the promoter region and recruiting transcriptional regulators to activate transcription (Xiao et al., 2018).

In this study, we used diploid sexual and apomict *Boechera* species from Brassicaceae family. *Boechera stricta* as a sexually reproducing species is one of the well-studied *model organisms,* native to North America (Al-Shehbaz et al., 2003; Anderson et al., 2015). On the other hand, diploid *B. divaricarpa* reproduces asexually through seeds (Koch et al., 2003; Dobeš et al., 2004). The genome assembly of *B. stricta* is available in Phytozome (v13) database (Goodstein et al., 2012; Lee et al., 2017) and Sequence Read Archive (SRA) data on NCBI (National Center for Biotechnology Information) is

available for *B. divaricarpa*. We obtained the ortholog protein sequences corresponding to *SUVH7* in *Boechera spp.* using available genome data and used to construct a phylogenetic tree in angiosperms. We compared the expression levels of *SUVH7* in apomict and sexual *Boechera spp*. with quantitative RT-PCR in the tissues during seed development. Therefore, we aimed to elucidate how epigenetic mechanisms might affect apomictic seed development.

MATERIALS and METHODS

Plant Material and Self-Pollination

B. divaricarpa (ES:9, 500209; BS) and *B. stricta* (ES:6, 500206; DG; DQ013050) seeds were kindly provided by M. Eric Schranz (Wageningen University, The Netherlands) and grown according to Schranz et al. (2005) method. After surface sterilization, seeds were placed on sterile filter papers in sterile Petri dishes. Sealed Petri dishes were conserved in dark at 4°C for three weeks to break the dormancy. Subsequently, the Petri dishes were placed in a growth chamber at +21°C following a 16-hour light/8-hour dark long-day photoperiod. After germination, seedlings were transferred in pots containing peat: perlite (4:1) mix. About seven weeks later, the plants were vernalized for three weeks at 10°C to induce flowering.

After flowering, the unopened immature flower buds were emasculated and hand-pollinated manually with their own pollens. Then, following crossing, we collected fertilized siliques 24, 48, 72, and 120 hours after pollination (HAP). For each day, we collected 20 pistils from different plants with sterile forceps in microcentrifuge tubes and frozen them in liquid nitrogen and stored them at -80°C until use.

Bioinformatic Studies

We downloaded *B. stricta* genome, transcriptome, and protein data (Phytozome genome ID: 278) from the Phytozome database (Goodstein et al., 2012) and *B. divaricarpa* transcript reads (SRX712111, SRX712112, SRX4617982) and genomic reads (SRX12498101) from the SRA database to search for the SUVH7 sequences. Then we uploaded these databases to Geneious R8 software (http://www.geneious.com) (Kearse et al., 2012) and constructed our local database.

We downloaded the SUVH7 protein sequence of *Arabidopsis thaliana* from The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/) and used this sequence as a reference for BLAST analysis of *B. stricta* SUVH7 protein sequence search in Geneious. As a result of BLAST analysis, we found Bostr.7128s0386.1p protein sequence with an E-value of 0 and 99,86% query coverage. Then to verify this protein represents the SUVH7 protein of *B. stricta*, we downloaded all available SUVH family proteins (SUVH1-10) of best known Brassicaceae family members, such as *A. thaliana, Capsella rubella, Eutrema salsugineum, Camelina sativa, Brassica napus, Raphanus sativus*, from NCBI database. We also added available SUVH family members of *B. stricta* from the Pytozome

database and aligned the sequences with the MUSCLE algorithm (Edgar, 2004) in Geneious R8, with default parameters. Finally, we constructed a phylogenetic tree of the SUVH protein family in Brassicaceae with the UPGMA algorithm and used the Jukes-Cantor genetic distance model and bootstrap resampling method with 1000 replicates. In this phylogenetic tree, Bostr.7128s0386.1p protein is grouped with other species' SUVH7 proteins (Figure 1). After confirming Bostr.7128s0386.1p is the SUVH7 protein of *B. stricta* via the phylogenetic tree, we used *B. stricta* protein sequence (Bostr.7128s0386.1p) as query and searched for *B. stricta SUVH7* transcript sequence with tBLASTn analysis. After we obtained the transcript sequence (Bostr.7128s0386.1) which encodes *B. stricta* SUVH7 protein, we used this transcript as a query and obtained the genomic sequence of *SUVH7* in *B stricta* (MLHT01000465). We also saved the -1000bp upstream sequence of the genome for analyzing the regulatory elements in the promoter region. Then, we used *B. stricta SUVH7* transcript sequence (Bostr.7128s0386.1) as a query and searched for the *SUVH7* transcript in *B. divaricarpa* using the "Map to references" option in Geneious software with its default parameters. After obtaining a partial SUVH7 transcript sequence for *B. divaricarpa,* we translated it into protein with Geneious. We also used -1000 bp promoter sequence of *B. stricta* as a reference and mapped *B. divaricarpa* genomic reads to obtain the -1000 bp promotor region of *B. divaricarpa*.

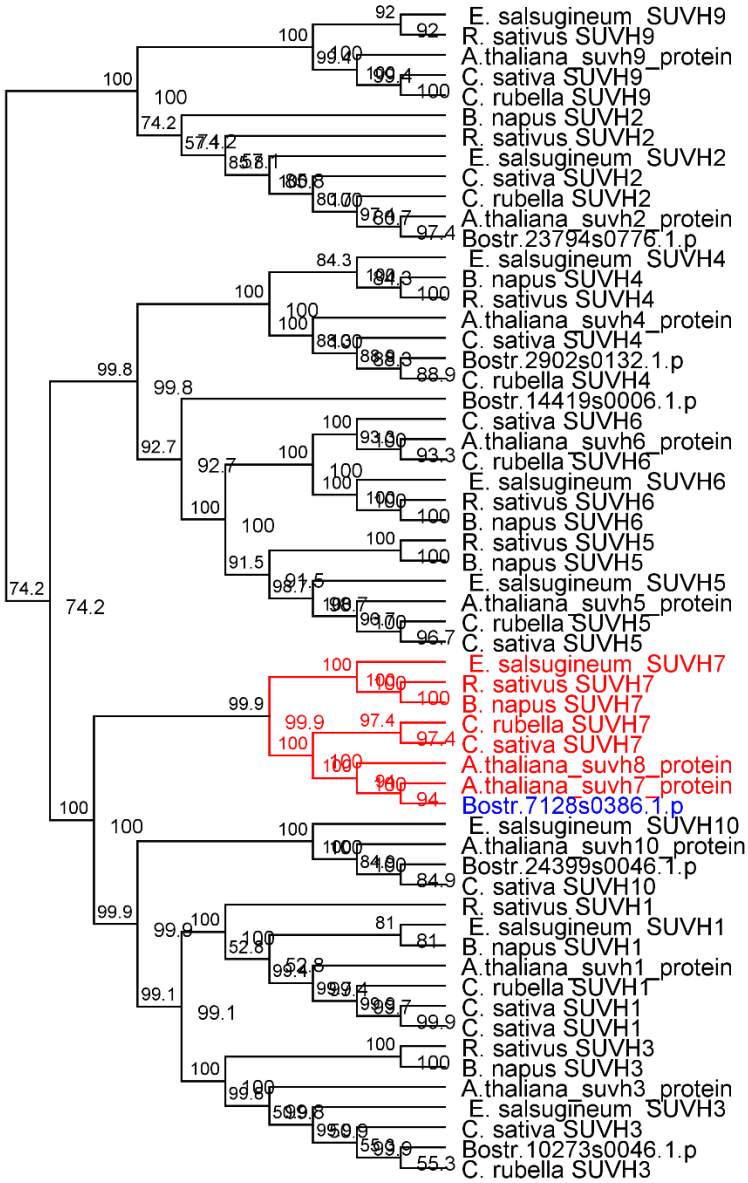

Figure 1. Phylogenetic tree of SUVH protein family in Brassicaceae (Red branch shows the SUVH7 proteins and the blue branch represents the Bostr.7128s0386.1.p protein of *Boechera stricta*)

We analyzed the subcellular localization, molecular weight, isoelectric point, promoter region of *B. stricta* and *B. divaricarpa* SUVH7 sequences with available bioinformatics tools. We predicted the subcellular localizations with CELLO v.2.5: subcellular localization predictor (http://cello.life.nctu.edu.tw/) (Yu et al., 2006) and the molecular weights and isoelectric points of SUVH7 proteins with ExPASy's ProtParam server (http://web. expasy.org/protparam/) (Gasteiger et al., 2005). -1000bp upstream promoter regions for regulatory elements were analyzed with PlantCARE (Plant Cis-Actin Regulatory Element) database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al., 2002). Then, we searched for the conserved domains in SUVH7 proteins of various plant species including Brassicaceae,

Rubiaceae, Cucurbitaceae, Rutaceae, Malvaceae, Fabaceae, Poaceae, Salicaceae, Solanaceae and Vitaceae family members with NCBI Conserved domain search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

We also constructed a phylogenetic tree using SUVH7 protein sequences of various plant species. For this, we used *B. stricta* SUVH7 protein sequence as a query in the BLAST tool in NCBI and Phytozome databases and searched for other plant species protein sequences. Then, we recorded the highly matched sequences of other well-known plant species and aligned the sequences with the MUSCLE algorithm in Geneious R8, with default parameters. Then, we constructed a phylogenetic tree with the UPGMA algorithm and used the Jukes-Cantor genetic distance model and bootstrap resampling method with 1000 replicates.

Expression Studies

To reveal the expression levels of *SUVH7* in diploid apomict *B. divaricarpa* and sexual *B. stricta*, we purified total RNA from growing siliques (24, 48, 72, and 120 HAP) and unopened immature flower buds using PureLink® RNA Mini Kit (12183020, ThermoFischer). The RNA integrities were checked on agarose gel, and we determined the quantities of RNAs with Qubit 2.0 Fluorometer and stored them at -80°C until use. Before cDNA synthesis, we applied DNase I (RNase-free, EN0521, ThermoFischer) to the total RNAs to avoid DNA contamination. Then, cDNAs were synthesized with a High-Capacity cDNA Reverse Transcription Kit (4368814; Applied Biosystems, Thermo Fischer) according to the manufacturer's instructions.

In the study, we performed quantitative RT-PCR (qPCR) reactions to determine the expression levels of *SUVH7*. The gene-specific primers were designed using available genomic and transcriptome data in Geneious R8 software (Kearse et al., 2012) (Table 1).

Table 1. Primer sequences for qRT-PCR

	Primer sequence 5'-3'
UBQ F	GGCTAAGATCCAGGACAAGGAAGGTAT
UBQ R	CTGGATGTTATAGTCAGCCAAAGTGCG
SUVH7 F	GTGAGGAAACCAGACCAACCTAATT
SUVH7 R	GTATGAAACCCTCTCTCGAATCAATCA

In each well, 50 ng of cDNA, 0.12 µmol of each primer, and Power SYBR1 Green PCR Master Mix (#4367659; Thermo Fischer) are included in a 10 µl total volume. *UBQ* (Polyubiquitin) was used for normalization (Pellino et al., 2011). The *UBQ* primers were also used for -RT controls, with the cDNA template but without reverse transcriptase enzyme. Negative controls without a template were also performed with either the *SUVH7* or the *UBQ* primers. All qRT-PCR reactions are performed with

3 technical replicates. The qPCR conditions were pre-denaturation at 95 °C for 10 min, 40 cycles for denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s and elongation at 72 °C for 60 s and then melt curve analysis with the 7500 Real-Time PCR (Applied Biosystems). The results were analyzed with DataAssist™ (Applied Biosystems) Software.

RESULTS

Protein domains of SUVH7 are conserved among different plant species

After constructing our local *B. stricta* database in the Geneious software, we obtained the *B. stricta* SUVH7 protein sequence (Bostr.7128s0386.1p), which consists of 668 amino acids and has 70% pairwise identity with *A. thaliana* protein. Then, we acquired the transcript and genomic sequences for *B. stricta* SUVH7 and analyzed the -1000bp upstream region of the genomic sequence for promoter regulatory elements. As for *B. divaricarpa*, we assembled 14,888 SRA transcript reads and obtained 1560 bp partial transcript and 520 aa partial protein. This protein has a %66,02 pairwise identity with *A. thaliana*. We also used *B. stricta* promotor sequence as reference and mapped 711 SRA genomic reads to obtain the -1000bp promotor region of *B. divaricarpa*.

We detected 15 different cis-acting elements that are common in both species and response to light, auxin, and gibberellin hormones and environmental stress conditions in the promoter region of the *Boechera spp. SUVH7* genes (Table 2).

Table 2. Cis-acting regulatory elements in *SUVH7* promoter region of *Boechera spp*.

Cis-acting Element	Sequence	Function
ARE	AAACCA	cis-acting regulatory element essential for the anaerobic induction
AT~TATA-box	TATATA	core promotor element
CAAT-box	CAAT	common cis-acting element in promoter and enhancer regions
GA-motif	ATAGATAA	part of a light responsive element
GATA-motif	GATAGGA	part of a light responsive element
LS7	CAGATTTATTTTTA	part of a light responsive element
MYB	CAACCA	myb recognition motif
P-box	CCTTTTG	gibberellin-responsive element
STRE	AGGGG	stress response element
TATA	TATAAAAT	core promotor element
TATA-box	ATTATA	core promoter element around -30 of transcription start
TCT-motif	TCTTAC	part of a light responsive element
TGA-element	AACGAC	auxin-responsive element
Unnamed__1	CGTGG	-
Unnamed__4	CTCC	-

We also constructed a phylogenetic tree using SUVH7 orthologues in various plant species to confirm that the histon methyltransferase SUVH7 is conserved among plant families. As a result, two main branches were formed from the phylogenetic tree. One of these branches contains species from the Brassicaceae family (red branch in Figure 2), including *B. stricta* and *B. divaricarpa* (*Boechera spp*. are indicated in blue color in Figure 2). Besides, *B. stricta* SUVH7 protein has a molecular weight of 74821.63 Da while *B. divaricarpa* protein is 55816.04 Da. Their theoretical pI is 5.94 and 7.81, respectively and they both mainly have a nuclear localization according to the CELLO (v.2.5) and ProtParam server (Yu et al., 2006; Gasteiger et al., 2005) analysis.

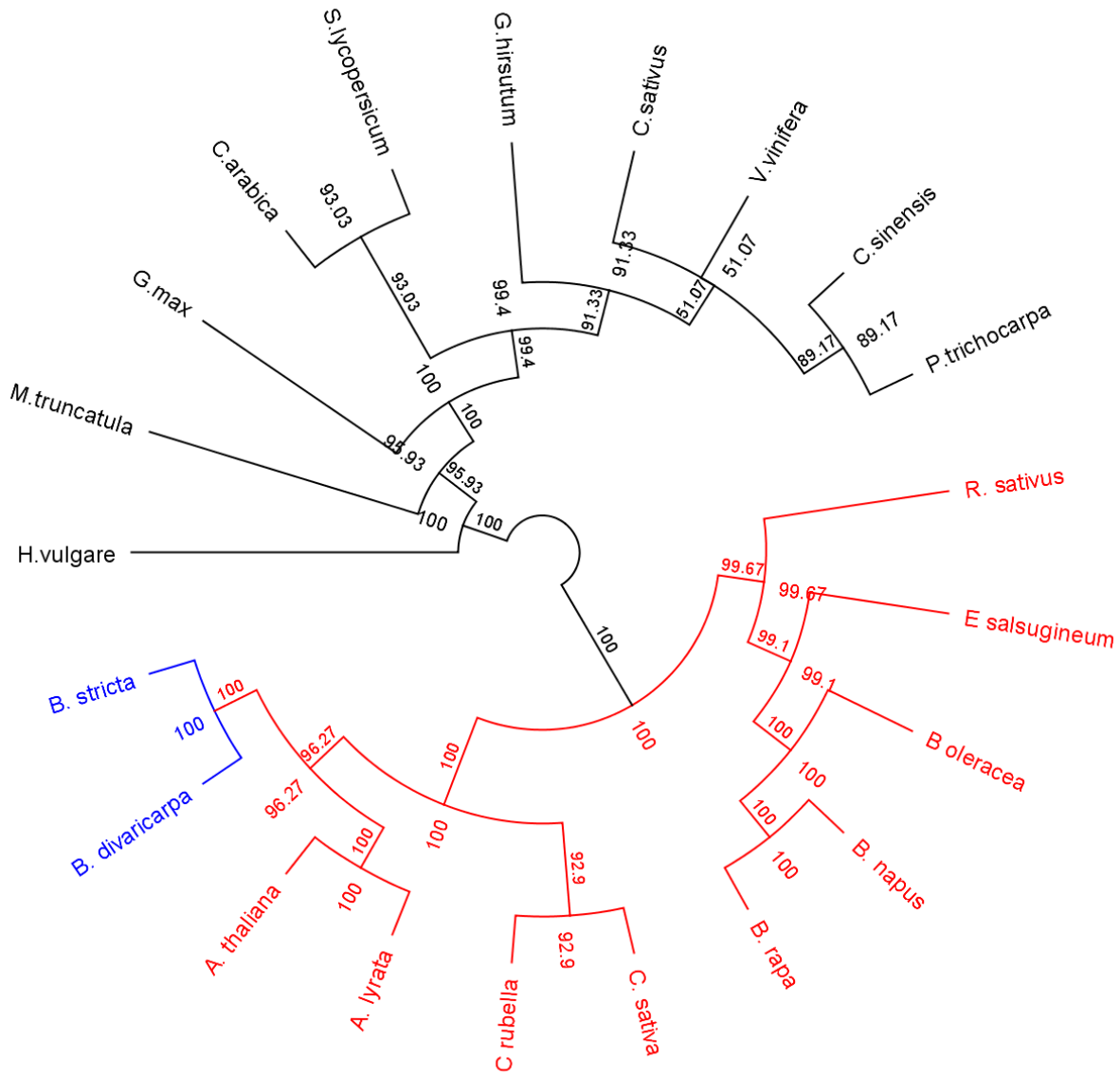

Figure 2. Phylogenetic tree of SUVH7 protein sequences that belongs to various plant species (Red branch shows the Brassicaceae family and the blue branches show *Boechera* spp., numbers on the nodes represent iterations as bootstrap values)

We analyzed the conserved domains in the SUVH7 proteins. We have found that all SUVH7 orthologues have the "ccd:cd10545" conserved domain belongs to "SET (Su(var)3-9, Enhancer-of-zeste, Trithorax) domain superfamily (cl40432)" (Figure 3). Additionally, all species, except R. sativus, have the conserved domain "ccd:smart00466". R. sativus has the "ccd:pfam02182". Both conserved domains belong to the "SAD_SRA superfamily (cl02620)". Moreover, A thaliana and B. napus have an extra Pre-SET motif (ccd:smart00468) while C. sativa has a similar motif (pfam05033) and the motifs both belong to the "Pre-SET superfamily (cl02622)". Furthermore, only B. oleraceae has an Atrophin-1 domain (ccd:pfam03154) which belongs to the "Athropin-1 super family (cl38111)".

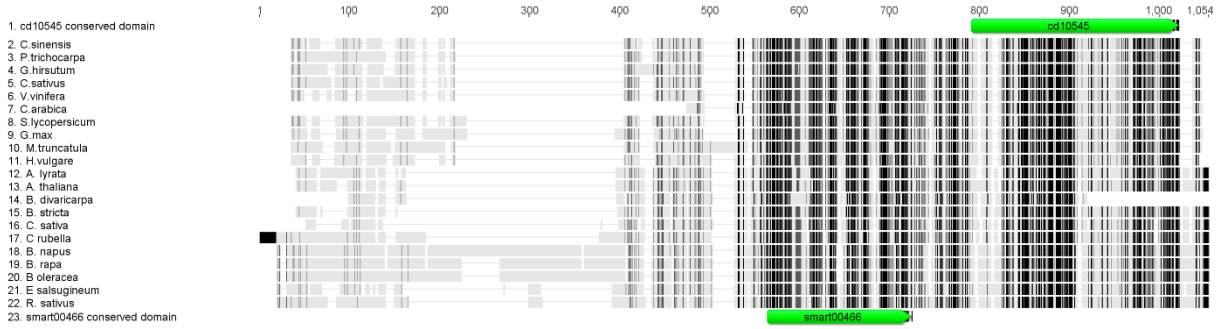

Figure 3. Conserved domain placements (cd10545 and smart00466, represented by green lines) of SUVH7 proteins of well-known plant species including B. stricta

SUVH7 has differentially expressed in apomict and sexual species

We investigated the expression levels of *SUVH7* in before/after fertilization tissues of sexual and apomict Boechera species. *SUVH7* showed differential expression profiles in apomict and sexual plants. In apomict *B divaricarpa*, the expression of *SUVH7* is similar and slightly higher at 24, 72, and 120 HAP than in the unopened flower buds before fertilization while the expression dramatically increases in siliques (~3.5 fold) at 48 HAP. However, in sexual *B. stricta*, *SUVH7* expression gradually decreases during 48 HAP and reaches the lowest level at 48th hour in siliques (~5.5 fold) compared to unopened buds, and then increases again at 72 and 120 HAP (Figure 4).

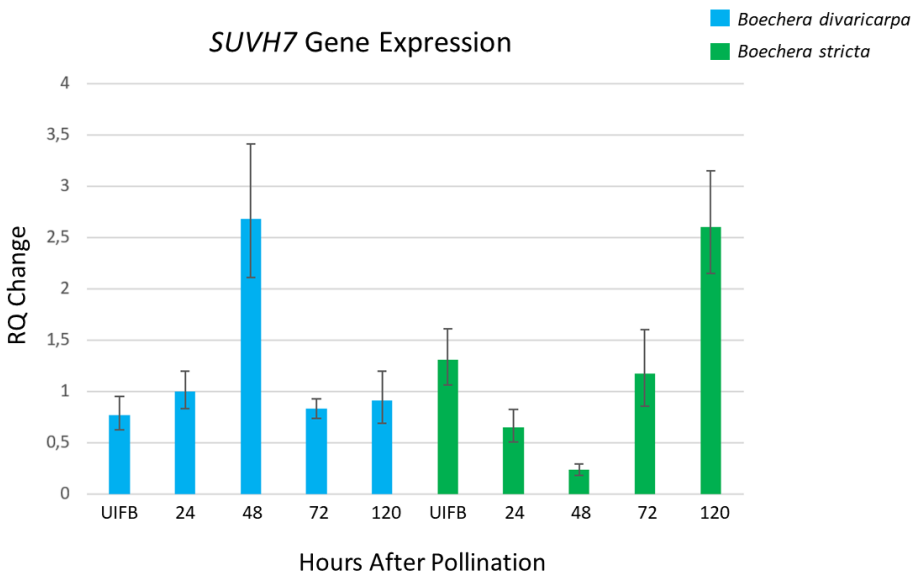

Figure 4. Expression profile of *SUVH7* in apomict and sexual plants in pre/post-pollination stages (UIFB: Unopened immature flower buds, RQ: Relative quantitation, Error bars represent the standard error of the mean)

Compared to unopened buds before fertilization, the expression level of *SUVH7* was similar at 24, 72, and 120 HAP in apomict *B. divaricarpa*, but there was a significant increase in expression level only at 48 HAP. However, in sexual *B. stricta*, *SUVH7* expression levels in the first days after pollination were determined to be lower than the level before pollination. The level of expression is at its lowest at the 48 HAP while it increased at 120 HAP compared to the pre-pollination stage.

DISCUSSION

We determined the expression profiles of *SUVH7* in apomict and sexual *Boechera* species during seed development. Understanding of the epigenetic processes that control apomict reproduction will help us to harness apomixis in commercially important plants (Hanna and Bashaw, 1987; Koltunow et al., 1995).

SUVH7 encodes a histone-lysine N-methyltransferase (H3K9) in *A. thaliana* (Baumbusch et al., 2001; Hsieh et al., 2011) and has a catalytic SET domain that is responsible for epigenetic control of gene expression (Baumbusch et al., 2001). In the promoter region of *Boechera spp.* SUVH7, we found regulatory cis-acting elements related to light, hormone, and environmental stress responses which are consistent with its role in histone modification and epigenetic control of other genes (Baumbusch et al., 2001).

We constructed a phylogenetic tree using the *Boechera spp.* and well-known plant species' SUVH7 protein sequences. In the tree, members of the Brassicaceae family such as *B. stricta*, *B. divaricarpa*, and *A. thaliana* are grouped together in the same branch, indicating that SUVH7 proteins

are highly conserved in Brassicaceae. Additionally, we found that *Boechera spp.* SUVH7 is localized in the nucleus, which is consistent with its role in histone modification.

Conserved domain analysis showed that each plant species studied have the SET domain and SAD_SRA superfamily domains in common. SET domains exist in lysine methyltransferases which catalyse the methylation of lysine residues in histones that are fundamental in epigenetic regulation of gene activation/silencing (Thorstensen et al., 2011) and SAD_SRA domain is a DNA binding domain that can bind directly to the methylated sites (Johnson et al., 2007).

In our study, we also revealed the expression profiles of *SUVH7* with qRT-PCR in diploid apomict and sexual *Boechera* species in unopened young flower bud (before pollination) and silique tissues. At the 48th hour after pollination, *SUVH7* expression was in the highest level for *B. divaricarpa* and in the lowest level in *B. stricta* in the tissues studied.

In flowering plants, the primary endosperm nucleus occurs by divisions following the fusion of central cells with sperm. Although embryo and endosperm developmental stages have variations from species to species, they share a common mechanism.

In our study, the expression of *SUVH7* is slightly increased in apomicts and decreased in sexual plants 24 HAP compared to the unopened buds. In *A. thaliana* as a close relative of *Boechera*, endosperm has the sixth syncytial mitosis while the embryo has 2-8 cells following 24 HAP (Boisnard-Lorig et. al. (2001). Between 36-60 HAP, the eighth syncytial mitosis is restricted to the peripheral endosperm, and chalazal endosperm has large and small nuclei while the embryo is at dermatogen/globular stage (Boisnard-Lorig et. al., 2001). At the 48th hour, when the differential expression is at the maximum, the endosperm is at the early-globular stage in *A. thaliana* (Luo et al., 2000). While the embryo is at the beginning of the heart stage at 72 HAP, endosperm cellularization begins, and at the late torpedo stage of the embryo at about 144 HAP, cytokinesis is completed in *A. thaliana* endosperm (Li, 2011). In our study, we also observed an increase for the *SUVH7* mRNAs 72 HAP only in sexual *B. stricta.* This can be explained by the initiation of the endosperm cellularization, which starts 72 hours later when the embryo is at the heart stage in *A. thaliana*.

In apomict *B. divaricarpa*, the *SUVH7* expression is almost similar at 24, 72, and 120 HAP but there is a dramatic increase at 48 HAP, which suggests *SUVH7* might be active for only a short time in apomicts, and during this short time that switch is likely to be off in sexual *B. stricta*.

Wolff et al. (2015)'s study shows that *suvh7* mutant is able to rescue triploid seed abortion in *A. thaliana*, indicating that SUVH7 establishes postzygotic hybridization barriers for the formation of triploid seeds. The paternally expressed role of *SUVH7* for establishing postzygotic hybridization barriers for normal seed development in *A. thaliana* may also control normal endosperm development

in early hours following pollination in apomict *B. divaricarpa*, which has a pentaploid endosperm (4m:1p).

Likewise, in one of our previous studies, we found that DRM2, an RNA-directed DNA methyltransferase, is upregulated in apomict *B. divaricarpa* compared to the *B. Stricta* throughout 120 HAP, indicating deregulation of DNA methylation machinery during seed development (Taşkın et al., 2017). Upregulation of paternally expressed imprinted histone methyltransferase *SUVH7* expression in *B. divaricarpa* pentaploid endosperm (4m:1p, 5C) suggests an epigenetic reprogramming in apomicts.

In apomict dicots, 5C endosperm is rare, but can be found in a pseudogamous and facultatively apomictic/sexual genotype, in an obligate apomict genotype and, in a pseudogamous facultatively meiotic–parthenogenetic/apomictic/sexual genotype of *Hypericum perforatum* (Matzk et al., 2000, 2001). Other *Hypericum* or *Taraxacum* species can have 3C, 4C, 6C, 10C or 12C endosperm while the embryo is 2C (Matzk et al., 2000, 2001). In our study, *B. stricta* has 2C:3C and *B. divaricarpa* has 2C:5C embryo: endosperm ratio, respectively. Therefore, it is likely to be gene expression differences between these species and functional studies should have been done to reveal the mission of SUVH7 in apomict seed development.

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Declaration of Interest Statement

The authors declare no conflict of interest.

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