



Article

# Identification of SWI2/SNF2-Related 1 Chromatin Remodeling Complex (SWR1-C) Subunits in Pineapple and the Role of Pineapple SWR1 COMPLEX 6 (*AcSWC6*) in Biotic and Abiotic Stress Response

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**Abstract:** Chromatin remodeling complex orchestrates numerous aspects of growth and development in eukaryotes. SWI2/SNF2-Related 1 chromatin remodeling complex (SWR1-C) is a member of the SWI/SNF ATPase-containing chromatin remodeling complex responsible for the exchange of H2A for H2A.Z. In plants, SWR1-C plays a crucial role by transcriptionally regulating numerous biological and developmental processes. However, SWR1-C activity remains obscure in pineapple. Here, we aim to identify the SWR1-C subunits in pineapple. By genome-wide identification, we found a total of 11 SWR1-C subunits in the pineapple. The identified SWR1-C subunits were named and classified based on the sequence similarity and phylogenetic analysis. RNA-Seq analysis showed that pineapple SWR1-C subunits are expressed differentially in different organs and at different stages. Additionally, the qRT-PCR of pineapple SWR1-C subunits during abiotic stress exposure showed significant changes in their expression. We further investigated the functions of pineapple SWR1 COMPLEX 6 (*AcSWC6*) by ectopically expressing it in *Arabidopsis*. Interestingly, transgenic plants ectopically expressing *AcSWC6* showed susceptibility to fungal infection and enhanced resistance to salt and osmotic stress, revealing its involvement in biotic and abiotic stress. Moreover, the complementation of mutant *Arabidopsis swc6* by pineapple SWC6 suggested the conserved function of SWC6 in plants.

**Keywords:** SWR1-C; chromatin remodeling complex; SWC6/SEF; biotic stress; pineapple

## 1. Introduction

Epigenetics is one of the major processes that orchestrate gene regulation in plants, animals, and eukaryotes in general. It involves DNA methylation, covalent modification of DNA, and histone

modifications [1]. It is also linked with DNA methylation resulting in transcriptional silencing [2–4]. In plants, epigenetics is essential for reproduction, the viability of seeds, development of the pollen grain, and it ensures proper regulation of genes [2]. Development of eukaryotes from embryo to mature organism is regulated by sophisticated biological processes, including chromatin remodeling [5]. Epigenetic regulation at nucleosomes is largely dependent on multiprotein covalent alteration of histones or ATP-dependent chromatin remodeling proteins. Changes in gene expressions are governed by chromatin remodeling that ensures successful developmental and physiological responses [6]. This phenomenon is regulated by ATP-dependent chromatin remodelers such as INO80, CHD, and SWI/SNF [7].

SWI2/SNF2-RELATED 1 is largely known as SWR1 chromatin remodeling complex (SWR1-C) and it is conserved throughout the eukaryotes [6,8]. SWR1-C is reported to facilitate the exchange of histone H2A with variant histone H2A.Z [9]. Exchange of H2A.Z has a great influence on gene expression due to its peculiar physicochemical characteristics [10]. H2A.Z affects nucleosome stability and changes chromatin architecture to fine tune gene expression positively or negatively [9]. H2A.Z governs transcription through modification of chromatin accessibility in the eukaryotes [11–13]. In yeast, fourteen subunits of the SWR1-C subunits have been identified [10]. These are SWR1 a homologue of PHOTOPERIOD INDEPENDENT AND EARLY FLOWERING (PIE1) in *Arabidopsis*, SWR1 COMPLEX2 (SWC2), SWR1 COMPLEX 3 (SWC3), SWR1 COMPLEX 5 (SWC5), SWR1 COMPLEX 6 (SWC6) which is a homologue of SERRATED LEAF AND EARLY FLOWERING (SEF) in *Arabidopsis*, SWR1 COMPLEX 7 (SWC7), ACTIN-RELATED PROTEIN 6 (ARP6), YEAST ALL1 FUSED GENE FROM CHROMOSOME 9 (YAF9), BROMODOMAIN-CONTAINING FACTOR 1 (BDF 1) which is also known as GTE8 in *Arabidopsis*, ACTIN 1 (ACT1), ACTIN-RELATED PROTEIN 4 (ARP4), SWR1 COMPLEX 4 (SWC4), RUVB LIKE PROTEIN 1 (RVB1), and RUVB LIKE PROTEIN 2 (RVB2) [4,14]. Some of the SWR1-C subunits are shared members between SWR1-C and other chromatin remodeling complexes. ACT1, ARP4, RVB1, and, RVB2, are also subunits of the INO80 chromatin-remodeling complex. Similarly, SWC4, YAF9, ACT1, and ARP4 are also subunits shared with NuA4 acetyl transferase complex [4,10].

SWR1-C subunits, such as ARP6 and SWC6, facilitate binding and histone replacement while SWC2, together with ARP6, SWC6, and SWR1, acts as a subcomplex. Similarly, they associate with each other and are required for the association of other SWR1-C subunits [10,15]. SWC2 is important for H2A.Z transfer, association, and nucleosome binding [15]. SWC6 and ARP6 are required for H2A.Z deposition, while other components such as SWC5 and YAF9 function in H2A.Z transfer but not nucleosome binding. Association of several subunits, such as BDF1, YAF9, and SWC4, requires ARP4 for their activity. Null mutation of a number of nonessential SWR1-C subunits has clearly demonstrated that H2A.Z deposition in vivo is linked to YAF9, ARP6, SWC2, and SWC6, as well as PIE1 [4]. In plants, developmental processes such as flower development, stress tolerance, immunity, DNA damage repair, and transition from the vegetative phase to reproductive phase require the action of SWR1-C [10,16,17]. SWR1 also represses anthocyanin accumulation by depositing H2A.Z at anthocyanin biosynthetic genes which negatively regulates the expression of these genes in *Arabidopsis* [18].

Loss of function of key SWR1-C subunits such as ARP6, SWC6 (SEF), or PIE1 in *Arabidopsis* is characterized by a pleiotropic phenotype affecting vegetative and reproductive phases of plant developmental processes [6]. SWC6 mutation in *Arabidopsis* triggers developmental irregularities by reducing transcript levels of MADS AFFECTING FLOWERING 4 (MAF4) and FLOWERING LOCUS C (FLC) and accelerates the expression of SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1) which are important genes in the flowering pathway [12]. SWC6 mutants exhibit leaf serration, absence of internodes in the floral organs, bushy phenotype, and inflorescence with the irregular number of floral parts [19]. In *Arabidopsis*, *swc6* phenotype is similar to that of the *pie1* and the *arp6* mutants but *pie1* mutants show a severe phenotype compared to *arp6* and *swc6* [17,19–21]. Functional studies suggest the genetic association and interaction between ARP6, SWC6, and PIE1 [5,21]. Moreover, ARP6 mutation directly affects meiosis during female gametogenesis by controlling meiotic genes [22]. SWR1-C is also involved in immune response. Mutation in SEF and PIE1, core SWR1-C subunits,

triggers immune responsive genes, revealing the importance of the SWR1-C complex in immunity and biotic interaction [10]. SWC6 is a conserved member of plant SWR1 complex and a homolog of SEF in *Arabidopsis* [10,19]. It is implicated in the substitution of H2A with H2A.Z variant, thus affecting chromatin structure which in turn facilitates gene expression through turning on and turning off of a number of genes [5,19]. Mutation in SWC6 in *Arabidopsis* is characterized by early flowering, leaf serration, irregular numbers of petals, and reduced apical dominance as a result of reduced expression of FLC, MAF4, and MAF5 [5,23]. SWR1-C has been well characterized in yeast, *Arabidopsis*, and humans [5].

Pineapple (*Ananas comosus* L. Merr) is among the popular exotic fruits worldwide and is a member of the *Bromeliaceae* family domesticated in northern and southern America about 6000 years ago [24–27]. It is one of the most significant tropical fruits after banana and citrus [28,29]. Pineapple fruit is well known for its great flavor, taste, and fragrance and is cultivated on a continental scale with medicinal and nutritional value [24]. It is rich in vitamins such as vitamin A, B, and C [29]. Among the *Bromeliaceae* family, only pineapple has active global trade due to its flavor. Thirty million tonnes of pineapple are annually produced in the world [29].

In this study, we have characterized the SWR1-C chromatin remodeling complex in pineapple. Although SWR1-C has been well studied in *Arabidopsis*, its investigation in pineapple has not been reported. Here, we have identified 11 SWR1-C subunits in pineapple and characterized their features using different bioinformatic methods. In addition, we investigated the expression profile of identified SWR1-C subunits in different organs and developmental stages. We further validated the function of AcSWC6 during biotic and abiotic stress by ectopically expressing it in *Arabidopsis*. Finally, we complemented the *Arabidopsis* (dicot) *swc6* mutant using pineapple (monocot) AcSWC6. Collectively, our results suggest that the SWR1-C subunit SWC6 has a conserved function in plants.

## 2. Materials and Methods

### 2.1. Plant Material, Growth Conditions, and Treatments

Pineapple plants were MD2 propagated by tissue culture [30] and were supplied by Haixia institute of science and technology Centre for Genomics and Biotechnology, Fujian Agriculture and Forestry University, Fuzhou, China. Plastic pots containing soil mixture (peat moss: perlite = 2:1 (*v/v*)) were used for pineapple plants and grown in a growth house at 30 °C temperature and 60–70 mMOL<sup>-1</sup> photons m<sup>-2</sup> s<sup>-1</sup> with 70% humidity and 16/8 h light cycle [9,31,32]. All the *Arabidopsis* plants used in this research were in background Colombia. *Arabidopsis thaliana* (L.) Col-0; (CS60000) was referred to as wild type (WT) and the T-DNA insertional mutants *Atswc6* (SAIL\_536\_A05) were obtained from the *Arabidopsis* Biological Resource Center (ABRC) and confirmed by genotyping. To grow plants, *Arabidopsis* seeds were planted on a modified Hoagland's medium in round 9 cm petri plates with 1% (*w/v*) sucrose and 1% bacto agar (*w/v*) [33]. They were then kept at 4 °C in the dark for 48 h to stratify the seeds [34] before they were transferred to growth room with 22 °C, 16 h illumination of light and 8 h of darkness under the light strength of 100 μmol m<sup>-2</sup> s<sup>-1</sup>.

For the abiotic stress treatments, 30-day old pineapple plants were subjected to different abiotic stress of NaCl 150 mM, mannitol 350 mM, heat at 45 °C and cold at 4 °C for 24, 48, and 72 h, respectively. For *Arabidopsis* abiotic treatment, wild type, *Atswc6* mutant, *Atswc6* complemented line, and the AcSWC6 overexpression line were assayed for growth on Hoagland's medium supplemented with or without NaCl 150 mM, mannitol 300 mM, and kept in a growth room. To observe the growth phenotype photographs were taken after 4 d and 6 d and analyzed.

The biotic stress for pineapple and *Arabidopsis* was carried out as described by Seifi et al., [35] with some modifications. The necrotrophic pathogen *Sclerotinia sclerotiorum* was provided by the Haixia institute of science and Technology, Fujian Agriculture and Forestry University, Fujian China, and was cultured on 2% potato dextrose agar at a temperature of 25 to 28 °C for three days. The plants were then treated with fungus for 24 h by placing it on the adaxial surface of the leaves of the plants using

the agar plug method [35,36] followed by 3,3'-diaminobenzamide (DAB) staining to detect the H<sub>2</sub>O<sub>2</sub> activity [37].

### 2.2. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Pineapple plants exposed to biotic and abiotic stresses as stated above were used for RNA isolation, followed by cDNA preparation using a standard protocol. The qPCR was done using TranStart Top Green qPCR superMix in a Bio-Rad qPCR set CFX96 Touch™ real time machine (Bio-Rad, Singapore). Real-time PCR in 20 µL volume was carried out with the following steps: for 2 min at 95 °C, then 95 °C for 10 s for 40 cycles, then 15 s at 60 °C, followed by 15 s at 72 °C. The pineapple EFl $\alpha$  gene was used as the internal control [38,39]. The Livak method ( $-\Delta\Delta CT$ ) was used for the calculation of fold change [38,39]. The experiments were carried out in three biological and three technical replicates. Primers used in real time are listed in Supplementary Table S2.

### 2.3. RNA Seq of Pineapple

RNA from gynoecium, ovule, stamen, petal, and sepal of pineapple (MD2 variety) at various stages of development were isolated for library preparation and Illumina sequencing as previously described by Chen et al., [32]. Total RNA was isolated by Plant RNeasy Mini (Qiagen, Strasse 1, Hilden, Germany), followed by cDNA library preparation using NEBNext Ultra™ RNA library prep (NEB, Ipswich, MA, USA) following standard protocols. After quality checking and sequencing, the reads were aligned to the genome of pineapple using TopHat v2.1.1 [40] with default parameters. The transcript abundance of pineapple SWR1-C subunits were calculated as fragments per kilobase of exon model per million mapped reads (FPKM) and a heatmap was generated using pheatmap, a package of R software based on the log<sub>2</sub> (FPKM+1).

### 2.4. Phylogenetic Analysis and Identification of Homologue Sequences in Pineapple

SWR1-C subunit protein sequences of *Arabidopsis* were downloaded from TAIR (<http://www.arabidopsis.org>) and of *Oryza sativa* from the rice data center of China (<http://www.ricedata.cn/gene/index.htm>). For the identification of SWR1-C subunits in pineapple, SWR1-C keywords were used to blast against the genome of pineapple in the phytozome database (<https://phytozome.jgi.doe.gov>); then, we performed a search in the pineapple genome database by using the HMM profiles to carryout BLAST-P with 0.01 set as the e-value. Parameters of MUSCLE 3.7 were set at default to generate the multiple sequence alignment of SWR1-C subunits in pineapple, *Arabidopsis*, and rice. The phylogenetic trees were generated using MEGA 7 by the maximum likelihood (ML) method with default parameters and settings with exception of the bootstrap option n = 1000 plus pairwise gaps removal. The neighbor-joining (NJ) method was used to generate phylogenetic trees. ExpASy (<http://web.expasy.org>) was used to determine the isoelectric point (pI) and molecular weight (MW) of all the SWR1-C subunits in pineapple [41–43]. The details of the locus of *Arabidopsis* and rice SWR1-C subunits are provided in Supplementary Table S1.

### 2.5. Gene Structure Analysis and Chromosomal Location

For the mapping of SWR1-C subunits on the various linkage groups in pineapple, MapChart [44] was used based on their start and stop identification numbers identified from the phytozome (<https://phytozome.jgi.doe.gov>) database using the pineapple genome. GSDS (gene structure and display server) was used to make the exon–intron structural organization in pineapple SWR1-C [45].

### 2.6. Vector Construction

Pineapple genomic DNA was used to amplify 5058 bp of complete *AcSWC6* gene from start codon and without stop codon using the primers listed in Supplementary Table S2. The amplified *AcSWC6* was then moved to the vector pENTR/D-TOPO (Invitrogen). The positive clones were then recombined

with the destination vector pGWB505 using LR recombination clonase (Invitrogen, Carlsbad, CA, USA) [9]. The final cassette was transformed into GV3101 strain of *Agrobacterium tumefaciens* and used for transformation of *Atswc6* and Col-0 using the floral dip method [46].

### 2.7. Microscopy

For live microscopy, 5 d old seedlings were mounted on a cover glass slide for observation using a Leica TCS SP8 microscope (Leica, Wetzlar, Germany) and imaged.

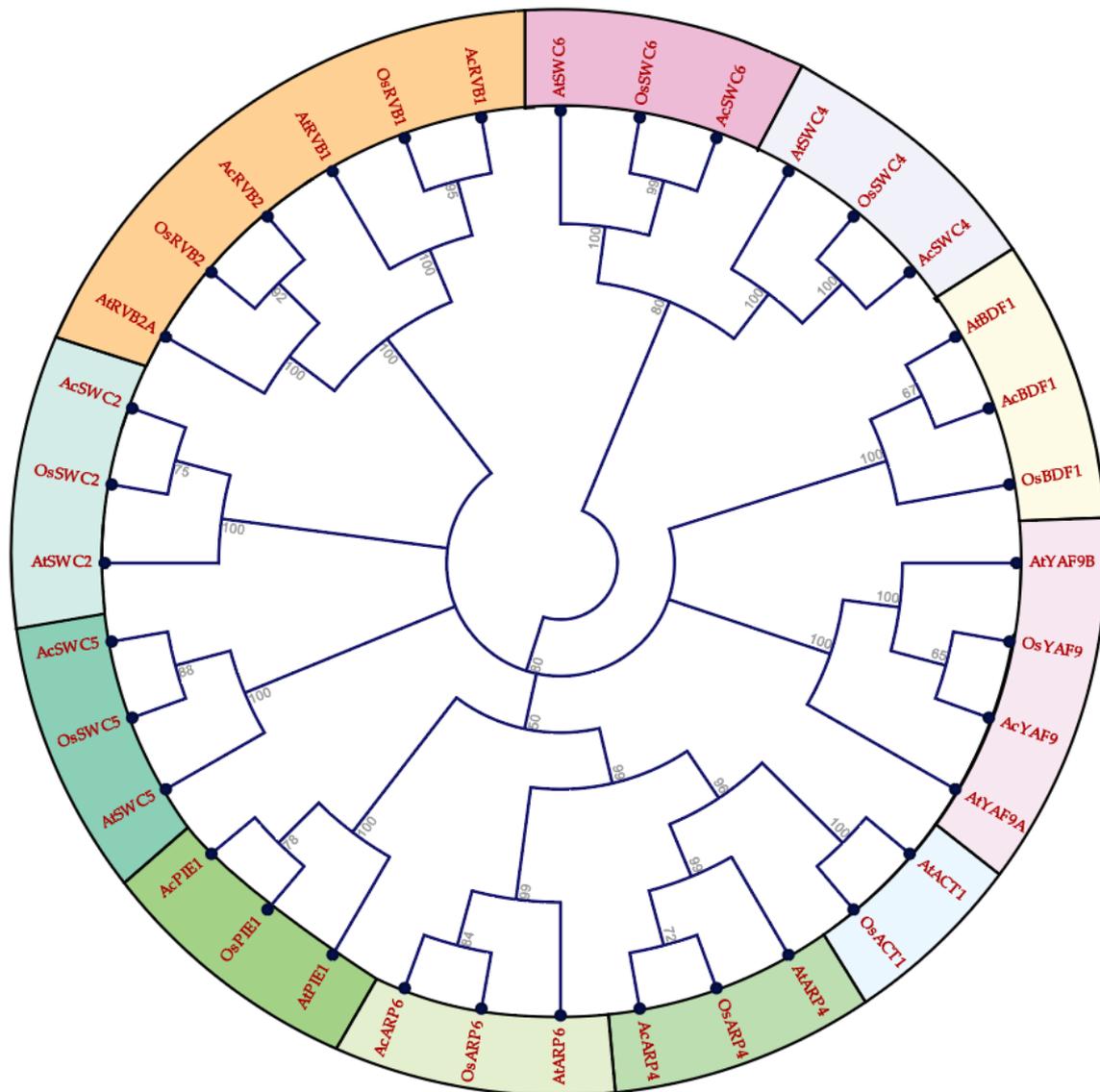
### 2.8. Statistical Analysis

Results are expressed as the means  $\pm$  standard error (SE) from at least three experiments. The statistical significance was analyzed by two-tailed Student's *t*-test.

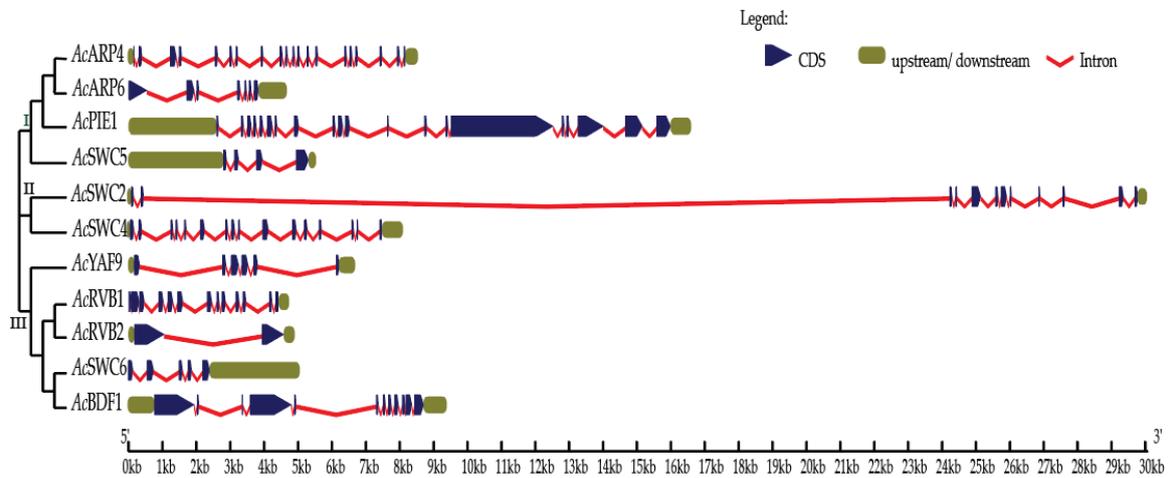
## 3. Results

### 3.1. Identification, Phylogenetic Analysis, and Gene Structure of SWR1-C Subunits

We identified 11 SWR1-C subunits in pineapple using the genome of pineapple, BLAST, HMM, and available pineapple annotation based on the SWR1-C in yeast and *Arabidopsis* [10,14,19]. To study the evolutionary and functional relationship of identified SWR1-C subunits, a phylogenetic tree of multiple species was built using the full-length amino acid sequences of pineapple, *Arabidopsis*, and rice using the maximum likelihood parameter. The pineapple SWR1-C components were then annotated based on their sequence similarity and phylogeny with other species. However, most of the individual subunits formed an independent group, with their corresponding homolog in rice and *Arabidopsis* revealing their conserved nature between monocots and dicots (Figure 1). Additionally, another phylogenetic tree was generated based on the gene structure between the individual subunits in pineapple which divided the complex into three groups: Group I, Group II, and Group III, respectively (Figure 2). Group I has four members, whereas Group III has five members. The smallest group is Group II with only two members. This suggests that structural diversity is a common phenomenon among the pineapple SWR1-C subunits which could be the reason they have nonredundant function in some biological processes.



**Figure 1.** Phylogenetic tree indicating the relationships among SWI2/SNF2-Related 1 chromatin remodeling complex (SWR1-C) subunits from pineapple, rice, and *Arabidopsis*. Prefixes ‘At’, Os, and ‘Ac’ indicate SWR1-C proteins from *Arabidopsis*, *Oryza sativa*, and *Ananas comosus* respectively.



**Figure 2.** Exon–intron structure of pineapple SWR1-C subunits.

For structural divergence of the SWR1-C subunits in pineapple, the exon–intron structures were further investigated. An exon–intron structure was constructed using the Gene Structure Display Server 2.0 (GSDS) (Figure 2). The result revealed that among the SWR1 complex subunits, only *AcSWC2* and *AcBDF1* have the same numbers of exons and introns, while other subunits of the complex exhibit variation of exon and intron numbers. The difference in exons, introns, and UTR length in the SWR1-C suggests that the subunits may have different roles to play during the growth and developmental processes of pineapple [43].

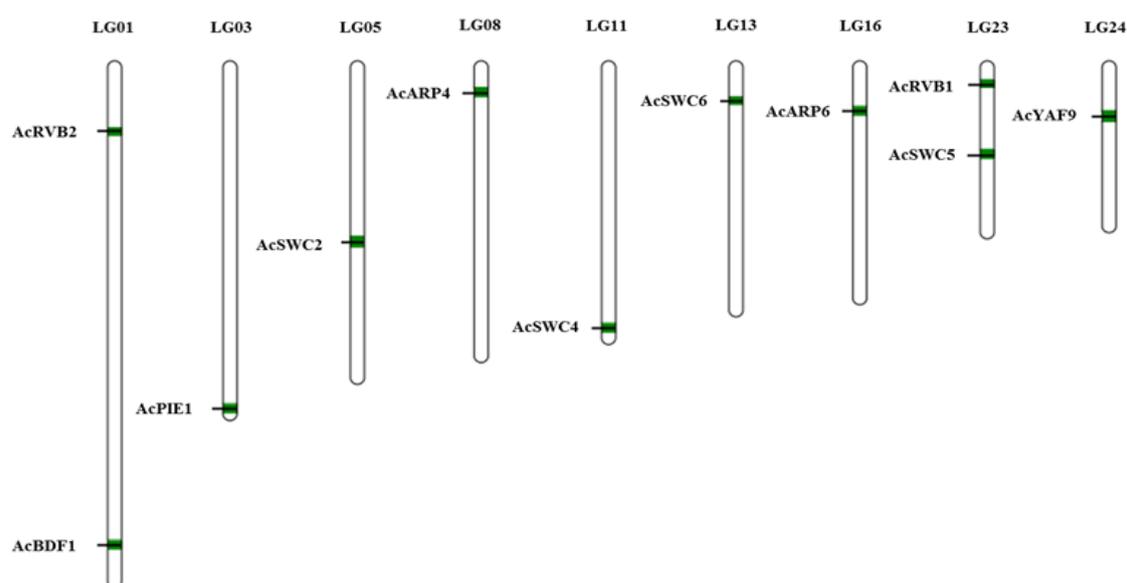
SWR1-C subunits in pineapple were further characterized based on the length of their open reading frame (ORF), protein sequence size, protein molecular weight (MW), and isoelectric point (pI) (Table 1).

**Table 1.** Characterization of SWR1-C subunits of pineapple.

| Gene          | Gene ID   | MW (kDa) | pI   | Amino Acid | Location           | ORF  | Chr |
|---------------|-----------|----------|------|------------|--------------------|------|-----|
| <i>AcPIE1</i> | Aco017256 | 230.32   | 5.44 | 2021       | 16521947..16538538 | 6066 | 03  |
| <i>AcARP4</i> | Aco007686 | 48.75    | 5.09 | 444        | 1249997..1258550   | 1335 | 08  |
| <i>AcARP6</i> | Aco018334 | 42.98    | 5.97 | 382        | 2117532..2122193   | 1149 | 16  |
| <i>AcYAF9</i> | Aco027612 | 32.38    | 6.72 | 295        | 2382105..2388792   | 888  | 24  |
| <i>AcRVB1</i> | Aco015484 | 56.28    | 6.94 | 511        | 847770..852493     | 1536 | 23  |
| <i>AcRVB2</i> | Aco012319 | 55.51    | 5.69 | 507        | 3080033..3084927   | 1524 | 01  |
| <i>AcSWC2</i> | Aco016372 | 43.23    | 5.69 | 376        | 8471728..8501804   | 1131 | 05  |
| <i>AcSWC4</i> | Aco005752 | 50.48    | 9.49 | 449        | 12625273..12633418 | 1350 | 11  |
| <i>AcSWC5</i> | Aco007381 | 28.71    | 8.65 | 256        | 4261067..4266596   | 771  | 23  |
| <i>AcSWC6</i> | Aco012501 | 28.51    | 9.03 | 252        | 1632792..1637849   | 759  | 13  |
| <i>AcBDF1</i> | Aco006628 | 126.34   | 6.93 | 1127       | 23130832..23140236 | 3384 | 01  |

### 3.2. Chromosomal Location of SWR1-C Components

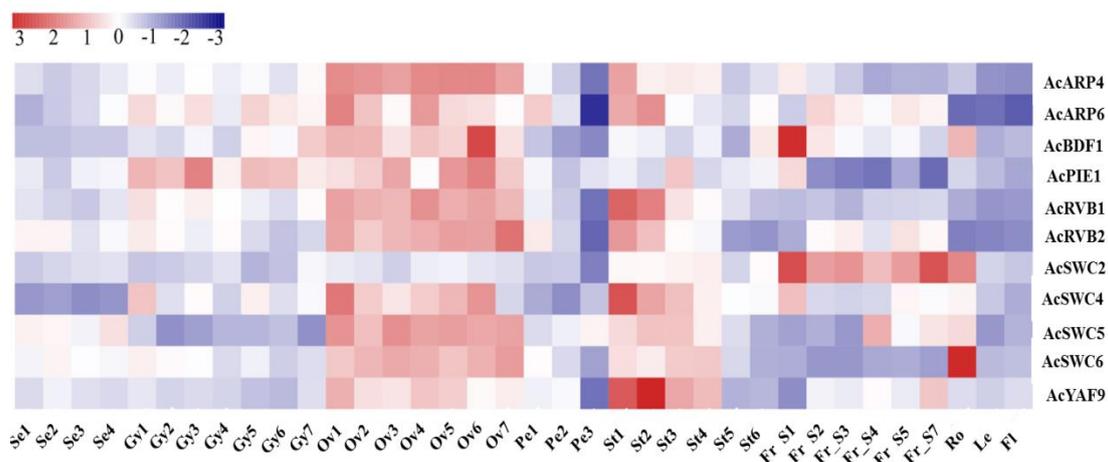
SWR1-C subunits genes were unevenly mapped on nine linkage groups (LG) out of the total 25 linkage groups present in pineapple. Based on our mapped data, we found two genes located on linkage groups 1 and 23, while linkage group 3, 5, 8, 11, 13, 16, and 24 had one gene each (Figure 3). Our data suggest that the length of the linkage group has no relation with the number of genes on it.



**Figure 3.** Chromosomal locations of pineapple SWR1-C subunits. The 11 SWR1 subunits of pineapple were mapped to different chromosomes using MapChart. The position of pineapple SWR1-C subunits are represented in green.

### 3.3. AcSWR1-C Subunits Show Differential Expression

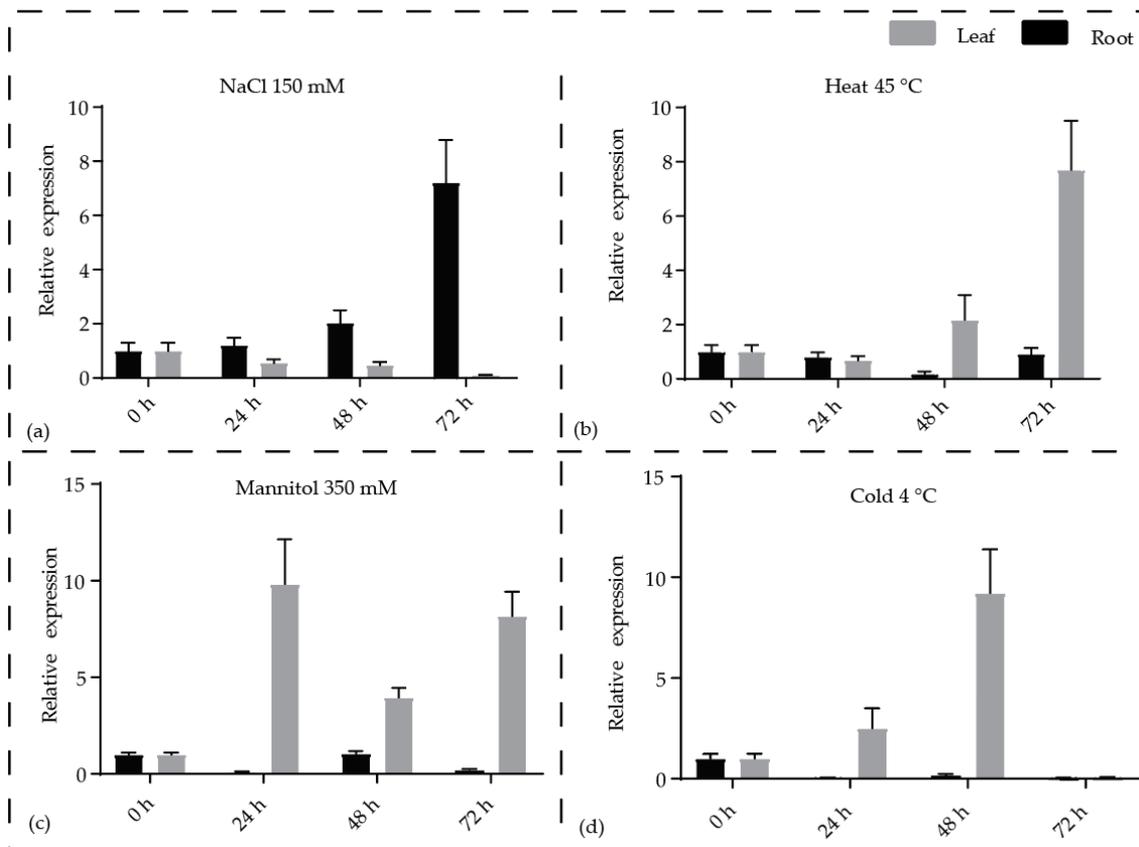
Transcriptome data obtained from different developmental stages and organs was used to examine the transcriptional divergence of SWR1-C. From the RNA-Seq data and average log values of each of the pineapple SWR1-C subunits, an expression heatmap was generated (Figure 4). According to the expression data, almost all the subunits showed low expression level in the sepal except AcSWC5 which was slightly more expressed. In the gynoecium, AcARP6 and AcPIE1 were highly expressed, while AcARP4, AcBDF1, AcRVB1, AcRVB2, AcSWC4, and AcSWC6 were less expressed. All the subunits were significantly expressed in the ovule except for AcSWC2. Similarly, in the stamen, most of the subunits were highly expressed except AcBDF1 and AcSWC2; however, AcPIE1 shows a reduced expression in stage three of stamen development. In the fruit, AcARP4, AcPIE1, AcRVB1, AcSWC6, and AcYAF9 were less expressed, while AcSWC2 was expressed more compared to AcARP6, AcBDF1, AcRVB2, AcSWC4, AcSWC5, and AcYAF9. This suggests that the pineapple SWR1-C subunits are differentially expressed in different organs during growth and development.



**Figure 4.** Expression profiles of pineapple SWR1-C subunits in different organs and developmental stages. Red color indicates high levels of transcript abundance, and blue color indicates low transcript abundance. The color scale is shown at top left side of the figure. Sample details are mentioned at the bottom of each lane: sepal Se1–Se4, gynoecium Gy1–Gy7, ovule Ov1–Ov7, petal Pe1–Pe3, stamen St1–St6, fruit Fr\_S1–Fr\_S7, root Ro, leaf Le and flower Fl. “S” is the abbreviation for “stage.” pineapple SWR1-C subunit names are shown on the right side of the figure.

### 3.4. Expression of SWR1-C Components is Regulated Under Abiotic Stress

The influence of abiotic stress was examined in pineapple SWR1-C on the basis of their expression during exposure to stress. All the pineapple SWR1-C subunits expression was examined by quantitative real-time (qRT) PCR under NaCl, mannitol, heat, and cold stresses, respectively. Our qRT-PCR data indicate that all the SWR1-C subunits of pineapple are regulated by the selected stress treatments. Most of the subunits showed significantly high expression to NaCl in roots, while AcSWC2, AcSWC5, AcRVB1, and AcRVB2 were significantly upregulated in both leaf and the root at different time points. All the SWR1-C subunits were significantly upregulated in the root compared to leaf under NaCl and mannitol treatments. Similarly, when subjected to heat treatment, all subunits were upregulated in leaf and the root except AcSWC6, AcSWC2, AcSWC4, and AcSWC5, which were upregulated in the leaves only. Similarly, during the cold stress, AcARP6, AcBDF1, AcSWC6, and AcSWC2 were upregulated in the leaf, while only AcARP4 was upregulated in the root. AcPIE1, AcSWC4, AcSWC5, and AcYAF9 were less expressed in the cold treatment (Figure S1). The expression of AcSWC6 was increased in the root under NaCl treatment, while it was higher in the leaf under mannitol, heat, and cold treatments when compared to the untreated plants (Figure 5a–d). Taken together, these results suggest that the SWR1-C subunits play essential functions under abiotic stress conditions.



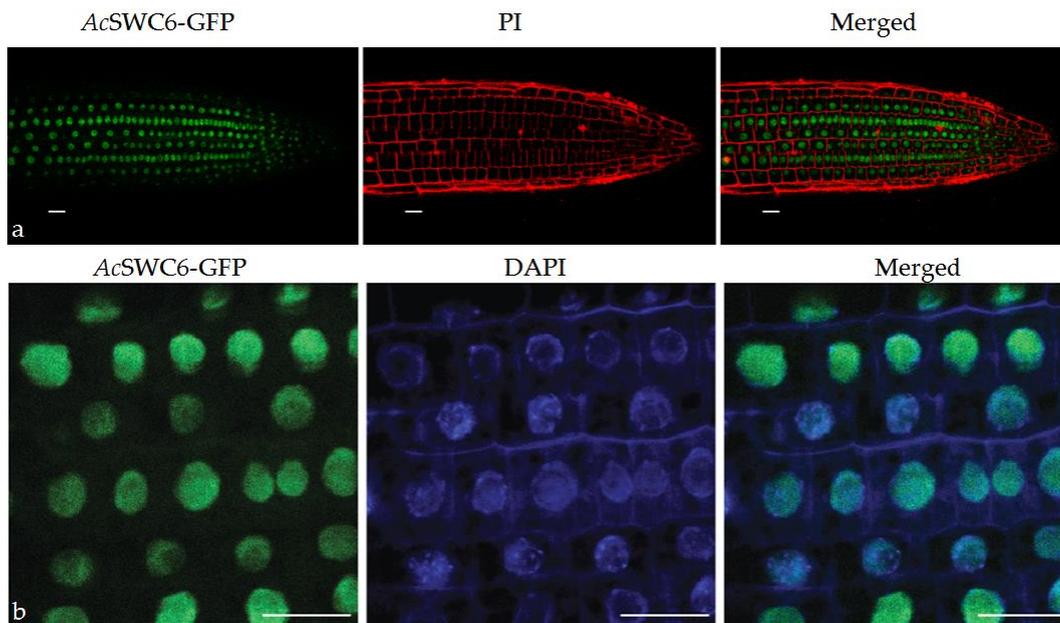
**Figure 5.** Expression profiles of the pineapple *AcSWC6* subunit in response to different stress treatments. qRT-PCR of *AcSWC6* subunit at different time point (0 h, 24 h, 48 h, and 72 h) and samples (leaf and root) after (a) Salt (NaCl 150 mM), (b) Heat (45 °C) stress (c) (Mannitol 350 mM), and (d) Cold (4 °C) stress. Data were normalized with pineapple *EF1a* gene and vertical bars indicate standard error (SE). All experiments were performed with three technical and three biological repeats.

### 3.5. *AcSWC6* Gets Localized to the Nucleus

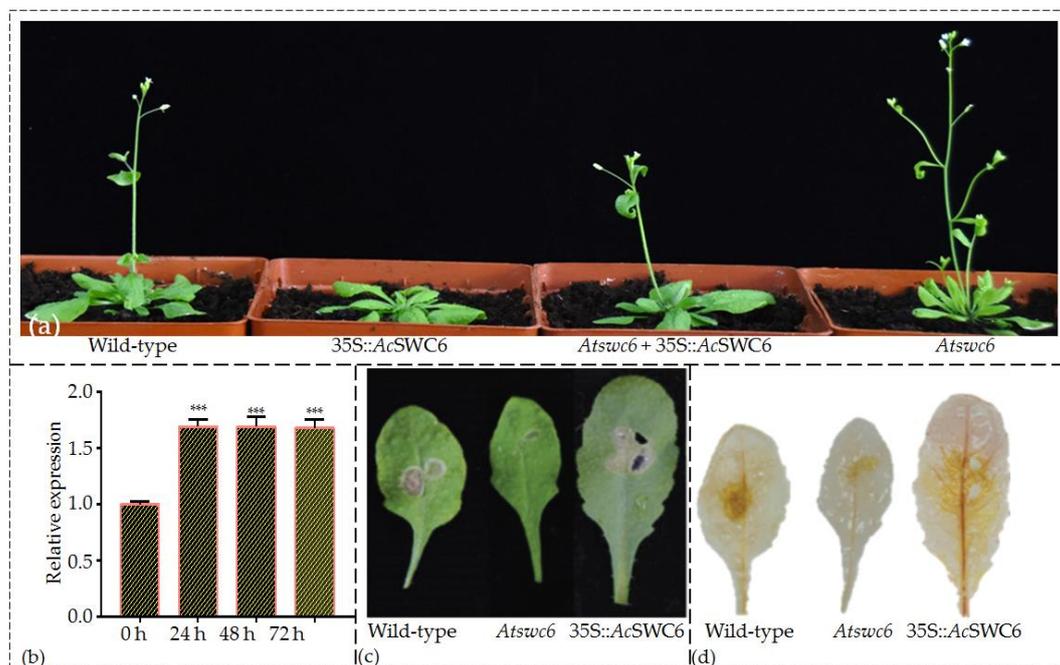
Roots of the *AcSWC6* transgenic plants were examined under a confocal microscope in order to understand the localization and expression of the *AcSWC6* protein. We found that *AcSWC6*-GFP localizes in the nucleus of transgenic lines (Figure 6).

### 3.6. *AcSWC6* Regulates the Biotic and Abiotic Stress Tolerance in *Arabidopsis*

To examine the role of *AcSWC6* in immunity and pathogen resistance, we infected the leaves of the pineapple plants with the fungus *Sclerotinia sclerotiorum* and relative transcript abundance of *AcSWC6* was measured at different time points. We found that the fungal infection increased the *AcSWC6* transcript significantly (Figure 7b). This finding clearly indicates that the *AcSWC6* is involved in the biotic stress response in pineapple. We then checked the performance of *AcSWC6* overexpressing *Arabidopsis* plants compared to wild type and *Atswc6* mutant against fungal infection. Rosette leaves of the plants were incubated for 24 h to allow fungal infection. The infected leaves were then photographed (Figure 7c), followed by DAB staining (Figure 7c). The *Atswc6* mutant showed higher resistance to the fungal attack with the least damage to leaves and  $H_2O_2$  activity, while the 35S::*AcSWC6* overexpression line was susceptible with increased damage to leaves and higher  $H_2O_2$  activity compared to the mutant and the wild type (Figure 7c,d).

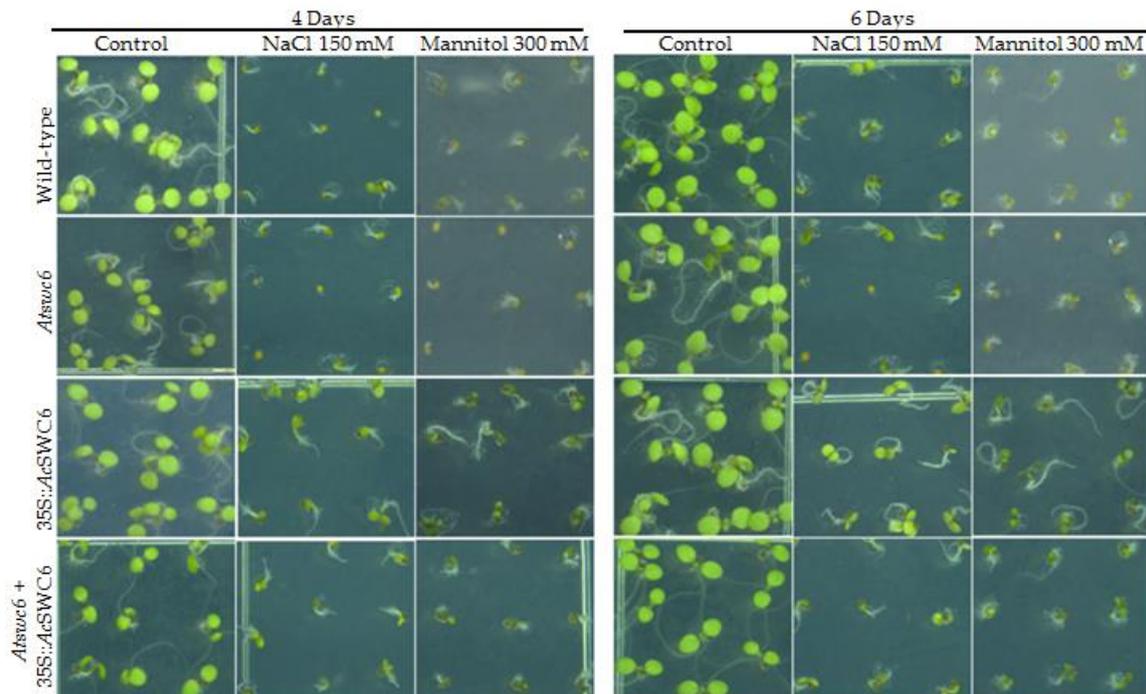


**Figure 6.** *AcSWC6-GFP* localizes to the nucleus. (a) *AcSWC6-GFP* localization in the nucleus of seven-day-old roots of transgenic *Arabidopsis* plants. GFP fluorescence is represented in green and propidium iodide (PI) fluorescence channel is represented in red (b) *AcSWC6-GFP* localization in the nucleus of seven-day-old roots of transgenic *Arabidopsis* plants. GFP fluorescence is represented in green and DAPI fluorescence channel is represented in blue. Scale bars = 20  $\mu$ m.



**Figure 7.** *AcSWC6* regulates the biotic stress tolerance in *Arabidopsis*: (a) Morphology of four-week-old wild-type (Col-0) and *AcSWC6* overexpressing plant; (b) Relative expression of *AcSWC6* in pineapple leaf at different time points after infection with fungus (*Sclerotinia sclerotiorum*); (c) Damage in leaves by fungus (*Sclerotinia sclerotiorum*) to mature rosette leaves of wild-type and *AcSWC6* overexpressing plants after 24 h infection; (d) Detection of  $H_2O_2$  by DAB staining in leaves of wild-type (Col-0) and *AcSWC6* overexpressing plants after 24 h infection. The brown color reflects the reactive oxygen species (ROS) accumulation after the fungal treatment.

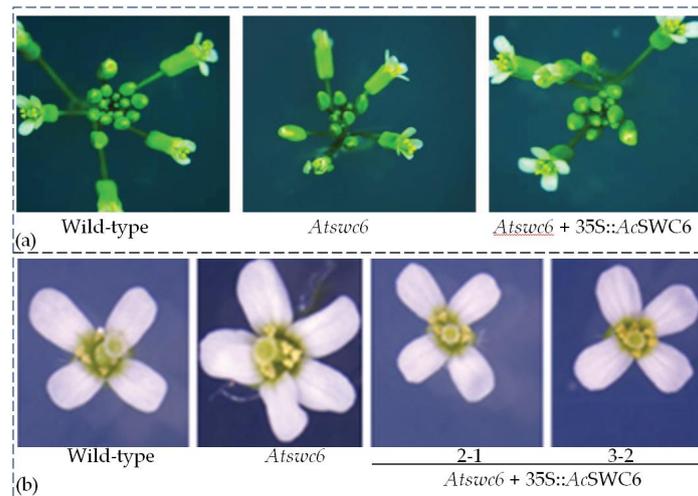
Previous reports have shown that SWR1-C plays a crucial role during growth and environmental responses [6,11]. To further examine the role of AcSWC6 during abiotic stress we used different AcSWC6 *Arabidopsis* lines and studied their growth phenotype after 4 d and 6 d of salinity (NaCl 150 mM) and osmotic (mannitol 300 mM) stress treatment. The *Atswc6* mutant showed susceptibility to salinity and osmotic stress compared to wild-type plants. The plants ectopically expressing AcSWC6 displayed better performance in terms of germination and growth phenotype during salinity and osmotic stress (Figure 8). These findings clearly indicate that the SWR1-C subunit SWC6 contributes to biotic and abiotic stress responses in plants.



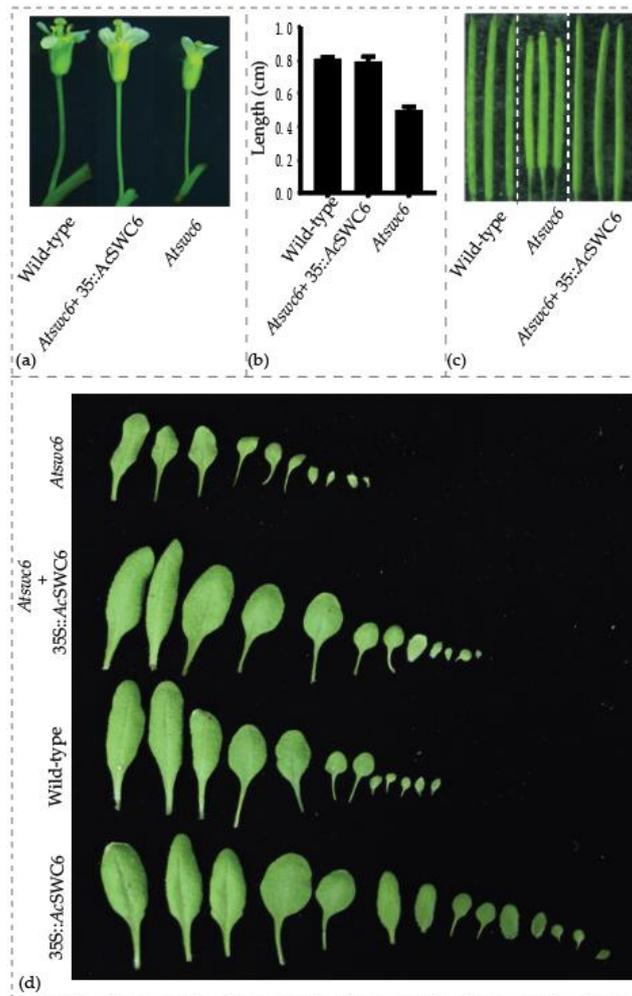
**Figure 8.** Overexpression of AcSWC6 results in resistance to salinity and osmotic stress. Phenotype of 4 days and 6 days old wild type, *Atswc6*, 35S::AcSWC6 and complemented line germinated under salinity (150 mM NaCl) and osmotic stress (300 mM mannitol).

### 3.7. SWC6 Function Is Conserved in Plants

The *Atswc6* complementation plasmid was constructed by amplifying a 5058 bp coding region of AcSWC6 from pineapple genomic DNA, using primers listed in the Supplementary Table S2. It was then used to transform the *Arabidopsis swc6* mutant plants via the floral dip method. Ten different lines from the initial screening were selected for further generation advancement. Finally, plants from three independent T3 generation lines were used for phenotypic assessment. All of the transformed lines plants showed that AcSWC6 can rescue the phenotype of the *Atswc6* plants (Figure 7a). There was no obvious difference in the root growth between the complemented and the wild type plants. The complemented plants exhibited a growth pattern similar to that of the wild type and the small and serrated leaves phenotype along with early flowering phenotype was rescued by AcSWC6. Moreover, the small flowers and irregular floral organs phenotype, and the flower architecture phenotype were also restored (Figure 9a,b). The silique size and seed setting phenotypes were also rescued by AcSWC6 (Figure 10a,c).



**Figure 9.** *AcSWC6* rescues the *Atswc6* mutant floral architecture. (a) Flower architecture phenotype of wild type, *Atswc6*, and *Atswc6* 35S::AcSWC6 complemented lines. (b) Petal phenotype of wild type, *Atswc6*, and *Atswc6* 35S::AcSWC6.



**Figure 10.** *AcSWC6* flower phenotype in *Arabidopsis*. (a) and (b) Pedicel length phenotype, (c) Silique and, (d) leaf phenotype of wild type, *Atswc6*, and *Atswc6* 35S::AcSWC6 complemented lines.

#### 4. Discussion

Chromatin remodelers perform the task of chromatin remodeling and chromatin dynamics has significant involvement in almost every cellular activity by regulating gene expression [6,47]. Eukaryotic cells possess an evolutionary conserved ATP-dependent chromatin remodeling complex, SWR1-C, belonging to the SWI/SNF class of remodelers [48,49]. The subunits of SWR1-C have been studied in *Arabidopsis* [6,10]; however, the complex is still unexplored in pineapple. Here, we identified the subunits of SWR1-C in pineapple and studied their properties in detail. We further characterized one subunit, *AcSWC6*, by overexpressing it and complementing the *Atswc6* mutant in *Arabidopsis*. Our results suggest the existence of SWR1-C in pineapple and a functionally conserved role of *AcSWC6* in plants.

In *Arabidopsis*, *PIE1*, *ARP6*, *ARP4*, *SWC6*, and *SWC4* have been studied and characterized as SWR1-C subunits [6,21,50,51]. Here, we identified the homologs of the SWR1-C subunits from pineapple with high sequence similarity between that of *Arabidopsis*, rice, and yeast. The multispecies phylogenetic tree (Figure 1) revealed the conserved nature of SWR1-C between pineapple, *Arabidopsis*, and rice. The SWR1-C subunits formed the independent groups with their homologs in *Arabidopsis* and rice. Based on the phylogenetic relationship, the subunits could be further divided into three categories (Figure 2). Group I consisted of *AcARP4*, *AcARP6*, *AcPIE1*, and *AcSWC5*. Group II had *AcSWC2* and *AcSWC4*, and Group III had *AcYAF9*, *AcRVB1*, *AcRVB2*, *AcSWC6*, and *AcBDF1*. *ACT1* was present in *Arabidopsis* and rice but not in pineapple. These findings support the existence of SWR1-C in plants. SWR1-C is expected to act as a single entity in various aspects of development and environmental cues. However, the subunits show independent function, especially in immunity and during abiotic stresses [8,10]. The possible explanation could be the presence of a high degree of exon–intron variation in the gene structure of each subunit in the complex. The final conformational structure and composition of the SWR1-C subunits affect nucleosome and H2A.Z deposition, which could influence the recruitment of transcriptional machinery [52].

We next examined the expression pattern of SWR1-C subunits in pineapple using the RNAseq data and generated a hierarchical expression map. We found that most of the SWR1-C subunits are highly expressed in the ovule and stamen except *AcSWC2*, which shows higher expression in the fruit. This could be because SWR1-C regulates flower and gametophyte development [11]. Mutants of the core subunits of SWR1-C, *PIE1*, *SWC6*, and *ARP6*, exhibit defects in flowering with bushy appearance, early bolting, and defects in floral organs, indicating their involvement in floral development [19]. Among the pineapple SWR1-C subunits, *AcPIE1* was expressed specifically in ovule, gynoecium, and slightly in stamen stage 3, suggesting its involvement in pineapple flower development similarly indicated by *Arabidopsis pie1* mutants with severe flowering defects when compared to *swc6* and *arp6* mutants [53]. *AcARP6* was expressed in the same manner as *AcPIE1*; however, it was additionally expressed in stamen and fruit. Similarly, *AcSWC6* also showed additional expression in sepal, stamen, and root compared to *AcPIE1* (Figure 4). These results are in agreement to previous studies on the role of SWR1-C in gametophyte and flower development [5,14,22,54]. Increasing evidence suggests that SWR1-C plays a major role in plants under both optimal and stressed conditions [8,10]. We investigated the transcript level of *AcSWC6* under different abiotic stress conditions by qRT-PCR (Figure 5). Our data showed that the transcript level of *AcSWC6* changed significantly during the stress treatments. An increase in the level of the transcript of *AcSWC6* was noticed in the root but it decreased in the leaf during NaCl treatment (Figure 5a). However, during osmotic stress, a rise in the level of the transcript was observed in the leaf but it decreased in the root (Figure 5c). Similarly, during heat and cold stresses, an increase in the transcript level of *AcSWC6* was observed (Figure 5b,d). Altogether, the data suggest that *AcSWC6* is involved in the stress response in plants.

Plants exhibit different strategies to respond to pathogen attack and adapt or escape harsh environmental conditions due to their sessile nature [10]. SWR1-C plays a significant role in abiotic and biotic stresses by regulating gene expression during pathogen attack and environmental stress [8,10]. In *Arabidopsis*, mutation in *PIE1*, *ARP6*, and *SWC6* confers resistance to pathogens [14,17].

We investigated the role of pineapple *AcSWC6* during biotic (fungal attack) and abiotic (salinity and osmotic) stress in *AcSWC6* overexpressing *Arabidopsis* plants, consistent with previous reports, we found that *AcSWC6* regulates the immunity of the plants during pathogen attack. The *AcSWC6*-expressing plants resulted in pathogen susceptibility, while the *Arabidopsis swc6* mutant showed enhanced resistance to the pathogen compared to wild type and the *AcSWC6*-expressing plant (Figure 7c,d). Additionally, during abiotic stress treatment, we found that the *Arabidopsis swc6* mutant is sensitive to salt and osmotic stress, whereas the plants ectopically expressing the pineapple *SWC6* displayed increased resistance to abiotic stresses. Ectopic expression of *AcSWC6* may affect the deposition of H2A.Z, which in turn regulates key genes contributing to biotic and abiotic stress.

To further investigate the conserved nature of *SWC6*, we complemented *Arabidopsis* mutant *Atswc6* with pineapple *AcSWC6*. The results showed that the pineapple *AcSWC6* could complement the *Arabidopsis Atswc6* and completely rescue the mutant phenotype. The serrated leaf, early flowering, extra petals (from 4–6), and length of the flower pedicel phenotypes were all rescued (Figure 10a–d). Flower architecture is an important determinant of reproduction in plants and it is controlled by *SWR1* directly or indirectly. Pedicel elongation is controlled by the *PRE1* (PACLOBUTRAZOL RESISTANCE1) bHLH transcription factor through coordination of the ER-MAPK signaling pathway and the *SWR1* complex. When *PRE*'s expression is reduced, pedicel length is also reduced. Similarly, this phenomenon is associated with phytohormones such as brassinosteroid (BR), gibberellin (GA), and auxin. Cai et al., (2017) reported that *SWR1* controls the expression of *PREs* and this regulates flower architecture and pedicel length [9]. Previously, Lazaro et al., (2008) reported that *AtSWC6* overexpression showed no additional phenotype [21]. However, we found that the ectopic expression of *AcSWC6* delayed the flowering and increased susceptibility to biotic stress (Figure 7a,c,d). This delayed flowering could result from the variation in gene structure between the *SWC6* of pineapple and *Arabidopsis*. The expression of *AcSWC6* may cause a change in the deposition of H2A.Z, which could have resulted in the delay of flowering by regulating the expression of key genes involved in biotic and abiotic stress response.

## 5. Conclusions

In the present study, we comprehensively studied *SWR1-C* in pineapple and identified a total of 11 *SWR1-C* subunits. The expression profiles of the *SWR1-C* subunits in pineapple suggest that *SWR1-C* plays an important role in pineapple development and environmental stress response. The findings of the present study support the conserved nature and role of *AcSWC6* in plants during growth and development. This information could form a basis for further research to utilize *SWR1-C* subunits for development of next-generation crops.

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**Author Contributions:** M.A. and Y.Q. conceived and designed the research. B.H.J., M.A., B.F., performed the experiments. J.G.G., Z.L., W.L., L.L., and O.A.A., helped in the experiments S.C., G.L., Y.C. and J.-M.H. analyzed data. B.H.J., M.A., and Y.Q. wrote the manuscript. M.A., and Y.Q. revised the manuscript. All the authors read and approved the final manuscript.

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**Conflicts 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.

## Abbreviations

|      |   |
|------|---|
| SWR1 | SWI2/SNF2-RELATED 1                     |
| DAB  | 3,3-diaminobenzidine                    |
| SWC2 | SWR1 COMPLEX 2                          |
| SWC3 | SWR1 COMPLEX 3                          |
| SWC5 | SWR1 COMPLEX 5                          |
| SWC6 | SWR1 COMPLEX 6                          |
| SEF  | SERRATED LEAF AND EARLY FLOWERING       |
| SWC7 | SWR1 COMPLEX 7                          |
| ARP6 | ACTIN-RELATED PROTEIN 6                 |
| YAF9 | YEAST ALL1 FUSED GENE FROM CHROMOSOME 9 |
| BDF1 | BROMODOMAIN-CONTAINING FACTOR 1         |
| ACT1 | ACTIN 1                                 |
| ARP4 | ACTIN-RELATED PROTEIN 4                 |
| SWC4 | SWR1 COMPLEX 4                          |
| RVB1 | RUVB LIKE PROTEIN 1                     |
| RVB2 | RUVB LIKE PROTEIN 2                     |
| UTR  | Untranslated region                     |
| CDS  | Coding sequence                         |

## References

- Conde, D.; Perales, M.; Sreedasyam, A.; Tuskan, G.A.; Lloret, A.; Badenes, M.L.; Gonzalez-Melendi, P.; Rios, G.; Allona, I. Engineering Tree Seasonal Cycles of Growth Through Chromatin Modification. *Front. Plant Sci.* **2019**, *10*, 412. [[CrossRef](#)] [[PubMed](#)]
- Kim, M.Y.; Ono, A.; Scholten, S.; Kinoshita, T.; Zilberman, D.; Okamoto, T. DNA demethylation by ROS1a in rice vegetative cells promotes methylation in sperm. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 9652–9657. [[CrossRef](#)] [[PubMed](#)]
- Forgione, I.; Woloszynska, M.; Pacenza, M.; Chiappetta, A.; Greco, M.; Araniti, F.; Abenavoli, M.R.; Van Lijsebettens, M.; Bitonti, M.B.; Bruno, L. Hypomethylated *drm1 drm2 cmt3* mutant phenotype of *Arabidopsis thaliana* is related to auxin pathway impairment. *Plant Sci.* **2019**, *280*, 383–396. [[CrossRef](#)] [[PubMed](#)]
- Wu, W.H.; Wu, C.H.; Ladurner, A.; Mizuguchi, G.; Wei, D.; Xiao, H.; Luk, E.; Ranjan, A.; Wu, C. N terminus of Swr1 binds to histone H2AZ and provides a platform for subunit assembly in the chromatin remodeling complex. *J. Biol. Chem.* **2009**, *284*, 6200–6207. [[CrossRef](#)] [[PubMed](#)]
- Choi, K.; Park, C.; Lee, J.; Oh, M.; Noh, B.; Lee, I. Arabidopsis homologs of components of the SWR1 complex regulate flowering and plant development. *Development* **2007**, *134*, 1931–1941. [[CrossRef](#)] [[PubMed](#)]
- Gomez-Zambrano, A.; Crevillen, P.; Franco-Zorrilla, J.M.; Lopez, J.A.; Moreno-Romero, J.; Roszak, P.; Santos-Gonzalez, J.; Jurado, S.; Vazquez, J.; Kohler, C.; et al. Arabidopsis SWC4 Binds DNA and Recruits the SWR1 Complex to Modulate Histone H2A.Z Deposition at Key Regulatory Genes. *Mol. Plant* **2018**, *11*, 815–832. [[CrossRef](#)] [[PubMed](#)]
- Paul, S.; Bartholomew, B. Regulation of ATP-dependent chromatin remodelers: Accelerators/Brakes, anchors and sensors. *Biochem. Soc. Trans.* **2018**, *46*, 1423–1430. [[CrossRef](#)] [[PubMed](#)]
- Sura, W.; Kabza, M.; Karlowski, W.M.; Bieluszewski, T. Dual Role of the Histone Variant H2A.Z in Transcriptional Regulation of Stress-Response Genes. *Plant Cell* **2017**, *29*, 791–807. [[CrossRef](#)]
- Cai, H.; Zhao, L.; Wang, L.; Zhang, M.; Su, Z.; Cheng, Y.; Zhao, H.; Qin, Y. ERECTA signaling controls Arabidopsis inflorescence architecture through chromatin-mediated activation of PRE1 expression. *New Phytol.* **2017**, *214*, 1579–1596. [[CrossRef](#)]
- Berriri, S.; Gangappa, S.N.; Kumar, S.V. SWR1 Chromatin-Remodeling Complex Subunits and H2A.Z Have Non-overlapping Functions in Immunity and Gene Regulation in Arabidopsis. *Mol. Plant* **2016**, *9*, 1051–1065. [[CrossRef](#)]
- Kumar, S.V. H2A.Z at the core of transcriptional regulation in plants. *Mol. Plant* **2018**, *11*, 1112–1114. [[CrossRef](#)] [[PubMed](#)]

12. Choi, K.; Kim, J. Regulation of MicroRNA-Mediated Developmental Changes by the SWR1 Chromatin Remodeling Complex. *Plant Physiol.* **2016**, *171*, 1128–1143. [[PubMed](#)]
13. Dai, X.; Bai, Y.; Zhao, L.; Dou, X.; Liu, Y.; Wang, L.; Li, Y.; Li, W.; Hui, Y.; Huang, X.; et al. H2A.Z Represses Gene Expression by Modulating Promoter Nucleosome Structure and Enhancer Histone Modifications in Arabidopsis. *Mol. Plant* **2017**, *10*, 1274–1292. [[CrossRef](#)] [[PubMed](#)]
14. March-Diaz, R.; Reyes, J.C. The beauty of being a variant: H2A.Z and the SWR1 complex in plants. *Mol. Plant* **2009**, *2*, 565–577. [[CrossRef](#)] [[PubMed](#)]
15. Wu, W.H.; Alami, S.; Luk, E.; Wu, C.H.; Sen, S.; Mizuguchi, G.; Wei, D.; Wu, C. SWC2 is a widely conserved H2AZ-binding module essential for ATP-dependent histone exchange. *Nat. Struct. Mol. Biol.* **2005**, *12*, 1064–1071. [[CrossRef](#)] [[PubMed](#)]
16. Morrison, A.J.; Shen, X. Chromatin remodelling beyond transcription: The INO80 and SWR1 complexes. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 373–384. [[CrossRef](#)]
17. March-Diaz, R.; Garcia-Dominguez, M.; Lozano-Juste, J.; Leon, J.; Florencio, F.J.; Reyes, J.C. Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. *Plant J.* **2008**, *53*, 475–487. [[CrossRef](#)]
18. Cai, H.; Zhang, M.; Chai, M.; He, Q.; Huang, X.; Zhao, L.; Qin, Y. Epigenetic regulation of anthocyanin biosynthesis by an antagonistic interaction between H2A.Z and H3K4me3. *New Phytol.* **2019**, *221*, 295–308. [[CrossRef](#)]
19. March-Diaz, R.; Garcia-Dominguez, M.; Florencio, F.J.; Reyes, J.C. SEF, a new protein required for flowering repression in Arabidopsis, interacts with PIE1 and ARP6. *Plant Physiol.* **2007**, *143*, 893–901. [[CrossRef](#)]
20. Rosa, M.; Von Harder, M.; Cigliano, R.A.; Schlogelhofer, P.; Mittelsten Scheid, O. The Arabidopsis SWR1 chromatin-remodeling complex is important for DNA repair, somatic recombination, and meiosis. *Plant Cell* **2013**, *25*, 1990–2001. [[CrossRef](#)]
21. Lazaro, A.; Gomez-Zambrano, A.; Lopez-Gonzalez, L.; Pineiro, M.; Jarillo, J.A. Mutations in the Arabidopsis SWC6 gene, encoding a component of the SWR1 chromatin remodelling complex, accelerate flowering time and alter leaf and flower development. *J. Exp. Bot.* **2008**, *59*, 653–666. [[CrossRef](#)] [[PubMed](#)]
22. Qin, Y.; Zhao, L.; Skaggs, M.I.; Andreuzza, S.; Tsukamoto, T.; Panoli, A.; Wallace, K.N.; Smith, S.; Siddiqi, I.; Yang, Z.; et al. ACTIN-RELATED PROTEIN6 Regulates Female Meiosis by Modulating Meiotic Gene Expression in Arabidopsis. *Plant Cell* **2014**, *26*, 1612–1628. [[CrossRef](#)] [[PubMed](#)]
23. Cui, Z.; Tong, A.; Huo, Y.; Yan, Z.; Yang, W.; Yang, X.; Wang, X.X. SKIP controls flowering time via the alternative splicing of SEF pre-mRNA in Arabidopsis. *BMC Biol.* **2017**, *15*, 80. [[CrossRef](#)] [[PubMed](#)]
24. Ming, R.; VanBuren, R.; Wai, C.M.; Tang, H. The pineapple genome and the evolution of CAM photosynthesis. *Nat. Genet.* **2015**, *47*, 1435–1442. [[CrossRef](#)] [[PubMed](#)]
25. Redwan, R.M.; Saidin, A.; Kumar, S.V. The draft genome of MD-2 pineapple using hybrid error correction of long reads. *DNA Res.* **2016**, *23*, 427–439. [[CrossRef](#)] [[PubMed](#)]
26. Ur Rahman, Z.; Azam, S.M.; Liu, Y.; Yan, C.; Ali, H.; Zhao, L.; Chen, P.; Yi, L.; Priyadarshani, S.; Yuan, Q. Expression profiles of Wuschel-related homeobox gene family in pineapple (*Ananas comosus* L.). *Trop. Plant Biol.* **2017**, *10*, 204–215. [[CrossRef](#)]
27. Ming, R.; Wai, C.M.; Guyot, R. Pineapple Genome: A Reference for Monocots and CAM Photosynthesis. *Trends Genet.* **2016**, *32*, 690–696. [[CrossRef](#)] [[PubMed](#)]
28. Xie, T.; Chen, C.; Li, C.; Liu, J.; Liu, C.; He, Y. Genome-wide investigation of WRKY gene family in pineapple: Evolution and expression profiles during development and stress. *BMC Genom.* **2018**, *19*, 490. [[CrossRef](#)]
29. Nguyen, B.T.; Bujna, E.; Fekete, N.; Tran, A.T.M.; Rezessy-Szabo, J.M.; Prasad, R.; Nguyen, Q.D. Probiotic Beverage from Pineapple Juice Fermented with Lactobacillus and Bifidobacterium Strains. *Front. Nutr.* **2019**, *6*, 54. [[CrossRef](#)] [[PubMed](#)]
30. Priyadarshani, S.; Hu, B.; Li, W.; Ali, H. Simple protoplast isolation system for gene expression and protein interaction studies in pineapple (*Ananas comosus* L.). *Plant Methods* **2018**, *14*, 95. [[CrossRef](#)]
31. Ali, H.; Liu, Y.; Azam, S.M.; Rahman, Z.U.; Priyadarshani, S.; Li, W.; Huang, X.; Hu, B.; Xiong, J.; Ali, U.; et al. Genomic Survey, Characterization, and Expression Profile Analysis of the SBP Genes in Pineapple (*Ananas comosus* L.). *Int. J. Genom.* **2017**, *2017*, 1032846. [[CrossRef](#)] [[PubMed](#)]

32. Chen, P.; Li, Y.; Zhao, L.; Hou, Z.; Yan, M.; Hu, B.; Liu, Y.; Azam, S.M.; Zhang, Z.; Rahman, Z.U.; et al. Genome-Wide Identification and Expression Profiling of ATP-Binding Cassette (ABC) Transporter Gene Family in Pineapple (*Ananas comosus* (L.) Merr.) Reveal the Role of AcABCG38 in Pollen Development. *Front. Plant Sci.* **2017**, *8*, 2150. [[CrossRef](#)] [[PubMed](#)]
33. Baskin, T.I.; Wilson, J.E. Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules. *Plant Physiol.* **1997**, *113*, 493–502. [[CrossRef](#)] [[PubMed](#)]
34. Rahman, A. Auxin: A regulator of cold stress response. *Physiol. Plant* **2013**, *147*, 28–35. [[CrossRef](#)] [[PubMed](#)]
35. Seifi, H.S.; Zarei, A.; Hsiang, T.; Shelp, B. Spermine is a potent plant defense activator against gray mold disease on *Solanum lycopersicum*, *Phaseolus vulgaris* and *Arabidopsis thaliana*. *Phytopathology* **2019**. [[CrossRef](#)] [[PubMed](#)]
36. Raad, M.; Glare, T.R.; Brochero, H.L.; Muller, C.; Rostas, M. Transcriptional Reprogramming of *Arabidopsis thaliana* Defence Pathways by the Entomopathogen *Beauveria bassiana* Correlates with Resistance Against a Fungal Pathogen but Not Against Insects. *Front. Microbiol.* **2019**, *10*, 615. [[CrossRef](#)]
37. Daudi, A.; O'Brien, J.A. Detection of Hydrogen Peroxide by DAB Staining in *Arabidopsis* Leaves. *Plant Cell* **2012**, *2*, e263. [[CrossRef](#)]
38. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [[CrossRef](#)]
39. Chen, H.; Hu, B.; Zhao, L.; Shi, D.; She, Z.; Huang, X.; Priyadarshani, S.; Niu, X.; Qin, Y. Differential Expression Analysis of Reference Genes in Pineapple (*Ananas comosus* L.) during Reproductive Development and Response to Abiotic Stress, Hormonal Stimuli. *Trop. Plant Biol.* **2019**, *12*, 67–77. [[CrossRef](#)]
40. Trapnell, C.; Roberts, A.; Goff, L.; Pertea, G.; Kim, D.; Kelley, D.R.; Pimentel, H.; Salzberg, S.L.; Rinn, J.L.; Pachter, L. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* **2012**, *7*, 562–578. [[CrossRef](#)]
41. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2—Approximately maximum-likelihood trees for large alignments. *PLoS ONE* **2010**, *5*, e9490. [[CrossRef](#)] [[PubMed](#)]
42. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [[CrossRef](#)] [[PubMed](#)]
43. Li, W.; Yan, M.; Hu, B.; Priyadarshani, S.; Hou, Z.; Ojolo, S.P.; Xiong, J.; Zhao, H.; Qin, Y. Characterization and the Expression Analysis of Nitrate Transporter (NRT) Gene Family in Pineapple. *Trop. Plant Biol.* **2018**, *11*, 177–191. [[CrossRef](#)]
44. Voorrips, R.E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* **2002**, *93*, 77–78. [[CrossRef](#)] [[PubMed](#)]
45. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics* **2015**, *31*, 1296–1297. [[CrossRef](#)] [[PubMed](#)]
46. Clough, S.J.; Bent, A.F. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J. Cell Mol. Biol.* **1998**, *16*, 735–743. [[CrossRef](#)]
47. Potok, M.E.; Wang, Y.; Xu, L.; Zhong, Z.; Liu, W.; Feng, S.; Naranbaatar, B.; Rayatpisheh, S.; Wang, Z.; Wohlschlegel, J.A.; et al. *Arabidopsis* SWR1-associated protein methyl-CpG-binding domain 9 is required for histone H2A.Z deposition. *Nat. Commun.* **2019**, *10*, 3352. [[CrossRef](#)] [[PubMed](#)]
48. Henderson, I.R.; Jacobsen, S.E. Epigenetic inheritance in plants. *Nature* **2007**, *447*, 418–424. [[CrossRef](#)]
49. Sarnowska, E.; Gratkowska, D.M.; Sacharowski, S.P.; Cwiek, P.; Tohge, T.; Fernie, A.R.; Siedlecki, J.A.; Koncz, C.; Sarnowski, T.J. The Role of SWI/SNF Chromatin Remodeling Complexes in Hormone Crosstalk. *Trends Plant Sci.* **2016**, *21*, 594–608. [[CrossRef](#)] [[PubMed](#)]
50. Deal, R.B.; Kandasamy, M.K.; McKinney, E.C.; Meagher, R.B. The nuclear actin-related protein ARP6 is a pleiotropic developmental regulator required for the maintenance of FLOWERING LOCUS C expression and repression of flowering in *Arabidopsis*. *Plant Cell* **2005**, *17*, 2633–2646. [[CrossRef](#)]
51. Deal, R.B.; Topp, C.N.; McKinney, E.C.; Meagher, R.B. Repression of flowering in *Arabidopsis* requires activation of FLOWERING LOCUS C expression by the histone variant H2A.Z. *Plant Cell* **2007**, *19*, 74–83. [[CrossRef](#)] [[PubMed](#)]
52. Nguyen, V.Q.; Ranjan, A.; Stengel, F.; Wei, D.; Aebersold, R.; Wu, C.; Leschziner, A.E. Molecular architecture of the ATP-dependent chromatin-remodeling complex SWR1. *Cell* **2013**, *154*, 1220–1231. [[CrossRef](#)] [[PubMed](#)]

53. Choi, J.; Hyun, Y.; Kang, M.J.; In Yun, H.; Yun, J.Y.; Lister, C.; Dean, C.; Amasino, R.M.; Noh, B.; Noh, Y.S.; et al. Resetting and regulation of Flowering Locus C expression during Arabidopsis reproductive development. *Plant J. Cell Mol. Biol.* **2009**, *57*, 918–931. [[CrossRef](#)] [[PubMed](#)]
54. Ojolo, S.P.; Cao, S.; Priyadarshani, S.; Li, W.; Yan, M.; Aslam, M.; Zhao, H.; Qin, Y. Regulation of Plant Growth and Development: A Review from a Chromatin Remodeling Perspective. *Front. Plant Sci.* **2018**, *9*, 1232. [[CrossRef](#)] [[PubMed](#)]



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