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# Genome-Wide Identification and Expression Analysis of the *DA1* Gene Family in Sweet Potato and Its Two Diploid Relatives

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**Abstract:** The *DA1-like* gene family plays a crucial role in regulating seed and organ size in plants. The *DA1* gene family has been identified in several species but has not yet been reported in sweet potatoes. In this study, nine, eleven, and seven *DA1*s were identified in cultivated sweet potato (*Ipomoea batatas*,  $2n = 6x = 90$ ) and its two diploid wild relatives, *I. trifida* ( $2n = 2x = 30$ ) and *I. triloba* ( $2n = 2x = 30$ ), respectively. The *DA1* genes were classified into three subgroups based on their phylogenetic relationships with *Arabidopsis thaliana* and *Oryza sativa* (rice). Their protein physiological properties, chromosomal localization, phylogenetic relationships, gene structure, promoter cis-elements, and expression patterns were systematically analyzed. The qRT-PCR results showed that the expression levels of four genes, *IbDA1-1*, *IbDA1-3*, *IbDA1-6*, and *IbDA1-7*, were higher in the sweet potato leaves than in the roots, fiber roots, and stems. In our study, we provide a comprehensive comparison and further the knowledge of *DA1-like* genes in sweet potatoes, and provide a theoretical basis for functional studies.

**Keywords:** sweet potato; organ size; *DA1-like* gene; qRT-PCR



**Citation:** Zhou, Z.; Huang, J.; Wang, Y.; He, S.; Yang, J.; Wang, Y.; Li, W.; Liu, Y.; Xu, R.; Li, Y.; et al. Genome-Wide Identification and Expression Analysis of the *DA1* Gene Family in Sweet Potato and Its Two Diploid Relatives. *Int. J. Mol. Sci.* **2024**, *25*, 3000. <https://doi.org/10.3390/ijms25053000>

Academic Editors: Pedro Martínez-Gómez and Tibor Janda

Received: 8 January 2024  
Revised: 28 February 2024  
Accepted: 4 March 2024  
Published: 5 March 2024



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## 1. Introduction

The sizes of plant seeds and organs are important yield traits. An important topic in developmental biology is size regulation. Multiple genes involved in seed size regulation have been identified in plants, including *DA1* [1], *SGD1* [2], *GS3* [3], *OsLG3* [4], *LGY3* [5], *GW5* [6], *GW2* [7], *GW8* [8], *qGL3* [9], *GS10* [10], *GL4* [11], *TGW2* [12], and *GS9* [13]. *DA1* is an important gene that regulates seed size. This gene encodes a ubiquitin receptor. A protein sequence analysis has indicated that *DA1* contains two typical ubiquitin interaction motifs (UIMs) and a single zinc-binding (LIM) domain. Through ubiquitination, ubiquitin proteins regulate transcription factors' and regulatory proteins' stability and activity, which is a critical regulatory function in the growth and determination of seed size [14].

The *DA1-like* gene family has been identified in several crops: four genes in rapeseed (*Brassica napus*) [15], eleven genes in soybean (*Glycine max*) [16], six genes in maize (*Zea mays* L.) [17], three genes in oat (*Avena sativa* L.) [18], twenty-one genes in cotton (*Gossypium* spp.) [19], and four genes in rice (*Oryza sativa* L.) [20]. The *DA1* gene was initially discovered to regulate the size of leaves, seeds, flowers, and siliques in *Arabidopsis thaliana*. Additionally, abscisic acid (ABA) upregulates *AtDA1* expression, thereby positively regulating seed size [1]. The *DA2* gene encodes a ring-type E3 ubiquitin ligase that interacts with *DA1*,

thereby influencing seed size. Decreasing the expression level of *BnDA1* in rapeseed significantly increased seed size, yield, and biomass [15]. In rice, *HDR3*, as a UIM-type active ubiquitin receptor, increases its ubiquitination level by interacting with GW6a. This slows down the degradation of the 26S proteasome. This effect promotes the grain filling rate, which increases the final grain size and weight [21]. The interaction of *TaDA1* and *TaGW2*, homologous genes of *Arabidopsis thaliana DA1*, has been found in wheat. Both genes regulate grain size and have a genetic additive effect. The excellent haplotypes *TaDA1-2A* and *TaGW2-6B* are associated with a high 1000-grain weight, and they also exhibit additive effects [22]. The upregulation of the *ZmDA1* or *ZmDAR1* genes promotes grain development and enlargement, leading to increased yield in maize. In addition, *ZmDR1* regulates starch synthesis, promotes starch accumulation, and enhances its quality [17]. Therefore, improving crop yield and quality can be achieved by varying the *DA1-like* gene family's expression levels.

*Ipomoea batatas* (L.) Lam. is a type of sweet potato that belongs to the Convolvulaceae group. It is known for its high yield, strong adaptability, and wide cultivation areas, making it a significant staple crop and industrial raw material [23,24]. The completion of the genome sequencing of sweet potato (*I. batatas* (L.) Lam.,  $2n = B_1B_1B_2B_2B_2B_2 = 6x = 90$ ) [25] and its two diploid species, *I. trifida* NCNSP0306 ( $2n = 2x = 30$ ) and *I. triloba* NCNSP0323 ( $2n = 2x = 30$ ) [26], has made it possible to analyze the sweet potato gene family using bioinformatics [27–32]. However, the *DA1-like* gene family in sweet potatoes has not been reported in these whole-genome studies.

In this study, we identified *DA1-like* genes in sweet potato and two diploid species at the whole-genome level. We examined the gene architectures, promoter cis-acting elements, expression patterns, and evolutionary relationships of these *DA1-like* genes in several tissues. Moreover, the expression profiles of particular *DA1-like* genes were confirmed using quantitative real-time polymerase chain reaction (qRT-PCR) tests in four different tissue sites. These results supply crucial information for examining the roles of *DA1-like* genes in sweet potatoes.

## 2. Results

### 2.1. Identification of *DA1-like* Genes in Sweet Potato and Two Diploid Relatives

We used three strategies to comprehensively identify all of the members of the *DA1-like* gene family in sweet potato and its two diploid relatives. There are 27 genes in the *DA1-like* gene family that have been identified. Respectively, in the sweet potato and its two diploid relatives, there are 9, 7, and 11 *DA1-like* family members. By sequencing these *DA1-like* genes, the physicochemical properties were determined in *I. batatas*. The *IbDA1* CDS lengths ranged from 471 bp (*IbDA1-9*) to 1992 bp (*IbDA1-2*). There were 157 amino acids in *IbDA1* (*IbDA1-9*) and 664 amino acids in *IbDA1-2*; the MW values differed from 17.91 kDa to 74.59 kDa; and the isoelectric point values ranged from 5.09 (*IbDA1-6*) to 7.49 (*IbDA1-2*). Among all of the *DA1-like* genes, only *IbDA1-2* had a PI value greater than seven, indicating that it was a basic protein. There were Thr, Ser, and Tyr phosphorylation sites on all of the *IbDA1*s. The aliphatic index varied from 64.49 (*IbDA1-6*) to 85.64 (*IbDA1-9*). *IbDA1-2* and *IbDA1-9* had aliphatic indices exceeding 85, suggesting thermophilia. The GRAVY scores of all of the *IbDA1* proteins were less than 0, which indicates that they are hydrophilic proteins. The subcellular localization predictions indicated that *IbDA1-1*, *IbDA1-3*, *IbDA1-6*, *IbDA1-7*, and *IbDA1-8* would be found in the nucleus; *IbDA1-4*, *IbDA1-5*, and *IbDA1-9* would be detected in the cytoplasm; and *IbDA1-2* would be detected in the endoplasmic reticulum (Table 1).

**Table 1.** Characterization of *IbDA1* gene family members in sweet potato.

Gene ID	Gene Name	CDS (bp)	Protein (aa)	MW/kDa	pI	Phosphorylation Site			Aliphatic Index	GRAVY	Subcellular Locations
						Ser	Thr	Tyr			
g41815	<i>IbDA1-1</i>	1407	469	53.15	6.23	41	22	18	65.9	−0.607	nucleus
g46658	<i>IbDA1-2</i>	1992	664	74.59	7.49	52	30	26	85.41	−0.111	endoplasmic reticulum
g60449	<i>IbDA1-3</i>	1386	462	52.51	6.29	29	21	18	75.97	−0.413	nucleus
g15267	<i>IbDA1-4</i>	657	219	25.40	6.22	8	11	7	77.34	−0.2	cytoplasm
g15269	<i>IbDA1-5</i>	729	243	28.00	6.7	9	12	9	75.74	−0.147	cytoplasm
g17266	<i>IbDA1-6</i>	1614	538	61.55	5.09	52	23	21	64.49	−0.75	nucleus
g1178	<i>IbDA1-7</i>	1590	530	59.80	5.2	45	26	21	74.1	−0.45	nucleus
g1183	<i>IbDA1-8</i>	795	265	29.96	6.5	30	14	9	75.76	−0.422	nucleus
g46660	<i>IbDA1-9</i>	471	157	17.91	5.22	7	7	7	85.64	−0.049	cytoplasm

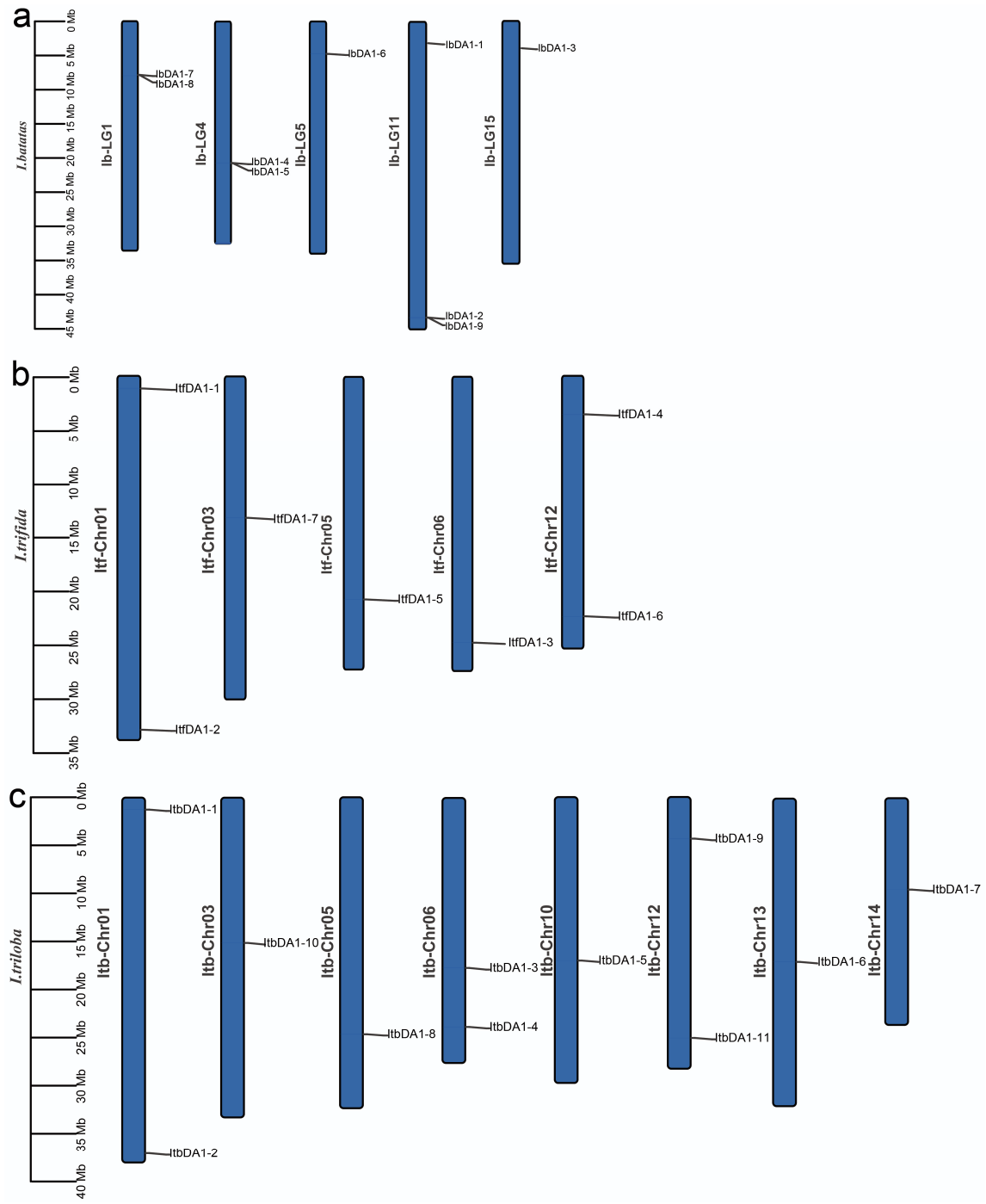
A *DA1-like* gene can be found on chromosomes 5, 5, and 8 of *I. batatas*, *I. trifida*, and *I. triloba*, respectively. LG11 contained three *IbDA1*s (*IbDA1-1*, *-2*, and *9*), two were on LG1 (*IbDA1-7* and *-8*) and LG4 (*IbDA1-4* and *-5*), and LG5 (*IbDA1-6*) and LG15 (*IbDA1-3*) had one each in *I. batatas*. Two *ItfDA1*s were detected on LG1 (*ItfDA1-1* and *-2*) and LG12 (*ItfDA1-4* and *-6*), and one each on LG3 (*ItfDA1-7*), LG5 (*ItfDA1-5*), and LG6 (*ItfDA1-3*) in *I. trifida*. The *DA1-like* genes in *I. triloba* had the highest number of members, with one or two genes on each chromosome (Figure 1).

## 2.2. Evolutionary Analysis of the *DA1-like* Genes

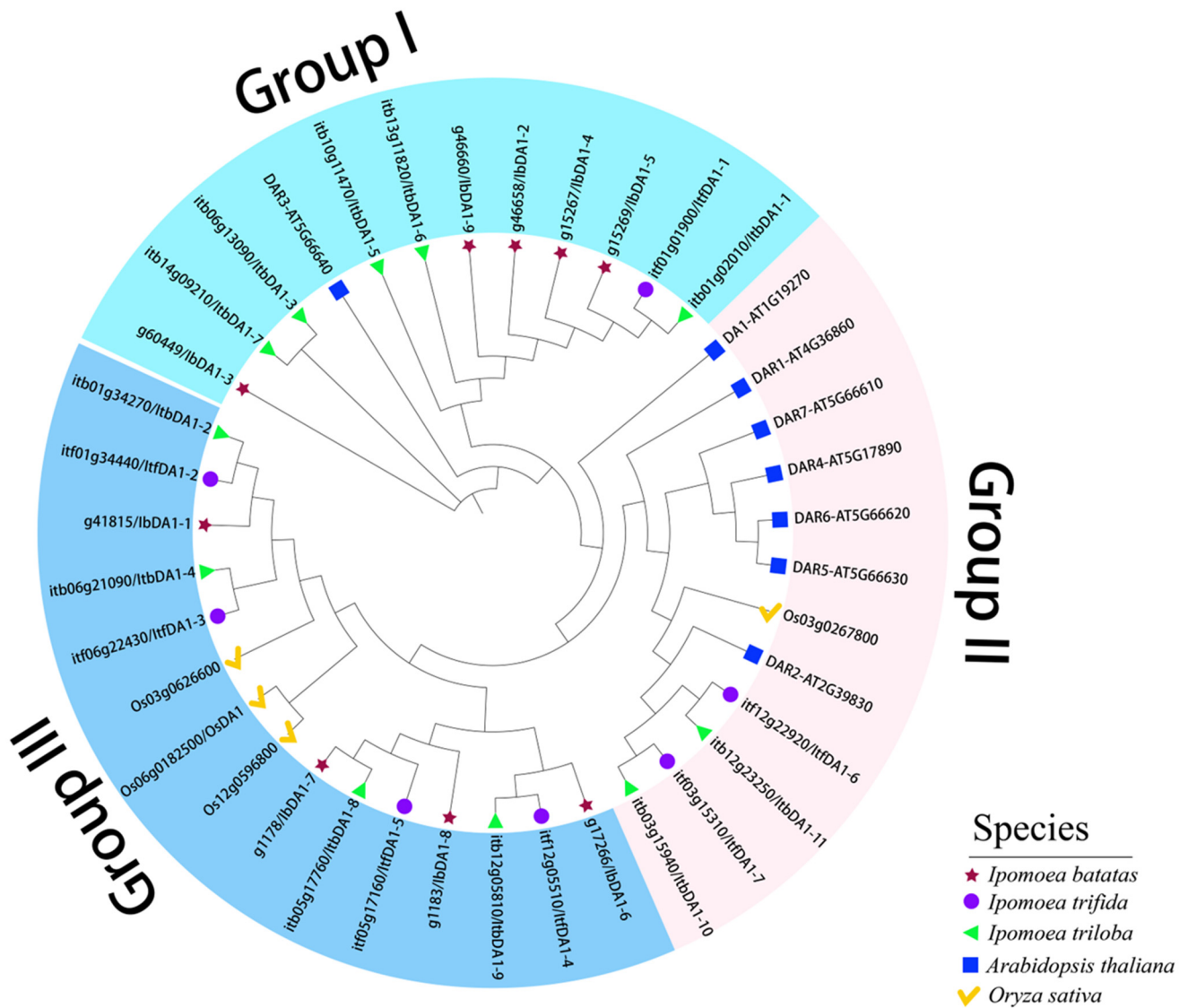
We constructed a phylogenetic tree for thirty-nine *DA1* proteins (i.e., nine in *I. batatas*, seven in *I. trifida*, eleven in *I. triloba*, eight in *Arabidopsis thaliana*, and four in *Oryza sativa*) to study the evolutionary relationship of *DA1* in *I. batatas*, *I. trifida*, *I. triloba*, *A. thaliana*, and *O. sativa* (Figure 2). Considering their evolutionary divergence, the *DA1* proteins found in sweet potatoes and their diploid relatives can be classified into three subclasses (Groups I to III). As a result, (for *I. batatas*, *I. trifida*, *I. triloba*, *A. thaliana*, and *O. sativa*) the *DA1*s were distributed in three groups: Group I (12: 5, 1, 5, 1, 0), Group II (12: 0, 2, 2, 7, 1), and Group III (15: 4, 4, 4, 0, 3).

## 2.3. An Analysis of the Conserved Motif and Exon and Intron Structures of *DA1* Genes from Sweet Potato and Its Two Closest Relatives

An analysis of the sequence motifs in nine *IbDA1*s, seven *ItfDA1*s, and eleven *IbDA1*s was performed using the MEME website. Ten highly conserved motifs were also identified. The number of motifs varied among the members, ranging from zero to nine. Over half of the members (16 *DA1*s) contained all ten motifs. Most of the *DA1*s contained eight of these conserved motifs, and only a few *DA1*s differed in their number and types of motifs, such as *IbDA1-9* (without a motif), *IbDA1-3* (lacking motifs 3, 6, and 10), and *IbDA1-1* (lacking motifs 4 and 10) (Figure 3a). We analyzed the exon and intron structures to comprehensively understand the structural characteristics of the *DA1* genes. In the cultivated sweet potato, *IbDA1-2* had the largest number of exons (thirteen), whereas *IbDA1-4* and *IbDA1-5* had the smallest number of exons (three). Comparing the cultivated sweet potato and its two diploid wild relatives, we observed a substantial variation in the number of exons per gene, ranging from a maximum of thirteen to a minimum of only two exons. The *DA1-like* genes had distinct functional differences during evolution, according to these results (Figure 3b).



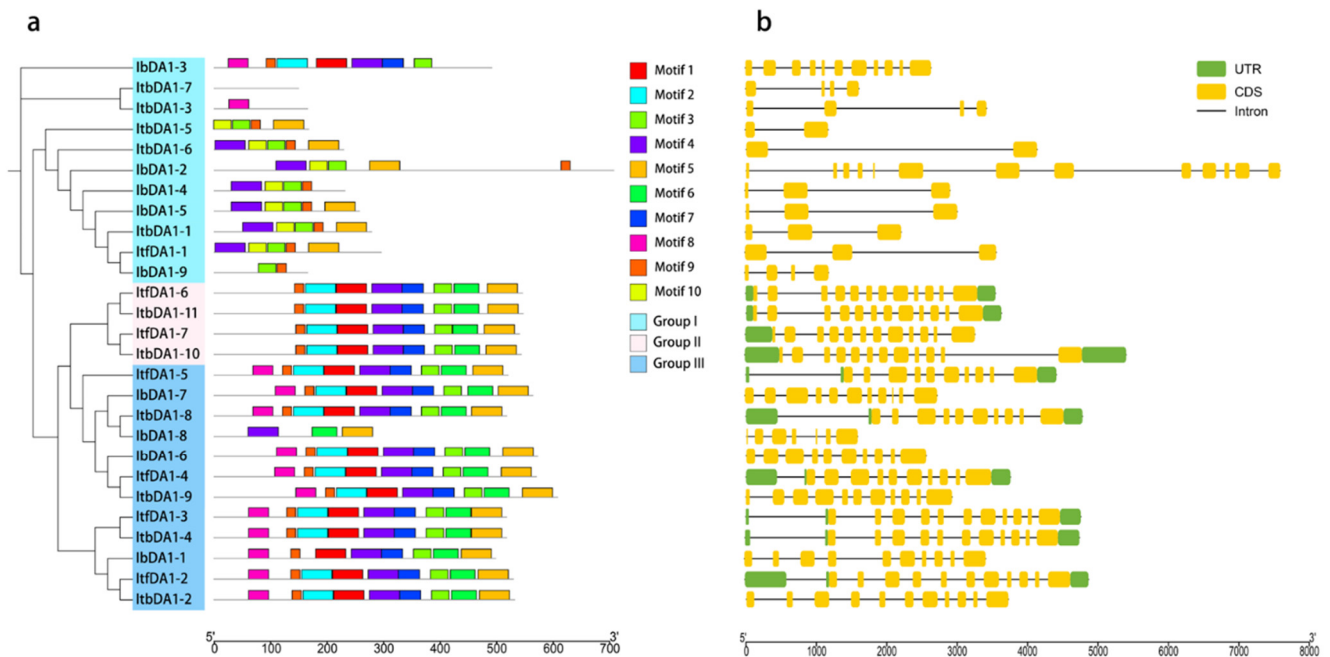
**Figure 1.** Chromosomal localization and distribution of DA1-like genes in *I. batatas* (a), *I. trifida* (b), and *I. triloba* (c).



**Figure 2.** Phylogenetic analysis of the DA1 proteins from five plant species (i.e., *I. batatas*, *I. trifida*, *I. triloba*, *Arabidopsis thaliana*, and *Oryza sativa*). In total, 39 DA1s were divided into three subgroups (Groups I to III) according to their evolutionary distance. The red stars, purple circles, green triangles, blue rectangles, and yellow check mark represent the 9 IbDA1s in *I. batatas*, the 7 ItfDA1s in *I. trifida*, the 11 ItbDA1s in *I. triloba*, the 8 AtDA1s in *Arabidopsis thaliana*, and the 4 OsDA1s in *Oryza sativa*, respectively.

#### 2.4. Sweet Potato and Its Two Diploid Relatives: A Cis-Element Analysis

*Cis*-elements participate in the regulation of gene expression. We analyzed the *cis*-regulatory elements present in the 2000 bp promoter regions of the *IbDA1s*, *ItbDA1s*, and *ItfDA1s* in order to investigate the molecular regulatory mechanisms of the *DA1s*. According to Figure 4, the elements can be grouped into five groups based on their predicted functions: core elements, binding sites, developmentally regulated elements, hormone-responsive elements, stress-responsive elements, and light-responsive elements.



**Figure 3.** Conserved motifs and analysis of the exon–intron structure of the *DA1* family in *I. batatas*, *I. trifida*, and *I. triloba*. (a) The phylogenetic tree shows that the *DA1*s are distributed in three subgroups (left), and the ten conserved motifs are shown in different colors. (b) Exon–intron structures of *DA1*s. The green boxes, yellow boxes, and black lines represent the UTRs, exons, and introns, respectively.

All of the *IbDA1* members contained the core elements CAAT-box, TATA-box, MYC, and TCA; however, TCA was absent in *IbDA1-4*. The developmental transcription factor MYB was identified in all *IbDA1*s, whereas the majority of the *IbDA1*s contained P-box (*IbDA1-1*, -2, -4, -5, and -6) and O2-site (*IbDA1-2*, -3, -6, and -7) elements. Among these, *IbDA1-6* exhibited the highest diversity of developmental elements, including MYB, P-box, O2-site, WUN-motif, ATCT-motif, GC-motif, and HD-zip. In most of the *IbDA1*s, hormone- and light-responsive elements were observed, including CGTCA motifs for the MeJA themes, TCA themes for the SA themes, and AE-box motifs for the light motifs; however, these elements were absent from *IbDA1-3*. Additionally, the *IbDA1* promoters exhibited a high abundance of drought-responsive MYC elements among the abiotic elements.

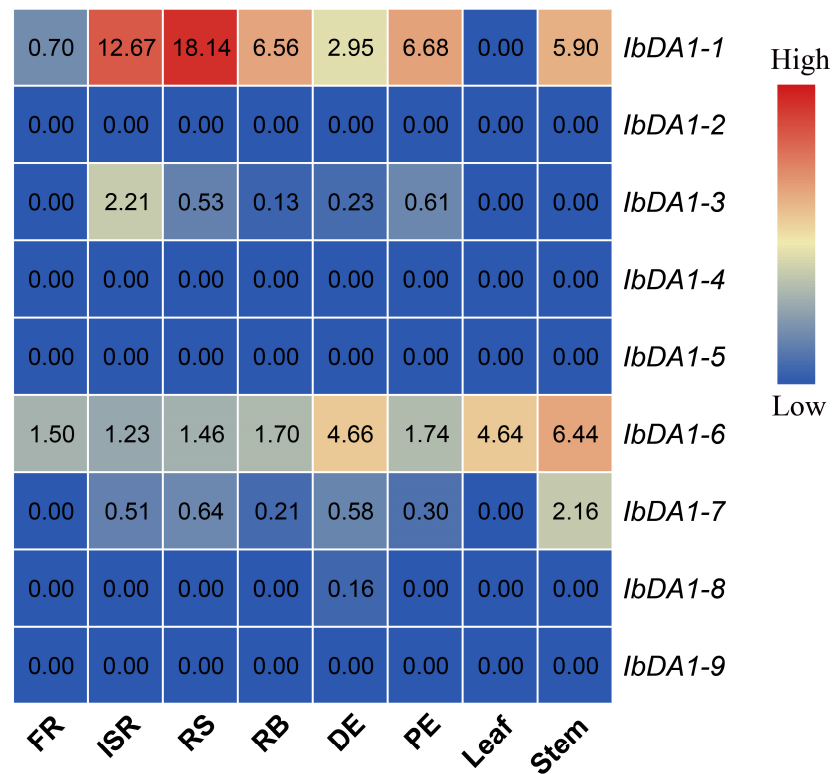
We analyzed two wild sweet potato relatives to clarify the characteristics of cis-acting elements in the *DA1*s' promoter regions. Similar to the *IbDA1*s, the *ItbDA1*s and *ItfDA1*s have CAAT-boxes and TATA-boxes in their promoter regions as well as the developmental element MYB. In addition, most of the *ItbDA1*s and *ItfDA1*s had the developmental elements O2-site and P-box, the hormone elements CGTCA motif and TCA, the abiotic stress elements MYB, MYC, and STRE, and the light-responsive elements GATA-motif and AE-box.

### 2.5. Different Tissues Containing *DA1*-like Genes Analyzed via RNA-Seq

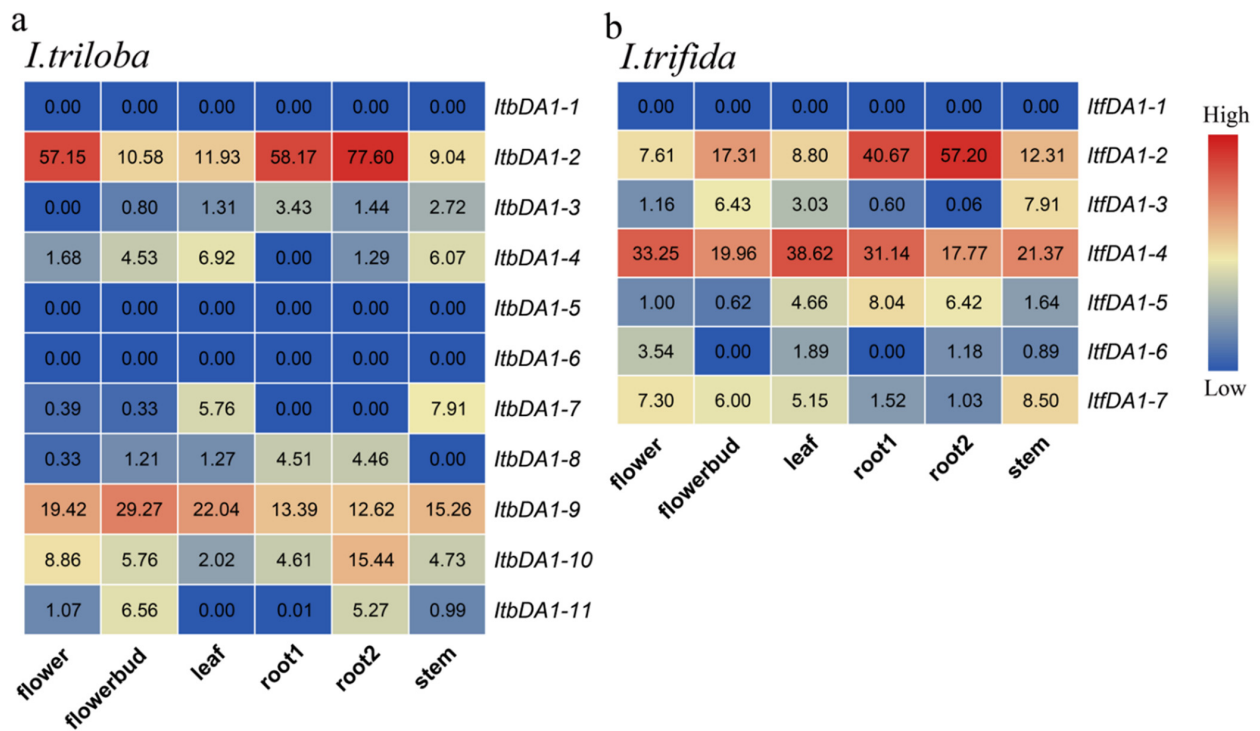
To identify the expression of the *DA1* gene in different tissues, we analyzed the published transcriptome data of “Xushu 18”. This was performed to clarify the potential function of the *DA1* gene in sweet potatoes. A heat map was created using fragments per kilobase of the exon model per million mapped fragments (FPKM). Usually, a gene's FPKM value is greater than 1, indicating its expressibility and functionality. In this study, the FPKM of *IbDA1-6* in the sweet potatoes was greater than 1 in eight different tissues, indicating that *IbDA1-6* is expressible and functional. *IbDA1-1* was highly expressed in six tissues but not in the fibrous root (FR) or leaf. The FPKM values of *IbDA1-2*, -3, -4, -7, -8, and -9 were almost all less than 1 in the eight sweet potato tissues, indicating that they were non-functional (Figure 5).



10). Moreover, three *ItbDA1*s (i.e., *ItbDA1-1*, 5, and 6) showed low expression levels in all of the tissues (Figure 6a).



**Figure 5.** Expression pattern of the *DA1*-like genes in “Xushu 18” in different tissues. The eight tissues comprise stem, leaf, fibrous root (FR), initiated storage root (ISR), root stock (RS), root body (RB), distal end (DE), and proximal end (PE) tissues.



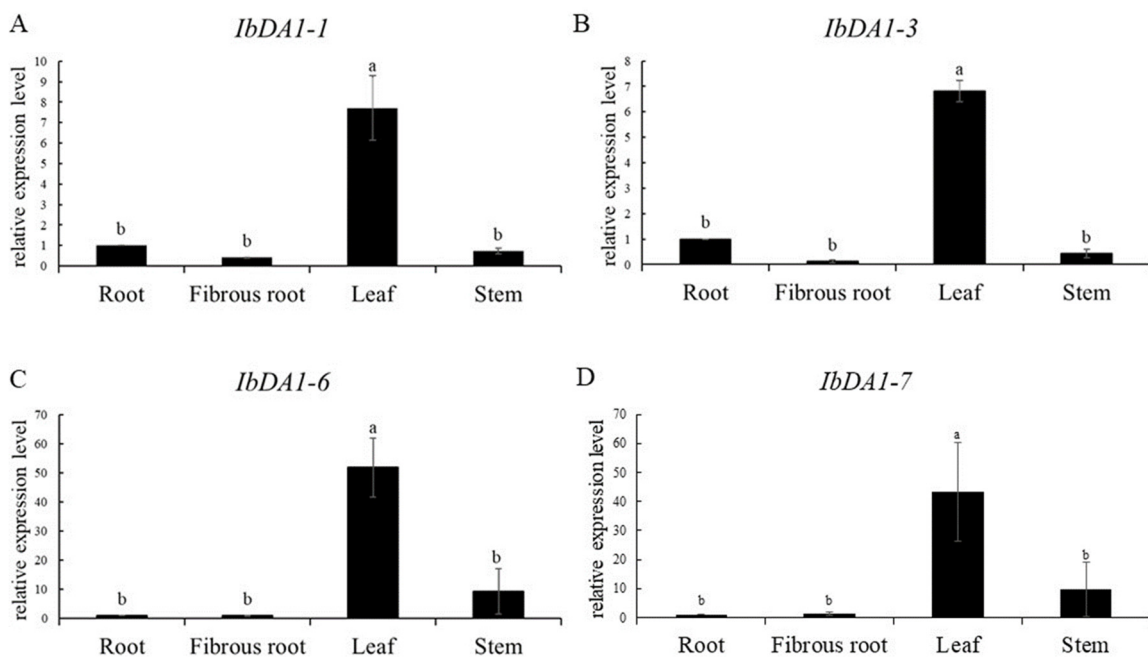
**Figure 6.** Gene expression patterns of *ItbDA1*s (a) and *ItfDA1*s (b) in different organs (root 1, root 2, stem, leaf, flower, and flower bud).



In all tissues of *I. trifida*, *ItfDA1-1* showed a low level of expression. The expression levels of six *ItfDA1s* (i.e., *ItfDA1-2*, *-3*, *-4*, *-5*, and *-7*) were high across all of the tissues. Furthermore, *ItfDA1-6* showed a low level of expression in all of the tissues and *ItfDA1-3* showed a low level of expression in the flowers and roots (Figure 6b).

### 2.6. QRT-PCR of the Sweet Potato DA1-like Gene in Different Tissues

We analyzed the expression patterns of four genes (*IbDA1-1*, *IbDA1-3*, *IbDA1-6*, and *IbDA1-7*) in four different tissues to better understand the role of *IbDA1* in sweet potato growth and development (Figure 7). The gene expression was high in leaves, but there were no significant differences in the expression in the roots, fibrous roots, and stems. The expression of *IbDA1-1* was higher in the roots and fibrous roots than that of the other three genes.



**Figure 7.** Analysis of *IbDA1* gene expression in different tissues of “HD7798” using qRT-PCR. Note: data are shown as mean  $\pm$  SEM ( $n = 3$ ). (A–D) Expression levels of *IbDA1-1* (A), *IbDA1-3* (B), *IbDA1-6* (C), and *IbDA1-7* (D) in roots, fibrous roots, leaves, and stems. Different letters means significant at 5% probability levels according to Duncan’s new multiple range method.

### 3. Discussion

*AtDA1* is one of the smaller gene families in *Arabidopsis thaliana*, containing only eight genes. These genes not only play a regulatory role in grain size, but also play an important role in stress in *Arabidopsis thaliana* [33,34]. DA1 encodes a ubiquitination receptor, so DA1 may interact with the ubiquitination substrate in the ubiquitination protease pathway to promote its degradation. Recent studies have shown that the *DA1* gene family is involved in the ubiquitin protease pathway to regulate grain size [20,35]. At present, studies on the control of plant organ size by DA1 have been reported in a variety of crops, such as maize [17], rice [21], rape [15], wheat [22], and so on. These results indicate that DA1 has a relatively conservative function in crops and plays an important role in plant growth and development.

The *DA1* gene family members have been systematically analyzed in a variety of crops such as *Arabidopsis thaliana* [1], rice [20], rapeseed [15], maize [17], cotton [19], oat [18], and soybean [27], but the *DA1-like* genes of sweet potato have not been analyzed. With the rapid development of sweet potato sequencing technology and genetic engineering, it is convenient to mine candidate genes related to sweet potato yield and quality. Although the polyploid genome of sweet potato hindered the process of identifying grain size

genes by mapping cloning, the use of the regulators of grain size identified in the model rice and *Arabidopsis thaliana* plants was effective as a candidate gene validation method. Therefore, this study systematically identified the *DA1-like* gene family in sweet potato, which provides a favorable theoretical and genetic reference for the subsequent study of *IbDA1* gene function.

In this study, we discovered *DA1-like* genes in sweet potatoes and their diploid relatives. Based on the evolutionary distance, the *DA1-like* genes were divided into three subgroups. According to the evolutionary tree, some *DA1* proteins in sweet potatoes are closely related to *OsDA1* proteins in rice and may have similar functions. These results suggest that *DA1* proteins have differentiated significantly during long-term evolution; however, some *DA1* proteins may still regulate organ size. According to the chromosomal location results, the sweet potato and its two diploid relatives have significantly different chromosomal distributions of *DA1-like* genes, suggesting the evolution of distinct variations among the species. In addition, we found that *IbDA1-7* and *IbDA1-8* are clustered in LG1; *IbDA1-4* and *IbDA1-5* are clustered in LG4; and *IbDA1-2* and *IbDA1-9* are clustered in LG11. Clustering has been extensively characterized in organisms such as fungi and plays an important role in transcriptional regulation mechanisms [36]. This suggests that clustering is conserved in Eukaryotes [37]. However, clustering exists only in sweet potatoes, but not in *I. batatas* and *I. trifida*, which is worth considering. We analyzed that the possible reason for this is that sweet potato has been artificially domesticated in the evolution of clustering so that its traits meet the needs of production. In the future, the functional verification of these cluster genes can be carried out to analyze the causes of the occurrence of these cluster genes. The physiological characteristics, phylogenetic relationships, gene structures, and promoter cis-elements of the *DA1* proteins were systematically studied. The expression patterns and qRT-PCR analyses suggested that *IbDA1-1* and *IbDA1-6* might regulate sweet potato traits.

### 3.1. Evolution of the *DA1-like* Gene Family in Sweet Potato and Its Two Diploid Relatives

The studied sweet potato and its two diploid relatives contained 27 *DA1s*, i.e., 9 *IbDA1s*, 11 *ItbDA1s*, and 7 *ItfDA1s* (Figure 1). These genes were relatively stable in terms of their structure and physical and chemical properties and had high functional conservation. Some genes had closer evolutionary relationships with rice *OsDA1*, such as *IbDA1-1*, *IbDA1-7*, and *IbDA1-6*. *OsDA1* is homologous to *AtDA1*, has a high affinity for ubiquitin, and interacts with *OsUBP15* to regulate rice grain size [20]. These results suggest that *IbDA1-1*, *IbDA1-7*, and *IbDA1-6* may regulate organ size in sweet potatoes. The *IbDA1* gene family in sweet potatoes comprises nine members, surpassing the numbers found in rapeseed, corn, oat, rice, and *Arabidopsis thaliana*; however, this is less than in cotton and soybean. In addition, more *DA1-like* genes were identified in *I. triloba* than in *I. trifida*, out of the two diploid wild relatives of sweet potatoes. The genome size may influence the diversity of *DA1-like* gene numbers and positions among species.

Over half of the *DA1s* contained ten conserved motifs. This indicates that these motifs have been relatively conserved (Figure 3a). The role of exons in gene diversity evolution is crucial [38]. Sweet potato and its two diploid relatives differ in the number of exons and introns in some *DA1s*. (Figure 3b). The Group I *IbDA1-1* genes contained 10 exons. This group's homologous genes, *ItfDA1-1* and *ItbDA1-1*, contained 3 exons, and *IbDA1-2* possessed 13 exons, whereas *ItbDA1-5* contained the smallest number (only 2). These results suggest that *DA1s* evolved into genes with different functions in sweet potatoes and their diploid relatives. This is due to the differences in the structure of these exons.

### 3.2. Differences in the Functions of the *DA1-like* Gene Family in Growth and Development between Sweet Potato and Its Two Diploid Relatives

The regulation of plant organ size by *DA1-like* genes has been reported in several species [21,39–41] However, the function of *DA1-like* genes in regulating organ size in sweet potatoes has not been reported. We analyzed the cis-elements of these genes to explore the

potential function of *DA1-like* genes in the growth and development of sweet potato and its two diploid relatives. As an extensively expressed transcription factor family in plants, the MYB transcription factor family actively participates in plant development and stress responses by binding specifically to MYB cis-elements in target gene promoters [42]. In this study, we found that the *DA1-like* promoter region contained MYB cis-acting elements in both the cultivated sweet potatoes and its two diploid relatives, indicating that the *DA1-like* gene family may interact with MYB transcription factors and thus participate in the growth, development, and stress resistance responses of sweet potatoes. In addition, we found that most of the *DA1-like* gene promoter regions contained many elements related to the response to hormonal and abiotic stresses. This provides a basis for studying the *DA1-like* gene family in sweet potatoes under stress conditions.

We focused on analyzing the expression pattern of DA1 in different tissues to further explore the potential function of *DA1-like* genes in the growth and development of sweet potato and its two diploid relatives. The results showed that the expressions of *IbDA1-2*, *IbDA1-4*, *IbDA1-5*, and *IbDA1-9* were downregulated in the eight tissues (stem, leaf, FR, ISR, RS, RB, DE, and PE). The stem showed high levels of *IbDA1-6* expression, and it was expressed in all the tissues. The expression levels of the *ItfDA1s* and *ItbDA1s* exhibited significant variation across six tissues in *I. trifida* and *I. triloba*. For instance, although *ItbDA1-1* was not detected in any tissue, *ItbDA1-2* was highly expressed in all of the tissues. These results suggest that *DA1-like* genes may play different roles in the growth and development of sweet potatoes. Furthermore, qRT-PCR was used to validate the expression profiles of DA1s across various sweet potato tissues. Interestingly, *IbDA1-1*, *IbDA1-3*, *IbDA1-6*, and *IbDA1-7* were highly expressed in the sweet potato leaves, indicating that they might be important functional genes involved in regulating sweet potato leaf development.

## 4. Material and Methods

### 4.1. Identification of DA1s

From the following websites: <http://sweetpotato.plantbiology.msu.edu/> (accessed on 9 October 2023) and <http://sweetpotato.plantbiology.msu.edu/> (accessed on 9 October 2023), we downloaded the genomic data of the sweet potato and two wild species. The protein sequences of the *DA1* gene family in *Arabidopsis thaliana* were obtained from the TAIR11 database (<https://www.arabidopsis.org/>, accessed on 9 October 2023), and the protein sequences of the *DA1* gene family in sweet potato and *Arabidopsis thaliana* (AT1G19270, AT4G36860, AT2G39830, AT5G66640, AT5G17890, AT5G66630, AT5G66620, and AT5G66610) were compared via BLAST. Meanwhile, Hidden Markov Model data of typical DA1 family protein knots were downloaded from the PFAM database (<http://pfam.sanger.ac.uk>, accessed on 10 October 2023), and the protein sequences containing characteristic domains were searched for using HMMER 3.0 software. The candidate proteins screened through the above two methods were merged, and multiple comparisons were performed using SnapGene 4.3.6 software. Incomplete or redundant sequences were removed manually, and screening results were checked using Pfam and SMART online analysis (<http://smart.emblheidelberg.de/>, accessed on 11 October 2023). Gene sequences without the *DA1* gene family domains or containing incomplete DA1 domains were removed, and the sweet potato *DA1* genes were finally obtained.

### 4.2. Distribution of DA1s on Chromosomes

Gene family members *IbDA1*, *ItfDA1*, and *ItbDA1* were located using annotation files for *I. batatas* genomes (<https://ipomoea-genome.org/>, accessed on 9 November 2023), *I. trifida*, and *I. triloba* (<http://sweetpotato.plantbiology.msu.edu/>, accessed on 9 November 2023).

### 4.3. Prediction of DA1 Protein Characteristics

We calculated DA1's molecular weight, theoretical PI, instability index, and hydrophilicity with ExPASy (<https://www.expasy.org/>, accessed on 15 October 2023). Plant-

mPLOC (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>, accessed on 15 October 2023) was used to predict DA1 subcellular localization.

#### 4.4. Analysis of the Phylogenetic Relationships among DA1s

CLUSTALX 1.8.3 was used to perform multiple sequence alignments of the inferred amino acid sequences of the DA1s from *I. batatas*, *I. trifida*, *I. triloba*, *A. thaliana*, and *O. sativa*. After importing the alignment findings into MEGA11, 1000 bootstrap repeats and the greatest likelihood approach were used to build a phylogenetic tree ([www.megasoftware.net](http://www.megasoftware.net), accessed on 19 November 2023). Then, using iTOL (<http://itol.embl.de/>, accessed on 19 November 2023), the phylogenetic tree was created.

#### 4.5. Detection of Domains and Analysis of Conserved Motifs in DA1s

Using MEME 5.3.0 software (<https://meme-suite.org/meme/>, accessed on 21 November 2023), a conserved domain analysis of the DA1s was carried out, with a maximum motif count of 10. The conserved domain and exon–intron structures of the DA1s were visualized by applying Tbtools v2.056. PlantCARE was used to anticipate the DA1 cis-elements.

#### 4.6. Transcriptome Analysis

We used the published RNA-seq data of “Xushu 18” (PRJNA511028) to explore the expression pattern of DA1s in different tissues and organs of sweet potatoes [43]. From the Sweet Potato Genomics Resource (<http://sweetpotato.plantbiology.msu.edu/>, accessed on 25 November 2023), RNA-seq data for *ItfDA1s* and *ItbDA1s* were downloaded [26]. We plotted gene expression levels using TBtools.

#### 4.7. Plant Material and qPCR

The sweet potato variety “HD7798”, independently bred by Hainan University, was utilized as the experimental material in this study, and four DA1-like genes were selected to analyze their expression patterns in leaves, stems, roots, and fibrous roots. *IbDA1* primers were produced using Primer 6.0 (Table S1). Reverse transcription was performed using an M-MLV RTase cDNA Synthesis Kit (TaKaRa, Kyoto, Japan). qRT-PCR was performed using the Roche LightCycler<sup>®</sup> 480II (Applied Biosystems, Foster City, CA, USA) System under the following conditions: 95 °C for 15 s, followed by 40 cycles of 95 °C for 15 s each, then 55 °C for 15 s, and 72 °C for 15 s. Relative quantification was performed using the  $2^{-\Delta\Delta C_t}$  method [44].

#### 4.8. Statistical Analysis

The student’s two-tailed t test was used for significant difference analysis between two samples. One-way ANOVA analyses followed by Tukey’s test ( $p < 0.05$ ) were used for pairwise multiple comparisons. All of the analyses were performed using SPSS 24.0 software.

## 5. Conclusions

In this study, DA1-like genes are presented for the first time in sweet potato. Its two diploid relatives *I. trifida* and *I. triloba* were shown to have abundant genetic variation and diverse expression patterns, suggesting that the DA1 gene family members may play different roles in the growth and development in these plants. This study provides a theoretical basis for further exploration of the function of the DA1-like genes in the growth and organ size regulation of sweet potatoes. However, further functional analyses are needed to clarify their role.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms25053000/s1>.

**Author Contributions:** L.W., R.X. and Y.L. (Yunhai Li) designed the experiments, L.W. wrote the manuscript. Z.Z. and J.H. performed most of the experiments and analyzed the data. S.H., J.Y., and Y.W. (Ying Wang) collected and downloaded the data. Y.L. (Yi Liu), W.L. and Y.W. (Yuehui Wang) designed the quantitative primers and performed the RT-PCR experiments. All authors have read and agreed to the published version of the manuscript.

**Funding:** The Project of Sanya Yazhou Bay Science and Technology City (SCKJ-JYRC-2022-64), the Natural Science Foundation of Hainan Province (322RC592), and the National Natural Science Foundation of China (31960159 and 32360083) supported this study.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data and conclusions are included in this paper.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

- Li, Y.; Zheng, L.; Corke, F.; Smith, C.; Bevan, M.W. Control of final seed and organ size by the DA1 gene family in *Arabidopsis thaliana*. *Genes Dev.* **2008**, *22*, 1331–1336. [[CrossRef](#)]
- Tang, S.; Zhao, Z.Y.; Liu, X.T.; Sui, Y.; Zhang, D.D.; Zhi, H.; Gao, Y.Z.; Zhang, H.; Zhang, L.L.; Wang, Y.N.; et al. An E2-E3 pair contributes to seed size control in grain crops. *Nat. Commun.* **2023**, *14*, 3091. [[CrossRef](#)]
- Fan, C.; Xing, Y.; Mao, H.; Lu, T.; Han, B.; Xu, C.; Li, X.; Zhang, Q. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.* **2006**, *112*, 1164–1171. [[CrossRef](#)]
- Yu, J.P.; Xiong, H.Y.; Zhu, X.Y.; Zhang, H.L.; Li, H.H.; Miao, J.L.; Wang, W.S.; Tang, Z.S.; Zhang, Z.Y.; Yao, G.X.; et al. OsLG3 contributing to rice grain length and yield was mined by Ho-LAMap. *BMC Biol.* **2017**, *15*, 28. [[CrossRef](#)]
- Liu, Q.; Han, R.X.; Wu, K.; Zhang, J.Q.; Ye, Y.F.; Wang, S.S.; Chen, J.F.; Pan, Y.J.; Li, Q.; Xu, X.P.; et al. G-protein  $\beta\gamma$  subunits determine grain size through interaction with MADS-domain transcription factors in rice. *Nat. Commun.* **2018**, *9*, 852. [[CrossRef](#)]
- Krizek, B.A. Making bigger plants: Key regulators of final organ size. *Curr. Opin. Plant Biol.* **2009**, *12*, 17–22. [[CrossRef](#)] [[PubMed](#)]
- Song, X.-J.; Huang, W.; Shi, M.; Zhu, M.-Z.; Lin, H.-X. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat. Genet.* **2007**, *39*, 623–630. [[CrossRef](#)]
- Wang, S.K.; Wu, K.; Yuan, Q.B.; Liu, X.Y.; Liu, Z.B.; Lin, X.Y.; Zeng, R.Z.; Zhu, H.T.; Dong, G.J.; Qian, Q.; et al. Control of grain size, shape and quality by OsSPL16 in rice. *Nat. Genet.* **2012**, *44*, 950–954. [[CrossRef](#)]
- Gao, X.Y.; Zhang, J.Q.; Cai, G.; Du, H.Y.; Li, J.B.; Wang, R.Q.; Wang, Y.J.; Yin, J.; Zhang, W.C.; Zhang, H.S.; et al. qGL3/OsPPKL1 induces phosphorylation of 14-3-3 protein OsGF14b to inhibit OsBZR1 function in brassinosteroid signaling. *Plant Physiol.* **2022**, *188*, 624–636. [[CrossRef](#)]
- Chen, E.R.; Hou, Q.Q.; Liu, K.; Gu, Z.L.; Dai, B.X.; Wang, A.H.; Feng, Q.; Zhao, Y.; Zhou, C.C.; Zhu, J.J.; et al. Armadillo repeat only protein GS10 negatively regulates brassinosteroid signaling to control rice grain size. *Plant Physiol.* **2023**, *192*, 967–981. [[CrossRef](#)]
- Wu, W.G.; Liu, X.Y.; Wang, M.H.; Meyer, R.S.; Luo, X.J.; Ndjiondjop, M.N.; Tan, L.B.; Zhang, J.W.; Wu, J.Z.; Cai, H.W.; et al. A single-nucleotide polymorphism causes smaller grain size and loss of seed shattering during African rice domestication. *Nat. Plants* **2017**, *3*, 17064. [[CrossRef](#)]
- Ruan, B.P.; Shang, L.G.; Zhang, B.; Hu, J.; Wang, Y.X.; Lin, H.; Zhang, A.P.; Liu, C.L.; Peng, Y.L.; Zhu, L.; et al. Natural variation in the promoter of TGW2 determines grain width and weight in rice. *New Phytol.* **2020**, *227*, 629–640. [[CrossRef](#)]
- Zhao, D.-S.; Li, Q.-F.; Zhang, C.-Q.; Zhang, C.; Yang, Q.-Q.; Pan, L.-X.; Ren, X.-Y.; Lu, J.; Gu, M.-H.; Liu, Q.-Q. GS9 acts as a transcriptional activator to regulate rice grain shape and appearance quality. *Nat. Commun.* **2018**, *9*, 1240. [[CrossRef](#)]
- Sharma, B.; Joshi, D.; Yadav, P.K.; Gupta, A.K.; Bhatt, T.K. Role of Ubiquitin-Mediated Degradation System in Plant Biology. *Front. Plant Sci.* **2016**, *7*, 806. [[CrossRef](#)]
- Wang, J.L.; Tang, M.Q.; Chen, S.; Zheng, X.F.; Mo, H.X.; Li, S.J.; Wang, Z.; Zhu, K.M.; Ding, L.N.; Liu, S.Y.; et al. Down-regulation of BnDA1, whose gene locus is associated with the seeds weight, improves the seeds weight and organ size in Brassica napus. *Plant Biotechnol. J.* **2017**, *15*, 1024–1033. [[CrossRef](#)]
- Zhao, M.; He, L.; Gu, Y.; Wang, Y.; Chen, Q.; He, C. Genome-Wide Analyses of a Plant-Specific LIM-Domain Gene Family Implicate Its Evolutionary Role in Plant Diversification. *Genome Biol. Evol.* **2014**, *6*, 1000–1012. [[CrossRef](#)]
- Xie, G.; Li, Z.; Ran, Q.; Wang, H.; Zhang, J. Over-expression of mutated ZmDA1 or ZmDAR1 gene improves maize kernel yield by enhancing starch synthesis. *Plant Biotechnol. J.* **2017**, *16*, 234–244. [[CrossRef](#)] [[PubMed](#)]
- Lee, J.S.; Ko, C.S.; Seo, Y.W. Oat AsDA1-2D enhances heat stress tolerance and negatively regulates seed-storage globulin. *J. Plant Physiol.* **2023**, *284*, 153981. [[CrossRef](#)] [[PubMed](#)]

19. Yang, S.X.; Huang, L.; Song, J.K.; Liu, L.S.; Bian, Y.Y.; Jia, B.; Wu, L.Y.; Xin, Y.; Wu, M.; Zhang, J.F.; et al. Genome-Wide Analysis of DA1-Like Genes in *Gossypium* and Functional Characterization of GhDA1-1A Controlling Seed Size. *Front. Plant Sci.* **2021**, *12*, 647091. [[CrossRef](#)] [[PubMed](#)]
20. Shi, C.; Ren, Y.; Liu, L.; Wang, F.; Zhang, H.; Tian, P.; Pan, T.; Wang, Y.; Jing, R.; Liu, T.; et al. Ubiquitin Specific Protease 15 Has an Important Role in Regulating Grain Width and Size in Rice. *Plant Physiol.* **2019**, *180*, 381–391. [[CrossRef](#)] [[PubMed](#)]
21. Gao, Q.; Zhang, N.; Wang, W.Q.; Shen, S.Y.; Bai, C.; Song, X.J. The ubiquitin-interacting motif-type ubiquitin receptor HDR3 interacts with and stabilizes the histone acetyltransferase GW6a to control the grain size in rice. *Plant Cell* **2021**, *33*, 3331–3347. [[CrossRef](#)]
22. Liu, H.; Li, H.F.; Hao, C.Y.; Wang, K.; Wang, Y.M.; Qin, L.; An, D.G.; Li, T.; Zhang, X.Y. TaDA1, a conserved negative regulator of kernel size, has an additive effect with TaGW2 in common wheat (*Triticum aestivum* L.). *Plant Biotechnol. J.* **2020**, *18*, 1330–1342. [[CrossRef](#)]
23. Liu, Q. Improvement for agronomically important traits by gene engineering in sweetpotato. *Breed. Sci.* **2017**, *67*, 15–26. [[CrossRef](#)]
24. El Sheikha, A.F.; Ray, R.C. Potential impacts of bioprocessing of sweet potato: Review. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 455–471. [[CrossRef](#)] [[PubMed](#)]
25. Yang, J.; Moeinzadeh, M.H.; Kuhl, H.; Helmuth, J.; Xiao, P.; Haas, S.; Liu, G.; Zheng, J.; Sun, Z.; Fan, W.; et al. Haplotype-resolved sweet potato genome traces back its hexaploidization history. *Nat. Plants* **2017**, *3*, 696–703. [[CrossRef](#)] [[PubMed](#)]
26. Wu, S.; Lau, K.H.; Cao, Q.; Hamilton, J.P.; Sun, H.; Zhou, C.; Eserman, L.; Gemenet, D.C.; Olukolu, B.A.; Wang, H.; et al. Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. *Nat. Commun.* **2018**, *9*, 4580. [[CrossRef](#)] [[PubMed](#)]
27. Zhao, M.; Gu, Y.; He, L.; Chen, Q.; He, C. Sequence and expression variations suggest an adaptive role for the DA1-like gene family in the evolution of soybeans. *BMC Plant Biol.* **2015**, *15*, 120. [[CrossRef](#)] [[PubMed](#)]
28. Zhang, C.; Liu, S.; Liu, D.; Guo, F.; Yang, Y.; Dong, T.; Zhang, Y.; Ma, C.; Tang, Z.; Li, F.; et al. Genome-wide survey and expression analysis of GRAS transcription factor family in sweetpotato provides insights into their potential roles in stress response. *BMC Plant Biol.* **2022**, *22*, 232. [[CrossRef](#)] [[PubMed](#)]
29. He, P.; Zhang, J.; Lv, Z.; Cui, P.; Xu, X.; George, M.S.; Lu, G. Genome-wide identification and expression analysis of the polygalacturonase gene family in sweetpotato. *BMC Plant Biol.* **2023**, *23*, 300. [[CrossRef](#)] [[PubMed](#)]
30. Amoanimaa-Dede, H.; Shao, Z.; Su, C.; Yeboah, A.; Zhu, H. Genome-wide identification and characterization of F-box family proteins in sweet potato and its expression analysis under abiotic stress. *Gene* **2022**, *817*, 146191. [[CrossRef](#)] [[PubMed](#)]
31. Yang, Z.; Sun, J.; Chen, Y.; Zhu, P.; Zhang, L.; Wu, S.; Ma, D.; Cao, Q.; Li, Z.; Xu, T. Genome-wide identification, structural and gene expression analysis of the bZIP transcription factor family in sweet potato wild relative *Ipomoea trifida*. *BMC Genet.* **2019**, *20*, 41. [[CrossRef](#)] [[PubMed](#)]
32. Li, Y.; Zhang, L.; Zhu, P.; Cao, Q.; Sun, J.; Li, Z.; Xu, T. Genome-wide identification, characterisation and functional evaluation of WRKY genes in the sweet potato wild ancestor *Ipomoea trifida* (H.B.K.) G. Don. under abiotic stresses. *BMC Genet.* **2019**, *20*, 90. [[CrossRef](#)] [[PubMed](#)]
33. Peng, Y.; Chen, L.; Lu, Y.; Wu, Y.; Dumenil, J.; Zhu, Z.; Bevan, M.W.; Li, Y. The Ubiquitin Receptors DA1, DAR1, and DAR2 Redundantly Regulate Endoreduplication by Modulating the Stability of TCP14/15 in Arabidopsis. *Plant Cell* **2015**, *27*, 649–662. [[CrossRef](#)]
34. Srivastava, V.; Verma, P.K. The plant LIM proteins: Unlocking the hidden attractions. *Planta* **2017**, *246*, 365–375. [[CrossRef](#)]
35. Du, L.; Li, N.; Chen, L.L.; Xu, Y.X.; Li, Y.; Zhang, Y.Y.; Li, C.Y.; Li, Y.H. The Ubiquitin Receptor DA1 Regulates Seed and Organ Size by Modulating the Stability of the Ubiquitin-Specific Protease UBP15/SOD2 in Arabidopsis. *Plant Cell* **2014**, *26*, 665–677. [[CrossRef](#)] [[PubMed](#)]
36. Cittadino, G.M.; Andrews, J.; Purewal, H.; Avila, P.E.A.; Arnone, J.T. Functional Clustering of Metabolically Related Genes Is Conserved across Dikarya. *J. Fungi* **2023**, *9*, 523. [[CrossRef](#)] [[PubMed](#)]
37. Arnone, J.T.; Robbins-Pianka, A.; Arace, J.R.; Kass-Gergi, S.; McAlear, M.A. The adjacent positioning of co-regulated gene pairs is widely conserved across eukaryotes. *BMC Genom.* **2012**, *13*, 546. [[CrossRef](#)]
38. Fedorova, L.; Fedorov, A. Introns in gene evolution. *Genetica* **2003**, *118*, 123–131. [[CrossRef](#)]
39. Gong, P.; Demuyneck, K.; De Block, J.; Aesaert, S.; Coussens, G.; Pauwels, L.; Nelissen, H. Modulation of the DA1 pathway in maize shows that translatability of information from Arabidopsis to crops is complex. *Plant Sci.* **2022**, *321*, 111295. [[CrossRef](#)]
40. Chen, Y.; Inzé, D.; Vanhaeren, H. Post-translational modifications regulate the activity of the growth-restricting protease DA1. *J. Exp. Bot.* **2021**, *72*, 3352–3366. [[CrossRef](#)] [[PubMed](#)]
41. Keren, I.; Lacroix, B.; Kohrman, A.; Citovsky, V. Histone Deubiquitinase OTU1 Epigenetically Regulates DA1 and DA2, Which Control Arabidopsis Seed and Organ Size. *iScience* **2020**, *23*, 100948. [[CrossRef](#)]
42. Ambawat, S.; Sharma, P.; Yadav, N.R.; Yadav, R.C. MYB transcription factor genes as regulators for plant responses: An overview. *Physiol. Mol. Biol. Plants* **2013**, *19*, 307–321. [[CrossRef](#)] [[PubMed](#)]

43. Zhang, J.-Z.; He, P.-W.; Xu, X.-M.; Lü, Z.-F.; Cui, P.; George, M.S.; Lu, G.-Q. Genome-Wide Identification and Expression Analysis of the Xyloglucan Endotransglucosylase/Hydrolase Gene Family in Sweet Potato [*Ipomoea batatas* (L.) Lam]. *Int. J. Mol. Sci.* **2023**, *24*, 775. [[CrossRef](#)] [[PubMed](#)]
44. Arocho, A.; Chen, B.; Ladanyi, M.; Pan, Q. Validation of the 2-DeltaDeltaCt calculation as an alternate method of data analysis for quantitative PCR of BCR-ABL P210 transcripts. *Diagn. Mol. Pathol. Am. J. Surg. Pathol. Part B* **2006**, *15*, 56–61. [[CrossRef](#)] [[PubMed](#)]

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