agronomy

Article

Identification of Carrot Expansin Gene Family and Its Regulation of Carrot Growth and Development

Rong-Rong Zhang [1], Ya-Hui Wang [1], Jian-Ping Tao [1,2], Jian-Hua Zhou [3], Nan Zhang [1], Xue-Feng Peng [1], Li Zhang [3], Xiaoe Xiang [4], Yu-Jie Sun [1], Guo-Fei Tan [5] and Ai-Sheng Xiong [1,*]

[1] State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Ministry of Agriculture and Rural Affairs Key Laboratory of Biology and Germplasm Enhancement of Horticultural Crops in East China, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China; 2021204032@stu.njau.edu.cn (R.-R.Z.); wangyahui@njau.edu.cn (Y.-H.W.); 2019404002@stu.njau.edu.cn (J.-P.T.); 2024204050@stu.njau.edu.cn (N.Z.); 2023104063@stu.njau.edu.cn (X.-F.P.); 2023104062@stu.njau.edu.cn (Y.-J.S.)
[2] Institute of Agricultural Information, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
[3] Zhengzhou Agricultural Science and Technology Research Institute, Zhengzhou 450015, China; jianhuazhou2023@163.com (J.-H.Z.); zznkyzl@126.com (L.Z.)
[4] National Experimental Teaching Center for Animal Sciences, Nanjing Agricultural University, Nanjing 210095, China; xxxiang@njau.edu.cn
[5] Institute of Horticulture, Guizhou Academy of Agricultural Sciences, Guizhou Horticultural Engineering Technology Research Center, Guiyang 550006, China; tgfei@foxmail.com
* Correspondence: xiongaisheng@njau.edu.cn

Abstract

Carrot (*Daucus carota* L.) is an important root vegetable crop in the Apiaceae and is widely cultivated around the world. Expansins play crucial roles in the growth and development of plants. Here, a total of 35 carrot expansins were identified from carrot. Sequence alignment and phylogenetic analysis revealed that carrot expansins could be classified into four subfamilies, each with similar exon/intron structures and motif compositions, indicating that carrot expansins were relatively conserved during evolution. Chromosomal localization and gene duplication analysis indicated that *DcEXP* genes were unevenly distributed across carrot nine chromosomes and had evolved predominantly under purifying selection. Measurements of key agronomic characters of carrots at different developmental stages (30, 60, and 90 days after sowing) indicated significant positive correlations among root fresh weight, aboveground fresh weight, root–shoot ratio, root length, and root diameter. The period from 30 to 60 days after sowing was identified as the primary phase of taproot enlargement. Analysis of spatiotemporal expression patterns revealed that most *DcEXP* genes were specifically expressed in the taproots, and only one gene, *DcEXP18*, was specifically expressed in leaves. During the rapid growth period of carrot taproots (30 and 60 days after sowing), the genes *DcEXP2*, *DcEXP3*, *DcEXP5*, *DcEXP8*, *DcEXP11*, *DcEXP13*, *DcEXP17*, *DcEXP19*, *DcEXP20*, *DcEXP22*, *DcEXP26*, *DcEXP28*, and *DcEXP33* exhibited high expression levels, suggesting that they played potential important roles in carrot taproot enlargement. These findings will advance our knowledge of the molecular mechanisms underlying expansin regulation of carrot growth and development.

Keywords: carrot; expansin; growth and development; gene expression; tissue specificity

Academic Editor: Matthew Hegarty

Received: 13 August 2025
Revised: 28 September 2025
Accepted: 4 October 2025
Published: 5 October 2025

Citation: Zhang, R.-R.; Wang, Y.-H.; Tao, J.-P.; Zhou, J.-H.; Zhang, N.; Peng, X.-F.; Zhang, L.; Xiang, X.; Sun, Y.-J.; Tan, G.-F.; et al. Identification of Carrot Expansin Gene Family and Its Regulation of Carrot Growth and Development. *Agronomy* **2025**, *15*, 2338. https://doi.org/10.3390/agronomy15102338

1. Introduction

Carrot (*Daucus carota* L.), a biennial herbaceous plant belonging to the genus *Daucus* in the Apiaceae family, is a cultivated variant of its wild counterpart. As a prominent member of the world's top ten most widely grown vegetables, carrot is extensively cultivated across the globe [1,2]. The taproot of carrot, being the main edible part, is highly favored by consumers for its significant nutritional and economic value [3]. During the growth and development of carrots, the growth of the aerial parts and the underground parts is interdependent and mutually restrictive. Poor growth of the aerial parts can negatively impact the development of the taproot. Conversely, if the aerial parts grow excessively vigorous, the translocation of assimilates to the underground parts is reduced, which also affects the development and nutrient accumulation of the taproot. Only when the aerial stem and leaves grow robustly can it be conducive to the development and nutrient accumulation of the taproot, achieving a strong plant with a large root [4,5].

McQueen-Mason et al. isolated two proteins, CsEXP1 and CsEXP2, with molecular weights of 29–30 kDa from the hypocotyls of cucumber seedlings [6]. These proteins were found to possess the ability to restore the expansibility of cell walls in vitro under acidic conditions; they were consequently named expansins [6]. Expansins are widely distributed in both monocotyledonous and dicotyledonous plants. To date, various expansins have been identified in different plant species, such as maize [7,8], rice [9,10], tobacco [11], *Arabidopsis thaliana* [12,13], soybean [14], grape [15], and tomato [16–19]. Expansins constitute a large superfamily of genes. Based on the results of gene sequence analysis, expansins can be divided into four independent subfamilies, namely α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB) [20]. Among them, the EXPA and EXPB families are the most diverse and extensively studied expansins. Research has shown that expansins are low-molecular-weight proteins composed of 250–270 amino acid residues, featuring three distinct domains: a signal peptide domain, a catalytic domain, and a binding domain. The signal peptide domain is located at the N-terminus, consisting of 20–30 amino acids. It directs the expansin protein into the endoplasmic reticulum, where it undergoes processing and modification before being transported to the plasma membrane and subsequently released into the cell wall [21,22]. Catalytic domain is composed of 120–135 amino acids and shares homology with endoglucanase GH45. Its core sequence includes the HFD (His-Phe-Asp) motif and cysteine residues (Cys, C), which are capable of weakening the hydrogen bond connections between cell wall polysaccharides [23]. The binding domain consists of 90–120 amino acids and exhibits sequence homology with Group II grass pollen allergens. It contains four conserved tryptophan residues (Trp, W) that enable it to interact with the cellulose framework, thereby exerting its function [24,25]. Expansin genes typically contain four conserved introns (A, C, B, F), whose positions within the gene are generally fixed [25,26]. The function of expansins relies on the synergistic action of these domains, which weaken the hydrogen bonds between cell wall polysaccharides, thereby promoting cell wall loosening and expansion [24,26].

Expansins are of great significance in plant growth and development, participating in nearly the whole process of plant growth and development, such as seed germination, leaf development, stem elongation, root growth, and the development of flowers and fruits [26]. Choi et al. introduced the *OsEXPA4* gene into rice and observed that the length of the coleoptile and mesocotyl in sense transgenic plants was significantly increased, with a notable elevation in the expression level of the *OsEXPA4*. Conversely, in antisense transgenic seedlings, the opposite effects were seen [27]. Muller et al. discovered that 19 expansin genes in maize could influence leaf growth and development by regulating cell division, cell wall differentiation, and leaf expansion [28]. The soybean expansin gene *GmEXPB2* has been shown to promote root cell differentiation and root elongation [29]. In carrot, previous research has characterized the phenotypes and agronomic traits at different growth and development stages [3]. Based on proteome and transcriptome data combined with carrot phenotype analysis, Wang et al. preliminarily identified the *DcEXP* gene family associated with carrot root development [30]. However, research on the *DcEXP* gene family in carrot remains relatively limited. It is currently unclear which *DcEXP* members participate in the critical period of carrot taproot enlargement, nor whether their expression levels correlate with yield traits such as root fresh weight and root diameter.

In this study, the expansin gene family in carrot were identified. To identify potential regulatory nodes where *DcEXP* genes may participate in carrot development at the phenotypic level, we first profiled five agronomic traits (root fresh weight, aboveground fresh weight, root–shoot ratio, root length, and root diameter) at 30, 60 and 90 days after sowing and performed trait correlation analyses. Subsequently, we conducted whole-family expression profiling of *DcEXP* genes in taproot and leave samples from this period, integrating “trait variation” with “gene expression” to provide a foundation for further research into the molecular mechanisms underlying the regulation of taproot swelling in carrots by *DcEXP* genes.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The carrot cultivar ‘Kurodagosun’ (‘KRD’) was cultivated in the artificial climate chamber of the State Key Laboratory of Crop Genetics and Germplasm Enhancement and Utilization at Nanjing Agricultural University. The growth conditions were as follows: a daytime temperature of 25 °C, a nighttime temperature of 18 °C, a photoperiod of 16 $h \cdot d^{-1}$, and a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The planting substrate consisted of a mixture of organic soil, vermiculite, and perlite in a volume ratio of 2:2:1. A total of 20 pots were sown, with 100 plants in total. Leaf and taproot samples were collected at 30, 60, and 90 days after sowing (DAS) from 30 well-growing plants per sample type, mixed thoroughly, and then immediately frozen in liquid nitrogen and stored at −80 °C for subsequent experiments. Three independent biological replicates were prepared for each carrot plant sample.

2.2. Extraction of Total RNA and Synthesis of cDNA

Total RNA was extracted from plant tissues using the polysaccharide polyphenol plant total RNA extraction kit (Proteinssci, Shanghai, China). The extracted RNA samples were reverse-transcribed into cDNA using the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China). The synthesized cDNA was diluted 15-fold with sterile ddH_2O and stored at −20 °C for subsequent use.

2.3. Identification of the Carrot DcEXP Gene Family

To identify the *DcEXP* gene family in carrot, we obtained the carrot genome data from the Phytozome database (JGI) (https://phytozome.jgi.doe.gov/pz/portal.html) (accessed on 10 January 2025). The sequences of the expansin gene family from *Arabidopsis thaliana* were downloaded from the TAIR database (https://www.arabidopsis.org/) [31]. An HMM (Hidden Markov Model) for the expansin family was constructed using the HMMER 3.4 software. The DcEXP proteins in the carrot genome were identified using the HMM and the Pfam online tool (http://pfam.xfam.org/), specifically by intersecting the PF01357 and PF03330 domains. Finally, redundant sequences were removed using the Blastp program to obtain the final identification results.

2.4. Multiple Sequence Alignment and Phylogenetic Tree Construction of the Carrot DcEXP Gene Family

Multiple sequence alignment of DcEXP proteins in carrot was performed using ClustalX 1.81 software and DNAMAN 6.0 software. The phylogenetic tree was constructed by the Neighbor-Joining (NJ) method in MEGA 7.0 with the parameters set for 1000 bootstrap replications [32]. Based on the phylogenetic tree topology and the classification of homologous genes in *Oryza sativa* and *Arabidopsis thaliana*, the DcEXP proteins in carrot were classified into different subfamilies.

2.5. Protein Properties and Sequence Analysis of the Carrot DcEXP Gene Family

Genomic DNA, CDS, amino acid sequences, and gene annotations were extracted from the carrot genome database. The amino acid count (aa), molecular weight (MW), isoelectric point (*pI*), and hydropathy (GRAVY) of DcEXP protein sequences were analyzed using the online tool Expasy (https://web.expasy.org/protparam/) (accessed on 10 January 2025). The gene structure of *DcEXP* genes was analyzed using the Gene Structure Display Server (GSDS) 2.0 (http://gsds.cbi.pku.edu.cn/). Conserved motifs within the *DcEXP* gene family were identified using the MEME Suite (https://meme-suite.org/meme/tools/meme) (accessed on 10 January 2025). The results were visualized using TBtools-II software [33].

2.6. Chromosomal Localization, Collinearity and Selection Pressure Analysis of the Carrot DcEXP Gene Family

Chromosomal localization information of the *DcEXP* gene family was obtained from the carrot genome database, and chromosomal localization maps were drawn using Tbtools software. Synteny analysis of the *DcEXP* gene family was completed in MCScanX. Non-synonymous substitution rate (*Ka*), synonymous substitution rate (*Ks*), and *Ka/Ks* analyses of homologous genes were performed using *KaKs*_calculator2.0 software and Tbtools software.

2.7. Cis-Acting Element Analysis of the Carrot DcEXP Gene Family

The genomic DNA sequences 2000 bp upstream of the start sites of the *DcEXP* genes were defined as promoter regions. The *cis*-acting elements in the promoter regions of the *DcEXP* genes were analyzed using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (accessed on 12 January 2025). The results were visualized using TBtools software.

2.8. Measurement of Agronomic Characters and Correlation Analysis

To gain further insight into how the *DcEXP* genes participate in and regulate carrot growth and development, we first analyzed the phenotypes and agronomic characters during key developmental stages of carrots. Agronomic characters of carrots were measured at 30, 60, and 90 days after sowing. The root fresh weight and aboveground fresh weight were measured using an electronic analytical balance. The root–shoot ratio was calculated as the ratio of the fresh weight of the taproot to that of the aerial parts. Root length was measured with a ruler, and root diameter (the diameter at the root head) was measured using a vernier caliper with automatic reading. Each of these measurements was conducted with three biological replicates. Statistical analysis, significant difference analysis, and Pearson's correlation analysis were performed using SPSS 25.0 software, and the results were visualized with TBtools software.

2.9. Gene Expression Analysis

The expression levels of *DcEXP* genes in carrot at different growth stages and in various tissues were determined using Hieff qPCR SYBR Green Master Mix (Yeason, Shanghai, China). The experiments were conducted in 96-well plates using the Real-Time PCR detection system (Bio-rad, CFX96, California, USA). Carrot *DcActin* was used as the reference gene [34], and specific quantitative primers were designed using Primer Premier 6.0 software (Supplemental Table S1). The total reaction volume for RT-qPCR was 20 µL, which included 10 µL of SYBR Premix *Ex Taq*, 2 µL of cDNA template, 7.2 µL of ddH$_2$O, and 0.4 µL of each forward and reverse primer. The thermal cycling program was as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for

10 s and annealing at 60 °C for 30 s. Fluorescence signals were continuously measured during a stepwise increase in temperature from 65 °C to 95 °C to generate melting curves. The relative expression levels of the genes were calculated using the $2^{-\Delta\Delta CT}$ method [35]. Each sample was analyzed in triplicate. Statistical analysis was performed using SPSS 25.0 software, and significant differences were determined by Duncan's multiple range test at $p < 0.05$.

3. Results

3.1. Identification and Analysis of the Carrot DcEXP Gene Family

Based on the carrot genome database, a total of 35 *DcEXP* genes were identified. According to the nomenclature proposed by Wang et al. [30], these genes were designated as *DcEXP1~DcEXP26* and *DcEXP28~DcEXP36*. A comparative sequence analysis of the *DcEXP* gene family members was conducted (Table 1). The results indicated that the 35 *DcEXP* genes were distributed across all 9 carrot chromosomes. The lengths of the *DcEXP* gene sequences varied significantly, within the range of 671~4266 bp. The amino acid sequence of DcEXP19 was the longest (293 aa), while that of DcEXP18 was the shortest (186 aa). The molecular weights of the DcEXP proteins were within the range of 20.60~31.36 kDa, and their theoretical isoelectric points (*pI*) were within the range of 4.89~9.92. Except for DcEXP2, DcEXP9, DcEXP10, DcEXP16, DcEXP19, DcEXP24, and DcEXP31, all other DcEXP proteins were predicted to be hydrophilic. The multiple sequence alignment results showed that the 35 DcEXP amino acid sequences share 45% identity (Figure 1).

Table 1. Information of *DcEXP* family members and physicochemical properties of DcEXP proteins in carrot.

Gene Name	Gene ID	Chromosome Location	gDNA Length (bp)	Amino Acid Length (aa)	Molecular Weight (kDa)	Theoretical *pI*	Grand Average of Hydropathicity
DcEXP1	DCAR_100846	Chr1: 6,339,073–6,340,335	1263	256	28.19	9.46	−0.02
DcEXP2	DCAR_103393	Chr1: 42,300,842–42,302,755	1914	266	28.40	8.84	0.08
DcEXP3	DCAR_206001	Chr2: 27,997,411–27,998,682	1272	260	28.11	9.65	−0.08
DcEXP4	DCAR_206376	Chr2: 32,762,836–32,763,816	981	262	28.88	9.46	−0.05
DcEXP5	DCAR_206704	Chr2: 36,196,798–36,198,305	1508	260	28.14	9.92	−0.04
DcEXP6	DCAR_208090	Chr2: 47,500,356–47,501,332	977	249	26.42	8.63	−0.14
DcEXP7	DCAR_209418	Chr2: 56,592,221–56,593,616	1396	249	27.65	6.37	−0.17
DcEXP8	DCAR_310739	Chr3: 8,031,000–8,031,983	984	255	27.55	9.51	−0.01
DcEXP9	DCAR_310740	Chr3: 8,040,221–8,041,199	979	255	27.59	9.43	0.01
DcEXP10	DCAR_310999	Chr3: 10,333,932–10,335,287	1356	257	27.79	5.32	0.02
DcEXP11	DCAR_311357	Chr3: 14,013,746–14,017,096	3351	264	28.67	7.53	−0.08

Table 1. *Cont.*

Gene Name	Gene ID	Chromosome Location	gDNA Length (bp)	Amino Acid Length (aa)	Molecular Weight (kDa)	Theoretical *pI*	Grand Average of Hydropathicity
DcEXP12	DCAR_312580	Chr3: 47,099,297–47,100,658	1362	242	26.36	9.60	−0.12
DcEXP13	DCAR_313707	Chr3: 58,306,829–58,310,084	3256	262	28.35	9.62	−0.02
DcEXP14	DCAR_417312	Chr4: 43,592,653–43,593,579	927	247	26.57	9.32	−0.12
DcEXP15	DCAR_417091	Chr4: 42,059,527–42,060,504	978	256	27.38	9.22	−0.01
DcEXP16	DCAR_519135	Chr5: 7,163,308–7,164,252	945	255	27.13	8.53	0.01
DcEXP17	DCAR_519597	Chr5: 11,841,355–11,843,154	1800	251	27.87	9.67	−0.16
DcEXP18	DCAR_520240	Chr5: 27,149,749–27,150,419	671	186	20.60	8.85	−0.49
DcEXP19	DCAR_520709	Chr5: 31,877,033–31,878,497	1465	293	31.36	6.37	0.01
DcEXP20	DCAR_521959	Chr5: 42,405,577–42,407,092	1516	261	28.19	9.20	−0.08
DcEXP21	DCAR_626607	Chr6: 40,060,705–40,061,644	940	246	26.54	9.39	−0.04
DcEXP22	DCAR_625076	Chr6: 28,787,956–28,789,696	1741	262	28.29	9.04	−0.08
DcEXP23	DCAR_728217	Chr7: 19,885,069–19,886,970	1902	258	28.24	8.03	−0.19
DcEXP24	DCAR_729133	Chr7: 31,691,116–31,692,131	1016	240	25.61	8.13	0.00
DcEXP25	DCAR_729937	Chr7: 38,481,461–38,482,393	933	244	26.07	6.27	−0.07
DcEXP26	DCAR_832117	Chr8: 24,892,236–24,893,322	1087	247	26.48	9.02	−0.18
DcEXP28	DCAR_830574	Chr8: 2,025,127–2,026,785	1659	255	27.68	9.31	−0.14
DcEXP29	DCAR_830374	Chr8: 402,868–403,956	1089	253	27.23	8.72	−0.19
DcEXP30	DCAR_935972	Chr9: 44,050,963–44,052,416	1454	250	26.58	9.03	−0.15
DcEXP31	DCAR_207623	Chr2: 44,225,282–44,226,327	1046	264	29.08	9.27	0.00
DcEXP32	DCAR_726983	Chr7: 2,203,016–2,207,281	4266	240	25.40	8.09	−0.07
DcEXP33	DCAR_416279	Chr4: 35,958,787–35,960,231	1445	287	30.75	6.44	−0.09
DcEXP34	DCAR_726980	Chr7: 2,137,458–2,138,746	1289	252	26.88	4.91	−0.16
DcEXP35	DCAR_728680	Chr7: 26,399,400–26,401,031	1632	257	27.60	4.89	−0.10
DcEXP36	DCAR_728679	Chr7: 26,395,737–26,397,225	1489	256	27.65	7.99	−0.11

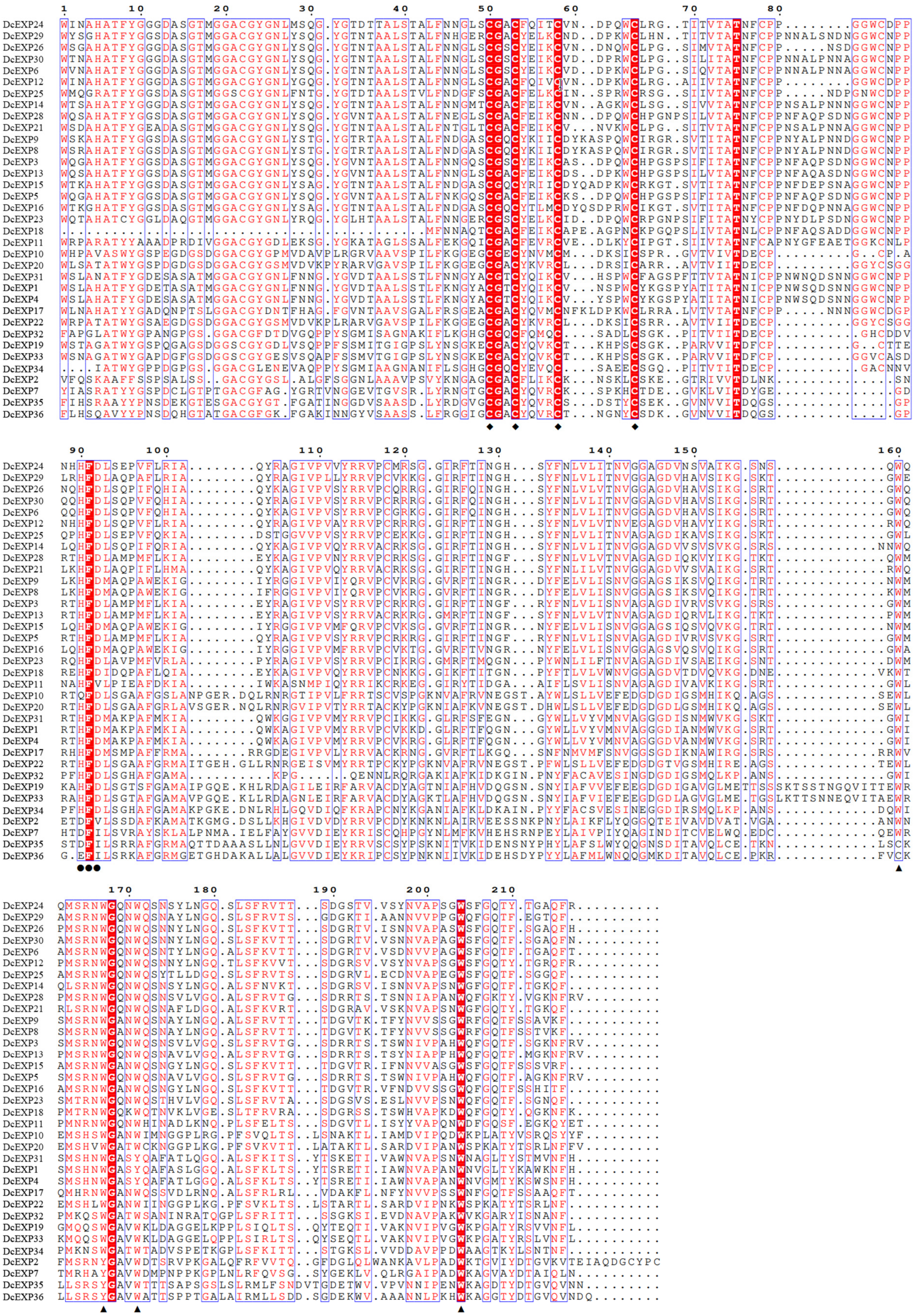

Figure 1. Multiple sequence alignment of DcEXP protein amino acid sequences in carrot. '♦' represent conserved cysteine (Cys, C) residues, '●' represent the HFD (His-Phe-Asp) motif, and '▲' represent tryptophan (Trp, W) residues.

3.2. Phylogenetic Analysis of the Carrot DcEXP Gene Family

The amino acid sequences of EXP proteins from carrot, rice, and *Arabidopsis* were aligned, and a phylogenetic tree was constructed using the Neighbor-Joining method (Figure 2). The results showed that the carrot, rice, and *Arabidopsis* EXP proteins were divided into four subfamilies: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB), with members of each subfamily forming their own distinct clade. Among them, the EXPA subfamily contained the most DcEXP proteins, with 24 members; the EXPB subfamily included 7 DcEXP proteins; only DcEXP2 (DCAR_103393) belonged to the EXLA subfamily; and the EXLB subfamily comprised three DcEXP proteins, namely DcEXP7 (DCAR_209418), DcEXP35 (DCAR_728680), and DcEXP36 (DCAR_728679).

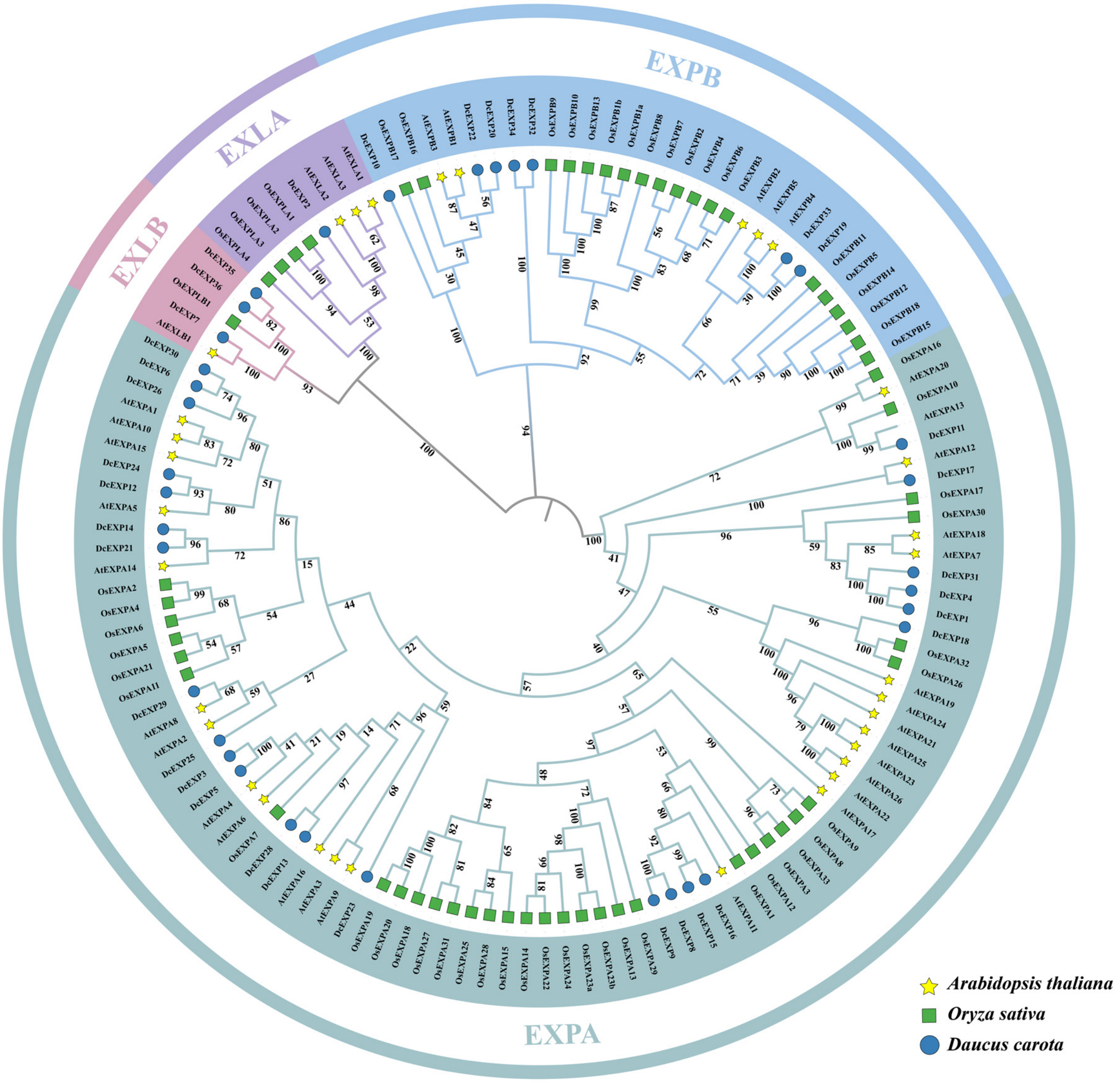

Figure 2. Phylogenetic tree of EXP protein in carrot, rice, and *Arabidopsis*.

3.3. Conserved Motif and Gene Structure Analysis of the Carrot DcEXP Gene Family

Based on the phylogenetic relationships of EXP proteins in carrot and *Arabidopsis thaliana*, the DcEXP proteins were classified into four distinct subfamilies (Figure 3A). The conserved motifs of the *DcEXP* family members were analyzed using the MEME online tool, resulting in the identification of 10 conserved motifs, which were distributed among the different subfamilies as shown in Figure 3B and Supplemental Figure S1. Genes with high homology share the same motif composition. Motif 1, motif 2, and motif 9 were present only in the EXPA subfamily, while motif 10 was exclusive to the EXPB subfamily. Except for the EXPA subfamily, all other subfamilies contain motif 7. The domain analysis results (Figure 3C) indicated that the EXPA subfamily genes possessed the PLN00050 (DPBB_1 domain) and PLN00193 (Pollen_allerg_1 domain). The EXPB subfamily genes mainly contained the DPBB_EXPB_N, Expansin_C, and PLN03023 (DPBB_1 and Pollen_allerg_1 domains). Members of the EXLA and EXLB subfamilies shared the same domain composition, with a conserved PLN03023 domain. The gene structure analysis results (Figure 3D) showed that, except for *DcEXP18*, all EXPA subfamily genes had two introns. EXPB subfamily genes contained three introns, and EXLA and EXLB subfamily genes had the same number of introns, which was four.

Figure 3. Phylogenetics, conserved motif, conserved domain, and gene structure analysis of the DcEXP protein in carrot. (**A**): Phylogenetic tree; (**B**): Conserved motif distribution; (**C**): Conserved domain distribution; (**D**): Gene structure.

3.4. Chromosomal Localization and Collinearity Analysis of the Carrot DcEXP Gene Family

A chromosomal localization map was drawn based on genomic annotations to investigate the distribution of *DcEXP* genes on carrot chromosomes (Figure 4A). As shown in the figure, the 35 *DcEXP* genes were located on all nine chromosomes. The highest number of genes, seven, was found on chromosome 7, while the lowest number, one, was found on chromosome 9. To explore the potential relationships and possible gene duplications among *DcEXP* genes in carrot, a collinearity analysis was performed. As shown in Figure 4B and Supplemental Table S2, among the 35 members of the *DcEXP* gene family, 24 pairs of homologous gene pairs were identified as segmental duplications. The selection pressure analysis of these 24 pairs of homologous genes revealed that the *Ka/Ks* ratios were all less than 1, indicating that the evolution of *DcEXP* genes within the species was primarily driven by purifying selection.

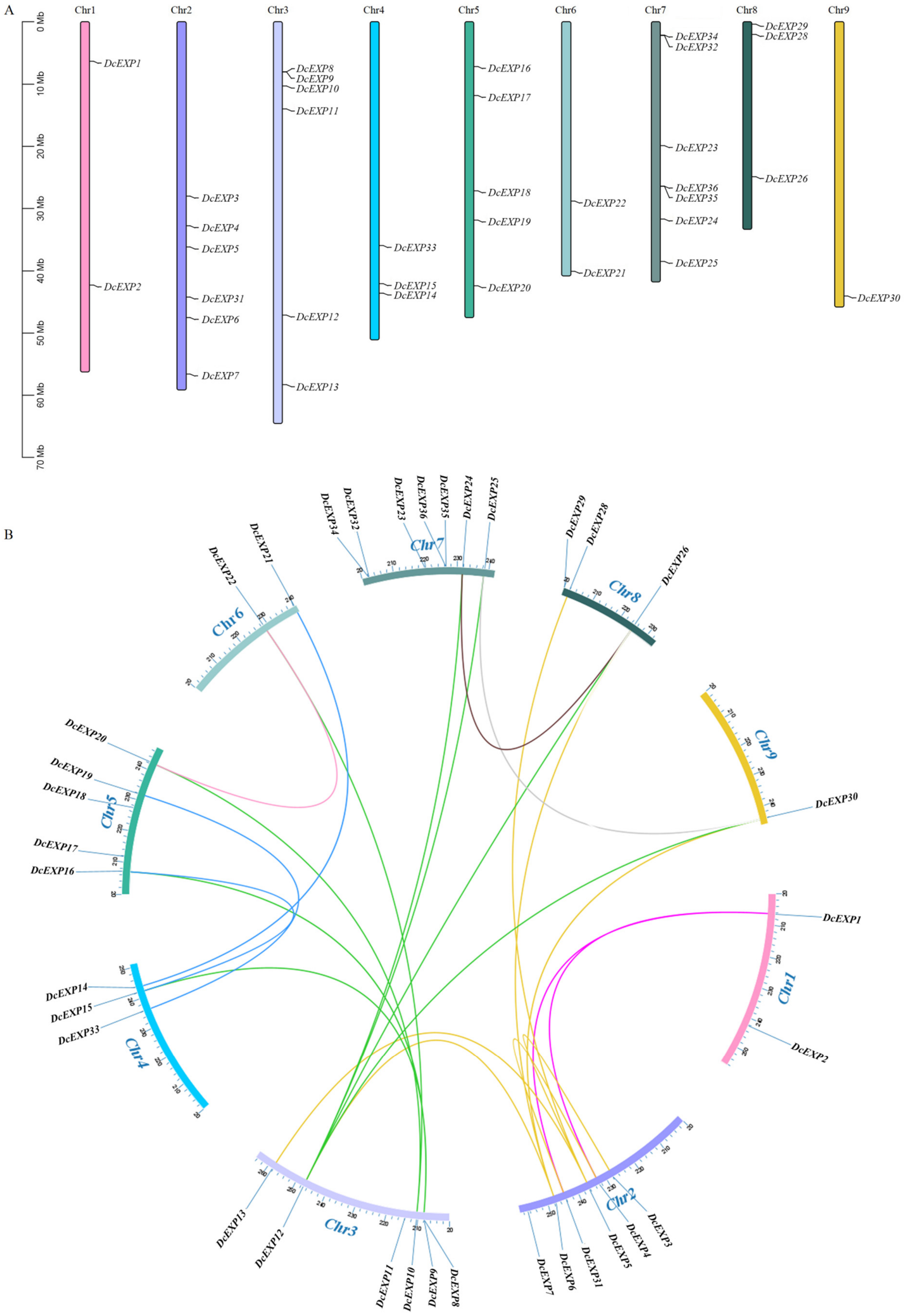

Figure 4. Chromosomal determination and collinearity analysis of *DcEXP* genes in carrot. (**A**): Chromosomal determination; (**B**): Collinearity analysis. Lines represent collinear gene pairs.

3.5. Cis-Acting Elements Analysis of the Carrot DcEXP Gene Family

To explore the potential regulatory mechanisms of *DcEXP* genes in carrot, a *cis*-acting element analysis was conducted on the 2000 bp upstream sequences from the start codons of the *DcEXP* gene family members. The results showed that the promoter regions of *DcEXP* genes contained a variety of *cis*-acting elements, which were categorized into three classes based on their functions: elements related to plant growth and development, plant hormone response, and abiotic and biotic stress response (Figure 5). Among these, light-responsive elements (GT1-motif), abscisic acid-responsive elements (ABRE), methyl jasmonate-responsive elements (CGTCA-motif and TGACG-motif), anaerobic induction elements (ARE), and low-temperature responsive elements (LTR) were widely present in *DcEXP* genes. The majority of the genes had a predominance of plant growth and development-related elements in their promoter regions. *DcEXP11* and *DcEXP28* had the highest number of plant growth and development-related elements in their promoter regions, with 23 elements each. The promoter region of *DcEXP32* did not contain any plant hormone-responsive elements. The auxin-responsive element (AuxRR-core) was only found in the promoter regions of *DcEXP23* and *DcEXP35*.

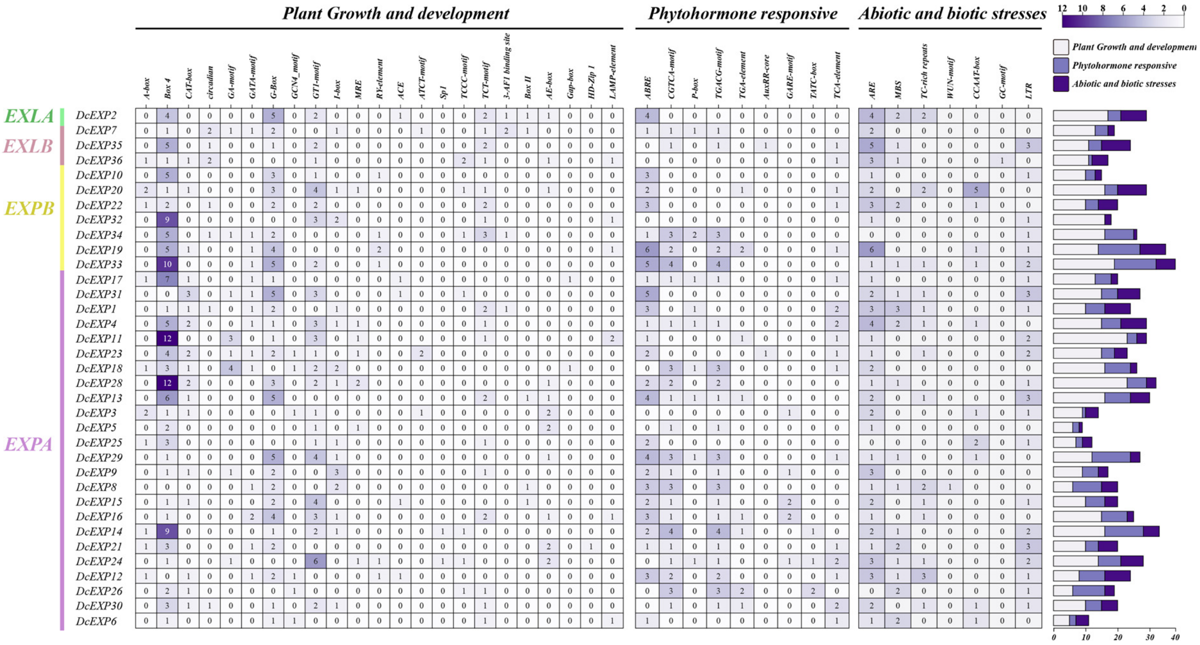

Figure 5. Distribution of *cis*-elements of *DcEXP* gene promoters in carrot.

3.6. Agronomic Characters and Correlation Analysis of Carrot at Different Growth and Development Stages

The phenotypes of 'Kurodagosun' ('KRD') carrot plants at 30, 60, and 90 days after sowing were shown in Figure 6A. As the carrot plants grew, the aerial parts of 'KRD' became more lush, and the taproots gradually enlarged. The taproots showed significant enlargement between 30 and 60 days after sowing. The root fresh weight, aboveground fresh weight, root–shoot ratio, root length, and root diameter were measured at 30, 60, and 90 days after sowing (Figure 6B). The results indicated that during the growth and development of carrots, the root fresh weight, root–shoot ratio, and root diameter all increased significantly. There were no significant differences in the aboveground fresh weight and root length between 60 and 90 days after sowing.

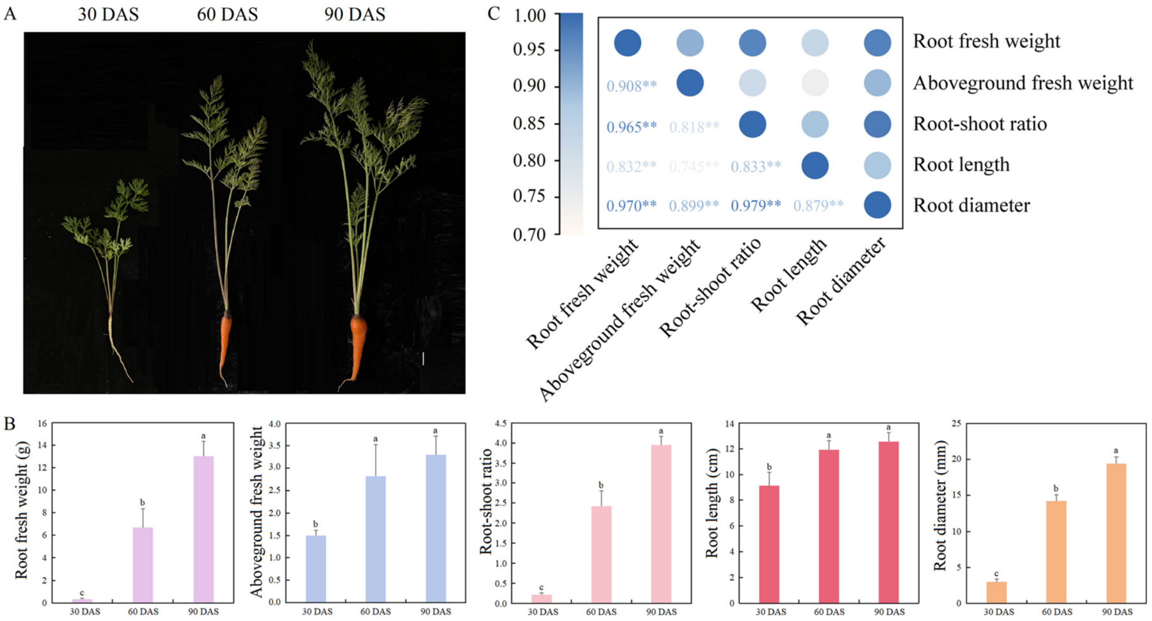

Figure 6. Growth status and analysis of agronomic characters of carrot 'Karodagosun' at three growth stages. (**A**): The status of carrot 'Karodagosun' at three growth stages. The white line represent 2 cm; (**B**): Root fresh weight, aboveground fresh weight, root–shoot ratio, root length, and root diameter at 30, 60, 90 days after sowing of carrot 'Karodagosun'. Error bars represent mean standard deviation (SD). Different lowercases indicate the significant difference at $p < 0.05$ level. (**C**): Correlation coefficient analysis of agronomic characters of carrot. ** indicate significant correlation at the level of $p < 0.01$.

To further investigate the relationships between different agronomic characters during the growth and development of carrots, a Pearson's correlation analysis was conducted on the five agronomic characters. As shown in Figure 6C, all five agronomic characters exhibited highly significant positive correlations. The strongest correlations were found between root diameter and root–shoot ratio (0.979 **), root diameter and root fresh weight (0.970 **), and root fresh weight and root–shoot ratio (0.965 **). These results suggested that as the taproot diameter increases, the root fresh weight and root–shoot ratio also increase, indicating that a thicker taproot was associated with a greater root fresh weight and a higher root–shoot ratio.

3.7. Temporal and Spatial Expression Pattern Analysis of DcEXP Genes in Carrot

The expression levels of *DcEXP* genes in leaves and taproots of 'Kurodagosun' ('KRD') carrot plants at 30, 60, and 90 days after sowing were shown in Figure 7. The results indicated that *DcEXP18* was specifically expressed in leaves, with the highest expression level at 60 days after sowing, which was 7.95 and 5.57 times higher than that at 30 and 90 days after sowing, respectively. The expression levels of *DcEXP6*, *DcEXP16*, *DcEXP24*, *DcEXP25*, and *DcEXP29* in leaves at 30 days after sowing were significantly higher than those at other stages. The genes of *DcEXP2*, *DcEXP3*, *DcEXP5*, *DcEXP8*, *DcEXP11*, *DcEXP13*, *DcEXP17*, *DcEXP19*, *DcEXP20*, *DcEXP22*, *DcEXP26*, *DcEXP28*, and *DcEXP33* were mainly expressed in taproots, with very low or undetectable expression levels in leaves at all three time points. All 35 *DcEXP* genes exhibited relatively low expression levels in taproots at 90 days after sowing.

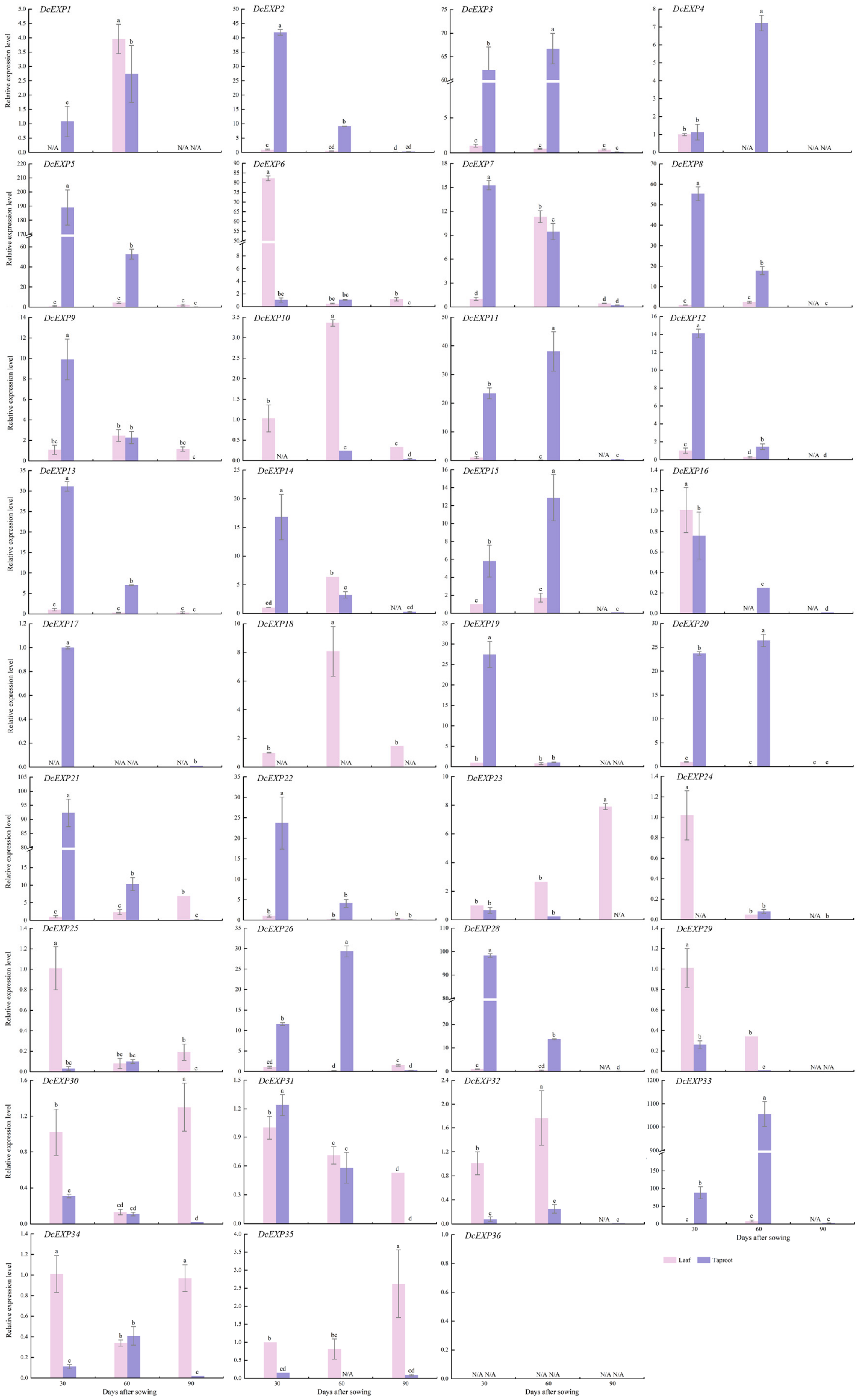

Figure 7. The relative expression levels of *DcEXP* genes in different tissues of carrot at different growth stages. Error bars represent mean standard deviation (SD). Different lowercase letters indicate significant differences ($p < 0.05$) for each gene among the combinations of three growth stages (30, 60, and 90 days after sowing) and two tissues (leaf and taproot).

4. Discussion

Expansins play crucial roles in plant growth and development [24]. In recent years, with the advancement of genomics and bioinformatics, expansin gene families have been identified in multiple plant species [36]. For instance, the soybean genome contained 75 expansin family members [14], there were 52 expansin family members in tobacco [37], 88 expansin genes in maize [38], and 29 expansin genes in grape [15]. In this study, based on the carrot genome database, we identified 35 expansin genes in carrot, designated as *DcEXP*s. The variation in the number of expansin family members among different species may be related to genome duplication, chromosomal recombination, and translocation events. According to phylogenetic analysis, plant expansin genes can be divided into four subfamilies: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB) [20]. Previous studies have indicated that during plant evolution, the EXPA subfamily emerged first, followed by the EXPB subfamily, while the EXLA and EXLB subfamilies appeared most recently [6,9,22,24,26,39]. The EXPA subfamily first emerged before the divergence of algae and bryophytes, and its DPBB_1 domain has never been lost in land plants, consistently preserving the "acid-growth" cell-wall-loosening function [6,9]. In this study, all carrot DcEXPA members retained this domain intact and clustered with rice and *Arabidopsis* EXPA genes in the same clade, suggesting that DcEXPA proteins likely regulate the enlargement of parenchyma cells in the carrot taproot and thereby influence carrot growth and development. EXPB has been shown to function in plant reproduction. In *Arabidopsis*, *AtEXPB5* was highly expressed in reproductive organs and facilitates pollen-tube elongation [40]. *DcEXP33* and *DcEXP19* were most closely related to *AtEXPB5*, suggesting that they might retain their reproductive tissue expression characteristics, which can be validated in the future through flower and root RNA-seq ratios. The EXPA subfamily has the most members, followed by the EXPB subfamily, while the EXLA and EXLB subfamilies have the fewest members. For example, in *Arabidopsis thaliana*, the EXPA subfamily had the highest number of genes (26), while the EXPB, EXLA, and EXLB subfamilies had 6, 3, and 1 genes, respectively [20,25]. In rice, the expansin genes were distributed among the four subfamilies as follows: EXPA (34 genes), EXPB (19 genes), EXLA (4 genes), and EXLB (1 gene) [41]. In tomato, the expansin gene family included 25 EXPA, 8 EXPB, 1 EXLA, and 4 EXLB members [37]. In this study, based on the phylogenetic tree topology and the classification of homologous genes in rice and *Arabidopsis*, the 35 *DcEXP* genes in carrot were classified into four subfamilies: EXPA, EXPB, EXLA, and EXLB, containing 24, 7, 1, and 3 genes, respectively. The classification results and the number of genes in each subfamily were consistent with the findings of previous studies, the members of the subfamily gather in groups, revealing the regular origin and evolutionary relationships between subfamilies.

The exon/intron structure is typically conserved among members of homologous gene families, and its analysis can help reveal the evolutionary history of gene families [42]. A comprehensive analysis of the exon/intron structure of expansin gene families in grape, soybean, tobacco, wheat, and cabbage [14,15,37,43,44] revealed that the typical number of introns in the four expansin subfamilies are as follows: EXPA (2 introns), EXPB (3 introns), EXLA (4 introns), and EXLB (3 or 4 introns). In this study, most EXPA subfamily genes in carrot contained 2 introns, all EXPB subfamily genes contained 3 introns, and all EXLA and EXLB subfamily genes had the same number of introns, which was 4. These results were consistent with previous studies, indicating a high degree of similarity in exon/intron structure within the same subfamily. In addition to the DPBB_1 and Pollen_allerg_1 domains, expansin gene family sequences also contain multiple conserved motifs. Previous studies have identified 10 motifs in soybean expansin genes and 11 motifs in tobacco expansin genes [14,37]. The analysis results showed that the type and number of motifs are similar within the same subfamily but differ among different subfamilies. In this study, we analyzed the conserved motifs of *DcEXP* family members in carrot and identified 10 conserved motifs. Genes within the same subfamily had similar motif compositions, which was consistent with previous findings, indicating that the gene structure and domain composition of expansin subfamilies are highly conserved during evolution.

Genome-wide phylogeny showed that the expansin subfamilies originated before the monocot-eudicot divergence and were repeatedly expanded by successive whole-genome duplications. Branch-specific analyses revealed that positively selected sites cluster in the catalytic HFD motif and the cellulose-binding face carrying conserved tryptophans, indicating that evolutionary fine-tuning of these domains alters their polysaccharide affinity. Consequently, duplicated genes with novel functions acquired unique expression domains, participating in diverse cell wall relaxation events [36]. This tightly links gene family diversification with the functional diversity required for complex plant morphologies. Expansins are stable and widely present in plants, and they are localized on most chromosomes of plants. However, their distribution on chromosomes is uneven [45]. Studies have shown that expansin genes in rice were distributed across 10 chromosomes. In *Arabidopsis thaliana* and Chinese cabbage, expansin genes were found on every chromosome [25,46]. In this study, the 35 carrot expansin genes were distributed across all nine chromosomes, indicating that the expansin gene family may have certain selective advantages during the evolution of carrots. Moreover, the differences in the chromosomal distribution patterns of expansins may be related to the genomic evolutionary history and functional requirements of different species. During the course of evolution, plants frequently undergo gene duplication events to adapt to changes in external environmental conditions. Gene duplication is one of the main drivers of genome and gene family evolution [47]. Among these, tandem duplication and segmental duplication are the primary modes of expansion in gene families [48]. After the formation of new genes, they may undergo functional divergence due to different types of selective pressures [49]. Studies have shown that tandem duplication was the main expansion mode of the expansin gene family in monocots, such as rice and wheat [40,50]. In dicots, the expansion mode of the expansin gene family was mainly through segmental duplication [14,25,41,43]. Additionally, during the study of the chromosomal localization patterns of expansin gene families, previous research has found that some genes distributed on different chromosomes had collinear relationships [14], indicating that chromosomal rearrangements may have occurred in the gene family during evolution. In this study, among the 35 members of the carrot *DcEXP* gene family, 24 pairs of collinear gene pairs were identified, all of which were segmental duplications. The *Ka*/*Ks* ratios were less than 1, indicating that the *DcEXP* gene family has evolved in a relatively conservative manner, which may be related to the similar regulatory functions of expansin genes in plant growth and development.

Expansins, key cell wall-loosening proteins, play a pivotal role in the enlargement of carrot storage roots [30]. To elucidate the relationship between the *DcEXP* gene family and phenotypic development, we first analyzed five core agronomic characters (root fresh weight, aboveground fresh weight, root–shoot ratio, root length, and root diameter) at three critical developmental stages: 30, 60, and 90 days after sowing. The results revealed that 30~60 days after sowing constituted the critical period for rapid taproot enlargement. All five characters exhibited highly significant positive correlations, consistent with previous reports [3,30], indicating this stage as the golden period for carrot morphological establishment and yield accumulation. Based on this phenotypic temporal pattern, we further examined the expression differences of *DcEXP* family members in taproots and leaves across developmental stages. This approach aims to integrate the "gene family-critical taproot enlargement period-trait correlation" framework to elucidate, at the molecular level, how *DcEXP* regulates rapid carrot taproot enlargement and to deliver actionable gene targets for high-yield carrot breeding. Numerous studies have shown that expansins are primarily present in growing plant tissues and organs, and their gene expression exhibits temporal and spatial specificity [24]. Lee et al. analyzed the transcriptional level of the soybean expansin gene *GmEXP1* and found that *GmEXP1* was specifically expressed in roots, with varying expression levels at different stages of root development, peaking in the root system at 5 days after seed germination [51]. In this study, most carrot expansin genes were specifically expressed in the taproot, with only one gene, *DcEXP18*, being specifically expressed in leaves. *DcEXP36* was not expressed in either the taproot or leaves of carrots, suggesting that it may be expressed during the reproductive growth stage of carrots. During the taproot expansion period of carrots (30~60 days after sowing), the expression levels of genes such as *DcEXP2*, *DcEXP3*, *DcEXP5*, *DcEXP8*, *DcEXP11*, *DcEXP13*, *DcEXP17*, *DcEXP19*, *DcEXP20*, *DcEXP22*, *DcEXP26*, *DcEXP28*, and *DcEXP33* were relatively high, indicating that they may play roles in taproot development. Pacifici et al. found that inhibiting the expression of the *Arabidopsis EXPA1* gene resulted in impaired cell wall elongation in *atexpa1* mutants, thereby affecting cell differentiation in the root apical meristem [52]. It was closely related to carrot *DcEXP26*, and *DcEXP26* was highly expressed in taproot, speculating that *DcEXP26* might play a similar role to *AtEXPA1* in carrot taproot by maintaining the extensibility of root apical meristem cell walls, promoting the differentiation and expansion of thin-walled cells, and regulating the development of carrot taproot. The *Arabidopsis AtEXLA2* gene was associated with lateral root formation [53] and was most closely related to the carrot *DcEXP2*. In this study, *DcEXP2* exhibited the highest expression levels in 30 days after sowing taproots. It was hypothesized that *DcEXP2* might influence storage root branching and final morphogenesis by regulating the initiation and elongation of lateral root primordia during early carrot taproot development. Moreover, all 35 *DcEXP* genes exhibited lower expression levels in the taproot at 90 days after sowing, demonstrating that the expression of carrot expansin genes was temporally and spatially specific and mainly involved in taproot development. Wang et al., based on proteomic and transcriptomic analyses, found that *DcEXP* genes were involved in regulating taproot development in carrots [30], which was consistent with the results of this study. It was inferred that *DcEXP* genes are key regulators of taproot enlargement in carrots and their functions can be further explored through functional validation. With the rapid development of carrot genomics, further research and verification will be carried out [54–56].

This study systematically identified the expansin gene family in carrot and analyzed the temporal and spatial expression patterns of these genes in conjunction with growth phenotypes. The findings provide a reference for further research and validation of the regulatory functions of *DcEXP* genes in carrot taproot enlargement, and lay the foundation for regulating carrot growth and improving yield and quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15102338/s1, Figure S1. Predicted conserved motifs of carrot DcEXP proteins. Table S1. Nucleotide sequences of primers used for RT-qPCR. Table S2. The duplicate *DcEXP* gene pairs in carrot.

Author Contributions: Conceptualization, A.-S.X. and R.-R.Z.; methodology, R.-R.Z. and Y.-H.W.; investigation, R.-R.Z. and N.Z.; formal analysis, R.-R.Z., J.-P.T., J.-H.Z., X.-F.P., L.Z., X.X., Y.-J.S. and G.-F.T.; resources, A.-S.X.; writing—original draft preparation, R.-R.Z.; writing—review and editing, A.-S.X.; visualization, R.-R.Z.; supervision, A.-S.X.; project administration, A.-S.X. and R.-R.Z.; funding acquisition, A.-S.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Fundamental Research Funds for the Central Universities (KYLH2025002), Key Laboratory of Vegetable Genetics and Physiology of Qinghai Province Open Research Project (2020-SCSYS-01), Priority Academic Program Development of Jiangsu Higher Education Institutions Project (PAPD), and the Bioinformatics Center of Nanjing Agricultural University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: In this section, all the other data sets supporting the conclusions of this article are included within this article.

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

References

1. Brandenburg, W.A. Possible relationships between wild and cultivated carrots (*Daucus carota* L.) in the Netherlands. *Genet. Resour. Crop Evol.* **1981**, *29*, 369–375. [CrossRef]
2. Que, F.; Hou, X.L.; Wang, G.L.; Xu, Z.S.; Tan, G.F.; Li, T.; Wang, Y.H.; Khadr, A.; Xiong, A.S. Advances in research on the carrot, animportant root vegetable in the Apiaceae family. *Hortic. Res.* **2019**, *6*, 69. [CrossRef]
3. Wang, G.L.; Jia, X.L.; Xu, Z.S.; Wang, F.; Xiong, A.S. Sequencing, assembly, annotation, and gene expression: Novel insights into the hormonal control of carrot root development revealed by a high-throughput transcriptome. *Mol. Genet. Genom.* **2015**, *290*, 1379–1391. [CrossRef] [PubMed]
4. Que, F.; Wang, G.L.; Li, T.; Wang, Y.H.; Xu, Z.S.; Xiong, A.S. Genome-wide identification, expansion, and evolution analysis of homeobox genes and their expression profiles during root development in carrot. *Funct. Integr. Genom.* **2018**, *18*, 685–700. [CrossRef]
5. Wang, X.J.; Luo, Q.; Li, T.; Meng, P.H.; Pu, Y.T.; Liu, J.X.; Zhang, J.; Liu, H.; Tan, G.F.; Xiong, A.S. Origin, evolution, breeding, and omics of Apiaceae: A family of vegetables and medicinal plants. *Hortic. Res.* **2022**, *9*, uhac076. [CrossRef]
6. McQueen-Mason, S.; Durachko, D.M.; Cosgrove, D.J. Two endogenous proteins that induce cell wall extension in plants. *Plant Cell* **1992**, *4*, 1425–1433. [PubMed]
7. Wu, Y.; Meeley, R.B.; Cosgrove, D.J. Analysis and expression of the alpha-expansin and beta-expansin gene families in maize. *Plant Physiol.* **2001**, *126*, 222–232. [CrossRef] [PubMed]
8. Sabirzhanova, I.B.; Sabirzhanov, B.E.; Chemeris, A.V.; Veselov, D.S.; Kudoyarova, G.R. Fast changes in expression of expansin gene and leaf extensibility in osmotically stressed maize plants. *Plant Physiol. Biochem.* **2005**, *43*, 419–422. [CrossRef]
9. Cho, H.T.; Kende, H. Tissue localization of expansins in deepwater rice. *Plant J.* **1998**, *15*, 805–812. [CrossRef]
10. Huang, J.; Takano, T.; Akita, S. Expression of alpha-expansin genes in young seedlings of rice (*Oryza sativa* L.). *Planta* **2000**, *211*, 467–473. [CrossRef]
11. Wang, G.; Gao, Y.; Wang, J.; Yang, L.; Song, R.; Li, X.; Shi, J. Overexpression of two cambium-abundant Chinese fir (*Cunninghamia lanceolata*) α-expansin genes *ClEXPA1* and *ClEXPA2* affect growth and development in transgenic tobacco and increase the amount of cellulose in stem cell walls. *Plant Biotechnol. J.* **2011**, *9*, 486–502. [CrossRef] [PubMed]
12. Cho, H.T.; Cosgrove, D.J. Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9783–9788. [CrossRef]
13. Cho, H.T.; Cosgrove, D.J. Regulation of root hair initiation and expansin gene expression in *Arabidopsis*. *Plant Cell* **2002**, *14*, 3237–3253. [CrossRef]
14. Zhu, Y.; Wu, N.; Song, W. Soybean (*Glycine max*) expansin gene superfamily origins: Segmental and tandem duplication events followed by divergent selection among subfamilies. *BMC Plant Biol.* **2014**, *14*, 93. [CrossRef]
15. Dal Santo, S.; Vannozzi, A.; Tornielli, G.B.; Fasoli, M.; Venturini, L.; Pezzotti, M.; Zenoni, S. Genome-wide analysis of the expansin gene superfamily reveals grapevine-specific structural and functional characteristics. *PLoS ONE* **2013**, *8*, e62206. [CrossRef]
16. Di Sansebastiano, G.P.; Fornaciari, S.; Barozzi, F.; Piro, G.; Arru, L. New insights on plant cell elongation: A role for acetylcholine. *Int. J. Mol. Sci.* **2014**, *15*, 4565–4582. [CrossRef]
17. Catalá, C.; Rose, J.K.; Bennett, A.B. Auxin regulation and spatial localization of an endo-1,4-beta-D-glucanase and a xyloglucan endotransglycosylase in expanding tomato hypocotyls. *Plant J.* **1997**, *12*, 417–426. [CrossRef]
18. Caderas, D.; Muster, M.; Vogler, H.; Mandel, T.; Rose, J.K.; McQueen-Mason, S.; Kuhlemeier, C. Limited correlation between expansin gene expression and elongation growth rate. *Plant Physiol.* **2000**, *123*, 1399–1414. [CrossRef] [PubMed]
19. Arru, L.; Rognoni, S.; Poggi, A.; Loreti, E. Effect of sugars on auxin-mediated *LeEXPA2* gene expression. *Plant Growth Regul.* **2008**, *55*, 11–20. [CrossRef]
20. Sampedro, J.; Carey, R.E.; Cosgrove, D.J. Genome histories clarify evolution of the expansin superfamily: New insights from the poplar genome and pine ESTs. *J. Plant Res.* **2006**, *119*, 11–21. [CrossRef] [PubMed]

21. Nakai, K.; Kanehisa, M. A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics* **1992**, *14*, 897–911. [CrossRef] [PubMed]
22. Lee, Y.; Kende, H. Expression of alpha-expansin and expansin-like genes in deepwater rice. *Plant Physiol.* **2002**, *130*, 1396–1405. [CrossRef] [PubMed]
23. Cosgrove, D.J. Enzymes and other agents that enhance cell wall extensibility. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 391–417. [CrossRef]
24. Cosgrove, D.J. New genes and new biological roles for expansins. *Curr. Opin. Plant Biol.* **2000**, *3*, 73–78. [CrossRef]
25. Sampedro, J.; Lee, Y.; Carey, R.E.; dePamphilis, C.; Cosgrove, D.J. Use of genomic history to improve phylogeny and understanding of births and deaths in a gene family. *Plant J.* **2005**, *44*, 409–419. [CrossRef] [PubMed]
26. Cosgrove, D.J. Plant expansins: Diversity and interactions with plant cell walls. *Curr. Opin. Plant Biol.* **2015**, *25*, 162–172. [CrossRef]
27. Choi, D.; Lee, Y.; Cho, H.T.; Kende, H. Regulation of expansin gene expression affects growth and development in transgenic rice plants. *Plant Cell* **2003**, *15*, 1386–1398. [CrossRef]
28. Muller, B.; Bourdais, G.; Reidy, B.; Bencivenni, C.; Massonneau, A.; Condamine, P.; Rolland, G.; Conéjéro, G.; Rogowsky, P.; Tardieu, F. Association of specific expansins with growth in maize leaves is maintained under environmental, genetic, and developmental sources of variation. *Plant Physiol.* **2007**, *143*, 278–290. [CrossRef]
29. Guo, W.; Zhao, J.; Li, X.; Qin, L.; Yan, X.; Liao, H. A soybean β-expansin gene *GmEXPB2* intrinsically involved in root system architecture responses to abiotic stresses. *Plant J.* **2011**, *66*, 541–552. [CrossRef]
30. Wang, Y.H.; Que, F.; Wang, G.L.; Hao, J.N.; Li, T.; Xu, Z.S.; Xiong, A.S. iTRAQ-based quantitative proteomics and transcriptomics provide insights into the importance of expansins during root development in carrot. *Front. Genet.* **2019**, *10*, 247. [CrossRef]
31. Lee, Y.; Choi, D.; Kende, H. Expansins: Ever-expanding numbers and functions. *Curr. Opin. Plant Biol.* **2001**, *4*, 527–532. [CrossRef]
32. Sudhir, K.; Glen, S.; Koichiro, T. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874.
33. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef]
34. Wang, G.L.; Tian, C.; Jiang, Q.; Xu, Z.S.; Wang, F.; Xiong, A.S. Comparison of nine reference genes for real-time quantitative PCR in roots and leaves during five developmental stages in carrot (*Daucus carota* L.). *J. Hortic. Sci. Biotech.* **2016**, *91*, 264–270. [CrossRef]
35. Pfaffl, M.W. A new mathematical model for relative quantification in realtime RT-PCR. *Nucleic Acids Res.* **2001**, *29*, e45. [CrossRef]
36. Wang, Z.; Cao, J.; Lin, N.; Li, J.; Wang, Y.; Liu, W.; Yao, W.; Li, Y. Origin, evolution, and diversification of the expansin family in plants. *Int. J. Mol. Sci.* **2024**, *25*, 11814. [CrossRef]
37. Ding, A.; Marowa, P.; Kong, Y. Genome-wide identification of the expansin gene family in tobacco (*Nicotiana tabacum*). *Mol. Genet. Genom.* **2016**, *291*, 1891–1907. [CrossRef] [PubMed]
38. Zhang, W.; Yan, H.; Chen, W.; Liu, J.; Jiang, C.; Jiang, H.; Zhu, S.; Cheng, B. Genome-wide identification and characterization of maize expansin genes expressed in endosperm. *Mol. Genet. Genom.* **2014**, *289*, 1061–1074. [CrossRef] [PubMed]
39. Li, Y.; Darley, C.P.; Ongaro, V.; Fleming, A.; Schipper, O.; Baldauf, S.L.; McQueen-Mason, S. Plant expansins are a complex multigene family with an ancient evolutionary origin. *Plant Physiol.* **2002**, *128*, 854–864. [CrossRef] [PubMed]
40. Mollet, J.C.; Leroux, C.; Dardelle, F.; Lehner, A. Cell wall composition, biosynthesis and remodeling during pollen tube growth. *Plants* **2013**, *2*, 107–147. [CrossRef]
41. Shi, Y.; Xu, X.; Li, H.; Xu, Q.; Xu, J. Bioinformatics analysis of the expansin gene family in rice. *Hereditas* **2014**, *36*, 809–820.
42. Lecharny, A.; Boudet, N.; Gy, I.; Aubourg, S.; Kreis, M. Introns in, introns out in plant gene families: A genomic approach of the dynamics of gene structure. *J. Struct. Funct. Genom.* **2003**, *3*, 111–116. [CrossRef]
43. Li, N.; Pu, Y.; Gong, Y.; Yu, Y.; Ding, H. Genomic location and expression analysis of expansin gene family reveals the evolutionary and functional significance in *Triticum aestivum*. *Genes Genom.* **2016**, *38*, 1021–1030. [CrossRef]
44. Krishnamurthy, P.; Muthusamy, M.; Kim, J.A.; Jeong, M.J.; Lee, S.I. *Brassica rapa* expansin-like B1 gene (*BrEXLB1*) regulate growth and development in transgenic *Arabidopsis* and elicits response to abiotic stresses. *J. Plant. Biochem. Biotechnol.* **2019**, *28*, 437–446. [CrossRef]
45. Liu, W.; Lyu, T.; Xu, L.; Hu, Z.; Xiong, X.; Liu, T.; Cao, J. Complex molecular evolution and expression of expansin gene families in three basic diploid species of *Brassica*. *Int. J. Mol. Sci.* **2020**, *21*, 3424. [CrossRef] [PubMed]
46. Krishnamurthy, P.; Hong, J.K.; Kim, J.A.; Jeong, M.J.; Lee, Y.H.; Lee, S.I. Genome-wide analysis of the expansin gene superfamily reveals *Brassica rapa*-specific evolutionary dynamics upon whole genome triplication. *Mol. Genet. Genom.* **2015**, *290*, 521–530. [CrossRef]
47. Moore, R.; Purugganan, M. The early stages of duplicate gene evolution. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15682–15687. [CrossRef]

48. Cannon, S.B.; Mitra, A.; Baumgarten, A.; Young, N.D.; May, G. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* **2004**, *4*, 10. [CrossRef] [PubMed]
49. Nadeau, J.H.; Sankoff, D. Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution. *Genetics* **1997**, *147*, 1259–1266. [CrossRef] [PubMed]
50. Sampedro, J.; Cosgrove, D. The expansin superfamily. *Genome Biol.* **2005**, *6*, 242. [CrossRef] [PubMed]
51. Lee, D.K.; Ahn, J.H.; Song, S.K.; Choi, Y.D.; Lee, J.S. Expression of an expansin gene is correlated with root elongation in soybean. *Plant Physiol.* **2003**, *131*, 985–997. [CrossRef] [PubMed]
52. Pacifici, E.; Di Mambro, R.; Dello Ioio, R.; Costantino, P.; Sabatini, S. Acidic cell elongation drives cell differentiation in the *Arabidopsis* root. *EMBO J.* **2018**, *37*, e99134. [CrossRef]
53. Boron, A.K.; Van Loock, B.; Suslov, D.; Markakis, M.N.; Verbelen, J.P.; Vissenberg, K. Over-expression of *AtEXLA2* alters etiolated arabidopsis hypocotyl growth. *Ann. Bot.* **2015**, *115*, 67–80. [CrossRef]
54. Wang, Y.H.; Liu, P.Z.; Liu, H.; Zhang, R.R.; Liang, Y.; Xu, Z.S.; Li, X.J.; Luo, Q.; Tan, G.F.; Wang, G.L.; et al. Telomere-to-telomere carrot (*Daucus carota*) genome assembly reveals carotenoid characteristics. *Hortic. Res.* **2023**, *10*, uhad103. [CrossRef]
55. Wang, H.R.; Zhang, R.R.; Wang, Y.H.; Zhou, J.H.; Sun, M.; Wang, L.X.; Zhang, Y.Q.; Liang, Y.; Li, X.J.; Xu, Z.S.; et al. Carotene hydroxylase DcCYP97A3 affects carotenoid metabolic flow and taproot color by influencing the conversion of α-carotene to lutein in carrot. *Hortic. Res.* **2025**, *12*, uhaf054. [CrossRef] [PubMed]
56. Coe, K.; Bostan, H.; Rolling, W.; Turner-Hissong, S.; Macko-Podgórni, A.; Senalik, D.; Liu, S.; Seth, R.; Curaba, J.; Mengist, M.F.; et al. Population genomics identifies genetic signatures of carrot domestication and improvement and uncovers the origin of high-carotenoid orange carrots. *Nat. Plants* **2023**, *9*, 1643–1658. [CrossRef] [PubMed]