Overexpression of AcEXPA23 Promotes Lateral Root Development in Kiwifruit

Zhiyong Wu †, Ming Li †, Yunpeng Zhong ©, Lan Li, Dawei Cheng, Hong Gu, Xizhi Guo, Xiujuan Qi * and Jinyong Chen *

Key Laboratory of Fruit Breeding Technology of Ministry of Agriculture and Rural Affairs, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou 450009, China; 8210195086@caas.cn (Z.W.); liming07@caas.cn (M.L.); zhongyupeng@caas.cn (Y.Z.); lilan01@caas.cn (L.L.); chengdawei@caas.cn (D.C.); guhong@caas.cn (H.G.); guoxizhi@caas.cn (X.G.)
* Correspondence: qixiujuan@caas.cn (X.Q.); chenjinyong@caas.cn (J.C.)
† These authors contributed equally to this work.

Abstract: Kiwifruit is loved by consumers for its unique taste and rich vitamin C content. Kiwifruit are very sensitive to adverse soil environments owing to fleshy and shallow roots, which limits the uptake of water and nutrients into the root system, resulting in low yield and poor fruit quality. Lateral roots are the key organs for plants to absorb water and nutrients. Improving water and fertilizer use efficiency by promoting lateral root development is a feasible method to improve yield and quality. Expansins proteins plays a major role in lateral root growth; hence, it is important to identify expansin protein family members, screen key genes, and explore gene function in root development. In this study, 41 expansin genes were identified based on the genome of kiwifruit (‘Hongyang’, Actinidia chinensis). By clustering with the Arabidopsis thaliana expansin protein family, the 41 AcExpansin proteins were divided into four subfamilies. The AcExpansin protein family was further analysed by bioinformatics methods and was shown to be evolutionarily diverse and conserved at the DNA and protein levels. Based on previous transcriptome data and quantitative real-time PCR assays, we screened the candidate gene AcEXPA23. Overexpression of AcEXPA23 in kiwifruit increased the number of kiwifruit lateral roots.

Keywords: kiwifruit; expansin; lateral root

1. Introduction

Actinidia L., belonging to the family Actinidiaceae, comprises a total of 75 taxa, including 54 species and 21 varieties [1]. Cultivars in production are mainly derived from the original variety of Actinidia chinensis Planch. var. chinensis, A. chinensis Planch. var. delicosa (A. Chev.), and Actinidia arguta [2]. Kiwifruit is known as the “king of fruits” because of its unique taste and rich vitamin C content and is popular among consumers [2–4]. As of 2019, the kiwifruit planting area in China reached 250,000 ha, far exceeding the sum of the planting areas of other countries in the world and increasing annually [5]. Although the kiwifruit harvested area and output of China rank first in the world, the output per unit area ranks only 20th in the world, with clear import and export trade deficits [5]. Fruit yield and quality are closely related to the efficiency of water and nutrient uptake by plant roots. The root system of kiwifruit is composed of fleshy roots, is mainly distributed in the upper layer of the soil, and is easily affected by the surrounding soil environment [1]. To a certain extent, this limits the moisture and nutrient absorption efficiency of kiwifruit roots, resulting in low fruit production and poor quality [6].

Lateral roots not only promote water absorption and the efficiency with which plants obtain nutrients from the surrounding soil but also provide sufficient mechanical support for the aboveground parts of plants [7]. Lateral root formation is divided into five stages: pre-branch site priming, lateral root initiation, lateral root patterning, lateral root
emergence, and lateral root elongation [8,9]. Currently, four models have been identified that regulate lateral root initiation and patterning in Arabidopsis thaliana: (i) the IAA28-ARF module [10]; (ii) the SOLITARY-ROOT/IAA14-ARF7-ARF19 module [11–14]; (iii) the BODENLOS/IAA12-MONOPTEROS/ARF5 module [15,16]; and (iv) the SHY2/IAA3-ARF module [17]. LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE (LBD/ASL) proteins play an important role in the development of lateral organs in plants, including lateral root formation [18]. ARF7 and ARF19 regulate lateral root formation via activation of LBD16/ASL18 and LBD29/ASL16 [14]. LBD18 regulates lateral root formation in conjunction with LBD16 downstream of ARF7 and ARF19 [19,20]. LBD18/ASL20 acts as a specific DNA-binding transcriptional activator that directly regulates EXPANSIN14 (EXP14), a gene encoding a cell wall-loosening factor that promotes lateral root emergence in A. thaliana [18].

Expansins are cell-wall-loosening proteins that directly induce the extension of the cell wall by disrupting non-covalent bonding between cellulose microfibrils and associated matrix polysaccharides in the cell wall [21–23]. Expansin is encoded by a multigenic superfamily in plants divided into four subfamilies: EXPA, α-expansin; EXPB, β-expansin; EXLA, expansin-like A; and EXLB, expansin-like B [24]. Expansins have been shown to be involved in different plant developmental processes, including root development and root hair initiation [25,26], stem internode elongation [27], leaf development [28,29], flower development [30], fruit development and ripening [31], seed germination [32], stomatal movement [33], organ abscission [34], and biotic/abiotic stress response [35–38].

Our previous study showed that exogenous brassinosteroid (BR) treatment in kiwifruit did significantly promote lateral root development [39]. For in-depth research, we focused, herein, on the expansin gene, the most downstream regulatory gene in current lateral root research. We performed stable genetic transformation of the selected candidate gene AcEXP A23 in kiwifruit. Our results showed that overexpression of AcEXP A23 significantly promoted the increase in lateral roots in kiwifruit. This is important for kiwifruit to absorb more water and nutrients through the lateral roots to improve yield and quality.

2. Results

2.1. Phylogenetic Tree Analysis of the AcExpansin Protein Family

We identified 41 expansin protein family members in kiwifruit. To further classify the expansin gene in kiwifruit, a phylogenetic tree was constructed using 41 AcExpansin protein sequences and with all 36 Arabidopsis expansin protein sequences as references (Figure 1, Table S1). The results showed that the AcExpansin protein family was divided into four subfamilies: AcEXPA, AcEXPB, AcEXLA, and AcEXLB. According to the classification results, the identified AcExpansin protein family members were renamed (Table 1). The subfamily AcEXPA contained 28 genes (AcEXPA1–AcEXPA28), subfamily AcEXPB contained 6 genes (AcEXPB1–AcEXPB6), subfamily AcEXLA had 3 genes (AcEXLA1–AcEXLA3), and subfamily AcEXLB had 4 genes (AcEXLB1–AcEXLB4).
Figure 1. Phylogenetic tree of expansins in kiwifruit and *Arabidopsis*. The phylogenetic tree was constructed using the neighbour-joining method in MEGA11 (related parameter settings: bootstrap: 1000, model/method: p-distance, gaps/missing data treatment: partial deletion). The four expansin subfamilies are: EXPA, α-expansin; EXPB, β-expansin; EXLA, expansin-like A; and EXLB, expansin-like B.

Table 1. Physicochemical characterization of expansin proteins in kiwifruit.

| Subfamily | Gene Sequence Number | Gene Name | Number of Amino Acids | Molecular Weight | Theoretical pI | Instability Index | Aliphatic Index | GRAVY |
|-----------|----------------------|-----------|-----------------------|------------------|----------------|------------------|----------------|-------|
| AcEXP A1  | AcEXP A1             | 249       | 26,626.76             | 7.53             | 26.28          | 67.03            | −0.116         |
| AcEXP A2  | AcEXP A2             | 344       | 37,499.78             | 9.31             | 33.13          | 66.40            | −0.074         |
| AcEXP A3  | AcEXP A3             | 210       | 22,935.07             | 9.20             | 24.52          | 57.67            | −0.136         |
| AcEXP A4  | AcEXP A4             | 236       | 27,231.61             | 8.12             | 22.97          | 81.48            | −0.078         |
| AcEXP A5  | AcEXP A5             | 210       | 22,278.80             | 9.72             | 31.12          | 67.81            | −0.100         |
| AcEXP A6  | AcEXP A6             | 247       | 27,400.70             | 10.16            | 40.00          | 63.20            | −0.431         |
| AcEXP A7  | AcEXP A7             | 255       | 27,589.41             | 8.94             | 41.09          | 70.42            | −0.060         |
| AcEXP A8  | AcEXP A8             | 259       | 28,304.18             | 9.74             | 35.26          | 76.45            | 0.026          |
| AcEXP A9  | AcEXP A9             | 210       | 22,278.80             | 9.72             | 31.12          | 67.81            | −0.100         |
| AcEXP A10 | AcEXP A10            | 247       | 27,400.70             | 10.16            | 40.00          | 63.20            | −0.431         |
| AcEXP A11 | AcEXP A11            | 255       | 27,589.41             | 8.94             | 41.09          | 70.42            | −0.060         |
| AcEXP A12 | AcEXP A12            | 259       | 28,304.18             | 9.74             | 35.26          | 76.45            | 0.026          |
| AcEXP A13 | AcEXP A13            | 259       | 28,122.98             | 9.47             | 36.04          | 67.88            | −0.066         |
| AcEXP A14 | AcEXP A14            | 255       | 27,616.01             | 8.89             | 27.10          | 64.27            | −0.101         |
| AcEXP A15 | AcEXP A15            | 255       | 27,616.01             | 8.89             | 27.10          | 64.27            | −0.101         |
| AcEXP A16 | AcEXP A16            | 255       | 27,616.01             | 8.89             | 27.10          | 64.27            | −0.101         |
| AcEXP A17 | AcEXP A17            | 255       | 27,616.01             | 8.89             | 27.10          | 64.27            | −0.101         |
| AcEXP A18 | AcEXP A18            | 255       | 27,616.01             | 8.89             | 27.10          | 64.27            | −0.101         |
| AcEXP A19 | AcEXP A19            | 193       | 20,731.32             | 9.08             | 30.18          | 64.20            | −0.202         |
| AcEXP A20 | AcEXP A20            | 281       | 31,079.42             | 9.40             | 32.99          | 68.01            | −0.441         |
| AcEXP A21 | AcEXP A21            | 250       | 26,443.57             | 8.98             | 33.57          | 65.56            | −0.096         |
| AcEXP A22 | AcEXP A22            | 248       | 26,471.56             | 8.88             | 36.38          | 61.73            | −0.157         |
| AcEXP A23 | AcEXP A23            | 222       | 23,767.37             | 8.69             | 35.10          | 83.11            | 0.131          |
| AcEXP A24 | AcEXP A24            | 259       | 27,821.58             | 9.39             | 44.33          | 70.85            | −0.008         |
| AcEXP A25 | AcEXP A25            | 255       | 27,415.04             | 9.06             | 26.35          | 65.41            | −0.074         |
| AcEXP A26 | AcEXP A26            | 247       | 26,284.66             | 8.76             | 28.93          | 70.32            | 0.029          |
| AcEXP A27 | AcEXP A27            | 240       | 25,567.64             | 8.64             | 38.18          | 67.08            | −0.049         |
| AcEXP A28 | AcEXP A28            | 240       | 25,567.64             | 8.64             | 38.18          | 67.08            | −0.049         |
Table 1. Cont.

| Subfamily | Gene Sequence Number | Gene Name | Number of Amino Acids | Molecular Weight | Theoretical pI | Instability Index | Aliphatic Index | GRAVY |
|-----------|----------------------|-----------|----------------------|------------------|----------------|-------------------|----------------|-------|
| AcEXPB    | Actinidia09861        | AcEXPB1   | 266                  | 28,632.73        | 8.79           | 37.55             | 77.74          | −0.021 |
|           | Actinidia11045        | AcEXPB2   | 266                  | 28,861.98        | 8.86           | 38.09             | 75.53          | −0.064 |
|           | Actinidia13599        | AcEXPB3   | 197                  | 21,192.05        | 8.97           | 37.32             | 72.79          | −0.307 |
|           | Actinidia22767        | AcEXPB4   | 231                  | 23,757.54        | 6.21           | 32.77             | 57.92          | −0.082 |
|           | Actinidia33485        | AcEXPB5   | 248                  | 25,863.81        | 4.82           | 35.78             | 67.62          | −0.119 |
|           | Actinidia39310        | AcEXPB6   | 265                  | 28,448.37        | 8.46           | 33.87             | 76.87          | −0.070 |
| AcEXLA    | Actinidia18015        | AcEXLA1   | 457                  | 50,895.06        | 7.04           | 32.07             | 81.93          | −0.082 |
|           | Actinidia20552        | AcEXLA2   | 259                  | 28,282.42        | 8.81           | 25.72             | 81.70          | 0.026  |
|           | Actinidia24612        | AcEXLA3   | 783                  | 86,023.25        | 6.88           | 43.40             | 82.95          | −0.258 |
|           | Actinidia10878        | AcEXLB1   | 256                  | 27,956.06        | 4.73           | 32.56             | 72.34          | −0.200 |
|           | Actinidia25907        | AcEXLB2   | 236                  | 25,757.25        | 7.52           | 30.21             | 79.28          | −0.072 |
|           | Actinidia25941        | AcEXLB3   | 254                  | 27,840.00        | 4.81           | 30.87             | 67.56          | −0.254 |
|           | Actinidia31474        | AcEXLB4   | 256                  | 28,035.36        | 5.13           | 36.77             | 71.99          | −0.186 |

2.2. Physicochemical Properties of AcExpansin Protein Family

We further analysed the physicochemical properties of the 41 AcExpansins. As shown in Table 1, the length of the AcExpansin proteins ranged from 193 to 783 aa. The molecular weight and theoretical pI of the identified AcExpansin proteins ranged from 20.73 kDa to 86.02 kDa and 4.73 to 10.16, respectively. The average isoelectric point was 8.37 and 80% of the expansin proteins had isoelectric points greater than 8.00, indicating that most of the expansin proteins were alkaline. The expansin protein instability index was 22.97–46.03. The lipid solubility index of expansin proteins was 57.67–83.11, with an average value of 70.66. Among them, the lipid solubility index of five AcExpansin proteins exceeded 80.00, indicating that they belonged to the class of thermophilic proteins. The total average hydrophobicity of the 41 AcExpansin genes was −0.441–0.131, indicating they belonged to amphiprotic proteins with comparable hydrophobicity (>0 for hydrophobicity, <0 for hydrophilicity, and ±0.5 for amphiprotic proteins).

2.3. AcExpansin Protein Family Chromosomal Location

The kiwifruit V3 genome contains 29 chromosomes. To determine the genomic distribution, physical location analysis of 41 expansin genes was performed using the online tool MG2C. Our results showed that 38 AcExpansin genes were unevenly distributed on 22 chromosomes, whereas 3 members of the AcExpansin protein family were attributed to chromosomes that were undetermined (Figure 2). Among the 38 AcExpansin genes, chromosome 3 contained 4 genes; chromosomes 9, 22, and 25 contained 3 genes each; chromosomes 1, 4, 8, 12, 19, 21, and 23 contained 2 genes each; and the other chromosomes contained 1 gene each.

2.4. Analysis of Conserved Domains and Gene Structure of the AcExpansin Protein Family

In total, eight conserved motifs (named motif 1–8) in AcExpansin proteins were identified using the TBTools software (v.1098696) (Figure 3A). Figure 3 shows that genes from the same subfamily have similar motifs, indicating structural similarities between genes in the same group. Except for motif 8, the other motifs were widely distributed in the AcEXPB subfamily. Motif 2 was only present in the AcEXPA subfamily. Motif 8 was unique to the AcEXPB, AcEXLA, and AcEXLB subfamilies.
Figure 2. Schematic representations of the chromosomal distributions of the kiwifruit expansin genes. Centromeric positions are shown according to location of each AcExpansin.
The gene structure of the 41 AcExpansin genes was analysed using TBtools and genomic DNA sequences (Figure 3B). Most of the AcEXPA genes included three exons. The number of exons in AcEXPB genes was either three or four. The exon number of AcEXLB genes was either four or five. The exon numbers of the three AcEXLA genes were very different. AcEXLA1, AcEXLA2, and AcEXLA3 contained 11, 5, and 13 exons, respectively.

2.5. Intraspecies Collinearity Analysis of Expansion Genes in Kiwifruit

Tandem and segmental duplication events were identified to investigate gene duplication events. As a result, 43 gene pairs were generated from the 34 segmental duplicated genes (Figure 4). Most segmental duplication genes were found in the EXPA subfamily (AcEXPA5, AcEXPA6, AcEXPA9, AcEXPA11, AcEXPA14, AcEXPA16, AcEXPA18, AcEXPA21, AcEXPA22, AcEXPA23, AcEXPA24, and AcEXPA28). Two tandem duplicated genes were identified, forming one pair (Figure 4).
Figure 4. Analysis of collinearity of the expansin genes from kiwifruit. Gray lines in the background indicate collinear blocks within the kiwifruit genome and the lines with different colours highlight syntenic expansin gene pairs.

2.6. Screening of Candidate Expansin gene under BR and Brassinazole Treatment

Based on our previous transcriptome sequencing data (Submission ID: SUB9537634, BioProject ID: PRJNA726005), a heatmap was constructed to analyse the expression patterns of the AcExpansin protein family members with BR and brassinazole treatment (Figure 5A). The results indicated that genes that were upregulated were mainly from the AcEXPA sub-family. Three AcExpansin genes (AcEXPA14, AcEXPA18, and AcEXPA23) were markedly induced by BR and were markedly reduced by brassinazole treatment. We further performed qRT-PCR assays for these three AcExpansin genes, among which AcEXPA23 had the highest expression level of 78-fold (Figure 5B). Therefore, we selected AcEXPA23 as a candidate gene to further explore its function in lateral root development.
Figure 5. Screening of the candidate gene AcEXPA23. (A) Heatmap analysis of the expansin protein family genes in kiwifruit. (B) Fluorescence quantitative expression of AcEXPA14, AcEXPA18, and AcEXPA23. Asterisks indicate significant differences among treatments (n = 3, Fisher’s LSD, **p ≤ 0.05, ***p ≤ 0.001).

2.7. Subcellular Localisation of AcEXPA23

To determine the subcellular localisation of AcEXPA23, we fused the terminator-removed CDS of AcEXPA23 to green fluorescent protein (GFP) under the control of the CaMV35S constitutive promoter. Using a polyethylene glycol-mediated procedure, 35S::AcEXPA23::GFP fusion proteins and 35S::GFP (Control) were transferred into Arabidopsis protoplasts. The results showed that control 35S::GFP was distributed throughout the whole cell, whereas 35S::AcEXPA23::GFP was detected in the cytoplasm of the Arabidopsis protoplasts (Figure 6).

Figure 6. Subcellular localisation of AcEXPA23. Vector control (35S::GFP) and fusion protein construct 35S::AcEXPA23::GFP were introduced into the Arabidopsis protoplast.
2.8. Transient Overexpression of AcEXPA23 in Kiwifruit

To investigate the role of AcEXPA23 in lateral root development, transient overexpression was performed in kiwifruit by hairy root infection technology. Laser confocal microscopy was used and a PCR assay was performed to identify positive seedlings (Figure 7A,B). Seedlings in which the fluorescence signal of hairy roots could be observed and amplified from the eGFP sequence fragment were considered as positive plants. Compared with the control, plant overexpression AcEXPA23 increased by 2.2-times in the number of lateral roots in the hairy roots (Figure 7C,D). The results indicated that AcEXPA23 plays an important role in the lateral root development of kiwifruit.

2.9. AcEXPA23 Overexpression in Kiwifruit Enhanced the Number of Lateral Roots

To confirm the roles of AcEXPA23 in kiwifruit, we obtained AcEXPA23-overexpressing plants of kiwifruit by transforming explants produced from leaf strips. Similar to transient overexpression, seedlings in which the fluorescence signal of hairy roots was observed and amplified from the eGFP sequence fragment were considered as positive plants (Figure 8A,B). Finally, two overexpression lines were obtained. We observed that overexpression of AcEXPA23 significantly increased the number of lateral roots compared with that in wild-type kiwifruit (Figure 8C). The number of lateral roots of both Line 1 and Line 2 was 2.45-times higher than that of the wild-type seedlings (Figure 8D). The expression levels of AcEXPA23 in Line 1 and Line 2 were 17- and 63-times that in wild-type plants, respectively (Figure 8E). Therefore, we conclude that AcEXPA23 plays an important role in regulating lateral root development in kiwifruit.
3. Discussion

The expansin gene is widespread in plants, ranging from algae to higher plants, indicating that the expansin protein family is important for the growth and development of plants [40]. Evolutionary analysis of expansins can provide valuable insights into the regulation of important agronomic traits in kiwifruit genetics and breeding. Our study identified 41 expansin genes in kiwifruit containing two conserved domains, double psi-beta barrel and pollen allergen domains, which are characteristic of other expansins identified to date [41].

In kiwifruit, out of the 41 expansins, the number of AcEXPA was dominant compared to the other expansin categories (AcEXPB; AcEXLA and AcEXLB), in line with findings of earlier studies in other species [42–50]. Segmental and tandem duplications have been reported to be two of the main causes of gene family expansion in plants [51]. This conclusion is further supported by the finding that most members of the AcExpansin subfamily undergo tandem and segmental duplications. Genes that undergo duplication have three evolutionary outcomes: maintaining the original conserved function, generating new functions, or forming pseudogenes [52]. AcEXPA1–AcEXPA16 are a pair of tandem duplicated genes located on chromosome 3, which are from the EXPA subfamily (Figures 1, 2 and 4). The expression of AcEXPA1 and AcEXPA16 was obviously different (Figure 5), indicating that they may have evolved into two genes with different functions. Furthermore, the motif compositions of these two genes were found to be consistent (Figure 3), suggesting that the difference in function may originate from the difference in cis-acting elements in the promotor region.

The expression of expansin genes is not only regulated by plant development processes but is also affected by plant hormones. For example, BR induces elongation of excised epicotyl segments and the levels of all EXPA transcripts increased significantly in *Cicer arietinum* [53]. BR promotes *AtEXP A5* expression and positively affects root cell elongation [54]. The transcript level of *EXP1* was upregulated in response to BR treatment in sweet potato [55]. Previously, with heat mapping and qRT-PCR analysis, we also found that BR treatment upregulates *AcEXP A23* expression in kiwifruit roots (Figure 5A) [39]. We analysed the interaction network of *AcEXP A23* using STRING based on AcExpansin ortho-
logues in Arabidopsis. This could help us to understand gene function and efficiency [56]. The similarity between AcEXP A23 and AtEXP A14 was 83.2%. Studies showed that overexpression of AtEXP A14 in Arabidopsis stimulated the formation of emerged lateral roots, whereas loss of function of AtEXP A14 reduced auxin-stimulated lateral root formation [18]. We speculate that the AcEXP A23 gene plays an important role in the regulation of lateral root development.

In this study, transformation experiments demonstrated that overexpression of AcEXP A23 could significantly enhance the number of lateral roots in kiwifruit (Figures 7 and 8), which was in line with findings of previous studies [18,57–60]. A recent study on maize yield found that 48% of the yield gain was associated with a decadal climate trend, 39% with agronomic improvements, and only 13% with improvement in genetic yield potential [61]. These findings differed from those of most previous studies, which attributed a much greater weight to genetic yield potential improvement. The continuous activities of human beings have gradually intensified climate change, and it has become more and more important to improve the adaptability of plants [62–64]. The present study shows that overexpression of AcEXP A23 promotes the development of increased lateral roots in kiwifruit. Therefore, BR can be developed into a root regulation product as an improved agronomic measure to improve the root system of kiwifruit, improve the utilisation of water and nutrients, and improve yield and quality.

Expansins are cell-wall-loosening proteins. Most studies showed that expansins are localized in the cell wall, such as EXPB2 from rice [65], EXPB2 from Heterodera avenae [66], and EXP A4 from Chrysanthemum morifolium [67]. However, some expansins are also reportedly localized to the plasma membrane. For example, the subcellular localisation of epidermal cells in tobacco and onions revealed that EXPB7 was localised in the plasma membrane of Hordeum vulgare [68], EXP A1 was located in the plasma membrane [69], EXLA2 was also located in the plasma membrane of tobacco [70]. In the present study, the 35S::AcEXP A23:GFP fusion protein was transiently expressed in Arabidopsis protoplasts and the results showed that the protein was located in the cytoplasm (Figure 6), which may be related to the specific functions of expansins.

4. Materials and Methods

4.1. Identification of Kiwifruit Expansin Genes

We identified the expansin protein family genes in kiwifruit using the following four steps. First, the hidden Markov model (HMM) of the two characteristic domains of the expansin gene, pfam01357 (Pollen_allerg_1) and pfam03330 (DPBB_1), from the Pfam database (http://pfam.xfam.org/ (accessed on 4 January 2022)) was downloaded. The expansin protein family genes of kiwifruit were then identified using the Simple HMM Search function in the TBtools software [71]. Second, the Arabidopsis expansin protein family protein sequences were downloaded from the TAIR website (www.arabidopsis.org (accessed on 5 January 2022)). The expansin protein family genes of kiwifruit were identified using the Blast Compare Two Seqs function in the TBtools software [71]. Third, the intersection of the genes obtained in the above two steps was considered. Finally, according to the two characteristic domains of the expansin gene, pfam01357 (Pollen_allerg_1) and pfam03330 (DPBB_1), the final screening was performed on the website of InterPro (https://www.ebi.ac.uk/interpro/ (accessed on 6 January 2022)). In addition, the kiwifruit genome protein data used in the first and second identification processes were downloaded from the kiwifruit genome database (http://kiwifruitgenome.org (accessed on 4 January 2022)).

4.2. Analysis of AcExpansin Protein Family Characteristics

ClustalW in MEGA11 was used to align the relatedness of AcExpansin protein family gene sequences and a phylogenetic tree was constructed using the neighbour-joining method in MEGA11 (related parameter settings: bootstrap, 1000; model/method, p-distance; gaps/missing data treatment, partial deletion) [72]. Further adjustments and annotations to the evolutionary tree were performed using EVOLVIEW (http://www.
Chromosome localisation maps were generated online using MG2C (http://mg2c.iask.in/mg2c_v2.1/ (accessed on 21 January 2022)). Motif and gene structures were analysed and visualised using the TBtools software [71]. The physicochemical properties of the proteins were analysed using the online tool Expasy (https://www.expasy.org/ (accessed on 19 January 2022)). Intraspecies collinearity analysis was performed and visualised using the TBtools software [71].

4.3. Transient Expression Hairy Root

We performed the following steps for instantaneous conversion. The AcEXP A23 ORF was first cloned into the pART-CAM-EGFP vector under the control of the CaMV 35S promoter (Table S2). The recombinant vector was then transformed into K599 Agrobacterium. The K599 Agrobacterium containing the target gene was shaken to make the OD_{600} value reach 0.5–0.8 and the volume of the bacterial solution was approximately 10 mL. The bacterial solution was then centrifuged twice at 8000 rpm for 10 min each and then resuspended in MES buffer (10 mmol/L MES-KOH, pH = 5.2, 10 mmol/L MgCl_2 and 10 µmol/L acetosyringone). The wild-type kiwifruit (rooted tissue culture seedlings) in good growth condition was selected, and 100–150 µL of bacterial liquid was injected into the young stem with a 0.5 mL syringe, and the infested area is wrapped with degreasing cotton then and placed into the soil. Eighteen plants were used per treatment. After two weeks, the degreasing cotton was removed. Over time, the plants continued to grow. To prevent growing roots from being exposed, they were buried with vermiculite. When hairy roots grew at the infected site and their length was more than 2 cm, the original root system below it was cut off to facilitate rapid growth, and the leaves on the shoots were removed to facilitate survival.

4.4. Transformation of A. Chinensis Leaves

The AcEXP A23 ORF was cloned into the pART-CAM-EGFP vector driven by the CaMV 35S promoter and the recombinant plasmid was subsequently transformed into A. chinensis leaves, according to the protocol outlined by Wang [73] (Table S2). Transgenic plants were obtained after approximately 6 months. Transformed plants were identified using PCR and qRT-PCR for the successful verification of transgene incorporation. Two transgenic kiwifruit lines with high AcEXP A23 mRNA expression were selected for morphological analysis.

4.5. Subcellular Localisation

The AcEXP A23 ORF with a mutated stop codon was cloned between the XbaI and SalI sites of the pBI221-GFP vector using T4 DNA ligase (Thermo Scientific, Waltham, MA, USA) (Table S2). The recombinant and control plasmids were transformed into Arabidopsis leaf protoplasts as described previously [74]. After 18 h, GFP fluorescence was observed under a laser scanning confocal microscope (FV1000 viewer; Olympus, Tokyo, Japan) at 488 nm with argon-ion laser excitation and GFP was detected at 507 nm. Chloroplast autofluorescence was analysed using 488 nm argon-ion laser excitation, SP 630 nm IR detection, a pinhole of approximately 1.0 units, and an optical section thickness of approximately 0.5 µm.

4.6. Quantitative Real-Time Polymerase Chain Reaction

Primer Premier 5 software was used to design qRT–PCR primers for target genes (the primers used are listed in Table S2). The RNA extraction method and qRT-PCR were performed as described by Wu [39]. Actin (GenBank EF063572) was used as the normalised control gene [75] (Table S2). Three biological replicates were analysed. The relative expression was calculated using the 2^{–ΔΔCt} method [76].

4.7. Statistical Analysis

Excel 2010 (Microsoft Corporation, Redmond, WA, USA), IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA) and Origin 2021 (OriginLab Corporation, Northampton,
MA, USA) were used for statistical analyses of the data. Differences between treatments were determined using ANOVA and mean comparisons were made using Fisher’s least significant LSD.

5. Conclusions

We identified and analysed the AcExpansin gene family using bioinformatic methods. As such, 41 expansin genes were identified from kiwifruit and classified into four subfamilies, including AcEXPA, AcEXPB, AcEXLA, and AcEXLB. These genes were further analysed for physicochemical properties, chromosomal location, conserved domains, gene structure, and intraspecies collinearity. We found that 41 expansin genes were evolutionarily diverse and conserved at the DNA and protein levels. Finally, we used genetic transformation technology in the kiwifruit to demonstrate that overexpression of AcEXPA23 can promote the development of increased lateral roots in kiwifruit. This is of great significance for promoting the absorption of water and nutrients in kiwifruit to improve yield and quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23148026/s1.

Author Contributions: X.Q. and J.C. conceived the research. Z.W. and M.L. performed the experiments, analysed the data, and wrote the manuscript. Y.Z., L.L., D.C., H.G. and X.G. provided scientific suggestions. Z.W., M.L., X.Q. and J.C. revised the manuscript. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the earmarked fund for CARS (CARS-26); the Technical System of Bulk Fruit Industry in Henan Province (HARS-22-09-S); the Modern Agricultural Industrial Technology System of Henan Province (Grant No. S2014-11); the Agricultural Science and Technology Innovation Program, Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2022-ZFRI-03); the Scientific and Technological Research in Henan Province (Grant No. 212102110431); the National Natural Science Foundation of China (32102325); the Academician (expert) Workstation project of Yunnan Province (2021YFD1200202-08); and the Rescue protection of rare and endangered kiwifruit germplasm resources (2021YFD1200202-08).

Institutional Review Board Statement: The research content of the manuscript does not involve ethical issues.

Informed Consent Statement: The research content of the manuscript does not involve humans.

Data Availability Statement: The original data for the RNA-seq data were submitted to the SRA database (Submission ID: SUB9537634, BioProject ID: PRJNA726005).

References
1. Fang, J.B.; Zhong, C.H. Fruit scientific research in new China in the past 70 years: Kiwifruit. J. Fruit Sci. 2019, 36, 1352–1359.
2. Xu, X.B.; Zhang, Q.M. Research and utilizations of germplasm resource of kiwifruit in china. Chin. Bull. Bot. 2003, 20, 648–655.
3. Iwasawa, H.; Morita, E.; Yui, S.; Yamazaki, M. Anti-oxidant effects of kiwi fruit in vitro and in vivo. Biol. Pharm. Bull. 2011, 34, 128–134. [CrossRef]
4. He, J.L.; Wu, D.T.; Zhang, Q.; Chen, H.; Li, H.Y.; Han, Q.H.; Lai, X.Y.; Wang, H.; Wu, Y.X.; Yuan, J.G.; et al. Efficacy and mechanism of cinnamon essential oil on inhibition of colletotrichum acutatum isolated from ‘hongyang’ kiwifruit. Front. Microbiol. 2018, 9, 1288. [CrossRef]
5. Qi, X.J.; Guo, D.D.; Wang, R.; Zhong, Y.P.; Fang, J.B. Development status and suggestions on Chinese kiwifruit industry. J. Fruit Sci. 2020, 37, 754–763.
6. Chen, J.Y.; Fang, J.B.; Qi, X.J.; Gu, H.; Lin, M.M.; Zhang, W.Y.; Wei, C.G. Research progress on rootstock of kiwifruit. J. Fruit Sci. 2015, 32, 959–968.
7. Lynch, J. Root architecture and plant productivity. Plant Physiol. 1995, 109, 7–13. [CrossRef]
8. Malamy, J.E.; Benfey, P.N. Organization and cell differentiation in lateral roots of Arabidopsis thaliana. Development 1997, 124, 33–44. [CrossRef]
9. Peret, B.; De Rybel, B.; Casimiro, I.; Benkova, E.; Swarup, R.; Lapinte, L.; Beeckman, T.; Bennett, M.J. Arabidopsis lateral root development: An emerging story. *Trends Plant Sci.* **2009**, *14*, 399–408. [CrossRef]

10. De Rybel, B.; Vassileva, V.; Parizot, B.; Demeulemaere, M.; Grunewald, W.; Audenaert, D.; Van Campenhout, J.; Overvoorde, P.; Jansen, L.; Vanneste, S.; et al. A novel AUX/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. *Curr. Biol.* **2010**, *20*, 1697–1706. [CrossRef]

11. Fukushima, Y.; Tameda, S.; Masuda, H.; Tasaka, M. Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. *Plant J.* **2002**, *29*, 153–168. [CrossRef]

12. Fukushima, H.; Nakao, Y.; Okushima, Y.; Theologis, A.; Tasaka, M. Tissue-specific expression of stabilized SOLITARY-ROOT/IAA14 alters lateral root development in Arabidopsis. *Plant J.* **2005**, *44*, 382–395. [CrossRef]

13. Okushima, Y.; Overvoorde, P.J.; Arima, K.; Alonso, J.M.; Chan, A.; Chang, C.; Ecker, J.R.; Hughes, B.; Lui, A.; Nguyen, D.; et al. Functional genomic analysis of the AUXIN RESPONSE FACTOR gene family members in Arabidopsis thaliana: Unique and overlapping functions of ARF7 and ARF19. *Plant Cell* **2005**, *17*, 444–463. [CrossRef]

14. Okushima, Y.; Fukushima, H.; Onoda, M.; Theologis, A.; Tasaka, M. ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in Arabidopsis. *Plant Cell* **2007**, *19*, 118–130. [CrossRef]

15. De Smet, I.; Lau, S.; Voss, U.; Vanneste, S.; Benjamins, R.; Rademacher, E.H.; Schlereth, A.; De Rybel, B.; Vassileva, V.; Grunewald, W.; et al. Bimodular auxin response controls organogenesis in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 2705–2710. [CrossRef]

16. Smet, I.D. Multimodular auxin response controls lateral root development in Arabidopsis. *Plant Signal. Behav.* **2010**, *5*, 580–582. [CrossRef]

17. Goh, T.; Kasahara, H.; Mimura, T.; Kamiya, Y.; Fukushima, H. Multiple AUX/IAA-ARF modules regulate lateral root formation: The role of Arabidopsis SHY2/IAA3-mediated auxin signalling. *Philos. Trans. R. Soc. B Biol. Sci.* **2012**, *367*, 1461–1468. [CrossRef]

18. Lee, H.W.; Kim, M.J.; Kim, N.Y.; Lee, S.H.; Kim, J. LBD18 acts as a transcriptional activator that directly binds to the EXPANSIN14 promoter in promoting lateral root emergence of Arabidopsis. *Plant J.* **2013**, *73*, 212–224. [CrossRef]

19. Lee, D. Genome-wide analysis of the transcriptome downstream of iaa1 during early auxin response and expression analysis of iaa1-regulated auxin-response genes. *J. Exp. Bot.* **2009**, *60*, 3935–3957. [CrossRef]

20. Lee, H.W.; Kim, N.Y.; Lee, D.J.; Kim, J. LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in Arabidopsis. *Plant Physiol.* **2009**, *151*, 1377–1389. [CrossRef]

21. McQueen-Mason, S.J.; Cosgrove, D.J. Expansin mode of action on cell walls (analysis of wall hydrolysis, stress relaxation, and binding). *Plant Physiol.* **1995**, *107*, 87–100. [CrossRef]

22. 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. [CrossRef]

23. McQueen-Mason, S.; Cosgrove, D.J. Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 6574–6578. [CrossRef]

24. Kende, H.; Bradford, K.; Brumnell, D.A.; Cho, H.T.; Cosgrove, D.J.; Fleming, A.J.; Gehring, C.; Lee, Y.; McQueen-Mason, S.; Rose, J.K. Nomenclature for members of the expansin superfamily of genes and proteins. *Plant Mol. Biol.* **2004**, *55*, 311–314. [CrossRef]

25. Che, J.; Yamaji, N.; Shen, R.F.; Ma, J.F. An Al-inducible expansin gene, OsEXPA10 is involved in root cell elongation of rice. *Plant J.* **2016**, *88*, 132–142. [CrossRef]

26. Cho, H.T.; Cosgrove, D.J. Regulation of root hair initiation and expansin gene expression in Arabidopsis. *Plant Cell* **2002**, *14*, 3237–3253. [CrossRef]

27. Cho, H.T.; Kende, H. Expression of expansin genes is correlated with growth in deepwater rice. *Plant Cell* **1997**, *9*, 1661–1671. [CrossRef]

28. Goh, H.H.; Sloan, J.; Dorca-Fornell, C.; Fleming, A. Inducible repression of multiple expansin genes leads to growth suppression during leaf development. *Plant Physiol.* **2012**, *159*, 1759–1770. [CrossRef]

29. Kuluev, B.R.; KnyaZEv, A.V.; Mikhaylova, E.V.; Chemeris, A.V. The role of expansin genes PtrEXPA3 and PnEXPA3 in the regulation of leaf growth in poplar. *Russ. J. Genet.* **2017**, *53*, 651–660. [CrossRef]

30. Saito, T.; Pham Anh, T.; Katsumi-Horigane, A.; Bai, S.; Ito, A.; Sekiyama, Y.; Ono, H.; Moriguchi, T. Development of flower buds (*Pyrus pyrifolia*) from late autumn to early spring. *Tree Physiol.* **2015**, *35*, 653–662. [CrossRef]

31. Civello, P.M.; Powell, A.L.T.; Sabateh, A.; Bennett, A.B. An expansin gene expressed in ripening strawberry fruit. *Plant Physiol.* **1999**, *121*, 1273–1279. [CrossRef]

32. Chen, Y.; Han, Y.; Zhang, M.; Zhou, S.; Kong, X.; Wang, W. Overexpression of the wheat expansin gene TaEXP2 improved seed production and drought tolerance in transgenic tobacco plants. *PloS ONE* **2016**, *11*, e0153494. [CrossRef]

33. Wei, P.; Chen, S.; Zhang, X.; Zhao, P.; Xiong, Y.; Wang, W.; Chen, J.; Wang, X. An alpha-expansin, VfEXP1A, is involved in regulation of stomatal movement in *Vicia faba* L. *Chin. Sci. Bull.* **2011**, *56*, 3531–3537. [CrossRef]

34. 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]

35. 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]

36. Wu, Y.J.; Thorne, E.T.; Sharp, R.E.; Cosgrove, D.J. Modification of expansin transcript levels in the maize primary root at low water potentials. *Plant Physiol.* **2001**, *126*, 1471–1479. [CrossRef]
37. Shao, Y.; Feng, X.H.; Nakahara, H.; Irshad, M.; Eneji, A.E.; Zheng, Y.; Ping, A. Apical-root apoplastic acidification affects cell wall extensibility in wheat under salinity stress. *Physiol. Plant.* 2021, 173, 1850–1861. [CrossRef]

38. Chen, Y.; Han, Y.; Kong, X.; Kang, H.; Ren, Y.; Wang, W. Ectopic expression of wheat expansin gene TaEXP2A improved the salt tolerance of transgenic tobacco by regulating Na\(^{+}\)/K\(^{+}\) and antioxidant competence. *Physiol. Plant.* 2017, 159, 161–177. [CrossRef]

39. Wu, X.; Gu, S.; Gu, H.; Cheng, D.; Li, L.; Guo, X.; Wang, M.; He, S.; Li, M.; Chen, J. Physiological and transcriptomic analyses of brassinosteroid function in kiwifruit root. *Environ. Exp. Bot.* 2022, 194, 104685. [CrossRef]

40. Sun, W.; Yu, H.; Liu, M.; Ma, Z.; Chen, H. Evolutionary research on the expansin protein family during the plant transition to land provides new insights into the development of Tarryt buckwheat fruit. *BMC Genom.* 2021, 22, 252. [CrossRef]

41. Bordoloi, K.S.; Dihingia, P.; Krishnatreya, D.B.; Agarwala, N. Genome-wide identification, characterization and expression analysis of the expansin gene family under drought stress in tea (*Camellia sinensis* L.). *Plant Sci.* 2021, 8, 32–44. [CrossRef]

42. Han, Z.; Liu, Y.; Deng, X.; Liu, D.; Liu, Y.; Hu, Y.; Yan, Y. Genome-wide identification and expression analysis of expansin gene family in common wheat (*Triticum aestivum* L.). *BMC Genom.* 2019, 20, 101. [CrossRef]

43. Lee, Y.; Choi, D.; Kende, H. Expansins: Ever-expanding numbers and functions. *Curr. Opin. Plant Biol.* 2001, 4, 527–532. [CrossRef]

44. 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]

45. 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]

46. Zhu, Y.; Wu, N.; Song, W.; Yin, G.; Qin, Y.; Yan, Y.; Hu, Y. 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]

47. 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]

48. Lu, Y.; Liu, L.; Wang, X.; Han, Z.; Ouyang, B.; Zhang, J.; Li, H. Genome-wide identification and expression analysis of the expansin gene family in tomato. *Mol. Genet. Genom.* 2016, 291, 597–608. [CrossRef]

49. Zhang, S.; Xu, R.; Gao, Z.; Chen, C.; Jiang, Z.; Shu, H. A genome-wide analysis of the expansin genes in *Malus* *x* *Domestica*. *Mol. Genet. Genom.* 2014, 289, 225–236. [CrossRef]

50. Dal Santo, S.; Vannozzi, A.; Torrelli, 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]

51. Qiao, X.; Li, Q.; Yin, H.; Qi, K.; Li, L.; Wang, R.; Zhang, S.; Paterson, A.H. Gene duplication and evolution in recurring polyploidization-diploidization cycles in plants. *Genome Biol.* 2019, 20, 38. [CrossRef]

52. Nan, Q.; Qian, D.; Niu, Y.; Qi, K.; Li, L.; Wang, R.; Zhang, S.; Paterson, A.H. Gene duplication and evolution in recurring polyploidization-diploidization cycles in plants. *Genome Biol.* 2019, 20, 38. [CrossRef]

53. Sanchez, M.A.; Mateos, I.; Labrador, E.; Dopico, B. Brassinolides and IAA induce the transcription of *four a-expansin* genes related to development in *Cicer arietinum*. *Plant Physiol. Biochem.* 2004, 42, 709–716. [CrossRef]

54. Park, C.H.; Kim, T.W.; Son, S.H.; Hwang, J.Y.; Lee, S.C.; Chang, S.C.; Kim, S.H.; Kim, S.W.; Kim, S.K. Brassinosteroids control *Brassinosteroid* function in kiwifruit root. *Front. Plant Sci.* 2019, 107, 252. [CrossRef]

55. Bae, J.M.; Kwak, M.S.; Noh, S.A.; Oh, M.J.; Kim, Y.S.; Shin, J.S. Overexpression of sweetpotato expansin cDNA (*IpEXP1*) increases seed yield in *Arabidopsis*. *Transgenic Res.* 2019, 28, 395–408. [CrossRef]

56. Zhao, F.; Li, G.; Hu, P.; Zhao, X.; Li, L.; Wei, W.; Feng, J.; Zhou, H. Identification of basic/ helix-loop-helix transcription factors reveals candidate genes involved in anthocyanin biosynthesis from the strawberry white-flesh mutant. *Sci. Rep.* 2018, 8, 2721. [CrossRef]

57. Lee, H.W.; Kim, J. EXPANSINA17 up-regulated by *LBD18/ASL20* promotes lateral root formation during the auxin response. *Plant Cell Physiol.* 2013, 54, 1600–1613. [CrossRef]

58. Li, A.X.; Han, Y.Y.; Wang, X.; Chen, Y.H.; Zhao, M.R.; Zhou, S.-M.; Wang, W. Root-specific expression of wheat expansin gene TaEXP2B3 enhances root growth and water stress tolerance in tobacco. *Environ. Exp. Bot.* 2015, 110, 73–84. [CrossRef]

59. Kong, Y.; Wang, B.; Du, H.; Li, W.; Li, X.; Zhang, C. *GmEXLB1*, a soybean expansin-like B gene, alters root architecture to improve phosphorus absorption in *Arabidopsis*. *Plant Cell Physiol.* 2019, 10, 808. [CrossRef]

60. Yang, Z.; Gao, Z.; Zhou, H.; He, Y.; Liu, Y.; Lai, Y.; Zheng, J.; Li, X.; Liao, H. *GmPTF1* modifies root architecture responses to phosphate starvation primarily through regulating *GmEXP2* expression in soybean. *Plant J.* 2021, 107, 525–543. [CrossRef]

61. Cortes, A.J.; Blair, M.W. Genotyping by sequencing and genome-environment associations in wild common bean predict widespread divergent adaptation to drought. *Front. Plant Sci.* 2018, 9, 128. [CrossRef]

62. Wu, X.; Islam, A.S.M.F.; Limpot, N.; Mackasmiel, L.; Mierzwia, J.; Cortes, A.J.; Blair, M.W. Genome-wide SNP identification and association mapping for seed mineral concentration in mung bean (*Vigna radiata* L.). *Front. Genet.* 2020, 11, 656. [CrossRef]

63. Buitrago-Bitar, M.A.; Cortes, A.J.; Lopez-Hernandez, F.; Londono-Caicedo, J.M.; Munoz-Florez, J.E.; Munoz, L.C.; Blair, M.W. Allelic diversity at abiotic stress responsive genes in relationship to ecological drought indices for cultivated tepary bean, *Phaseolus acutifolius* A. gray, and its wild relatives. *Genes* 2021, 12, 556. [CrossRef]

64. Rizzo, G.; Monzon, J.P.; Tenorio, F.A.; Howard, R.; Cassman, K.G.; Grassini, P. Climate and agronomy, not genetics, underpin recent maize yield gains in favorable environments. *Proc. Natl. Acad. Sci. USA* 2022, 119, e2113629119. [CrossRef]
65. Zou, H.; Wenwen, Y.; Zang, G.; Kang, Z.; Zhang, Z.; Huang, J.; Wang, G. OsEXPB2, a β-expansin gene, is involved in rice root system architecture. *Mol. Breed.* 2015, 35, 1–14. [CrossRef]

66. Liu, J.; Peng, H.; Cui, J.; Huang, W.; Kong, L.; Clarke, J.L.; Jian, H.; Wang, G.L.; Peng, D. Molecular characterization of a novel effector expansin-like protein from heterodera avenae that induces cell death in *Nicotiana benthamiana*. *Sci. Rep.* 2016, 6, 35677. [CrossRef]

67. Ren, H.; Wen, L.Z.; Guo, Y.H.; Yu, Y.Y.; Sun, C.H.; Fan, H.M.; Ma, F.F.; Zheng, C.S. Expressional and functional verification of the involvement of CmEXP A4 in chrysanthemum root development. *J. Plant Growth Regul.* 2019, 38, 1375–1386. [CrossRef]

68. He, X.; Zeng, J.; Cao, F.; Ahmed, I.M.; Zhang, G.; Vincze, E.; Wu, F. HvEXP B7, a novel β-expansin gene revealed by the root hair transcriptome of Tibetan wild barley, improves root hair growth under drought stress. *J. Exp. Bot.* 2015, 66, 7405–7419. [CrossRef]

69. Liu, W.; Feng, X.; Chen, Z.H.; Zhang, G.; Wu, F. Transient silencing of an expansin HvEXP A1 inhibits root cell elongation and reduces Al accumulation in root cell wall of Tibetan wild barley. *Environ. Exp. Bot.* 2019, 165, 120–128. [CrossRef]

70. He, X.; Zeng, J.; Cao, F.; Ahmed, I.M.; Zhang, G.; Vincze, E.; Wu, F. HvEXP B7, a novel β-expansin gene revealed by the root hair transcriptome of Tibetan wild barley, improves root hair growth under drought stress. *J. Exp. Bot.* 2015, 66, 7405–7419. [CrossRef]

71. 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. Biol. Evol.* 2021, 38, 3022–3027. [CrossRef]

72. Tamura, K.; Stecher, G.; Kumar, S. MEGA11 molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 2021, 38, 3022–3027. [CrossRef]

73. Wang, T.; Atkinson, R.; Janssen, B. The choice of agrobacterium strain for transformation of kiwifruit. *Acta Hortic.* 2007, 753, 227–232. [CrossRef]

74. Wang, N.; Zhang, W.; Qin, M.; Li, S.; Qiao, M.; Liu, Z.; Xiang, F. Drought tolerance conferred in soybean (*Glycine max.* L) by GmMYB84, a novel R2R3-MYB transcription factor. *Plant Cell Physiol.* 2017, 58, 1764–1776. [CrossRef]

75. Li, M.; Ma, F.; Liang, D.; Li, J.; Wang, Y. Ascorbate biosynthesis during early fruit development is the main reason for its accumulation in kiwi. *PLoS ONE* 2010, 5, e14281. [CrossRef]

76. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods* 2001, 25, 402–408. [CrossRef]