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ARTICLE

Morphological characteristics, anatomical structure, and gene expression: novel insights into gibberellin biosynthesis and perception during carrot growth and development

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Gibberellins (GAs) are considered potentially important regulators of cell elongation and expansion in plants. Carrot undergoes significant alteration in organ size during its growth and development. However, the molecular mechanisms underlying gibberellin accumulation and perception during carrot growth and development remain unclear. In this study, five stages of carrot growth and development were investigated using morphological and anatomical structural techniques. Gibberellin levels in leaf, petiole, and taproot tissues were also investigated for all five stages. Gibberellin levels in the roots initially increased and then decreased, but these levels were lower than those in the petioles and leaves. Genes involved in gibberellin biosynthesis and signaling were identified from the carrotDB, and their expression was analyzed. All of the genes were evidently responsive to carrot growth and development, and some of them showed tissue-specific expression. The results suggested that gibberellin level may play a vital role in carrot elongation and expansion. The relative transcription levels of gibberellin pathway-related genes may be the main cause of the different bioactive GAs levels, thus exerting influences on gibberellin perception and signals. Carrot growth and development may be regulated by modification of the genes involved in gibberellin biosynthesis, catabolism, and perception.

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INTRODUCTION

Plant growth and development is a complex process that involves differentiation and morphogenesis. Hormonal regulation is recognized as a key process in plant growth and development[1]. For example, gibberellins (GAs) are phytohormones that play essential roles in plant growth and developmental stages, including seed germination, flowering, sex expression, and leaf and fruit senescence[2,3]. It has been reported that GA-mediated plant growth is mainly achieved by promoting cell elongation in plants[4]. Further studies indicate that GAs also regulate cell production to control plant growth[5]. Inadvertently, GA was first discovered in the fungus *Gibberella fujikuroi*[6]. To date, more than 100 GAs have been identified in plants, fungi, and bacteria; however, only a few GAs exhibit biological activities. GA_1, GA_3, GA_4, and GA_7 are the major bioactive GAs; other GAs are non-bioactive and act as precursors of the bioactive forms[7]. To understand the roles of GAs in plant growth and development, researchers should investigate the regulation and response of GAs in plants.

In higher plants, GAs originate from geranylgeranyl diphosphate (GGPP), which is synthesized from isopentenyl pyrophosphate (IPP)[8]. GGPP is then converted into *ent*-kaurene by *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS). *Ent*-kaurene is oxidized to GA_{12}-aldehyde, a precursor of all GAs. Finally, GA_{12}-aldehyde is then transformed into various GAs in a process catalyzed by GA20-oxidase (GA20ox), GA3-oxidase (GA3ox), GA2-oxidase (GA2ox), and other enzymes[9,10] (Figure 1). Biochemical, molecular, and genetic studies have shown that genes that encode the enzymes in this pathway are essential for GA accumulation and plant growth[11–13]. The downregulation of the *StGA3ox* genes in potato alters GA content and affects plant and tuber growth[14]. Similarly, *PsGA3ox1* transgene expression exhibits higher GA_1 levels and alters GA biosynthesis and catabolism gene expression as well as plant phenotype[15]. Gibberellin metabolism, stem growth, and biomass production in tobacco (*Nicotiana tabacum*) either increase or decrease when *AtGA20ox* or *AtGA2ox* is overexpressed[16]. Thus, the genes involved in GA biosynthesis and catabolism should be identified to better control GA accumulation and plant growth.

Hormone-mediated control of plant growth and development involves both synthesis and response[17]. DELLA proteins are major inhibitors of plant growth and development[18]. A bioactive GA binds to a GA receptor, namely, GIBBERELLIN INSENSITIVE DWARF1 (GID1), and forms a GA-GID1 complex; as a result, the degradation of DELLAs is triggered[19,20]. This mechanism is also called de-repression of GA. Further studies on *Arabidopsis* and rice have found that a specific ubiquitin E3 ligase complex ($SCF^{SLY1/GID2}$) is required for this process[21]. In recent years, positive and negative factors of GA signaling were identified in higher plants[22]. SLEEPY1 (SLY1), GAMYB, and PICKLE act as positive regulators of gibberellin signaling[23,24]. Negative regulators of gibberellin signaling include SHORT INTERNODE (SHI), SPINDLY (SPY), and other proteins[25,26]. Chitin-inducible gibberellin-responsive protein (CIGR) may be involved in GA-mediated phosphorylation/dephosphorylation of DELLAs[27].

Carrot (*Daucus carota* L.), an Apiaceae plant, is commonly consumed worldwide for its nutritional value[28]. Carrot has also been the focus of many studies aimed at investigating the molecular biology of plants[29–31]. GA is essential for carrot somatic embryogenesis and is believed to play important roles in stem elongation, root growth, and flower initiation in carrot[32–36]. However, GA biosynthesis and response in carrot remain unclear. The regulation of

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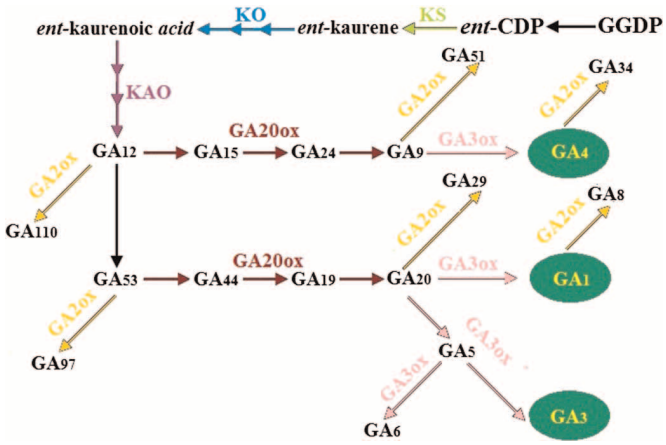

Figure 1. Proposed pathway for gibberellin (GA) biosynthesis in plants.

GA biosynthesis and response may also differ among organs in the carrot. Thus, GA-mediated plant growth and development in carrot should be studied. Our work aimed to investigate GA metabolism and signaling during carrot growth and development. We attempted to gain more insight into GA biosynthesis and response in carrot. The results from this study also provide useful information for GA-mediated plant growth and development.

MATERIALS AND METHODS

Plant material and growth conditions

The seeds of *D. carota* L. cv. 'Kurodagosun' were cultivated in an artificial chamber at the Nanjing Agricultural University (32°02′N, 118°50′E). The plants were grown at 25℃ for 16 h during the daytime followed by 18℃ for 8 h in the dark. Samples were collected at 25, 42, 60, 75, and 90 days after sowing (DAS). Morphological characteristics and age were considered to verify the developmental stages. To ensure the accuracy of biochemical and molecular research, whole carrot taproot was collected at each developmental stage. For upper ground tissues, petioles and leaves from whole carrot plants were separately harvested at each developmental stage. The samples of whole taproot, petioles, and leaves were ground in a mortar. The samples were then randomly divided into two groups for GA determination and RNA isolation. Three biological replicates were performed at each collection time point.

Anatomical structure analysis

To examine the growth status of each plant, we investigated the changes in cell structure during carrot growth and development. Fresh samples were cut into small pieces and immediately stored in phosphate buffer (pH 7.2) with 2.5% glutaraldehyde. The slices were dehydrated with ethanol and then treated with epoxy propane. Subsequently, the samples were soaked and embedded in Spurr resin[37]. A Leica ultramicrotome (Germany) was used to cut the samples into thin sections (~1 μm), which were stained with 0.5 % methyl violet for 10 min. Then, the slices were observed and then photographed under a Leica DMLS microscope (Germany).

Assay of bioactive GA levels

Samples were ground in a mortar with 10 mL of 80% methanol extraction solution containing 1 mM butylated hydroxytoluene. The extract was incubated at 4℃ for 4 h and centrifuged at 3500*g* for 10 min. Supernatants were then filtered through Chromoseq C18 columns. Efflux was collected and dried with N_2. Residues were dissolved in a phosphate-buffered saline (PBS) solution containing 0.1 % (v/v) Tween 20 and 0.1 % (w/v) gelatin (pH 7.5). The endogenous levels of bioactive GAs were then determined using an indirect enzyme-linked immunosorbent assay (ELISA) as previously described[38–40].

Total RNA extraction and cDNA synthesis

Total RNA was isolated from carrot roots, petioles, and leaves at different stages using an RNAsimple Total RNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. RNA quality and concentration was then assessed by gel electrophoresis and the use of a One-Drop spectrophotometer. To eliminate contaminating genomic DNA, we treated total RNA with gDNA Eraser for 2 min at 42℃ (TaKaRa, Dalian, China).cDNA was synthesized using PrimeScript RT reagent kit (TaKaRa, Dalian, China) according to the manufacturer's protocols. The cDNA was then diluted 10 times for quantitative real-time PCR (qRT-PCR) analysis.

Gene expression analysis by quantitative real-time PCR

To identify the genes involved in GA biosynthesis and response in carrot, GA-related genes of *Arabidopsis* and other plant species were aligned with the sequences in carrotDB, a genomic and transcriptomic database for carrot, which was built by our group (Lab of Apiaceae Plant Genetics and Germplasm Enhancement, http://apiaceae.njau.edu.cn/carrotdb/index.php)[29] (Figure S1–S29). Primers used for qRT-PCR were designed with Primer Premier 6 software (Tables 1and 2). qRT-PCR was performed using SYBR Premix *Ex Taq* (TaKaRa, Dalian, China). Each reaction contained 2 μL of diluted cDNA strand, 10 μL of SYBR Premix *Ex Taq*, 7.4 μL of deionized water, and 0.4 μL of each primer, accumulating a final volume of 20 μL. PCR was strictly performed according to the following standards: 95℃ for 30 s, followed by 40 cycles of 95℃ for 5 s and 60℃ for 30 s. The experiments were repeated with three independent samples, and the results were normalized against the carrot reference gene, namely, *DcActin*[31]. Data from *DcGA2ox2* in carrot root at 22 DAS were selected as calibrators for gene expression analysis.

Statistical analysis

Differences in the GA levels during carrot development were detected using Duncan's multiple-range test at a 0.05 probability level.

Table 1. Description of pathway-related genes and primers of genes used for qRT-PCR

Name	Gene ID	Primer sequences (forward/reverse)
DcKS	ID49567	GCGATGGGATGTTGGCGAAGAA/CCGATTGGTGAACTCTGATTGTTGTC
DcKO	ID32899	ATGGTCGCAACAAGTGATTATGATGAG/TCTCTGTTATTACGATGTCGCTTCTGA
DcKAO1	ID50166	CACAAGCGGCTGAGACGATTAACA/TTCGACCACTTATCCAATGCAGACTT
DcKAO2	ID21746	AAGAAGAGGAAGAAGATGAGTGATGGT/TCGTATCCTGCAATAACAAGACTGACA
DcGA20ox1	ID43121	AACCTAATATCGGATGCTCACAAGTCT/AGGTGGATGAGGTCTTCTTAGTAGAGT
DcGA20ox2	ID18860	AACACCAGAGAAGAACCAGAGTAACAT/GCCTGCCATGACTATGAAGGATGAA
DcGA20ox3	ID44911	CTTGGTATAGGTCCGTCGTATCTTAGG/ATGTAGGATCACAATGAGGTCCAGTTC
DcGA3ox1	ID40044	GGAAGAAATGGGATGGGTCACTGT/CCGTTGGTTAGTATGTGGAGCAGAT
DcGA3ox2	ID17101	AGACTCCCTGCTGCTCACCATTT/CCGATGCTCCTTCTCACTCACGAT
DcGA2ox1	ID44237	TGTTGATGACTGCCTACAGGTAATGAC/CATGAGTGAAGTTGATGGTGCAATCTT
DcGA2ox2	ID47688	ACTTATAATCAGAGCCTGCGAAGAACA/GGAAGGATTGGCGTCAAGTAAGAGAT
DcGA2ox3	ID47590	GCCGTTGATAGCGACCTAATGTTC/CCGTTGGATCTCAAGATGGTGAAGA
DcGA2ox4	ID30452	TTCAGTTCCAGCAGACCAAGACTC/GCTTGAGCAGTGAAGGCAATGG
DcGA2ox5	ID34042	TAACCAGCAGTCCCGAATCTCCAT/AAGCGTCCAGAATACACAGCCTTC
DcActin	ID41767	CGGTATTGTGTTGGACTCTGGTGAT/CAGCAAGGTCAAGACGGAGTATGG

Table 2. Description of selected genes related to gibberellin perception and primers of genes for qRT-PCR

Name	Gene ID	Primer sequences (forward/reverse)
DcGID1a	ID427505	GCAGCGGAATCAGGAATTGAAGTG/ATGCTCTCCAATACCAATCTCTGTCTC
DcGID1b	ID427507	ATGCTTCGCCGTCCTGATGG/GCTGACCTATAAACACGATTGAGAAG
DcGID1c	ID427506	AACATGCTTCGCCGTCCTGATG/GAACTGCGTTGGGAGGGACTTTG
DcDELLA1	ID48205	GTCGGATCTTGATGCTTCTATGCTTGA/TTGCTCCACAACCGTGACAATCTC
DcDELLA2	ID31936	CCTTCGCAGGATAACACGGATCATT/CCACAGCAACCATCACCTTCTCAA
DcDELLA3	ID43703	TTGAGCGACACGAGACACTGACT/GAGGTAGCAATAAGCGAGCGAGTG
DcSLY1	ID28764	GATAATTTCGCCGACAATTTCGCTGAT/GCCGTCTTGTTCCACTGCTTGT
DcCIGR	ID15901	CGATAGATGTTAGCCTGCCGAGAG/CTCCAACTTGTAATGCTCCGAGTAAGA
DcPICKLE1	ID46539	ATGTCCAACTGCTGCTGCTGATAG/TTCCACTTCACAAGATACTGCTTCACA
DcPICKLE2	ID48322	AAGCGAGCTAGAACGAAGACAACC/CGATGGACTGAGTGAGATGAGATGAC
DcSPY	ID47859	TGGAGAGTTGGAGTCTGCTATCACT/AATATGCCACGCCTTGGTTAATATCG
DcGAMYB	ID43195	ACTATTCCAGCCAGTTGACTTCTCCT/GCGTCGTCTAATGAACTTCCACTAACA
DcSHI1	ID460846	GCCATTCAGCAGCCACTTAATCTC/AAGCATTGAGCGGAGTTGGATAGA
DcSHI2	ID460843	GGCAACCAAGCGAAGAAGGATTGTATA/CCAAGAATGTTCACCTGCTGTCTCT
DcSHI3	ID34142	CAACCTGGTCTCGGATCACTCAAG/CCGCAGTCCTGACAGCTTATGG

RESULTS

Plant growth analysis

Over the course of growth and development, carrot tissues were harvested at 25, 42, 60, 75, and 90 DAS, respectively (Figure 2). The developmental stages were identified by age and growth indices. The root was white, and the fresh weight of the root was less than that of the shoot at 25 DAS. Up to 42 DAS, the root surface appeared orange, and the root, together with the petioles, was evidently elongated. Root weight and diameter significantly increased between 42 and 60 DAS. Subsequently, the root continued to enlarge, and the root became heavier than the shoot. However, root length presented no evident change (Figure 2).

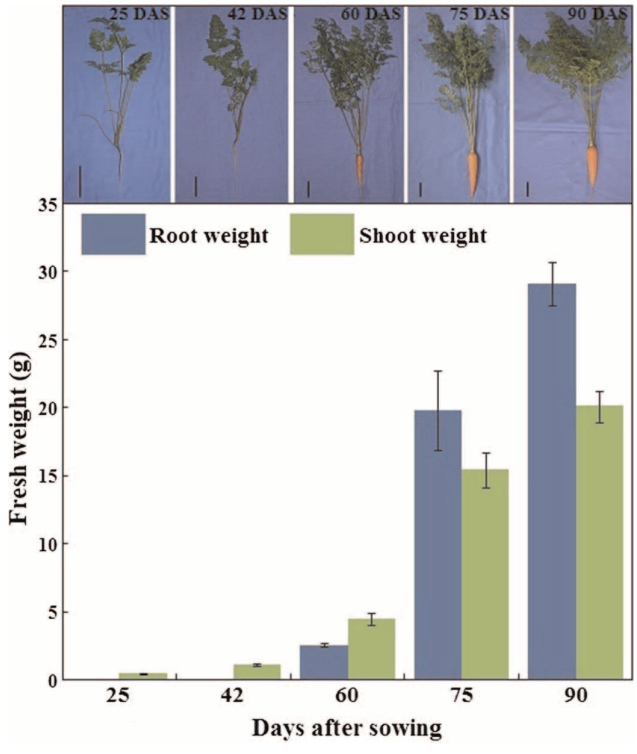

Figure 2. Description of root weight and shoot weight during carrot growth and development. Carrot samples at 25, 42, 60, 75, and 90 days after sowing were harvested. Black lines in the lower left corner of each plant represent 3 cm in that pixel, whereas error bars represent standard deviation among three independent replicates. Data are the mean ± SD of three replicates.

Structural changes in the roots, petioles, and leaves

In the roots. At 25 DAS, the root was white, and the vascular cambium (VC) located between primary phloem (PP) and protoxylem (Px) did not show evident thickness (Figure 3A). At 42 DAS, the root surface appeared orange, and VC differentiated outward and inward, forming the secondary phloem (SP) and the secondary xylem (SX), respectively (Figure 3B). Subsequently, the root continued to enlarge (Figure 3C, F, and J).

In the petioles. The collenchyma (Co), which provides structural support for carrot growth, was very conspicuous under the microscope (Figure 4). As the plant grew, regions of Co, phloem (P), and xylem(X) were evidently enlarged, suggesting that constant thickness developed in the petioles.

In the leaves. At 25 DAS, the numbers of palisade and spongy cells were limited, and the cell arrangement was disordered (Figure 5A). Subsequently, the leaves expanded, and the cells in the palisade tissue (Pt) and spongy tissue (St) were compactly arranged (Figure 5B–E).

Changes in bioactive GAs

The levels of bioactive GAs (GA_1, GA_3, GA_4, and GA_7) were analyzed in the roots, petioles, and leaves during carrot growth and development (Figure 6). For each plant, the bioactive GA contents in the leaves and petioles were higher than those in the roots. During carrot growth, the highest GA levels in the roots were observed at 42 DAS; this level subsequently decreased. However, GA levels in the petioles slightly changed, and the GA levels in the leaves relatively fluctuated.

Expression profiles of genes in the GA biosynthetic pathway during carrot growth and development

DcKS, *DcKO*, *DcKAO1*, *DcKAO2*, *DcGA20ox1*, *DcGA20ox2*, *DcGA20ox3*, *DcGA3ox1*, *DcGA3ox2*, *DcGA2ox1*, *DcGA2ox2*, *DcGA2ox3*, *DcGA2ox4*, and *DcGA2ox5* were identified from our database. These genes were then selected for qRT-PCR to investigate their expression levels. Biosynthetic pathway-related genes were evidently regulated by carrot growth and development (Figure 7).

In the roots, transcript levels of *DcKS*, *DcKO*, *DcKAO1*, *DcGA3ox1*, and *DcGA2ox5* exhibited a similar pattern, in which an initial increase and a subsequent decrease were observed. In contrast, the mRNA level of *DcGA20ox3* initially decreased and then

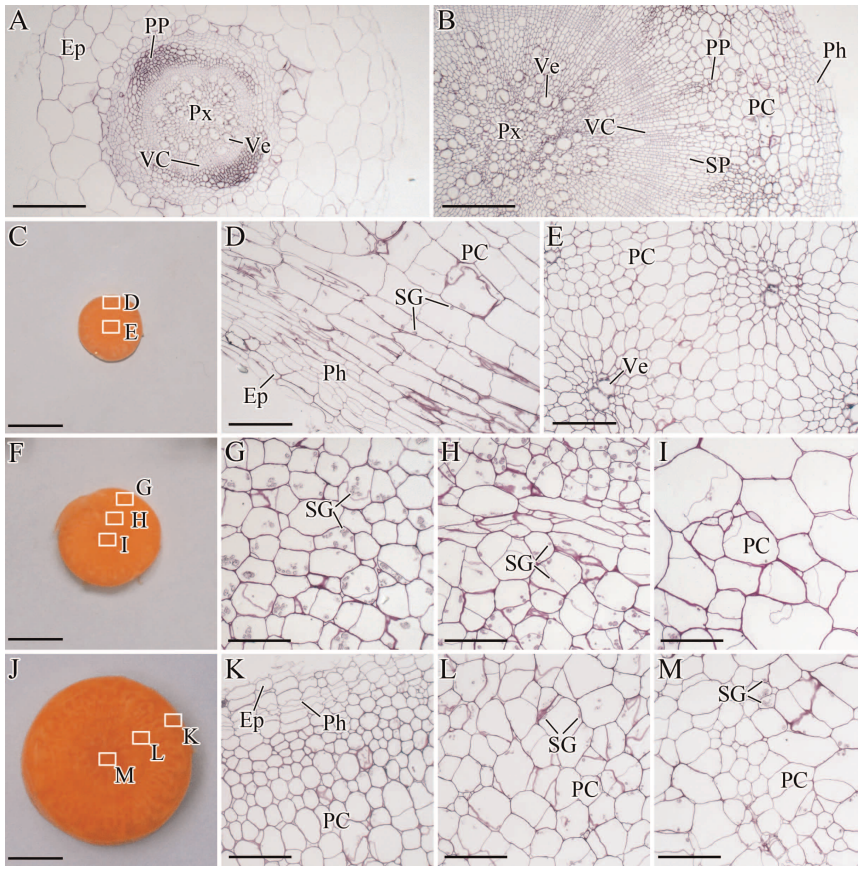

Figure 3. Structural changes in the roots during carrot growth and development. The roots were harvested at 25 (A), 42 (B), 60 (C, D, and E), 75 (F, G, H, and I), and 90 (J, K, L, and M) days after sowing. Epidermis (Ep), parenchymalcell (PC), phellogen (Ph), primary phloem (PP), protoxylem (Px), starch granule (SG), secondary phloem (SP), vascular cambium (VC), and vessel (Ve) are marked in the figure. Scale bars in A, B, D, E, G, H, I, K, L, and M are 100 μm in length, whereas bars in C, F, and J are 1 cm in length.

increased. The mRNA levels of *DcKAO2*, *DcGA20ox1*, *DcGA2ox2*, and *DcGA2ox3* initially decreased then increased and finally decreased again. Conversely, the mRNA level of *DcGA2ox1* showed completely opposite results. However, *DcGA20ox2*, *DcGA3ox2*, and *DcGA2ox4* constantly decreased over the course of the experiment. In the petioles and leaves, biosynthetic pathway-related genes were also significantly regulated by growth and development.

For the same plant, the expression levels of these genes differed in different tissues. For example, the mRNA levels of *DcKAO2* and *DcGA3ox1* in the roots were lower than those in the petioles or leaves, whereas *DcKO* showed the highest level in the roots during carrot growth and development (Figure 7).

Expression profiles of GA-responsive genes during carrot growth and development

GA perception and subsequent signal transduction are essential for GA functions during plant growth. Thus, GA receptors should be identified to further increase our understanding of the GA signaling pathway. The genes involved in GA response, namely, *GID1a*, *GID1b*, *GID1c*, *DcDELLA1*, *DcDELLA2*, *DcDELLA3*, *DcSLY1*, *DcCIGR*, *DcPICKLE1*, *DcPICKLE2*, *DcSPY*, *DcGAMYB*, *DcSHI1*, *DcSHI2*, and *DcSHI3* showed marked changes in mRNA levels during plant growth (Figure 8). In the roots, the transcript levels of *DcGID1b* and *DcSLY1* were higher at 90 DAS and lower at 25 and 42 DAS. *DcCIGR*, *DcSHI2*, and *DcSHI3* showed high expression at 25 and 42 DAS and consistently low expression at the last three time points. *DcGID1a*, *DcGID1c*, *DcCIGR*, *DcPICKLE1*, *DcPICKLE2*, *DcSPY*, *DcGAMYB*, and *DcSHI1* were highly expressed at 60 DAS, whereas transcription of *DcDELLA1* was highest at 75 DAS. In the petioles, transcription of *DcDELLA3* was highest at 40 DAS. *DcGID1b*, *DcGID1c*, *DcSLY1*, *DcCIGR*, *DcPICKLE2*, *DcSPY*, and *DcSHI1* showed high expression at 90 DAS. In the leaves, *DcGID1c*, *DcDELLA1*, *DcDELLA2*, *DcCIGR*, *DcCIGR*, *DcPICKLE2*, and *DcSHI2* exhibited the highest mRNA abundance at 25DAS, whereas *DcGID1a* and *DcGID1b* showed high expression at 90 DAS.

For the same plant, some genes, including *DcDELLA1*, *DcDELLA2*, *DcDELLA3*, *DcCIGR*, *DcPICKLE1*, *DcSPY*, *DcSHI1*, and *DcSHI3*, showed the lowest expression in the roots across all growth stages. However, expression patterns of other genes in different tissues may change during plant growth. *DcGID1a*, *DcGID1b*, *DcDELLA1*, *DcDELLA2*, *DcDELLA3*, *DcSLY1*, and *DcCIGR* were expressed at higher levels than other genes (Figure 8).

DISCUSSION

Hormonal regulation is essential for plant growth and development[41,42]. Several classes of hormones have been identified, which are ascribed to growth regulation[43]. Among these hormones, GAs usually promote cell elongation[44]. Carrot, a root vegetable, is commonly consumed worldwide for its nutritional value. Carrot undergoes significant alterations in its tissues during plant growth. Our previous work suggested that some GA-related genes are differentially expressed at different carrot root developmental stages, indicating that GAs may play important roles in carrot root development[29,45]. Thus, GA accumulation and its potential role in carrot should be further investigated.

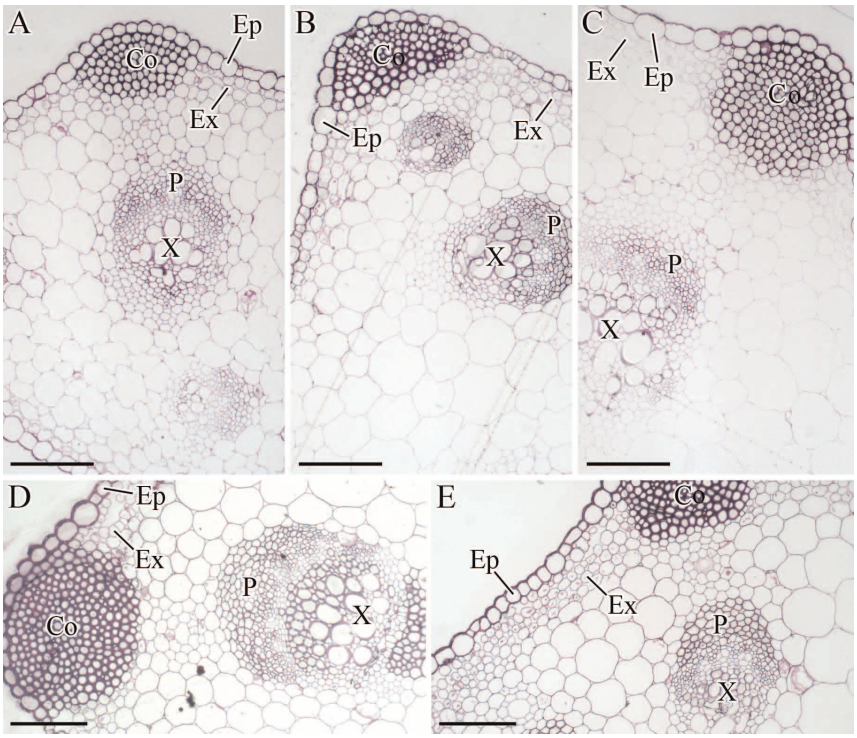

Figure 4. Structural changes in the petioles during carrot growth and development. The petioles were harvested at 25 (A), 42 (B), 60 (C), 75 (D), and 90 (E) days after sowing. Collenchyma (Co), epidermis (Ep), exodermis (Ex), phloem (P), and xylem (X) were marked in the figure. Scale bars in A, B, C, D, and E are 100 μm in length.

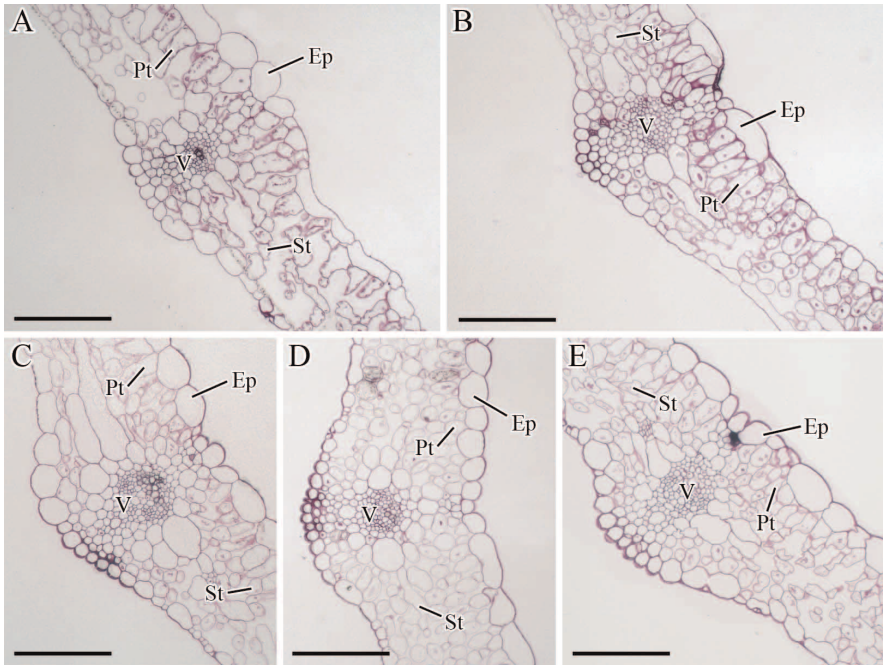

Figure 5. Structural changes in the leaves during carrot growth and development. The leaves were harvested at 25 (A), 42 (B), 60 (C), 75 (D), and 90 (E) days after sowing. Epidermis (Ep), palisade tissue (Pt), spongy tissue (St), and vascular (V) were marked in figure. Scale bars in A, B, C, D, and E are 100 μm in length.

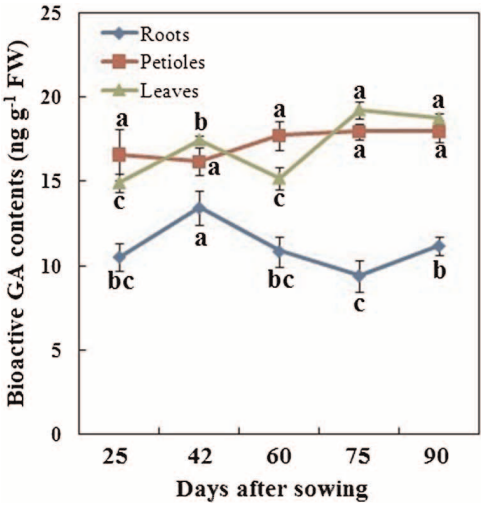

Figure 6. Bioactive GAs levels in different tissues during carrot growth and development. Error bars represent the standard deviation among three independent replicates. Data are expressed as the mean ± SD of three replicates. Different lowercase letters represent significant differences at $P < 0.05$.

Carrot growth and structural development

In this study, marked elongation and differentiation was observed at 42 DAS, suggesting that this time point may be crucial for root development. Root enlargement can be attributed to the continuous differentiation of VC. A 42-day-old carrot may also be active in pigment accumulation because the root surface first appeared orange during this time[46,47]. The petiole, as a transport organ, was elongated and thickened during plant growth (Figure 4). The leaves also evidently expanded (Figure 5). Therefore, we aimed to investigate whether GAs are implicated in these processes and to determine the response of carrots to GAs.

GA content and functions

Our results found that the levels of bioactive GAs were higher in the leaves and petioles than in other parts (Figure 6). These findings indicated that GA biosynthesis and catabolism might be tissue-specific[48]. Previous studies have suggested that GAs are the major promoters of cell elongation[49–51]. In the current work, the existence of GAs in different tissues may provide constant stimuli for structure formation and development (Figures 3–5)[52]. In the roots, the highest levels of bioactive GAs were observed at 42 DAS when an enlargement occurred at the same time (Figure 3). Thus, GAs may play important roles in cell proliferation in carrot growth and development, which is consistent with the results reported by Ubeda-Tomás and colleagues[5].

GA biosynthesis and catabolism

GAs are present in all tissues and are essential for carrot growth. However, our results found that GA levels may differ in various carrot tissues. GA levels were also significantly altered during carrot growth. Thus, GA production and regulation should be understood. In this study, the expression levels of 14 genes involved in GA biosynthesis were studied during carrot growth and development. All of these genes responded to the growth stages, thereby eliciting evident influence on the GA levels. However, the expression patterns were incompletely consistent with the GA levels possibly because of the feedback mechanisms of GAs[53–55]. Other hormones may also play vital roles in GA biosynthesis and metabolism, indicating a complex mechanism of GA biosynthesis and catabolism[56,57]. *DcKAO2*, *DcGA3ox1*, and *DcGA2ox3* also showed tissue-specific expression patterns. This interesting observation may partly explain the different GA levels in the different tissues.

GA response and regulators

GA perception and signaling transduction are also important for GA-mediated plant growth and development. A GA-GID1-DELLA signaling module has been extensively described[21,58]. A total of 15 receptors or acting components in this module were investigated using qRT-PCR during carrot growth. These genes were evidently regulated by carrot growth and development, and this result indicated that this module is also present in carrot.

The transcripts of *DcDELLA1*, *DcDELLA2*, *DcDELLA3*, *DcCIGR*, *DcPICKLE1*, *DcSPY*, *DcSHI1*, and *DcSHI3* were higher in the petioles and leaves than in the roots. This observation was consistent with the GA levels among different tissues, which indirectly suggested that bioactive GAs are produced at their site of action[59,60].

Interestingly, genes encoding DELLAs in the current study were expressed at high levels. Two main factors support this finding. First, high *DcDELLA* transcript levels suggested that DELLAs may be the main restraints in plant growth[61,62]. Second, DELLA homeostasis could balance or relieve the cues induced by GAs or other hormones[63], thereby stabilizing plant growth.

CONCLUSIONS

The morphological and anatomical structure of roots, petioles, and leaves were significantly altered during carrot plant growth. GAs may play important roles in the elongation and expansion of carrot tissues. The relative transcript levels of pathway-related genes may be the main cause for the different levels of bioactive GAs, therefore exerting influences on gibberellin perception and signals. Carrot growth and development may be regulated by modification of the genes involved in gibberellin biosynthesis, catabolism, and perception.

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: Ai-Sheng Xiong and Guang-Long Wang. Performed the experiments: Guang-Long Wang, Fei Xiong, Feng Que, Zhi-Sheng Xu, and Feng Wang. Analyzed the data: Guang-Long Wang. Contributed reagents/materials/analysis tools: Ai-Sheng Xiong. Wrote the paper: Guang-Long Wang. Revised the paper: Guang-Long Wang and Ai-Sheng Xiong. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that there are no competing interests.

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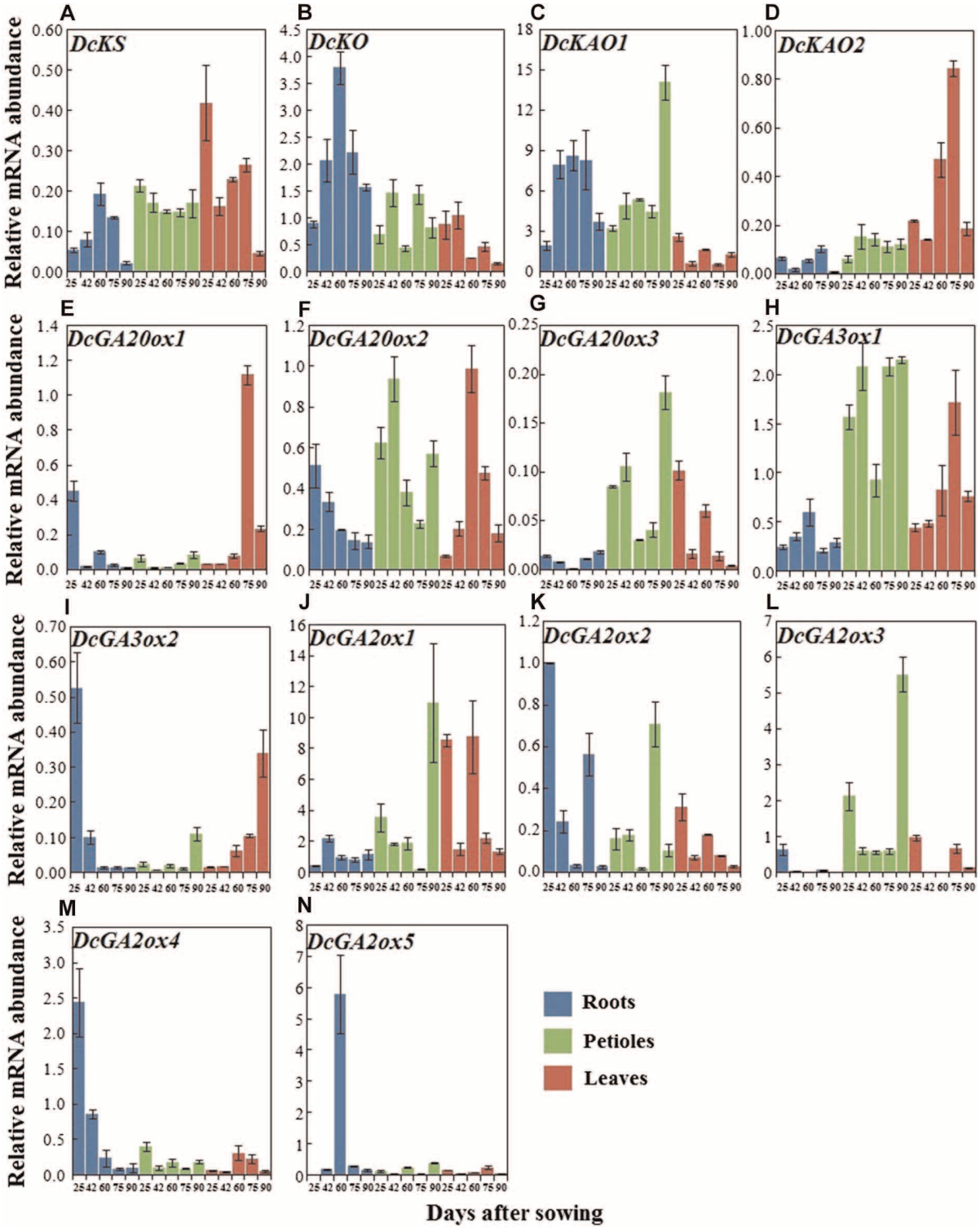

Figure 7. qRT-PCR analysis of genes involved in GA biosynthesis in different tissues during carrot growth and development. Error bars represent the standard deviation among three independent replicates. Data are expressed as the mean ± SD of three replicates.

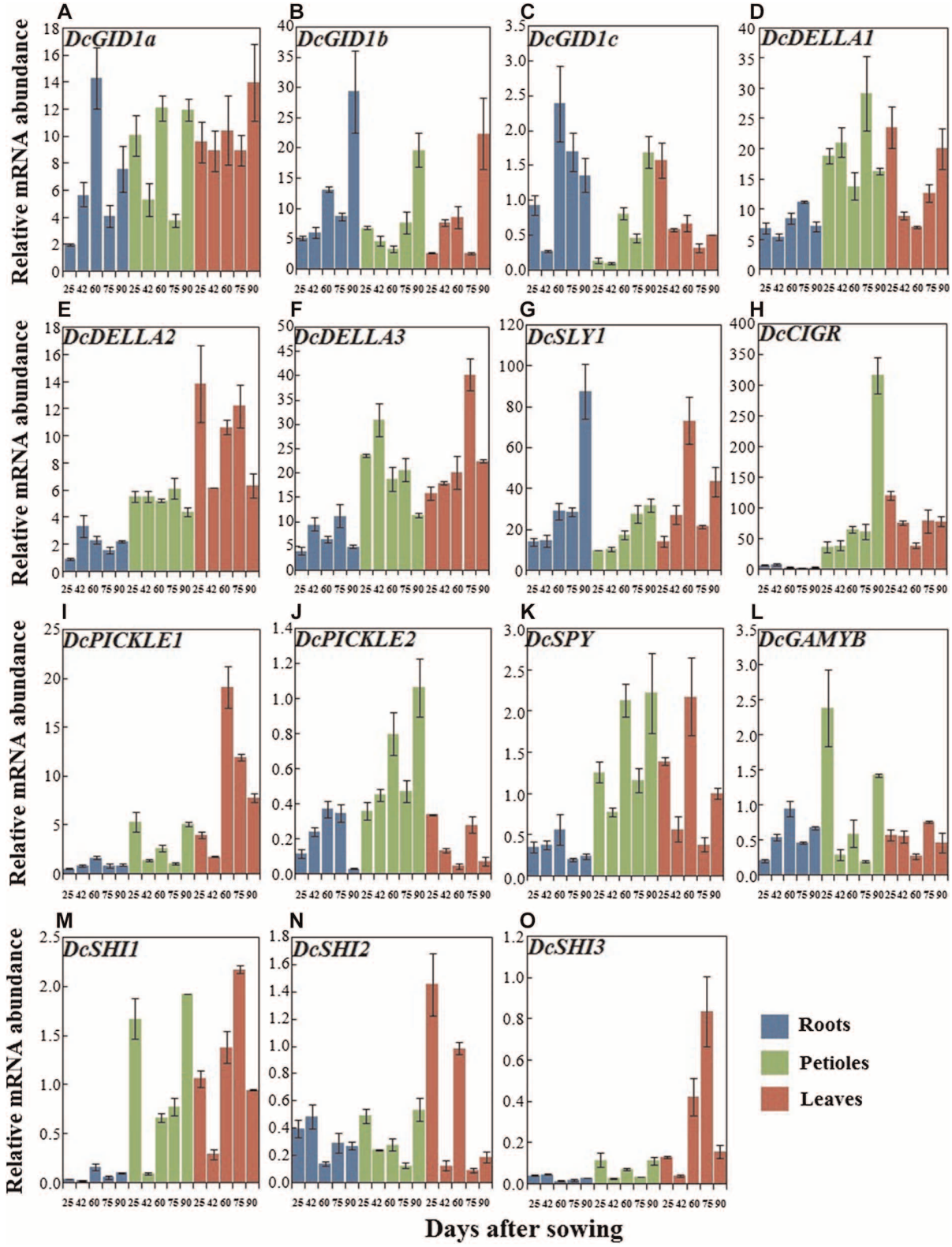

Figure 8. qRT-PCR analysis of genes involved in GA response in different tissues during carrot growth and development. Error bars represent standard deviation among three independent replicates. Data are expressed as the mean ± SD of three replicates.

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