Mol Genet Genomics (2015) 290:1379–1391
DOI 10.1007/s00438-015-0999-5

ORIGINAL PAPER

Sequencing, assembly, annotation, and gene expression: novel insights into the hormonal control of carrot root development revealed by a high-throughput transcriptome

Guang-Long Wang · Xiao-Ling Jia · Zhi-Sheng Xu · Feng Wang · Ai-Sheng Xiong

Received: 12 December 2014 / Accepted: 22 January 2015 / Published online: 11 February 2015

Abstract Previous studies have indicated that hormonal control is essential for plant root growth. The root of the carrot is an edible vegetable with a high nutritional value. However, molecular mechanisms underlying hormone-mediated root growth of carrot have not been illustrated. Therefore, the present study collected carrot root samples from four developmental stages, and performed transcriptome sequencing to understand the molecular functions of plant hormones in carrot root growth. A total of 160,227 transcripts were generated from our transcriptome, which were assembled into 32,716 unigenes with an average length of 1,453 bp. A total of 4,818 unigenes were found to be differentially expressed between the four developmental stages. In total, 87 hormone-related differentially expressed genes were identified, and the roles of the hormones are extensively discussed. Our results suggest that plant hormones may regulate carrot root growth in a phase-dependent manner, and these findings will provide valuable resources for future research on carrot root development.

Keywords Root development · Anatomical structure · Transcriptome · Hormonal regulation · *Daucus carota* L.

Communicated by S.Hohmann.

G.-L. Wang · X.-L. Jia · Z.-S. Xu · F. Wang · A.-S. Xiong (✉)
State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China
e-mail: xiongaisheng@njau.edu.cn

Introduction

The carrot (*Daucus carota* L.), a plant of the Apiaceae family, is one of the most economically important vegetables worldwide known for its edible and nutritious root (Luby et al. 2014). The carrot root undergoes a notable size change during its growth process, but only limited information regarding carrot root development is available. In higher plants, the root is an essential organ that absorbs nutrients and water for plant growth (Petricka et al. 2012). It is also involved in plant anchorage, hormone synthesis, and storage (Schiefelbein and Benfey 1991). However, root development has not been as extensively investigated as that of other plant organs, although the root exhibits several unique characteristics.

Transcriptome sequencing has been a useful method of identifying novel transcripts and splice isoforms as well as performing expression analysis. Indeed, numerous sequences and plant molecular information have been obtained using transcriptome sequencing (Klaus et al. 2000; Lu et al. 2010; Torti et al. 2012; Duarte et al. 2014; Li et al. 2014), but such studies have not been conducted on carrot development.

Previous work has suggested that hormones are the major intrinsic regulators of plant growth (Durbak et al. 2012). Auxin derived from both shoots and roots contributes to normal root development (Reed et al. 1998; Saini et al. 2013), and several lines of evidence indicate that auxin promotes proliferation, elongation, and vascular tissue formation in the roots (Aloni 2013; Takatsuka and Umeda 2014). As previously reported, auxin transport toward the root tip seems to contribute largely to taproot elongation (Blilou et al. 2005). However, excess auxin results in the severe inhibition of root growth (López-Bucio et al. 2005). Cytokinins are critical regulators of root vascular

Fig. 1 Growth status of carrots at four different developmental stages. **a** Stage 1, 22 days old; **b** Stage 2, 40 days old; **c** Stage 3, 56 days old; and **d** Stage 4, 95 days old. *Vertical white lines* in the *lower right corner* of each plant represent 3 cm

development (Yokoyama et al. 2007), but may also act as growth inhibitors by affecting the root apical meristem (Werner et al. 2003; Ren et al. 2009). Gibberellins appear to control root growth through promoting cell elongation (Inada and Shimmen 2000; Shani et al. 2013). Recent studies report that gibberellins also regulate cell production to control root growth (Ubeda-Tomás et al. 2009). Ethylene and abscisic acid are believed to have similar effects on root growth, with low levels stimulating root growth and higher levels inhibiting it (Joshi-Saha et al. 2011; Luo et al. 2014). Furthermore, ethylene-induced inhibition of root growth appears to require the involvement of abscisic acid (Ghassemian et al. 2000; Luo et al. 2014). The newly identified hormones, brassinosteroids and jasmonates also play important roles in root growth (Müssig et al. 2003; Hacham et al. 2011).

As shown above, these hormones have overlapping functions and often interact with each other to form a complex regulatory network for root growth (Jung and McCouch 2013). Using advanced molecular genetic analysis, the crosstalk and cooperation between their biosynthesis and signaling pathways have been extensively observed, thus substantially improving our understanding of hormone-mediated root growth (Mouchel et al. 2006; Zhou et al. 2011). Plant hormones are clearly essential for root growth and development (Jung and McCouch 2013; Liu et al. 2014), and such regulation may be achieved in a developmental stage-dependent manner (Sharp et al. 2004; Joshi-Saha et al. 2011).

The present study investigated the growth characteristics and anatomical structure of the carrot root during root enlargement. Transcriptome analysis of the carrot root was also conducted, and genes involved in hormone biosynthesis and signaling pathways were extensively investigated to help elucidate the hormonal control of root growth based on digital gene expression.

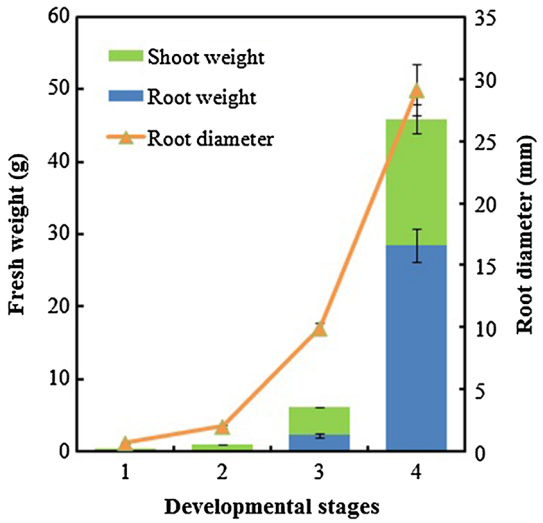

Fig. 2 Shoot weight, root weight, and root diameter during carrot root development. Data are means ± standard errors (*SE* shown by *error bars*) of three independent replicates

Materials and methods

Plant material and tissue preparation

'Kurodagosun' carrot seeds were sown at Nanjing Agricultural University (32°02′N, 118°50′E) in December 2013. The plants were cultivated in a container with a mixture of vermiculite and organic soil. The artificial weather was maintained at 25 °C for 14 h in the daytime with 320 μmol $m^{-2}s^{-1}$ light intensity and at 18 °C for 10 h during the night. Plants were divided into four stages of 22 (Stage 1), 40 (Stage 2), 56 (Stage 3), and 95 days (Stage 4) after the seeds were planted. The roots from these four stages were harvested and stored at −80 °C until analysis.

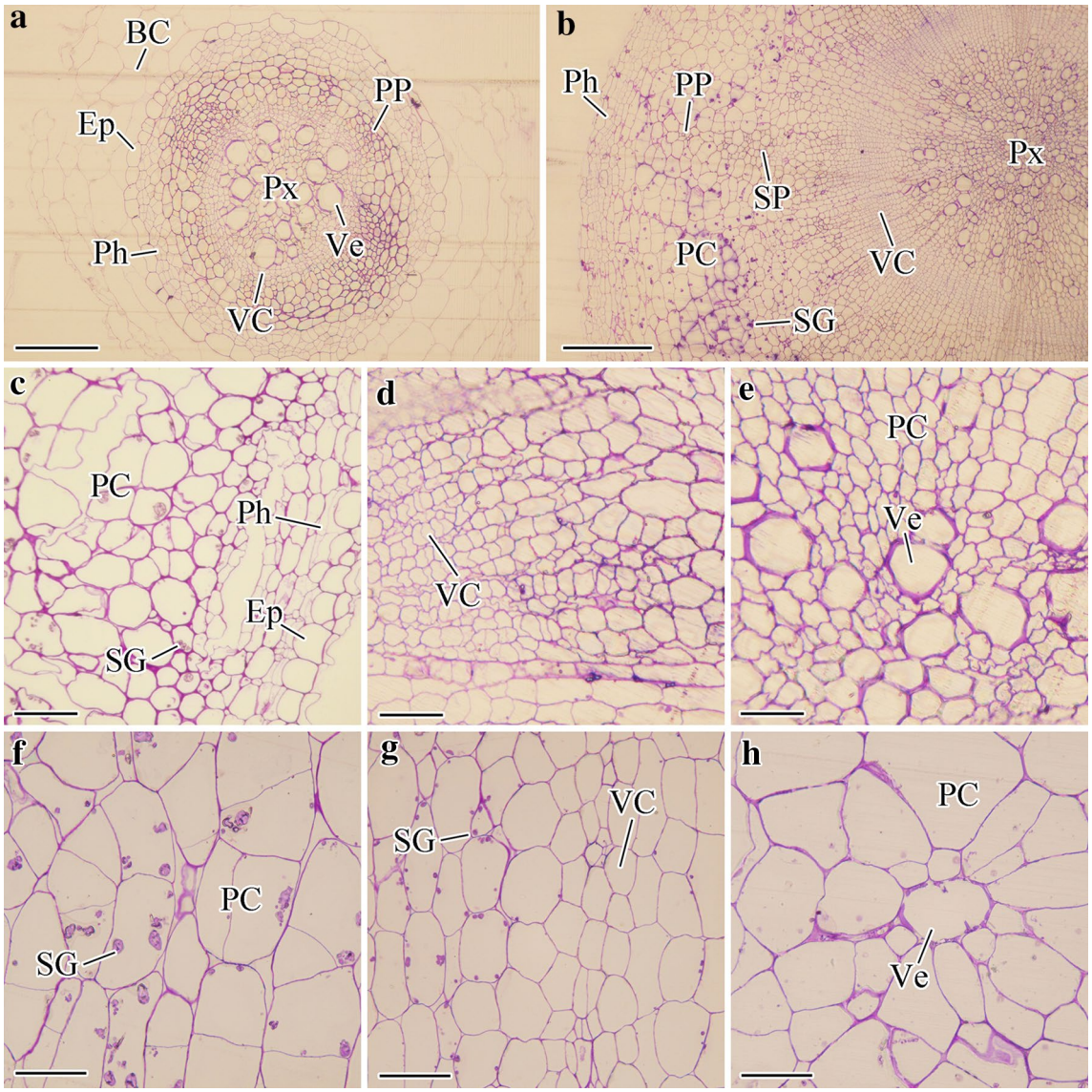

Fig. 3 Anatomical structure of carrot roots from **a** Stage 1, **b** Stage 2, (**c**–**e**) Stage 3, and (**f**–**h**) Stage 4. **c** and **f** show the outer vascular parenchyma cells close to the epidermis; **d** and **g** represent the inner vascular parenchyma cells; **e** and **h** represent the central part of carrot roots. *BC* border cells, *Ep* epidermis, *PC* parenchymal cell, *Ph* phellogen, *PP* primary phloem, *Px* protoxylem, *SG* starch granule, *SP* secondary phloem, *VC* vascular cambium, *Ve* vessels. *Scale bars* in **a** and **b**, 100 μm; *scale bars* in **c**–**h**, 50 μm

Anatomical structure analysis

To investigate the changes in anatomical structure of the carrot root, fresh root samples were cut into small pieces and stored in phosphate buffer (pH 7.2) with 2.5 % glutaraldehyde. The slices were dehydrated with ethanol and treated with epoxy propane the soaked and embedded with Spurr resin (Spurr 1969). Using a Leica ultramicrotome (Germany), we cut the samples into thin sections (~1 μm) and stained them with methyl violet for 3 min. The slices were observed and then photographed using a Leica DMLS microscope (Germany).

RNA extraction and mRNA-seq library construction

Total RNA from roots of all four stages was extracted using an RNAprep pure plant kit (Tiangen, Beijing, China) according to the manufacturer's instructions. An Illumina HiSeq™ library was constructed according to the manufacturer's instructions. Briefly, mRNA was purified by attaching beads containing oligo-dT to the poly-A. mRNAs were then cut into short fragments using a chemical reagent and applying a high temperature. Subsequently, these short fragments were used as templates to synthesize first-strand cDNA. Buffer, dNTPs, RNase H, and DNA polymerase I were used

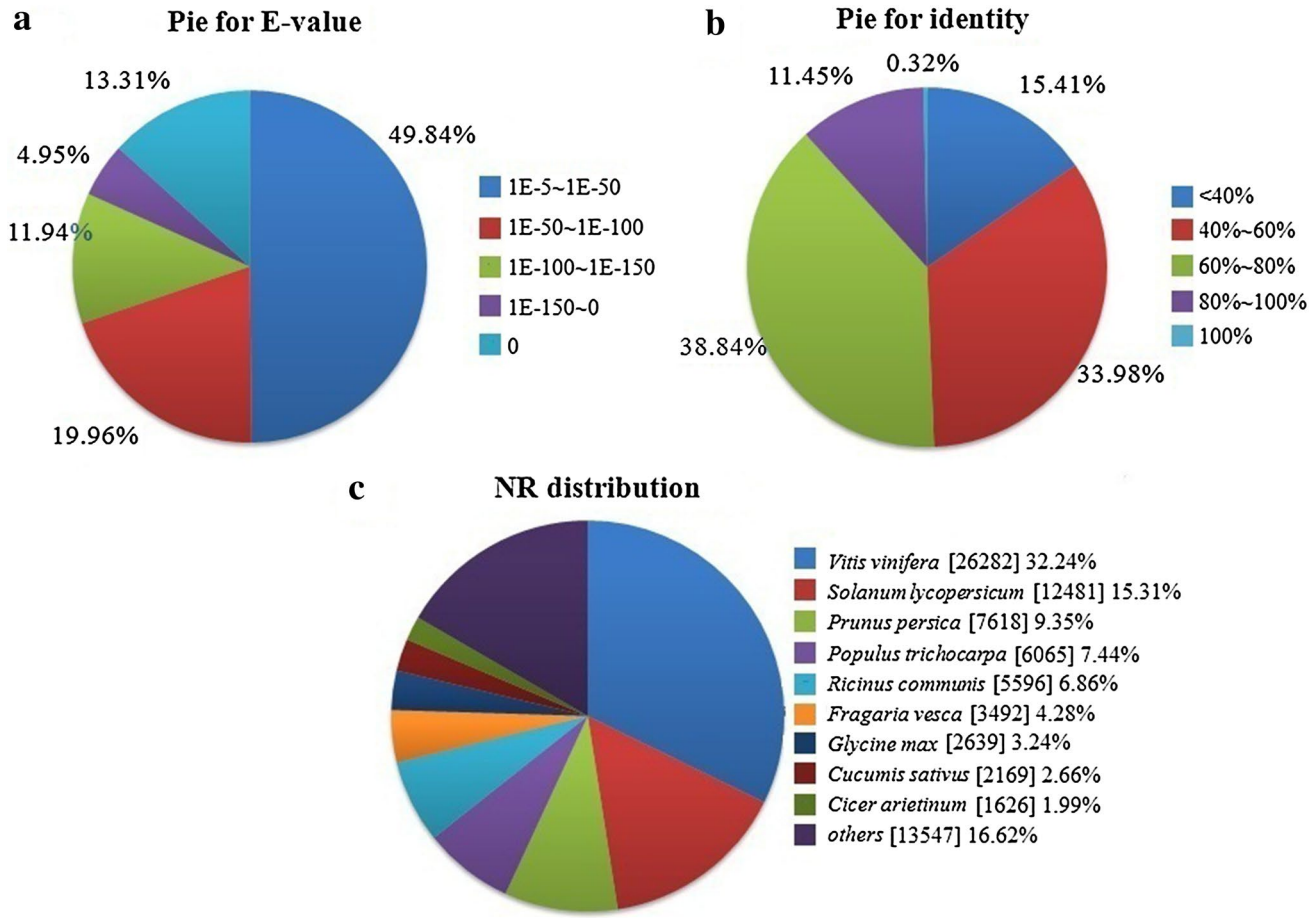

Fig. 4 Distribution for **a** *E* value, **b** identity, and **c** NR species

to synthesize second-strand cDNA from first-strand cDNA. The overhangs from fragmentation were converted into blunt ends using an end-repair mix (Illumina). Indexing adapters were ligated to the ends of double stranded cDNA when single A nucleotides were added to the 3′ ends of blunt fragments to prepare these adapters for hybridization in a flow cell. The short fragments with adapters ligated on both ends were enriched by PCR amplification and establishment of a cDNA library. After checking and quantifying, we mixed the multiplexed DNA libraries with normalized 10 nM concentration in equal volumes. The library was then sequenced using an Illumina HiSeq™ 2000 platform.

Data filtering and de novo assembly

Raw reads from four samples were collected to remove unsuitable reads and to perform subsequent de novo assembly. Data filtering was performed according to standard procedures. In brief, the adapter sequences and reads with a low quality score or a final length <50 were removed. High quality reads were assembled into contigs, transcripts, and unigenes using Trinity (http://trinityrnaseq.sourceforge.net/), non-redundant (NR) protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and NCBI Blast software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Unigenes were submitted to BLASTX search to identify homology against the NR database with a cut-off *E* value of $1e^{-5}$, and top-hit species were indicated.

Gene annotation and analysis

We used the evolutionary genealogy of genes: nonsupervised orthologous groups (eggNOG), a database of orthologous groups of genes, to identify and annotate unigene distribution. Gene ontology (GO) was also introduced to analyze the complicated biological behaviors of these unigenes during root enlargement. A Venn plot was constructed in which numbers in a circle denoted phase-specific genes, while numbers in two or more intersecting circles indicated phase overlapping genes.

Identification of differentially expressed genes (DEGs)

Reads per kilobase per million mapped reads (RPKM) were used to quantify gene expression. RPKM values were

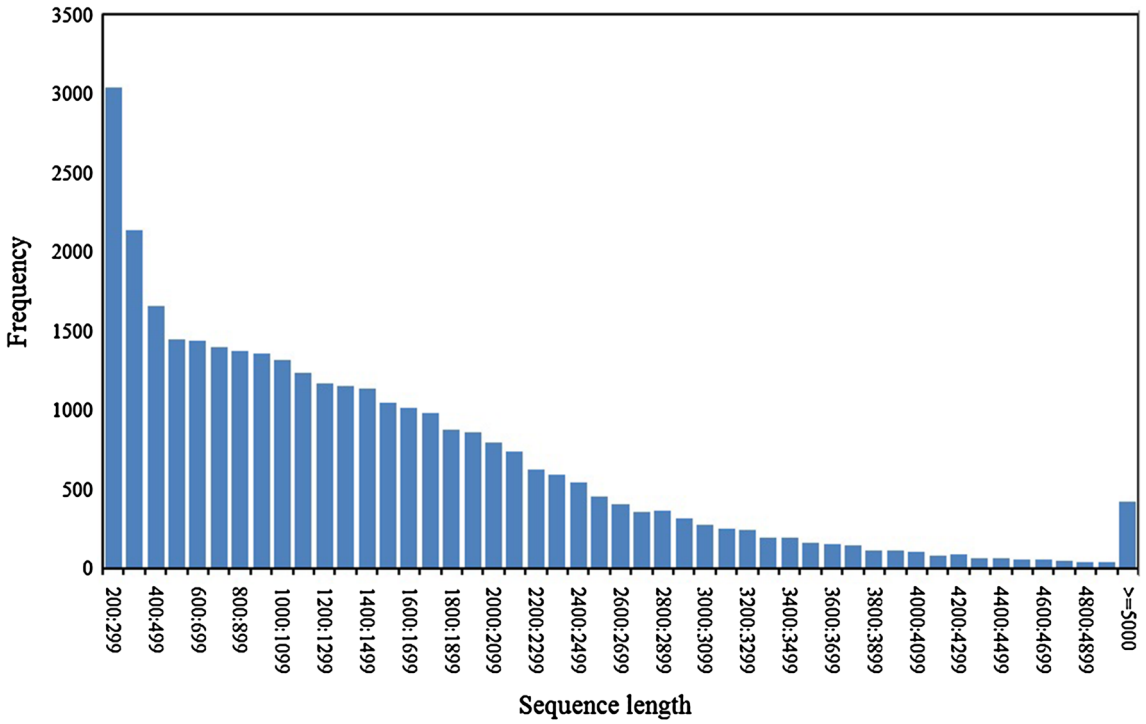

Fig. 5 Unigene length distribution in carrot root development

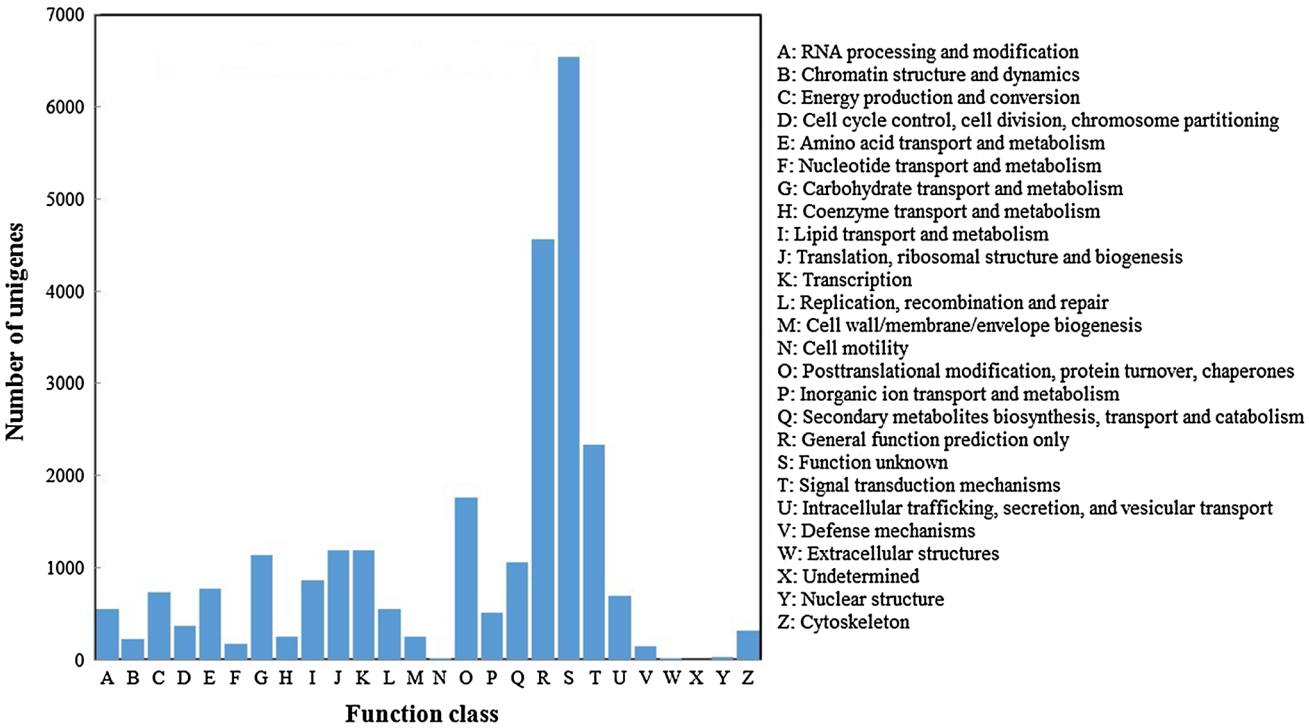

Fig. 6 Function classification of unigenes in evolutionary genealogy of genes: non-supervised orthologous groups (eggNOG). A total of 26, 188 unigenes showed significant similarity to sequences in eggNOG database and were classified into 26 groups. Letters on *x*-axis indicates groups (shown on *right of histogram*), and *y*-axis represents unigene number

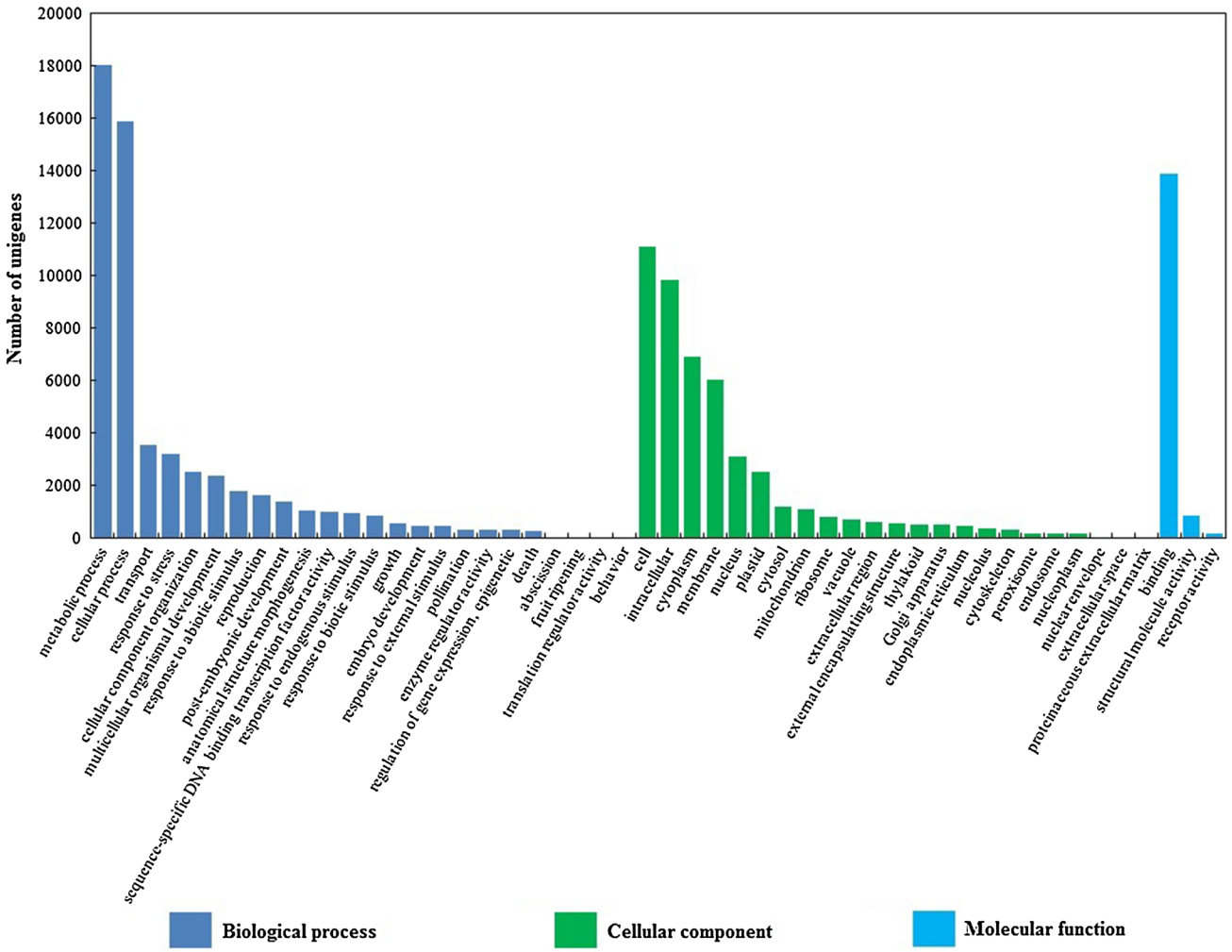

Fig. 7 Gene ontology (*GO*) classification of assembled unigenes. Each unigene was classified into at least one GO term and all unigenes were grouped into three categories: molecular function, cellular component and biological process

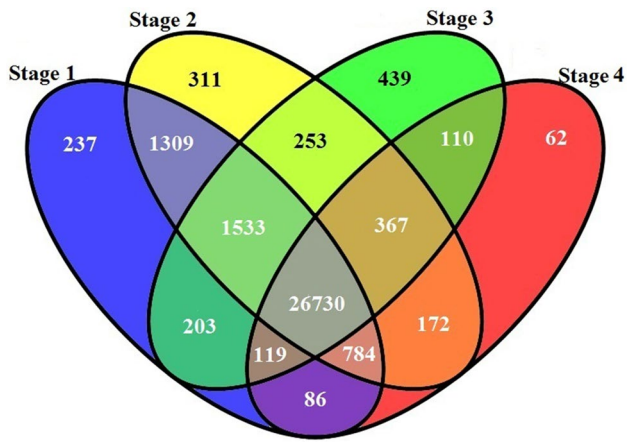

Fig. 8 *Venn diagram* showing the number of genes commonly and uniquely expressed at each sampling point. Numbers in an *oval* denote phase-specific genes, and numbers in two or more intersecting *ovals* represent overlapped genes

normalized, and genes with expression levels exhibiting more than twofold changes between two phases were clustered into DEGs. GO enrichment was adopted to further investigate the DEGs (significant difference at the 0.05 and 0.01 level). Relative RPKM was $\log_2$ (RPKM + 1) transformed to perform cluster analysis, and a heat map was then generated from $\log_2$ 'relative RPKM' using average linkage analysis.

Results

Plant growth analysis

We harvested carrots at all four stages of growth: 22 days old (Stage 1), 40 days old (Stage 2), 56 days old (Stage 3), and 95 days old (Stage 4) (Fig. 1). In Stage 1, the root was white, but had become orange in color and increased in size

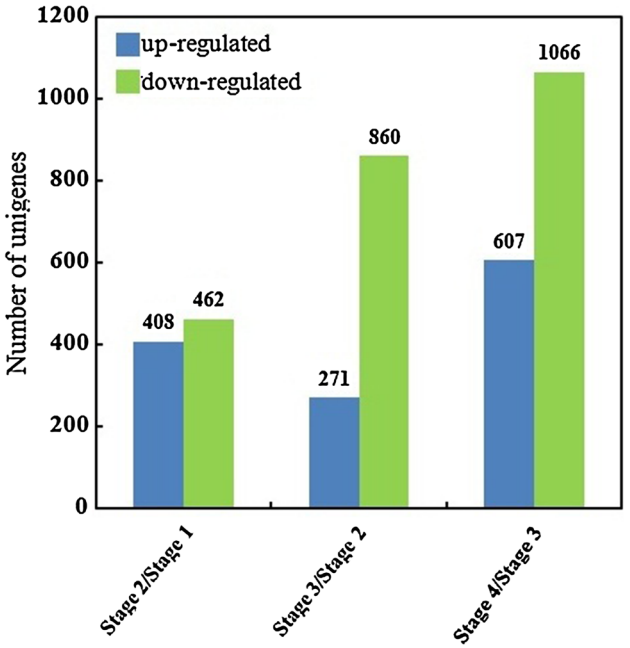

Fig. 9 Distribution of DEGs among different stages of carrot root development. Stage 2/Stage1 represents a comparison of up-regulated or down-regulated genes in Stage 2 compared with stage 1. The labeling is similar for other stages

by Stage 2. Fresh root and shoot weights increased over the different stages (Fig. 2), while the middle diameter of the root gradually increased during root development.

Anatomical observation of carrot roots

Leica DMLS microscopy revealed the internal structure of the roots at different developmental stages. In Stage 1, the protoxylem and the primary phloem were relatively thin (Fig. 3a), but the number and size of cells in these parts had increased by Stage 2, resulting in thickening of the carrot roots. This thickening could also be attributed almost exclusively to the constant division of the vascular cambium (VC), located between the xylem and the phloem (Fig. 3b). The VC differentiated inwards to form the secondary xylem, and outwards to form the secondary phloem (SP). After this time point, expansion of the outer vascular parenchyma cells was visible (Fig. 3b, c, f), and starch granules (SGs) were also seen during the later stages of growth (Fig. 3b–d, f, g).

Assembly, functional annotation, and classification

A total of 22,940,679 clean reads were obtained for Stage 1, 48,885,725 for Stage 2, 36,758,033 for Stage 3, and 35,189,581 for Stage 4. The Trinity software generated 160,227 transcripts by de novo assembly, and each transcript was aligned with the NR database (Fig. 4). Based on the results of the top-hits from BLASTX, we assembled the transcripts into 32,716 unigenes with an average length of 1,453 bp and a 41.03 % GC content. Among these unigenes, 13,832 (42.28 %) were in the size range 200–1,000 bp, and only 419 (1.28 %) were >2 kb (Fig. 5).

All unigenes were predicted and classified on the basis of eggNOG database, which is a database that can explore the ancestry of a protein. A total of 26,188 unigenes were found to be similar with eggNOG, and were then clustered into 26 categories (Fig. 6). The largest category was "function unknown", followed by "general function prediction only" (4,564, 17.43 %), and "signal transduction mechanisms" (2,329, 8.89 %).

Based on GO annotations, the unigenes were classified into different functional categories (Fig. 7). "Molecular process" and "cellular process" were the most abundant GO slims within the biological process category, while "cell" and "binding" were the most highly represented groups within the cellular component category and molecular function category, respectively. Genes involved in other important biological processes such as stress response, anatomical structure, and growth were also identified.

Figure 8 shows the Venn diagrams presenting the commonly or uniquely expressed genes detected at each developmental stage. A total of 26,730 genes were expressed in all four stages, with approximately 237, 311, 439, and 62 genes exclusively expressed in Stages 1, 2, 3, and 4, respectively.

Analysis of differentially expressed genes

Compared with Stage 1, there were 408 up-regulated and 462 down-regulated genes in Stage 2. For each comparison, the number of down-regulated genes exceeded that of up-regulated genes (Fig. 9). To gain more insights into the functions of differentially expressed genes (DEGs), we searched for markedly enriched GO terms compared with the reference gene background (Fig. 10). According to molecular function, the DEGs mapped to "binding" were enriched during the later stages of development (Stages 3 and 4). According to cellular component, the GO term "extracellular region" was enriched in all developmental stages. Considering biological processes, the DEGs that mapped to "sequence-specific DNA binding transcription factor activity" and "response to stress" were enriched in all stages. The DEGs in later developmental stages (Stages 2, 3, and 4) constituted a high proportion of "metabolic process" genes, suggesting that a substantial number of this type of gene may be involved in metabolic processes.

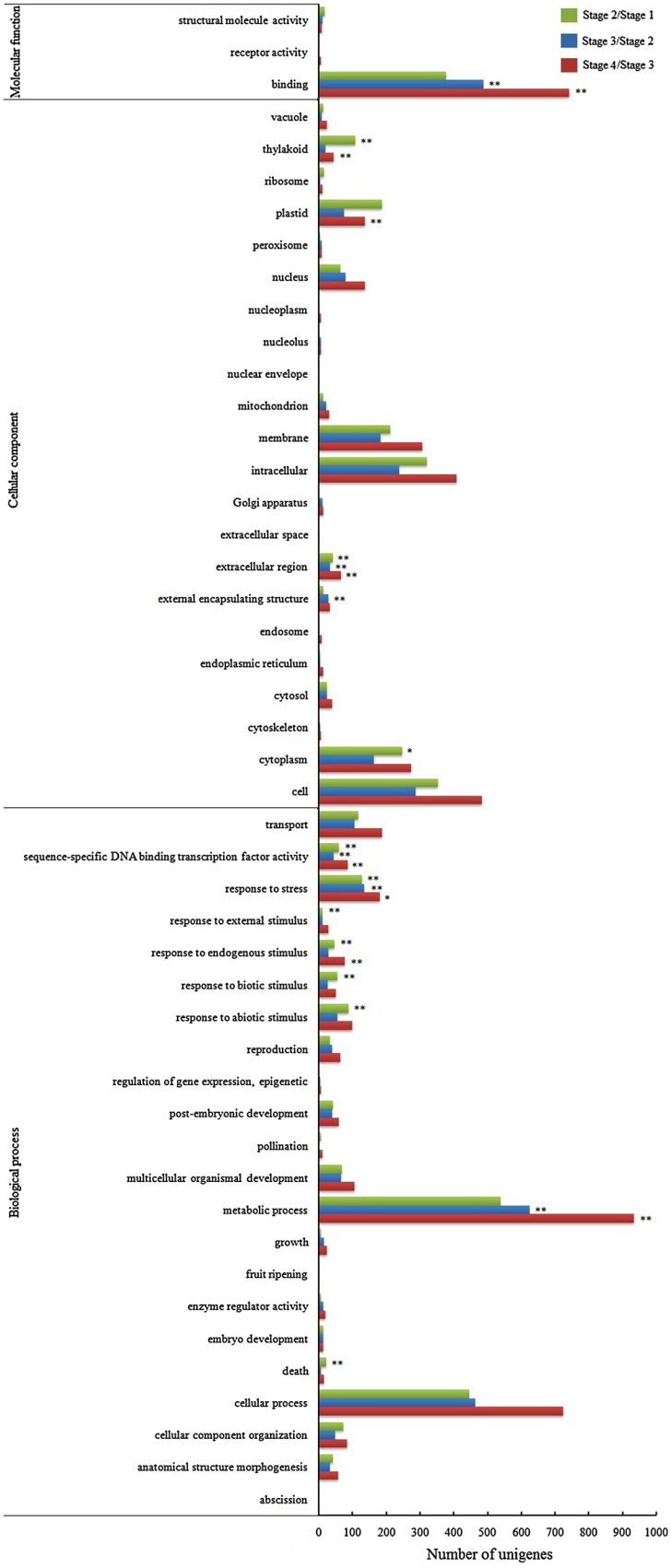

Fig. 10 GO enrichment analysis of DEGs. *Asterisk* and *double asterisk* indicate significant difference at the 0.05 and 0.01 probability levels, respectively

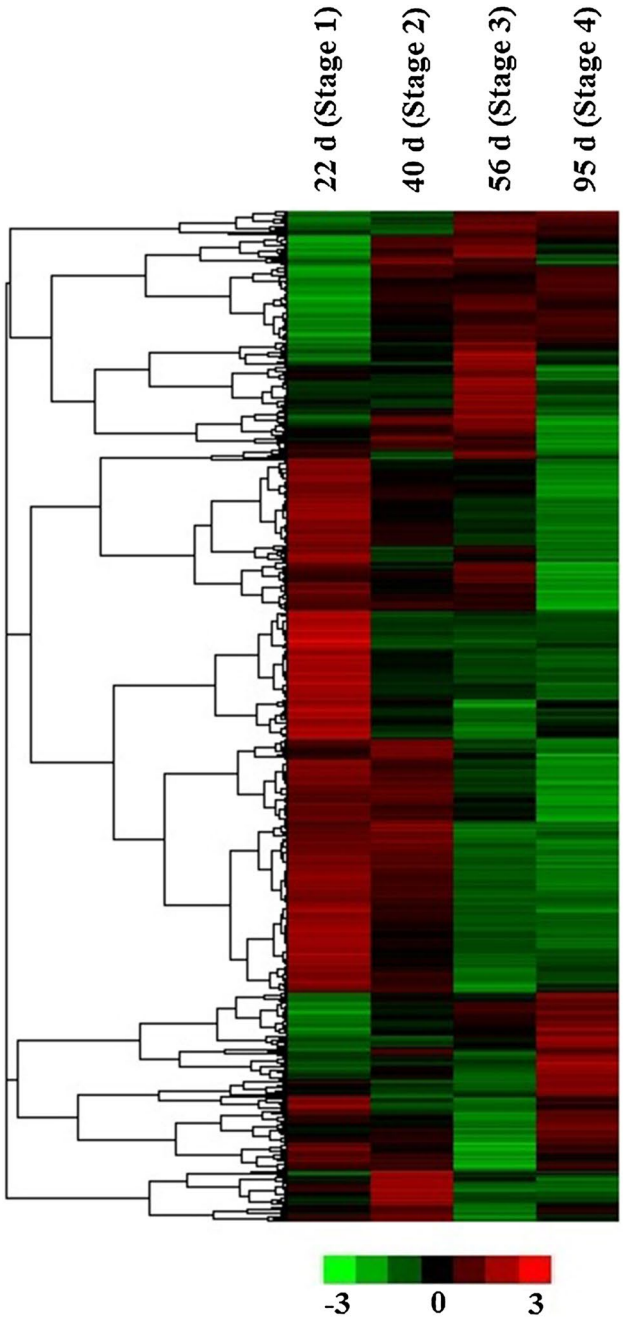

Fig. 11 Expression changes and cluster analysis of DEGs in different developmental stages. Cluster analysis was conducted on the basis of average linkage clustering. *Red*, *green*, and *black boxes* represent genes with high, low, and moderate expressions, respectively (color figure online)

A total of 4,818 DEGs were identified in root enlargement, which were then clustered using hierarchical cluster analysis (Fig. 11). DEGs in Stage 1 were located close to those of Stage 2, while a similar pattern was observed between DEGs of Stages 3 and 4.

Hormonal control of carrot root growth

Figure 12 shows the number of DEGs involved in plant hormone metabolism and signaling during carrot root development. A total of 87 DEGs were hormone-related, involving auxin, cytokinin, abscisic acid, gibberellins, ethylene, brassinosteroids, and jasmonic acid pathways (Table 1).

In the auxin metabolism and signaling pathway, 10 of 15 DEGs showed down-regulated trends, whereas only one was up-regulated. Similar results were observed in the cytokinin, gibberellin, jasmonic acid, and brassinosteroid pathways. In the abscisic acid biosynthesis pathway, where some unigenes also regulate the accumulation of lycopene, carotenes, and lutein, six of 11 DEGs showed up-regulated trends, while five of nine DEGs in the abscisic acid signaling pathway were down-regulated during root growth. In the ethylene pathway, *ACS1* and *ACS3* encoding 1-aminocyclopropane-1-carboxylic acid (ACC) synthase showed a similar pattern, indicating redundant roles for *ACS1* and *ACS3*. However, two unigenes, *ACO1* and *ACO5*, encoding ACC oxidase showed opposite trends (Fig. 12).

Discussion

Molecular mechanisms play vital roles in plant growth and development (Wu and Cheng 2014), and it is important to identify the genes responsible for controlling plant development to fully comprehend these mechanisms. To date, studies based on increasingly available sequence data have detected numerous genes and expression profiles that occur during the development of plant tissues such as flowers, leaves, and fruits (Ando et al. 2012; Sweetman et al. 2012; Singh et al. 2013; Lou et al. 2014). The root provides the plant growth with water, nutrients, and anchorage (Rich and Watt 2013), and an understanding of how root growth is controlled by hormones is essential for crop production, especially for fleshy root plants such as the carrot (Herder et al. 2010; Remans et al. 2012). However, the dynamics of the genes involved in carrot root development remain unclear.

'Kurodagosun' is a carrot variety widely cultivated for its high and stable production, which is also used as a model for genetic and breeding research (Huang et al. 2014; Xu et al. 2014a, b). Its root undergoes a significant size change during plant growth, providing useful material for our present work. As previously described, root growth is a consequence of an increase in both the number of cells in the root and their size (Perilli et al. 2012). Our results show that carrot root enlargement can be attributed to the differentiation of

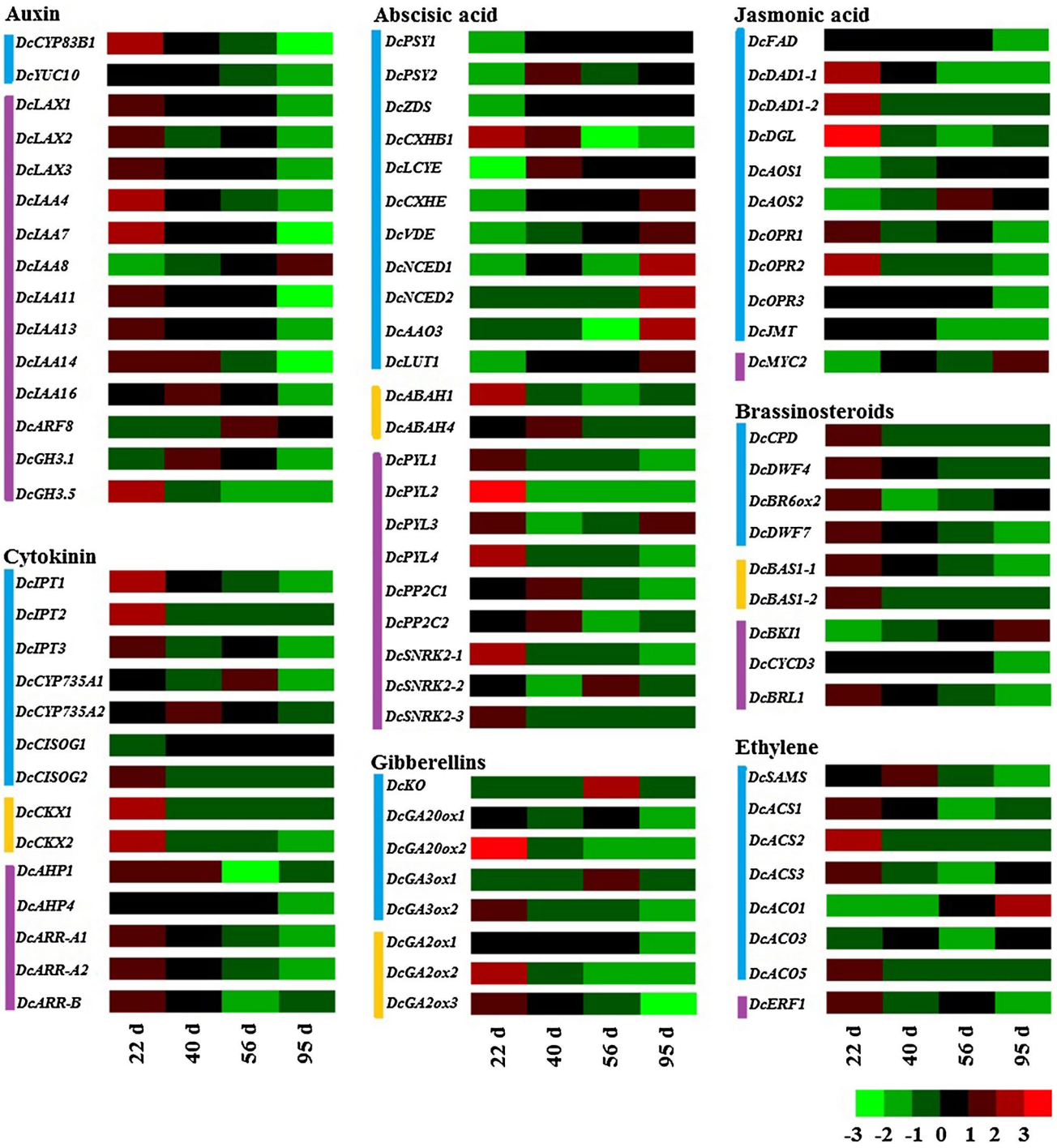

Fig. 12 Expression profiles of genes related to plant hormone metabolism and signaling during different developmental stages. *Blue*, *yellow*, and *purple vertical lines* represent biosynthesis, degradation, and signaling genes, respectively (color figure online)

VC, a cylindrical meristematic tissue that gives rise to the secondary xylem toward the inner part, and to SP toward the outer part. We also observed SGs deposition in the carrot root; its distribution is similar to that of sugar and carotene, which are located in the cortex (Vilaine et al. 2013).

To gain a better understanding of root growth and development, carrot roots from four stages were analyzed by high-throughput Illumina sequencing. A total of 32,716 unigenes were assembled, of which 26,730 were co-expressed during all four developmental stages, indicating that they are essential for root development. The genes that

Table 1 List of differentially expressed genes (DEGs) related to plant hormone metabolism and signaling

Hormone	Gene ID	Gene name	Putative function
Auxin	gene43558	*DcCYP83B1*	Biosynthesis
	gene47813	*DcYUC10*	Biosynthesis
	gene40028-1	*DcLAX1*	Transport
	gene40028-3	*DcLAX2*	Transport
	gene37261	*DcLAX3*	Transport
	gene43753	*DcIAA4*	Signaling
	gene32603	*DcIAA7*	Signaling
	gene38111	*DcIAA8*	Signaling
	gene42857	*DcIAA11*	Signaling
	gene42568	*DcIAA13*	Signaling
	gene38082	*DcIAA14*	Signaling
	gene40549	*DcIAA16*	Signaling
	gene48599	*DcARF8*	Signaling
	gene46571	*DcGH3.1*	Conjugation
	gene49187	*DcGH3.5*	Conjugation
Cytokinin	gene48943	*DcIPT1*	Biosynthesis
	gene32166	*DcIPT2*	Biosynthesis
	gene34617	*DcIPT3*	Biosynthesis
	gene38662	*DcCYP735A1*	Biosynthesis
	gene39794	*DcCYP735A2*	Biosynthesis
	gene46960	*DcCISOG1*	Biosynthesis
	gene45592	*DcCISOG2*	Biosynthesis
	gene47359	*DcCKX1*	Degradation
	gene47985	*DcCKX2*	Degradation
	gene45447	*DcAHP1*	Signaling
	gene35677	*DcAHP4*	Signaling
	gene46768	*DcARR-A1*	Signaling
	gene46802	*DcARR-A2*	Signaling
	gene41088	*DcARR-B*	Signaling
Abscisic acid	gene37860	*DcPSY1*	Biosynthesis
	gene26551	*DcPSY2*	Biosynthesis
	gene46397	*DcZDS*	Biosynthesis
	gene40661	*DcCHXB1*	Biosynthesis
	gene41045	*DcLCYE*	Biosynthesis
	gene30694	*DcCXHE*	Biosynthesis
	gene45567	*DcVDE*	Biosynthesis
	gene41395	*DcNCED1*	Biosynthesis
	gene46542	*DcNCED2*	Biosynthesis
	gene47183	*DcAAO3*	Biosynthesis
	gene30694	*DcLUT1*	Biosynthesis
	gene46720	*DcABAH1*	Catabolism
	gene24738	*DcABAH4*	Catabolism
	gene41467	*DcPYL1*	Signaling
	gene45542	*DcPYL2*	Signaling
	gene50119	*DcPYL3*	Signaling
	gene34677	*DcPYL4*	Signaling
	gene41116	*DcPP2C1*	Signaling
	gene48703	*DcPP2C2*	Signaling
	gene47229	*DcSNRK2-1*	Signaling
	gene50213	*DcSNRK2-2*	Signaling
	gene44744	*DcSNRK2-3*	Signaling
Gibberellin	gene34720	*DcKO*	Biosynthesis
	gene43121	*DcGA20ox1*	Biosynthesis
	gene18860	*DcGA20ox2*	Biosynthesis
	gene48493	*DcGA3ox1*	Biosynthesis
	gene17101	*DcGA3ox2*	Biosynthesis
	gene44237	*DcGA2ox1*	Deactivation
	gene47688	*DcGA2ox2*	Deactivation
	gene47590	*DcGA2ox3*	Deactivation
Jasmonic acid	gene44168	*DcFAD*	Biosynthesis
	gene46390	*DcDAD1-1*	Biosynthesis
	gene48786	*DcDAD1-2*	Biosynthesis
	gene48258	*DcDGL*	Biosynthesis
	gene48840	*DcAOS1*	Biosynthesis
	gene38648	*DcAOS2*	Biosynthesis
	gene45214	*DcOPR1*	Biosynthesis
	gene49464	*DcOPR2*	Biosynthesis
	gene35807	*DcOPR3*	Biosynthesis
	gene43477	*DcJMT*	Biosynthesis
	gene43883	*DcMYC2*	Signaling
Brassinosteroids	gene44962	*DcCPD*	Biosynthesis
	gene42688	*DcDWF4*	Biosynthesis
	gene33014	*DcBR6ox2*	Biosynthesis
	gene45116	*DcDWF7*	Biosynthesis
	gene34693	*DcBAS1-1*	Deactivation
	gene47463	*DcBAS1-2*	Deactivation
	gene50635	*DcBKI1*	Signaling
	gene46510	*DcCYCD3*	Signaling
	gene49332	*DcBRL1*	Signaling
Ethylene	gene41021	*DcSAMS*	Biosynthesis
	gene46706	*DcACS1*	Biosynthesis
	gene44542	*DcACS2*	Biosynthesis
	gene27965	*DcACS3*	Biosynthesis
	gene34303	*DcACO1*	Biosynthesis
	gene42469	*DcACO3*	Biosynthesis
	gene32565	*DcACO5*	Biosynthesis
	gene39464	*DcERF1*	Signaling

were expressed in one to three developmental stages may instead correlate with phase-specific processes. In total, 4,818 DEGs were identified in four developmental stages, which can provide novel genetic material for further studies aimed to measure and characterize root development.

Plant hormones are intrinsic regulators of plant growth in response to environmental cues (Malamy 2005), and have been shown to be necessary for carrot root growth

and development (Michalczuk et al. 1992; Mitsuhashi et al. 2003; Kulka 2008). We identified 87 DEGs involved in hormone biosynthesis, deactivation, and signaling pathways, indicating that plant hormones regulate carrot root growth in a stage-dependent manner (Michalczuk et al. 1992). Most DEGs showed down-regulated trends, leading us to hypothesize that hormones play vital roles in root formation and initiation (Aloni et al. 2006). Alternatively, they may work actively in metabolism regulation and pigmentation (Woeste et al. 1999; Titapiwatanakun and Murphy 2009), which is supported by the observed roles of hormones in sugar and anthocyanin metabolism (Shan et al. 2009; LeClere et al. 2010). On the other hand, jasmonic acid and cytokinin inhibit root growth (Creelman 1998; Kuderová et al. 2008), which may explain the observed down-regulated trends of the unigenes associated with these two hormones. Furthermore, the similar expression profiles in different hormones reveal that extensive hormonal crosstalk occurs during the regulation of root development (Nemhauser et al. 2006).

In conclusion, root development appears to be a complex process involving several changes. Our current work has provided a novel view of carrot root development, indicating that 4,818 DEGs are involved in root enlargement, and that 87 unigenes are hormone-related. As a result, plant hormones may regulate root growth in a developmental stage-dependent manner. These transcriptome sequencing data will increase the genetic resources available for scientists working on root enlargement.

Acknowledgments The research was supported by the New Century Excellent Talents in University (NCET-11-0670); Jiangsu Natural Science Foundation (BK20130027); China Postdoctoral Science Foundation (2014M551609), Priority Academic Program Development of Jiangsu Higher Education Institutions Project.

Conflict of interest The authors declare that there are no competing interests.

References

Aloni R (2013) Role of hormones in controlling vascular differentiation and the mechanism of lateral root initiation. Planta 238:819–830

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Ando K, Carr K, Grumet R (2012) Transcriptome analyses of early cucumber fruit growth identifies distinct gene modules associated with phases of development. BMC Genomics 13:518

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433:39–44

Creelman RA (1998) Jasmonate perception: characterization of COI1 mutants provides the first clues. Trends Plant Sci 3:367–368

Duarte J, Rivière N, Baranger A, Aubert G, Burstin J, Cornet L, Lavaud C, Lejeune-Hénaut I, Martinant JP, Pichon JP, Pilet-Nayel ML, Boutet G (2014) Transcriptome sequencing for high throughput SNP development and genetic mapping in Pea. BMC Genomics 15:126

Durbak A, Yao H, McSteen P (2012) Hormone signaling in plant development. Curr Opin Plant Biol 15:92–96

Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. Plant Cell 12:1117–1126

Hacham Y, Holland N, Butterfield C, Ubeda-Tomas S, Bennett MJ, Chory J, Savaldi-Goldstein S (2011) Brassinosteroid perception in the epidermis controls root meristem size. Development 138:839–848

Herder GD, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600–607

Huang Y, Li MY, Wang F, Xu ZS, Huang W, Wang GL, Ma J, Xiong AS (2014) Heat shock factors in carrot: genome-wide identification, classification, and expression profiles response to abiotic stress. Mol Biol Rep. doi:10.1007/s11033-11014-13826-x

Inada S, Shimmen T (2000) Regulation of elongation growth by gibberellin in root segments of Lemna minor. Plant Cell Physiol 41:932–939

Joshi-Saha A, Valon C, Leung J (2011) Abscisic acid signal off the STARTing block. Mol Plant 4:562–580

Jung JKH, McCouch S (2013) Getting to the roots of it: genetic and hormonal control of root architecture. Front Plant Sci 4:186

Klaus M, Aaron L, Thomas E, Allen M, Jürg S, Kay AL, Jeffery LD, Robert AD (2000) The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nat Genet 26:403–410

Kuderová A, Urbánková I, Válková M, Malbeck J, Brzobohatý B, Némethová D, Hejátko J (2008) Effects of conditional IPT-dependent cytokinin overproduction on root architecture of *Arabidopsis* seedlings. Plant Cell Physiol 49:570–582

Kulka RG (2008) Hormonal control of root development on epiphyllous plantlets of *Bryophyllum* (*Kalanchoë*) *marnierianum*: role of auxin and ethylene. J Exp Bot 59:2361–2370

LeClere S, Schmelz EA, Chourey PS (2010) Sugar levels regulate tryptophan-dependent auxin biosynthesis in developing maize kernels. Plant Physiol 153:306–318

Li MY, Wang F, Jiang Q, Ma J, Xiong AS (2014) Identification of SSRs and differentially expressed genes in two cultivars of celery (*Apium graveolens* L.) by deep transcriptome sequencing. Hort Res 1:10

Liu J, Rowe J, Lindsey K (2014) Hormonal crosstalk for root development: a combined experimental and modeling perspective. Front Plant Sci 5:116

López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Pérez-Torres A, Rampey RA, Bartel B, Herrera-Estrella L (2005) An auxin transport independent pathway is involved in phosphate stress-induced root architectural alterations in *Arabidopsis*. Identification of BIG as a mediator of auxin in pericycle cell activation. Plant Physiol 137:681–691

Lou Q, Liu Y, Qi Y, Jiao S, Tian F, Jiang L, Wang Y (2014) Transcriptome sequencing and metabolite analysis reveals the role of delphinidin metabolism in flower colour in grape hyacinth. J Exp Bot 65:3157–3164

Lu T, Lu G, Fan D, Zhu C, Li W, Zhao Q, Feng Q, Zhao Y, Guo Y, Li W, Huang X, Han B (2010) Function annotation of the rice transcriptome at single-nucleotide resolution by RNA-seq. Genome Res 20:1238–1249

Luby CH, Maeda HA, Goldman IL (2014) Genetic and phenological variation of tocochromanol (vitamin E) content in wild (*Daucus carota* L. var. *carota*) and domesticated carrot (*D. carota* L. var. *sativa*). Hort Res 1:15

Luo X, Chen Z, Gao J, Gong Z (2014) Abscisic acid inhibits root growth in *Arabidopsis* through ethylene biosynthesis. Plant J 79:44–55

Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. Plant Cell Environ 28:67–77

Michalczuk L, Cooke TJ, Cohen JD (1992) Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry 31:1097–1103

Mitsuhashi W, Toyomasu T, Masui H, Katho T, Nakaminami K, Kashiwagi Y, Akutsu M, Kenmoku H, Sassa T, Yamaguchi S, Kamiya Y, Kamada H (2003) Gibberellin is essentially required for carrot (*Daucus carota* L.) somatic embryogenesis: dynamic regulation of gibberellin 3-oxidase gene expressions. Biosci Biotechnol Biochem 67:2438–2447

Mouchel CF, Osmont KS, Hardtke CS (2006) *BRX* mediates feedback between brassinosteroid levels and auxin signalling in root growth. Nature 443:458–461

Müssig C, Shin G-H, Altmann T (2003) Brassinosteroids promote root growth in *Arabidopsis*. Plant Physiol 133:1261–1271

Nemhauser JL, Hong F, Chory J (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell 126:467–475

Perilli S, Di Mambro R, Sabatini S (2012) Growth and development of the root apical meristem. Curr Opin Plant Biol 15:17–23

Petricka JJ, Winter CM, Benfey PN (2012) Control of *Arabidopsis* root development. Annu Rev Plant Biol 63:563–590

Reed RC, Brady SR, Muday GK (1998) Inhibition of auxin movement from the shoot into the root Inhibits lateral root development in *Arabidopsis*. Plant Physiol 118:1369–1378

Remans T, Thijs S, Truyens S, Weyens N, Schellingen K, Keunen E, Gielen H, Cuypers A, Vangronsveld J (2012) Understanding the development of roots exposed to contaminants and the potential of plant-associated bacteria for optimization of growth. Ann Bot 110:239–252

Ren B, Liang Y, Deng Y, Chen Q, Zhang J, Yang X, Zuo J (2009) Genome-wide comparative analysis of type-A *Arabidopsis* response regulator genes by overexpression studies reveals their diverse roles and regulatory mechanisms in cytokinin signaling. Cell Res 19:1178–1190

Rich SM, Watt M (2013) Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. J Exp Bot 64:1193–1208

Saini S, Sharma I, Kaur N, Pati P (2013) Auxin: a master regulator in plant root development. Plant Cell Rep 32:741–757

Schiefelbein JW, Benfey PN (1991) The development of plant roots: new approaches to underground problems. Plant Cell 3:1147–1154

Shan X, Zhang Y, Peng W, Wang Z, Xie D (2009) Molecular mechanism for jasmonate-induction of anthocyanin accumulation in *Arabidopsis*. J Exp Bot 60:3849–3860

Shani E, Weinstain R, Zhang Y, Castillejo C, Kaiserli E, Chory J, Tsien RY, Estelle M (2013) Gibberellins accumulate in the elongating endodermal cells of *Arabidopsis* root. Proc Natl Acad Sci USA 110:4834–4839

Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT (2004) Root growth maintenance during water deficits: physiology to functional genomics. J Exp Bot 55:2343–2351

Singh VK, Garg R, Jain M (2013) A global view of transcriptome dynamics during flower development in chickpea by deep sequencing. Plant Biotechnol J 11:691–701

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43

Sweetman C, Wong D, Ford C, Drew D (2012) Transcriptome analysis at four developmental stages of grape berry (*Vitis vinifera* cv. Shiraz) provides insights into regulated and coordinated gene expression. BMC Genom 13:691

Takatsuka H, Umeda M (2014) Hormonal control of cell division and elongation along differentiation trajectories in roots. J Exp Bot 65:2633–2643

Titapiwatanakun B, Murphy AS (2009) Post-transcriptional regulation of auxin transport proteins: cellular trafficking, protein phosphorylation, protein maturation, ubiquitination, and membrane composition. J Exp Bot 60:1093–1107

Torti S, Fornara F, Vincent C, Andrés F, Nordström K, Göbel U, Knoll D, Schoof H, Coupland G (2012) Analysis of the *Arabidopsis* shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. Plant Cell 24:444–462

Ubeda-Tomás S, Federici F, Casimiro I, Beemster GTS, Bhalerao R, Swarup R, Doerner P, Haseloff J, Bennett MJ (2009) Gibberellin signaling in the endodermis controls *Arabidopsis* root meristem size. Curr Biol 19:1194–1199

Vilaine F, Kerchev P, Clément G, Batailler B, Cayla T, Bill L, Gissot L, Dinant S (2013) Increased expression of a phloem membrane protein encoded by *NHL26* alters phloem export and sugar partitioning in *Arabidopsis*. Plant Cell 25:1689–1708

Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H, Schmülling T (2003) Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant Cell 15:2532–2550

Woeste KE, Ye C, Kieber JJ (1999) Two Arabidopsis mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. Plant Physiol 119:521–530

Wu W, Cheng S (2014) Root genetic research, an opportunity and challenge to rice improvement. Field Crop Res 165:111–124

Xu ZS, Huang Y, Wang F, Song X, Wang GL, Xiong AS (2014a) Transcript profiling of structural genes involved in cyanidin-based anthocyanin biosynthesis between purple and non-purple carrot (*Daucus carota* L.) cultivars reveals distinct patterns. BMC Plant Biol 14:262

Xu ZS, Tan HW, Wang F, Hou XL, Xiong AS (2014) CarrotDB: a genomic and transcriptomic database for carrot. Database 2014:bau096

Yokoyama A, Yamashino T, Amano Y-I, Tajima Y, Imamura A, Sakakibara H, Mizuno T (2007) Type-B ARR transcription factors, ARR10 and ARR12, are implicated in cytokinin-mediated regulation of protoxylem differentiation in roots of *Arabidopsis thaliana*. Plant Cell Physiol 48:84–96

Zhou ZY, Zhang CG, Wu L, Zhang CG, Chai J, Wang M, Jha A, Jia PF, Cui SJ, Yang M, Chen R, Guo GQ (2011) Functional characterization of the *CKRC1/TAA*1 gene and dissection of hormonal actions in the *Arabidopsis* root. Plant J 66:516–527