Comparative transcriptome analysis of roots at different development stages reveals the mechanism of root expansion of *Callerya speciosa* Champ.

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Research article

Keywords: *C. speciosa*, Tuberous roots, Different development stages, Transcriptome

Posted Date: November 18th, 2019

DOI: <https://doi.org/10.21203/rs.2.17390/v1>

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Abstract

Background *Callerya speciosa* Champ., a perennial shrub medicinal plant of Fabaceae, is one of the most commonly used in traditional Chinese medicine and food that has been widely cultivated in South China. The tuberous roots of *C. speciosa* transferred from fibrous roots but few fibrous roots can transfer into tuberous roots. To date, the underlying mechanisms for tuberous root formation and development of *C. speciosa* have not yet been elucidated.

Introduction

Callerya speciosa Champ., commonly used for food and medicine, is a perennial shrub medicinal plant of the *Fabaceae* family. This species is mainly distributed in Fujian, Guangdong, Guangxi, Guizhou and Hainan Provinces in China and has a widely cultivation area in South China. The tuberous roots of *C. speciosa* produce more than 41 primary medicinal compositions, such as maackiain, formononetin, and a variety of polysaccharides *etc* [1], and used extensively in many traditional Chinese medicines. Modern pharmacological studies have demonstrated that *C. speciosa* have significant contributions to immunity enhancement, hepatoprotection, arresting cough, expectorant and anti-asthmatic effects [2]. The swelling of storage roots is affected by the balance of cambium propagation and lignification. Only those possessed high ability of cambium propagation and low-lignification develop into tuberous roots, which is determined by genetic, physiological, and environmental factors [3]. Few fibrous roots can transfer into storage roots, but mechanisms underlying tuberous root development have not yet been elucidated.

Some progress has been made in the morphoanatomical and molecular studies of storage-organ formation for several tuber crops *Ipomoea batatas* (sweet potato) [4], *Solanum tuberosum* (potato) [5], *Manihot esculenta* (cassava) [6], and medicinal plant *Rehmannia glutinosa* [7] and *Trichosanthes kirilowii* Maxim [8] in recent years. In particular, the anatomical process of storage root formation in sweet potato has been well described since the 1910s by McCormick [9]. The vigorous secondary xylem and phloem is constantly developed by the differentiation of vascular cambia including both primary cambia and secondary vascular cambia, which promotes the cell division and expansion of parenchyma cells for storage of starch granules. Stele lignification during the early phase of storage root development is generally identified as the main factor to limit the expansion of roots [10]. The anatomical properties of medicinal plant *R. glutinosa* are similar to those of sweet potato [3], but yet has never been studied in *C. speciosa*.

Endogenous phytohormones are also closely related to the initiation and development of tuberous roots. The synergistic actions of various phytohormones finally result in the bulking of tuberous roots, such as auxin (IAA), cytokinins (CKs), abscisic acid (ABA), gibberellin (GAs), ethylene and jasmonic acid (JA) [11]. Auxins can be considered as a general coordinator of growth and development, who play an important role in tuberization and stress resistance [12], proliferation of metaxylem and cambium cells, cell expansion [13]. CKs control numerous developmental processes throughout the plant life cycle, including gametogenesis, root meristem specification, vascular development, shoot and root growth,

meristem homeostasis, senescence, and so on [14]. ABA was also essential for tuber formation and it might play roles in storage root bulking at the later stage of storage root development [13]. Previous studies have shown that ABA and GA antagonistically mediate the tuberization, and GA is considered as a dominant negative regulator for stolon elongation and tuber formation [16]. The literatures also revealed that JA might promote the initiation of storage root formation by enlarging the meristematic cells [15].

Many key genes regulating storage root formation, especially transcription factors (TFs), have been intensively studied. Several *MADS-box* genes (*lbMADS1*, 3, 4, 10, 79; *lbMBP1~9*; *lbAGL17*, 20; *StMADS1*, 3, 11, 13, 16, 29; *SRD1*) have been found to significantly express in root tissues of sweet potato and potato, and their possible functions in tuber development have subsequently been studied [13,17]. Three sweet potato class 1 knotted1-like homeobox (*KNOX1*) genes (*lbkn1*, *lbkn2* and *lbkn3*) all expressed in the storage roots, but only *lbkn1* and *lbkn2* were obviously up-regulated in storage roots [18]. Tanaka et al. suggested that seven members of *SRFs* genes developmentally up-regulated during storage root formation but three of those down-regulated [19]. *SRF6*, a gene encoded a receptor-like kinase (*RLK*) and high-expressed around the primary cambium and meristem in the xylem, was related to the thickening of storage root. The *Dof* zinc finger transcriptional factor *SRF1* modulated the carbohydrate metabolism and highly expressed in the storage roots of sweet potato[20]. A cDNA library of early stage storage roots in sweet potato was constructed and 22 differentially expressed genes between early stage storage root and fibrous root were identified, including *NAM-like* genes and *MADS-box* genes, and *Extensin-like* genes[21]. However, an expansin gene (*lbEXP1*) was identified to decrease significantly at the young storage root stage in sweet potato and play a negative role in the formation of storage root by suppressing the proliferation of metaxylem and cambium cells to inhibit the initial thickening growth of SRs [22]. In potato, *CONSTANS (CO)*//*FLOWERING LOCUS (FT)* module has been proven to participate in controlling tuberization. Two different FT-like paralogues (*StSP3D* and *StSP6A*) could control the tuberization transition [23], while a *CO-like* gene *StCOL2* repress tuberization in a photoperiod-dependent manner by affecting the mRNA levels of *StBEL5* (a tuberization promoter) [24]. In addition, several miRNAs, like *miR172/miR3946*[3], have been identified to be involved in tuberization. However, little is known about the molecular regulatory mechanism in the development and the expansion of *C. speciosa* tuberous roots.

The expression profiles of differential genes involving in tuberous roots are efficiently detected by using transcriptome techniques. Among the adventitious root (AR), thickening adventitious root (TAR), and the developing tuberous root (DTR) of *Rehmannia glutinosa*, 6794 differentially expressed unigenes involved in phenylpropanoid biosynthesis, starch and sucrose metabolism, and plant hormone biosynthesis were identified during root development by using Illumina sequencing and de novo assembly strategies [7]. Wang et al. revealed the temporal patterns of gene expression associated with tuberous root formation and development in sweet potato through genome-wide microarray analysis [25]. Phytohormone action and 11 agronomic traits have been correlated with growth processes associated with tuberization in cassava through genome-wide analysis [6,26]. The putative genes involved in photoperiodic tuberization in potato were identified by using digital gene expression (DGE) tag profiling analysis [27]. Although some differentially expressed genes between storage root and fibrous root at its seedling stage of *C. speciosa*

were identified [28], the profile of differentially expressed genes at different tuberous root development stages is still poorly understood.

In this study, morphological and anatomical analysis, physiological and biochemical indexes of tuberous roots at three different development stages were performed. A different phenotype that is incapable of forming tuberous roots is valuable for illustrating the transition mechanisms of tuberous roots. Therefore, the phenotype of unexpanded fibrous roots (UFRs) was also collected. We made use of the samples (UFRs and tuberous roots at three representative tuberous root development stages) that Illumina sequencing was offering to identify the differential expressed genes. The objective of the study was to gain insight into the development process by identifying genes that are differentially expressed and bringing out specific features which are reflections to each representative development stage. A particular attention has been paid to the differential expressed genes between UFRs and TRs, as it will provide the evidence to elucidate the mechanism underlying the development switch for tuber formation.

Methods

Plant material

Seeds of *C. speciosa* (Champ.) Schot were incubated in wet perlite under dark conditions at 26 ± 2 °C until germination (approximately 4 d). Two days after germination, seedlings were cultured in a greenhouse which was equipped with a system for monitoring temperature ($25^\circ \pm 4$ °C), relative humidity ($80 \pm 5\%$) and natural photoperiod in the Guangxi Botanical Garden of Medicinal Plants. Every plant was supplied with about 500 mL of water per day and fertilized with 5 g fertilizer (N: P: K = 15:15:15) per month. The soil was a mixture of peat soil, washed sand and yellow loam with equal parts. Samples at the initiated tuberous stage (ITRs), middle tuberous stage (MTRs) and late tuberous stage (LTRs) were collected when seedlings were cultured from one-year to three-year and the diameter was satisfied with 5–12 cm, 12–20 cm and more than 20 cm, respectively. These unexpanded fibrous roots (UFRs) were selected from 3-year seedlings of unexpanded phenotype (the diameter less than 5 cm). The roots of five plants were collected for each sample at 10 am, immediately freezed in liquid nitrogen and stored at -80 °C before RNA isolation. Samples were identified by Prof. Ding Fang and Yunfeng Huang from Guangxi Institute of traditional Chinese Medicine & Pharmaceutical Science. The voucher specimens were deposited in Guangxi Botanical Garden of Medicinal Plants (No. 12110401).

Anatomical and physiological indexes analyses

The samples of tuberous roots and UFRs were cut into some pieces with surgical blades immediately after collection and fixed in 70% osmotically balanced formalin/acetic acid/ alcohol (FAA) fixative solution buffer for more than 12 h for anatomical analyses, in order to understand the developmental character of tuberous roots and define the key stages of tuberous root development. After samples were rinsed in ddH₂O three times, they were dehydrated using an increasing ethanol series (70, 80, 85, 90, 95

and 100%, 1 h for each step), and permeabilized in turpentine oil. The paraffin section method and optical microscope pictures photographed were performed as previously described [29] and the roots were cut into transection (10 μm thick). Serial tissue sections were stained with aqueous safranin O followed by fast green FCF in absolute alcohol. Cellulose content was determined using the anthrone method [30]. Lignin content was determined using the Klason method [31]. Phenol-sulfuric acid method was used to determine the content of polysaccharide [2]. Starch content was determined according to Firon [10].

Total RNA extraction

Total RNA (5 μg) was isolated from roots by following the method described by Liu [32] with slight modifications. Briefly, 1.0 mL TRIzol reagent was added into 100 mg of root sample, the sample was homogenized and centrifuged at $12,000\times g$ for 10 min at 4 °C. Then, the supernatant was transferred into a new tube after the fatty layer was removed. The tube was mixed with 0.2 mL chloroform, shaken for 15 s, incubated for 3–5 min at room temperature and centrifuged $12,000\times g$ for 15 min at 4 °C. The aqueous phase was transferred into a new tube to precipitate RNA. RNase-free glycogen (10 μg) and 100% isopropanol (0.5 mL) were added into the tube, mixed and incubated for 10 min at room temperature. Samples were centrifuged at $12,000\times g$ for 10 min at 4 °C. The supernatant was discarded and the pellet was washed with 1 mL 75% ethanol. After vortexed the tube gently, the tube was centrifuged at $7500\times g$ for 3 min at 4 °C and the wash was discarded. The RNA pellet was air-dried, dissolved with 50 μL nuclease-free water. The purity and concentration of RNA were determined using Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of RNA was analyzed by agarose gel electrophoresis.

Library construction and transcriptome sequencing

For each sample, equal amount (20 μg) of total RNA (RIN > 8.0) was applied to construct cDNA library and sequence using BGISEQ–500 platform. In briefly, the Oligo (dT) selection was used to enrich the poly (A) mRNAs. The mRNAs were then chemically fragmented to double-strand cDNA (dscDNA) by N6 random primers. The synthesized cDNA was subjected to end-repair and then was 3' adenylated. Adaptors were ligated to the ends of these 3' adenylated cDNA fragments. The ligation products were purified and many rounds of PCR amplification were performed to enrich the purified cDNA template using PCR primer. Denature the PCR product by heat and the single strand DNA is cyclized by splint oligo and DNA ligase. Finally, the final cDNA libraries were sequenced using BGISEQ–500 platform. A total of twelve libraries (three replicates per sample) were sequenced and the results were analyzed by the BGI Tech Solutions Co., Ltd. (BGI Tech) (BGI, Shenzhen, PR China). The sequence reads were submitted into the NCBI Short Read Archive (SRA) database and included in NCBI BioProject database with accession number PRJNA574557 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA574557>).

***De novo* assembly and annotation of transcriptome**

Adaptor sequences and low-quality reads were filtered to generate high-quality reads, whose quality were controlled by FASTQ format [33]. The *de novo* assembly and analysis of transcriptome was carried out using the superTranscripts function of Trinity software (v2.4.0) (release 2014-07-17) according to the protocol [34], to construct the possible 'gene' sequences of *C. speciosa*. To annotate the gene ontology (GO) and biology pathway, several public databases, including NCBI non-redundant (NR), Kyoto Encyclopedia of Genes and Genomes (KEGG) and UniProt were performed. Firstly, all the assembled genes were aligned in databases mentioned above using the BLAST software (version: v2.2.23) [35], and a cut-off of e-value (1×10^{-5}) could filter the matched transcripts. Secondly, we used BLAST2GO software (v2.5.0) [36] to retrieve the resulting transcripts associated GO items classifying to biological processes (BPs), cellular components (CCs) and molecular functions (MFs). Using an e-value $\leq 1e^{-5}$, the corresponding enzyme commission (EC) numbers for unigenes were produced. The transcripts with corresponding ECs were mapped to KEGG metabolic pathway database. Finally, the candidate coding areas in the transcriptome were identified using TransDecoder (v3.0.1) [37]. ORF of each unigene was searched using getorf (v EMBOS6.5.7.0) [38] and then aligned ORF to TF domains (from PlntfDB) using hmmsearch (v3.0) [39] to identify transcription factors (TFs).

Transcriptome profiling and DEGs analysis

To profile the gene expression in different development stage, Bowtie2 (v2.2.5) [40] and RSEM (v1.2.12, RNA-Seq by Expectation-Maximization) [41] tools were applied in this study to mapped to the Trinity assembled clean reads of ITRs (ITR1, ITR2 and ITR3), MTRs (MTR1, MTR2 and MTR3), LTRs (LTR1, LTR2 and LTR3) and UFRs (UFR1, UFR2 and UFR3), and estimate the abundance of reads that aligned to the transcripts, respectively. Differential expression of transcripts in different samples was also evaluated by fragments per kilobase of transcript per million mapped reads (FPKM) in order to normalize the calculation of gene expression. The differential expression of transcripts among samples was directly compared by using DEGseq software [42], with a threshold of FPKM >10 in at least one tuberous sample, Log_2FC (Fold Change) >1 (up-regulated) or Log_2FC <-1 (down-regulated), *q-value* <0.05 and *p-value* <0.5.

GO and KEGG analysis of DEGs

We performed GO and KEGG pathway classification and functional enrichment on DEGs. GO has three ontologies: molecular biological function, cellular component and biological process, which were classified respectively for all the candidate genes.

p-value (Fisher's exact test) and *q-value* are both values that indicate the degree of enrichment and *q-value* is corrected *p-value* [43]. The smaller they are, the more significantly DEGs enriched to this pathway. Significant GO items and KEGG pathways should satisfy *p-value* < 0.5 and *q-value* < 0.05. Those not

related to plant GO items and KEGG pathways were filtered. CDSs were also predicted by BLASTx (E -value $\leq 10^{-5}$) based on the four public protein databases (NR, SwissProt, KOG and KEGG).

Quantitative real-time PCR analysis

To validate the expression patterns of candidate genes, quantitative real-time PCR (qRT-PCR) experiment was performed to confirm the expression patterns in the different development stage of roots, as previously described [44]. Briefly, total RNA was extracted by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and quantified with *NanoDrop 2000c* spectrophotometer (*Thermo Scientific*, Waltham, MA, USA). Only those RNA preparations, which satisfied the requirement for an A260/A280 ratio of 1.9 to 2.1 and an A260/A230 ratio greater than 2.0, were used for further analysis. cDNA was synthesized from total RNA (1 μ g) using Prime ScriptTM RT reagent kit with gDNA eraser (Perfect Real Time) kit (Takara, Dalian, China) after Genomic DNA was cleaned up using the gDNA eraser procedure. The forward and reverse primers of sixteen randomly selected DEGs were designed using Primer 5.0 (Additional file 1: Table S1a) and synthesized by BGI-Shenzhen. Real-time reactions were performed with a total 20 μ L of reaction mixture, including SYBR[®] Primix Ex TaqTM (Bio-Rad) (10 μ L), cDNA template (200 ng, 2.0 μ L), each primer (10 mM) (1.0 μ L) and ddH₂O (6.0 μ L). No-template controls (NTC, ddH₂O instead of cDNA) were also carried out for every primer pair to judge the template contamination. All PCR reactions were amplified via the CFX 96TM real-time system (Bio-Rad, Hercules, CA, USA) under following conditions: 1 cycle at 95 °C for 3 min, 40 cycles (each consisting of 10 s at 95 °C, 30 s at 60 °C, and 10 s at 95 °C), and a melting curve analysis by heating from 65 to 95 °C with a speed of 0.5 °C per 5 s. Each candidate gene in each sample was conducted in three biological replicates with three technique repeats. 60S ribosomal protein L34 (60S) was selected as a reference gene, because of its relatively stable expression in root transcriptions of *C. speciosa* (data unpublished) (Additional file 1: Table S1b). Therefore, the expression level of each gene was also normalized to those of 60S. The relative expression of each candidate gene was calculated by the $2^{-\Delta\Delta C_q}$ formula [45].

Statistical analyses

Three repetitions were performed to determine each value and their standard deviations. Data were analyzed using IBM SPSS Statistics 19.0 software (Ehningen, Germany) and presented as the means $\pm$ standard deviations (SD). The statistical significance was determined using Student's *t* test. Values in figures marked with different lowercase and capital letters are significantly different at 0.05 and 0.01 probability levels, respectively.

Results

Anatomical characteristics of key developmental stages of tuberous roots

Three representative development stages (ITRs, MTRs, LTRs) and the unexpanded fibrous roots (UFRs) were defined (Fig.1 a-b). With the cell divisions and expansions in the primary cambium, secondary meristems, including vascular cambiums, meristems around the vessels and accessory cambiums differentiate in the secondary xylem and phloem, together with the scattered tertiary structures differentiated by accessory cambiums, trigger the expansion process. ITRs represent a critical point in the transition from adventitious roots to storage roots, in which secondary meristems appear in the fibrous roots (Fig. 1c, h). Since the tuberous root formation from ITRs stage, the percentage of secondary xylem in secondary vascular cambiums is much larger than that of phloem, in which mostly consist of parenchyma cells, and it increase along with the expansion process (Fig 1 d, i). Furthermore, the smaller proliferative cells proliferate next to the larger, mature parenchyma cells. In the LTRs stage, more vascular tissues are distributed in secondary xylem (Fig 1 e). UFRs are special phenotypes that lack the ability to trigger the expansion process, which are an optimal control material and different from the fibrous roots at the seedling stage. The anatomical structure of UFRs characterizes sclerenchyma cells, lignified cells and seldom starch grains, which hindered the swelling of roots (Fig. 1 f, j). In addition, more and more starch grains are stored in the parenchyma cells at LTRs stage of tuberous roots. The clustered crystals of calcium oxalate are also observed in the parenchyma cells in the secondary xylem (Fig. 1 g).

Only a few fibrous roots could develop into tuberous roots. Furthermore, the swelling stage of tuberous roots were also not synchronous (Fig. 1 i). The tuberous roots may be initiated early, late, or never, which is uncertain and difficult to regulate. Therefore, the root systems usually are composed of tuberous roots at different development stages and the unexpanded fibrous roots.

Transcriptome analysis of UFRs and TRs at different stages

A total of 782.57 M bases high-quality clean reads was obtained after eliminated the low-quality reads. All the sequence read data were uploaded to NCBI SRA database (PRJNA574557). After *de novo* assembly by Trinity program, 153,153 unigenes were finally acquired with a total length of 217.31 M bases and N50 value of 2,167 bp. A detailed overview of the sequencing and assembly results are shown in additional file 2: Table S2. Among these, the unigenes were with an average length and GC content were 1,418 bp and 41.67%, respectively (Table 1). In addition, the length distribution of all unigenes told us there were a total of 81,510 (53.22%) unigenes longer than 1,000 bp, with 15,684 (10.24%) unigenes longer than 3,000 bp (Additional file 3: Fig. S1a). Additionally, CDSs of 84,585(55.23%) were also predicted, in which 3,305 (3.91%) were longer than 3000 bp, 60,545 (71.58%) ranged from 500 bp to 3000 bp, and 20,735 (24.51%) were shorter than 500 bp. The average length of these CDSs was 1,120 bp. The statistics of gene numbers distributed in different FPKM range in all the samples was shown in additional file 3: Fig. S1b. More than 60% unigenes were commonly expressed (PPKM>1) in different samples within the group (Additional file 3: Fig. S1c). These results indicated that the quality of assembly sequences was sufficient for further analyses.

Functional annotation and classification of the assembled unigenes

All the assembled unigenes were annotated by mapping to seven public databases (NR, NT, SwissProt, KOG, KEGG, Pfam and GO) (Additional file 4: Table S3 and Additional file 5: Fig. S2a). A total of 106,882(69.79%), 100,406 (65.56%), 79,007(51.59%), 86,701 (56.61%), 86,688 (56.60%), 80,297 (52.43%) and 75,729 (49.45%) were aligned to these seven databases, respectively. The annotation of unigenes provides valuable information for the functional classifications as well as for unique genes discovery. The similarity distribution indicated that there were 21.30% of all the unigenes mapped to *Cicer arietinum* by aligning to NR database, followed by *Cajanus cajan* (15.96%), *Glycine max* (14.56%), belonged to the same Leguminosae family (Additional file 5: Fig. S2b). A heat map (Additional file 5: Fig. S2c) showed that the high correlation of RNA-Seq results among biological replicates from same sample.

All the sequences were divided into three categories (biological process, cellular component, and molecular function) using Blast2GO program, to describe their involvement in root growth and development process. All the unigenes were assigned to 41 GO terms, including 16, 12 and 13 groups in biological process, cellular component, and molecular function, respectively, and the eight-top for each category was listed in additional file 5: Fig. S2d. Among biological process, unigenes assigned to “cellular process” and “biological regulation” occupied the highly percentage, followed by the “metabolic process”. The functional genes may be induction, transduction and modulation during biological process of root development, which are potentially important topics to explore the mechanism of tuberous roots formation.

86,701 unigenes were further classified to 25 functional classifications in KOG database to differentiate the assembled sequences. The dominated classifications was “General function prediction only” (17,861 unigenes) and “Signal transduction mechanisms” (13,227 unigenes) (Additional file 6: Fig. S3a). Moreover, 81,412 unigenes were involved in the KEGG pathways (Additional file 6: Fig. S3b), which were categorized into five functional groups, including metabolism (58.77%), organismal systems (23.64%), environmental information processing (5.15%), genetic information processing and cellular processing (4.99%). The metabolism pathways assigned the most unigenes to global and overview maps (22.38%) and carbohydrate metabolism (10.01%), followed by amino acid metabolism (4.96%) and lipid metabolism (4.22%). Carbohydrate metabolism was identified to the most important pathway during tuberous root development, in which many carbohydrates as energy stored in tuberous roots. For carbohydrate metabolism, sub-pathways of starch and sucrose metabolism, amino sugar and nucleotide sugar metabolism, pentose and glucuronate interconversions were the three-top represented (Additional file 6: Fig. S3c), while phenylpropanoid biosynthesis was the most highly represented sub-pathways of biosynthesis of other secondary metabolites (Additional file 6: Fig. S3d).

Identification of differentially expressed genes (DEGs) TRs at different stages compared with UFRs

To identify the genes that are related to root expansion, three different development stages of tuberous roots (ITRs, MTRs and LTRs) and UFRs libraries were compared using RNA-Seq technology. Compared to UFRs, we identified 5,064 (3,726 up-regulated and 1,338 down-regulated), 5,623 (4,067 up-regulated and 1,556 down-regulated), 3,835 (3,087 up-regulated and 748 down-regulated) and 4,379 (3,086 up-regulated and 1,293 down-regulated) DEGs among ITRs, MTRs, LTRs and TRs (mixture of ITRs + MTRs + LTRs) respectively, with a threshold of FPKM >10 in at least one tuberous sample and the absolute value of Log_2FC >1 compared to UFRs (Fig. 2a). The complete list of DEGs is provided in Additional file 7: Table S4.

Venn diagram of DEGs could differentiate the commonly and specifically up-regulated and down-regulated unigenes in the three different stages of tuberous roots growth and development (Fig. 2b). 233 unigenes were found to exhibit commonly up-regulated and 133 unigenes were found to exhibit commonly at least 10-fold higher FPKM values compared to UFRs (Additional file 7: Table S4). Notably, some unigenes such as CL4275.Contig2_All (ethylene-responsive factor 2), CL4583.Contig1_All (elongation factor 1-beta 1) and CL5055.Contig3_All (sugar porter family MFS transporter) kept increasing, but others such as CL1398.Contig14_All (extensin-like protein), CL3473.Contig9_All (elongation factor 1-alpha) and CL15142.Contig1_All (60S ribosomal protein L10) kept decreasing during the process of root swelling.

Functional analysis of DEGs

For the commonly upregulated DEGs, they were mapped to 94 KEGG pathways, which were summarized to 17 represented categories (Fig. 2c). More than half categories were related to metabolism pathways, and these metabolic pathways may be closely involved to the development of tuberous roots and bioactive compounds synthesis, such as “metabolic pathways” (ko01100), “starch and sucrose metabolism” (ko00500) and “biosynthesis of secondary metabolites” (ko01110). Notably, 14 and 33 DEGs were involved in the pathway “plant hormone signal transduction” (ko04075) and “MAPK signaling pathway” (ko04016) in the expansion activities of roots, respectively. In biosynthesis of secondary metabolites pathway, 2 and 10 DEGs were identified to “flavonoid biosynthesis” (ko00941) and “phenylpropanoid biosynthesis” (ko00940), respectively, which is related to accumulation of medicinal ingredients, indicating that those ingredients might be accumulated along with the process of tuberization, especially at the LTRs stage. Heat maps and KEGG function enrichment analysis were also used to show the expression profiles and functions of the commonly DEGs (Additional file 9: Fig. S4). Detailed information of functional analysis for commonly upregulated and downregulated DEGs (346) was showed in Additional file 8: Table S5.

For the DEGs in TRs-vs-UFRs (4,351 unigenes), most of them were also involved in “metabolism” and “genetic information processing” pathways, such as “ribosome”, “flavonoid biosynthesis” and “glycolysis/gluconeogenesis” *etc.* (Additional file 10: Fig. S5 and Additional file 7: Table S4). Subsequently, we focused on these DEGs in several classical families whose products have been reported to be involved in metabolic processes and regulation of metabolic processes as well as functions related to tuberization development, such as transcription factor, hormone metabolism and signal transduction, sucrose and starch synthase, cell expansion and cell-wall synthesis, and calcium signaling transduction. Here we showed the statistics of these families as detailed below.

Transcription factors

The transcription factors family might be the largest gene family identified in this study. We identified 2427 unigenes encoding transcription factors (TFs) but only 90 of them were differentially expressed (64 up-regulated and 26 down-regulated) in TRs samples compared to UFRs (Table 2 and Additional file 11–12: Table S6-S7). These 90 DEGs mainly encode *AP2-EREBP* TFs, *WARY* TFs, *MAD* TFs, *NAC* TFs, *bHLH* TFs, *MYB* TFs, *bZIP* TFs. Several TFs, such as *AP2-EREBP* TFs, *WARY* TFs and *MYB* TFs, were specifically up-regulated expressed while others, including *bHLH* TFs, *HSF* TFs and *C2C2-GATA* TFs, were specifically down-regulated expressed in TRs compared to UFRs. In addition, the number of up-regulated DEGs encoded TFs in MTRs (93) and LTRs (77) samples was much more than that in ITRs (44), indicating that more transcription factors may play a positive role in the rapid-expansion and mature of tuberous roots. A heat map (Fig. 3) showed that all the *MYB* TFs had relatively higher expression in the expansion and elongation of roots, especially in MTRs stage. Most of the *bHLH* TFs were down-regulated along with the process of tuberous d development. However, the opposite trend was identified in most of the *WRKY* TFs and *NAC* TFs. It is difficult to determine the regulation of *AP2-EREBP* TFs, because they have different expression patterns during tuberous root development. Furthermore, the expression profile of differentially expressed TFs in LTRs and UFRs which were at the same growing period were compared and the result showed that many TFs were up-regulated in LTRs.

Hormone metabolism and signal transduction

We annotated 1241 unigenes that was related to hormone metabolism, of which 129 were differentially expressed (107 up-regulated and 22 down-regulated) in TRs vs UFRs group. The number of DEGs in ITRs and TRs was much more than that of others (Table 2). The DEGs mainly related to the biosynthesis and response to GA, auxin, CKs, ABA, ethylene and jasmonic acid (JA) were demonstrated (Additional file 11: Table S6). Auxin-related DEGs, including the Aux/IAA family and auxin response factor (ARF), were mainly detected in tuberous roots. All the unigenes encoding ARFs were up-regulated expressed and peaked at LTRs, whereas others are mainly especially up-regulated in ITRs and MTRs compared to UFRs. The expression of CL7973.Contig3_All and CL7973.Contig4_All was significantly higher than other unigenes in tuberous roots (Fig. 4a). Most DEGs encoding CKs were also up-regulated during the

development of tuberous roots and peaked at ITRs and MTRs stages. Unigene35772_All was especially up-regulated in ITRs, which might be involved in the initiation of tuberous roots (Fig. 4b). Some DEGs that associated with abscisic acid (ABA), such as CL2304.Contig5_All and CL2304.Contig7_All, were most highly expressed at the later stage of storage root development (MTRs and LTRs) while DEGs related to gibberellin (GA) synthesis were at relatively high levels at the earlier stage (Fig. 4c-d). A lipoxygenase related to JA synthesis was more highly expressed in ITRs compared with UFRs, whereas CL418.Contig22_All was significantly induced at ITRs stage (Fig. 4e). Nearly all the DEGs related to ethylene metabolism and signaling had the high expression in all stages of tuberization. Interestingly, A transcript (Unigene900_All) functioned to encoding ethylene-responsive transcription factor was markedly up-regulated at MTRs stage, suggesting that it's important to tuberization (Fig. 4f). In total, the number of up-regulated DEGs related to hormone metabolism and signal transduction in tuberous samples was more than that in UFRs, indicating that the synthesis and metabolism, as well as the signal transduction of hormones may play an important role in the initiation, elongation and mature stage of tuberous roots. Some phytohormones (JA, GA) might essential for tuber initiation and formation, whereas ABA might be more critical for storage root bulking and mature. The auxins, ethylene and CKs continuously played roles from early to late stages of tuberization.

Cell expansion and cell-wall synthesis

We next analyzed the genes that related to cell expansion and cell-wall synthesis during the development of tuberous roots. Among the 485 expansion-related transcripts, 83 were differentially expressed and only 29 (10 up-regulated and 19 down-regulated) in TRs compared to UFRs, including *XET* (6), *Extensin* (4), *Expansin* (6) and *Elongation* (13) (Table 2 and Additional file 11: Table S6). We found that some DEGs, such as Unigene45810_All, Unigene45828_All and CL47.Contig1_All, were activated at MTRs and LTRs, suggesting they might be involved in development of tuberous roots. Meanwhile, some down-regulated DEGs related to cell extension and elongation were also identified in TRs vs UFRs. The transcript CL1398.Contig5_All and CL3473.Contig8_All were highly expressed at UFRs stage, indicating that they might be tuberization-limited factors (Fig. 4g). There were 103 transcripts that have the capacity of encoding cell wall synthesis, of which 16 were differentially expressed in TRs-vs-UFRs and peaked in ITRs or MTRs (Table 2 and Additional file 11: Table S6). The up-regulated gene CL8402.Contig5_All was highly expressed in both ITRs and MTRs stage and peaked at MTRs (Fig. 4g).

Calcium signaling transduction

The expression patterns of calcium signaling-related genes at different development stages of tuberous roots and un-expansion roots were also observed, including *CBL*, *CaM/CaM-binding*, *CBP* and *CSES*. Among the 381 transcripts, 27 (7.09%) were differentially expressed, in which 10 up-regulated and 12 down-regulated DEGs in TRs compared to UFRs (Table 2 and Additional file 11: Table S6). The expression of three *CaM/CaM-bindings*, five *CBPs* and two *CSEs* peaked in MTRs sample, in which the number of

DEGs was also more than other samples. The expression of Unigene42383_All (peaked in MTRs) and Unigene786_All (peaked in UFRs) were much higher than others (Fig. 4h). We suggested that Unigene42383_All might play a key role in the development of tuberous root.

Sucrose and starch synthase

Genes related to sucrose and starch syntheses were also selected for profiling during the development of tuberous roots. There were 59 transcripts that were involved in sucrose and starch biosynthesis respectively, and 19 sucrose synthases and 7 starch synthases were differentially up-expressed in TR samples compared to UFRs (Table 2 and Additional file 11: Table S6). The expression of starch synthase genes kept high expression in MTRs and LTRs, *e.g.* Unigene35652_All and Unigene49607_All (Fig. 4i). Most of sucrose synthase genes were highly expressed in ITRs and MTRs, suggesting that they played important roles in the initiation and swelling of tuberous roots. Some genes, such as CL3807.Contig7_All, CL3807.Contig36_All and Unigene25361_All are especially high-expressed in ITRs stage, which might contribute to the sucrose formation and accumulation in the initiation of tuberous root formation. CL3807.Contig32_All might participate in the swelling of tuberous roots (Fig. 4j). Generally, DEGs related to starch synthesis might play a greater role in later stages of tuberous root formation (MTRs and LTRs) accompanied by carbohydrate metabolism, whereas sucrose rapid accumulation might occur in the earlier stages (ITRs and MTRs) in tuberous roots of *C. speciosa*.

Lignin and cellulose-related

We identified 20 genes encoding lignin-related proteins but only 4 of them were differentially expressed in tuberous roots compared to UFRs (Table 2 and Additional file 11: Table S6). These four genes were all annotated as cinnamyl alcohol dehydrogenase and up-regulated in UFRs stage (Fig. 4k). Among the 239 cellulose-related genes, only six were differentially expressed in TRs vs UFRs, in which three genes encoding the cellulose synthase-like protein were all up-regulated in the earlier stage of tuberous roots. CL14823.Contig2_All was activated during the process of tuberous root formation but the expression in UFRs sample was much higher than other samples (Fig. 4l).

Validation of candidate DEGs involved in tuberization

The relative expression levels of candidate DEGs associated with tuberization of roots were performed to assess the accuracy of transcriptome sequencing data. As shown in Fig. 5, the relative expression levels ($2^{-\Delta\Delta C_t}$) of all the selected genes were consistent with the transcriptome data (FPKM values). Nearly all the person correlation (R^2) between relative expression levels and FPKM values were more than 0.868 except

for CL6957.Contig8_All ($R^2 = 0.665$), which was significant at the 0.01 level (2-tailed), indicating that the qRT-PCR data confirmed the validity of the transcriptome.

Assays of physiological indexes related to storage root formation

To further discuss the consistency of gene expression and related physiological characters, four physiological indexes related to storage root formation were performed. Cellulose content in different samples was evaluated (Fig 6a). There was no significant difference between ITRs and MTRs, with a lower level at the initial and mid-stage of expansion. Cellulose content increased rapidly in the late-stage of expansion and reached 33.48%, which was more than 2-fold change. The expression of CL14823.Contig2_All could match the change in the content of cellulose at UFRs and LTRs, which was the best candidate gene for cellulose biosynthesis (Fig 4l). Coincidentally, the lignin content in UFRs was also significantly higher than others, with 4.68-fold compared to LTRs (Fig 6b), which may attribute to the high-expression of three lignin-related genes (Fig 4k). Polysaccharide and starch content were gradually increased during the expansion of roots. The data indicated a peak of polysaccharide formation (a nearly 2-fold increase) and starch accumulation (an over 3-fold increase) at the mature of tuberous roots. Polysaccharide content was found to be at a relatively high level (31.32%) but starch content at the lowest level (2.57%) in UFRs (Fig 6c-d). The regularity of starch and sucrose related gene expression was also respectively consistent with the content of starch and polysaccharide in our work (Fig. 4i-j). Therefore, the accumulation trend of cellulose, lignin, polysaccharide and starch were also highly in agreement with the expression patterns of the identified key genes related to their biosynthesis.

Discussion

The formation and development of tuberous roots is a complexly and genetically programmed process, which involves the proliferation of secondary meristems and the accumulation of carbohydrates. Previous studies suggested that vascular cambiums are formed at the beginning of tuberous root development. Subsequently secondary meristems differentiate in the xylem, together with divisions of large parenchyma cells in the xylem, finally lead to an expansion of root diameter. Accordingly, the anatomical analysis of three representative development stages (ITRs, MTRs and LTRs) and the unexpanded fibrous roots (UFRs) were performed in this study (Fig. 1). Our result showed that higher activity of secondary meristems and lower degree of lignification in the stele were key to increase the diameter of tuberous roots, which is consistent with the results of previous studies. In addition, more starch grains and other dry matter were accumulated in parenchymal cells at the LTRs stage, suggesting they might facilitate the expansion of cells and formation of medicinal component. Moreover, similarly to medicinal plant *Rehmannia glutinosa* [3], there are no obvious and distinct boundaries among the three tuberous stages, indicating that the initiation and expansion of tuberous roots are inseparable processes.

Some tuberous roots induction and initiation still occurs when others have been initiation at rapid-expansion or mature stage.

In this study, the transcriptome data revealed that a total of 153,153 unigenes (N50 = 2,167 bp) were identified, of which 86,701 unigenes were further classified 25 functional classifications (Additional file 6: Fig. S3). Compared to UFRs, 5,064, 5,623, 3,835 and 4,379 DEGs among ITRs, MTRs, LTRs and TRs (Mixture of ITRs + MTRs + LTRs) (FPKM>10 and Log2FC >1) respectively were also identified, including 233 and 133 commonly up- and down- regulated DEGs during tuberization process (Fig. 2). In TRs-vs-UFRs (4,351 unigenes), functional analysis showed that DEGs were mainly involved in “metabolism” and “genetic information processing” pathways (Additional file 9: Fig. S4). Our further analysis revealed that several gene families, such as transcription factors, hormone-regulated, sucrose and starch synthase, cell expansion and cell-wall synthesis, and calcium signaling regulated genes, might be participate in the tuberous root formation and development.

A series of transcription factors trigger the tuberous root initiation and development

Previous studies have revealed that many transcription factors are the important regulators of tuberous root formation [3]. Our transcriptome data identified a series of transcription factors trigger the tuberous root initiation and development of *C. speciosa*, including *MYB* (5), *bHLH* (15), *WRKY* (19), *NAC* (5), *AP2-EREBP* (20), *bZIP* (5), and 21 other transcription factors (Additional file 11–12: Table S6-S7), which were found differently expressed in TRs compared with UFRs. Moreover, the number of up-regulated DEGs encoded TFs in MTRs (93) and LTRs (77) samples was much more than that in ITRs (44), indicating that more transcription factors may play a positive role in the rapid-expansion and mature of tuberous roots. *MYB* genes are involved in various physiological and developmental processes. Li et al reported that most *R. glutinosa* *MYB* genes were differentially expressed in the tuberous root [3]. Similarly, all the *MYB* TFs were upregulated in the tuberous root of *C. speciosa* in the present study, especially at MTRs stage. In particular, CL7184.Contig1_All was strongly upregulated in the tuberous roots at the rapid-swelling stage (MTRs) compared with fibrous roots, which indicated that this gene might be a positive regulator during tuberous root development (Fig. 3). The basic helix-loop-helix (*bHLH*) was usually reported to form complexes with *MYB* families as MBW (*MYB*, *bHLH* and *WD40/WDR*) transcriptional regulatory complex and synergistically regulate that either activate or repress the expression of sets of target genes [46]. *MYB* activators are able to passively activate anthocyanin/ flavonoid biosynthesis and hormone signaling by coupling with *bHLH* proteins to compete with *MYB* repressors in the present study. At the MTRs and LTRs stage of tuberous root development, the expression of *bHLH* TFs exhibited the downregulation trend, indicating they might play the repressive function (Fig. 3). It has been reported that *WRKY* family transcription factors are one of the largest families of transcriptional regulators and act as transcriptional activators and repressors, which might be involved in plant responses to biotic and abiotic stress, development and senescence, hormone responses and calcium signaling [47]. For example, 111 *WRKY*

TFs were differentially expressed between storage roots and fibrous roots at the seedling stage of *C. speciosa* [28]. In our data, 19 genes annotated as *WRKYs* were differentially expressed in the TRs compared with UFRs library and most of them were upregulated (Fig. 3). Furthermore, six members of *WRKY* TFs were always differentially up-regulated expression in three tuberous development stages (ITRs, MTRs and LTRs) compared with UFRs (Additional file 11: Table S6). Further research is necessary to determine whether the expression of these genes is correlated with the regulation of tuberous root development. It has been suggested that *AP2-EREBP* are mainly involved in xylem/phloem differentiation, cell proliferation, tuberization, cell wall synthesis and secondary metabolism [3]. Our transcriptome data showed 9 *AP2-EREBP* TFs were upregulated during the process of tuberous root development, especially at the late stage (Fig. 3), suggesting these genes might play critical roles in the development and mature of tuberous roots by regulating the differentiation of xylem/phloem and accumulation of secondary metabolism. In sweet potato, *lbNAC1* has multiple biological functions in the JA response, including the inhibition of root formation, accumulation of anthocyanin *etc*, and might positively correlate with sporamin formation [48]. In our work, a *NAC* gene (Unigene5276_All) was highly expressed in LTRs stage (a 4.20 log₂fold increase with FPKM values from 5.67 to 104.77) and showed lower expression in the MTRs stage (Additional file 7: Table S4 and Fig. 5). In addition, other transcription factors, such as *bZIP*, *BTF3b-like* and *CCAAT-binding etc*, might also play roles in tuberous root initiation and development, perhaps at a certain stage.

Phytohormones synergistically participate in regulating the initiation and development of tuberous roots

Studies have demonstrated the initiation and development of tuberous roots regulated by endogenous phytohormones [11]. In this study, obvious changes were detected in the expression of genes related to auxins, CKs, GA, ABA, ethylene, JA and BRs in TRs, and majority of these genes were up-regulated in more than one tuberous development stage (Table 2 and Additional file 11: Table S6). More up-regulated DEGs related to hormone metabolism in tuberous samples was identified than in UFRs. Not only genes related to CKs and ethylene, but also those related to auxins showed the different up-regulated trends from early to late stages of tuberous root development (Fig. 4a-b, f and Additional file 11: Table S6), which might be considered essential in both initiation and thickening of tuberous roots. CL7973.Contig3_All and CL7973.Contig4_All related to auxins were thought to play vital roles in the development of tuberous roots. Unigene35772_All related to UDP-glucose flavonoid 3-O-glucosyltransferase peaked at ITRs, suggesting it might play an important role in the balance of CKs. Ethylene-responsive transcription factors (*ERFs*) usually combine with *APETALA2 (AP2)* to regulate expression of a wide variety of downstream target genes related to development and stress response [49]. The expression profile of Unigene900_All related to *ERFs* increased from early to late stages of tuberous root development and peaked at MTRs stage. ABA and GA antagonistically mediate the tuberization [9, 10], and high expression

of genes ABA was detected in the late stage of tuberous root development. CL2304.Contig5_All and CL2304.Contig7_All encoding abscisic stress ripening-like protein might induce the mature of tuber roots (Fig. 4c). Nevertheless, GA was reported to play the most prominent role in tuber initiation and genes involved in GA degradation do exhibit an upregulated profile during early stages of tuber development, leading to a rapid decrease of active GA content, thereby facilitating stolon-tip swelling [50]. Our result found most genes related to GA were involved in the initial stage. Two DEGs encoding GA (gibberellin 2-beta-dioxygenase CL3126.Contig4_All and gibberellin-regulated protein 1-like CL2301.Contig1_All) were upregulated during tuber initiation and swelling (Fig. 4d). Additionally, genes related to JA peaked at ITRs stage and are thought to be important in enlarging the meristematic cells and serve as promoters of tuber initiation (Fig. 4e). Furthermore, the ability of hormones synthesis and the balance of them are believed to determine the rate of storage root formation. The synergistically regulation of various phytohormones, such as JA & CKs [15], ABA and GA [16] have been found to play a critical role on the initiation and development of storage roots. Therefore, we hypothesized that there were hormone signals dominated by JA, GA and ABA during the formation and expansion of tuberous roots of *C. speciosa*, in which JA and GA might essential for tuber initiation and formation, whereas ABA might be more critical for storage root bulking, which warrants further investigation.

Multiple pathways are activated in regulating the initiation and development of tuberous roots

The initiation and development of storage roots constitute a highly complicated process that involves multiple pathways, which regulate the development of secondary anomalous cambia and the accumulation of starch, sucrose and other chemical components [11]. Accordingly, our study showed that carbohydrate metabolism, which mainly involved metabolic pathways (ko01100), starch and sucrose metabolism (ko00500) and biosynthesis of secondary metabolites (ko01110), was identified to the most important pathway during tuberous root development (Fig. 2 and Additional file 8: Table S5). Additionally, some DEGs in carbohydrate metabolism pathways, including 21 commonly up-regulated DEGs and 10 commonly down-regulated DEGs compared to UFRs, substantially regulated at three different storage root stages (Additional file 8: Table S5).

Similar to the result in *R. glutinosa* [3], several genes of MAPK and hormone signaling pathways were also up-regulated in TRs (Additional file 7–8: Table S4-S5), indicating that they participated in the induction and development of tuberous roots. Moreover, many studies have demonstrated that calcium signaling played important roles in the process of plant growth and development, including cell division, cell differentiation, and tuber initiation [51–52]. We also found that the Ca²⁺-binding proteins CaM, CBP, and CBL recognize and transduce Ca²⁺ signals to downstream genes as inhibitors or promoters in tuber swelling, in which *CBP* might be the promoters for tuberization (Additional file 11: Table S6). Unlike the result by Li [3], who suggested Ca²⁺ signals were involved in the initiation of tuberous root formation, we found that the expression of several DEGs related to Ca²⁺ signaling significantly increased at MTRs stage

of *C. speciosa* and speculated that the development of tuberous roots might attributable to them, e.g. Unigene42383_All. More evidence is needed to demonstrate the role of Ca^{2+} signaling-related genes.

In addition, plant cell walls consist mostly of crystalline cellulose fibrils embedded in a matrix of complex polysaccharides, and the cell enlargement was attributed to the growth of cell walls [53]. In this study, the transcriptome data also revealed that some genes involved in cell expansion and cell-wall synthesis were also identified to be activated in three different storage stages compared to UFRs (Additional file 11: Table S6). In general, genes encoding *XET* and cell wall synthesis were up-regulated in development of tuberous roots while genes encoding expansions and elongations were down-regulated (Additional file 11: Table S6). *XET* genes were reported to act as a signal switch in the feedback regulation of ethylene signaling [54] and several *XET* genes in *C. speciosa* were activated during the process of tuberization. Expansins are classified by sequence-based phylogeny into two classes: *EXPLA* and *EXPLB*. *EXPLA*s were identified as mediators of acid-induced wall loosening while little has been known about *EXPLB*s [55]. Homologs of *EXPLA1*, *EXPLA2* and *EXPLA4* were down-regulated and that of *EXPLB15* was up-regulated, which need further research in *C. speciosa*.

Down-regulated of lignin biosynthesis and up-regulated of carbohydrates accumulation facilitate the tuberous process

Genes encoding cellulose synthase enzymes are involved in cellulose biosynthesis to form secondary cell wall, such as UDP-arabinopyranose mutases (*UAMs*) [56]. In this study, cellulose deposition increased rapidly in the late-stage of expansion, whereas the content of cellulose in UFRs was markedly higher than in three stages of tuberous roots (Fig. 6a), which might be attribute to the expression of CL14823.Contig2_All (related to *UAMs*) (Fig. 4l). Lignin deposition in roots significantly inhibited tuberous root formation [57]. Coincidentally, the lignin content in UFRs was also significantly higher than others, with 4.68-fold compared to LTRs (Fig 6b), in agreement with up-expression of three key enzyme genes (cinnamyl alcohol dehydrogenase, *CAD*) of lignin biosynthesis (Fig 4k).

Sucrose is likely to serve as nutrient and signal molecule at the same time. The surplus of sugar is identified as one of the main factors resulted in tuberization, and sucrose has been reported as a promoter to stimulate the formation of tuberous roots in several plants [58–59]. In the starch and sucrose metabolic pathway, the synthesis of sucrose is catalyzed by sucrose phosphate synthase, while sucrose could convert into UDP-glucose and fructose by sucrose synthase. The expression of sucrose synthase was mainly up-regulated in the earlier stages (ITRs and MTRs) (Fig. 4j and Additional file 11: Table S6), which may play a key role in the initiation of tuberous roots of *C. speciosa*.

Starch and polysaccharide are both important form of carbohydrates stored in tuberous roots of *C. speciosa*. The polysaccharide of *C. speciosa* mainly consists of glucose, fructose, and galactose *etc.* The

content of polysaccharide was gradually increased along with the development process of tuberous roots (Fig 6c) and up-regulated by some sucrose synthase genes, e.g. CL3807.Contig32_All (Fig 4j). Several enzymes including granule-bound starch synthase (GBSS), starch branching enzyme (SBE) and UDP-glucose pyrophosphorylase (UDPGP), are pivotal in sweet potato starch biosynthesis [60–62]. Unigene35652_All and Unigene49607_All related to GBSS were significantly upregulated in the MTRs and LTRs stages of tuberous root development in our work (Fig. 4i and Additional file 11: Table S6), meanwhile significant levels of starch accumulation were detected the MTRs and LTRs stages (Fig. 6d). These findings indicated that the accumulation of starch increased along with the process of tuberous root development. Together with the anatomical, physiological and transcriptome results in this study, we speculated that down-regulated of lignin biosynthesis and up-regulated of carbohydrates (starch, cellulose and polysaccharide) accumulation facilitate the tuberous process of *C. speciosa*.

Conclusions

Tuberous root initiation and development are very important for the quantity and quality of *C. speciosa*. A combination of morphological, physiological and transcriptome analysis was performed in this study. Multiple tuberous root development-associated genes, including transcription factors, Ca^{2+} and MAPK signaling, phytohormones, conversion of sugars and starches, cell expansion and cellulose biosynthesis, were differentially upregulated between TRs and UFRs, which might participate in either the whole process or one certain stage of tuberous roots (Additional file 13: Fig. S6). This is the first transcriptome study of *C. speciosa* between tuberous roots at three different stages and unexpanded fibrous roots. A large gene dataset generated in the present study, which is valuable for further research, will facilitate the underlying of molecular mechanisms regulating the development switch for tuber formation and contribute to the breeding of *C. speciosa*.

Additional Files

Additional file 1: Table S1. a. Primers used for qRT-PCR analysis. *b.* Ranking of reference genes stability analysis by three different algorithms. (XLS 33 kb)

Additional file 2: Table S2. Summary of the BGISEQ–500 RNA-Seq data analysis. (XLS 28 kb)

Additional file 3: Fig. S1. Distribution and Venn diagram analysis of transcripts. (PDF 329 kb)

Additional file 4: Table S3. BLAST analysis of the full-length transcripts against public databases. (XLS 25 kb)

Additional file 5: Fig. S2. Annotation of the assembled transcriptome and gene expression profiling. (PDF 329 kb)

Additional file 6: Fig. S3. Functional analysis of the assembled transcriptome. (PDF 349 kb)

Additional file 7: Table S4. DEGs at the different developmental stages of tuberous roots compared to UFRs. (XLS 3875 kb)

Additional file 8: Table S5. Functional analysis of commonly upregulated and downregulated DEGs. (XLS 335 kb)

Additional file 9: Fig. S4. Heat maps and KEGG function enrichment analysis for the commonly upregulated and downregulated differentially expressed genes (DEGs). (PDF 483 kb)

Additional file 10: Fig. S5. Function enrichment analysis of DEGs in the tuberous roots (mixture of ITRs + MTRs + LTRs) (TRs) compared to UFRs. (PDF 480 kb)

Additional file 11: Table S6. Several differentially expressed families of DEGs in TRs compared with UFRs. (XLS 116 kb)

Additional file 12: Table S7. TFs that are differentially expressed in TRs compared with UFRs. (XLS 28 kb)

Additional file 13: Fig. S6. A preliminary scenario for the mechanism of tuberous roots initiation and development in *C. speciosa*. Genes in red frames and dots were up-regulated genes. (PDF 454 kb)

Abbreviations

ABA: abscisic acid;

CKs: cytokinins;

COG: Clusters of Orthologous Groups;

FPKM: fragments per kilobase of transcript per million mapped reads;

GA: gibberellin;

GO: Gene ontology;

TF: transcription factor;

ITRs: the initiation stage of tuberous roots;

JA: jasmonic acid;

KEGG: Kyoto encyclopedia of genes and genomes;

KOG: EuKaryotic Orthologous Group;

Log₂FC: Log₂ fold change;

LTRs: the late-swelling stage of tuberous roots;

MTRs: the mid-swelling stage of tuberous roots;

NR: NCBI non-redundant database;

qRT-PCR: quantitative real-time polymerase chain reaction;

RSEM: RNA-Seq by Expectation-Maximization;

SRA: sequence read archive;

TRs: tuberous roots;

UFRs: unexpanded fibrous roots.

Declarations

Acknowledgements

We acknowledge Mr. Dajie Zhou from Beijing Genomics Institute (Shenzhen) for the assistance of transcriptome sequencing.

Funding

This work was supported by the National Science Foundation of China (81760689), the National Science Foundation of Guangxi (2018GXNSFAA281287, 2016GXNSFBA380008), Fund of Guangxi Innovation-driven Development (AA17204056), Key Research and Development Program of Guangxi (AB16380210), and Youth Innovation research team project of Guangxi University of traditional Chinese medicine (2018QT001). The Funding bodies had no influence over the experimental design, data analysis or interpretation, or manuscript writing.

Authors' contributions

SY, RH and CP designed the research; SY, ZL and JG performed the sample preparation, total RNA extraction, anatomical and physiological data collection; SY, RH and CP performed the RNA-seq data and statistical analysis; SY and CP wrote the manuscript; RH and YT revised the manuscript; All authors read and approved the final manuscript.

Ethics approval and consent to participate

No specific permits were required for the described field studies. The location is not privately-owned or protected in any way, and the field studies did not involve endangered or protected species.

Consent for publication

Not applicable.

Availability of data and materials

Data supporting the results of this study can be obtained from [NCBI], but the availability of these data is limited (accession number: SAMN12859809, SAMN12859810, SAMN12859811, SAMN12859812, SAMN12859813, SAMN12859814, SAMN12859815, SAMN12859816, SAMN12859817, SAMN12859818, SAMN12859819, SAMN12859820). These data are used under the permission of the current study and are therefore not publicly available. We chose to publish our data on October 20, 2020. Until such a time, the data is available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Statement

The authors have the appropriate permissions to use the samples for experimental research.

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References

1. Wang C, Wang Y, Wang G, Ya J, Zhang X, Ye Wen-Cai. Chemical constituents from roots of *Millettia speciosa*. Chinese Traditional & Herbal Drugs. 2008; 39(7):972–975.
2. Yao S, Bai L, Lan Z, Tang M, Zhai Y, Hao H, Wei R. Hairy root induction and polysaccharide production of medicinal plant *Callerya speciosa* Champ. Plant Cell Tissue & Organ Culture. 2016; 126(1):177–186.

- 3.Li M, Yang Y, Li X, Gu L, Wang F, Feng F, Tian Y, Wang F, Wang X, Lin W, Chen X, Zhang Z. Analysis of integrated multiple 'omics' datasets reveals the mechanisms of initiation and determination in the formation of tuberous roots in *Rehmannia glutinosa*. J Exp Bot. 2015; 66(19): 5837–5851.
- 4.Wang H, Yang J, Zhang M, Fan W, Firon N, Pattanaik S, Yuan L, Zhang P. Altered phenylpropanoid metabolism in the maize Lc-expressed sweet potato (*Ipomoea batatas*) affects storage root development. Sci Rep. 2016; 6:18645.
- 5.Lakhotia N, Joshi G, Bhardwaj AR, Katiyar-Agarwal S, Agarwal M, Jagannath A, Goel S, Kumar A. Identification and characterization of miRNAome in root, stem, leaf and tuber developmental stages of potato (*Solanum tuberosum* L.) by high-throughput sequencing. BMC Plant Biol. 2014; 14(1):6–22.
- 6.Zhang S, Chen X, Lu C, Ye J, Zou M, Lu K, Feng S, Pei J, Liu C, Zhou X, Ma P, Li Z, Liu C, Liao Q, Xia Z, Wang W. Genome-Wide association studies of 11 agronomic traits in cassava (*Manihot esculenta* Crantz). Front Plant Sci. 2018; 9:503–518.
- 7.Sun P, Xiao X, Duan L, Guo Y, Qi J, Liao D, Zhao C, Liu Y, Zhou L, Li X. Dynamic transcriptional profiling provides insights into tuberous root development in *Rehmannia glutinosa*. Front Plant Sci. 2015; 6:396–410.
- 8.Zhao F, Wang R, Xue J, Duan Y. Efficient callus-mediated regeneration and in vitro root tuberization in *Trichosanthes kirilowii* Maxim., a medicinal plant. In Vitro Cell Dev-Pl. 2018; 54(6):621–625.
- 9.McCormick F. A. Notes on the anatomy of the young tuber of *Ipomoea batatas* Lam. Bot Gaz. 1916; 61(5):388–398.
- 10.Firon N, LaBonte D, Villordon A, Kfir Y, Solis J, Lapis E, Perlman TS, Doron-Faigenboim A, Hetzroni A, Althan L, Adani Nadir L. Transcriptional profiling of sweetpotato (*Ipomoea batatas*) roots indicates down-regulation of lignin biosynthesis and up-regulation of starch biosynthesis at an early stage of storage root formation. BMC Genomics. 2013; 14(1):460–485.
- 11.Dong T, Zhu M, Yu J, Han R, Tang C, Xu T, Liu J, Li Z. RNA-Seq and iTRAQ reveal multiple pathways involved in storage root formation and development in sweet potato (*Ipomoea batatas* L.). 2019; 19(1):136–151.
- 12.Kolachevskaya OO, Lomin SN, Arkhipov DV, Romanov GA. Auxins in potato: molecular aspects and emerging roles in tuber formation and stress resistance. Plant Cell Rep. 2019; 38(6):681–698.
- 13.Noh SA, Lee HS, Huh EJ, Huh GH, Paek KH, Shin JS, Bae JM. *SRD1* is involved in the auxin-mediated initial thickening growth of storage root by enhancing proliferation of metaxylem and cambium cells in sweetpotato (*Ipomoea batatas*).. J Exp Bot. 2010; 61(5):1337–1349.
- 14.Zurcher E, Muller B. Cytokinin synthesis, signaling, and function—advances and new insights. Int Rev Cell Mol Biol. 2016; 324:1–38.

- 15.Ku AT, Huang YS, Wang YS, Ma D, Yeh KW: IbMADS1 (*Ipomoea batatas* MADS-box 1 gene) is involved in tuberous root initiation in sweet potato (*Ipomoea batatas*).. Ann Bot. 2008; 102(1):57–67.
- 16.Grandellis C, Fantino E, Muniz Garcia MN, Bialer MG, Santin F, Capiati DA, Ulloa RM. *StCDPK3* phosphorylates in vitro two transcription factors involved in GA and ABA signaling in potato: *StRSG1* and *StABF1*. PloS One. 2016; 11(12):167389–167408.
- 17.Dong TT, Song WH, Tan CT, Zhou ZL, Yu JW, Han RP, Zhu MK, Li ZY. Molecular characterization of nine sweet potato (*Ipomoea batatas* Lam.) MADS-box transcription factors during storage root development and following abiotic stress. Plant Breeding. 2018; 137(5):790–804.
- 18.Tanaka M, Kato N, Nakayama H, Nakatani M, Takahata Y. Expression of class I knotted1-like homeobox genes in the storage roots of sweetpotato (*Ipomoea batatas*).. J Plant Physiol. 2008; 165(16):1726–1735.
- 19.Tanaka M, Takahata Y, Nakatani M. Analysis of genes developmentally regulated during storage root formation of sweet potato. J Plant Physiol. 2005; 162(1):91–102.
- 20.Tanaka M, Takahata Y, Nakayama H, Nakatani M, Tahara M. Altered carbohydrate metabolism in the storage roots of sweet potato plants overexpressing the SRF1 gene, which encodes a Dof zinc finger transcription factor. Planta. 2009; 230(4):737–746.
- 21.You MK, Hur CG, Ahn YS, Suh MC, Jeong BC, Shin JS, Bae JM. Identification of genes possibly related to storage root induction in sweet potato. FEBS Lett. 2003; 536 (1–3):101–105.
- 22.Noh SA, Lee HS, Kim YS, Paek KH, Shin JS, Bae JM. Down-regulation of the IbEXP1 gene enhanced storage root development in sweetpotato. J Exp Bot. 2013; 64(1):129–142.
- 23.Cristina N, Abelenda JA, Cruz-Oro E, Cuéllar CA, Tamaki S, Silva J, Shimamoto K, Prat S. Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature. 2011; 478(7367):119–122.
- 24.González-Schain ND, Díaz-Mendoza M, Zurczak M, Suárez-López P. Potato CONSTANS is involved in photoperiodic tuberization in a graft-transmissible manner. Plant J. 2012; 70(4):678–690.
- 25.Wang Z, Fang B, Chen X, Liao M, Chen J, Zhang X, Huang L, Luo Z, Yao Z, Li Y: Temporal patterns of gene expression associated with tuberous root formation and development in sweetpotato (*Ipomoea batatas*).. BMC Plant Biol. 2015; 15:180.
- 26.Sojikul P, Saithong T, Kalapanulak S, Pisuttinusart N, Limsirichaikul S, Tanaka M, Utsumi Y, Sakurai T, Seki M, Narangajavana J. Genome-wide analysis reveals phytohormone action during cassava storage root initiation. Plant Mol Biol. 2015; 88(6):531–543.

27. Shan J, Song W, Zhou J, Wang X, Xie C, Gao X, Xie T, Liu J. Transcriptome analysis reveals novel genes potentially involved in photoperiodic tuberization in potato. *Genomics*. 2013; 102(4):388–396.
28. Xu L, Wang J, Lei M, Li L, Fu Y, Wang Z, Ao M, Li Z. Transcriptome analysis of storage roots and fibrous roots of the traditional medicinal herb *Callerya speciosa* (Champ.) ScHot. *PloS One*. 2016; 11(8):160338–160358.
29. Yao S, Huang W, Pan C, Zhan J, He LF. Caspase-like proteases regulate aluminum-induced programmed cell death in peanut. *Plant Cell Tiss Organ Cult*. 2016; 127(3):691–703.
30. Abidi N, Hequet E, Cabrales L. Changes in sugar composition and cellulose content during the secondary cell wall biogenesis in cotton fibers. *Cellulose*. 2010; 17(1):153–160.
31. Velasquez AV, Martins CMMR, Pacheco M Dos Santos P, Zuniga M, Fukushima RS. Comparative study of some analytical methods to quantify lignin concentration in tropical grasses. *Asian-Australas J Anim Sci*. 2018; doi: 10.5713/ajas.17.0450.
32. Liu X, Zhang R, Ou H, Gui Y, Wei J, Zhou H, Tan H, Li Y. Comprehensive transcriptome analysis reveals genes in response to water deficit in the leaves of *Saccharum narenga* (Nees ex Steud.) hack. *BMC Plant Biol*. 2018; 18(1):250–266.
33. Cock PJ, Fields CJ, Goto N, Heuer ML, Rice PM. The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. *Nucleic Acids Res*. 2010; 38(6):1767–1771.
34. Wei S, Ma X, Pan L, Miao J, Fu J, Bai L, Zhang Z, Guan Y, Mo C, Huang H, Chen M. Transcriptome analysis of *Taxillus chinensis* (DC.) Danser seeds in response to water loss. *PloS One*. 2017; 12(1):169177–169203.
35. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. 1997; 25(17):3389–3402.
36. Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*. 2005; 21(18):3674–3676.
37. Li M, Liang Z, Zeng Y, Jing Y, Wu K, Liang J, He S, Wang G, Mo Z, Tan F, Li S, Wang L. De novo analysis of transcriptome reveals genes associated with leaf abscission in sugarcane (*Saccharum officinarum* L.). *BMC Genomics*. 2016; 17:195–213.
38. Rice P, Longden I, Bleasby A. EMBOSS: the European molecular biology open software suite. *Trends Genet*. 2000; 16(6):276–277.

39. Mistry J, Finn RD, Eddy SR, Bateman A, Punta M. Challenges in homology search: HMMER3 and convergent evolution of coiled-coil regions. *Nucleic Acids Res.* 2013; 41(12):121–131.
40. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat methods.* 2012; 9(4):357–359.
41. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics.* 2011; 12: 323–339.
42. Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics.* 2010; 26(1):136–138.
43. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)*, 1995, 57(1):289–300.
44. Yao SC, Zhan J, Pan CL, Xiong WJ, Xiao D, Wang YL, Wang AQ, He LF. Identification and validation of reference genes for real-time qPCR normalization during Al-induced programmed cell death in peanut. *Biologia Plantarum.* 2019; 63: 237–246.
45. Kundu A, Patel A, Pal A. Defining reference genes for qPCR normalization to study biotic and abiotic stress responses in *Vigna mungo*. *Plant Cell Rep.* 2013; 32(10):1647–1658.
46. Liu Y, Lin-Wang K, Espley RV, Wang L, Li Y, Liu Z, Zhou P, Zeng L, Zhang X, Zhang J, Allan AC. *StMYB44* negatively regulates anthocyanin biosynthesis at high temperatures in tuber flesh of potato. *J Exp Bot.* 2019; 70(15):3809–3824.
47. Rushton PJ, Somssich IE, Ringler P, Shen QJ. WRKY transcription factors. *Trends Plant Sci.* 2010; 15(5):247–258.
48. Chen SP, Kuo CH, Lu HH, Lo HS, Yeh KW. The sweet potato NAC-domain transcription factor *lbNAC1* is dynamically coordinated by the activator *lbbHLH3* and the repressor *lbbHLH4* to reprogram the defense mechanism against wounding. *PLoS Genet.* 2016; 12(10):1006397–1006423.
49. Phukan UJ, Jeena GS, Tripathi V, Shukla RK. Regulation of *apetala2*/ethylene response factors in plants. *Front Plant Sci.* 2017; 8:150–168.
50. Roumeliotis E, Visser RG, Bachem CW. A crosstalk of auxin and GA during tuber development. *Plant Signal Behav.* 2012; 7(10):1360–1363.
51. Gargantini PR, Giammaria V, Grandellis C, Feingold SE, Maldonado S, Ulloa RM. Genomic and functional characterization of *StCDPK1*. *Plant Mol Biol.* 2009; 70(1–2):153–172.
52. Reddy AS, Day IS, Narasimhulu SB, Safadi F, Reddy VS, Golovkin M, Harnly MJ. Isolation and characterization of a novel calmodulin-binding protein from potato. *J Biol Chem.* 2002; 277(6):4206–

4214.

- 53.Yilmaz N, Kodama Y, Numata K. Revealing the architecture of the cell wall in living plant cells by bioimaging and enzymatic degradation. *Biomacromolecules*. 2019; doi: 10.1021/acs.biomac.9b00979.
- 54.Zhang Z, Wang N, Jiang S, Xu H, Wang Y, Wang C, Li M, Liu J, Qu C, Liu W, Wu S, Chen X, Chen X. Analysis of the xyloglucan endotransglucosylase/hydrolase gene family during apple fruit ripening and softening. *J Agric Food Chem*. 2017; 65(2):429–434.
- 55.Cosgrove DJ. Plant expansins: diversity and interactions with plant cell walls. *Curr Opin Plant Biol*. 2015; 25:162–172.
- 56.Sena JS, Lachance D, Duval I, Nguyen TTA, Stewart D, Mackay J, Seguin A. Functional analysis of the PgCesA3 white spruce cellulose synthase gene promoter in secondary xylem. *Front Plant Sci*. 2019; 10:626–639.
- 57.Li M, Yang Y, Feng F, Zhang B, Chen S, Yang C, Gu L, Wang F, Zhang J, Chen A, Lin W, Chen X, Zhang Z. Differential proteomic analysis of replanted *Rehmannia glutinosa* roots by iTRAQ reveals molecular mechanisms for formation of replant disease. *BMC Plant Biol*. 2017; 17(1):116–137.
- 58.Sonnewald S, Sonnewald U. Regulation of potato tuber sprouting. *Planta*. 2014; 239(1):27–38.
- 59.Chincinska I, Gier K, Kruegel U, Liesche J, He H, Grimm B, Harren FJM, Cristescu SM, Kuehn C. Photoperiodic regulation of the sucrose transporter *StSUT4* affects the expression of circadian-regulated genes and ethylene production. *Front in Plant Sci*. 2013; 4:26–38.
- 60.Wang X, Chang L, Tong Z, Wang D, Yin Q, Wang D, Jin X, Yang Q, Wang L, Sun Y, Huang Q, Guo A, Peng M. Proteomics profiling reveals carbohydrate metabolic enzymes and 14–3–3 proteins play important roles for starch accumulation during cassava root tuberization. *Sci Rep*. 2016; 6:19643–19658.
- 61.Zhang B, Zhou W, Qiao D, Zhang P, Zhao S, Zhang L, Xie F. Changes in nanoscale chain assembly in sweet potato starch lamellae by downregulation of biosynthesis enzymes. *J Agric Food Chem*. 2019; 67(22):6302–6312.
- 62.Zhang K, Wu Z, Tang D, Luo K, Lu H, Liu Y, Dong J, Wang X, Lv C, Wang J, Lu K. Comparative transcriptome analysis reveals critical function of sucrose metabolism related-enzymes in starch accumulation in the storage root of sweet potato. *Front Plant Sci*. 2017; 8:914–929.

Tables

Due to technical limitations, tables are only available as a download in the supplemental files section

Figures

Figure 1

The key developmental stages of tuberous roots and unexpanded fibrous roots. a. The phenotype of tuberous roots and unexpanded fibrous roots. b. The cross section of tuberous roots and unexpanded fibrous roots. c-g. The anatomical characteristics of tuberous roots and unexpanded fibrous roots: c. Initiated tuberous roots (ITRs). d. The mid-swelling stage of tuberous roots (MTRs). e. The late tuberous roots (LTRs). f. The unexpanded fibrous roots (UFRs). g. Starch grain and calcium oxalate crystal in

parenchymal cell of LTRs. h-j. The phenotype of root systems at different age of seedlings. h. One-year seedlings. i. Three-year seedlings with tuberous roots. j. Three-year seedlings with unexpanded fibrous roots. Ac, accessory cambium; CC, cork cambium; Coc, calcium oxalate crystal; Msv, meristems around the vessel; P, periderm; PC, parenchymal cell; Sc-sclerenchyma cells; SG, starch grain; Sph, secondary phloem; Sx, secondary xylem; TS, the scattered tertiary structure; Vc, vascular cambium.

Figure 2

Transcriptome profiling and differential expression. a. Number of up- and down-regulated transcripts in ITRs, MTRs, LTRs and TRs compared to UFRs. b. Venn diagram of differentially expressed genes (DEGs) identified in all comparisons. Numbers in red represent commonly up- (233) and down-regulated (113) transcripts in ITRs, MTRs and LTRs compared to UFRs. c. KEGG annotation of the commonly up- and down-regulated DEGs. Red means up-regulated and blue means down-regulated genes.

Figure 3

The expression levels of DEGs in transcription factors family.

Figure 4

The expression levels of DEGs in several families. a. Auxin-related genes; b. CKs-related genes; c. ABA-related genes; d. GA-related genes; e. JA-related genes; f. ethylene-related genes; g. Cell expansion and cell-wall synthesis; h. Calcium signaling transduction; i. Starch synthase; j. Sucrose synthase; k. Lignin-related genes; l. Cellulose-related genes.

Figure 5

Validation of the transcriptomic data with qRT-PCR. Sixteen transcripts were selected in this validation. The detail information of selected transcripts was shown in Additional file 1: Table S3. The relative expression levels ($2^{-\Delta\Delta C_t}$) were calculated by qRT-PCR and FPKM values identified by transcriptome. Results represent the mean of three biological replicates (mean $\pm$ SD, $n=3$) and error bar represents the standard deviation.

Figure 6

The physiological indexes related to storage root formation. a. Cellulose content; b. Lignin content; c. Polysaccharide content; d. Starch content.

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