Integrated mRNA and miRNA transcriptome analysis reveals regulatory network of yam tuber expansion

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Abstract
Yam tuber is a storage organ, derived from modified stem. Tuber expansion is a complex process, it depends on the expression of genes that can be influenced by environmental and endogenous factors. However, little is known about the mechanism that regulates tuber expansion. In order to identify genes and miRNAs involved in tuber enlargement, we examined the transcriptome and small RNA in yam tuber during its initiation and expansion stages. A total of 14238 differentially expressed transcripts in yam tuber at its expansion stage were identified by using transcriptome technology. Among them, 5723 genes were up-regulated and 8515 genes were down-regulated, respectively. Functional analysis revealed the coordination of tuber plant involved in processes of cell events, metabolism, biosynthesis, and signal transduction pathways at transcriptional level, suggesting that these DEGs are somehow involved in response of tuber expansion. In addition, 536 transcription factor genes showed differential expression during the expansion stage at transcriptional level. REVEILLE 6-like was identified to be up-regulated in the circadian rhythm pathway. REVEILLE 6-like LHY protein, zinc finger CCCH domain-containing protein 14, and DELLA genes were up-regulated in expansion stage. Moreover, these genes were involved in circadian rhythm pathway, starch and sucrose metabolism pathway, and GA pathway by KEGG analysis respectively. Noteworthy, data analysis revealed that 23 known tuber miRNAs belong to 11 miRNA families, and 50 novel miRNAs were identified. miRNA160, miRNA396, and miRNA535 may be involved in complex network to regulate cell division and differentiation in yam during its expansion stage. The integrated analysis of miRNA-mRNA identified to be preferentially expressed in hormone signaling in expansion stage, miRNA160 was down-regulated in expansion stage, ARF18 and ARF17 were up-regulated in auxin signal transduction of tuber expansion in yam, highlighting the involvement of miRNA-mRNA in the regulation the hormone. The transcriptome and miRNA datasets presented here identified a subset of candidate genes and miRNAs that are putatively associated with tuber expansion in yam, thus a hypothetical model of genetic regulatory network associated with tuber expansion in yam was put forward, which may provide a foundation for molecular regulatory mechanism researching on tuber expansion in Dioscorea species.
Background
Yams are monocotyledonous plant belonging to the family Dioscoreaceae, and tuber is its harvested organ. Tuber originates from the expansion of underground stem, are suitable for nutrients storage, with many large parenchyma cells. Tuber morphogenesis involves three general processes: induction, initiation, and formation; starch and accumulated proteins are two main processes of tuber growth[1]. The tuber morphogenesis of yam can be divided into three stages: initiation stage, expansion stage, and maturation stage. The expansion stage can be divided into three periods: early expansion stage, middle expansion stage, late expansion stage[2, 3]. Tuber morphogenesis is a complex physiological process regulated by heredity, environment, hormones, etc[4]. Great efforts have done to explore the physiological factors affecting the morphogenesis of yam tubers. Short days promoted tuber expansion at the initiation tuber stage of water yam (Dioscorea alata), whereas long days inhibited tuber expansion[5, 6]. Endogenous hormones including gibberellins(GA), acetic acid(IAA) and abscisic acid(ABA) performed a key role at the beginning of tuber expansion stage, and trans-zeatin(tZ), jasmonic acid(JA) were also involved in tuber expansion[2, 7, 8]. Exogenous hormones had been used to study tuber expansion, GAs could promote tuber expansion and yield through in vitro and in vivo treatment [9, 10]. Exogenous GA application combined with ABA have promoted microtuber growth and expansion[11]. Exogenous JA was found to be essential for yam tuberization and induced an increase in the number of tuber in vitro and in vivo[12, 13]. However, fundamental knowledge about endogenous metabolic networks is utterly lacking in tuber expansion.

The induction and growth of microtubers in vitro are controlled by nutrients, and sucrose concentration is the most important factor affecting tuberization and frequency of proliferation in yam [7, 14]. Yam tuber morphology was significantly correlated with nutrient accumulation and enzymatic activity. Sucrose, soluble sugars, and proteins increased significantly during tuber expansion stage, then subsequently decreased at maturity stage. Starch content increased throughout tuber morphogenesis, and sucrose synthase, sucrose phosphate synthase, and AGPase were significantly correlated with these nutrient accumulation[15]. Although many DNA molecular markers have been used to uncover the genetic diversity and relationship among yam germplasms[16-18], little is known
about specific genes involved in tuber morphogenesis. The sucrose synthase 4 and sucrose-phosphate synthase 1 were associated with the earliest stages of starch biosynthesis and storage, a SCARECROW-LIKE gene was involved in formation of adventitious roots[19]. PE2.1 and PE53 are members of the pectinesterase(PE) superfamily, which may be involved in the regulation of starch and sucrose metabolism and signaling pathways. Therefore, they may play an important role in microtuber formation[20]. Tuber morphogenesis is a complex biological process involving many specific genes and proteins, especially yam tuber expansion stage. Transcriptome techniques can efficiently find and detect these genes and proteins. Potato is a tuber crop, many transcriptome analyses revealed that many genes are regulated in early stages of stolon-to-tuber transitions, or tuberization by nutrients, photoperiodic conditions, exogenous hormones, and stress[21-24]. Former transcriptome study revealed that some putative genes were involved in dioscin biosynthesis[25], and chalcone isomerase (CHS), flavanone 3-hydroxylase (F3H), flavonoid 3'-monooxygenase(F3'H), dihydroflavonol 4-reductase(DFR), leucoanthocyanidin dioxygenase(LDOX), and flavonol 3-O-glucosyltransferase(UF3GT) were significantly expressed in flavonoid biosynthesis[26]. However, there are no reports of transcriptome study on tuber expansion. Therefore, the study of key genes involved in the metabolism of yam tuber expansion will provide a clear network to elucidate the molecular mechanism of tuber growth in the transcriptome.

microRNAs (miRNAs) are small, endogenous, non-coding RNAs that have important functions in many biological processes, such as the regulations of growth and development, stress response, and metabolism. Many studies have shown that miRNAs play important roles in root and tuber formation or development[27-29]. MiR165/166 regulates root growth by determining the fate of root cells in Arabidopsis combined with phytohormone crosstalk, by negatively regulating its target genes auxin response factor ARF10, ARF16 and ARF17[30]. miRNA 172 and miR156 are involved in tuberization process, either as a component or a regulator of long distance gibberellin signaling pathways[31, 32]. Potato specific miRNA193, miRNA152, and conserved miR172-1, miRNA172-5 showed significant expression during developmental stages of tuberization[28]. Although many studies have found that miRNAs are involved in tuber and root development, the miRNA-mediated regulatory network during
tuber expansion is still unclear.

Although whole-genome sequencing of the heterozygous diploid Guinea yam (*D. rotundata*) has been performed for sex determination at the seedling stage[33], a detailed comparative transcriptome and miRNA analysis during yam tuber expansion stage need to be detected. In this study, transcriptome and small RNA sequencing were performed to thoroughly investigate the key genes and miRNA in yam tuber expansion stage.

**Results**

Overview of transcriptome dynamics and small RNA sequencing

To identify the regulation of transcriptome and miRNAs co-regulatory network during tuber expansion, we examined the transcriptomes and small RNA during tuber initiation stage (GH16_I) and expansion stage (GH16_E) (Fig.1). Meanwhile, transcriptome library was constructed from a pool of mixed RNA consisting of initiation and expansion stages in order to construct small RNA and transcriptomes. Approximately 6.67 Gb bases in total on BGISEQ-500 sequencing platform were gained by transcriptome de novo analysis (Table 1). After filtering low-quality reads and adaptor sequences, 7.7% of the total unigenes longer than 2000bp in all assembled yams transcripts were obtained, indicating that it is difficult to calculate the number of genes in yams due to deficient information of its genome (Fig.2). A total of 32207 genes were functionally annotated with 7 functional database (NR, NT, GO, KOG, KEGG, SwissProt, and InterPro): 25694 (79.78%), 16891 (52.45%), 17603 (54.66%), 19472 (60.46%), 20191 (62.69%), 22159 (68.80%), 8270 (25.68%) reads were annotated functionally (Fig.3). 13566 genes were commonly annotated in NR, KOG, KEGG, SwissProt and InterPro databases.

Based on the function annotation results of NR database (Fig.4), the best matching species distribution for each sequence matched the gene for *Elaeis guineensis* (33.21%), followed by *Phoenix dactylifera* (23.35%), *Ananas comosus* (7.47%), *Musa acuminate* subsp. *malaccensis* (7.31%).

A total of 32026 genes were detected, accounting for approximately 6.6 Gb of clean reads of each mRNA library by RNA-Seq analysis with three biological repeats respectively (Table 1). The average mapping rate of transcriptome library was 82.57%. Based on heatmap cluster analysis, both tuber initiation (GH16_I) and expansion stage (GH16_E) samples showed good correlations with each other.
(Fig.5). The expression levels of 6 cDNA libraries were delineated by boxplot profiles (Fig.6), indicating that the samples are highly reproducible. Genes with expression levels >5 FPKM were retained for statistical analysis.

Furthermore, the corresponding six small RNA libraries at the three time points were also constructed for deep sequencing. Initially, a total of 170,957,171 reads were generated (Table 2). After filtering low-quality reads and adaptor sequences, 157,958,048 clean reads longer than 18nt for six libraries with an average of 26.32 M clean reads, and length distribution of clean reads showed the classes of sRNA were 21-24nt (Additional file 2: FigS1). Subsequently, 4593044 (17.48%), 5032588 (18.58%), 4642869 (17.58%) tags in tuber initiation and 6388211 (25.11%), 5872589 (22.36%), 6086348 (22.98%) in tuber expansion were mapped to sRNA database (rRNA, tRNA, snRNA and snoRNA), respectively.

Differentially expressed genes annotation by GO term and KEGG pathway

To identify differentially regulated genes in tuber expansion stage, DESeq software was used to compare the gene expression between initiation and expansion stages. Among them, 5765 and 8515 genes were up-regulated and down-regulated, respectively, differentially expressed in expansion stage (GH16_E), compared to initiation stage (GH16_I) (Additional file 1: Table S1).

In order to better understand the function of the DEGs, 44 significant GO categories were identified. For cellular component, 15 GO categories were enriched in DEGs, including cell (24%), membrane (20%), membrane part (18%), and organelle (18%) (Fig.7). For biological processes, DEGs associated with cellular process (33%), metabolic process (31%), and biological regulation (9%) were enriched during expansion stage (Fig.7). The molecular functions of the DEGs were mainly associated with catalytic activity (44%), binding (41%), transporter activity (5%), structural molecular activity (4%).

Among the significant GO term analysis, 15 genes were enriched in cell wall polysaccharide metabolic process (GO:0010383), 15 genes were involved in hemicellulose metabolic process (GO:0010410), and 13 genes were related to xyloglucan metabolic process (GO:0010411) related to cell wall formation during expansion stage(Table 3). In addition, the results also revealed a number of significant expression genes involved in tissue development, root morphogenesis, root system
development, and root development (Table 4).

KEGG is a signal pathway database with an extremely rich signal pathway map, identifying 21 pathways. Interestingly, KEGG pathway analysis showed that plant hormone signal transduction (ko04075), biosynthesis of amino acids (ko01230) were enriched with DEGs during expansion stage (Fig.8). Other pathways such as MAPK signaling pathway (ko04016), starch and sucrose metabolism (ko00500), and carbon metabolism (ko01200) were also identified as involving 283, 204, and 236 DEGs, respectively. The metabolic pathways may be closely related to the development of tuber expansion and bioactive compound synthesis.

Comprehensive analysis of differentially expressed genes in expansion stages

Compared with initiation stage, there were a large number of DEGs in tuber expansion stages using NR, GO and KEGG annotation. Signal transduction, cell wall, cell division, starch and sucrose metabolism were selected for profiling during the expansion of yam tuber (Additional file 1: Table S2).

Hormone signal

A total of 242 DEGs were identified to be highly similar to many plant hormone signal pathways, including 131 down-regulated and 111 up-regulated DEGs in expansion stage (Additional file 1: Table S1). Interestingly, most of plant hormone-related genes in GA, IAA, and ABA signal pathways were discovered during expansion stage.

In the auxin transduction pathway, the transcriptional level of auxin influx carrier /auxin-responsive protein IAA (AUX/IAA) and small auxin up RNA (SAUR) were significantly down-regulated during the expansion stage, while auxin-responsive GH3 gene family (GH3) was up-regulated. In contrast, two auxin response factor ARFs (CL2135.Contig1_Total_1, and Unigene5660_Total_1) were been shown high expression level during expansion stage, while other two ARFs (CL2887.Contig2_Total_1, Unigene5486_Total_1) were low expression level during expansion stage(Additional file 1: Table S2).

In gibberellin transduction pathway, the expression of gibberellin receptor GID2 was low expression during expansion stage. In contrast, DELLA proteins were highly expressed during the expansion stage. Meanwhile, protein phosphatase 2C (PP2C) was highly expressed during expansion stage.

MAPK and calcium signaling
To investigate the effect of MAPK and calcium signaling during the expansion stage, 6 mitogen-activated protein kinases (MAPK) genes showed up-regulated during expansion stage, while MPK6 and MPK8 were down-regulated. In summary, 48 DETs were homologous with calcium signal-related genes (Additional file 1: Table S1), including calcium-dependent protein kinases (CDPKs), calcium-binding proteins (CBPs), and calreticulin (CBL). It is worth noting that CBLs were down-regulated during expansion stage (Additional file 1: Table S2).

Cell wall and cell cycle
A total of 98 transcripts homologous to the genes associated with cell wall and cell cycle were observed as differentially regulated during expansion stage (Additional file 1: Table S1), including xyloglucan endotransglucosylase/hydrolase (XTH), expansin, extension, cyclin-dependent kinases (CKS), cell division protease (ftsHs), cell division cycle 5-like protein (CDC5), cell division control protein (CDC), cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitor (CDKIs). All of the expansin, extension, cell wall synthesis, and CKS genes were down-regulated during expansion stage. Meanwhile, most of the cell cytoskeleton and XTH were down-regulated during expansion stage in yam (Additional file 1: Table S2).

Starch and sucrose metabolism
The major constituents of starch and sucrose metabolism during expansion stage are sucrose synthase genes (SuSy), sucrose phosphate synthase genes (SPS), starch synthase (SS), and invertase genes (INV) (Additional file 1: Table S1). Among them, SuSy were down-regulated during expansion stage. Interestingly, dioscorins, the major storage proteins in yam tubers, were significantly up-regulated during the expansion stage (Additional file 1: Table S2). These results indicated that many functional genes were involved in expansion stage of yam tuber.

Transcription factor
Transcription factors are widely involved in various biological processes. 1254 TF-encoding genes were found and classified into 55 different families in yam tuber, of which MYB, MYB-related, and AP2-EREP were enriched in tuber (Fig.9). Further analysis revealed that 541 TF-encoding genes belonging to 48 TF families were differentially expressed during the expansion stage. 286 TF encoding genes
were up-regulated and 255 TF encoding genes were down-regulated, respectively (Additional file 1: Table S3). The most abundant TF gene families with the highest number of expression during expansion stage were depicted by heat map (Fig.10). Interestingly, REVEILLE 6-like LHY protein (Unigene12250_Total_1), zinc finger CCCH domain-containing protein 14 (CL256.Contig1_Total_1), and DELLA genes (Unigene5584_Total_1, Unigene22110_Total_1, CL1653.Contig2_Total_1, CL1340.Contig2_Total_1) were up-regulated during expansion stage (Additional file 1: Table S3). Moreover, these genes were involved in circadian rhythm pathway, starch and sucrose metabolism pathway, and GA pathway by KEGG analysis respectively.

Detection of known and novel miRNAs expressed in tuber initiation and expansion stages

The investigation of both known miRNA and novel putative miRNAs was performed by miRDeep2 program. This program combined the position and frequency of small RNAs with the secondary structure of miRNA precursor to provide novel miRNA that can specifically be in the tubers. To compare miRNA expression in six libraries, the number of clean reads was used as background for normalization, and transcripts per million reads (TPM) was used to present the expression levels of miRNAs. Data analysis showed that there were 23 known miRNAs (21 and 20 in tuber initiation and expansion stage, respectively) and 50 novel miRNAs in yam tuber (Additional file 1: Table S4) and 69, 69, 72 total miRNAs were detected in tuber initiation stage (GH16_I), and 68,66,70 total miRNAs were detected in tuber expansion stage (GH16_E), respectively (Table 2). Distribution of normalized miRNAs expression showed that approximately 75%-80.56% of the total detected miRNA expression exceeded 10 TPM (Additional file 1: Table S4).

Further analysis revealed that 23 known miRNAs belong to 11 miRNA families, miRNA168, miRNA159, and novel miRNA16 were the most extensively represented families. All miRNAs were analysed to detect differential miRNA expressions (DEM). The results showed that miRNAs expression was dynamically regulated during expansion stages. A total of 44 differentially expressed miRNAs were identified including 11 known and 33 novel miRNAs, it showed that 40 miRNAs were down-regulated, and 4 miRNAs were up-regulated during expansion stage (Table 5). Interestingly, miR160 family (miRNA160, miRNA160a-5p, miRNA160b-1, miRNA160h-1) and miR535a_1 were down-regulated...
during expansion stage, miRNA396b and miRNA168 were up-regulated compared to other miRNA families (Table 5).

Identification of target genes of differentially expressed miRNA in tuber expansion compared with initiation stage

The miRNA regulates target mRNA through translational repression or mRNA degradation. To identify the correlation between the expression of DEMs and DEGs, a total of 11 DEMs were putatively targeted 34 DEGs (Additional file 1: Table S5). Furthermore, based on GO and KEGG analysis of the targets, it was revealed that several key genes involved in expansion stage were completely regulated by miRNAs. GO analysis revealed differentially expressed miRNAs that were regulated during expansion stage by binding, cell, membrane, organelle, membrane part (Fig.11). KEGG pathways analysis were involved in transport and catabolism, signal transduction, biosynthesis of other secondary metabolites, energy metabolism, carbohydrate metabolism, and lipid metabolism. Notably, three genes which were targets of two differentially expressed miRNAs (miR535a_1, miR160) were identified in plant hormone signal transduction pathway (Fig.12). miRNA160 was down-regulated during expansion stage, ARF18 (CL2135.Contig1_Total_1), ARF17 (Unigene5660_Total_1) were up-regulated in auxin signal transduction. Log2 (GH16-E/GH16-I) of three miRNAs and their target genes showed their differential expression was significant, but these miRNAs also regulated their target expression during expansion stage. miR535a_1 participated in brassinosteroid signal transduction pathway by targeting SBP. Moreover, miR396b and miR396a-5b were involved in metabolic processes and functional proteins through targeting genes, including IST1-like protein, growth-regulating factor 4, photosystem II oxygen-evolving enhancer protein 2, and acetyl-CoA carboxylase biotin carboxyl carrier protein(Fig.12, Additional file 1: Table S5).

Some target genes and corresponding miRNA were involved in cellular event, regulation of phytohormone levels, carbohydrate metabolism and other metabolism, implying that they may play crucial roles during tuber expansion (Fig .13).

Validation of the DEGs and DEMs data using RT-qPCR

To identify the accuracy and reliability of transcriptome and miRNA data, RT-qPCR was used to
measure the expressions of some DEGs and DEMs, including 17 mRNAs, and 11 miRNAs, so specific primers were designed (Additional file 1: Tables S6 and S7). All mRNA and miRNA expressions have confirmed the accuracy of RNA-Seq data. Overall, these results indicate that 16 out of 17 mRNAs showed similar expression patterns compared to DEGs analysis (Fig.14), and 9 out of 11 miRNAs also showed very similar patterns compared to DEMs analysis (Fig.15).

Discussion
In tuber plants, tuber formation is based on successive gene expression during development, and the expression of genes depends on specific tissues or developmental stages by transcriptome analysis, such as Solanum tuberosum, Miscanthus lutariariparius, Raphanus sativus, Nelumbo nucifera and Aconitum heterophyllum[28, 34-37]. Yam is one of the most marketable stem tuber crops. Yam tuber expansion stage is an attractive theoretical model for studying the development of underground organs because it is the main storage organ as nutrients reservoir. Therefore, understanding of candidate genes involved in the expansion stage is of predominant importance. In this study, 6.6 Gb clean reads were generated and 14238 transcripts were identified that have shown significant differential expression during expansion stage.

Multiple signaling pathways regulation
KEGG enrichment analysis revealed that a large number of genes were involved in pathways related to MAPK signaling pathway, plant hormone signal transduction, starch and sucrose metabolism, and carbon metabolism (Fig.8). These genes are involved in cell wall biosynthesis, cell proliferation, and expansion, nutrient accumulation, primary metabolism, and hormone signal transduction[34, 38, 39]. Cellular processes are triggered by specific stimuli and hormones that are involved in a series of signaling pathways. Calcium signaling is a major essential nutrient requirement for normal growth and development of plants. As a second messenger, it plays a role in many fundamental cellular processes such as cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell differentiation, photomorphogenesis, plant defense and various stress responses [40-42]. A potato Ca²⁺-dependent protein kinase, StCDPK1, which was reported to be expressed during tuberization, may possibly be involved in potato tuberization through transcriptional activation of certain genes[40,
In addition, StCDPK1 was affected by JA, ABA, GA treatment[43-45], suggested that CDPK may possibly be mediator of hormone-to-tuber stimulation. Moreover, majority of studies have demonstrated that mRNA abundance of CBL, CBP, and GaM were affected by cell division during swelling of potato tubers[40, 43, 46]. In this study, expression of calcium signaling-related genes, including CDPK and CBPs, increased during expansion stage. Interesting, CBLs were down-regulated (Additional file 1: Table S2). The role of CBL in root development is characterized by a strong gene expression during cell proliferation in root, and its expression in root is regulated by auxin and cytokinin[47, 48]. These results demonstrated that calcium signal is regulated during expansion stage. In addition, MAPK signal pathways are known to play a central role in cell proliferation, differentiation and hormones, but it is not known to be involved in tuber or root formation[49]. In this study, MAPK signal-related genes were up-regulated during expansion stage. Cell number and cell size are two key determinants of plant and organ size, while involved in cell division, cell expansion and cell cycle[50, 51]. In this study, XTH, expansin, extension, CKS, ftsHs, CDC5, CDC, CDKs, and CDKIs involved in cell division, cell expansion and cell cycles were differentially regulated during expansion stage. These differentially regulated genes involved in cell wall and cell cycle metabolism were identified in Arabidopsis, Rehmannia glutinosa, radish[35, 36, 52].

Transcription factors regulation

Transcription factors have been identified to play an important role in regulation of plant growth, development, and secondary metabolism. Most transcription factors have been identified to play critical roles in organogenesis, including MADS, bHLH, and GRAS. In sweet potato, MADS were preferentially expressed in root at initiation of tuberization, and in the vascular cambium region where the active cell proliferation regulated by jasmonic acid, cytokinins and stress response[53, 54]. In Arabidopsis, bHLH family, such as PIF3, PIF4, and PIF5 are key regulators for cell elongation during seedling development, antagonistically regulated by light and gibberellins [55]. GRAS transcription factor family specifically regulated the initiation of axillary meristem in Arabidopsis, such as SCARECROW (SCR) is expressed in cortex/endodermal initial cells in the root system and played a key role in regulating the radial organization of the root[56]. DELLA, such as RGA and GAI, controls cell
expansion and cell division in hypocotyl, shoot, root, and floral induction[57]. In this study, a large number of transcription factor genes were found to be expressed differently during expansion stage at transcript level, including 66 MYB, 64 AP2-EREBP, 52 bHLH, and 37 WRKY (Fig.9), and REVEILLE 6-like were identified as up-regulated in circadian rhythm pathway. Zinc finger CCCH domain-containing protein 14 was identified as up-regulated in starch and sucrose metabolism pathway (Fig.9), which plays a variety of important roles in growth and development, hormone response, and response to biotic and abiotic stresses[58]. Three DELLA genes were up-regulated in GA pathway (Fig.10), which may play a key regulatory role in expansion stage, and participate in cell division and expansion[57]. All data suggest that these transcription factors may be potentially involved in the expansion stage.

Hormonal signaling regulation
A set of transcriptome evidence suggests that hormone signal pathway-related genes are enriched in regulation of onset and storage organ formation in sweet potato, potato, radish, lotus, and carrot, while may be potentially involved in cell division, differentiation, and expansion[1, 35, 37, 59, 60]. In KEGG pathway annotation, hormone signal pathway was the most enrich one, that was involved in eight signal pathways (Fig.8). In radish, most Aux/IAA, ARFs, and SAUR genes are abundantly expressed in the root cortex splitting and expansion stage, implying that these transcripts may be involved in cambium cell expansion[35]. Among auxin signal-related genes, Aux/IAA, ARFs were highly expressed in expansion stage in this study, and auxin has been identified to regulate cell division and expansion by altering genes expression[1, 61], suggesting that Aux/IAA, ARFs may offer excellent candidates during expansion stage.

Interestingly, in this study, DELLA genes were up-regulated, while GID1, like GID2 were down-regulated during expansion stage (Additional file 1: Table S2). DELLA proteins are nuclear transcriptional regulators that repress GA signaling and may restrict plant growth presumably by causing transcriptional reprogramming. Binding of GA to GID1 enhances the interaction between GID1 and DELLA, resulting in rapid degradation of DELLAs via the ubiquitin-proteasome pathway. The GA-GID1-DELLA signal control hypocotyl root elongation by reducing GA levels, and DELLA interacts directly with PIF4, PIF3 and SCL to mediate crosstalk between GA and light signals[55, 62]. DELLA is
significantly expressed in GA-mediated rhizomes, suggesting DELLAs can regulate rhizomatic expansion [37]. In addition, GA, IAA, and ethylene affect cell growth in roots via DELLA proteins[57]. Overall, these results indicate that hormone signaling-related genes have a complex regulatory network involved in tuber expansion.

Starch and sucrose metabolism regulation

Starch and sucrose are considered to be one of the major carbohydrates source, in which expansion tuber are highly coordinated with starch and sucrose metabolism genes in potato, radish, and lotus identified by transcriptome analysis [22, 35, 37]. In this study, alpha-amylase, beta-amylase and isoamylase were up-regulated(Additional file 1: Table S1), which is similar to increase in beta-amylase activity in swollen taproot in radish[35], where starch content decreased during the tuber expansion stage[15], and some sucrose metabolism genes were detected during expansion stage, including SuSy, SPS, INV, and invertase inhibitor(Additional file 1: Table S1). Evidence shows that sucrose can be converted to starch in storage root by SuSy and AGPase, which means that they play a key role in the early stage of radish expansion[35]. The SuSy gene was down-regulated during expansion stage. The results were similar to the taproot expansion stage in radish [35], implying that they may play a major roles in tuber expansion stage of yam. Yam tuber morphology is significantly correlated with sucrose and starch [15]. Therefore, these starch and sucrose metabolism genes are necessary for tuber expansion.

miRNAs regulation by targeting the potential genes

miRNA mediated gene regulation has been extensively studied in root and tuber development through transcriptional and post-transcriptional levels, which provides a better understanding of molecular regulatory network during tuber expansion stage. In this study, most miRNAs were down-regulated during expansion stage(Table 5). In maize leaves, miR160 was significantly up-regulated in meristem relative to the elongation and mature zones[63], however, miR160 was down-regulated during yam tuber expansion stage(Table 5). In general, miR160 expression is different in different plant. In this study, some miRNA-mRNA pairs were observed during expansion stage(Fig.12), miR160 was involved in root cell division and differentiation by regulating auxin response factors(ARFs)
affecting root development in *Arabidopsis thaliana* [30]. miRNA160 was down-regulated during expansion stage, auxin response factor ARF18 and ARF17 were up-regulated in auxin signaling (Fig.12), whereas, overexpression of miR160 in transgenic rice not only down-regulated the expressions of ARF10, ARF16, and ARF17, but also inhibited root-cap cells differentiation, lost control of cell division and led to ectopic expansion of the apical stem cell populations [64]. Previous studies have shown that the miRNA160-targeted ARF has been identified to be involved in cell expansion and cell differentiation in radish root and potato tuber [28]. In addition, miRNA396 play an important role in root growth and inhibit leaf cell division by UV-B radiation [65, 66]. miR535 is expressed in fruit development [67]. miRNA160, miRNA396 and miR535 may be involved in complex network of regulating cell division and differentiation during expansion stage.

Regulatory networks associated with tuber expansion

Tuber development and environmental responses involve gene regulatory networks [21-24, 34-37]. miRNA and target genes has been extensively studied in tuber development [21, 28], which greatly advanced our understanding of molecular regulatory networks underlying during yam tuber expansion. In this study, based on our transcriptome and small RNA analysis, previous transcriptomics analysis and results of other tuber crops, a putative model of the regulatory network associated with yam tuber expansion was proposed (Fig 13). In detail, it was found that CBL, CBP, and GaM may affect cell division and proliferation during potato tubers expansion [40, 43, 46]. The role of CBL and *StCDPK1* expressions were regulated by hormone in taproot development [43-45, 47, 48]. In this study, CDPKs, CBPs, and CBLs were widely expressed during expansion stage. MAPK signal related genes were up-regulated during expansion stage. MAPK signal pathway increased PI3K pathway, which in turn led to elevate GA levels, and generate calcium signals [68-70]. Moreover, MAPK signal pathway is known to play a central role in cell proliferation, differentiation and hormone signaling [50, 51]. XTH, expansin, extension, CKS, ftsHs, CDC5, CDC, CDKs, and CDKIs involved in cell division, cell expansion and cell cycles were differentially regulated during expansion stage, a finding similar to these differences in cell wall and cell cycle regulatory genes identify metabolism in *Arabidopsis*, *Rehmannia glutinosa*, radish [35, 36, 52]. Evidence shows that DELLA interacts directly with bHIH and
SCL, mediating cell division in hypocotyls, shoots and roots, and floral crosstalk between hormones and signals[55, 62]. DELLA were significantly expressed in GA-mediated rhizome development process, suggesting that these DELLA can regulate rhizomatic expansion[37]. DELLA can interact with PIF3, PIF4 to impact the expansion genes expression in hypocotyl elongation [71]. In sweet potato, MADS were preferentially expressed in root at the initiation of tuber, and in the vascular cambium region where the active cell proliferation regulated by jasmonic acid, cytokinins, and stress response[53, 54]. Among auxin signal-related genes, Aux/IAA, ARFs were enriched and expressed in tuber enlargement stage in this study. Most Aux/IAA, ARFs and SAUR genes were enriched and expressed in the root cortex during splitting and expanding stage in radish, suggesting that that these transcripts may be involved in cell expansion in the cambium[35]. SuSy, SPS, INV, SS, and beta-amylase, were highly correlated within the expansion tuber, such as potato, radish and lotus[22, 35, 37]. Dioscorinns, the major storage proteins in yam tubers, were significantly up-regulated in expansion stage. In this study, some miRNA and target genes were observed during yam tuber expansion stage, bHLH (target by miR5021), SBP (target by miR535a), and ARFs (targeted by miRNA160 and miR396), were identified to be involved in cell expansion and cell differentiation in radish root and potato tuber[28, 35]. In summary, environmental factors are the first signal to stimulate tuber growth, the signal transduction pathways (hormone, calcium and, MAPK signaling) and metabolism processes (cell wall, starch and sucrose metabolism) attributed to cell differentiation division, expansion by certain specific genes. Taken together, the result suggested that these DEGs may play a key role in the regulatory network of tuber expansion.

Conclusions
A total of 14238 transcripts expressed differentially during yam tuber expansion stage were first identified using transcriptome technology. These results reveal the coordination of tuber cells involved in the processes of cellular events, metabolism, biosynthesis, and signal transduction pathways at transcriptional levels. It is worth noting that the integrated analysis of miRNA-mRNA was identified to be preferentially expressed in auxin signal during expansion stage, highlighting the involvement of miRNA-mRNA in the hormonal regulation involved in tuber expansion development. In
summary, the transcriptome and miRNA datasets presented here identify a subset of candidate genes and miRNAs putatively associated with tuber enlargement in yam, and therefore propose a hypothetical model of the genetic regulatory network associated with tuber expansion in yam, which can provide the basis for molecular regulatory mechanism to study the expansion development in *Dioscorea* species.

**Materials And Methods**

**Plant Material**

*D. opposita* cultivar Guihuai 16 was planted in April at the farm of Guangxi University in Nanning, Guangxi Province, China (22°53’06.7”N 108°21’36.6”E). Its healthy tubers germination, planting patterns and tuber growth stages were consistent as described by Gong[2] in 2016. All the tuber growth stages were proved to have stable agronomic characteristic on three years filed observation. Tuber samples were collected at tuber initiation stage after field planting approximately forty days (initiation stage), additional samples were collected at sixty days (expansion stage). Five plants were selected randomly from every repetition each time. Then, the distal ends (5mm long) of five fresh tubers from a repetition were washed with distilled water, cut down into pieces, and mixed together as a biological repetition. All samples were immediately frozen in liquid nitrogen, and stored at -80°C until further use for the construction of various libraries. Two tuber developmental stages including initiation and expansion stage were used for the construction of various libraries. Three types of libraries were constructed, including one transcriptome *de novo* analysis libraries for mixed sample of initiation and expansion stage (named Total_1), six RNA-Seq analysis libraries (initiation stage named GH16_I, expansion stage named GH16_E), and small RNA analysis (initiation stage named C, and expansion stage named T in original data, in order to be consistent with the transcriptome data analyse, we renamed GH16_I, GH16_E for initiation and expansion stage, respectively). Three biological replicates for each of initiation and expansion stage were studied, respectively.

**Construction and sequencing of transcriptome *de novo*, RNA-Seq and small RNA libraries**

Information about the sample pool can be found on the subsections of plant materials above. Total
RNA was isolated from tuber samples (initiation and expansion stage) using MiniBEST reagent (TaKaRa), and RNA integrity was assessed by an Agilent 2100 BioAnalyzer. Construction of the transcriptome de novo and RNA-Seq libraries were followed from the manufacturer’s protocols. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads, fragmented, and reverse transcribed into cDNA, and cDNA was enriched by cycles of linear PCR to obtain final cDNA libraries. Finally, sequencing by synthesis was performed using a BGISEQ-500 sequencing platform. For the small RNA libraries, RNA bands of around 18-30nt in length were isolated. Libraries were prepared according to the small sample preparation protocol and sequenced using BGISEQ-500 sequencing platform. Raw reads from mRNA and small RNA were subjected to quality control (QC) to harvest high quality sequencing data. Raw reads were further filtered by a few steps to obtain clean reads.

Processing and mapping of mRNA-Seq and small RNA libraries sequencing data

After filtration, clean reads were performed de novo assembly to obtain unigenes. Functional databases are NT, NR, GO, KOG, KEGG, SwissProt, and InterPro used to annotate unigene function, Blastn, Blastx, Diamond, Blast2GO and InterProScan5 were used to align unigenes. RNA-Seq reads were mapped to the transcriptome de novo analysis data using bowtie2 results with default parameters after pre-processing mRNA-Seq data [72]. Gene expression levels were presented as FPKM values [73], and genes with expression levels >5 FPKM were retained for statistical analysis.

Enrichment analysis of DEGs was analysed using the GO and KEGG databases to obtain a detailed description of the DEGs. Small RNA reads were also screened from raw sequencing reads by removing adaptors, poly A sequences, and low-quality bases, sequences shorter than 18nt or longer than 32nt, after trimming, were removed. The high-quality clean reads were mapped to the reference genome and to other sRNA databases by Bowtie, Cmsearch, and Rfam [74].

Identification of known and novel miRNAs, and predicting the targets of miRNAs

To identify the isoforms of known miRNA, cleaned sRNA reads were performed using the miRProf tool and conserved miRNAs were identified using known plant miRNAs registered in miRBase[75]. To identify novel miRNAs and their precursors, unique reads were submitted to the RIPmiR[76]. Potential
miRNA targets were predicted using the psRoot and Target Finder[77].

Identification and functional annotation of differentially expressed genes and miRNAs

The expression levels of mRNA transcripts were measured as fragments per kilobase of exon per million fragments mapped (FPKM), and miRNA read counts were normalized to tags per million (TRM). Differentially expressed genes (DEGs) and miRNAs (DEMs) were identified by DEGseq[78]. Therefore, DEGs and DEMs were identified by stringent threshold ( |log2 (ratio) | >1). All DEGs were subjected to gene ontology and KEGG pathway analysis. Each DEG in tuber was predicted by aligning the gene sequences against the Plant Transcriptional Factor Database. The DEGs were classified according to their TF families. Significant GO and KEGG enrichment compared with the genomic background was determined using hypergeometric tests. Calculated p-values were subjected to FDR correction, and FDR ≤0.05 was applied as the threshold.

Validation of the DEGs and DEMs data using RT-qPCR

To identify the accuracy and reliability of transcriptome and miRNA data, RT-qPCR was used to measure the expressions of DEGs and DEMs. Total RNA used for RNA-Seq and small RNA analysis previously was reversely transcribed into cDNA with PrimeScript™ RT reagent kit and Mir-X™ miRNA first strand synthesis kit (Takara, Dalian, China), separately, according to the manufacture methods. RT-qPCR was performed using primers (Table S6, S7) on real-time PCR detection system (BIO-RAD). For DEGs quantification expression detected by the TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Dalian, China), 10 μl reaction solution containing 1x Green TB Green TB Premix Ex Taq II, 10μM primer, one-third dilution of the cDNA sample, and then the reaction conditions were: 30s at 95°C followed by 40 cycles of 30s at 95°C, and 30s at 60°C. For DEMs quantification expression were detected by the Mir-X miRNA qRT-PCR SYBR kit, 25μl reaction solution containing 2X SYBR advantage premix, 50XROX dye, 10μM miRNA-specific Primer, 2μl cDNA sample, and then the reaction conditions were: 10s at 95°C followed by 40 cycles of 5s at 95°C and 20s at 60°C. All mRNAs and miRNA expressions had three biological replicates with three technical replicates for each of biological replicate, ACTIN and U6 genes were used as reference genes, the relative expression levels were collected by the comparative Ct protocol.
Abbreviations
DEG: Differentially expressed gene; DEM: Differentially expressed microRNA; GO: Gene ontology;
KEGG: Kyoto encyclopedia of genes and genomes; miRNA: MicroRNA; qRT-PCR: Real-time quantitative
RT-PCR; RNA-Seq: RNA sequencing; TF: Transcription factor

Declarations

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Availability of data and materials
The materials of this study were provided by the College of Agriculture at Guangxi University.
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The raw sequencing data have been submitted to the NCBI SRA database (PRJNA533985).

Authors’ contributions
DX and LFH have designed experiments. YYZ, Saba and SZL have analyzed the sequencing data for
transcriptome assembly. JZ and AQW have developed the qPCR experiments. YYZ and LFH have
written the manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
We declare that the research was conducted in the absence of any commercial or financial relationships that could be a potential conflict of interest.

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Tables

Table 1 Statistic analysis of clean reads for mRNA in tuber initiation and expansion stages in yam

| Sample     | Total_1 | GH16_E_r1 | GH16_E_r2 | GH16_E_r3 | GH16_I_r1 | GH16_I_r2 | GH16_I_r3 |
|------------|---------|-----------|-----------|-----------|-----------|-----------|-----------|
| Total Raw Reads(Mb) | 74.71   | 66.43     | 66.43     | 66.35     | 66.43     | 66.42     | 66.52     |
| Total Clean Reads(Mb) | 66.74   | 65.56     | 65.68     | 65.61     | 65.7      | 65.7      | 65.83     |
| Total Clean Bases(Gb) | 6.67    | 6.56      | 6.57      | 6.56      | 6.57      | 6.57      | 6.58      |
| Clean Reads Q20(%)    | 96.4    | 98.52     | 98.5      | 98.57     | 98.63     | 98.56     | 98.54     |
| Clean Reads Q30(%)    | 88.22   | 92.67     | 92.63     | 92.95     | 93.12     | 92.86     | 92.78     |
| Clean Reads Ratio(%)  | 89.34   | 98.7      | 98.87     | 98.9      | 98.91     | 98.92     | 98.96     |
| Total Mapped Reads(%) | -       | 82.05     | 81.77     | 82.03     | 83.07     | 83.34     | 83.17     |

Table 2 Statistic analysis of clean reads for small RNA sequencing in tuber initiation and expansion stages in yam

| Sample name | Total tags | Clean tags | Mapped tags | Known miRNA | Novel miRNA | Total miRNA |
|-------------|------------|------------|-------------|-------------|-------------|-------------|
| GH16_E_r1   | 28256295   | 26276062   | 4593044     | 20          | 49          | 69          |
| GH16_E_r2   | 29343139   | 27085820   | 5032588     | 20          | 49          | 69          |
| GH16_E_r3   | 28395798   | 26406723   | 4642869     | 22          | 50          | 72          |
| GH16_I_r1   | 27687839   | 25438600   | 6388211     | 18          | 50          | 68          |
| GH16_I_r2   | 28663585   | 26268556   | 5872589     | 19          | 47          | 66          |
| GH16_I_r3   | 28610515   | 26482287   | 6086348     | 21          | 49          | 70          |

Table 3 GO enricher analysis of DEGs
| Type                        | ID          | Term                                      | Gene Number | Rich Ratio | P value      |
|-----------------------------|-------------|--------------------------------------------|-------------|------------|--------------|
| Molecular function          | GO:0005198  | Structural molecule activity               | 183         | 0.62       | 1.83867E-07 |
|                            | GO:0003735  | Structural constituent of ribosome         | 143         | 0.61       | 1.71036E-05 |
|                            | GO:0003700  | DNA binding transcription factor activity   | 116         | 0.59       | 0.001099451 |
| Cellular component          | GO:0005576  | Extracellular region                       | 91          | 0.70       | 2.22612E-07 |
|                            | GO:0043228  | Non-membrane-bounded organelle             | 285         | 0.58       | 1.68443E-06 |
|                            | GO:0043232  | Intracellular non-membrane-bounded organelle | 285       | 0.58       | 1.68443E-06 |
|                            | GO:0005840  | Ribosome                                  | 157         | 0.61       | 1.90586E-05 |
|                            | GO:0005618  | Cell wall                                 | 49          | 0.72       | 4.36888E-05 |
|                            | GO:0030312  | External encapsulating structure           | 49          | 0.72       | 4.36888E-05 |
|                            | GO:0048046  | Apoplast                                  | 32          | 0.78       | 0.000075892 |
|                            | GO:0022625  | Cytosolic large ribosomal subunit          | 22          | 0.81       | 0.000355453 |
| Biological process          | GO:0044262  | Cellular carbohydrate metabolic process    | 77          | 0.69       | 1.68372E-05 |
|                            | GO:0005975  | Carbohydrate metabolic process             | 171         | 0.60       | 0.00025216   |
|                            | GO:0044264  | Cellular polysaccharide metabolic process  | 53          | 0.70       | 0.000285538 |
|                            | GO:0045786  | Negative regulation of cell cycle          | 11          | 1.00       | 0.000452873 |
|                            | GO:0006073  | Cellular glucan metabolic process          | 50          | 0.69       | 0.000495823 |
|                            | GO:0010383  | Cell wall polysaccharide metabolic process | 15          | 0.88       | 0.001069447 |
|                            | GO:0010410  | Hemicellulose metabolic process            | 15          | 0.88       | 0.001069447 |
|                            | GO:0010411  | Xyloglucan metabolic process              | 13          | 0.93       | 0.000841262 |
### Table 4 Functional classification and pathway assignment of differentially expressed DEG by GO in expansion stage

| Tissue development [GO:0009888] | Gene ID | log2(GH16_E/GH16_I) |
|----------------------------------|---------|---------------------|
| Actin-related protein 3          | CL1179.Contig2_Total_1 | -2.00 |
| Anaphase-promoting complex subunit 10 | CL1997.Contig4_Total_1 | 1.82 |
| Alpha-tubulin                    | CL28.Contig3_Total_1  | -5.08 |
| Tubulin alpha chain              | CL3054.Contig1_Total_1 | -1.81 |
| Glutamine synthetase nodule isozyme | Unigene128_Total_1 | -4.98 |
| Phosphoenolpyruvate carboxylase 2 | Unigene13453_Total_1 | -1.23 |
| Phosphoenolpyruvate carboxylase 2 | Unigene13455_Total_1 | 1.32 |
| Anaphase-promoting complex subunit 10 | Unigene18039_Total_1 | -1.15 |
| Anaphase-promoting complex subunit 6 | Unigene2600_Total_1 | -1.06 |
| Actin-related protein 2          | Unigene4771_Total_1  | -1.10 |
| ATPase ASNA1 homolog             | Unigene5972_Total_1  | -1.32 |
| Homeobox protein knotted-1-like 3 | Unigene6778_Total_1 | 3.48 |
| Root morphogenesi, root system development, root development [GO:0009888,GO:0022622,GO:0048364] | | |
| Cytoplasmic tRNA 2-thiolation protein 2 | CL1237.Contig1_Total_1 | -1.02 |
| Cytoplasmic tRNA 2-thiolation protein 2 | CL1237.Contig2_Total_1 | -2.28 |
| Mediator of RNA polymerase II transcription subunit 32 | CL2787.Contig2_Total_1 | 2.02 |
| Succinate dehydrogenase assembly factor 2 | CL3034.Contig1_Total_1 | -1.04 |
| Guanine nucleotide-binding protein | Unigene18752_Total_1 | -3.18 |
| Enhanced ethylene response protein 5 | Unigene2193_Total_1 | -1.75 |
| ATPase ASNA1                      | Unigene5972_Total_1  | -1.32 |

### Table 5 Differentially expressed miRNAs involved in expansion stage

| miRNA id  | Read count(GH16_I) | Read count(GH16_E) | Expression(GH16_I) | Expression(GH16_E) | log2Ratio(GH16_E/GH16_I) | P value |
|-----------|--------------------|--------------------|--------------------|--------------------|------------------------|---------|
| novel_mir43 | 54974              | 33041              | 3682.196667        | 1731.716667        | -1.97                  | 0       |
| novel_mir35 | 52437              | 32873              | 3515.556667        | 1728.51            | -1.91                  | 0       |
| miR160a-5p  | 18032              | 121                | 1171.813333        | 6.4233333333       | -8.45                  | 0       |
| miRNA          | FPKM   | TPM    | RPKM   | TMM FPKM | DGE TMM | log2 FC | adj | p value | fold change |
|---------------|--------|--------|--------|----------|---------|---------|-----|---------|-------------|
| miR168a-5p    | 55653  | 59949  | 3708.9 | 3153.8   | 3153.8 | -1.13   | 0   |         |             |
| miR168        | 20208  | 106873 | 1334.1 | 5618.4   | 1.17    | 0       |     |         |             |
| novel mir1    | 16275  | 7760   | 1085.6 | 407.5    | -2.30   | 0       |     |         |             |
| miR396b       | 1097   | 21915  | 72.8   | 224.5    | 1.17    | 0       |     |         |             |
| miR535a_1     | 24978  | 25221  | 1654.3 | 1315.0   | -1.22   | 0       |     |         |             |
| miR396a-5p    | 12295  | 7317   | 816.3  | 224.5    | -1.98   | 0       |     |         |             |
| novel mir33   | 6408   | 4269   | 425.4  | 224.5    | -1.82   | 0       |     |         |             |
| novel mir15   | 9666   | 10433  | 549.1  | 1315.0   | -1.12   | 0       |     |         |             |
| novel mir11   | 4852   | 3769   | 323.0  | 199.1    | -1.60   | 0       |     |         |             |
| novel mir25   | 5833   | 6318   | 333.3  | 1315.0   | -1.12   | 0       |     |         |             |
| novel mir32   | 3196   | 2381   | 212.7  | 1315.0   | -1.66   | 0       |     |         |             |
| novel mir36   | 1859   | 1246   | 124.1  | 212.7    | -1.27   | 0       |     |         |             |
| novel mir31   | 1632   | 1049   | 55.1   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir18   | 3381   | 3299   | 173.7  | 124.1    | -1.27   | 0       |     |         |             |
| novel mir19   | 1859   | 1246   | 124.1  | 212.7    | -1.27   | 0       |     |         |             |
| novel mir10   | 1632   | 1049   | 55.1   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir44   | 3381   | 3299   | 173.7  | 124.1    | -1.27   | 0       |     |         |             |
| novel mir36   | 1859   | 1246   | 124.1  | 212.7    | -1.27   | 0       |     |         |             |
| novel mir31   | 1632   | 1049   | 55.1   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir18   | 1701   | 1305   | 114.0  | 212.7    | -1.27   | 0       |     |         |             |
| novel mir19   | 1491   | 1071   | 99.1   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir12   | 1666   | 1308   | 111.3  | 212.7    | -1.27   | 0       |     |         |             |
| novel mir45   | 1478   | 1093   | 99.1   | 124.1    | -1.81   | 0       |     |         |             |
| miR160        | 926    | 451    | 62.6   | 212.7    | -2.27   | 0       |     |         |             |
| novel mir48   | 1107   | 690    | 74.5   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir26   | 1488   | 1201   | 99.8   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir46   | 1268   | 926    | 83.8   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir42   | 694    | 275    | 46.2   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir49   | 676    | 265    | 45.1   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir24   | 1045   | 779    | 69.8   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir23   | 377    | 109    | 25.1   | 124.1    | -1.81   | 0       |     |         |             |
| miR168a-3p    | 1330   | 1489   | 87.8   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir20   | 374    | 2206   | 24.7   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir30   | 964    | 999    | 63.4   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir37   | 173    | 45     | 11.5   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir47   | 90     | 10     | 6.0    | 124.1    | -1.81   | 0       |     |         |             |
| novel mir4    | 394    | 447    | 25.8   | 124.1    | -1.81   | 0       |     |         |             |
| novel mir34   | 10     | 200    | 0.6    | 124.1    | -1.81   | 0       |     |         |             |
| novel mir8    | 197    | 207    | 13.1   | 124.1    | -1.81   | 0       |     |         |             |
| miR168_1      | 30     | 5      | 1.9    | 124.1    | -1.81   | 0       |     |         |             |
Figures

Figure 1

A picture of yam tuber at different development stages A: Initiation stage B: Expansion

stage white bar is 10cm
Figure 2

Length distribution of assembled yam reads
Fig. 3 Number of genes aligned to different databases

Figure 3

Number of genes aligned to different databases

Figure 4

Distribution of species aligned by yam genes
Correlation analysis between the initiation and expansion stage
The boxplot of overall expression levels of lines initiation and expansion stage with three biological replicates, respectively. The y-axis displays the log10 (FPKM+1) of every sample.
Figure 7

Gene ontology classification of DEGs in expansion st

Figure 8

KEGG Pathway enrichment of top 20 DEGs
DEGs involved in IAA, ABA, GA and BR pathways of plant hormone signal transductions

The numbers of up and down regulated transcription factors in expansion stage
Figure 11

Heat map of the expression levels of highly expressed transcription factors
Figure 12

Functional classification and pathway assignment of differentially expressed miRNAs by GO analysis
Figure 13

Regulatory network from integrated analysis of miRNA-mRNA data Red stage represent negatively correlated, and blue represent positively correlated in each miRNA-mRNA
Figure 14
A proposed model of genetic and molecular interactions in the regulatory network during tuber expansion in yam
Verification of differential expressed genes by qRT-PCR of DEGs

Verification of differential expressed miRNAs by qRT-PCR
Supplementary Files

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