Integrated mRNA and miRNA transcriptome analysis on the regulational of mechanism tuber expansion in Yam

CURRENT STATUS: ACCEPTED

BMC Genomics  ▶ BMC Series

Yun-Yi Zhou  
Agriculture college of Guang Xi University  
zhouyunyi210@163.com
CORRESPONDING AUTHOR
ORCID: https://orcid.org/0000-0002-8885-7572

Shuzhen Luo  
Agriculture college of Guang Xi University

Dong Xiao  
Agriculture college of Guang Xi University

Jie Zhan  
Agriculture college of Guang Xi University

Aiqing Wang  
Agriculture college of Guang Xi University

Longfei He  
Agriculture college of Guang Xi University

DOI:  
10.21203/rs.2.9777/v1

SUBJECT AREAS
Epigenetics & Genomics

KEYWORDS
Yam, Expansion, Transcriptome, Small RNA
Abstract

Background

Tuber is the storage organ of yam derived from modified stems. The development of tube expansion is a complex process and depends on the expression of genes that are involved by environmental and endogenous factors. However, little is known about the mechanism that regulates tuber expansion. In this study, in order to identify genes and miRNAs involved in tuber enlargement, we examined the transcriptomes and small RNA in tuber initiation and expansion stages.

Results

A total of 14238 transcripts expressed differentially in the expansion stage were firstly identified using transcriptome technology. Of these, 5723 and 8515 genes were up and down regulated, respectively. The functional analysis revealed the coordination of tuber plant involved in processes of cell events, metabolism, biosynthesis and signalling transduction pathways at the transcriptional level, suggesting these DEGs are somehow involved in the responses of tuber expansion. In addition, 536 transcription factor genes were found differently expression in expansion stage at the transcript level. REVEILLE 6-like is identified to be up regulated in circadian rhythm pathway. REVEILLE 6-like LHY protein, zinc finger CCCH domain-containing protein 14, and DELLA genes were involved up regulated in expansion stage. Moreover, these genes were respectively involved in circadian rhythm pathway, starch and sucrose metabolism pathway, and GA pathway by KEGG analysis. Noteworthy, the analysis of the data showed that there were 23 known tuber miRNAs belonged to 11 miRNA families, and 50 novel miRNAs, miRNA160 miRNA396, and miR535 may be involved in complexity network to regulate cell division and differentiation in the expansion stage in yam.

The integrated analysis of miRNA-mRNA were identified to be preferentially expressed in hormone signalling in expansion stage, like the ARFs by miRNA160 highlighting the involvement of miRNA-mRNA in the regulation hormone of tuber expansion in yam.

Conclusion

The transcriptome and miRNA datasets presented here identified a subset of candidate genes and
miRNAs 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 of *Dioscoreaceae*, and the tuber is its harvested organ. Tuber originates from the expansion of underground stem, which is suitable for storage of nutrients, with lots of large parenchyma cells. Tuber morphogenesis, which involves three universal process: induction, initiation and formation; starch and stored protein of accumulation are two main processes in tuber growth[1]. The tuber morphogenesis of yam could be divided into three periods: initiation stage, expansion stage, and maturation stage, and the expansion stage could 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 hereditary, environment, hormones, etc[4]. Great efforts have been made to explore the physiological factors affecting tuber morphogenesis of yam, especially the initiation stage developing into expansion stage, short days promoting tuber expansion at the initiation tuber stage of water yam (*D. alata*), whereas long days inhibiting tuber expansion[5, 6].

Endogenous hormones played an important role in tuber morphogenesis, for example gibberellin (GA), acetic acid (IAA) and abscisic acid (ABA) playing 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]. To date, exogenous hormones have been used to study tuber expansion, GAs could promote tuber expansion and yield by treatment *in vitro* and *in vivo*[...
Exogenous GA combined with ABA promoted microtuber growth and expansion.

Exogenous JA was found to be essential for yam tuberization and induced an increase of tuber number in vitro and in vivo.

However, fundamental knowledge about endogenous metabolic networks is utterly lacking in tuber expansion.

The induction and growth of microtubers in vitro in yam have been found to be under the control of nutrients, sucrose concentration was the most important factor affecting tuberization and frequency of proliferation.

Yam tuber morphology were significantly correlated with nutrient accumulation and enzymatic activity, sucrose, soluble sugars, proteins are significantly increased in tuber expansion stage, subsequently decreased in maturity stage, starch content was increased in the whole tuber morphogenesis, and sucrose synthase, sucrose phosphate synthase, and AGPase were significantly correlated with these nutrient accumulation.

Although many DNA molecular markers were used to uncover the genetic diversity and relationship among yam germplasms, it is little known about specific genes involving 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.

PE2.1 and PE53 are the members of pectinesterase (PE) superfamily, which are likely to be associated with the regulation of starch and sucrose metabolism and signalling pathways.

Therefore, they may play important roles in microtuber formation.

The tuber morphogenesis is a complex biological process involving many specific genes and proteins, especially yam tuber expansion. Transcriptome techniques can efficiently find and detect these genes and proteins. Potato is a tuber crop, many transcriptome analysis revealed
that there were many genes were 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 gens 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 not the reports on the transcriptome study on tuber expansion. Thus,
to investigate the key genes involved in the metabolism of tuber expansion in yam will provide a clear
network to elucidate the molecular mechanism of tuber growth by transcriptome.

microRNAs (miRNAs) are endogenous small non-coding RNA with important functions in many
biological processes, such as the regulations of growth and development, stress response, and
metabolism. Many studies have indicated that miRNAs play important roles in root and tuber
formation or development[27-29]. MiR165/166 regulated root growth through determining the fate of root cells
combined with phytohormone crosstalk in Arabidopsis by negative regulating its target genes auxin
response factor ARF10, ARF16 and ARF17[30]. miRNA 172 and miR156 were involved in tuberization process, acting either as a
component or a regulator of long distance gibberellin signaling pathways[31, 32]. Potato specific miRNA193, miRNA152, and the conserved miR172-1, miRNA172-5
showed significant expression during developmental stages of tuberization[28]. Although a number of studies have been identified that miRNAs are involved in tuber and root development, the miRNA-mediated regulatory network during tuber expansion is still unclear. Although the whole genome sequencing of the heterozygous diploid Guinea yam (D. rotundata) had been done for sex determination at the seedling stage[33], a more detailed comparative transcriptome and miRNA analysis in the expansion stage should be detected. In this study, both transcriptome and small RNA sequencing were carried out to thoroughly investigate the crucial genes and miRNA in tuber expansion stages in yam.

Results
Overview of transcriptome dynamics and small RNA sequencing
In order to identify transcriptome and miRNAs co-regulated regulation in tuber expansion, we examined the transcriptomes and small RNA in tuber initiation and expansion stages (Fig.1). Meanwhile, the transcriptome library was constructed from mixed RNA pools consisting of initiation and expansion stages in order to construct small RNA and transcriptomes. Approximately 6.67Gb bases in total on BGISEQ-500 sequencing platform were gained by transcriptome de novo analysis (Additional file 1: Table S1). After filtering low-quality reads and adaptor sequences, 7.7% of the total unigenes longer than 2000bp in the length of distribution of all the assembled yams transcripts were gained, which indicated that it is difficult to calculate the number of genes in yams due to the deficiency information of its genome dates (Fig.2). A total of 32207 genes was performed functional annotation with 7 functional database (NR, NT, GO, KOG, KEGG, SwissProtand, InterPpro), 25694 (79.78%), 16891 (52.45%), 17603 (54.66%), 19472 (60.46%), 20191 (62.69%), 22159 (68.80%), 8270 (25.68%) reads were annotated function, respectively (Fig.3). 13566 genes were commonly annotated in NR, KOG, KEGG, SwissProt and InterPpro databases. Based on the NR database function
annotation results (Fig. 4), the species distributions of the best matches for each sequence matched the genes of *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 G of clean reads of each mRNA library by RNA-seq analysis (Additional file 1: Table S1). The average mapping rate of transcriptome library was 82.57% (Additional file 1: Table S1). Both the tuber initiation and expansion stages samples showed good correlations in each other based on the Pheatmap cluster analysis (Fig. 5).

Furthermore, the corresponding small RNA libraries at the three time points were also constructed for deep sequencing (Additional file 1: Table S1). 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, respectively. These tags were clustered into RNA classes (including miRNA, sncRNA), genome and unannotated group.

Differentially expressed genes annotation by GO term and KEGG pathway

To identify the genes regulated differentially in tuber expansion stage, DEseq software was used to compare the gene expression between initiation and expansion stages. Of these, 5765 and 8515 genes were up and down regulated, respectively (Additional file 1: Table S2).

To better understand the function of the DEGs, 44 significant GO categories were identified. For cellular component, 15 GO categories including cell, membrane and membrane part, organelle, and organelle part were enriched among DEGs (Fig. 6). For biological processes, DEGs related to cellular process, metabolic process and biological regulation were enriched in expansion stage (Fig. 6). The molecular functions of the DEGs were mainly associated with catalytic activity, binding, transporter activity, structural molecular activity. Among the significant GO term analysis, 15 genes were enriched in cell wall polysaccharide metabolic process, 15 genes were involved in hemicellulose metabolic process, and 13 genes were related to xyloglucan metabolic process related to cell wall formation in expansion stage (Table 1). Moreover, the results also showed a number of significantly expression genes involved in tissue development, root morphogenesis, root system development,
root development (Table 2). KEGG pathways analysis revealed that plant hormone signal transduction, biosynthesis of amino acids were enriched with DEGs in expansion stage (Fig. 7, Fig. 8). The other pathways like MAPK signalling pathway, starch and sucrose metabolism and carbon metabolism were also expressed in KEGG pathway. These metabolic pathways may be closely related to the development of tuber expansion and bioactive compound synthesis.

Comprehensive analysis of differentially expressed genes in tuber initiation and expansion stages

Compared with expansion stage, there were large number of DETs in tuber initiation 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 S3). 242 DETs were identified to show high similarity with many plant hormone signals, including 131 down-regulated and 111 up-regulated DETs. Interestingly, the majority of plant hormone related genes in GA, BRs, IAA, ABA signal pathways were found in expansion stage (Fig. 9). 14 mitogen activated protein kinases (MAPK) genes showed up-regulated in expansion stage, while some others including MAPKK17_18, MPK6 and MPK8 were down-regulated. In all, 48 DETs were homologous with calcium signal-related genes, including 27 calcium dependent protein kinases (CDPKs), 16 calcium binding proteins (CBPs), and 5 Calreticulins (CBL).

A total of 98 transcripts homologous to the genes associated with cell wall and cell cycle were observed as differentially regulated during expansion stage, 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), cyclin-dependent kinase inhibitor (CDKIs). All of the expansin, extension, cell wall synthesis and CKS genes were down-regulated in expansion stage. Meanwhile, the majority of cell cytoskeleton and XTH were down-regulated in expansion stage in yam.

The major constituents of starch and sucrose metabolism in expansion are sucrose synthase genes (SuSy), sucrose phosphate synthase genes (SPS), starch synthase (SS) and invertase genes (INV) (Additional file 1: Table S3). Among them, the majority of SPS were up-regulated, and SuSy were down-regulated in expansion stage. Interestingly, dioscorins, the major storage proteins in yam
tubers, were significantly up-regulated in expansion stage (Additional file 1: Table S3). These results indicated that many functional genes were involved in expansion stage in yam.

Transcription factor analysis

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.10). Further analysis revealed that 541 TF-encoding genes belong to 48 TF families were differentially expressed between initiation and expansion stage. There were 286 and 255 TF-encoding genes were up and down regulated, respectively (Additional file 1: Table S4). The TF gene families with the highest number were express by heat map in expansion stage (Fig.11).

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 involved up regulated in expansion stage (Additional file 1: Table S2). Moreover, these genes were respectively involved in circadian rhythm pathway, starch and sucrose metabolism pathway and GA pathway by KEGG analysis (Additional file 1: Table S2).

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 precursors to provide novel miRNA which may be specifically in tuber. 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 S5).

Further analysis revealed that 23 known miRNAs belonged to 11 miRNA families, miRNA168, miRNA159 and novel miRNA16 were the most extensively represented families. All miRNAs were analyzed to detect differential miRNA expressions (DEM). The results showed that miRNAs expression was dynamically regulated in expansion stages. A total of 44 differentially expressed miRNAs were identified including 11 known and 33 novel miRNAs. It showed that, 40 were down regulated, and 4 were up regulated in the expansion stage (Table 3). It is interesting that miR160
family(miRNA160, miRNA160a-5p, miRNA160b-1, miRNA160h-1) and miR535a_1 were down-regulated in expansion stage, miRNA396b and miRNA168 were up-regulated compared to their miRNA families (Table 3).

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 S6). Furthermore, based on GO and KEGG analysis of the targets, it revealed that several key genes involved in expansion stage were completely regulated by miRNAs. GO analysis revealed differentially expressed miRNAs regulated in expansion stage by binding, cell, membrane, organelle, membrane part (Fig. 12). KEGG pathways analysis were involved in transport and catabolism, signal transduction, biosynthesis of other secondary metabolites, energy metabolism, carbohydrate metabolism, lipid metabolism. It is notable that 3 genes which were targets of 2 differentially expressed miRNAs (miR535a_1, miR160) were identified in the pathway of plant hormone signal transduction (Fig. 13). miRNA160 was down regulated in expansion stage, ARF18 (CL2135.Contig1_Total_1), ARF17 (Unigene5660_Total_1) were up regulated in auxin signal transduction and Log2(GH16-E/GH16-I) of three miRNAs and their target genes showed not only all of them were differentially expressed significantly but also these miRNAs regulated the expression of their targets in expansion stage. miR535a_1 was involved in brassinosteroid signal transduction pathway by targeting SBP. Moreover, miR396b and miR396a-5b were involved in other metabolism processes and functional proteins by targeting genes, including IST1-like protein, growth-regulating factor 4, photosystem II oxygen-evolving enhancer protein 2, acetyl-CoA carboxylase biotin carboxyl carrier protein (Fig. 13, Additional file 1: Table S6).

Some target genes and corresponding miRNA were involved in cell event, regulation of plant hormone levels, carbohydrate metabolism, and other metabolism, implying that they may play crucial roles during the tuber expansion stage (Fig. 14).

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

**Discussion**

In stem tuber plant, the formation of tuber is based on successive gene expression during development, and the expression of genes depends on specific tissues or developmental stage by transcriptome analyze, such as *Solanum tuberosum, Miscanthus lutarioriparius, Raphanus sativus, Nelumbo nucifera* and *Aconitum heterophyllum* [28, 34-37]. Yam is one of the most marketable stem tuber crops. Tuber expansion stage in yam is attractive theoretical model to study the development of underground organs because it is the main storage organ for reservoirs of nutrients. Thus, the understanding of candidate genes involved in the expansion stage is of predominant importance. In this study, 6.6G clean reads were generated and 14238 transcripts were identified as significantly differentially expressed in the expansion stage by transcriptome.

**Multiple signalling pathways regulation**

KEGG enrichment analysis revealed that a large number of genes were involved in the pathways related to MAPK signalling pathway, plant hormone signal transduction, starch and sucrose metabolism and carbon metabolism (Fig.8), which 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 involving a series of
signal pathways. Calcium signal is a major essential nutrient required for normal growth and
development of plants. As a second messenger, it plays a role in a number of fundamental cellular
processes like cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell differentiation,
photomorphogenesis, plant defense and various stress responses [40-42]. A potato Ca^{2+}-dependent protein kinase, StCDPK1, which is reported to be expressed in tuberization, was possible involved in potato tuberization by transcriptional activation of some of the genes[40,43]. In addition, the StCDPK1 was impacted by the treatments with JA, ABA, GA[43-45], suggested the CDPK is a possible mediator of the response to tuberization stimuli by hormone. Moreover, majority of studies have demonstrated that mRNA abundance of CBL, CBP, GaM were impacted in cell division in the swelling of potato tubers[40,43,46]. In this study, there was an increased in expansion stage in the expressions of calcium signal related genes, including 27 CDPKs and 16 CBPs. Interesting, CBLs were down-regulated(Additional file 1: Table S3). The role of CBL in the root development displayed a strong gene expression in the cell proliferation in root, its expression in root was regulated by auxin and cytokinin[47,48]. These results demonstrated that calcium signal is regulated in expansion stage. In addition, MAPK signal pathways are known to play a central role in cell proliferation, differentiation and hormone, but it is not known to be involved in tuber or root formation[49]. In this study, MAPK signal related genes were up-regulated in expansion stage(Additional file 1: Table S3). Cell number and cell size are two key determinants of plant and organ size, while involved in cell division, cell expansion and cell cycles[50,
In this study, XTH, expansin, extension, CKS, ftsHs, CDC5, CDC, CDKs, CDKIs involved in cell division, cell expansion and cell cycles were differentially regulated in expansion stage (Additional file 1: Table S3). 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

The transcription factors have been identified to play an important role in regulation of plant growth, development and secondary metabolism. Majority of transcription factors have been identified to play critical roles in the formation of organ, including MADS, bHLH, GRAS. In sweetpotato, MADS were preferentially expressed in root at the 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, like PIF3, PIF4, PIF5, key regulators for cell elongation during seedling development, is antagonistically regulated by light and gibberellins [55]. GRAS transcription factor family specifically regulated the initiation of axillary meristem in *Arabidopsis*, like SCARECROW (SCR) was expressed in cortex/endodermal initial cells in root system and played key role in regulating the radial organization of the root [56]. DELLA, such as RGA and GAI controlled cell expansion and cell division in hypocotyl, shoot and root, and floral induction [51].
In this study, a lot of transcription factor genes were found differently expression in expansion stage at the transcript level, including 66MYB, 64AP2-EREBP, 52bHLH, 37WRKY(Fig.10), and REVEILLE 6-like were identified to up regulated in circadian rhythm pathway. Zinc finger CCCH domain-containing protein 14 is involved up regulation in starch and sucrose metabolism pathway(Fig.10), which play a variety of important roles in growth and development, hormone response, and response to biotic and biotic stresses.

3 DELLA genes were up regulated in GA pathway(Fig.10), which may play key regulatory functions in expansion stage, and are involved in cell division and expansion[57]. All the data suggest that these transcription factors may be potentially involved in the expansion stage.

Hormonal signaling regulation
A set of transcriptome evidences indicated that hormone signal pathway related-genes were enriched in regulation of the onset and formation storage organ in sweetpotato, potato, ridish, lotus and carrot, while may be potentially involved in cell division, differentiation and expansion[1, 35, 37, 59, 60]. In KEGG pathway annotation, the hormone signal pathway was the most enrich one, which was involved in 8 signal pathways (Fig.8). In radish, most Aux/IAA ,ARFs and SAUR genes were abundant expressed in the root cortex splitting and expanding stage, implying that these transcripts may be involved in cell expansion in the cambium[35]. Among auxin signal related genes, Aux/IAA, ARFs were highly expressed in expansion stage in this study(Additional file 1: Table S2), and auxin has been identified to regulate cell division and expansion by changing genes expression
implying that Aux/IAA, ARFs may be provided excellent candidates in expansion stage. Interestingly, in this study, DELLA genes were up regulated, while GIDI, like GID1 and GID2 were down regulated in expansion stage (Additional file 1: Table S3). DELLA proteins are nuclear transcriptional regulators, which repress GA signal and 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 controleds hypocotyl land root elongation by reducing GA levels, DELLA interacted directly with PIF4, PIF3 and SCL to mediate crosstalk between GA and light signal[55, 62]. The DELLA were significantly expressed in rhizome development processed by GA mediated, implying these DELLA could regulated rhizome expansion [37]. Moreover, GA, IAA and ethylene affected cell growth in the root by DELLA proteins[57]. Overall, these results imply that hormone signal related genes have complexity of regulatory networks involved in the expansion stage of tuber.

Starch and sucrose metabolism regulation

Starch and sucrose is considered as one of the major carbohydrates, which the expansion tuber is high coordination with starch and sucrose metabolism genes in potato, radish and lotus by transcriptome analyze [22].
In this study, alpha-amylase, beta-amylase and isoamylase were up-regulated (Additional file 1: Table S3), this results are similar to that beta-amylase activity was increased in the swelling taproot in the radish [35], where starch content decreased in tuber expansion stage [15], and SS was down-regulated in the expansion stage (Additional file 1: Table S3). Some sucrose metabolism genes were detected in expansion stage, including SuSy, SPS, INV and invertase inhibitor (Additional file 1: Table S3). Evidence shows that sucrose could invert into starch in storage root by SuSy and AGPase, implying that they play a key role in the early expansion stage in radish [35]. The SuSy and SPS genes were down and up regulated in the expansion stage, respectively. The results were similar to taproot expansion stage in radish [35], implying they may play a major in the tuber expansion stage in yam. Yam tuber morphology were significantly correlated with sucrose and starch in tuber expansion stage [15]. Therefore, these starch and sucrose metabolism genes were required for the tuber expansion development. The regulation miRNAs by targeting potential genes
miRNA mediated gene regulation has been extensively studied in root and tuber development by transcriptional and posttranscriptional levels, which is better understanding of the molecular regulatory networks in tuber expansion stage. In this study, majority of miRNA were down regulated in expansion stage(Table 3). In maize leaf, miR160 were significantly up regulated in the meristem relative to the elongation and mature zones[63]. However, miR160 was down regulated in yam tuber expansion stage(Table 3). In general, miR160 expression forms are differently in different plant. In this study, some miRNA-mRNA pairs were observed in expansion stage(Fig.13), miR160 was involved in root cell division and differentiation by regulating auxin response factors(ARFs), and influenced root development in Arabidopsis thaliana[30].

miRNA160 was down regulated in expansion stage, auxin response factor ARF18 and ARF17 were up regulated in auxin signal transduction(Fig.13), while over expressed miR160 in transgenic rice not only were down regulated the expressions of ARF10,ARF16, and ARF17, but also inhibited root cap cell differentiation, lost control of cell division and led to ectopic expansion of apical stem cell populations[64].

Former study revealed that the ARFs targeted by miRNA160, were identified to be involved in cell expansion and cell differentiation in radish root and potato tuber[28]. In addition, miRNA396 played important roles in root growth and inhibited leaf cell division by UV-B radiation[65, 66]. miR535 were expressed in fruit development[
miRNA160, miRNA396 and miR535 may be involved in complexity network to regulate cell division and differentiation in expansion stage.

The regulatory networks associated with tuber expansion

Tuber development and response to environment are involved in gene regulatory networks[21-24, 34-37]. miRNA and target genes has been extensively studies in tuber development[21, 28], which greatly advanced our understanding of molecular regulatory networks underlying the tuber expansion development in yam. In this study, a putative model of regulatory network associated with yam tuber expansion was proposed according to our transcriptome and small RNA analysis, previous other tuber crop transcriptomics analysis and research results(Fig 13). In detail, the CBL, CBP, GaM were found to be impacted in cell division and proliferation in the expansion of potato tubers[40, 43, 46]. The role of CBL and StCDPK1 expressions were regulated by hormone in taproot development [43-45, 47].
In this study, CDPKs, CBPs and CBLs were extensively expressed in the expansion stage. MAPK signal related genes were up-regulated in the expansion stage. MAPK signal pathway increased PI3K pathway, and then led to the increase of GA levels, generating calcium signal[68-70]. Moreover, MAPK signal pathways were known to play a central role in cell proliferation, differentiation and hormone [50,51]. XTH, expansin, extension, CKS, ftsHs, CDC5, CDC, CDKs, CDKIs involved in cell division, cell expansion and cell cycles were differentially regulated in expansion stage, the finding is similar that these differentially regulated genes involved in cell wall and cell cycle metabolism were identified in Arabidopsis, Rehmannia glutinosa, radish[35,36,52]. Evidence shows that DELLA interacted directly with bHIH and SCL to mediate cell division in hypocotyl, shoot and root, and floral crosstalk between hormone and signal[55,62]. The DELLA were significantly expressed in rhizome development processed by GA mediated, implying these DELLA could regulated rhizome expansion[
DELLA can interact with PIF3, PIF4 to impacted the expansion genes expression in hypocotyl elongation.

In sweetpotato, MADS were preferentially expressed in root at the initiation of tuberization, and in the vascular cambium region where the active cell proliferation regulated by jasmonic acid, cytokinins and stress response. Among auxin signal related genes, Aux/IAA, ARFs were enriched express in the enlargement stage in this study. most Aux/IAA, ARFs and SAUR genes were enrich expressed in the root cortex splitting and expanding stage in radish, implying that these transcripts may be involved in cell expansion in the cambium.

SuSy, SPS, INV, SS and beta-amylase, were high correlated with in the expansion tuber, like potato, radish and lotus.

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 in tuber expansion stage in yam, the ARF(target by miR396), the bHLH(target by miR5021), the SBP(target by miR535a), and
the ARFs targeted by miRNA160, were identified to be involved in cell expansion and cell differentiation in radish root and potato tuber[28,35]. To sum up, environmental factors is the first signal to stimulate the tuber growth, the signal transduction pathways (hormone, calcium and MAPK signalling) and metabolism possesses (cell wall, starch and sucrose metabolism) are attributed to cell differentiation division, expansion by certain specific genes. Taken together, the result implied that these DEGs could play key roles in the regulatory network of tuber expansion.

Conclusions
A total of 14238 transcripts expressed differentially in expansion stage of yam were firstly identified using transcriptome technology. These results reveal the coordination of the tuber involved in the processes of cell events, metabolism, biosynthesis and signal transduction pathways at the transcriptional level. Noteworthy, the integrated analysis of miRNA-mRNA were identified to be preferentially expressed in auxin signal in expansion stage, highlighting the involvement of miRNA-mRNA in the hormone regulation of tuber expansion development. Taken together, the transcriptome and miRNA datasets presented here identified that a subset of candidate genes and miRNA putatively were associated with tuber enlargement 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 enlargement development in Dioscorea species.

Materials And Methods
Plant Material
*D. opposite* cultivar Guihuai 16 was planted at the farm of Guangxi University. Tuber samples were collected when tuber initiation after field planting approximately 40 days (initiation stage), additional samples were collected at 60 days (expansion stage). Two types of tuber developmental stages including initiation and expansion stage were used for the construction of various libraries, the three types of libraries were constructed, including one transcriptome *denovo* 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 were rename GH16_I, GH16_E for initiation and expansion stage, respectively). For three biological replicates of each of initiation and expansion stage were studied, respectively.

Construction and sequencing of transcriptome de novo, RNA-seq and small RNA libraries
For information about sample pooling, It can found the subsections of plant materials above. Total RNA were 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 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 were 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 were sequenced with BGISEQ-500 sequencing platform. The raw reads from mRNA and small RNA were subjected to quality control(QC) to harvest high quality of sequencing data. Raw reads were further filtered by a few of steps to obtain clean reads.

Processing and mapping of mRNA-Seq and small RNA libraries sequencing data
After filtering, the clean reads were performed de novo assembly to obtain unigenes, and functional databases are NT, NR, GO, KOG, KEGG, SwissProt and InterPro used to annotate unigene function, Blastn, Blastx, Diamond, Blast2GO and InterProScan5 were aligned unigenes to these functional data. RNA-seq reads were mapped to the transcriptome de novo analysis data using bowtie2 results with default parameters after preprocessing mRNA-seq data [72]. Gene expression levels were presented as FPKM values [73], genes with expression levels >1 FPKM were retained for further analysis. Small RNA reads also were 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 prediction of the targets of miRNAs

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

Identification and function 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), whereas miRNA read counts were normalized to tags per million (TRM). The differentially expressed genes (DEGs) and miRNAs (DEM) 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 sequences of genes against the Plant Transcriptional Factor Database. The DEGs were classified according to their TF families.

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 were 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 30 s at 95°C, and 30 s 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, then 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

Acknowledgements
Not applicable

Funding
This work was supported by the National Natural Science Foundation of China (Grant No. 81860670), the Guangxi Innovation Team Project of Tubers of Modern Agricultural Industrial Technology System of China(nycytxgcxtd-11-01), and the Guangxi Natural Science Foundation of China (Grant No. 2018GXNSFDA281029).

Availability of data and materials
The materials of this study were provided by College of Agriculture at Guangxi University. Correspondence and requests for materials should be addressed to Long-Fei He (lfhe@gxu.edu.cn).

The raw sequencing data have been submitted to the NCBI SRA database (PRJNA533985).

Authors' contributions.
DX and LFH designed experiments. YYZ and SZL analyzed the sequencing data for transcriptome
assembly. JZ and AQW developed the qPCR experiments. YYZ and LFH wrote 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.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Author details**

A College of Agriculture, Guangxi University, Nanning 530004, PR China. B Guangxi Key Laboratory for Agro-Environment and Agro-Product Safety, Nanning 530004, PR China. C Guangxi Colleges and Universities Key Laboratory of Crop Cultivation and Tillage, Nanning 530004, PR China

**References**

1. Aksenova NP, Konstantinova TN, Golyanovskaya SA, Sergeeva LI, Romanov GA: Hormonal regulation of tuber formation in potato plants. *Russ J Plant Physiol* 2012, 59(4):451-466.

2. Gong MX, Luo HL, Wang AQ, Zhou YY, Huang WJ, Zhu PC, He LF: Phytohormone profiling during tuber development of Chinese yam by ultra-high performance liquid chromatography-triple quadrupole tandem mass spectrometry. *J Plant Growth Regul* 2016, 36(2):362-373.

3. Matsumoto R, Kikuno H, Shiwachi H, Toyohara H, Takebayashi Y, Jikumaru Y, Kamiya Y: Growth of vine cuttings and fluctuations of concentrations of endogenous plant hormones in water yam (*Dioscorea alata* L.). *Trop Agric Develop* 2013, 57(1):23-30.

4. Yoshida Y, Takahashi H, Kanda H, Kanahamaz K: Effect of seed tuber weights on the development
of tubers and flowering spikes in Japanese yams (Dioscorea japonica) grown under different photoperiods and with plant growth regulators. J Jpn Soc Hortic Sci 2007, 76(3):230-236.

5. Chen SW, Shiwachi H, Sanada A, Toyohara H: Theobroxide and day length effects on the growth of yam (Dioscorea spp). Journal Issaass 2010, 16(1):22-30.

6. Shiwachi H, Ayankanmi T, Robert A: Effect of day length on the development of tubers in yams (Dioscorea spp.). Tropical Science, 2002, 42(1):162-170.

7. Ovono PO, Kevers C, Dommes J: Axillary proliferation and tuberisation of Dioscorea cayenensis-D-rotundata complex. Plant Cell Tiss Org 2007, 91(2):107-114.

8. Chen FQ, Fu Y, Wang DL, Gao X, Wang L: The effect of plant growth regulators and sucrose on the micropropagation and microtuberization of Dioscorea nipponica Makino. Journal of Plant Growth Regulation 2007, 26(1):38-45.

9. Gong MX, Luo HL, Yuan HJ, Wei SQ, Yang XD, He LF: Effects of exogenous gibberellin and paclobutrazol on tuber expansion and bulbil formation of Chinese yam. Acta Horticulturae Sinica 2015, 42(6):1175-1184.

10. Behera KK, Pani D, Sahoo S, Maharana T, Sethi BK: Effect of GA(3) and urea treatments on improvement of microtuber production and productivity of different types of planting material in greater yam (Dioscorea alata L.). Not Bot Horti Agrobo 2009, 37(2):81-84.

11. Hamadina El, Craufurd PQ, Battey NH, Asiedu R: In vitro micro-tuber initiation and dormancy in yam. Ann Appl Biol 2010, 157(2):203-212.

12. Bazabakana R, Wattiez R, Baucher M, Diallo B, Jaziri M: Effect of jasmonic acid on developmental morphology during in vitro tuberization of Dioscorea alata (L.). Plant Growth Regul 2003, 40(3):229-237.

13. Kim S, Jang S, Lee S: Exogenous effect of gibberellins and jasmonate on tuber enlargement of Dioscorea opposita. Aagronmoy Research 2005, 3(1):39-44.

14. Yan HB, Yang LT, Li YR: Axillary shoot proliferation and tuberization of Dioscorea fordii Prainet Burk. Plant Cell Tiss Org 2011, 104(2):193-198.

15. Liang RF, Li CZ, Zhang J, He LF, Wei BH, Gan XQ, He HY: Changes of matter accumulation and
relative enzymatic activity during yam tuber development. *Acta Agronomica Sinica* 2011, 37(5):903-910.

16. Loko YL, Bhattacharjee R, Agre AP, Dossou-Aminon I, Orobiyi A, Djedatin GL, Dansi A: Genetic diversity and relationship of Guinea yam (*Dioscorea cayenensis* Lam.-*D-rotundata* Poir. complex) germplasm in Benin (West Africa) using microsatellite markers. *Genet Resour Crop Ev* 2017, 64(6):1205-1219.

17. Girma G, Gedil M, Spillane C: Morphological, SSR and ploidy analysis of water yam (*Dioscorea alata* L.) accessions for utilization of aerial tubers as planting materials. *Genet Resour Crop Ev* 2017, 64(2):291-305.

18. Tamiru M, Yamanaka S, Mitsuoka C, Babil P, Takagi H, Lopez-Montes A, Sartie A, Asiedu R, Terauchi R: Development of genomic simple sequence repeat markers for yam. *Crop Sci* 2015, 55(5):2191-2200.

19. Akakpo R, Scarcelli N, Chair H, Dansi A, Djedatin G, Thuillet AC, Rhone B, Francois O, Alix K, Vigouroux Y: Molecular basis of African yam domestication: analyses of selection point to root development, starch biosynthesis, and photosynthesis related genes. *Bmc Genomics* 2017, 18(1):782.

20. Zhao XT, Zhang XL, Guo XB, Li SJ, Han LL, Song Zi, Wang YY, Li JH, Li MJ: Identification and validation of reference genes for qRT-PCR studies of gene expression in *Dioscorea opposita*. *BioMed research international* 2016.

21. Kondhare KR, Malankar NN, Devani RS, Banerjee AK: Genome-wide transcriptome analysis reveals small RNA profiles involved in early stages of stolon-to-tuber transitions in potato under photoperiodic conditions. *BMC Plant Biol* 2018, 18(1):284.

22. Van Harsselaar JK, Lorenz J, Senning M, Sonnewald U, Sonnewald S: Genome-wide analysis of starch metabolism genes in potato (*Solanum tuberosum* L.). *Bmc Genomics* 2017, 18.

23. Gong L, Zhang H, Gan X, Zhang L, Chen Y, Nie F, Shi L, Li M, Guo Z, Zhang G et al: Transcriptome profiling of the potato (*Solanum tuberosum* L.) plant under drought stress and water-stimulus conditions. *Plos One* 2015, 10(5):e0128041.

24. 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.

25. Li J, Liang Q, Li C, Liu M, Zhang Y: Comparative transcriptome analysis identifies putative genes involved in dioscin biosynthesis in *Dioscorea zingiberensis*. *Molecules* 2018, 23(2).

26. Wu ZG, Jiang W, Mantri N, Bao XQ, Chen SL, Tao ZM: Transcriptome analysis reveals flavonoid biosynthesis regulation and simple sequence repeats in yam (*Dioscorea alata* L.) tubers. *Bmc Genomics* 2015, 16:346.

27. Liu C, Liu X, Xu W, Fu W, Wang F, Gao J, Li Q, Zhang Z, Li J, Wang S: Identification of miRNAs and their targets in regulating tuberous root development in radish using small RNA and degradome analyses. *3 Biotech* 2018, 8(7):311.

28. 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, 14:6.

29. Sun YY, Qiu Y, Zhang XH, Chen XH, Shen D, Wang HP, Li XX: Genome-wide identification of microRNAs associated with taproot development in radish (*Raphanus sativus* L.). *Gene* 2015, 569(1):118-126.

30. Mallory AC, Bartel DP, Bartel B: MicroRNA-directed regulation of Arabidopsis AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. *Plant Cell* 2005, 17(5):1360-1375.

31. Sarkar D: Photoperiodic inhibition of potato tuberization: an update. *Plant Growth Regul* 2010, 62(2):117-125.

32. Martin A, Adam H, Diaz-Mendoza M, Zurczak M, Gonzalez-Schain ND, Suarez-Lopez P: Graft-transmissible induction of potato tuberization by the microRNA miR172. *Development* 2009, 136(17):2873-2881.

33. Tamiru M, Natsume S, Takagi H, White B, Yaegashi H, Shimizu M, Yoshida K, Uemura A, Oikawa K, Abe A et al: Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. *BMC Biol* 2017, 15(1):86.

34. Hu RB, Yu CJ, Wang XY, Jia CL, Pei SQ, He K, He G, Kong YZ, Zhou GK: De novo transcriptome
analysis of miscanthus iutarioiriparius identifies candidate genes in rhizome development. *Front Plant Sci* 2017, 8.

35. Yu RG, Wang J, Xu L, Wang Y, Wang RH, Zhu XW, Sun XC, Luo XB, Xie Y, Everlyne M et al: Transcriptome profiling of taproot reveals complex regulatory networks during taproot thickening in radish (*Raphanus sativus* L.). *Front Plant Sci* 2016, 7.

36. Malhotra N, Sood H, Chauhan RS: Transcriptome-wide mining suggests conglomerate of genes associated with tuberous root growth and development in *Aconitum heterophyllum* Wall. 3 *Biotech* 2016, 6(2):152.

37. Yang M, Zhu LP, Pan C, Xu LM, Liu YL, Ke WD, Yang PF: Transcriptomic analysis of the regulation of rhizome formation in temperate and tropical lotus (*Nelumbo nucifera*). *Sci Rep-Uk* 2015, 5.

38. Ursache R, Nieminen K, Helariutta Y: Genetic and hormonal regulation of cambial development. *Physiologia plantarum* 2013, 147(1):36-45.

39. Bhalerao RP, Fischer U: Environmental and hormonal control of cambial stem cell dynamics. *Journal of Experimental Botany* 2017, 68(1):79-87.

40. Nookaraju A, Pandey SK, Upadhyaya CP, Heung JJ, Kim HS, Chun SC, Kim DH, Park SW: Role of Ca2+-mediated signaling in potato tuberization: An overview. *Botanical Studies* 2012, 53(2):177-189.

41. Chen T, Wu X, Chen Y, Li X, Huang M, Zheng M, Baluska F, Samaj J, Lin J: Combined proteomic and cytological analysis of Ca2+-calmodulin regulation in *Picea meyeri* pollen tube growth. *Plant physiology* 2009, 149(2):1111-1126.

42. Feng JL, Yang ZJ, Chen SP, El-Kassaby YA, Chen H: Signaling pathway in development of camellia oleifera nurse seedling grafting union. *Trees-Structure and Function* 2017, 31(5):1543-1558.

43. 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.

44. Yu XC, Li MJ, Gao GF, Feng HZ, Geng XQ, Peng CC, Zhu SY, Wang XJ, Shen YY, Zhang DP: Abscisic acid stimulates a calcium-dependent protein kinase in grape berry. *Plant physiology* 2006, 140(2):558-579.

45. Ulloa RM, Raices M, MacIntosh GC, Maldonado S, Tellez-Inon MT: Jasmonic acid affects plant
morphology and calcium-dependent protein kinase expression and activity in *Solanum tuberosum*. *Physiologia plantarum* 2002, 115(3):417-427.

46. Kim MS, Kim HS, Kim YS, Baek KH, Oh HW, Hahn KW, Bae RN, Lee IJ, Joung H, Jeon JH: Superoxide anion regulates plant growth and tuber development of potato. *Plant Cell Rep* 2007, 26(10):1717-1725.

47. Kumar Meena M, Kumar Vishwakarma N, Tripathi V, Chattopadhyay D: CBL-interacting protein kinase 25 contributes to root meristem development. *Journal of Experimental Botany* 2018.

48. Cui XY, Du YT, Fu JD, Yu TF, Wang CT, Chen M, Chen J, Ma YZ, Xu ZS: Wheat CBL-interacting protein kinase 23 positively regulates drought stress and ABA responses. *Bmc Plant Biology* 2018, 18.

49. Hirt H: Connecting oxidative stress, auxin, and cell cycle regulation through a plant mitogen-activated protein kinase pathway. *P Natl Acad Sci USA* 2000, 97(6):2405-2407.

50. Polyn S, Willems A, De Veylder L: Cell cycle entry, maintenance, and exit during plant development. *Current opinion in plant biology* 2015, 23:1-7.

51. Guo M, Simmons CR: Cell number counts--the fw2.2 and CNR genes and implications for controlling plant fruit and organ size. *Plant Sci* 2011, 181(1):1-7.

52. Paque S, Mouille G, Grandont L, Alabadi D, Gaertner C, Goyallon A, Muller P, Primard-Brisset C, Sormani R, Blazquez MA et al: AUXIN BINDING PROTEIN1 links cell wall remodeling, auxin signaling, and cell expansion in Arabidopsis. *Plant Cell* 2014, 26(1):280-295.

53. 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.

54. Song WH, Tan CT, Zhu MK, Xu T, Dong TY, Li ZY: Cloning and expression analysis of stress treatments of a MADS-box transcription factor in sweet potato. *Bulletin of Botanical Research* 2017, 37(4):587-595,627.

55. de Lucas M, Daviere JM, Rodriguez-Falcon M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blazquez MA, Titarenko E, Prat S: A molecular framework for light and gibberellin control of cell elongation. *Nature* 2008, 451(7177):480-484.
56. Heo JO, Chang KS, Kim IA, Lee MH, Lee SA, Song SK, Lee MM, Lim J: Funneling of gibberellin signaling by the GRAS transcription regulator SCARECROW-LIKE 3 in the Arabidopsis root. *P Natl Acad Sci USA* 2011, 108(5):2166-2171.

57. Daviere JM, Achard P: A pivotal role of DELLAs in regulating multiple hormone signals. *Mol Plant* 2016, 9(1):10-20.

58. Li WT, He M, Wang J, Wang YP: Zinc finger protein (ZFP) in plants-A review. *Plant Omics* 2013, 6(6):474-480.

59. Que F, Wang GL, Li T, Wang YH, Xu ZS, Xiong AS: Genome-wide identification, expansion, and evolution analysis of homeobox genes and their expression profiles during root development in carrot. *Functional & Integrative Genomics* 2018, 18(6):685-700.

60. Ponniah SK, Thimmapuram J, Bhide K, Kalavacharla V, Manoharan M: Comparative analysis of the root transcriptomes of cultivated sweetpotato (*Ipomoea batatas* L. Lam) and its wild ancestor (*Ipomoea trifida* Kunth G. Don). *Bmc Plant Biology* 2017, 17.

61. Ursache R, Nieminen K, Helariutta Y: Genetic and hormonal regulation of cambial development. *Physiol Plant* 2013, 147(1):36-45.

62. Zhang ZL, Ogawa M, Fleet CM, Zentella R, Hu J, Heo JO, Lim J, Kamiya Y, Yamaguchi S, Sun Tp: SCARECROW-LIKE 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in Arabidopsis. *P Natl Acad Sci USA* 2011, 108(5):2160-2165.

63. Aydinoglu F, Lucas SJ: Identification and expression profiles of putative leaf growth related microRNAs in maize (*Zea mays* L.) hybrid ADA313. *Gene* 2019, 690:57-67.

64. Yang SN, Wang J, Chen ZX, Tian XJ, Zhang JW, Long Y, Pei XW, Yuan QH: Clone and function analysis MiR160f in common wild rice(*Oryza rufipogon Griff.*). *Biotechnology Bulletin* 2014(11):114-118.

65. Bao ML, Bian HW, Zha YL, Li FY, Sun YZ, Bai B, Chen ZH, Wang JH, Zhu MY, Han N: miR396a-mediated basic Helix-Loop-Helix transcription factor bHLH74 repression acts as a regulator for root growth in Arabidopsis seedlings. *Plant and Cell Physiology* 2014, 55(7):1343-1353.

66. Casadevall R, Rodriguez RE, Debernardi JM, Palatnik JF, Casati P: Repression of growth regulating
factors by the microRNA396 inhibits cell proliferation by UV-B radiation in Arabidopsis leaves. Plant Cell 2013, 25(9):3570-3583.

67. Shi MY, Hu X, Wei Y, Hou X, Yuan X, Liu J, Liu YP: Genome-wide profiling of small RNAs and degradome revealed conserved regulations of miRNAs on auxin-responsive genes during fruit enlargement in peaches. International Journal of Molecular Sciences 2017, 18(12).

68. Manna A, De Sarkar S, De S, Bauri AK, Chattopadhyay S, Chatterjee M: Impact of MAPK and PI3K/AKT signaling pathways on Malabaricone-A induced cytotoxicity in U937, a histiocytic lymphoma cell line. International immunopharmacology 2016, 39:34-40.

69. Villasuso AL, Racagni GE, Machado EE: Phosphatidylinositol kinases as regulators of GA-stimulated alpha-amylase secretion in barley (Hordeum vulgare). Physiologia plantarum 2008, 133(2):157-166.

70. Liu Z, Wang B, He R, Zhao Y, Miao L: Calcium signaling and the MAPK cascade are required for sperm activation in Caenorhabditis elegans. Biochimica Et Biophysica Acta-Molecular Cell Research 2014, 1843(2):299-308.

71. Feng SH, Martinez C, Gusmaroli G, Wang Y, Zhou JL, Wang F, Chen LY, Yu L, Iglesias-Pedraz JM, Kircher S et al: Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature 2008, 451(7177):475-U479.

72. 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 research 2010, 38(6):1767-1771.

73. Langmead B, Salzberg SL: Fast gapped-read alignment with Bowtie 2. Nature methods 2012, 9(4):357-359.

74. Nawrocki EP, Eddy SR: Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 2013, 29(22):2933-2935.

75. Nawrocki EP, Burge SW, Bateman A, Daub J, Eberhardt RY, Eddy SR, Floden EW, Gardner PP, Jones TA, Tate J et al: Rfam 12.0: updates to the RNA families database. Nucleic acids research 2015, 43(Database issue):D130-137.

76. Breakfield NW, Corcoran DL, Petricka JJ, Shen J, Sae-Seaw J, Rubio-Somoza I, Weigel D, Ohler U,
Benfey PN: High-resolution experimental and computational profiling of tissue-specific known and novel miRNAs in Arabidopsis. *Genome research* 2012, 22(1):163-176.

77. Wu HJ, Ma YK, Chen T, Wang M, Wang XJ: PsRobot: a web-based plant small RNA meta-analysis toolbox. *Nucleic acids research* 2012, 40(Web Server issue):W22-28.

78. Wang L, Feng Z, Wang X, Zhang X: DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 2010, 26(1):136-138.

Tables

Table 1 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 2 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 3 The 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       |
| miR168a-5p    | 55653              | 59949              | 3708.9             | 3153.816667        | -1.13                    | 0       |
| miR168        | 20208              | 106873             | 1334.103333        | 5618.38            | 1.17                     | 0       |
| novel_mir1    | 16275 | 7760 | 1085.586667 | 407.58 | -2.30 | 0 |
| miR396b     | 1097  | 21915| 72.84333333| 1176.08| 3.09  | 0 |
| miR535a_1   | 24978 | 25221| 1654.31     | 1315.066667| -1.22 | 0 |
| miR396a-5p  | 12295 | 4269 | 425.4933333 | 224.5533333 | -1.82 | 0 |
| novel_mir33  | 6408  | 4297 | 816.34      | 549.1166667 | -1.12 | 0 |
| novel_mir15  | 9666  | 10433| 642.81      | 549.1166667 | -1.12 | 0 |
| novel_mir11  | 4852  | 3769 | 323.0733333 | 199.1266667 | -1.60 | 0 |
| novel_mir25  | 5833  | 6318 | 386.43      | 333.3666667 | -1.12 | 0 |
| novel_mir32  | 3196  | 2381 | 212.7166667 | 125.7166667 | -1.66 | 0 |
| novel_mir5   | 2039  | 1094 | 135.3133333 | 58.02666667 | -2.13 | 0 |
| novel_mir10  | 3197  | 3009 | 213.0166667 | 158.4866667 | -1.32 | 0 |
| novel_mir44  | 3381  | 3299 | 224.6533333 | 173.7233333 | -1.27 | 0 |
| novel_mir36  | 1859  | 1246 | 124.13      | 65.29333333 | -1.81 | 0 |
| novel_mir31  | 1632  | 1049 | 108.56      | 55.05666667 | -1.87 | 0 |
| novel_mir18  | 1701  | 1305 | 114.0466667 | 68.55  | -1.62 | 0 |
| novel_mir37  | 2777  | 2281 | 183.85      | 120.27 | -1.52 | 0 |
| novel_mir12  | 1666  | 1308 | 111.28      | 69.05  | -1.62 | 0 |
| novel_mir45  | 1478  | 1093 | 99.12       | 57.63  | -1.67 | 0 |
| miR160b_1   | 463   | 30   | 30.2666667  | 1.61666667 | -5.18 | 0 |
| novel_mir48  | 694   | 275  | 46.22333333 | 14.49666667 | -2.57 | 0 |
| novel_mir49  | 676   | 265  | 45.07333333 | 13.82333333 | -2.59 | 0 |
| novel_mir24  | 1045  | 779  | 69.79       | 40.94  | -1.66 | 0 |
| novel_mir23  | 377   | 109  | 25.15666667 | 5.67666667 | -3.03 | 0 |
| miR168_1    | 30    | 5    | 1.97666667  | 0.26033333 | -3.82 | 0 |
| novel_mir9  | 123   | 142  | 8.24333333  | 7.47666667 | -1.03 | 0 |
| miR160h_1   | 7     | 0    | 0.46333333  | 0.001  | -5.04 | 0.00041
Figures

Figure 1

A picture of yam tuber in 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

Fig. 4

Distribution of species aligned by yam genes
Correlation analysis between the initiation and expansion stage

| GH16_E_r1 | GH16_E_r2 | GH16_E_r3 | GH16_I_r1 | GH16_I_r2 | GH16_I_r3 |
|-----------|-----------|-----------|-----------|-----------|-----------|
| 1.0000    | 0.9999    | 0.9997    | 0.7480    | 0.7431    | 0.7439    |
| 0.9999    | 1.0000    | 0.9997    | 0.7442    | 0.7395    | 0.7403    |
| 0.9997    | 0.9997    | 1.0000    | 0.7480    | 0.7433    | 0.7440    |
| 0.7480    | 0.7442    | 0.7480    | 1.0000    | 0.9998    | 0.9992    |
| 0.7431    | 0.7395    | 0.7433    | 0.9998    | 1.0000    | 0.9998    |
| 0.7439    | 0.7403    | 0.7440    | 0.9992    | 0.9968    | 1.0000    |

Figure 5
Figure 6

Gene ontology classification of DEGs in expansion stage
Figure 7

Functional classification and pathway assignment of DEGs by KEGG in expansion stage

Figure 8

The top 20 in KEGG Pathway enrichment of the 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 represents negatively correlated, and blue represents positively correlated in each miRNA-mRNA pair.
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

This is a list of supplementary files associated with this preprint. Click to download.

additional file1.xlsx