LncRNA regulates tomato fruit cracking by coordinating gene expression in hormone-redox-cell wall network

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Abstract

Background Fruit cracking, occurs easily under unsuitable environmental conditions, is one of the main disorders in fruit production. It is widely accepted that plant have developed a defense mechanisms or regulatory networks in response to abiotic stress. This involves perceiving, integrating and responding to stress signals by modulating the expression of related genes. Fruit cracking is also a kind of physiological disease caused by abiotic stress. Previously reported a single or sevel genes may regulate fruit cracking. However, almost none of these efforts have involved cracking regulatory network. Results Here, 0, 8 and 30 h irrigation treatments resulted in differential expression of 1028 mRNAs and 87 lncRNAs in 'LA1698' (cracking resistant, CR) at 8 h_vs_0 h, 468 mRNAs and 15 lncRNAs at CR_30 h_vs_CR_0 h, 321 mRNAs and 19 lncRNAs at CR_30 h_vs_CR_8 h; 531 mRNAs and 75 lncRNAs in 'LA2683' (cracking susceptible, CS) at 8 h_vs_0 h, 420 mRNAs and 24 lncRNAs at CS_30 h_vs_CS_0 h, 270 mRNAs and 20 lncRNAs at CS_30 h_vs_CS_8 h; 339 mRNAs and 64 lncRNAs in the two contrasting tomato genotypes at 0 h, 338 mRNAs and 94 lncRNAs at 8 h, and 369 mRNAs and 77 lncRNAs at 30 h. The GO pathway of the differentially expressed mRNAs are mainly enriched in 'hormone metabolic process', 'cell wall organization', 'oxidoreductase activity' and 'catalytic activity'. In addition, lncRNAs regulated the expression of their neighboring genes and genes related to tomato cracking were selected to construct a lncRNA-mRNA network that influence tomato cracking. Conclutions This study provides insight into the responsive network for water-induced cracking in tomato fruit. specifically lncRNAs regulated hormone-redox-cell wall network, including plant hormone (including auxin, ethylene) and ROS (H_2O_2) signal transduction and many cell wall related mRNAs (EXP, PG, XTH), as well as some lncRNAs (XLOC_010878 and XLOC_016662, ect) .

Background

Fruit cracking, one of the main disorders in fruit production, can easily cause adverse impact in fruit marketability-like reducing the fruits quality for their poor appearance, decreasing shelf life, and even making the fruit unmarketable because of the fungal infection [1]. Cracking may occurs during fruit ripening or development. When there is a rapid flow into the fruit, and if skin loses strength and elasticity due to maturity, etc., cracking are most likely to occurs. Since 1930s, researchers have done a lot of theoretical and practice studies on cracking [2]. Cracking is the result of a combination of internal and external factors. The internal factor refers to the fruit's own characteristics (fruit size; shape; firmness; epidermal characteristics such as the deposition of cutin, wax; strength of the pericarp; arrangment of cells in the pericarp; quantity and status of stomata; accumulation of osmoregulation substances such as soluble sugar content and the growth stage of the fruit, etc.), external factors mainly include environment (humidity; light; temperature; wind, etc.), cultivation management (irrigation; mineral nutrition; plant regulation, etc.) [3-6]. Cortes [7] comprehensively analyzed 62 genotypes and found that the correlation between cracked fruit and heredity was significantly greater than that of the environment, indicating that the cracking characteristic can stable heritability, and there is difference among varieties.

Notably, cell wall components and modification appeared to be correlated with the strength of the skin and fruit cracking [8-11]. As ripening proceeded, the cell wall degradation gradually arised, simultaneously, the fruit cracking rate increased [8-9]. Cell wall is composed of cellulose-hemicellulose (Cel-Hem) network and pectin, which is essential to maintain the mechanical strength of the cell wall. As the fruit matures, enzymes and proteins that degrade the polysaccharide component of the cell wall are produced, such as polygalacturonase (*PG*), extended protein (*EXP*), pectin methylesterase (*PME*), β-galactosidase. (*β-gal*) and cellulase (*Cx*) [12-13]. The synergistic action of these enzymes leads to degradation of the cell wall polysaccharides and softening of the mature fruit peels [14]. Genes such as *EXP*, *PG,β-gal* and *XET* has proven to be associated with fruit cracking [15-20]. Inhibition of *β-gal* gene expression increases the rate of fruit cracking [18]. In tomato, inhibition of *LePG* expression slightly reduced the rate of fruit cracking [19]. Yang [21] found that the lower *PG, β-Gal*, and *Cx* activities, leading to greater protopectin and hemicelluloses, may make the skin of 'LA1698' firmer, increase bursting strength. Studies suggested that there is no single gene, but many genes work together to regulate fruit cracking [22-24]. Jiang et al's research illustrated that the simultaneous suppression of *SlPG* and *SlEXP1* in ripening fruits reduced cell wall disassembly and then reduced fruit cracking rate [24]. But it is still unclear whether there are other genes related to fruit cracking and which one is the major gene.

Recently, people gradually realize that non-coding RNAs (ncRNAs) have important biological function [25,26]. Researches illustrated that instead of about 2% protein-coding genes, there are more than 90% genes that do not have the ability to encode proteins and are transcribed into ncRNAs [25]. These ncRNAs originally thought to be "expression noise", "expression waste", but nowadays they have proven to be strictly regulated and played important roles in biological process of organisms and have extremely complex biological functions [27-29]. With much of the work about ncRNAs being published in humans and animals, studies on plants are limited to some model plants, such as Arabidopsis, maize, and wheat [30-33], and most of the annotated lncRNAs are related to the regulation of the development. Xin [30] identified 125 stress-related lncRNAs in wheat, of which 71 responded to powdery mildew and 77 responded to heat stress. Dai [31] found a specific expression of lncRNA *Zm401* in maize, and found that it regulates the expression of key genes involved in pollen development such as *ZmMADS2, MZm3-3* and *ZmC5*. Swiezewski [32] discovered that *COOLAIR* (cold induced long antisense intragenic RNA) is involved in the vernalization process and regulates the expression of plant flowering suppressor gene *FLC* (flowering locus C). In the plant field, most of the identified lncRNAs are able to respond to environmental stress and growth-related regulatory processes. Is lncRNAs play important roles in fruit cracking too?

This study aimed to explore a global view of transcriptional regulation (mRNAs and lncRNAs) of fruit cracking induced by irrigation in tomato. The RNAs expression in 0, 8 and 30 h irrigation-treated fruits from two contrasting tomato genotypes, 'LA1698' (cracking resistant, CR) and 'LA2683' (cracking susceptible, CS), were analyzed by mRNA and lncRNA sequencing. Differentially expressed mRNAs and lncRNAs related to fruit cracking were identified through transcriptome profiles and bioinformatics analysis. We then constructed a mRNA-lncRNA network. Finally, we determined a responsive network for water-induced cracking based on such high-throughput microarray data and previous results. The

findings reported here can increase our understanding of the transcriptional regulatory mechanisms of fruit cracking in tomato.

Methods

Plant Materials and Sample Collection

The cracking sensitive (CS) tomato 'LA2683' and the cracking resistance (CR) tomato 'LA1698' **(Fig. 1a)** were introduced from the tomato genetics resource centre (TGRC, University of California, Davis). The fruit cracking rate of 'LA2683' is 77.53%, and the fruit cracking rate of 'LA1698' is 20.17%. Both lines were selected and self-pollinated for more than 6 generations. All the seedlings were grown in 72-plug trays on 18 March, 2016. On 28 April, 2016, they were then transplanted to the same greenhouse at Kunshan Yuye leaf vegetable base (31°95′E, 119°16′N), Jiangsu province, China. The climate of this area belongs to a north subtropical south monsoon climate zone. Plants spacing followed a 30×50×100 cm pattern. When the tomato fruit entered the red ripening stage, the saturated irrigation was adopted to induce the cracking. Samples of fruits were taken from both genotypes at 0 h, 8 h and 30 h of the irrigation treatment, the samples were then immediately frozen in liquid nitrogen and stored at -80℃.

Transcriptome sequencing

Twelve fruit samples (2 genotypes, 3 time points, each with two replications) were collected and sequenced by Novogene, Beijing, China. Transcriptome sequencing was carried out on an Illumina HiSeq 2500 platform and paired-end raw reads were then generated.

RNA-seq data analysis

The raw image data was converted into raw reads by recognition system and were dumped to FASTQ format file. We calculated the Q20, Q30 and GC contents and then filtered to get clean reads. Finally, the clean reads were mapped to the tomato reference genome (https://solgenomics.net) by TopHat v2.0.9 [34], and transcriptome was assembled by Cufflinks [35].

Identification of mRNA and lncRNA

The transcripts that could compared to the known transcripts data were identified as annotated mRNA.

Then the transcripts were screened according to the following steps: (1) Exon number≥2, (2) Transcript length ≥200bp, (3) Screen out transcripts that overlap the database annotation exon area with Cuffcompare software. And the lncRNA overlapping with the exon region of the spliced transcript was included in the subsequent analysis, (4) The expression level of each transcript was calculated by Cuffquant, and transcripts with FPKM≥0.5 were selected, (5) Two protein-coding potential evaluation algorithms (CPC [36] and PFAM [37]) were used to predict the protein coding potential of the remaining transcripts. Only when these two algorithms simultaneously considered no protein-coding potential, were considered to be predicted lncRNAs. And finally the predicted lncRNA is obtained.

The transcripts were assessed by Protein coding potential evaluation algorithm, and the transcripts that have the potential to encode proteins were identified as novel mRNAs

Analysis of mRNA and lncRNA

mRNA and lncRNA abundance levels of the unigenes were normalized by the Fragments Per Kilobase of exon model per Million mapped reads (FPKM). The log2 (fold changes) between two samples were tested statistically to determine whether an individual gene expression with significantly altered expression. And *Q*-value≤0.05 & |log2 (fold change)|> 1 was considered to be a differentially expressed gene. The trend analyses of mRNA and lncRNA expression in tomato fruit after irrigation was then carried out **(Fig. 1b)**. To understand the function of differentially expressed mRNAs, these mRNAs were further employed to GO (Gene ontoligy) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis by using GOseq [38] and KOBAS [39] softwares, respectively.

Feature analysis of lncRNA

LncRNA mainly acts on the protein-encoding target gene by cis or trans to achieve its regulatory function. The principle of lncRNA acting on target genes by cis was that the function of lncRNA was related to its neighboring protein-coding genes [40]. In this study, the protein-coding gene within 100 k distance from the lncRNA was identified as its predicted target gene. The function of lncRNA was predicted by functional enrichment analysis of the target gene. Genomic characterization of the predicted lncRNA was performed and compared with mRNA. The comparison parameters included the number of exons, ORF length, transcript nucleic acid length and sequence conservation between species to understand the genomic characteristics of lncRNA.

qRT-PCR verification

Total RNA was extracted from the tomato fruit by RNAprep Pure Plant Kit (Tiangen BiotechCo.,Ltd. (Beijing, China)) following the manufacturer's instructions. The cDNA was generated using 5 ul of total RNA by abm's 5X All-In-One MasterMix. qRT-PCR was performed on a CFX 96 Touch RT-PCR detection system (Bio-Rad, USA) with abm's EvaGreen 2X qPCR MasterMix-Low ROX. Then 9 DEGs were randomly picked from the DEGs to verify the RNA-seq results. Gene-specific primers were designed using Premier 5.0 and the reference gene in this study was actin (GenaBank accession number is NM_001308447.1). The relative levels of gene expression were calculated using the $2^{-\Delta\Delta CT}$ [41] method. The sequences of primers were listed in **Additiition file 1.**

Result

RNA sequencing and identification of lncRNA and mRNA in tomato cracking

In total, we obtianed 0.82-1.19 billion raw reads from CR and CS tomatoes at various times points (0, 8 and 30 h of saturated irrigation treatments). And 0.79-1.14 billion clean reads in the samples were

obtained **(Table 1)**. Through genomic comparison, we identified 1 annotated lncRNA and 2406 novel lncRNAs **(Additional file 2)**, 33784 annotated mRNAs and 1453novel mRNAs.The candidate lncRNAs were further screened by cufflinks splicing **(Fig. 2a)**. In order to eliminate the possible protein-encoding transcripts, both CPC and PFAM protein coding potential evaluation algorithms were used to screen. Finally, 2406 novel lncRNAs were obtained **(Fig. 2b)**, including 2004 lincRNAs and 402 antisense lncRNAs. The transcript that is considered to have protein coding potential by any of the two algorithms was considered as the novel mRNA, which has a total of 1453.

Comparative features of mRNAs and lncRNAs

To gain insight into the genomic features of lncRNA, we calculated the exon number, transcript length, and ORF length of 2406 lncRNAs and compared them to annotated mRNAs (Fig. 3). The average length of the obtained lncRNA was 1535nt, which is similar to the mRNA (1221nt) ; the average number of exons of the identified lncRNA was 1.9, which was much lower than the exon number of the mRNA (4.7); in terms of ORF length, lncRNA has an average length of 98 bp, which is much lower than the mRNA (347bp). Consistent with previous studies, lncRNAs have shorter lengths, fewer exons, and lower expression levels. At the same time, we used phyloP to separately score lncRNA and mRNA, and the sequence conservation of lncRNA was lower than that of mRNA, which is consistent with previous studies [42]. lncRNAs can act in cis to regulate the expression of their neighboring genes, We identified 28126 pairs of lncRNA-mRNA with targeted relationships upstream and downstream of 2406 lncRNAs (Additional file 3).

Differential expression analysis

Differentially expressed mRNAs and lncRNAs were analyzed in CR tomato and CS tomato by using cufflinks software **(Fig. 4)**. mRNAs and lncRNAs with *Q*-value≤0.05 & |log2 (fold change)|> 1 were selected as differentially expressed genes. There were 1028 mRNAs and 87 lncRNAs differentially expressed at CR_8 h_vs_CR_0 h. Among them, 601 mRNAs and 16 lncRNAs were down-regulated, and 427 mRNAs and 71 lncRNAs were up-regulated. There were 468 mRNAs and 15 lncRNAs differentially expressed at CR_30 h_vs_CR_0 h, of which 313 mRNAs and 3 lncRNAs were down-regulated, 155 mRNA and 12 lncRNAs were up-regulated; There were 531 mRNAs and 75 lncRNAs differentially expressed at CS_8 h_vs_CS_0 h. Among them, 332 mRNA and 13 lncRNAs were down-regulated, and 199 mRNA and 62 lncRNAs were up-regulated. There were 420 mRNAs and 24 lncRNAs differentially expressed at CS_30 h_vs_CS_0 h, of which 126 mRNAs and 4 lncRNAs were down-regulated, 427 mRNA and 20 lncRNAs were up-regulated; There were 339 mRNAs and 64 lncRNAs differentially expressed at CS_0 h_vs_CR_0 h, and 338 mRNAs and 94 lncRNAs were differentially expressed at CS_8 h_vs_CR_8 h, and 369 mRNAs and 77 lncRNAs were differentially expressed at CS_30 h_vs_CR_30 h. Among them, at 0h of irrigation, 272 mRNAs and 34 lncRNAs were down-regulated, 77 mRNAs and 30 lncRNAs were up-regulated; at 8h of irrigation, 186 mRNAs and 55 lncRNAs were down-regulated, 152 mRNAs and 39 lncRNAs were up-regulated; at 30h of

irrigation, 147 mRNAs and 41 lncRNAs were down-regulated, 222 mRNAs and 36 lncRNAs were up-regulated.

Functional prediction of DEGs

In order to investigate trends in gene functions and enrichment for DEGs(differentially expressed genes), we performed the GO (Gene Ontology) analysis of the selected mRNA **(Additional file 4)**. The results showed that 810 DEGs in CR_8 h_vs_CR_0 h were involved in GO enrichment, among them, 29 DEGs were enriched into biological processes and cellular components. In CR_30 h_vs_CR_0 h, there are 357 DEGs involved in GO enrichment, most of which were enriched in biological processes. Among them, regulation of biological process and biological regulation have the largest number of genes, which account for 83 (23.25%) and 86 (24.09%), respectively, followed by 71 (19.89%) in regulation of cellular Progress. The DEGs in CR_30 h_vs_CR_8 h were mainly enriched in catalytic activity (60.9%). And for the CS tomato, 433 DEGs were obtained in 8h_vs_0h, and the number of single-organism metabolic progress was the highest, reaching 119 (27.48%), followed by the biological process-small molecule metabolic progress, 63 (14.55%); Most of the DEGs in CS_30 h_vs_CS_ 0h are enriched in biological processes such as regulation of metabolic progress, regulation of biosynthetic, etc. In CS_30 h_vs_CS_8 h, DEGs were mainly enriched in biological processes, 195 (79.59%). Between CR and CS tomato, the DEGs were mainly enriched in biological process, cell component and molecular function before irrigation treatment(0 h), and 44 (16.18%) DEGs were significantly enriched in oxidoreductase activity; after 8 hours of irrigation treatment, there were 5 (1.99%), 5 (1.99%) and 6 (2.39%) DEGs enriched in fruit ripening, anatomical strusture maturation, aging; after 30 hours of irrigation treatment, the number of DEGs enriched in catalytic was the highest, 146 (54.89%), followed by single-organism metabolic process, oxidation-reduction process, and fewer genes involved in cell components.

To further understand the DEGs function, significantly enriched KEGG pathways were analyzed **(Fig. 5, Additional file 5)**. The results show that the DEGs were mainly enriched in ‘Biosynthesis of secondary metabolites’, ‘cysteine and methionine metabolism’, ‘metabolic pathways’, ‘plant-pathogen interaction’, ‘photosynthesis-antenna protein’, ‘photosynthesis’, ‘histidine metabolism’ and ‘Cricadian rhythm-plant’ .

qRT-PCR validation of DEGs

The up-regulated expression gene and the down-regulated expression gene were randomly selected from the DEGs for qRT-PCR verification. The results of qRT-PCR revealed that most of these mRNAs share the similar expression tendencies with those from mRNA-Seq data, which might partially validate the reliability of our sequence data and our research results in the present study **(Fig. 6)**. The expression quantity detected by the two methods were slightly different, which may be due to the different detection range and sensitivity of the two detection methods. However, the same expression trend confirms the reliability of the RNA-Seq analysis results.

Discussion

Tomato is one of the most popular commercial vegetables, however, its fruit has high susceptibility to cracking. Cracks can occur throughout the fruit development stage during the ripening and post-harvest period [43-44], which may cause serious economic losses. Different hypotheses have been presented to explain the occurrence of tomato fruit cracking. Previous researches show that rapid fruit swelling and fruit cracking are closely related [45]. Irregular temperature or watering, especially going from lower temperature to very higher, or extremely dry to very humid conditions, will lead to a rapidly swelling. If the flesh grows faster than the pericarp and the skin is not strong enough, cracking can easily occures; Besides, cell senescence and apoptosis will influence skin strength and water absorbing, this in turn can affect fruit cracking; In addition, a large differential between day and night temperature will lead to the accumulation of carbohydrate. Fruits with lots of carbohydrate absorb more water, will grow large much sooner and are more likely to cracking. In general, fruit cracking is a complex problem. It is a mixture of nature and nurture. Previous studies have suggest that there is no single gene, but many genes work together to regulate fruit cracking [22-23]. Liu's research suggests that plants have been gradually developed a complex signal pathway to cope with adverse environmental stimulations [46]. That is, plants perceive different stress signals from circumstances, to integrate them and to respond to these different stresses by modulating the expression of related genes, plant perceives and responds to environmental stimulations. Is fruits cracking regulated by a complex network too?

LncRNA regulates tomato fruit cracking by coordinating gene expression in hormone-redox-cell wall network

LncRNA plays important roles in epigenetic regulation, cell cycle regulation and many other activities. Here, we identified several lncRNAs that are involved in fruit cracking. LncRNA mainly functions by cis or trans on protein-coding genes to achieve its regulatory function. The principle of cis action is that the function of lncRNA is related to its neighboring gene [40]. Most lncRNAs are not annotated, and we can't know their function. In order to predict the function of these lncRNAs, we performed functional analysis of lncRNA-targeted mRNAs, and constructed lncRNA-mRNA network **(Fig. 7, Additional file 6)**. The results showed that the mRNAs in the network **(Fig. 7a)** are mainly enriched in 'oxidation-reduction process', 'oxidoreductase activity', 'hormone metabolic process', 'response to hormone stimulus', 'catalytic activity', 'cell wall organization' and 'external encapsulating structure'. And we classify the target genes into four categories (cell wall polysaccharide metabolic, oxidation-reduction processe hormone and others) based on their function and amount..

Some lncRNAs are target for specifically functional mRNAs, we can assume that the lncRNAs perform the similar functions as their target mRNAs. For example, XLOC_010878, XLOC_016662, XLOC_033910 **(Fig. 7b)**, many of their target genes are enriched in 'dioxygenase activity', 'oxidation-reduction process' and 'oxidoreductase activity' ect., so we predicted their gene function as "redox regulation".

Some lncRNAs are targets for significantly differentially expressed mRNAs with various functions. Such as XLOC_16662 **(Fig. 7c)**, its target genes (Solyc07g026650.2, Solyc08g081000.2, Solyc03g031880.2, ect.) are enriched in 'oxidoreductase activity' and 'dioxygenase activity' term, For the other target genes,

Solyc08g008120.2 is enriched in 'negative regulation of abscisic acid mediated signaling pathway' term, and Solyc08g081010.2 is enriched in 'cell wall thickening', 'callose deposition in cell wall' and 'cellulose metabolic process' terms. Previous researches show that redox, hormone and cell wall are all very important factors that can influence fruit cracking, so we speculat that the lncRNA XLOC_16662 may play important role in regulating tomato fruit cracking. So did XLOC_008464, XLOC_033910, XLOC_007053, XLOC_025351 and XLOC_040425.

Key gene regulates tomato fruit cracking

According to the gene expressing analysis, GO and KEGG functional analysis, as well as the gene function annotated on the website (https://solgenomics.net), 16 significantly differentially expressed genes are predicted to related to fruit cracking on tomato, specifically Solyc07g026650.2, Solyc04g054830.2, Solyc07g017770.2, Solyc07g055990.2, Solyc04g072000.2, Solyc01g008710.2, ect **(Additional file 7)**. Hierarchical clustering analysis shows that the expression trends or quantity of these genes in the two varieties are completely different after the irrigation treatment (**Fig. 8a**). For instance, the expression of Solyc12g011030.1, Solyc04g072000.2, Solyc09g075330.2, Solyc02g080530.2, Solyc07g055990.2 and Solyc09g008720.1 in the CR tomato showed a downward trend, while the expression in the CS tomato presented an upward trend. The gene functions of these genes were pectin esterase, xyloglucan endotransglucosylase/ hydrolase, expansin, which play an important role in cell wall loosing and expansion and may also play a key regulatory role in tomato fruit cracking.

Finally, we mapped a pathway diagram (**Fig. 8b**) of fruit cracking based on these differentially expressed lncRNA, mRNA and previous studies [47-52], and also predicted key genes to play a key regulatory role in a certain pathway, such as Solyc09g008720.1, ethylene; Solyc02g080530.2, peroxide and Solyc09g075330.2, pectinase, ect . Previous research suggest that ethylene influences fruit development and ripening (regulating *PG* and *EXP* gene expression) [47], promotes programmed cell death of epithelial cells under ROS signaling [48]. Li et al [49] shows that ARFs serve as a cross talk point between the ethylene and auxin signaling. Furthermore, auxin induces the production of ROS, and H_2O_2 decomposes the polymer on the cell wall by producing ·OH [50]. And cell programmed death leads to reduced or lost permeability of the plasma membrane. This in turn influence fruit cell activity, water absorption and cracking. Simultaneously the increase of auxin can promote the accumulation of H_2O_2 and promote the elongation of cells [51]. Furthermore, Rayle and Cleland [52] proposed the acid growth theory. Which means on one hand, hydrogen ion exerts a purely chemical or physical effect, such as cleavage of some acid-labile bonds on the wall. Alternatively, it may activate the normal enzymatic processes directly or indirectly, which may lead to wall loosening. Based on these, we speculate the regulatory network of fruit cracking. And coexpression of cell wall, redox, hormone related mRNAs and its corresponding lncRNA influence fruit cracking.

Cell wall polysaccharide metabolic

The DEG Solyc08g077910.2 encodes an Expansin-like protein according to the gene function annotation of the tomato website. It breaks down the hydrogen bonds between its molecules to promote the depolymerization of the cell wall macromolecular network, which can lead to the relaxtion of the cell wall [53]. In this experiment, the expression level of Solyc08g077910.2 increased significantly at 8h of irrigation (log2(fold change)=7.13395) in CS tomato. The increased expression of expansin-like gene can relax the cell wall and that may influence fruit cracking.

Solyc07g055990.2 (Xyloglucan endotransglucosylase/ hydrolase7), Solyc12g011030.1 (xyloglucan endotransglucosylase/ hydrolase9) encodes a class of xyloglucan endotransglucosylase/hydrolase, which mediates the cleavage and polymerization of β-1,4-xyloglucan in the primary cell wall and is thought to play an important role in the formation and remodeling of xyloglucan. Xyloglucan usually fused in the cell wall, xyloglucan and its oligosaccharides determine tissue tension [54]. Jan [55] found that *OsXTH8* is involved in the cell wall modification process in rice, and is highly expressed in the vascular bundle of the sheath and the young roots in which the cells are vigorously elongated and differentiated, while it can respond to gibberellin, involved in the cell extension process. He [56] found that *OsXTH5, OsXTH19, OsXTH20, OsXTH24* and *OsXTH28* play important roles in the elongation of rice peduncle and can respond to drought stress. These studies indicate that the *OsXTH* gene family plays an important role in the regulation of the structural function of rice cell walls. In this experiment, the expression levels of Solyc12g011030.1 and Solyc07g055990.2 in CS tomato showed an upward trend, which showed a downward trend in the CR tomato **(Fig. 8a)**, and the expression level in CS tomato was significantly higher than that in the CR tomato. This illustrates that the CR tomato may have a possibly higher osmotic stress resistance ability with a down-regulation of the *XTH* gene that can strengthen the cell wall upon encountering the water stress.

Solyc10g080210.1 encodes a polygalacturonase whose main function is to hydrolyze α-1,4 glycosidic bonds in the cell wall polygalacturonic acid, polygalacturonic acid is a component of pectin, so it is also called pectinase [57]. Polygalacturonic acid exists in the cell wall in a highly methylated form, and the methyl group is removed from the pectin of the cell wall by the pectin methylesterase during tomato maturation, the degree of methylation decreased from 90% in the green ripening period to 35% in the red ripening period [58], which accelerated the degradation of the cell wall. In the antisense *PaPG1* transgenic study of strawberry, the expression level of *PG* was significantly inhibited, and the degree of fruit softening was also significantly delayed [59].

Redox process

Previous studies have shown that peroxidase in the cell wall leads to cell wall sclerosis by causing cross-linking of cell wall components, thereby inhibiting cell elongation [60-62]. Peroxidase can also directly regulates plant cell elongation by controlling H_2O_2 levels [63]. Solyc02g080530.2 encodes peroxide, which is significantly higher in CS tomato than in CR tomato. The expression of these gene may enhances the cell wall hardness and hinders the elongation of the cells of cell wall, resulting in the cracking of the fruit response to water swelling after the irrigation treatment. Solyc01g081250.2 encodes

Glutathione-S-transferase(*GST*). *GST* is a super-family enzyme with multiple functions in plants. It is not only involved in primary metabolism and secondary metabolism [64], it can also protect plants from oxidative damage and heterogeneous substances [65-67]. According to the data analysis, the gene expression of Solyc01g081250.2 in the CR tomato was significantly higher than that of CS tomato at the 0h, 8h and 30h treated with irrigation. The higher expression of *GST* in CR tomato can better maintain cell vigor and be beneficial to tomato fruits when coupling with water stress.

Hormone related

Previous research showed that hormone can regulate the expression of cell wall related genes. Trainotti [68] studied the expression of 32 genes in the development and softening of peach fruit, which are related to cell wall synthesis and degradation. The expression of these genes in unsoftened fruits can be inhibited by ethylene, while ethylene promotes the expression of these genes during fruit ripening and softening; Ethylene inhibits and promotes the dual regulation effect on the formation of plant secondary metabolites, which is the result of the interaction of ethylene with various factors inside and outside the cell [69-71]; *TAPG1,* the cell wall degrading enzyme gene, can be induced by ethylene at the transcriptional level in tomato[72]; Rose [73] identified that ethylene regulates *LeEXP1*, which is specifically expressed only during fruit ripening. The pathway of ethylene biosynthesis in plants is the methionine cycle, In this study, KEGG functional analysis of DEGs are significantly enriched in the methionine metabolic pathway. Solyc11g042560.1 encodes ethylene receptor, Solyc09g008720.1 is ethylene-responsive transcription factor, their expression levels are significantly up-regulated after irrigation and higher in CS tomato than in CR tomato.

In this study, only two biological replicates were used, and there may be a certain false positive rate. Therefore, we randomly selected 9 DEGs for real-time PCR, which proved to be consistent with high-throughput sequencing. It is indicated that the differential genes obtained by high-throughput sequencing are reliable.

Conclusions

In this study, the key genes in response to tomato cracking were identified by high-throughput sequencing, it has important guiding significance for the selection of new tomato varieties. We have also established the network of lncRNA-mRNA to learn about precise regulation of lncRNA on fruit cracking. To the best of our knowledge, this is the first discovery of lncRNA-mRNA network in tomato fruit cracking.

Abbreviations

CS: Cracking-susceptible

CR: Cracking-resistant

CS 0 h: Cracking-susceptible tomato fruit before irrigation (0 hours)

CS 8 h: Cracking-susceptible tomato fruit after 8 hours of irrigation

CS 30 h: Cracking-susceptible tomato fruit after 30 hours of irrigation

CR 0 h: Cracking-resistant tomato fruit before irrigation (0 hours)

CR 8 h: Cracking-resistant tomato fruit after 8hours of irrigation

CR 30 h: Cracking-resistant tomato fruit after 30 hours of irrigation

DEGs: Differential expressed genes

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

qRT-PCR: Quantitative real-time PCR

FPKM: Expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced

Declarations

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Availability of data and materials

The supporting data are included within the article and additional files.

Authors' contributions

JFL led and coordinated the project. XLZ, SMT, WZ and YL collected the plant materials and isolated the RNA. XLZ conducted the real-time quantitative PCR. XLZ, TYP and YQH conducted the bioinformatics analysis. XLZ, SMT, JFL wrote the paper. All authors have read and agree with the final manuscript. JFL is the corresponding author and is responsible for all contact and correspondence. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interest in the reported research.

References

1. Khadivikhub A , Zamani Z , Bouzari N . Evaluation of Genetic Diversity in Some Iranian and Foreign Sweet Cherry Cultivars by Using RAPD Molecular Markers and Morphological Traits. Horticulture Environment & Biotechnology, 2008;49(3):188-196. 2. Sawada E. Studies on the cracking of cherries. I. The cause of cracking. Agriculture and Horticulture. 1931;6: 964-892. 3. Khadivi-Khub A. Physiological and genetic factors influencing fruit cracking. Acta Physiologiae Plantarum. 2015;37:1718. 4. Balbontín, Cristián, Ayala, Héctor, M. Bastías, Richard, et al. Cracking in sweet cherries: A comprehensive review from a physiological, molecular, and genomic perspective. Chilean journal of agricultural research, 2013;73(1):66-72. 5. Beyer M, Hahn R, Peschel S, et al. Analysing fruit shape in sweet cherry (Prunus avium L.). Scientia Horticulturae. 2002;96:139-150. 6. Considine J, Brown K. Physical aspects of fruit growth. Theoretical analysis of distribution of surface growth forces in relation to cracking and splitting. Plant Physiology. 1981; 68:371-376. 7. Cortes C, Ayuso MC, Palomares G, et al. Relationship between radial and concentric cracking of tomato fruit. Scientia Horticulturae. 1983;21:323-328. 8. Domínguez E, Ferna´ndez MD, Herna´ndez JCL, Parra JP, Espan˜a L, Heredia A, Cuartero J. Tomato fruit continues growing while ripening, affecting cuticle properties and cracking. Physiol Plantarum. 2012;146(4):473-486. 9. Emmons CL W, Scott JW. Environmental and physiological effects on cuticle cracking in tomato. Am Soc Hortic Sci. 1997;122(6):797-801. 10. López-Casado G, Matas AJ, Domínguez E, Cuartero J, Heredia A. Biomechanics of isolated tomato (Solanum lycopersicumL.) fruit cuticles: the role of the cutin matrix and polysaccharides. Exp Bot. 2007;58:3875-3883. 11. Domínguez E, España L, López-Casado G, Cuartero J, Heredia A. Biomechanics of isolated tomato (Solanum lycopersicum) fruit cuticles during ripening: the role of flavonoids. Funct Plant Biol. 2009;36:613–620. 12. Ruiz-May E, Rose JKC. Progress toward the tomato fruit cell wall proteome. Front Plant Sci. 2013;29:7. 13. Carpita N, Mc Cann M. The cell wall. Am Soc Plant Physiol, Rockville. 2000. 14. Cantu D, Vicente A, Greve L, Dewey F, Bennett A, Labavitch J, Powell A. The intersection between cell wall disassembly, ripening, and fruit susceptibility to Botrytis cinerea. Proc Natl Acad Sci USA. 2008;105(3):859-864. 15. Wang Y, Lu WJ, Li JG, Jiang Y. Differential expression of two expansion genes in developing fruit of cracking-susceptible and resistant litchi cultivars. Am Soc Hortic Sci. 2006;131(1):118-121. 16. Kasai S, Hayama H, Kashimura Y, Kudo S, Osanai Y. Relationship between fruit cracking and expression of the expansin gene Md EXPA3 in ‘Fuji’ apples (Malus domesticaBorkh.). Sci Hortic. 2008;116(2):194-198. 17. Huang XM, Wang HC, Lu XJ, Yuan WQ, Lu JM, Li JG, Huang HB. Cell wall modifications in the pericarp of litchi (Litchi chinensis Sonn.) cultivars that differ in their resistance tocracking. Hortic Sci Biotech. 2006;81(2):231-237. 18. Moctezuma

E, Smith DL, Gross KC. Antisense suppression of a ß-galactosidase gene (TBG6) in tomato increases fruit cracking. Exp Bot. 2003;54:2025-2033 19. Schuch W, Kanczler J, Robertson D, Hobson G, Tucker G, Grierson D, Bright S, Bird C. Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. Hortscience. 1991;26(12):1517-1520. 20. Lu W, Wang Y, Jiang Y, Li J, Liu H, Duan X, Song L. Differential expression of litchi XET genes in relation to fruit growth. Plant Physiol Bioch. 2006;44(11):707–713 21. Yang Z, Wu Z, Zhang C. et al. The composition of pericarp, cell aging, and changes in water absorption in two tomato genotypes: mechanism, factors, and potential role in fruit cracking. Acta Physiol Plant. 2016;38: 215. 22. Peet M. Fruit cracking in tomato. Hort Technology. 1992; 2(2):216-223. 23. Cuartero J, Palomares G, Balasch S, Nuez F. Tomato fruit cracking under plastic-house and in the open air. Eucarpia Tomato Working, Avignon. 1981. 24. Jiang FL, Lopez A, Jeon S, De Freitas ST, Yu QH, Wu Z, Labavitch JM, Tian SK, et al. Disassembly of the fruit cell wall by the ripening-associated polygalacturonase and expansin influences tomato cracking. Horticulture Research. 2019; 6 :17. 25. Birney E, Stamato yannopoulos J A, Dutta A, et al. Indentification and analysis of function elements in 1% of the human genome by the Encode pilot project. Nature. 2007;447(7146):799-816. 26. Derrien T, Johnson R, Bussotti G, et al. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res. 2012;22(9):1775-1789. 27. Ponting CP, et al. Transcribed dark matter: meaning or myth? Hum Mol Genet. 2010;19(R2):R162-8. 28. Hutten HA, et al. Non-coding RNAs: hope or hype? Trends Genet. 2005; 21(5):289-97.□ 29. Wang JQ, Zhang YJ, Ren JM, Xu CD, Liu BY, Zhu ZG, Zhou T. Advances in research on biological functions and significance of Long non-coding RNA. Life science. 2012;24(06):543-548. 30. Xin M, Wang Y, Yao Y, et al. Identification and characterization of wheat long non-protein coding RNAs responsive to powdery mildew infection and heat stress by using microarray analysis and SBS sequencing[J]. BMC Plant Biol, 2011; 11: 61. 31. Dai XY, Yu JJ, Ma JX, Ao GM, Zhao Q. Overexpression of Zm401, an mRNA-like RNA, has distinct effects on pollen development in maize. Plant Growth Regulation. 2007;52(3): 229-239. 32. Swiezewski S, Liu F, Magusin A, Dean C. Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature. 2009;462(7274):799-802. 33. Wu HJ, Wang ZM, Wang M, et al. Widespread long noncoding RNAs as endogenous target mimics for micro RNAs in plants. Plant physiology. 2013;161(4): 1875-1884. 34. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, and Salzberg SL. TopHat2: a ccurate alignment of transcripto mes in the presence of insertions, deletions ang gene fusions. Genome Biol. 2013;14(4): R36. 35. Trapnell C, Williams B A, Pertea G, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature biotechnology. 2010;28:511-515. 36. Kong L, Zhang Y, Ye ZQ, et al. CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic Acids Research. 2007;35:W345-W349. 37. Punta M, Coggill PC, Ererhardt RY, et al. The Pfam protein families database. Nucleic Acids Research. 2011;gkr1065. 38. Young MD, Wakefield MJ, Smyth GK, et al. goseq: Gene Ontology testing for RNA-seq datasets. 2012. 39. Mao X, Cai T, Olyarchuk JG, et al. Automated genome annotation and pathway identification using the KEGG Orthology(KO) as a controlled vocabulary. Bioinformatics. 2005;21: 3787-3793. 40. Guli S, Esteller M. Cis-acting noncoding RNAs: friends and foes. Nature structural □ molecular biology. 2012;19:1068-1075. 41. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method.

Methods. 2001;25(4):402–8. 42. Johnsson P, Lipovich L, Grand RD, et al. Evolutionary conservation of long non-coding RNAs; sequence, structure, function. Biochimica et Biophysica Acta (BBA)-General Subjects. 2014;1840:1063-1071. 43. Walter JM. Hereditary resistance to disease in tomato. Annu Rev Phytopathol. 1967;5:131-162. 44. Cotner SD, Burns EE, Leeper PW. Pericarp anatomy of crackresistant and susceptible tomato fruits. J Am Soc Hort Sci. 1969;94:136-137. 45. Capel C, Yuste-Lisbona FJ, López-Casado G, et al. Multi-environment QTL mapping reveals genetic architecture of fruit cracking in a tomato RIL Solanum lycopersicum×S. pimpinellifolium population. Tag.theoretical & Applied Genetics. theoretische Und Angewandte Genetik. 2016;130(1):1-10. 46. Liu, JH, Peng T, Dai WS. Critical cis-Acting Elements and Interacting Transcription Factors: Key Players Associated with Abiotic Stress Responses in Plants. Plant Mol Biol Rep. 2014;32, 303-317. 47. Giovannoni J. Genetic regulation of fruit development and ripening. The Plant Cell, 2004, 16, 170–180. 48. Steffens B , Kovalev A , Gorb SN , et al. Emerging Roots Alter Epidermal Cell Fate through Mechanical and Reactive Oxygen Species Signaling. The Plant Cell. 2012;24(8):3296-3306. 49. Li JS, Dai XH, Zhao YD. A role for auxin response factor 19 in auxin and ethylene signaling in Arabidopsis. Plant physiology. 2006;140(3):899-908. 50. Liszkay, A. Production of Reactive Oxygen Intermediates (O2{middle dot-, H2O2, and {middle dotOH) by Maize Roots and Their Role in Wall Loosening and Elongation Growth. Plant Physiology. 2004;136(2):3114-3123. 51. Ivanchenko MG, Den OD, Monshausen GB, et al. Auxin increases the hydrogen peroxide (H2O2) concentration in tomato (Solanum lycopersicum) root tips while inhibiting root growth. Annals of Botany. 2013;112(6):1107-1116. 52. Rayle DL, Cleland R. Enhancement of wall loosening and elongation by Acid solutions. Plant physiology. 197;46(2):250-3. 53. Mc Queen-Mason SJ, Cosgrove DJ. Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. Proc Natl Acad Sci USA .1994;91(14): 6574-6578. 54. Park YB, Cosgrove DJ . A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases. Plant Physiol. 2012;158, 1933-1943. 55. Jan A, Yang GX, Nakamura H, Ichikawa H, Kitano H, Matsuoka M, Matsumoto H, Komatsu S. Characterization of a xyloglucan endotransglucosylase gene that is up-regulated by gibberellin in rice. Plant Physiology. 2004;136(3): 3670-3681. 56. He H, Serraj R, Yang Q. Changes in Os XTH gene expression, ABA content, and peduncle elongation in rice subjected to drought at the reproductive stage. Acta Physiology Plant. 2009;31(4): 749-756. 57. Lincoln T, Eduardo Z. Plant Physiology(Fourth Edition). Sunderland:Sinauer Associates, Inc.2006. 58. Tieman DM, Harriman RW, Ramamohan G, et al. An antisense pectin methylesterase fene alters pectin chemistry and soluble solids in tomato fruit. The Plant Cell. 1992;4(6): 667-679. 59. Quesada MA, Blanco-Portales R, Posé S, García-Gago JA, Jiménez-Bermúdez S, Muñoz-Serrano A, Caballero JL, Pliego-Alfaro F, Mercado JA. and Muñoz- Blanco J. Antisense down-regulation of the Fa PG1 gene reveals an unexpected central role for polygalacturonase in strawberry fruit softening. Plant Physiology. 2009;(150): 1022-1032. 60. Andrews J, Adams SR, Burton KS, Edmondson RN. Partial purification of tomato fruit peroxidase and its effect on mechanical properties of tomato fruit skin. J. Exo. Bot. 2002;53:2393-2399. 61. Cordoba-Pedregosa C, Cordoba F, Villalba JM, Gonzaez-Reyes JA. Zonal changes in ascorbate and hydrogen peroxide contents, peroxidase, and ascorbate-related enzyme activities in onion roots. Plant Physiol. 2003;131:.697-706. 62. Lin CC, Kao CH. Cell wall peroxidase against ferulic acid lignin, and NaCl-reddued root growth of rice seeding. J. Plant Physiol. 2001;158:667-671. 63. Passardi F, Cosio C, Penel C. Dunand C. Peroxidases have more functions than a

Swiss army knife. Plant Cell Rep., 2005;24:255-265. 64. Jablonkai I，Hatzios KK. In vitro conjugation of chloroacetanilide herbicides and atrazine with thiols and contribution of nonenzymatic conjugation to their glutathione mediated metabolism in corn. Journal of Agricultural and Food Chemistry. 1993;41(10) : 1736-1742． 65. Kampranis SC，Damianova R，Atallah M, et al. A novel plant glutathione S-transferase-peroxidase suppresses bax lethality in yeast. Journal of Biological Chemistry. 2000;275(38) : 29207-29216． 66. Marrs KA，Alfenito M R，Lloyd AM，et al，A glutathioneS-transferase involved in vacuolar transfer encoded bythe maize gene Bronze-2. Nature. 1995;375(6530) :397-400． 67. Bartling D, Radzio R, Steiner U, et al. A glutathione S-transferase with glutathione-peroxidase activity from Ara-bidopsis thaliana. European Journal of Biochemistry. 1993;216(2) : 579-586． 68. Trainotti L, Zanin D, Casadoro G. A cell wall-oriented genomic approach reveals a new and unexpected complexity of the softening in peaches. Journal of Experimental Botany. 2003;(54): 1821-1832. 69. Ruther J, Kleier S. Plant-plant signaling: ethylene synergizes volatile emission in Zea mays induced by exposure to (Z)-3-hexen-1-ol. J Chem Ecol. 2005;31:2217-2222. 70. Hossain MA, Kim S, Kim KH, Lee SJ, Lee H. Flavonoid compounds are enriched in lemon balm (Melissa officinalis) leaves by a high level of sucrose and confer increased antioxidant activity. Hortscience. 2009;44:1907- 1913. 71. Jeong SW, Das PK, Jeoung SC, Song JY, Lee HK, Kim YK, Kim WJ, Park YI, Yoo SD, Choi SB, Choi G, Park YI. Ethylene suppression of sugar-induced anthocyanin pigmentation in Arabidopsis. Plant Physiol. 2010;154:1514-1531. 72. Kalaitzis P, Koehler SM, Tucker ML. Cloning of a tomato polygalacturonase expressed in abscission. Plant Molecular Biology. 1995;(28):647-656. 73. Rose JK, Cosgrove DJ, Albersheim P, Darvill AG, Bennett AB. Detection of expansin proteins and activity duringtomato fruit ontogeny. Plant Physiol. 2000;123(4):1583-1592.

Table

Table 1 The overall assessment of sequencing data

Sample name	Raw reads	Clean reads	clean bases	Error rate(%)	Q20(%)	Q30(%)	GC content(%)
CR_0h	107964622	102656082	15.4G	0.01	98.13	95.03	42.82
CR_0h	102639560	97730610	14.66G	0.01	97.99	94.69	42.52
CS_0h	102699472	95783116	14.37G	0.01	97.58	93.81	42.95
CS_0h	108424258	103713024	15.56G	0.01	98.02	94.78	42.79
CR_8h	81919566	79478876	11.92G	0.02	96.41	91.23	42.94
CR_8h	100255896	94324174	14.15G	0.01	98.1	94.97	42.77
CS_8h	93474242	86534460	12.98G	0.01	97.79	94.28	42.96
CS_8h	119351634	114298442	17.14G	0.01	97.89	94.49	43
CR_30h	82958444	80567758	12.09G	0.02	96.32	91.05	42.29
CR_30h	104788296	100192938	15.03G	0.01	97.98	94.69	42.44
CS_30h	97274580	90916088	13.64G	0.02	96.86	92.29	42.84
CS_30h	105168826	98559578	14.78G	0.01	97.97	94.71	42.39

Additional Files

Additional file 1: Table S1. Detailed primer sequences for qPCR confirmation.

Additional file 2: Table S2. lncRNAs in 12 tomato fruit libraries

Additional file 3: Table S3. Target gene prediction based on the positional relationship between lncRNA and mRNA

Additional file 4: Table S4. GO analysis of DEGs between groups

Additional file 5: Table S5. KEGG analysis of DEGs between groups

Additional file 6: Table S6. Data of lncRNA-mRNA network

Additional file 7: Table S7. Key genes of tomato fruit cracking

Figures

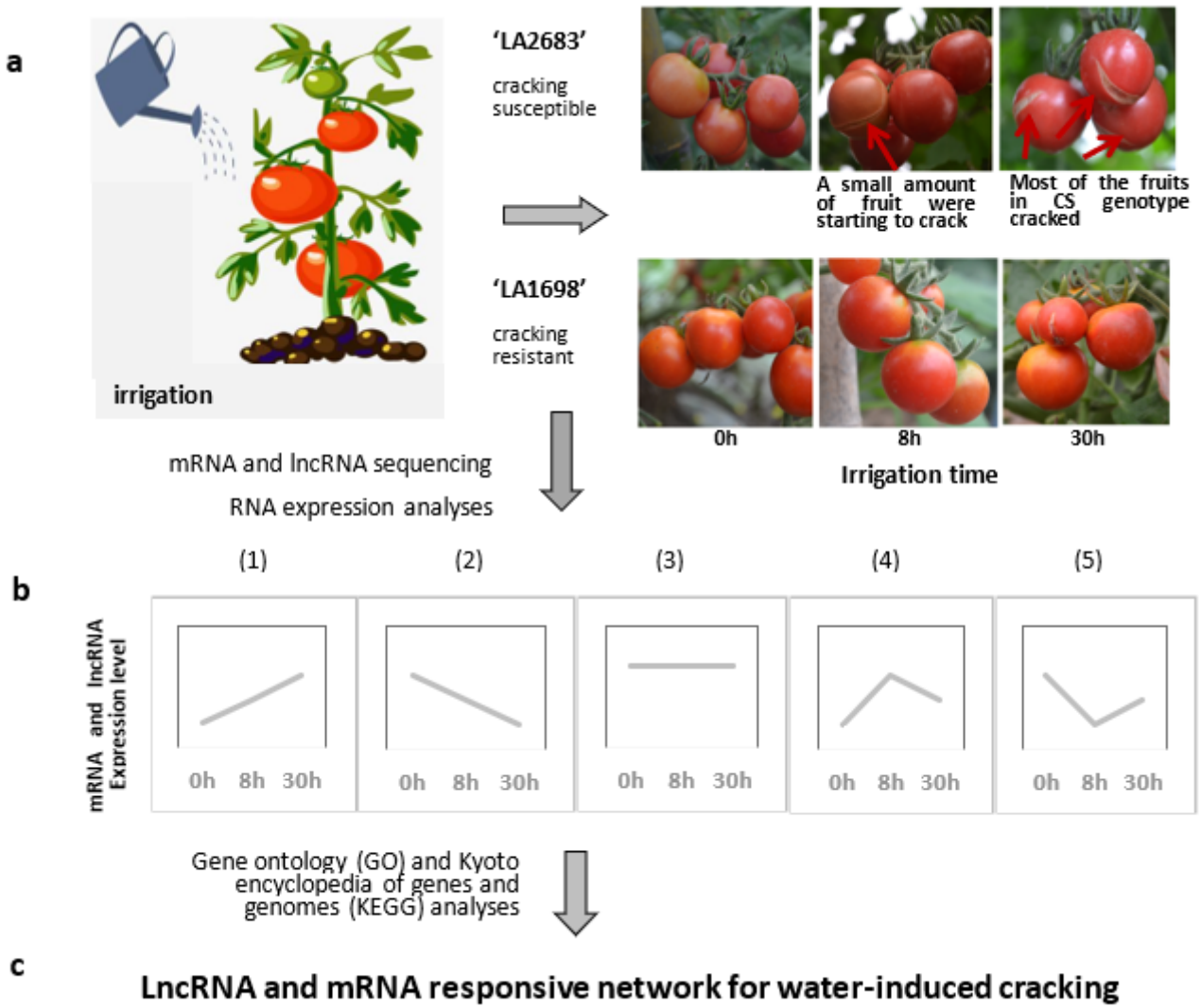

Figure 1

Work flow of present study. (a) Cracking status of two contrasting tomato genotypes after 0h, 8h and 30 h irrigation treatments. (b) The trend analyses of mRNA and lncRNA expression in tomato fruit after irrigation. The variation trends can be divided into five types. (1) Keep increasing (2) Keep decreasing (3) Keep constant (4) Increase first and then decrease (5) Decrease first and then increase. (c) Responsive network anaylyses.

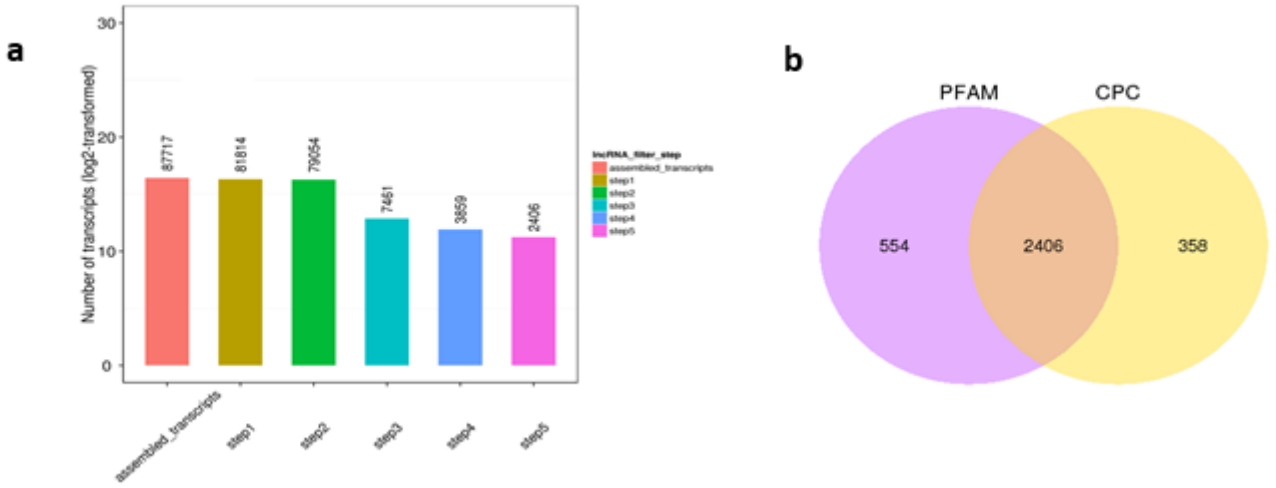

Figure 2

LncRNA screening. (a) The identification of lncRNA. (b) The assessment of the protein-coding potential of lncRNA

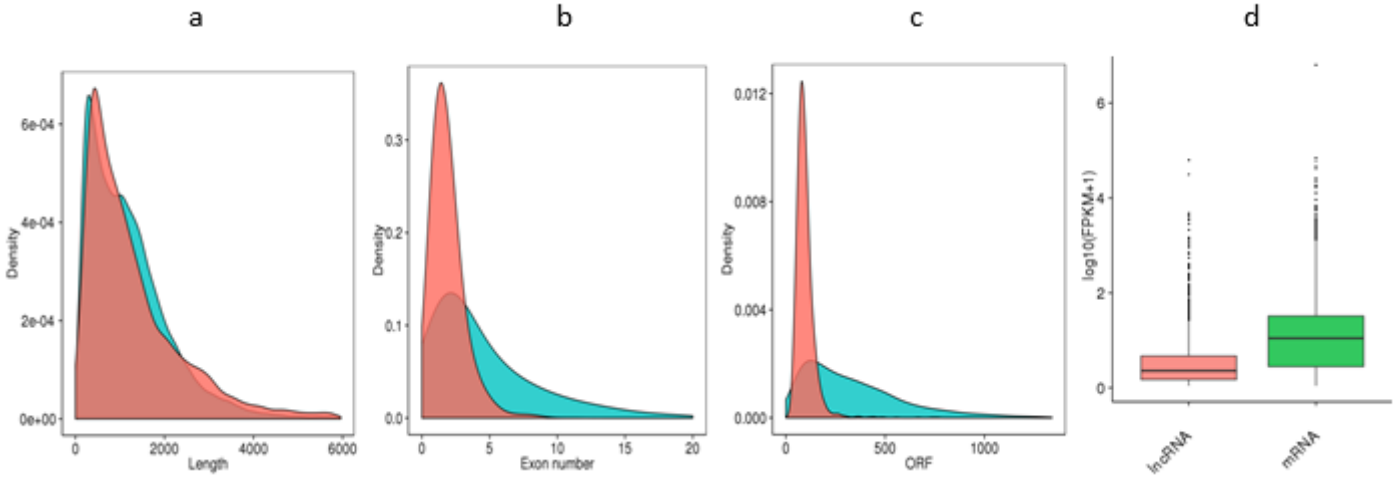

Figure 3

Comparative features of mRNAs and lncRNAs (a) Length distribution of mRNAs and lncRNAs . (b) Exon number distribution of mRNAs and lncRNAs. (c) ORF length distribution of mRNAs and lncRNAs. (d) Expression level indicated by log10 (FPKM + 1) in the mRNAs and lncRNAs.

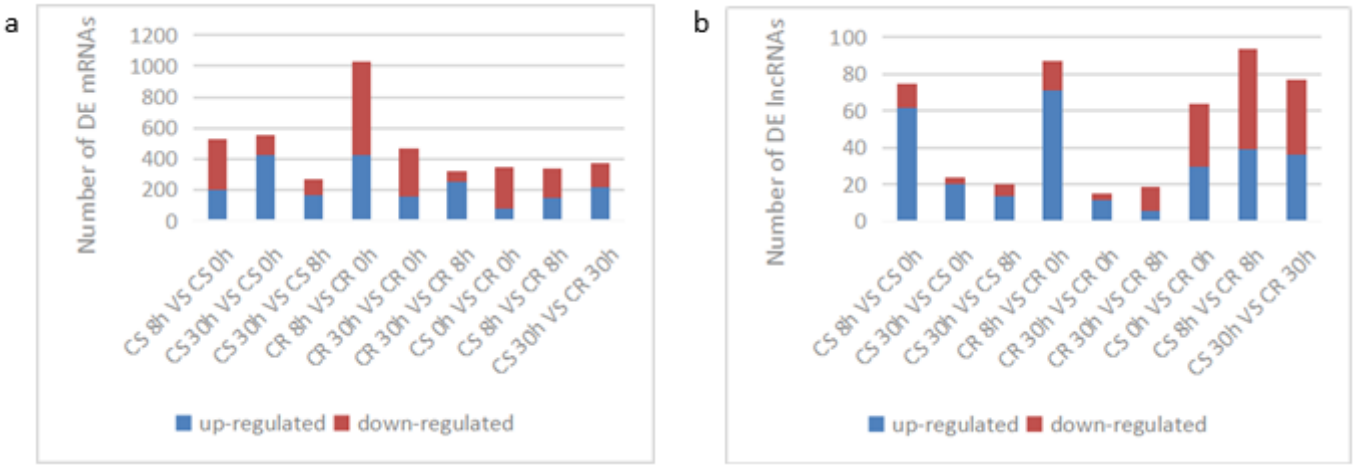

Figure 4

Differentially expressed mRNAs and lncRNA in different libraries. Up-regulated (blue) and down-regulated (red) mRNAs and lncRNA were quantified. The results of 9 comparisons were shown. (a) mRNAs (b) lncRNAs.

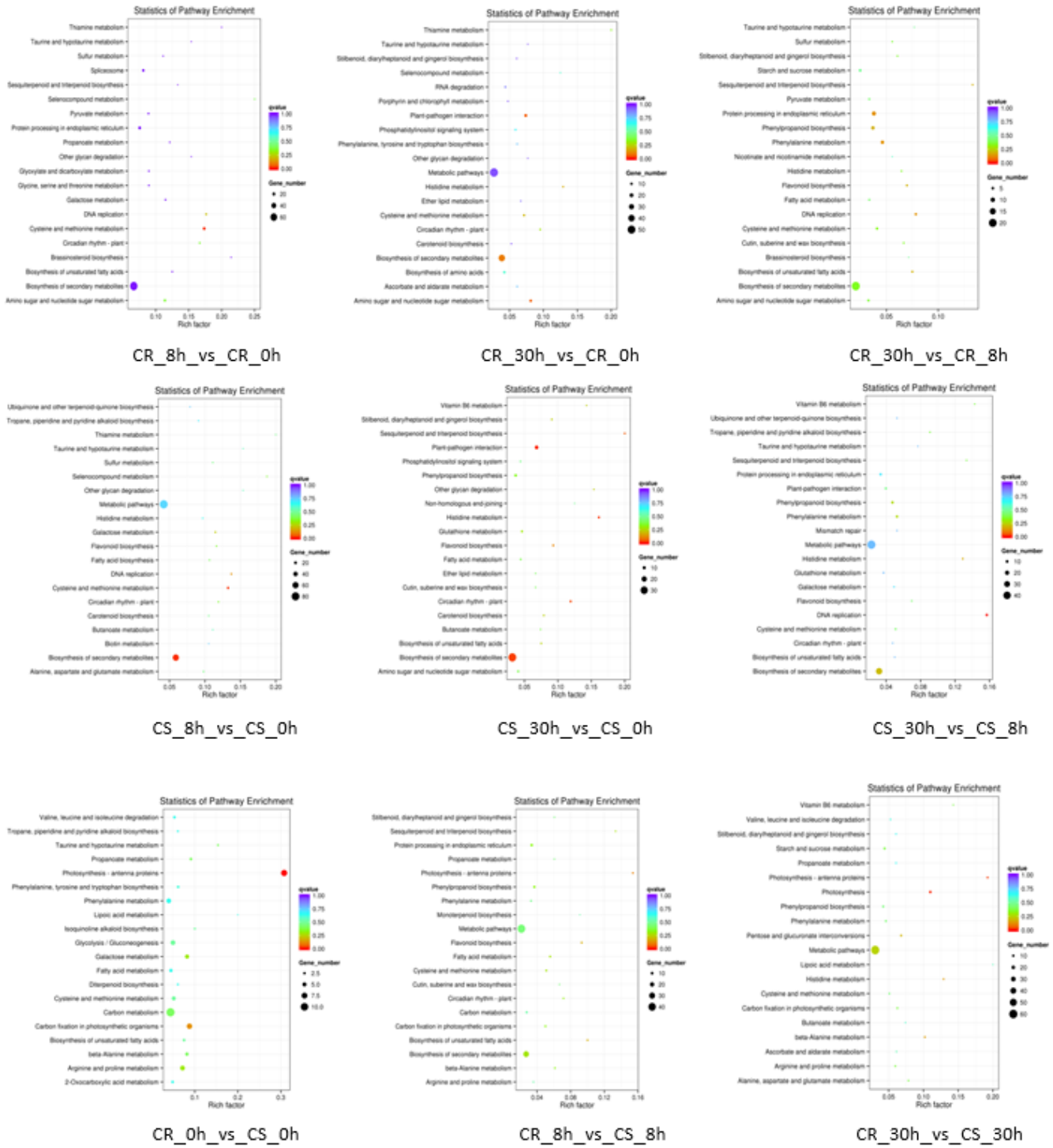

Figure 5

The top 20 KEGG pathways enrichment of DEGs. The x-axis indicates the rich factor and the y-axis indicates the pathway names

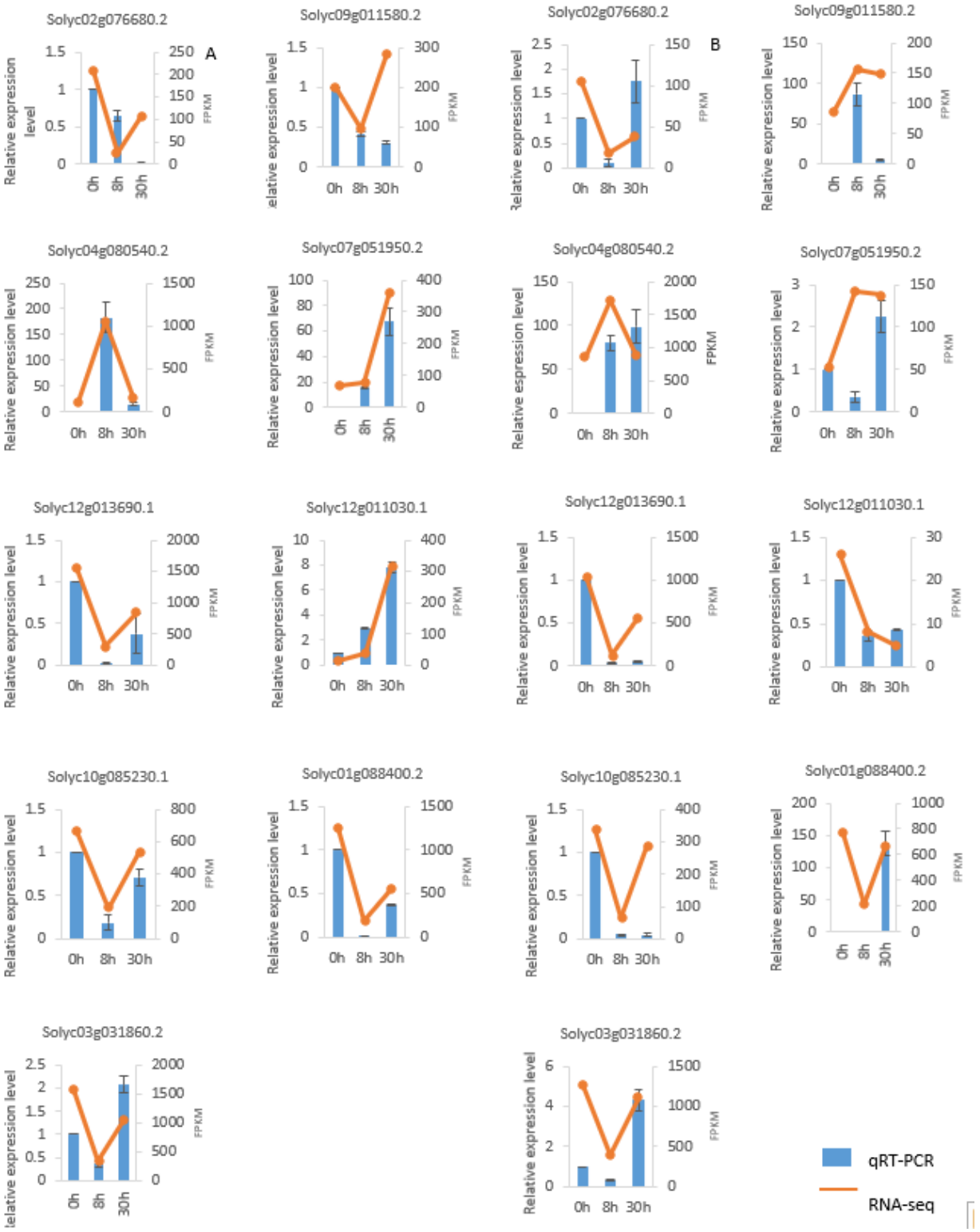

Figure 6

Real-time PCR validation of high-throughput sequencing data. The x-axis represents the time point of different sampling, the left y-axis represents relative expression level, and right y-axis represents FPKM. Blue bars represent data yielded by qRT-PCR, and red points represent data obtained by RNA sequencing. (a) LA2683 (b) LA1698

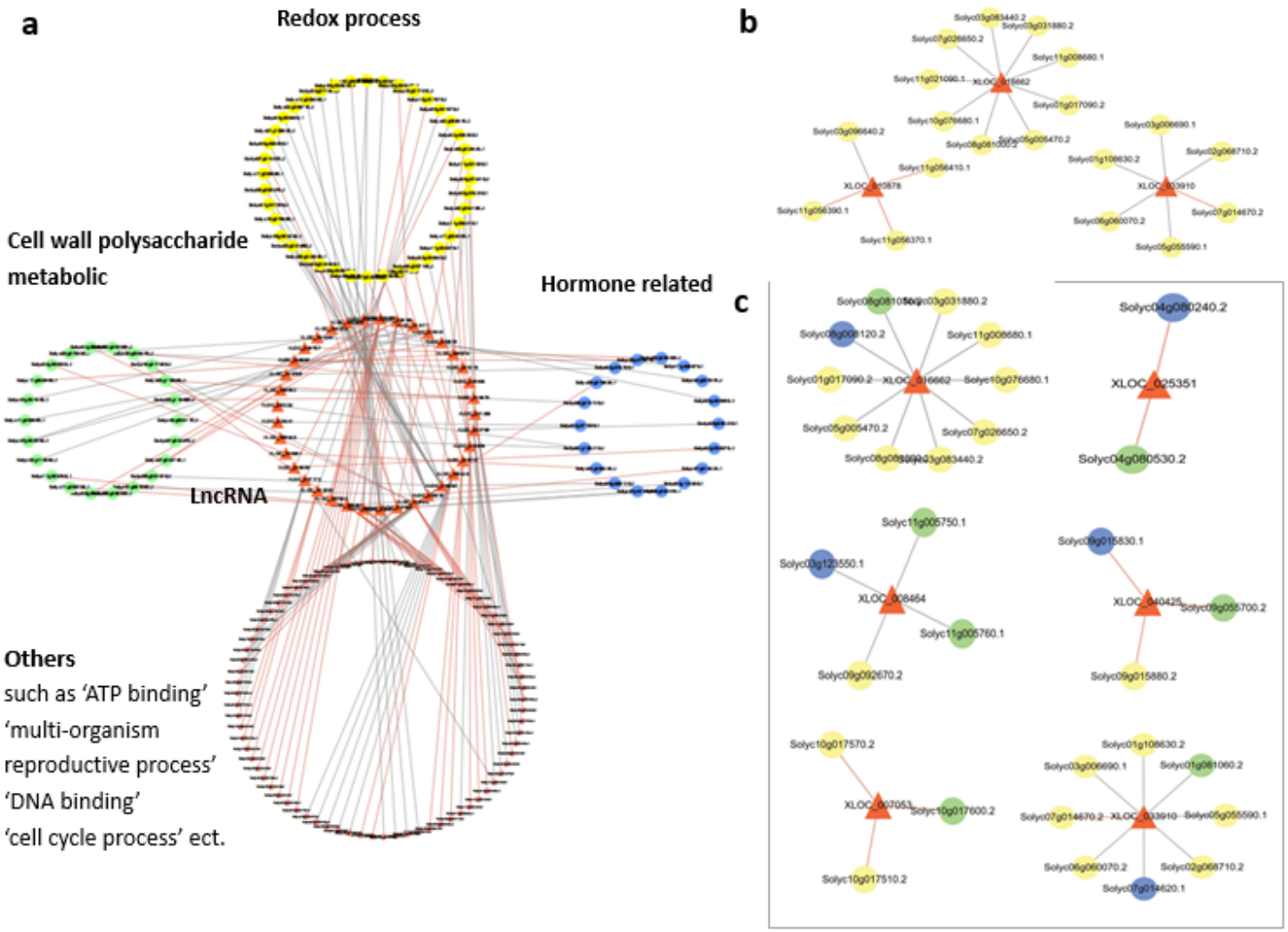

Figure 7

LncRNA-mRNA networks. The orange triangles represent lncRNAs, the circles represent mRNAs (green: cell wall polysaccharide metabolism, yellow: redox process, blue: hormone related, pink: others). The red edge represent the targeting mode of lncRNA and mRNA was co_location, and the gray edge represents co_expression. (a) LncRNA-mRNA network influences tomato fruit cracking. (b) LncRNAs that target the same kinds of mRNAs. (c) LncRNAs that target different kinds of mRNAs playing an important role in the network.

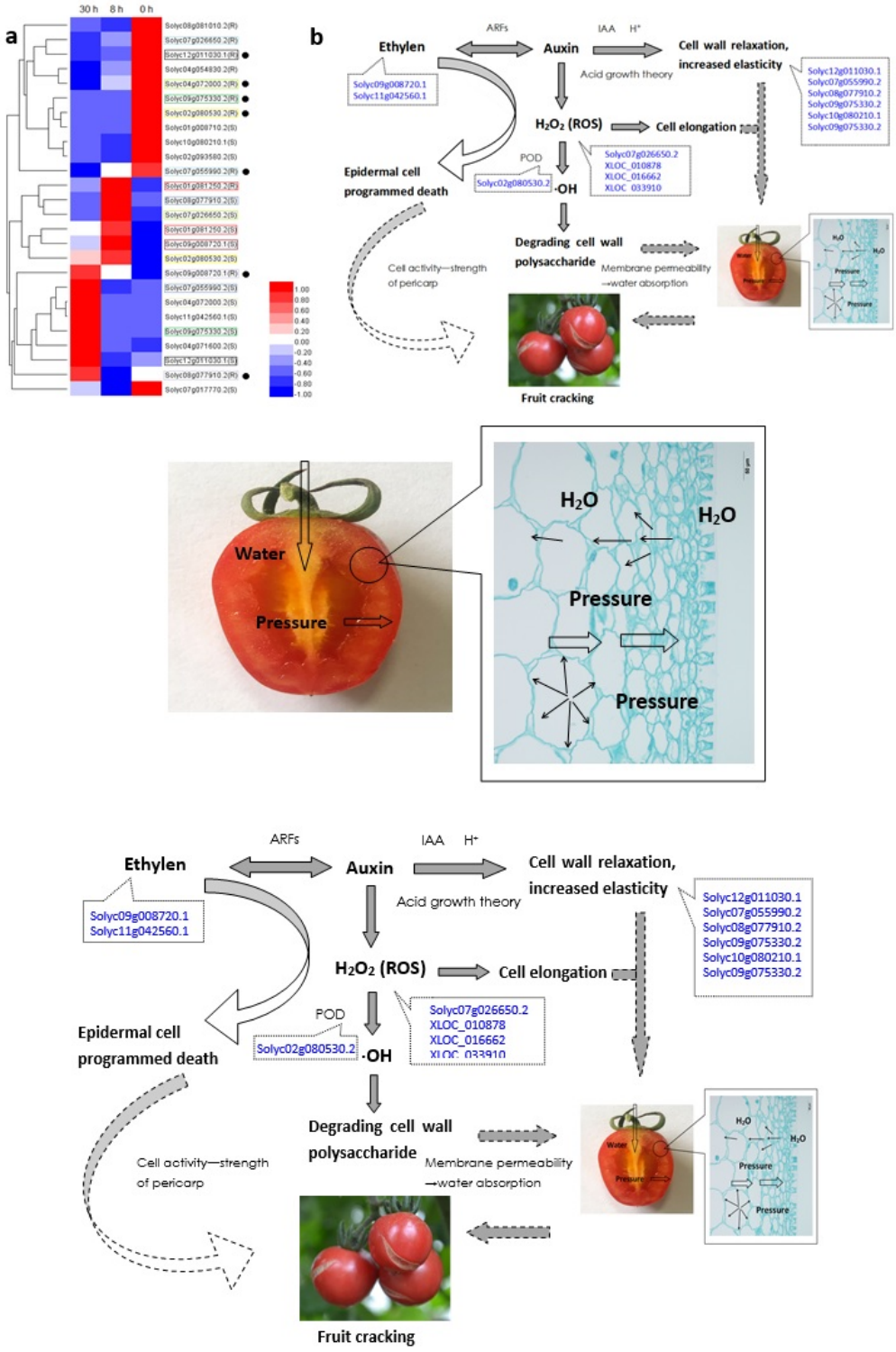

Figure 8

Hierarchical clustering analysis and fruit cracking regulating network speculation. (a) Hierarchical clustering analysis showed the key genes pression profiles in tomato fruit cracking. Based on euclidean distance, the minimum linkage method was used for cluster analysis. The same color boxes represent the same gene in CS and CR. S: cracking-susceptible, R: cracking-resistant. The solid dark circles represented genes that showed different expression trends in two genotypes. (b) The predicted pathway diagram of

fruit cracking in tomato, including hormones, reactive oxygen species, and cell wall polysaccharide metabolism. Solyc09g008720.1, ethylene receptor. Solyc11g042560.1, ethylene-responsive transcription factor . Solyc02g080530.2, peroxide. Solyc07g026650.2, 1-aminocyclopropane-1-carboxylate oxidase 5. Solyc07g055990.2, xyloglucan endotransglucosylase/ hydrolase 7. Solyc12g011030.1, xyloglucan endotransglucosylase/ hydrolase 9. Solyc08g077910.2, expansin. Solyc09g075330.2, pectinase. Solyc10g080210.1, polygalacturonase-2. Solyc09g075330.2, pectinesterase. XLOC_010878, XLOC_016662 and XLOC_033910 are predicted lncRNA that associated with the redox pathway. The dotted line indicates the speculation process.

Supplementary Files

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