

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

Lingzi Xue

Nanjing Agricultural University

Mintao Sun

Nanjing Agricultural University

Zhen Wu

Nanjing Agricultural University

Lu Yu

Nanjing Agricultural University

Qinghui Yu

Xinjiang Academy of Agricultural Sciences

Yaping Tang

Xinjiang Academy of Agricultural Sciences

Fangling Jiang (✉ jfl@njau.edu.cn)

Nanjing Agricultural University <https://orcid.org/0000-0002-1112-0580>

Research article

Keywords: Tomato, LncRNA, mRNA, Transcriptome, Network, Fruit cracking

Posted Date: October 8th, 2019

DOI: <https://doi.org/10.21203/rs.2.14406/v2>

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Version of Record: A version of this preprint was published at BMC Plant Biology on April 15th, 2020. See the published version at <https://doi.org/10.1186/s12870-020-02373-9>.

Abstract

Background Fruit cracking occurs easily under unsuitable environmental conditions and is one of the main types of damage that occurs in fruit production. It is widely accepted that plants have developed defence mechanisms and regulatory networks that respond to abiotic stress, which involves perceiving, integrating and responding to stress signals by modulating the expression of related genes. Fruit cracking is also a physiological disease caused by abiotic stress. It has been reported that a single or several genes may regulate fruit cracking. However, almost none of these reports have involved cracking regulatory networks. Results Here, 0, 8 and 30 h irrigation treatments resulted in the differential expression of 1028 mRNAs and 87 lncRNAs in 'LA1698' (cracking resistant, CR) at 8 h_vs_0 h, 468 mRNAs and 15 lncRNAs in CR_30 h_vs_CR_0 h, 321 mRNAs and 19 lncRNAs in 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 in CS_30 h_vs_CS_0 h, 270 mRNAs and 20 lncRNAs in CS_30 h_vs_CS_8 h; and 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 pathways of the differentially expressed mRNAs were mainly enriched in the 'hormone metabolic process', 'cell wall organization', 'oxidoreductase activity' and 'catalytic activity' categories. In addition, the lncRNAs regulated the expression of their neighbouring genes, and genes related to tomato cracking were selected to construct a lncRNA-mRNA network influencing tomato cracking. Conclusions This study provides insight into the responsive network for water-induced cracking in tomato fruit. Specifically, lncRNAs regulate the hormone-redox-cell wall network, including plant hormone (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, etc.).

Background

Fruit cracking, one of the main disorders in fruit production, can easily cause adverse impacts in fruit marketability such as reducing fruit quality due to a poor appearance, decreasing shelf life, and even making the fruit unmarketable because of fungal infection [1].

Fruit cracking occurs easily under unsuitable environmental conditions. For instance, under abiotic stress caused by dry to very wet conditions, there will be a rapid flow into the fruit, and if the skin loses strength and elasticity due to factors such as maturation, cracking is most likely to occur. It is widely accepted that plants have developed defence mechanisms and regulatory networks to respond to abiotic stress, which involve 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. Is there a regulatory network involved in fruit cracking?

Since the 1930s, researchers have performed many theoretical and practical studies on cracking [2]. Cracking is the result of a combination of internal and external factors. The internal factors are the fruit's own characteristics (fruit size, shape, firmness, deposition of cutin, wax, strength of the pericarp,

arrangement of cells in the pericarp, quantity and status of stomata, accumulation of osmoregulatory substances such as soluble sugars, growth stage of the fruit, etc.), while the external factors mainly include environmental factors (humidity, light, temperature, wind, etc.) and cultivation management measures (irrigation, mineral nutrition, plant regulation, etc.) [3-6]. Cortes [7] comprehensively analysed 62 genotypes and found that the correlation of cracked fruit with heredity was significantly greater than that with the environment, indicating that the cracking characteristic can show stable heritability and be regulated by certain genes.

Notably, cell wall components and modifications appear to be correlated with the strength of the skin and fruit cracking [8-11]. As ripening proceeds, cell wall degradation gradually occurs, and the fruit cracking rate increases [8-9]. The cell wall is composed of a 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 components 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 the degradation of cell wall polysaccharides and softening of the mature fruit peel [14]. Previous research has shown that genes such as *EXP*, *PG*, *β -gal* and *XET* are 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 reduces the rate of fruit cracking [19]. Simultaneous suppression of *SIPG* and *SIEXP1* expression in ripening fruits reduces cell wall disassembly and thereby reduces the fruit cracking rate by approximately 12% [21]. It is not a single gene but many genes working together that regulate fruit cracking [21-24]. It remains unclear whether there are other genes related to fruit cracking and which one is the major gene.

Recently, researchers have been gradually realizing that non-coding RNAs (ncRNAs) have important biological functions [25,26]. In contrast to the approximately 2% of protein-coding genes, more than 90% of genes do not have the ability to encode proteins and are transcribed into ncRNAs [25]. These ncRNAs were originally thought to represent "expression noise" or "expression waste", but they have now been proven to be strictly regulated to play important roles in the biological processes of organisms and exhibit extremely complex biological functions [27-29]. While much of the published work on ncRNAs has been conducted in humans and animals, the studies on plants are limited to certain model plants, such as *Arabidopsis*, maize, and wheat [30-33]. Xin [30] identified 125 stress-related lncRNAs in wheat, among which 71 responded to powdery mildew, and 77 responded to heat stress. Swiezewski [31] discovered that cold-induced long antisense intragenic RNA (*COOLAIR*) is involved in the vernalization process and regulates the expression of the plant flowering suppressor gene flowering locus C (*FLC*). However, none of these studies have focused on lncRNAs and fruit cracking. Is it possible that lncRNAs play important roles in fruit cracking too?

This study aimed to obtain a global view of the transcriptional regulation (mRNAs and lncRNAs) of fruit cracking induced by irrigation in tomato. RNA expression in 0, 8 and 30 h irrigation-treated fruits from two contrasting tomato genotypes, 'LA1698' (cracking resistant, CR) and 'LA2683' (cracking susceptible, CS), was analysed by mRNA and lncRNA sequencing. Differentially expressed mRNAs and lncRNAs related to

fruit cracking were then identified through transcriptome profiling and bioinformatic analysis. Finally, we determined a lncRNA-regulated hormone-redox-cell wall network for water-induced cracking in tomato. The findings reported here can increase our understanding of the transcriptional regulatory mechanisms of fruit cracking.

Methods

Plant materials and sample collection

The cracking-sensitive (CS) tomato cultivar 'LA2683' and the cracking-resistant (CR) tomato cultivar 'LA1698' (**Fig. 1a**) were introduced by the Tomato Genetics Resource Centre (TGRC, University of California, Davis). The fruit cracking rates of 'LA2683' and 'LA1698' are 77.53% and 20.17%, respectively. 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 transplanted to the same greenhouse of the Kunshan Yuye Leaf Vegetable Base (31°95'E, 119°16'N), Jiangsu Province, China. The climate of this area belongs to the north subtropical south monsoon climate zone. Plant spacing followed a 30×50×100 cm pattern. At the red ripening stage, saturated irrigation was adopted to induce fruit cracking. Samples of fruits were taken from both genotypes after 0 h, 8 h and 30 h of irrigation treatment. The samples were then immediately frozen in liquid nitrogen and stored at -80°C.

RNA-seq analysis

Twelve fruit samples (2 genotypes, 3 time points, each with two replications) were collected and sequenced by Novogene, Beijing, China. RNA was extracted from the tomato pericarp, and qualified RNA samples were used to construct a cDNA library. Transcriptome sequencing was carried out on the Illumina HiSeq 2500 platform. Sequences showing low quality, linker contamination or a high unknown base (N) content were filtered from the raw reads obtained after sequencing. All filtered clean reads were mapped to the tomato reference genome (<https://solgenomics.net>) using TopHat v2.0.9 [34], and the transcriptome was assembled by using Cufflinks [35].

Identification of mRNA and lncRNA

The transcripts that could be compared to known transcript data were identified as annotated mRNAs.

Then, the transcripts were screened according to the following criteria: (1) exon number ≥ 2 and (2) transcript length ≥ 200 bp. Additionally, (3) Transcripts that overlapped with the database annotation of the exon area according to Cuffcompare software were screened out. The lncRNAs overlapping with the exon region of a spliced transcript were included in the subsequent analysis. (4) The expression level of each transcript was calculated with Cuffquant, and transcripts with an FPKM ≥ 0.5 were selected. (5) Two algorithms for evaluating protein-coding potential (CPC [36] and PFAM [37]) were used to predict the protein-coding potential of the remaining transcripts. Only when these two algorithms simultaneously

indicated no protein-coding potential were the sequences considered to be predicted lncRNAs. Finally, the predicted lncRNAs were obtained.

The transcripts showing the potential to encode proteins by “CPC” and “PFAM” were identified as novel mRNAs.

Analysis of mRNAs and lncRNAs

The mRNA and lncRNA abundance of the unigenes was normalized via the fragments per kilobase of exon model per million mapped reads (FPKM) approach. The log₂ fold changes between two samples were tested statistically to determine whether the expression of an individual gene was significantly altered. A *Q*-value <0.05 and |log₂ fold-change| > 1 was considered to indicate a differentially expressed gene (DEG). Analyses of mRNA and lncRNA expression trends in tomato fruit after irrigation were then carried out (**Fig. 1b**). To understand the function of differentially expressed mRNAs, these mRNAs were further subjected to GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis by using GOseq [38] and KOBAS [39] software, respectively.

Feature analysis of lncRNAs

lncRNAs mainly acts on their protein-encoding target genes by cis-regulation or trans-regulation to achieve their regulatory functions. One of the functions of lncRNAs is cis-regulation of their neighbouring genes of the same allele; lncRNAs in less than 100 kb up/down stream of a gene are likely to be cis-regulators [40]. Another function of lncRNAs is the trans-regulation of co-expressed genes that are not adjacent to lncRNAs. Trans-regulation analysis is not be recommended for samples with a value < 6. In this study, the function of lncRNAs was predicted through functional enrichment analysis of the cis target gene.

Genomic characterization of the predicted lncRNAs was performed and compared with the mRNA results. The parameters for comparison to understand the genomic characteristics of the lncRNAs included the number of exons, ORF length, transcript nucleic acid length and sequence conservation between species

qRT-PCR verification

Total RNA was extracted from tomato fruit by using the RNeasy Pure Plant Kit (Qiagen Biotech Co., Ltd. (Beijing, China)) following the manufacturer’s instructions. cDNA was generated using 5 µl of total RNA and abm’s 5X All-In-One MasterMix. qRT-PCR was performed in 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 selected from the DEGs to verify the RNA-seq results. Gene-specific primers were designed using Premier 5.0. The relative levels of gene expression were calculated using the $2^{-\Delta\Delta CT}$ [41] method. The sequences of the primers are listed in **Additional file 1**.

Result

RNA sequencing and identification of lncRNA and mRNA

In total, we obtained 0.82 to 1.19 billion raw reads and 0.79 to 1.14 billion clean reads from CR and CS tomatoes at various time points (0, 8 and 30 h of saturated irrigation treatment) (**Table 1**). Through genomic comparison, Cufflinks splicing, and CPC and PFAM analysis, we identified 1 annotated lncRNA, 2406 novel lncRNAs (**Fig. 2; Additional file 2**), 33784 annotated mRNAs and 1453 novel mRNAs. The 2406 novel lncRNAs included 2004 lincRNAs and 402 antisense lncRNAs.

Comparison of mRNA and lncRNA features

The average length of the obtained lncRNAs was 1535 nt, which was similar to that of the mRNAs (1221 nt); the average number of exons and average ORF length of the identified lncRNAs were 1.9 and 98 bp, which were much lower values than those for the mRNAs (4.7 and 347 bp) (Fig. 3), consistent with previous studies. At the same time, we used phyloP to separately score the lncRNAs and mRNAs, and the sequence conservation of the lncRNAs was lower than that of mRNAs, which is consistent with previous studies [42]. We identified 28126 lncRNA-mRNA pairs with target relationships upstream and downstream of 2406 lncRNAs (Additional file 3).

Differential expression analysis

Differentially expressed mRNAs and lncRNAs were analysed in CR and CS tomatoes by using Cufflinks software (**Fig. 4**). mRNAs and lncRNAs with a Q -value < 0.05 and $|\log_2$ fold-change| > 1 were selected as differentially expressed genes. There were 1028 mRNAs and 87 lncRNAs that were differentially expressed in CR_8 h_vs_CR_0 h. Among these sequences, 601 mRNAs and 16 lncRNAs were downregulated, and 427 mRNAs and 71 lncRNAs were upregulated. There were 468 mRNAs and 15 lncRNAs that were differentially expressed in CR_30 h_vs_CR_0 h, among which 313 mRNAs and 3 lncRNAs were downregulated, while 155 mRNAs and 12 lncRNAs were upregulated. There were 531 mRNAs and 75 lncRNAs that were differentially expressed in CS_8 h_vs_CS_0 h. Among these sequences, 332 mRNA and 13 lncRNAs were downregulated, and 199 mRNAs and 62 lncRNAs were upregulated. There were 420 mRNAs and 24 lncRNAs that were differentially expressed in CS_30 h_vs_CS_0 h, among which 126 mRNAs and 4 lncRNAs were downregulated, 427 mRNAs and 20 lncRNAs were upregulated. There were 339 mRNAs and 64 lncRNAs that were differentially expressed in CS_0 h_vs_CR_0 h, while 338 mRNAs and 94 lncRNAs were differentially expressed in CS_8 h_vs_CR_8 h, and 369 mRNAs and 77 lncRNAs were differentially expressed in CS_30 h_vs_CR_30 h. Among these sequences, at 0 h of irrigation, 272 mRNAs and 34 lncRNAs were downregulated, while 77 mRNAs and 30 lncRNAs were upregulated; at 8 h of irrigation, 186 mRNAs and 55 lncRNAs were downregulated, while 152 mRNAs and 39 lncRNAs were upregulated; at 30 h of irrigation, 147 mRNAs and 41 lncRNAs were downregulated, while 222 mRNAs and 36 lncRNAs were upregulated.

Functional prediction of DEGs

To investigate the trends in gene functions and enrichment for DEGs, we performed GO (Gene Ontology) analysis of the selected mRNAs (**Additional file 4**). The results showed that 810 DEGs in CR_8 h_vs_CR_0 h exhibited GO enrichment; among these sequences, 29 DEGs were enriched in biological processes and cellular components. In CR_30 h_vs_CR_0 h, there were 357 DEGs, most of which were enriched in biological processes. Among these sequences, the regulation of biological process and biological regulation terms exhibited the largest number of genes, at 83 (23.25%) and 86 (24.09%), respectively, followed by 71 (19.89%) genes related to the regulation of cellular progress. The DEGs identified in CR_30 h_vs_CR_8 h were mainly enriched in catalytic activity (60.9%). For the CS tomato, 433 DEGs were obtained in the 8 h_vs_0 h comparison, and the number in the single-organism metabolic progress category was the greatest, a 119 (27.48%), followed by small molecule metabolic progress, at 63 (14.55%). Most of the DEGs identified in CS_30 h_vs_CS_0 h were enriched in biological processes such as the regulation of metabolic progress and regulation of biosynthesis. In CS_30 h_vs_CS_8 h, the DEGs were mainly enriched in biological processes, 195 (79.59%). Between the CR and CS tomatoes, the DEGs were mainly enriched in the biological process, cell component and molecular function categories before irrigation treatment (0 h), and 44 (16.18%) DEGs were significantly enriched in oxidoreductase activity; after 8 h of irrigation treatment, there were 5 (1.99%), 5 (1.99%) and 6 (2.39%) DEGs enriched in fruit ripening, anatomical structure maturation, and ageing, respectively; after 30 h of irrigation treatment, the number of DEGs enriched in the catalytic category was highest, at 146 (54.89%), followed by the single-organism metabolic process and oxidation-reduction process categories, while fewer genes were related to cell components.

To better understand the function of the DEGs, significantly enriched KEGG pathways were analysed (**Fig. 5; Additional file 5**). The results showed that the DEGs were mainly enriched in the 'biosynthesis of secondary metabolites', 'cysteine and methionine metabolism', 'metabolic pathways', 'plant-pathogen interaction', 'photosynthesis-antenna protein', 'photosynthesis', 'histidine metabolism' and 'circadian rhythm-plant' categories.

qRT-PCR validation of DEGs

Genes showing upregulated and the downregulated expression were randomly selected from the DEGs for qRT-PCR verification. The results of qRT-PCR revealed that most of these mRNAs shared similar expression tendencies to those indicated by the mRNA-Seq data, which might validate the reliability of our sequence data and our research results from the present study (**Fig. 6**). The expression levels detected by the two methods were slightly different, which might have been due to the different detection ranges and sensitivities of the two detection methods. However, the identification of the same expression trend confirmed the reliability of the RNA-Seq analysis results.

Discussion

Tomato is one of the most popular commercial vegetables; however, its fruit shows 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 studies have shown that rapid fruit swelling and fruit cracking are closely related [45]. Irregular temperatures or watering, especially a shift from a lower temperature to a much higher temperature or from extremely dry to very humid conditions, will lead to rapid swelling. If the flesh grows faster than the pericarp and the skin is not strong enough, cracking can easily occur. Cell senescence and apoptosis also influence skin strength and water absorption, which can in turn affect fruit cracking. In addition, a large differential between day and night temperatures will lead to the accumulation of carbohydrates. Fruits with high levels of carbohydrates absorb more water, grow much faster and are more likely to crack. In general, fruit cracking is a complex problem involving a mixture of nature and nurture. Previous studies have suggested it is not a single gene but many genes that work together to regulate fruit cracking [22-23]. Liu's research suggests that plants have gradually developed complex signalling pathways to cope with adverse environmental stimuli [46]. That is, plants perceive different stress signals from the circumstances in which they occur and then integrate these signals and respond to these different stresses by modulating the expression of related genes. Is it possible that cracking is also regulated by a complex network?

LncRNAs regulate tomato fruit cracking by coordinating gene expression in the hormone-redox-cell wall network

LncRNAs play important roles in epigenetic regulation, cell cycle regulation and many other activities. Here, we identified several lncRNAs that are involved in fruit cracking. LncRNAs mainly act in cis or trans on protein-coding genes to achieve their regulatory function. The principle of cis action is that the function of lncRNAs is related to their neighbouring genes [40]. Most lncRNAs are not annotated, and we do not know their functions. To predict the functions of these lncRNAs, we performed functional analysis of lncRNA-targeted mRNAs and constructed an lncRNA-mRNA network (**Fig. 7; Additional file 6**). The results showed that the mRNAs in the network (**Fig. 7a**) were mainly enriched in the 'oxidation-reduction process', 'oxidoreductase activity', 'hormone metabolic process', 'response to hormone stimulus', 'catalytic activity', 'cell wall organization' and 'external encapsulating structure' categories. We classified the target genes into four categories (cell wall polysaccharide metabolism, oxidation-reduction processes, hormones and others) based on their functions and amounts.

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

Some lncRNAs are targets of significantly differentially expressed mRNAs with various functions. For example, for XLOC_16662 (**Fig. 7c**), the target genes (Soly07g026650.2, Soly08g081000.2, Soly03g031880.2, etc.) are enriched in the 'oxidoreductase activity' and 'dioxygenase activity' terms. XLOC_16662 also has other target genes, such as Soly08g008120.2 ('negative regulation of abscisic

acid-mediated signalling pathway'), and Solyc08g081010.2 ('cell wall thickening'). Previous studies have shown that redox, hormone and cell wall terms are all very important factors that can influence fruit cracking, so we speculate that lncRNA XLOC_16662 may play an important role in regulating tomato fruit cracking, along with XLOC_008464, XLOC_033910, XLOC_007053, XLOC_025351 and XLOC_040425.

Key genes regulating tomato fruit cracking

According to the gene expression analysis, 16 significantly differentially expressed genes are predicted to be related to fruit cracking in tomato, specifically genes such as Solyc07g026650.2, Solyc04g054830.2, Solyc07g017770.2, Solyc07g055990.2, Solyc04g072000.2, Solyc01g008710.2, etc. (**Additional file 7**). Hierarchical clustering analysis showed that the expression trends or levels of these genes in the two varieties were 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. These genes encode proteins that function as pectin esterase, xyloglucan endotransglucosylase/hydrolase, and expansin, which play important roles in cell wall loosening 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 lncRNAs, mRNAs and previous studies [47-52]. Within this pathway, Solyc09g008720.1 (ethylene), Solyc02g080530.2 (peroxide) and Solyc09g075330.2 (pectinase) play important roles. Previous research suggests that ethylene influences fruit development and ripening (regulating cell wall-related *PG* and *EXP* gene expression) [47] and promotes programmed cell death of epithelial cells under ROS signalling [48]. Li et al. [49] showed that ARFs represent a point of cross-talk between ethylene and auxin signalling. Furthermore, auxin induces the production of ROS, and H₂O₂ decomposes polymers at the cell wall by producing ·OH [50]. Programmed cell death leads to a reduction in or loss of permeability of the plasma membrane, which in turn influences fruit cell activity, water absorption and cracking. Simultaneously, the increase of auxin can promote the accumulation of H₂O₂ and the elongation of cells [51]. Furthermore, Rayle and Cleland [52] proposed the acid growth theory indicating that hydrogen ions may exert a purely chemical or physical effect, such as cleavage of acid-labile bonds on the wall, or they may activate normal enzymatic processes directly or indirectly, potentially leading to wall loosening. Based on these findings, we speculate that the regulatory network of fruit cracking, especially the coexpression of cell wall-, redox-, and hormone-related mRNAs and their corresponding lncRNAs, influences fruit cracking.

Cell wall polysaccharide metabolic

The DEG Solyc08g077910.2 encodes an Expansin-like protein that breaks down the hydrogen bonds between molecules in the cell wall macromolecular network to promote the depolymerization of the network, which can lead to relaxation of the cell wall [53]. In this experiment, the expression level of Solyc08g077910.2 was increased significantly after 8 h of irrigation (log₂ fold-change=7.13395) in CS

tomato. The increased expression of the expansin-like gene can relax the cell wall and may influence fruit cracking.

Solyc07g055990.2 and Solyc12g011030.1 encode xyloglucan endotransglucosylase/hydrolases 7 and 9, respectively, which mediate the cleavage and polymerization of β -1,4-xyloglucan in the primary cell wall. Xyloglucan is usually fused to the cell wall, 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 rapidly elongated and differentiated. In addition, it can respond to gibberellin. He [56] found that *OsXTH5*, *OsXTH19*, *OsXTH20*, *OsXTH24* and *OsXTH28* play important roles in the elongation of rice peduncles 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, while they showed a downward trend in the CR tomato (**Fig. 8a**). Simultaneously, the expression level in CS tomato was significantly higher than that in CR tomato. This illustrates that the CR tomato may exhibit a greater osmotic stress resistance ability with downregulation of the *XTH* gene that can strengthen the cell wall upon encountering water stress.

The DEG Solyc10g080210.1 (pectinase) can remove the methyl group from polygalacturonic acid; during tomato maturation, the degree of methylation decreases from 90% in the green ripening period to 35% in the red ripening period [57], which accelerates the degradation of the cell wall. In an antisense *PaPG1* transgenic study of strawberry, the expression level of *PG* was significantly inhibited, and the degree of fruit softening was significantly delayed [58].

Redox processes

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 [59-61]. Peroxidase can also directly regulate plant cell elongation by controlling H_2O_2 levels [62]. Solyc02g080530.2 encodes peroxidase, whose levels are significantly higher in CS tomato than in CR tomato. The expression of these genes in CS tomato fruits may increase cell wall hardness and hinder the elongation of the cell wall, which will lead to fruit cracking when water absorption swelling occurs. Solyc01g081250.2 encodes glutathione-S-transferase (*GST*). The *GST* superfamily enzymes exhibit multiple functions in plants. They are not only involved in primary metabolism and secondary metabolism [63], but they can also protect plants from oxidative damage and heterogeneous substances [64-66]. According to the data analysis, the gene expression of Solyc01g081250.2 in the CR tomato was significantly higher than that in the CS tomato after 0 h, 8 h and 30 h of irrigation treatment. Higher expression of *GST* in CR tomato can better maintain cell vigour and be beneficial to tomato fruits when coupled with water stress.

Hormone-related

Previous research has shown that hormones can regulate the expression of cell wall-related genes. Trainotti [67] studied the expression of 32 genes related to cell wall synthesis and degradation. Their research showed that 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. At the same time, ethylene inhibits and promotes dual regulatory effects on the formation of plant secondary metabolites [68-70]; *TAPG1*, encoding a cell wall-degrading enzyme, can be induced by ethylene at the transcriptional level in tomato [71]; Rose [72] showed 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 revealed significant enrichment in the methionine metabolic pathway. Solyc11g042560.1 encodes an ethylene receptor, while Solyc09g008720.1 encodes an ethylene-responsive transcription factor, and their expression levels are significantly upregulated after irrigation and are higher in CS tomato than in CR tomato.

Conclusions

In this study, the key genes involved in the response to tomato cracking were identified by high-throughput sequencing, which has important significance for guiding the selection of new tomato varieties. We have also established an lncRNA-mRNA network to learn about the precise regulation of fruit cracking by lncRNAs. To the best of our knowledge, this is the first discovery of the lncRNA-mRNA network involved 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 8 hours of irrigation

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

DEGs: Differentially 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

Acknowledgements

Not applicable.

Funding

The work was supported by grants from the National Natural Science Foundation of China (No. 31701924), the Fundamental Research Funds for the Central Universities (No. KJQN201814, KYZZ201909) and Priority Academic Program Development of Jiangsu Higher Education Institutions.

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

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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-1	107964622	102656082	15.4G	0.01	98.13	95.03	42.82
CR_0h-2	102639560	97730610	14.66G	0.01	97.99	94.69	42.52
CS_0h-1	102699472	95783116	14.37G	0.01	97.58	93.81	42.95
CS_0h-2	108424258	103713024	15.56G	0.01	98.02	94.78	42.79
CR_8h-1	81919566	79478876	11.92G	0.02	96.41	91.23	42.94
CR_8h-2	100255896	94324174	14.15G	0.01	98.1	94.97	42.77
CS_8h-1	93474242	86534460	12.98G	0.01	97.79	94.28	42.96
CS_8h-2	119351634	114298442	17.14G	0.01	97.89	94.49	43
CR_30h-1	82958444	80567758	12.09G	0.02	96.32	91.05	42.29
CR_30h-2	104788296	100192938	15.03G	0.01	97.98	94.69	42.44
CS_30h-1	97274580	90916088	13.64G	0.02	96.86	92.29	42.84
CS_30h-2	105168826	98559578	14.78G	0.01	97.97	94.71	42.39

Additional Files

Additional file 1: Detailed primer sequences for qPCR confirmation.

Additional file 2: LncRNAs in 12 tomato fruit libraries

Additional file 3: Target gene prediction based on the positional relationships between lncRNAs and mRNAs

Additional file 4: GO analysis of DEGs between groups

Additional file 5: KEGG analysis of DEGs between groups

Additional file 6: Data for the lncRNA-mRNA network

Additional file 7: Key genes related to tomato fruit cracking

Figures

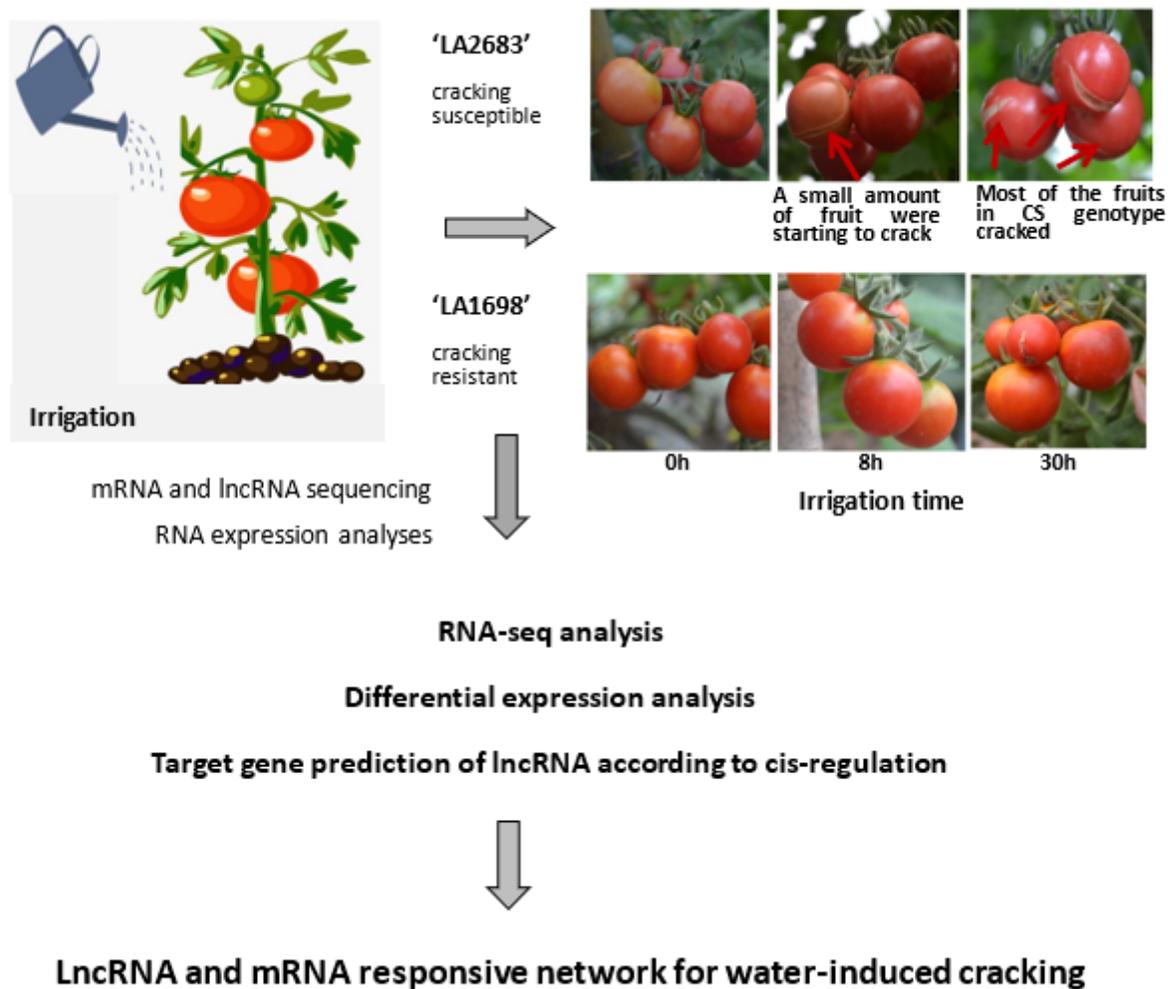


Figure 1

Experimental flow chart.

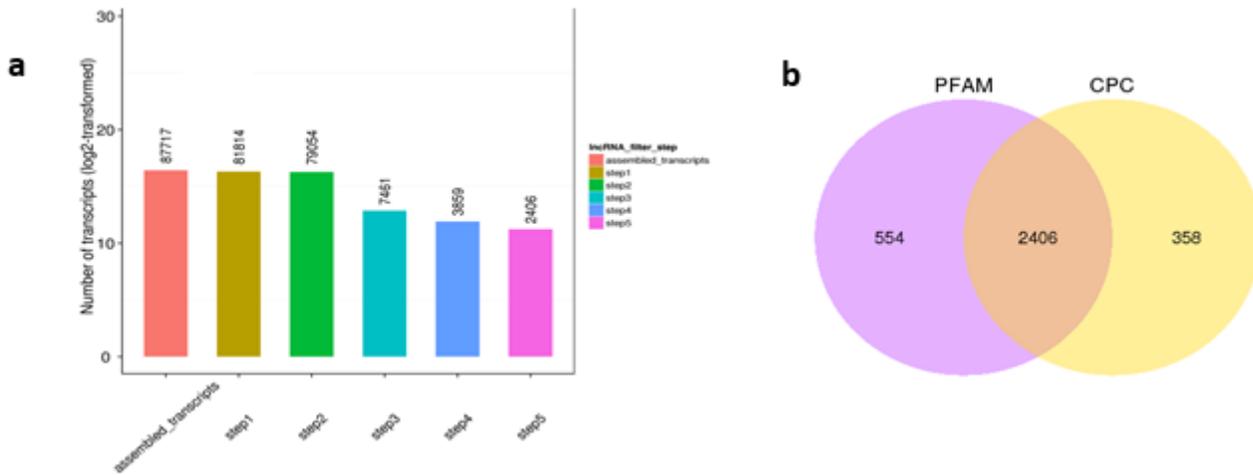


Figure 2

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

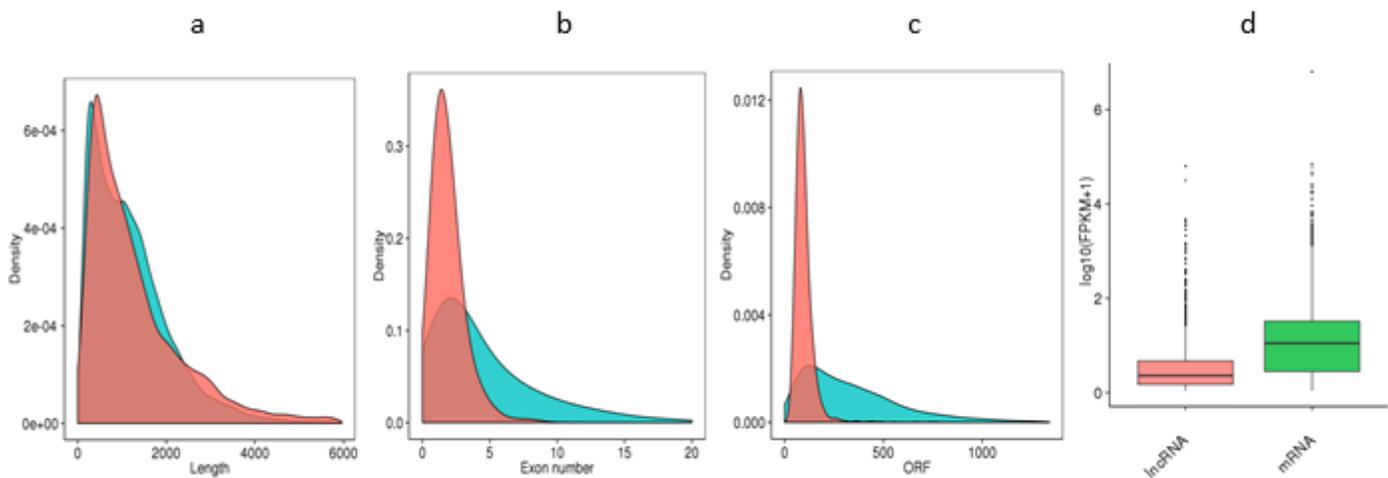


Figure 3

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 levels are indicated as $\log_{10}(\text{FPKM} + 1)$ values for the mRNAs and lncRNAs.

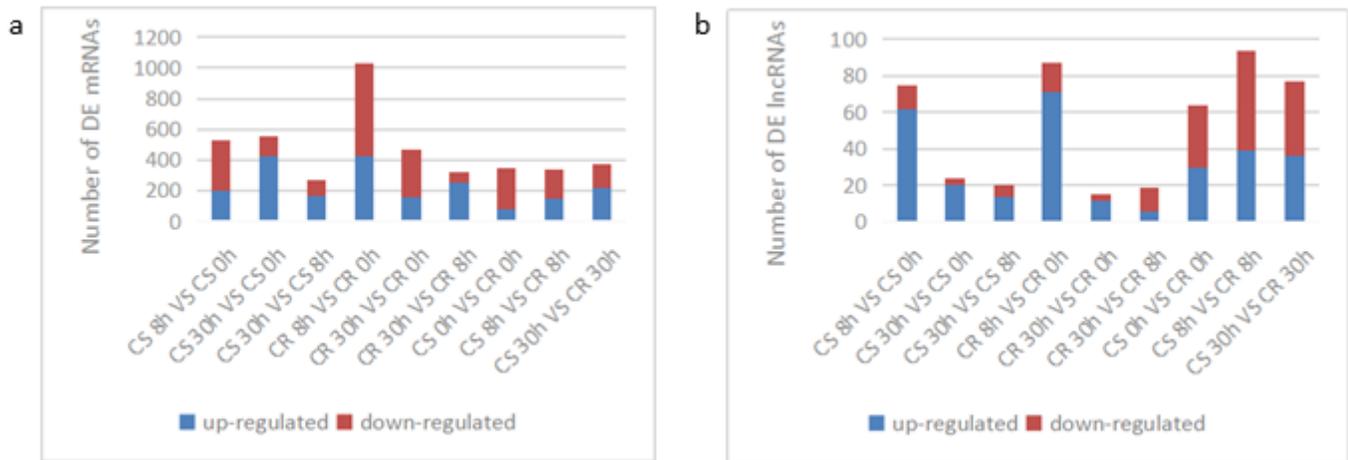


Figure 4

Differentially expressed mRNAs and lncRNAs in different libraries. Upregulated (blue) and downregulated (red) mRNAs and lncRNAs were quantified. (a) mRNAs. (b) lncRNAs. Note: CS is cracking-susceptible, CR is cracking-resistant. The same abbreviations are used below.



Figure 5

The top 20 KEGG pathways enriched by DEGs. The x-axis indicates the enrichment factor, and the y-axis indicates the pathway names.

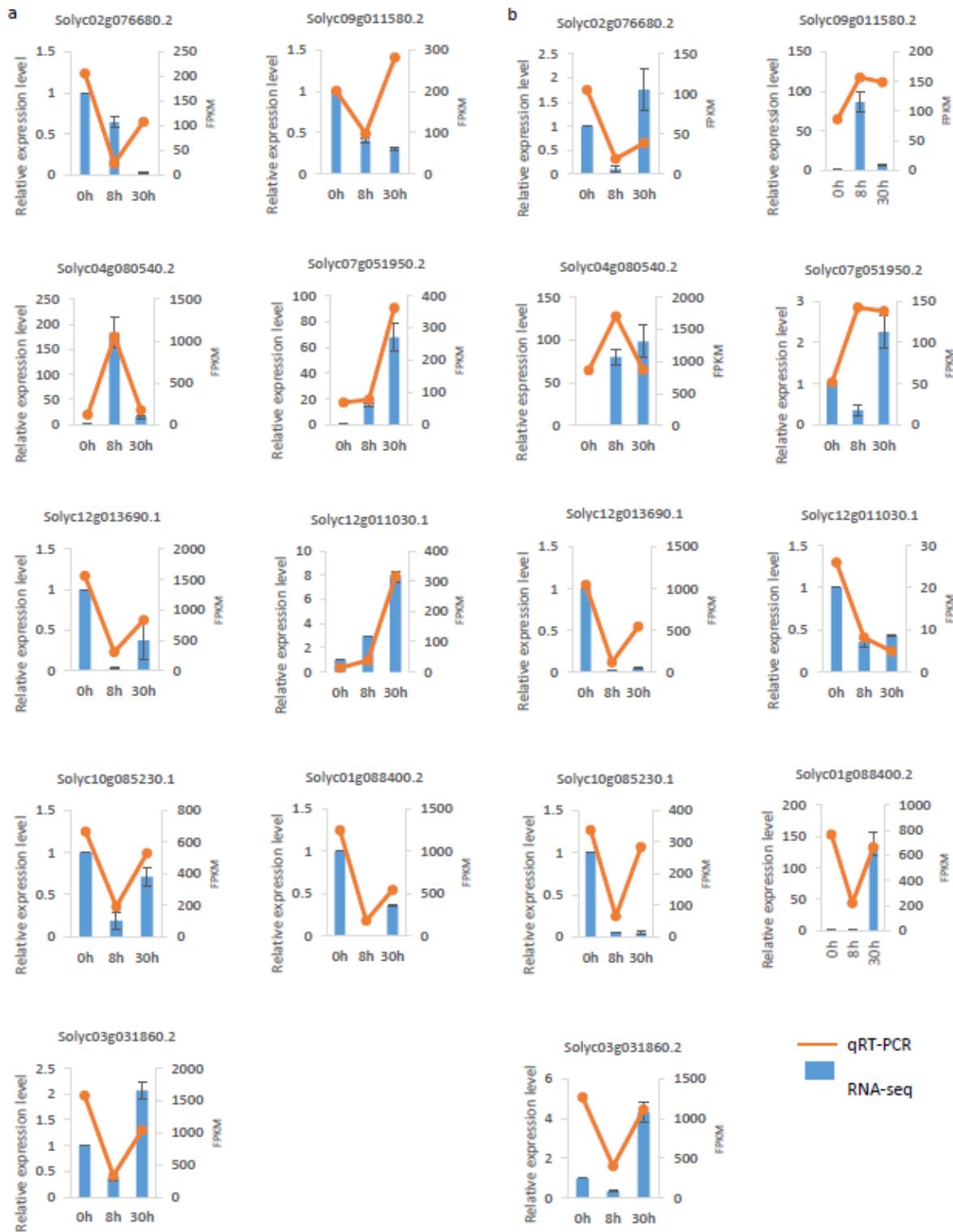


Figure 6

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

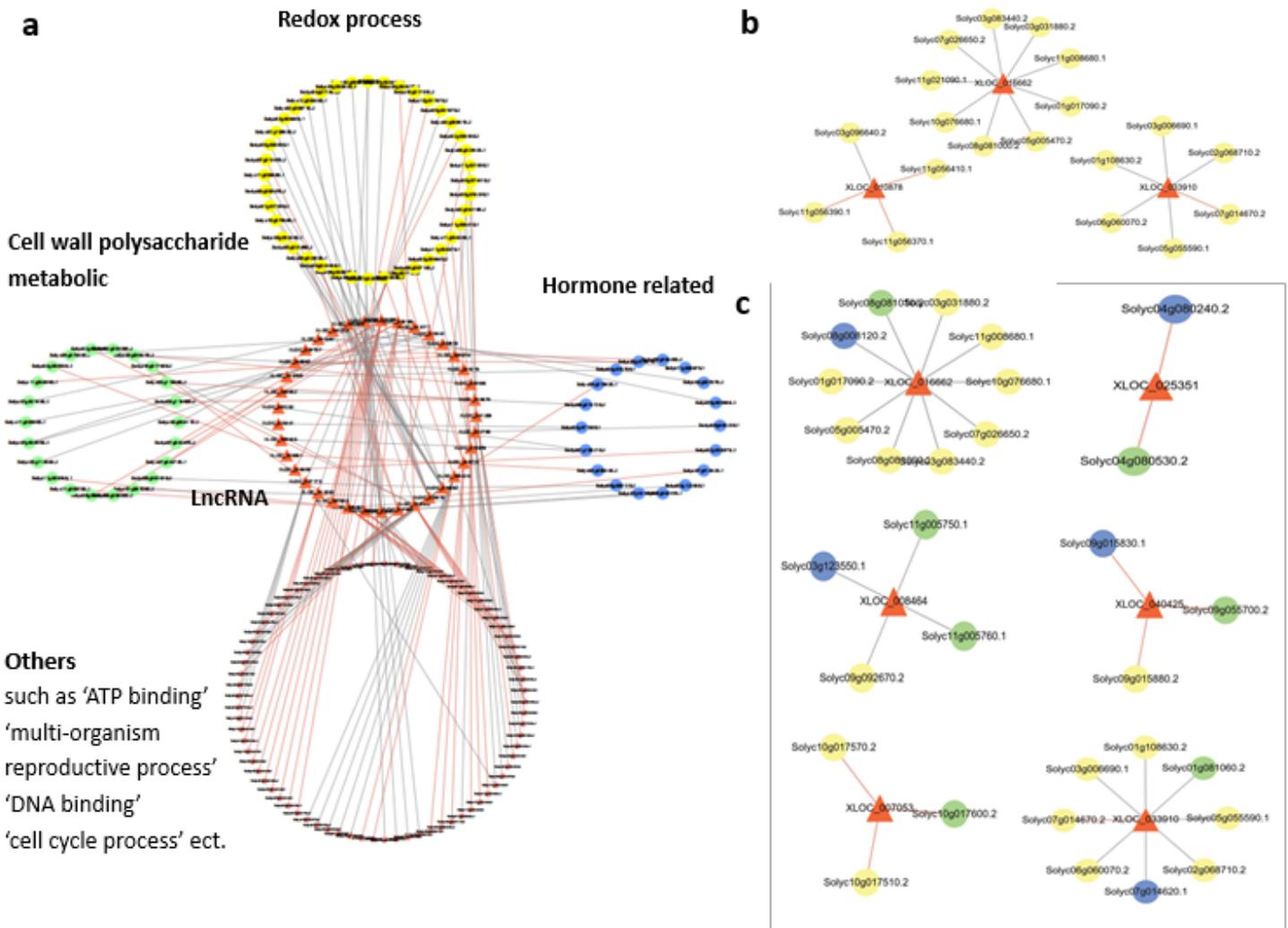


Figure 7

LncRNA-mRNA networks. The orange triangles represent LncRNAs, and the circles represent mRNAs (green: cell wall polysaccharide metabolism, yellow: redox process, blue: hormone-related, pink: other). The red edge represents the targeting mode of the LncRNA and the co-localized mRNA, and the grey edge represents co-expression. (a) The LncRNA-mRNA network influencing tomato fruit cracking. (b) LncRNAs that target the same kinds of mRNAs. (c) LncRNAs that target different kinds of mRNAs play an important role in the network.

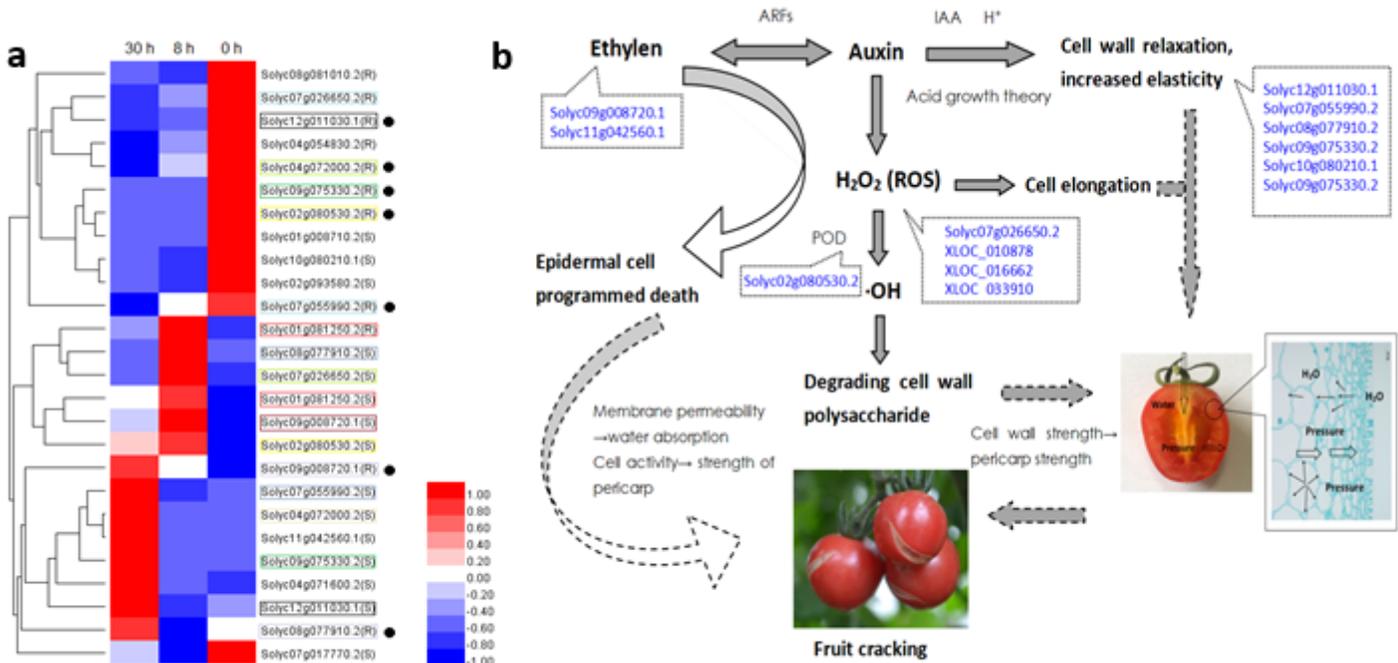


Figure 8

Hierarchical clustering analysis and proposed fruit cracking regulatory network. (a) Hierarchical clustering analysis showed the expression profiles of key genes involved in tomato fruit cracking. Based on the Euclidean distance, the minimum linkage method was used for cluster analysis. Boxes of the same colour represent the same gene in CS and CR. The solid dark circles represent genes that showed different expression trends in the two genotypes. (b) 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, peroxidase. 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 lncRNAs associated with the redox pathway. The dotted line indicates the speculation process.

Supplementary Files

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