scientific reports

Check for updates

OPEN

# Genome-wide identification of eQTL-associated lncRNAs in melon and their role in fruit cracking

Parisa Mohammadi & Aboozar Soorni✉

**Melon (*Cucumis melo* L.) is an economically important crop whose production is severely affected by fruit cracking, a disorder that leads to substantial post-harvest losses. While environmental factors are known contributors, the roles of long non-coding RNAs (lncRNAs), SNPs, and eQTLs in melon cracking remain poorly understood. This study aimed to characterize these molecular regulators to elucidate their functions in cracking susceptibility. Through transcriptome analysis of 112 melon recombinant inbred lines (RILs), we identified 341 high-confidence lncRNAs. Functional enrichment linked them to metabolic processes, cell wall remodeling, and stress responses. Co-expression networks revealed interactions between lncRNAs and key transcription factors (MYB, WRKY, NAC) and cell wall-related genes (*EXP*, *XTH*, *AQP*). eQTL analysis identified 10 lncRNAs associated with fruit cracking, with two (MSTRG.4395 and MSTRG.623) showing strong regulatory links to auxin-related (ILR1) and calcium-signaling (calmodulin) genes. SNPs in these loci significantly influenced lncRNA expression, suggesting roles in hormonal and structural pathways affecting cracking. This study provides the first genome-wide characterization of lncRNAs and eQTLs in melon fruit cracking, uncovering their regulatory roles in cell wall integrity, hormone signaling, and stress response. The identified lncRNA-mRNA networks and candidate SNPs offer potential molecular markers for breeding crack-resistant melon varieties, contributing to sustainable agriculture and reduced post-harvest losses.**

**Keywords** *Cucumis melo*, fruit cracking, lncRNAs, eQTLs, co-expression networks, SNP, cell wall metabolism

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is an important agricultural crop cultivated in both temperate and tropical regions[1,2]. According to the Food and Agriculture Organization (FAO), global production of melons surpasses 40 million tonnes each year[3]. According to reports published in 2018, China is the world's largest producing country for melons[4]. Moreover, Turkey, India and Iran are recognized as some of the leading melon producers globally[1].

Melon production is significantly limited by postharvest disorders, particularly fruit cracking, a physiological condition that severely reduces marketability and yield[5]. Fruit cracking severely impacts melon yield and quality globally, with economic losses reaching up to 70%. Contributing factors include environmental conditions such as light, temperature, and irrigation, as well as nutrient deficiencies of boron, calcium, zinc, and potassium. Additionally, cultivar susceptibility, fruit physical traits, and molecular mechanisms involving coding and non-coding RNAs play critical roles in this disorder[2,5–7]. Indeed, molecular mechanisms play a crucial role in fruit cracking. One of the most significant pathways involved in this process is the regulation of cell wall metabolism. This pathway encompasses the activity of enzymes such as polygalacturonase, pectin methylesterase, cellulase, and those responsible for the hydrolysis of xyloglucan. Xyloglucan, the most abundant hemicellulose in plant cell walls, plays a vital role in binding cellulose microfibrils, contributing to cell wall structure and integrity. Enzymes like xyloglucan endotransglucosylase/hydrolase (XTH), xyloglucan endo-transglycosylase (XET), and xyloglucan endo-hydrolase (XEH) regulate cell wall extension and fruit skin softening, impacting cracking susceptibility[6]. Polygalacturonase (PG), encoded by the *PG* gene family, degrades pectin, influencing fruit ripening and cracking, as shown in Atemoya fruit[8]. Structural components such as cutin, suberin, and lignin also affect skin integrity. Cutin-related genes (*DHDDS*, and *SULTR3*) are upregulated in crack-resistant watermelons, while cuticular wax biosynthesis genes (*GPAT*, *SMT*, and *KCS*) are downregulated in susceptible ones[9]. Suberin biosynthesis genes (*ABCG20*, *CYP86B1*, *NAC038*, and *NAC058*) increase suberin content, causing microcracks in apples[10], whereas lignin biosynthesis genes (*CAD9*, *CCR1*, and *OMT1*) prevent cracking[11]. Water and calcium transport mechanisms, involving genes like *PIP* (water transport) and *CALM*/*CARL* (calcium transport), are

Department of Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran. ✉email: soorni@iut.ac.ir

critical for cell wall stability and cracking resistance, as seen in jujube cultivars[12]. Expansin (EXP) proteins, encoded by the *EXP* gene family, contribute to cracking by breaking hydrogen bonds in cell walls, with *LcExp1* and *LcExp2* expression linked to litchi fruit cracking[13]. Hormonal balance, including ethylene, auxins, gibberellins, cytokinins, and abscisic acid (ABA), regulates cell turgor pressure and cracking susceptibility. Ethylene levels above 1–4 Pmol/kg/g in melons indicate cracking risk[5], while higher ABA levels in cracked fruits highlight hormonal regulation's importance[14]. Transcription factors (TFs) like *ERF4* in watermelon regulate ethylene production and skin firmness[15], and *MYB93* influences suberin synthesis and cracking susceptibility[16]. These molecular, structural, and hormonal factors collectively determine fruit susceptibility to cracking. Previous research in melon has also employed genetic models to describe the polygenic inheritance of cracking resistance[17]. Transcriptomic comparisons between crack-resistant and susceptible melon lines have further identified hormone- and cell wall-related differentially expressed genes[18]. In watermelon, a related cucurbit, QTL mapping has identified genomic regions associated with cracking tolerance[19].

Long non-coding RNAs (lncRNAs), single nucleotide polymorphisms (SNPs), and expression quantitative trait loci (eQTLs) are key molecular players in fruit cracking. lncRNAs regulate gene expression at epigenetic, transcriptional, and post-transcriptional levels, influencing fruit softening, ripening, and cell wall formation. In peaches, 1,500 differentially expressed lncRNAs (DELs) were linked to aromatic compound production, chlorophyll degradation, and tissue softening[20]. In tomatoes, lncRNAs like XLOC_16662 and XLOC_033910 influenced cracking-related genes, forming an lncRNA-mRNA regulatory network[21], while in pomegranates, lncRNAs were tied to calcium ion binding, hormone signaling, and sugar metabolism[22]. SNPs can alter lncRNA structure and function, impacting gene expression. eQTLs map genetic variations (SNPs) to gene expression changes, revealing their role in cracking-related traits. In a study on peaches, 133 eQTL regions were identified that regulate genes related to the hormone auxin, influencing fruit softening and ripening processes. These eQTLs can affect ripening speed and final fruit quality by modulating auxin signaling[23]. Similarly, in tomatoes, eQTLs were found to impact the biosynthesis of ethylene, pectate lyase, and the *SlWD40* gene, which are directly involved in the ripening process and affect traits such as ripening time and fruit quality[24].

Despite the growing body of research on the roles of lncRNAs, SNPs, and expression quantitative trait loci eQTLs in different traits across various species, there is a notable gap in studies focusing on melons. To date, no comprehensive research has been conducted to identify and characterize lncRNAs, SNPs, or perform eQTL analysis specifically in melons in the context of fruit cracking. Hence, this study aimed to address this gap by systematically identifying and characterizing lncRNAs and SNPs, as well as conducting eQTL analysis to uncover their regulatory roles in melon fruit cracking. By doing so, this research seeks to provide novel insights into the molecular mechanisms underlying cracking in melons and contribute to the development of strategies for mitigating this issue in agricultural practices.

## Results

### lncRNA identification

The transcriptome assembly performed using StringTie generated a total of 93,365 transcripts. From these, 5,453 transcripts with specific class codes "u," "x," "i," "o," and "e" identified as unannotated. Among these, 945 transcripts met the criteria of expression levels above 1 CPM and lengths ranging between 200 bp and 15 Kb. Further filtering revealed 936 transcripts that lacked homology to tRNA or rRNA. Subsequent analysis identified 634 transcripts with no significant matches in the UniProt, Pfam, or Rfam databases. Using CPC2, 591 noncoding transcripts detected and subjected to PLncPRO for the prediction of novel lncRNAs. Ultimately, 341 potential lncRNAs predicted through this pipeline, as summarized in Table 1.

## Distribution and functional analysis of lncRNA-associated target genes

The distribution of upstream and downstream target genes linked to lncRNAs was analyzed to investigate their potential roles in melon fruit cracking, with a focus on their regulation by lncRNAs. According to the results, a total of 2631 and 1997 potential target genes identified within 100 kb upstream and downstream of lncRNAs, respectively. For upstream target genes, the highest gene counts identified in critical pathways such as metabolic processes (327 genes), biosynthetic pathways (344 genes), and cellular growth and development (288 genes). These pathways are essential for maintaining cell wall integrity, osmotic balance, and structural stability, all of which are crucial in preventing fruit cracking. Additionally, genes involved in responses to environmental stresses (186 genes) and signaling processes (160 genes) prominently represented, underscoring their roles in

| Step | Number |
|---|---|
| Total transcripts assembled by StringTie | 93,365 |
| Potential novel transcripts (Class codes: i, u, x, o, e) | 5453 |
| Transcripts with CPM > 1, length > 200 bp and < 15 kb | 945 |
| Transcripts after filtering out tRNAs and rRNAs | 936 |
| Transcripts with no significant hit against UniProt, Pfam, and Rfam. | 634 |
| Noncoding transcripts predicted by CPC2 | 591 |
| LncRNAs predicted by PLncPRO | 341 |

**Table 1**. Results of the filtration step in the lncRNA identification pipeline, showing the total number of transcripts excluded at each stage.

enhancing fruit adaptability to external conditions and regulating cellular responses to stress. Lower gene counts observed in more specialized processes, such as morphogenesis (31 genes) and post-embryonic development (21 genes), which may influence mechanical stability and surface integrity during fruit development. For downstream target genes, a similar functional distribution was observed, albeit with reduced gene counts. Metabolic pathways (222 genes), biosynthetic processes (248 genes), and cellular growth and development (268 genes) remained the most represented categories, reflecting their fundamental roles in maintaining cellular homeostasis and structural integrity. Genes associated with responses to environmental stresses (131 genes) and signaling processes (104 genes) also identified, though in lower numbers compared to upstream genes. Specific processes such as morphogenesis (23 genes) and post-embryonic development (19 genes) showed minimal representation, suggesting their secondary role in cracking susceptibility. Overall, upstream genes were more abundant, particularly in metabolic, biosynthetic, and growth-related pathways, indicating that lncRNAs may exert stronger regulatory control over these genes (Fig. 1).

## Enrichment analysis of upstream and downstream target genes of lncRNAs

Enrichment analysis of upstream and downstream target genes of lncRNAs (Fig. 2) revealed their significant involvement in biological processes, molecular functions, and cellular components related to melon fruit cracking. Key biological processes included cellular and metabolic processes, particularly those involving polysaccharide metabolism, cell wall remodeling, and osmotic regulation, which are critical for maintaining cell wall integrity under mechanical stress. Molecular functions such as catalytic activity, hydrolase activity, and ion binding were highly enriched, highlighting their roles in cell wall synthesis, degradation, and osmotic balance. Catalytic activities, especially those related to cell wall remodeling, were the most frequent, suggesting their importance in enhancing cracking resistance. Additionally, cellular components like the cytoplasm and membrane were prominently represented, emphasizing their roles in material transport, ionic balance, and stress response regulation. The full list of target genes and their associated GO terms contributing to these enriched categories is provided in Supplementary Table S1.

## Interaction of expressed lncRNAs and target genes

Following the identification of potential target genes, the interaction between expressed long non-coding RNAs (E-lncRNAs), defined here as lncRNAs with expression levels > 1 CPM, and expressed genes (EGs) was evaluated using the lncTar tool. According to the results, ten high-confidence E-lncRNA–EG pairs identified based on two criteria: (1) a strong predicted interaction (standardized free energy threshold < -0.1 kcal/mol), and (2) the functional relevance of the target gene to known fruit cracking pathways (e.g., TF, cell wall modifiers, hormone-related enzymes). Among the E-lncRNAs, MSTRG.942, MSTRG.534, MSTRG.1196, and MSTRG.1003 were found to interact with the genes MELO3C001115, MELO3C000180.2, MELO3C000924.2, and MELO3C000729.2, respectively, all of which encode subunits of NAD+ oxidoreductase. Similarly, MSTRG.13,831, MSTRG.10,401,

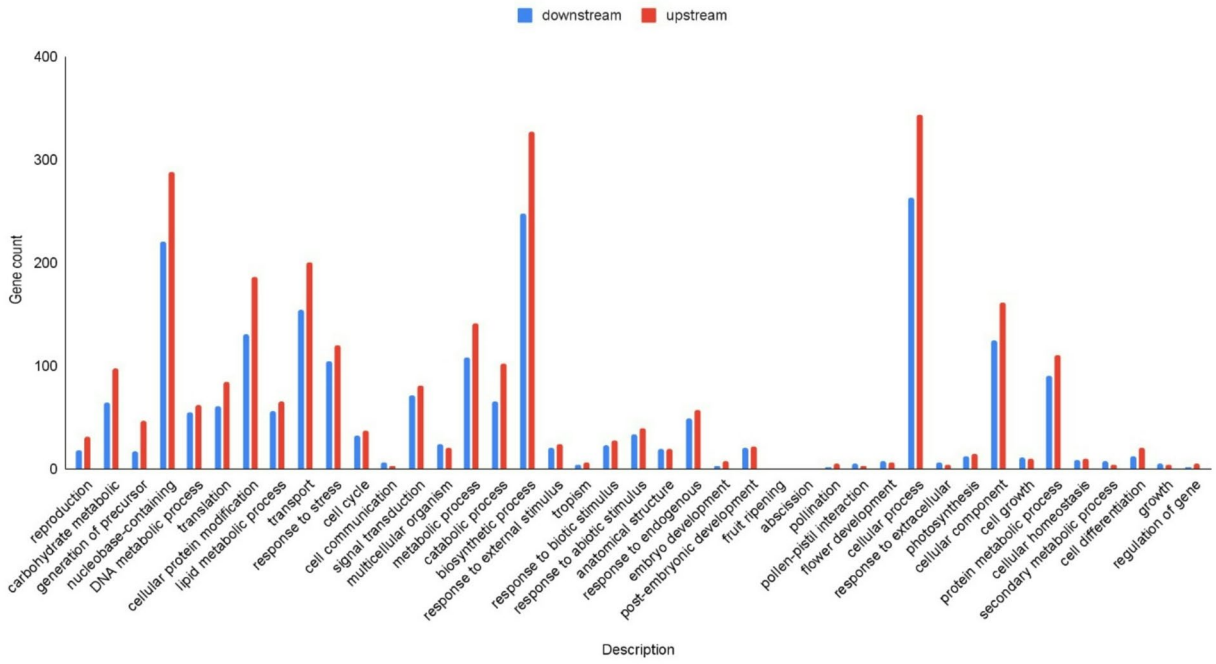


**Fig. 1**. Functional classification of putative cis-target genes for expressed long non-coding RNA (E-lncRNAs, lncRNAs with CPM > 1). Target genes were screened based on their location within a 100 kb genomic window upstream (blue) or downstream (orange) of each expressed E-lncRNAs. The bar chart shows the distribution of these gene counts across different functional categories.

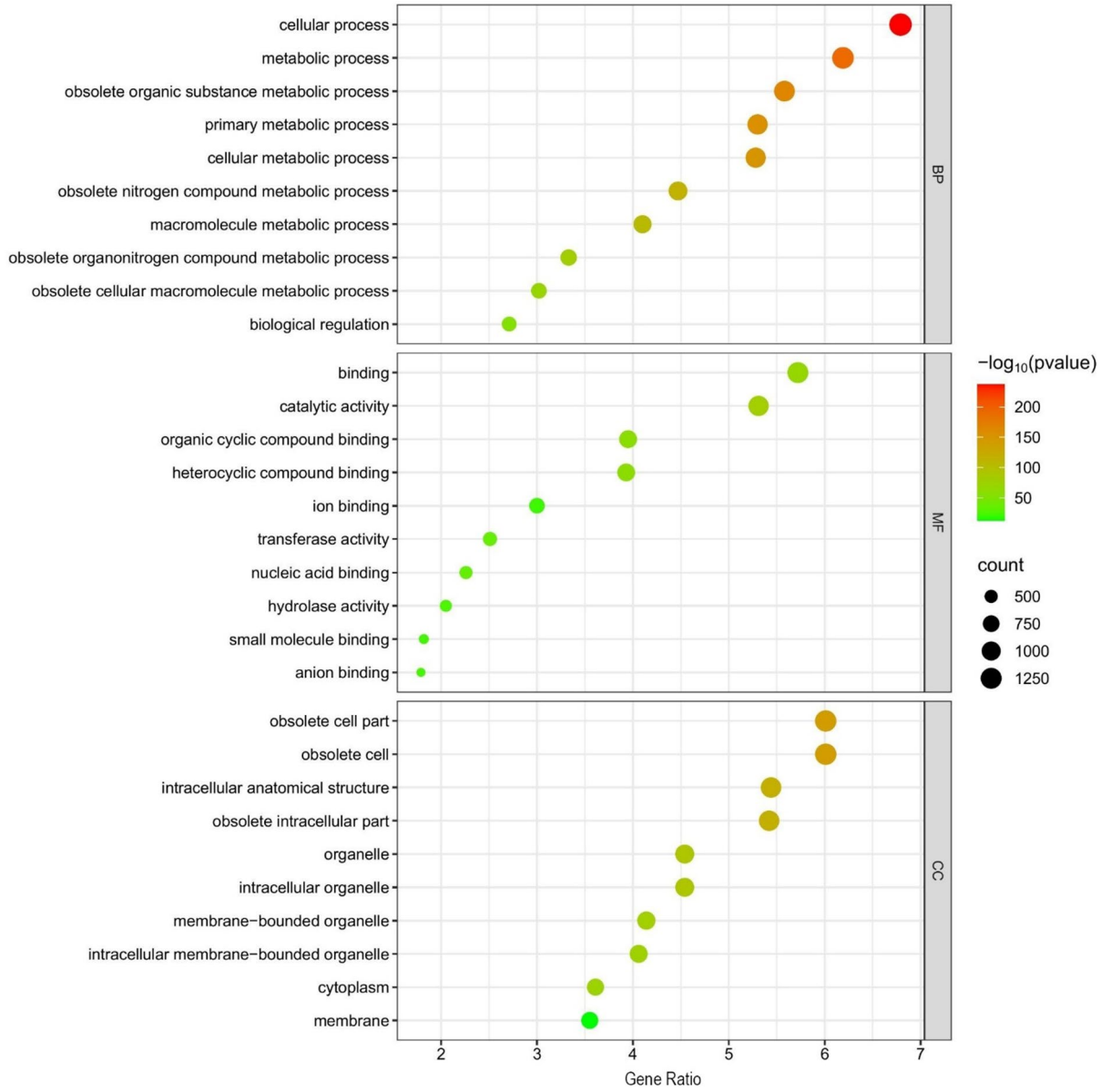


**Fig. 2**. Gene Ontology (GO) enrichment analysis of putative cis-target genes for E-lncRNAs (lncRNAs with CPM > 1). Enrichment was performed on the pool of upstream and downstream target genes located within a 100 kb range of E-lncRNAs. The bubble chart summarizes the significantly enriched GO terms (False Discovery Rate, FDR < 0.05) across three GO domains. The size of each bubble corresponds to the number of genes associated with the term, and the color intensity represents the statistical significance of the enrichment (-log10(p-value)).

and MSTRG.16,625 interacted with MELO3C010562.2, MELO3C031433.2, and MELO3C033441.2, respectively, encoding Myb/SANT-like DNA-binding domain proteins. Additionally, MSTRG.1768 interacted with MELO3C028133.2, which encodes a WRKY transcription factor, while MSTRG.623 interacted with MELO3C000247.2, encoding a NAC domain-containing protein. Another significant interaction was observed between MSTRG.15,609 and MELO3C007736.2, which encodes a homeobox-leucine zipper protein ATHB-6-like (HD-ZIP) transcription factor.

To validate the predicted interactions between E-lncRNAs and their target genes, we first visualized their coordinated expression patterns across the RIL population. A heatmap of expression levels (Fig. 3) reveals distinct co-expression clusters, confirming that these interacting pairs exhibit synchronized transcriptional activity across different samples.

Subsequently, we quantified these relationships through correlation analysis (Fig. 4), which identified several key lncRNA-mRNA pairs with significant positive correlations. Notably, the pairs MSTRG.534-MELO3C000180 and MSTRG.942-MELO3C001115 showed the strongest correlations, providing compelling evidence for their

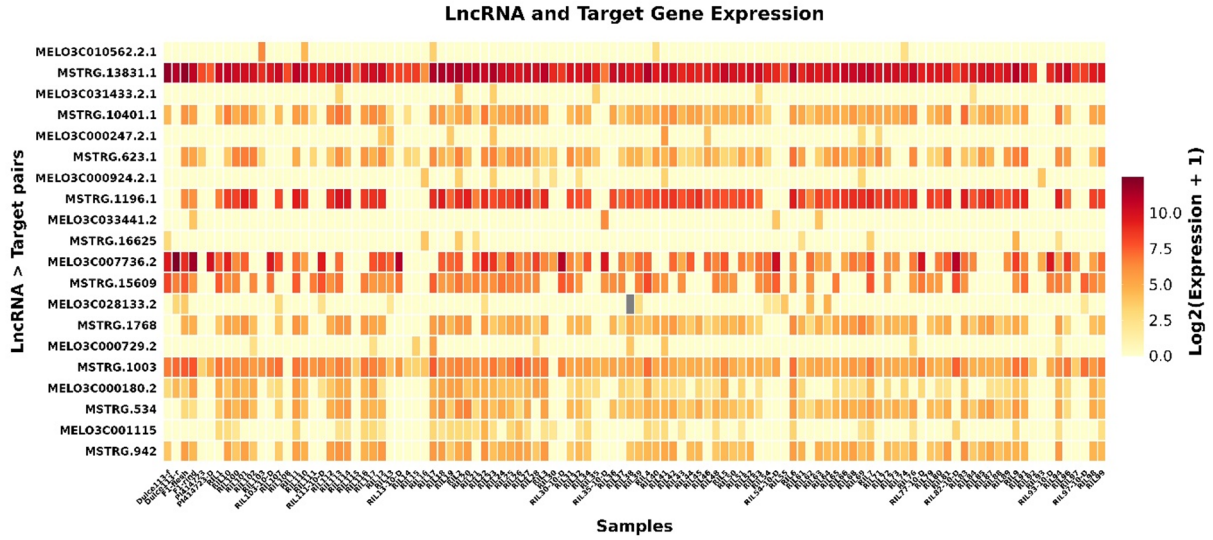


**Fig. 3**. Co-expression patterns of E-lncRNAs (lncRNAs with CPM > 1) and their target genes. The heatmap displays the normalized expression levels (as log2(expression + 1)) of key E-lncRNAs and their corresponding target expressed genes across 109 RIL samples. Rows represent individual E-lncRNAs (labeled on the right) and their predicted target genes (labeled on the left), while columns represent the individual RIL samples.

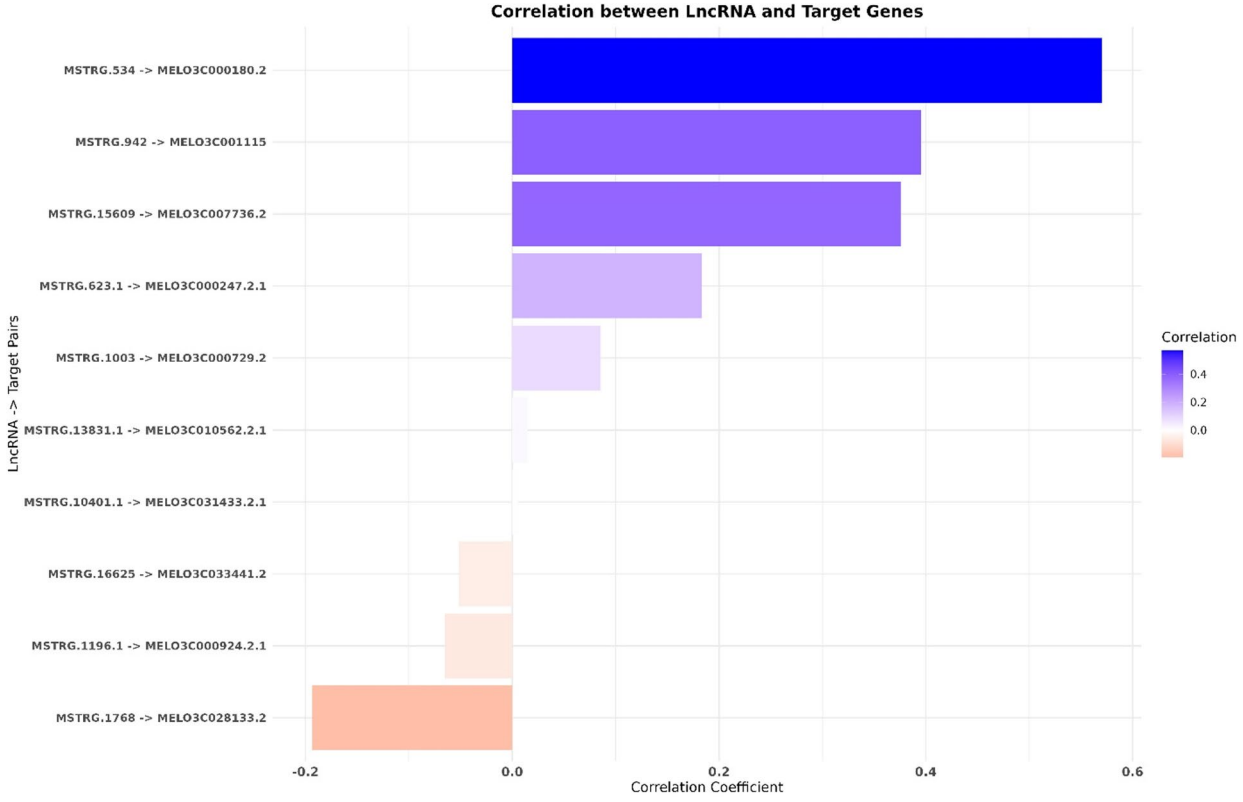


**Fig. 4**. The correlation plot illustrates the expression relationships for E-lncRNA-mRNA pairs across the RIL population. Correlation coefficients (r) are indicated, with positive values denoting coordinated expression and negative values indicating an inverse relationship.

potential regulatory relationships. Interestingly, MSTRG.1768 showed a significant negative correlation with MELO3C028133.2, suggesting this lncRNA may function to suppress the expression of its target gene.

## Co-expression network analysis

Co-expression network analysis (Fig. 5) identified four distinct modules, designated by the colors blue, brown, turquoise, and grey. To elucidate the relationships between protein-coding genes (PCGs) and lncRNAs associated with melon fruit cracking, the co-expression networks for each module visualized using Cytoscape and Gephi. The blue module, comprising 80 lncRNAs and 14 PCGs, revealed that a protein kinase gene (MELO3C000703) exhibited the highest number of interactions with multiple lncRNAs. Given that protein kinases are typically activated in response to stress or environmental changes, these lncRNAs may play a pivotal role in regulating cellular responses to environmental conditions and modulating cell wall structure, thereby influencing processes associated with fruit cracking. The brown module consists of 16 lncRNAs and 37 PCGs, with MYB transcription factors (MELO3C022997, MELO3C034442, MELO3C012926, MELO3C017925) demonstrating the highest degree of interaction with lncRNAs. Additionally, this module includes ethylene-responsive transcription factors (*ERFs*)(MELO3C005630, MELO3C012994, MELO3C017272), homeobox-leucine zipper proteins (*HD-ZIP*) (MELO3C012064, MELO3C022532), NAC domain proteins (MELO3C015427), and expansin proteins (*EXP*) (MELO3C021999), all of which exhibit significant interactions with other genes and various lncRNAs. In the turquoise module, which contains 106 lncRNAs and 110 PCGs, several transcription factors, including WRKY (MELO3C014305, MELO3C005937) and HD-ZIP (MELO3C003554, MELO3C007078), as well as genes involved in membrane and cell wall metabolism, such as *EXP* (MELO3C023866) and xyloglucan endotransglucosylase/hydrolase (*XTH*)(MELO3C017480), show the highest levels of interaction with lncRNAs. Furthermore, certain lncRNAs interact with genes involved in hormone metabolism, such as Gibberellin 2-oxidase (*GA2ox*) (MELO3C016152) and Auxin Responsive Factor (*ARF*)(MELO3C015762, MELO3C013003, MELO3C003701), which are critical regulators of cell growth and development. Dysregulation of these genes may lead to abnormal cell division and elongation, alterations in cell wall structure, and imbalanced growth between the fruit peel and flesh, thereby increasing mechanical stress and the likelihood of fruit cracking. The grey module, composed of 606 lncRNAs and 155 PCGs, features an lncRNA with the highest centrality in the network, interacting with Aquaporin (*AQP*) (MELO3C025165) and Cellulose synthase-like (*CSL*) (MELO3C017931) genes. Aquaporins are integral to the regulation of water and solute transport across cellular membranes, playing essential roles in maintaining water homeostasis, osmotic balance, and cellular turgor pressure. Cellulose synthase-like genes, on the other hand, are responsible for cellulose biosynthesis, a key structural component of plant cell walls, and are critical for plant cell growth and development.

## eQTL analysis of lncRNAs associated with fruit cracking

Matrix eQTL analysis identified 24 lncRNAs associated with 1574 SNPs (Fig. 6), among which 10 found in close genomic proximity to upstream or downstream genes implicated in fruit cracking (Table 2). This positional relationship suggests a potential regulatory role of these lncRNAs, possibly through interactions with neighboring cis-regulatory elements involved in cracking-related biological processes. Notably, MSTRG.4395 exhibited potential regulatory associations with genes encoding oxidoreductase, 1-aminocyclopropane-1-carboxylate synthase (*ACS*), and peroxidase enzymes. Similarly, MSTRG.623 showed possible regulatory links with *ARF*, *NAC*, and *OPRF3* genes. All details for SNP locus, lncRNAs, and associated genes are provided in Supplementary Table S2.

Further eQTL analysis revealed two biologically significant genes on chromosome 2 harboring SNPs with potential regulatory effects on lncRNA expression. The first, MELO3C015432.2, encodes an IAA-amino acid hydrolase ILR1-like 4 enzyme, which hydrolyzes auxin conjugates and modulates cell growth, differentiation, and stress responses. This gene was associated with MSTRG.4395, and multiple SNPs (positions: 1621958, 1622383, 1622481, 1622545, 1623626, 1622203, 1623988, 1624219, and 1624050) exhibited highly significant associations ($p < 10^{-6}$), suggesting their potential role in regulating lncRNA expression. The second gene, MELO3C015602.2, encodes a calmodulin protein, a key mediator of calcium-dependent signaling pathways involved in biotic and abiotic stress responses. SNPs at positions 3,223,350, 3,223,368, and 3,223,410 were significantly associated with MSTRG.623. Linear model analysis indicated that this calmodulin-encoding gene may exert a regulatory influence on the expression of the corresponding lncRNA.

## Discussion

While prior studies in melon have characterized the genetic architecture[17], physiological responses[25], and transcriptomic profiles[18] of fruit cracking, and while QTL analyses in watermelon have mapped cracking-related loci[19], our study represents the first integrative analysis of lncRNAs, SNPs, and eQTLs in melon cracking. This multi-omics approach enables us to link genetic variation to regulatory non-coding RNAs and their target networks, providing a mechanistic layer previously uncharacterized in this species. In this study, we conducted a comprehensive genome-wide analysis to identify and characterize eQTL-associated lncRNAs in melon, shedding new light on the molecular mechanisms involved in fruit cracking. Utilizing a rigorous bioinformatic pipeline, we identified 341 high-confidence lncRNAs from the melon transcriptome, highlighting a significant presence of non-coding RNAs within the melon genome.

Our initial functional characterization, based on enrichment analyses, revealed that lncRNA-associated target genes are predominantly involved in two broad and interconnected categories. First, we identified a substantial number of genes involved in metabolic processes, biosynthetic pathways, and cellular growth and development. These functional categories are known to contribute to the physiological basis of fruit cracking. The relevance of these metabolic and biosynthetic pathways to fruit cracking is corroborated by studies in

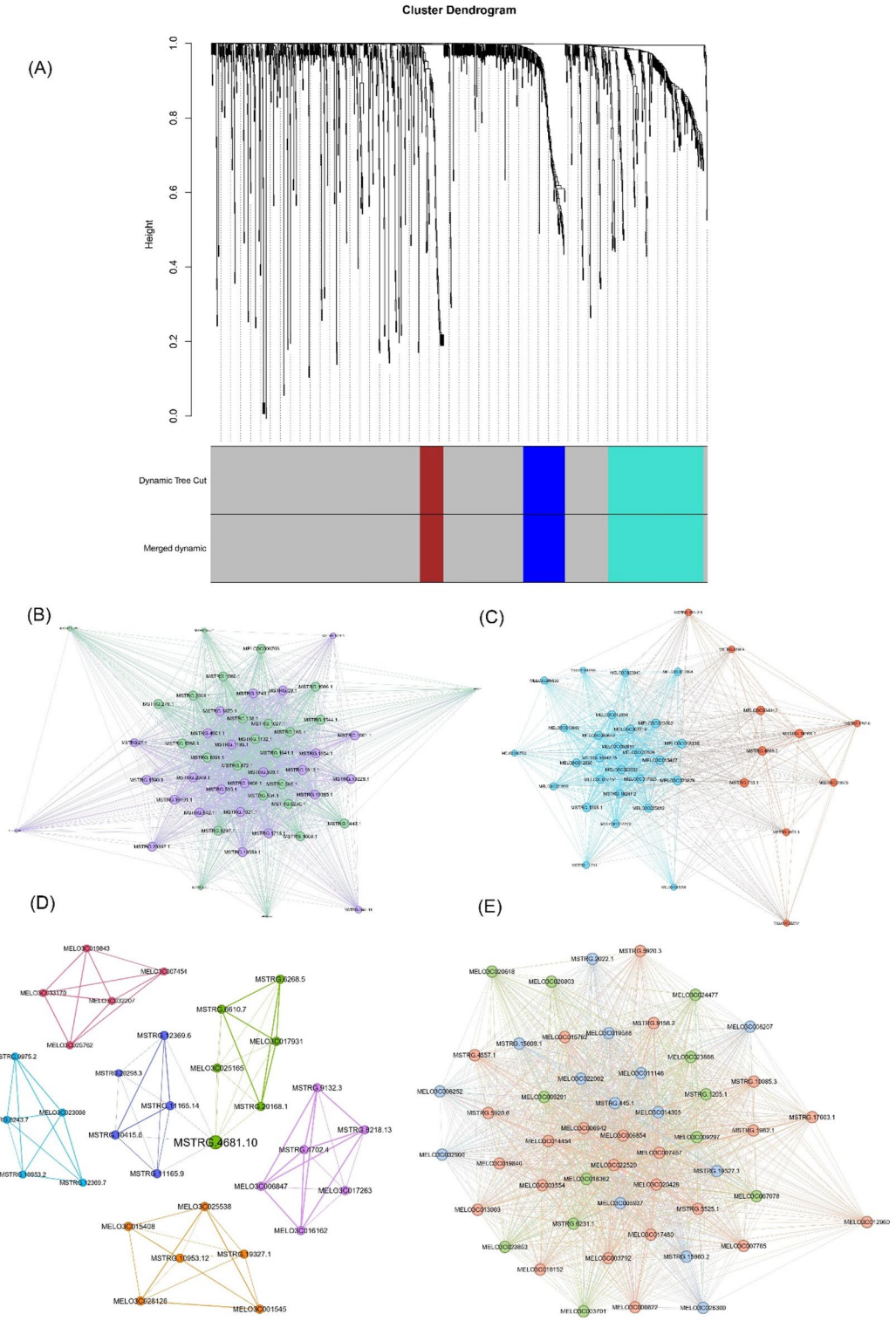


**Fig. 5**. WGCNA module characterization and identification of hub genes. (**A**) Clustering dendrogram of genes, with dissimilarity based on topological overlap, together with assigned module colors. (**B**) The co-expression network related to the blue module. (**C**) The co-expression network related to the brown module. (**D**) The co-expression network related to the greymodule. (**E**) The co-expression network related to the turquoise module.

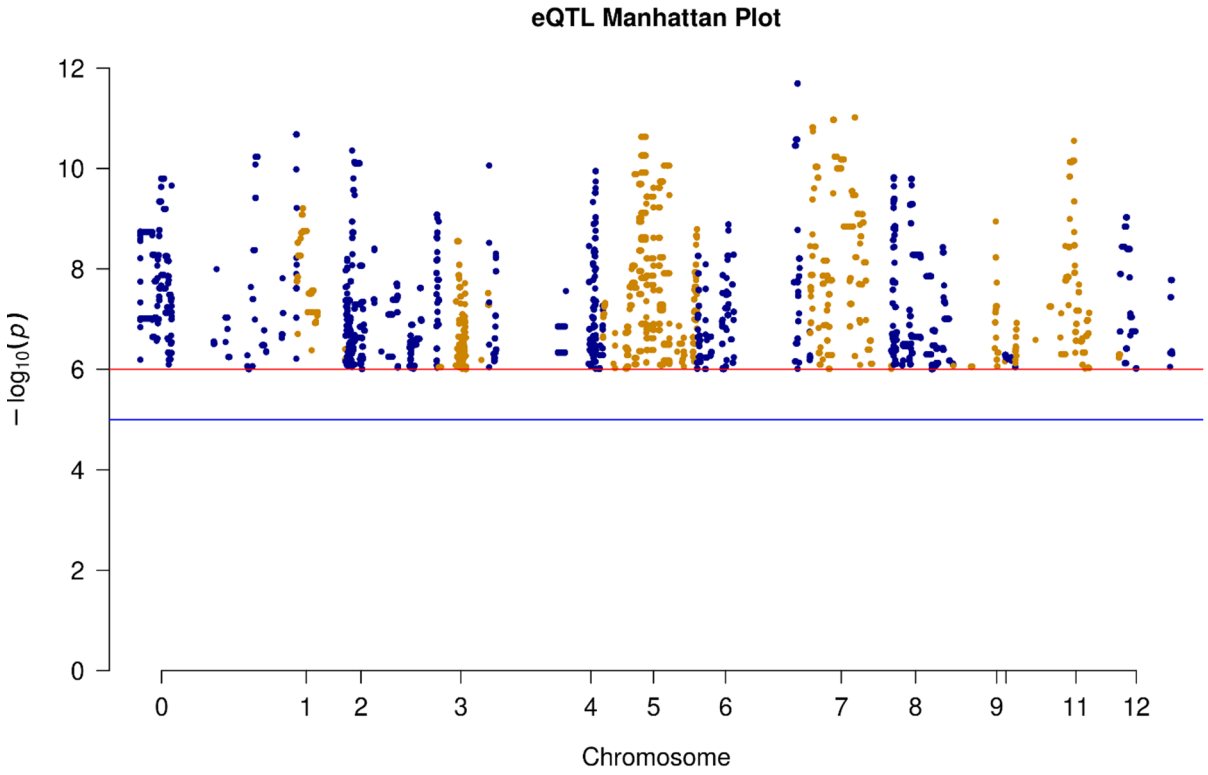


**Fig. 6**. Genome-wide distribution of cis-eQTLs associated with fruit cracking E-lncRNAs. The Manhattan plot displays the association strength (-log10 P-value) for all tested SNPs against their genomic position. Each dot represents an SNP-lncRNA association. The horizontal red line indicates the genome-wide significance threshold (FDR < 1e-5).

| lncRNA | Gene |
|---|---|
| MSTRG.13,975 | Homeobox leucine-zipper protein |
| | MYB-like DNA binding protein |
| MSTRG.4395 | oxidoreductase |
| | 1-aminocyclopropane-1-carboxylate synthase |
| | peroxidase |
| MSTRG.8371 | MYB-like DNA binding protein |
| | MYB-like DNA binding protein |
| | receptor-like protein kinase |
| | receptor-like protein kinase |
| MSTRG.623 | NAC domain-containing protein |
| | Auxin response factor |
| | 12-oxophytodienoate reductase 3 |
| MSTRG.8442 | pectinesterase |
| | pectinesterase-like |
| MSTRG.931 | acetyl-CoA carboxylase 1-like |
| | Lipoxygenase |
| MSTRG.16,081 | transcription factor bHLH101-like |
| | transcription factor bHLH100-like |
| MSTRG.1477 | Glycosyl hydrolase family 5 protein |
| MSTRG.19,634 | FAD/NAD(P)- binding oxidoreductase family protein |
| MSTRG.19,558 | transcription factor MYB44-like |

**Table 2**. Regulatory E-lncRNAs involved in the regulation of neighboring genes associated with fruit cracking.

other fruit species. Supporting this, transcriptomic studies in grapes have reported differential expression of genes associated with biosynthesis and metabolic regulation during the ripening period, suggesting their role in the onset of cracking[26]. Similarly, in jujube, metabolic genes related to growth regulation and biosynthetic activity were found to be differentially expressed between cracked and intact fruits[27]. Additionally, classification findings revealed that genes related to environmental stress responses and signaling pathways are predominantly associated with fruit cracking susceptibility. This observation is consistent with prior studies in tomato, where *SlGH9-15* was identified as a key gene contributing to cracking under hormonal and abiotic stress conditions[28]. Furthermore, research on fleshy fruit ripening highlights the involvement of intricate hormonal networks, transcriptional regulation, and epigenetic modifications in controlling gene expression during development[29].

To delineate specific regulatory pathways, we constructed co-expression networks, which uncovered diverse TFs and stress-responsive genes interacting with the identified lncRNAs. Notably, TFs such as MYB, WRKY, HD-ZIP, and NAC were significantly enriched. The prominence of these TF families is consistent with their established roles in fruit development and stress responses across species. In melon, MYB, WRKY, and HD-ZIP may regulate *CmEXPB1*, a gene implicated in resistance to fruit cracking[30]. The HD-ZIP family also exhibits lower expression in cracking-resistant watermelon varieties compared to susceptible ones[9]. NAC TFs, specific to plants, are linked to fruit growth and ripening. In Akebia, they co-express with cell wall-related genes associated with fruit cracking[31], contribute to cuticle formation in tomato (*P65*), and co-express with exocarp-related genes in apple and lignin biosynthesis in soybean[32,33]. Furthermore, our networks implicated key hormone-related TFs. In litchi, MYB TFs enhance the expression of genes involved in starch and sucrose metabolism, sugar and water transport, thereby weakening the pericarp[34]. Additionally, ARF (Auxin Response Factor) was highly expressed in cracked fruits of Chinese jujube, implicating its role in auxin-mediated cracking[35]. ERF TFs (Ethylene Response Factors) influence the fruit cuticle and epidermis structure, modulating cracking susceptibility[36]. Among the co-expressed genes with lncRNAs, *GA2ox* (Gibberellin 2-oxidase) plays a role in fruit development through gibberellin level regulation, as shown in mango following $GA_3$ and CPPU treatment[37].

Beyond transcriptional regulation, our co-expression analysis highlighted lncRNAs linked to genes that directly determine fruit mechanical properties. For instance, *EXP* genes, involved in cell wall loosening, were co-expressed with specific lncRNAs, suggesting a role in regulating tissue mechanical integrity. In 'Fuji' apples, *MdEXPA3* expression correlated with internal ring cracking during rapid expansion, with imbalance between mesocarp and pericarp *EXP* levels contributing to cracking[38]. Similarly, *XTH* (xyloglucan endotransglucosylase/hydrolase) genes, which affect tissue mechanics, co-expressed with lncRNAs in our study. In sweet cherry, *PavXTH14* and *PavXTH15* expression increased during ripening, reducing fruit firmness[39]. Genes involved in water transport and cell wall synthesis were also prominent. Elevated expression of aquaporin genes (*PIP1;1*, *PIP1;2*, *TIP1;1*, *NIP5;1*, and *NIP2;2*) in cracked melon fruits suggests their role in cracking via water and nutrient transport[40]. Their co-expression with lncRNAs supports this regulatory link. Similarly, *CSL* (cellulose synthase-like) genes, involved in cell wall formation, were co-expressed with lncRNAs. In grape berries, a cellulose synthase-like protein was upregulated in cracking-prone cultivars, pointing to its role in cell wall weakening[41].

Our eQTL analysis enabled us to move from correlation to causality, identifying two lncRNAs, MSTRG.4395 and MSTRG.623, as high-confidence regulatory hubs with a genetic basis for their association with fruit cracking. To identify SNPs with potential regulatory effects on these lncRNAs, two biologically significant genes detected, both located on chromosome 2. The first gene, *ILR1*, was reported by Wang et al. (2021) to be significantly upregulated in the edible aril of litchi fruit, where it plays a pivotal role in auxin metabolism. This elevated expression modulates auxin homeostasis, thereby influencing fruit tissue development. Differential hormonal regulation between the aril and the pericarp may contribute to observed variations in fruit growth and the mechanical integrity of the pericarp, factors potentially linked to fruit cracking susceptibility[34]. Consistent with these findings, our eQTL analysis identified *ILR1* as a key gene harboring multiple SNPs associated with the regulation of nearby MSTRG.4395, suggesting a potential cis-regulatory mechanism through which *ILR1* and this lncRNA may jointly influence hormonal pathways and structural traits relevant to fruit cracking. Additionally, the second gene, which encodes the calmodulin protein, is a well-known component of calcium-dependent signaling pathways and participates in numerous cellular responses to both biotic and abiotic stresses[12]. The association of calmodulin with MSTRG.623 in our eQTL analysis suggests a potential regulatory interaction, indicating that calmodulin and the linked lncRNA may collaboratively influence calcium-mediated signaling processes that could impact fruit cracking. Moreover, the eQTL results indicate that genes associated with oxidative stress responses, such as those encoding oxidoreductases and peroxidases, are located upstream and downstream of these lncRNAs, suggesting a possible involvement in redox-related regulatory networks. Previous studies have identified oxidoreductase as a key enzyme contributing to fruit cracking in tomato, Akebia, citrus and, jujube[21,35,42–44]. Among the additional upstream and downstream genes potentially exerting cis-regulatory effects on the identified lncRNAs are *ACS* and *OPRF3*. *ACS* is involved in the ethylene biosynthesis pathway, a hormone widely associated with fruit cracking, whereas *OPR3* participates in stress responses and cell growth regulation. The functional roles of these genes in hormonal signaling and stress-related pathways further support their possible contribution to the physiological processes underlying fruit cracking[27,45].

While our study identifies specific lncRNAs, which are associated with fruit cracking, future research should adopt a multi-tiered approach to validate and expand upon these findings. Initially, expression patterns of key candidates (e.g., MSTRG.4395 and MSTRG.623) should be confirmed via qRT-PCR in phenotypically extreme RILs, followed by broader lncRNA sequencing across contrasting genotypes. To establish causal relationships, CRISPR-Cas9-mediated functional characterization will be essential. Additionally, the regulatory influence of environmental variables, such as irrigation, humidity, and nutrient availability, on these genetic networks warrants investigation to understand the plasticity of fruit cracking and support the breeding of stably resistant cultivars under varied agronomic conditions. Finally, while this study focused on mesocarp tissue, future work

incorporating other fruit tissues (e.g., the exocarp, where cracking initiates) will provide a more spatially resolved understanding of the regulatory mechanisms underlying this complex trait.

## Conclusion

Fruit cracking is a complex physiological disorder that significantly reduces the marketability and yield of melons, yet its underlying molecular mechanisms remain poorly characterized. In this study, we conducted a comprehensive genomic and transcriptomic analysis to identify key regulatory players, lncRNAs, SNPs, and eQTLs, associated with melon fruit cracking. Our findings reveal that lncRNAs play a crucial role in modulating gene networks involved in cell wall metabolism, hormonal regulation, and stress responses, all of which contribute to cracking susceptibility. Through stringent bioinformatic filtering, we identified 341 high-confidence lncRNAs, many of which found to co-express with critical transcription factors (MYB, WRKY, NAC) and structural genes (*EXP*, *XTH*, *AQP*). These interactions suggest that lncRNAs may fine-tune the expression of genes responsible for maintaining cell wall integrity and osmotic balance, thereby influencing cracking resistance. Furthermore, eQTL analysis uncovered SNPs in regulatory regions of key genes (*ILR1*, calmodulin) that may influence lncRNA expression, providing mechanistic insights into how genetic variation contributes to phenotypic differences in cracking susceptibility. Our study bridges a critical knowledge gap by linking non-coding regulatory elements to the physiological processes underlying fruit cracking in melons.

## Materials and methods

### Data collection

In this study, RNA-seq data from 112 melon recombinant inbred lines (RILs) with the accession code PRJNA273910 were utilized. According to this study, the RIL population, designated as 414×Dul, was generated from a cross between two distinct melon subspecies: *Cucumis melo* var. *momordica* (PI 414723) and *Cucumis melo* var. *reticulatus* (Dulce). The PI 414,723 accession, an Indian melon typically used for cooking, is characterized by pale orange flesh, a non-sweet taste, and a strong, unpleasant aroma due to the accumulation of high levels of volatile sulfur compounds. In contrast, the Dulce accession, an American cantaloupe-type melon, exhibits dark orange flesh, a sweet flavor, and a pleasant fruity aroma. These pronounced phenotypic and compositional differences between the two subspecies make the 414×Dul RIL population an ideal model for exploring the genetic and molecular mechanisms underlying fruit traits. This population and its comprehensive phenotypic characterization for a wide range of fruit quality traits established in the original study by Galpaz et al. 2018 [46], which serves as the foundational resource for the data used here. Our analysis leveraged the entire dataset of 112 RILs to capture the full spectrum of genetic recombination and diversity, thereby enabling a powerful, genome-wide search for regulatory elements influencing complex traits. RNA-seq data obtained from the mesocarp tissue of mature fruits within the RIL population using the Illumina sequencing platform[46].

### lncRNA identification

To identify unannotated transcripts within the datasets, we employed a methodology adapted from established protocols[47–52]. Initially, quality control of paired-end RNA-Seq reads was performed using FastQC (available at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter sequences and low-quality bases then trimmed using Trimmomatic v0.30[53] with the parameters ILLUMINACLIP: TruSeq3-PE.fa:2:30:10, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:20, and MINLEN:50 to ensure high-quality data for subsequent analysis. The cleaned reads were then aligned to the melon reference genome, cultivar DHL92[54], available at http://cucurbitgenomics.org/v2/organism/23, using the STAR aligner v2.7.1[55]. The selection of this genome was underpinned by its direct phylogenetic alignment with biparental RIL population, with both being derived from *C. melo* ssp. *agrestis* and *melo* progenitors[46]. This close relationship guarantees a high degree of genomic synteny, thereby reducing reference bias and ensuring precise alignment. Alignment was guided by the melon annotated reference genome file (http://cucurbitgenomics.org/v2/ftp/genome/melon/DHL92/v4.0/DHL92_v4.gff3.gz) to ensure precise mapping. Transcript assembly was conducted using StringTie v2.0.6[56], and the resulting transcripts merged into a unified set using StringTie's merge function. These merged transcripts compared against the reference gene annotation file using gffcompare, enabling their classification into distinct categories. Unannotated transcripts extracted based on specific classification codes: "u" (intergenic lncRNAs), "x" (antisense lncRNAs), "i" (intronic lncRNAs), "o" (exonic lncRNAs overlapping reference transcripts), and "e" (single exon transfragments overlapping a reference exon).

To retrieve sequences of the unannotated transcripts, BEDTools v2.29.2[57] was used, with a BED file defining the transcript coordinates. A rigorous filtering process was applied to exclude coding sequences. Transcripts longer than 200 nucleotides with a count per million (CPM) greater than 1 retained. Potential transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) were filtered out using tRNAscan-SE 2.0[58] and Barrnap 0.9 (available at https://github.com/tseemann/barrnap). Transcripts with significant hits (E-value < 1e-5) against the UniProt (release 2021-02), Pfam (release 34.0), and Rfam (release 14.5) databases removed. To rigorously filter out transcripts with coding potential, we employed the Coding Potential Calculator (CPC2)[59] which assesses sequences based on intrinsic features and is recognized for its high accuracy and speed in distinguishing non-coding RNAs in eukaryotic genomes[60,61]. Finally, the remaining candidate lncRNAs validated using PLncPRO[62], a tool explicitly developed for predicting high-confidence lncRNAs in plants. PLncPRO was selected for its demonstrated superior performance in plant-specific transcriptomes compared to generic tools, as it utilizes a machine learning approach trained on plant data, thereby reducing false positives and increasing prediction reliability in our melon study system[63,64].

### Target gene prediction, functional analysis, and co-expression networks

To elucidate the functional roles of lncRNAs, we focused on identifying their potential regulatory targets. Specifically, EGs located within 100 kilobases (Kb) upstream or downstream of E-lncRNAs predicted as cis-regulatory targets[48,49,65–69]. Additionally, interactions between E-lncRNAs and EGs evaluated using LncTar[70] with default parameters. This tool predicts RNA-RNA interactions by assessing sequence complementarity to calculate the free energy (ΔG) and standardized free energy of the putative duplex. A potential target mRNA was identified if the free energy of its binding sites with a lncRNA fell below the standardized threshold of -0.1 kcal/mol. This specific, stringent threshold prioritizes the identification of high-confidence, stable interactions[65,70–73]. To further explore the biological functions of these target genes, Gene Ontology (GO) enrichment analysis was performed using the tools available in the Melonomics Database (http://cucurbitgenomics.org/v2). Significantly enriched GO terms identified using a Fisher's exact test, with a False Discovery Rate (FDR) correction for multiple testing and a significance threshold of p-value < 0.05. To investigate co-expression patterns between E-lncRNAs and EGs, we employed the Weighted Gene Co-Expression Network Analysis (WGCNA) package[74] in R, following parameters optimized in previous studies[47,49]. To construct a biologically meaningful scale-free network, we performed an analysis to determine the appropriate soft thresholding power (β). The pickSoftThreshold function was used to calculate the scale-free topology fit index (signed $R^2$) and mean connectivity for a range of powers[75]. A power of β = 8 was selected as the lowest value for which the scale-free topology fit index reached a saturation point above 0.80, balancing the achievement of a scale-free network with the preservation of high mean connectivity. This value was chosen based on the highest correlation between the empirical cumulative distribution function of node connectivity and the theoretical scale-free distribution, ensuring a balance between network connectivity and modularity. Subsequently, the topological overlap matrix (TOM) and its dissimilarity measure (1-TOM) were calculated. Modules identified through hierarchical clustering and the DynamicTree Cut algorithm[74]. An unsigned network approach was adopted to capture both positive and negative correlations, providing a comprehensive view of the co-expression relationships.

### eQTL_LncRNAs mapping

To identify eQTLs associated with lncRNAs, a filtered SNP dataset obtained from prior genetic analyses was utilized[76]. The variant calling for this dataset was performed using Platypus[77] under stringent parameters, including a minimum read depth of 2, a mapping quality score (MQ) ≥ 20, a base quality score ≥ 20, a minimum flanking distance of 5 bp, and a maximum of 80% missing data. Association mapping was performed using MatrixEQTL[78] with a linear model. This approach controls for spurious associations by testing the correlation between each SNP and the expression phenotype (lncRNA level) across the RILs, conditional on the genotypic state at that locus. In a biparental RIL population like ours, where the genetic background is a mosaic of the two founders, this model effectively accounts for the population structure, as the kinship and relatedness between lines are directly defined by their shared haplotype blocks. Cis-acting eQTLs defined as SNPs located within ± 1 Mb of the lncRNA transcriptional start or end sites, a conventional window size that captures local regulatory genetic variation. Statistical significance was determined using a false discovery rate (FDR) threshold, adjusted via the Benjamini-Hochberg method. To ensure high-confidence discoveries amidst the severe multiple-testing burden of genome-wide scans, stringent FDR thresholds of 1e-5 for both cis-eQTLs and trans-eQTLs (SNPs outside the 1 Mb window or on different chromosomes) were applied, consistent with the rigor of other high-resolution eQTL studies[79–82] To infer functional relationships, cis-regulated lncRNA-mRNA pairs identified by correlating lncRNA eQTLs with expression levels of nearby protein-coding genes (within 100 kb), which were predicted as putative regulatory targets.

## Data availability

All RNA-Seq data were deposited in the NCBI SRA database under the project PRJNA273910 ( [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA273910](https:/www.ncbi.nlm.nih.gov/bioproject/PRJNA273910) ).

Received: 6 February 2026; Accepted: 20 April 2026
Published online: 24 April 2026

## References

1. Lija, M. & Beevy, S. S. A Review on the diversity of Melon. *Plant. Sci. Today*. **8**, 995–1003 (2021).
2. Maryomana, L. & Beevy, S. S. Fruit Cracking in Melon. In *Biological and Abiotic Stress in Cucurbitaceae Crops* (IntechOpen, (2023).
3. Gómez-García, R., Campos, D. A., Aguilar, C. N., Madureira, A. R. & Pintado, M. Valorization of melon fruit (Cucumis melo L.) by-products: Phytochemical and Biofunctional properties with Emphasis on Recent Trends and Advances. *Trends food Sci. Technol.* **99**, 507–519 (2020).
4. Kubo, K., Pritchard, B. & Phyo, A. S. How Chinese demand for fresh fruit and vegetables is creating new landscapes of rural development and vulnerability in Southeast Asia: Insights from the Myanmar melon frontier. *Geoforum* **122**, 32–40 (2021).
5. Fernández-Trujillo, J. P. et al. Pre-and postharvest muskmelon fruit cracking: causes and potential remedies. *Horttechnology* **23**, 266–275 (2013).
6. Santos, M., Egea-Cortines, M., Gonçalves, B. & Matos, M. Molecular mechanisms involved in fruit cracking: A review. *Front. Plant. Sci.* **14**, 1130857 (2023).
7. Zhao, X. et al. Emerging roles and mechanisms of lncRNAs in fruit and vegetables. *Hortic. Res.* **11**, uhae046 (2024).
8. Chen, J. et al. Transcriptome analysis of atemoya pericarp elucidates the role of polysaccharide metabolism in fruit ripening and cracking after harvest. *BMC Plant. Biol.* **19**, 1 (2019).
9. Jiang HaiKun, J. H. et al. RNA-seq analysis of watermelon (Citrullus lanatus) to identify genes involved in fruit cracking. (2019).
10. Straube, J. et al. Russeting in apple is initiated after exposure to moisture ends: Molecular and biochemical evidence. *Plants* **10**, 65 (2020).

11. Joshi, M., Baghel, R. S., Fogelman, E., Stern, R. A. & Ginzberg, I. Identification of candidate genes mediating apple fruit-cracking resistance following the application of gibberellic acids 4+7 and the cytokinin 6-benzyladenine. *Plant. Physiol. Biochem.* **127**, 436–445 (2018).
12. Yong-Xiang, R. E. N. et al. study on the related cracking-resistant genes in chinese jujube. *Sci Pap Ser. B Hortic* **61**, (2017).
13. Yong, W., Wangjin, L., Jianguo, L. & Yueming, J. Differential expression of two expansin genes in developing fruit of cracking-susceptible and-resistant litchi cultivars. *J. Am. Soc. Hortic. Sci.* **131**, 118–121 (2006).
14. Zhang, M. et al. Progress in Fruit Cracking Control of Gibberellic Acid and Abscisic Acid. *Forests* vol. 15 at (2024). https://doi.org/10.3390/f15030547
15. Liao, N. et al. Ethylene-responsive factor 4 is associated with the desirable rind hardness trait conferring cracking resistance in fresh fruits of watermelon. *Plant. Biotechnol. J.* **18**, 1066–1077 (2020).
16. Falginella, L. et al. Differential regulation of triterpene biosynthesis induced by an early failure in cuticle formation in apple. *Hortic. Res.* **8**, 75 (2021).
17. Qi, Z. et al. Inheritance of fruit cracking resistance of melon (Cucumis melo L.) fitting E-0 genetic model using major gene plus polygene inheritance analysis. *Sci. Hortic. (Amsterdam)*. **189**, 168–174 (2015).
18. Fan, R. et al. Cytological, Phytohormone, and Transcriptomic Analyses Reveal the Key Genes and Pathways Involved in Melon Fruit Cracking. *Horticulturae* **11**, 227 (2025).
19. Zhan, Y. et al. A major QTL identification and candidate gene analysis of watermelon fruit cracking using QTL-seq and RNA-seq. *Front. Plant. Sci.* **14**, 1166008 (2023).
20. Zhou, H. et al. Genome-wide identification and characterization of long noncoding RNAs during peach (Prunus persica) fruit development and ripening. *Sci. Rep.* **12**, 11044 (2022).
21. Xue, L. et al. LncRNA regulates tomato fruit cracking by coordinating gene expression via a hormone-redox-cell wall network. *BMC Plant. Biol.* **20**, 162 (2020).
22. Wang, Y. et al. Transcriptional profiling of long non-coding RNAs regulating fruit cracking in Punica granatum L. under bagging. *Front. Plant. Sci.* **13**, 943547 (2022).
23. Carrasco-Valenzuela, T. et al. Expression QTL (eQTLs) analyses reveal candidate genes associated with fruit flesh softening rate in peach [Prunus persica (L.) Batsch]. *Front. Plant. Sci.* **10**, 1581 (2019).
24. Zhu, F. et al. A comparative transcriptomics and eQTL approach identifies SlWD40 as a tomato fruit ripening regulator. *Plant. Physiol.* **190**, 250–266 (2022).
25. Xue, Q., Li, H. & Du, T. Mild water deficit during maturity reduces cracking rate of greenhouse muskmelon while improving fruit quality. *Agric. Water Manag.* **313**, 109465 (2025).
26. Zhu, M., Yu, J., Zhao, M., Wang, M. & Yang, G. Transcriptome analysis of metabolisms related to fruit cracking during ripening of a cracking-susceptible grape berry cv. Xiangfei (Vitis vinifera L). *Genes Genomics*. **42**, 639–650 (2020).
27. Liu, Y., Zhang, P., Geng, Y., Xie, X. & Wen, P. Cracking of jujube fruits is associated with differential expression of metabolic genes. *FEBS Open. Bio*. **10**, 1765–1773 (2020).
28. Zhen, W. U., Rong, Z. & Bin, C. SlGH9-15 regulates tomato fruit cracking with hormonal and abiotic stress responsiveness cis-elements. *J. Integr. Agric.* **22**, 447–463 (2023).
29. Li, S., Chen, K. & Grierson, D. Molecular and hormonal mechanisms regulating fleshy fruit ripening. *Cells* **10**, 1136 (2021).
30. Hu, Y. et al. Genome-wide identification of the expansin gene family in netted melon and their transcriptional responses to fruit peel cracking. *Front. Plant. Sci.* **15**, 1332240 (2024).
31. Niu, J. et al. Integrative transcriptome and proteome analyses provide new insights into different stages of Akebia trifoliata fruit cracking during ripening. *Biotechnol. Biofuels*. **13**, 149 (2020).
32. Legay, S. et al. Apple russeting as seen through the RNA-seq lens: strong alterations in the exocarp cell wall. *Plant. Mol. Biol.* **88**, 21–40 (2015).
33. Dong, Y. et al. Pod shattering resistance associated with domestication is mediated by a NAC gene in soybean. *Nat. Commun.* **5**, 3352 (2014).
34. Wang, J., Wu, X. F., Tang, Y., Li, J. G. & Zhao, M. L. RNA-Seq provides new insights into the molecular events involved in Ball-Skin versus Bladder Effect on fruit cracking in litchi. *Int. J. Mol. Sci.* **22**, 454 (2021).
35. Lin, M. et al. Combined Transcriptome-and Proteome-based Analyses for Mining of Genes Associated with Fruit Cracking in Chinese Jujube (Ziziphus jujuba Mill). *Rom J. Hortic.* **2**, 9 (2021).
36. Li, S., Zhao, Y., Wu, P., Grierson, D. & Gao, L. Ripening and rot: How ripening processes influence disease susceptibility in fleshy fruits. *J. Integr. Plant. Biol.* **66**, 1831–1863 (2024).
37. Jiang, T. et al. Transcriptome analysis to identify candidate genes that response to GA3 and CPPU treatments for mango fruit development. *Plant. Growth Regul.* **104**, 885–897 (2024).
38. Kasai, S., Hayama, H., Kashimura, Y., Kudo, S. & Osanai, Y. Relationship between fruit cracking and expression of the expansin gene MdEXPA3 in 'Fuji'apples (Malus domestica Borkh). *Sci. Hortic. (Amsterdam)*. **116**, 194–198 (2008).
39. Zhai, Z. et al. Genome-wide identification of the xyloglucan endotransglucosylase/hydrolase (XTH) and polygalacturonase (PG) genes and characterization of their role in fruit softening of sweet cherry. *Int. J. Mol. Sci.* **22**, 12331 (2021).
40. Lopez-Zaplana, A., Bárzana, G., Ding, L., Chaumont, F. & Carvajal, M. Aquaporins involvement in the regulation of melon (Cucumis melo L.) fruit cracking under different nutrient (Ca, B and Zn) treatments. *Environ. Exp. Bot.* **201**, 104981 (2022).
41. Zhang, C., Cui, L., Liu, C., Fan, X. & Fang, J. Mining candidate genes of grape berry cracking based on high density genetic map. *Hortic. Plant. J.* **9**, 743–753 (2023).
42. Nazir, M. F. et al. Deciphering the genetic and biochemical drivers of fruit cracking in Akebia trifoliata. *Int. J. Mol. Sci.* **25**, 12388 (2024).
43. Huang, S. et al. Physiological mechanisms of citrus fruit cracking: study on cell wall components, osmoregulatory substances, and antioxidant enzyme activities. *Plants* **13**, 257 (2024).
44. Hou, L. et al. Comparative transcriptomic analyses of different jujube cultivars reveal the co-regulation of multiple pathways during fruit cracking. *Genes (Basel)*. **13**, 105 (2022).
45. Michailidis, M. et al. Genotype-and tissue-specific metabolic networks and hub genes involved in water-induced distinct sweet cherry fruit cracking phenotypes. *Comput. Struct. Biotechnol. J.* **19**, 5406–5420 (2021).
46. Galpaz, N. et al. Deciphering genetic factors that determine melon fruit-quality traits using RNA-Seq-based high-resolution QTL and eQTL mapping. *Plant. J.* **94**, 169–191 (2018).
47. Khoei, M. A., Karimi, M., Karamian, R., Amini, S. & Soorni, A. Identification of the Complex Interplay Between Nematode-Related lncRNAs and Their Target Genes in Glycine max L. *Front. Plant. Sci.* **12**, 779597 (2021).
48. Ghorbani, F. et al. Global identification of long non-coding RNAs involved in the induction of spinach flowering. *BMC Genomics* **22**, 704 (2021).
49. Soorni, A., Karimi, M., Al Sharif, B. & Habibi, K. Genome-wide screening and characterization of long noncoding RNAs involved in flowering/bolting of Lactuca sativa. *BMC Plant. Biol* **23**, 3 (2023).
50. Chen, X. et al. Comprehensive Transcriptome Analysis Uncovers Hub Long Non-coding RNAs Regulating Potassium Use Efficiency in Nicotiana tabacum. *Front. Plant. Sci.* **13**, 777308 (2022).
51. Chen, X. et al. Transcriptome Profiling of Transposon-Derived Long Non-coding RNAs Response to Hormone in Strawberry Fruit Development. *Front. Plant. Sci.* **13**, 915569 (2022).

52. Khemka, N., Rajkumar, M. S., Garg, R. & Jain, M. Genome-wide analysis suggests the potential role of lncRNAs during seed development and seed size/weight determination in chickpea. *Planta* **256**, 79 (2022).
53. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
54. Garcia-Mas, J. et al. The genome of melon (Cucumis melo L). *Proc. Natl. Acad. Sci. U S A*. **109**, 11872–11877 (2012).
55. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
56. Pertea, M. et al. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* **33**, 290–295 (2015).
57. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).
58. Chan, P. P., Lin, B. Y., Mak, A. J. & Lowe, T. M. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res.* **49**, 9077–9096 (2021).
59. Kang, Y. J. et al. CPC2: A fast and accurate coding potential calculator based on sequence intrinsic features. *Nucleic Acids Res.* **45**, W12–W16 (2017).
60. Kumar, B. et al. Genome-wide identification and characterization of tissue-specific non-coding RNAs in black pepper (Piper nigrum L). *Front. Plant. Sci.* **14**, 1079221 (2023).
61. Yu, S. et al. Genome-wide identification and characterization of lncRNAs in sunflower endosperm. *BMC Plant. Biol.* **22**, 494 (2022).
62. Khemka, N. & Singh, U. PLncPRO for prediction of long non-coding RNAs (lncRNAs) in plants and its application for discovery of abiotic stress-responsive lncRNAs in rice and chickpea. *Nucleic Acids Res.* **45**, 22 (2017).
63. da Negri, C., Paschoal, T. & Alves, W. A. A. R. L. Comparison tools for lncRNA identification: Analysis among plants and humans. in *IEEE Conference on Computational Intelligence in Bioinformatics and Computational Biology (CIBCB)* 1–8 (IEEE, 2020). 1–8 (IEEE, 2020). (2020).
64. Tian, X. et al. PlantLncBoost: key features for plant lncRNA identification and significant improvement in accuracy and generalization. *New Phytol.***247**, 3 (2025).
65. Ahmadi Khoei, M., Karimi, M., Karamian, R., Amini, S. & Soorni, A. Identification of the Complex Interplay Between Nematode-Related lncRNAs and Their Target Genes in Glycine max L. *Front. Plant. Sci.* **12**, 779597 (2021).
66. Jin, Z. et al. Identification and functional prediction of salt stress-related long noncoding RNAs in grapevine roots. *Environ. Exp. Bot.* **179**, 104215 (2020).
67. Chen, L. et al. Genome-wide analysis of long non-coding RNAs affecting roots development at an early stage in the rice response to cadmium stress. *BMC Genom.* **19**, 460 (2018).
68. Wan, S. et al. Integrated Analysis of Long Non-coding RNAs (lncRNAs) and mRNAs Reveals the Regulatory Role of lncRNAs Associated With Salt Resistance in Camellia sinensis. *Front. Plant. Sci.* **11**, 218 (2020).
69. Dhaka, B. et al. Functional identification of cis-regulatory long noncoding RNAs at controlled false discovery rates. *Nucleic Acids Res.* **52**, 2821–2835 (2024).
70. Li, J. et al. LncTar: a tool for predicting the RNA targets of long noncoding RNAs. *Brief. Bioinform.* **16**, 806–812 (2015).
71. Yan, X., Ma, L. & Yang, M. Identification and characterization of long non-coding RNA (lncRNA) in the developing seeds of Jatropha curcas. *Sci. Rep.* **10**, 10395 (2020).
72. Ghorbani, F. et al. Global identification of long non-coding RNAs involved in the induction of spinach flowering. *BMC Genom.* **22**, 704 (2021).
73. Mehralizade, Z., Soorni, A. & Meratian Esfahani, S. Regulatory Network of Spinach Under Salt Stress: An Integrated Study of Long Non-coding RNAs and mRNAs. *J. Plant. Growth Regul.* **44**, 2298–2314 (2025).
74. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform.* **9**, 559 (2008).
75. Zhang, B. & Horvath, S. A general framework for weighted gene co-expression network analysis. *Stat. Appl. Genet. Mol. Biol.* **4**, Article17 (2005).
76. Pereira, L. et al. QTL mapping of melon fruit quality traits using a high-density GBS-based genetic map. *BMC Plant. Biol.* **18**, 324 (2018).
77. Rimmer, A. et al. Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. *Nat. Genet.* **46**, 912–918 (2014).
78. Shabalin, A. A. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. *Bioinformatics* **28**, 1353–1358 (2012).
79. Mähler, N. et al. Gene co-expression network connectivity is an important determinant of selective constraint. *PLoS Genet.* **13**, e1006402 (2017).
80. Noble, J. D. et al. The genetic regulation of alternative splicing in Populus deltoides. *Front. Plant. Sci.* **11**, 590 (2020).
81. Zhu, G. et al. Rewiring of the fruit metabolome in tomato breeding. *Cell* **172**, 249–261 (2018).
82. Martínez-García, P. J., Mas-Gómez, J., Wegrzyn, J. & Botía, J. A. Bioinformatic approach for the discovery of cis-eQTL signals during fruit ripening of a woody species as grape (Vitis vinifera L). *Sci. Rep.* **12**, 7481 (2022).


## Acknowledgements
Not applicable.

## Author contributions
PM: investigation; methodology; formal analysis, writing-review. AS: Conceptualization; funding acquisition; investigation; project administration; methodology; formal analysis; validation; writing-original draft; writing-review and editing.

## Funding
No funding was received for conducting this study.

## Declarations

## Consent to Participate and Consent to Publish declarations
Not applicable.

## Competing interests
The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-50246-2.

**Correspondence** and requests for materials should be addressed to A.S.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.