Combined Transcriptome- and Proteome-based Analyses for Mining of Genes Associated with Fruit Cracking in Chinese Jujube (*Ziziphus jujuba* Mill.)

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ABSTRACT

Fruit dehiscence is a crucial physiological disorder in fruit crops. Fruit cracking may frequently cause more than 50% of yield losses in Chinese jujube (*Ziziphus jujuba* Mill.). In this study, we carried out a combined transcriptome sequencing and proteome analysis in Chinese jujube to reveal the molecular mechanisms involved in fruit cracking. A total of 31,272 transcripts and 5,480 proteins were identified. Of these, 5,425 genes were shared at both transcriptome and proteome levels. Compared to non-cracked fruits, 161 differentially expressed genes were revealed in cracked fruits, of which 39 upregulated and 122 downregulated genes. Proteome analysis revealed 182 differentially expressed proteins associated with fruit cracking, of which 68 upregulated and 114 downregulated. Validation of gene expression by real-time quantitative-polymerase chain reaction of 14 selected DEGs were found to regulate cell wall metabolism, unsaturated fatty acid oxidation, plant-pathogen interaction and hormone balance, endoglucanase, pectinesterase, peroxidases, and phytohormone signal transduction genes and calcium-binding protein. The expression levels of POD17, EG12, CML23, ARF, JAZ, AUX/IAA, and SAUR genes were consistent with the transcriptome analysis results. The gene expression levels of POD17 and POD51 were consistent with the proteomics data. However, the expression levels of genes CML23, PE17 and GH3.1 were contradictory with their proteome expression levels. Further analysis of the expression levels of the 14 genes from fruit skins of different samples at various stages of fruit cracking revealed that 11 genes were highly expressed in cracked fruits, suggesting these genes play important roles in fruit cracking. A putative calcium-binding protein CJML19 showed medium-low expression in cracked fruit skins, which may help regulate water entry/exit and may be associated with cracking resistance.

Keywords: Chinese jujube; fruit cracking; transcriptome; proteome; qRT-PCR

INTRODUCTION

Chinese jujube (*Ziziphus jujuba* Mill.) is an economically important fruit tree that originated in China and has been introduced to 48 countries across 5 continents (Liu *et al.*, 2020).

However, fruit dehiscence is the most important and common physiological disorder of Chinese jujube, it results in more than 50% of yield losses in severe cases (Chen *et al.*, 2012). Previous studies on fruit dehiscence mainly focused on morphological, anatomical and physiological characteristics. With the rapid development of omics technology, transcriptomics and proteomics analysis are more and more widely used in horticultural plant research. (Du, 2016; Jin, 2017; Chen, 2018; Chen, 2019). For example, transcriptome analysis has been used to analyse fruit dehiscence. Jiang (2019) identified 14 differentially expressed genes that are associated with fruit cracking in watermelons. In lychees, fruit cracking was associated with genes that regulate cell wall metabolism, unsaturated fatty acid oxidation and fruit skin hormone balance, including expansion (EXP) genes, pectinesterase (PE), and polyphenol oxidase (PPO) (Chen *et al.*, 2015; Wang *et al.*, 2019 Correia *et al.*, 2020) This study aimed to identify genes and examine molecular mechanisms that are related to fruit dehiscence of jujube to serve as guidance for breeding strategies of fruit cracking-resistant cultivar and effective prevention and control of fruit dehiscence in jujube.

MATERIALS AND METHODS

'Fucuimi' known to be sensitive to fruit dehiscence, was used as the test material, in the mature stage, its natural cracking rate can reach more than 90% (Wang *et al.*, 2020). At semi-red stage (80-90 days after flowering, Fruit on a secondary branch of 3-6 years old.), 100 fruits were picked from the jujube germplasm nursery at the Tarim University, Alar, Xinjiang, China and then rapidly classified as cracked fruit and non-cracked fruit samples before freezing in liquid nitrogen. The samples were stored at -78°C. Transcriptome and proteome analyses of the cracked and non-cracked fruit were performed at Novogene (Beijing, China). Three biological replicates were used for every sample.

RNA and protein extraction

The total RNA was extracted using the Tiangen DP441 Assay Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol. RNA concentration was measured using NanoDrop 2000 (Thermo Fisher Scientific, Massachusetts, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 System (Agilent Technologies, CA, USA). For protein extraction, four volumes of lysis buffer (8 M urea, 1% protease inhibitor cocktail) were added to the ground samples then sonicated three times on ice using a high intensity ultrasonic processor (Scientz, Ningbo, China). Debris were removed by centrifugation at 12,000 *g* at 4°C for 10 min. Finally, the supernatant was collected, and the protein concentration was determined with BCA kit (Beyotime, Shanghai, China) based on the manufacturer's instructions.

Transcriptome sequencing data analysis

For the RNA sample preparations, Sequencing libraries were generated using NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. The transcriptome data of cracked and non-cracked fruit skins were compared with the genome of 'Dongzao' jujube (Liu *et al.*, 2014). FPKM (Pertea *et al.*, 2015) was used as a marker for measuring transcript or gene expression levels and to calculate the fold change in gene expression between two samples. The DESeq (Leng *et al.*, 2013) method was used for screening the differentially expressed genes (DEGs) with a fold change ≥ 2 and an adjusted *P* value (FDR) was < 0.01 between two samples. Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway significant enrichment analyses (*Q* value ≤ 0.05) were carried out for DEGs to determine their biological functions and roles in metabolic pathways.

Protein identification and quantitative analysis

The tryptic peptides were divided into fractions by high pH reverse-phase HPLC using Thermo Betasil C18 column (5 μ m particles, 10 mm ID, 250 mm length). The resulting MS/MS data were processed using Maxquant search engine (v.1.5.2.8). For relative quantitation, a protein was considered as differentially expressed between skin samples when its fold change was > 1.3 and $P < 0.05$.

Validation of selected DEGs and proteins by real-time quantitative-polymerase chain reaction (qRT-PCR)

Table 1. Real-time quantitative-polymerase chain reaction (qRT-PCR) primers used for validation of selected differentially expressed genes (DEGs) in Chinese jujube

Gene/protein	Gene ID	Primer sequence (5' to 3')	Product size (bp)
POD17	LOC107425304	F: GACTTTGCGAAGGGGATGGA R: AGATTCCACACGGTGTTC	114
EG12	LOC107410658	F: TCGGTTTGACCAAGTTCCGAG R: CCAATCGCCAGCAGTTTTTGT	117
CML23	LOC107427847	F: GACAGCAATGGAGACGGGAA R: GCCTCCTCTTCCGTTAGCTC	86
CML23	LOC107427870	F: GACAGCAATGGAGACGGGAA R: GCCTCCTCTTCCGTTAGCTC	85
ARF	LOC107431430	F: TGCTCAATGGTTTCGGGTGTT R: AGGGAAAGGCGTAGACTCTTCT	142
JAZ	LOC107435541	F: ACCTGATGAGGATGGTCAATGT R: GGAGTTTAATGAAGCTTAAACCGGA	147
AUX/IAA	LOC107435327	F: TGGGCAGTATGTTTGTCTCCA R: CGACTCGCATCTTGTCTTGC	105
SAUR	LOC107414929	F: GGACAGGGTGAGGAACAACA R: GCGAAACTCCTCAACATGGC	150
XP_015869388.2 (PE17)	LOC107406730	F: TGCACTAGACCGAGCCGTGT R: TAGAGCTCGAGGCAGTCGGC	105
XP_015881810.1 (CML19)	LOC107417689	F: GAGGTGGAAGCTGCCGTGGA R: AACCCTAAAGCCTCTCTCAAGTCC	131
XP_015881810.1 (CML19)	LOC107417690	F: GAGCGGGTTTCTGCTTCTTCG R: TCCACGGCAGCTTCCACCTC	122
XP_024926183.1 (POD4)	LOC107411518	F: GGGTGCTTCTCTCCTTCGCCTT R: ACACAACGCCAGGGCAAACCTTG	110
XP_015890285.1 (POD51)	LOC107424909	F: CGGCGCACACCCTTGGATTTC R: TCGCTTGGAGCTGACCCACA	108
XP_015866400.1 (indole-3-acetic acid amido synthetase GH3.1)	LOC107403983	F: ACACCAGCGTCGTCGAGTACAC R: TCGGCGAGTTGGCTTGGTCTT	120
Actin	ZjACT	F: AGCCTTCCTGCCAACGAGT R: TTGCTTCTCACCCCTTGATGC	110

The FastQuant RT Super Mix reverse transcription kit (Tiangen Biotech, Beijing, China) was used for cDNA synthesis. The transcriptome sequencing results were used for qRT-PCR validation. The principles for designing qRT-PCR primers were used to construct target gene primers on NCBI and primers were submitted to Sangon Biotech (Shanghai, China) for synthesis. Table 1 shows the names of genes and proteins, primer sequences, and product size. The manufacturer instructions for the SuperReal PreMix Plus assay kit (Tiangen Biotech, Beijing, China) were used as reference for the qRT-PCR reaction system and amplification procedure. After the reaction was completed, amplification curve, melt curve, and gel electrophoresis analyses were carried out to test the specificity of the primers. Three biological replicates were set up for every reaction and the relative expression level of target genes in skins during fruit cracking were obtained based on the Ct value of the amplification curve. The internal reference gene *ZjACT* was used for correction and the $2^{-\Delta\Delta CT}$ and R software (gplots2 package) were used for analyses of relative expression and for plotting.

RESULTS AND DISCUSSIONS

Analysis of transcriptome and proteome differences between cracked and non-cracked fruit skins

The transcriptome and proteome sequencing results from skins of both cracked and non-cracked fruits were analyzed. Transcript-fold difference ≥ 2 and protein-fold difference ≥ 1.3 at $P < 0.05$ were the criteria for differentially expressed transcripts and proteins. A total of

161 DEGs were observed, of which 39 and 122 genes were upregulated and downregulated, respectively. Also identified were 182 differentially expressed proteins (DEPs), of which 68 were upregulated and 114 were downregulated (Figure 1). The DEGs and DEPs underwent functional annotation subsequently.

Fig. 1. Frequency distribution of differentially expressed transcripts and proteins quantified in transcriptome and proteome datasets

GO classification and KEGG enrichment analysis of DEGs

The DEGs of cracked and non-cracked fruit skins were compared and were aligned with the GO and KEGG databases. About 89 genes were annotated to the GO database and 27 DEGs were annotated to 19 metabolic pathways. All the enriched genes are involved in gene functions and metabolic processes, and they may take part in fruit skin cracking (Pertea *et al.*, 2015).

GO classification and KEGG enrichment analysis of DEPs

Figure 2 shows the results of GO functional annotation analysis of DEPs identified from cracked and non-cracked fruit skins. The DEPs mainly participated in the metabolic process, cellular process, and single-organism process in biological process, accounting for 35%, 24%, and 20%, respectively. About 21% accounted for all other processes.

In cellular component, DEPs mainly participate in cell, organelle, and macromolecular complex at 36%, 20%, 18%, respectively while all other processes accounted for 26%. For molecular function, DEPs were mainly enriched in catalytic activity and binding at 39% and 44%, respectively.

Figure 2. Results of gene ontology (GO) analysis of differentially expressed proteins identified in cracked fruit skins of Chinese jujube

KEGG pathway analysis of DEPs

Figure 3 shows the results of DEPs of cracked and non-cracked fruit skins that were aligned with the KEGG database for enrichment. The KEGG pathways that were enriched were photosynthesis-antenna proteins, photosynthesis, protein processing in endoplasmic reticulum, cyanoamino acid metabolism, flavonoid biosynthesis, phenylpropanoid biosynthesis, and ribosome.

Figure 3. Enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for the identified protein-differential proteins identified in cracked fruit skins of Chinese jujube

Analysis of differentially expressed transcripts and proteins

A combination of GO and KEGG enrichment analyses were used to identify DEGs and DEPs. Relevant physiological markers were noted as well to further screen for transcripts and proteins that may affect fruit skin cracking.

Differential expression analysis of fruit cracking-related genes

In phytohormone signal transduction pathways, three DEGs were annotated to auxin signal transduction and one DEG was annotated to jasmonic acid signal transduction. Among these genes, *AUX/IAA*, *ARF*, *SAUR*, and *JAZ4* were highly expressed in cracked fruit skins. In plant-pathogen interaction metabolic pathways, two *CML23* genes were highly expressed in cracked fruit skins that may be related to fruit cracking (Wang *et al.*, 2020b). GO functional analysis found that the expression level of genes regulating *endoglucanase* (*EG12*) and *peroxidase* (*POD17*) are low in non-cracked fruit skins. *EG12* regulates cellulase activity. It may play an important role in cellulose degradation, such as changes in cellulose content and fruit skin structure resulting in fruit skin cracking. *POD17* may indirectly regulate the lignin content, thus changing the peel structure and affect the peel cracking (Wang *et al.*, 2021).

Differential expression analysis of fruit cracking-related proteins

Table 2 shows four proteins that were differentially expressed in the phenylpropanoid synthesis pathway, namely two peroxidases (XP_024926183.1 and XP_015890285.1), a beta-glucosidase (XP_015879544.1), and a phenylalanine ammonia-lyase (XP_024922333.1). Among these proteins, the two peroxidases were highly expressed in cracked fruit skins and were mainly enriched in lignin catabolic and metabolic processes. While the expression level of the beta-glucosidase in non-cracked fruit skins was significantly lower than in cracked fruit skins, and the expression of the phenylalanine ammonia-lyase in non-cracked fruit skins was significantly upregulated compared with cracked fruit skins. Other proteins highly expressed in cracked fruit skins were laccases (XP_015878553.1, XP_015902437.1, and XP_015878552.1) that participate in lignin catabolic and metabolic processes as well as in phenylpropanoid synthesis. The expression levels of indole-3-acetic acid-amido synthetase GH3.1 (XP_015866400.1) that was upregulated in non-cracked fruit skins by 1.34-fold. Phytohormones play an important role in cracking and hormone interactions may affect fruit cracking. Additionally, two DEPs in the plant-pathogen interaction metabolic pathway (Table 2): a putative calcium-binding protein CML19 (XP_015881810.1) and a heat shock protein 82 (XP_015896451.1) both showed medium-low expression in cracked fruit skins. CML19 helps regulate stomata opening/closure and water entry/exit and may be associated with cracking resistance. DEPs identified in the α -linoleic acid metabolic pathway were 12-oxophytodienoate reductase (XP_015889050.1), allene oxide synthase (XP_015884706.1), and lipoxygenase 3 (XP_015872805.1). Both 12-oxophytodienoate reductase and allene oxide synthase had medium-low expression, while lipoxygenase 3 was highly expressed in cracked fruit skins. Table 3 summarizes the results of GO functional analysis. Cell wall/vacuolar inhibitor of fructosidase (XP_015867821.1) was highly expressed in cracked fruit skins and pectinesterase/pectinesterase inhibitor (XP_015869388.2) and miraculin (XP_015872399.1) showed medium-low expression in cracked fruit skins. In the cell wall macromolecular metabolism, xyloglucan endotransglucosylase/hydrolase protein

(XP_015899780.1 and XP_015887252.1) showed medium-low expression in cracked fruit skins. These DEPs are mainly cell wall substance metabolic enzymes.

Table 2. Differential accumulation of proteins related to plant-pathogen interaction, phenylpropanoid biosynthesis, alpha-linolenic acid metabolism and plant hormone signal transduction in fruit skins of Chinese jujube (*Ziziphus jujuba*)

Protein accession	Protein description	Cracked vs non-cracked	Cracked vs non-cracked	P value	Gene name
Plant-pathogen interaction					
XP_015881810.1	putative calcium-binding protein CML19 [Z. jujuba]	1.587	Up	0.00608	LOC107417689;
XP_015896451.1	heat shock protein 82 [Z. jujuba]	1.48	Up	0.000743	LOC107417690
Phenylpropanoid biosynthesis					
XP_015879544.1	beta-glucosidase 13-like [Z. jujuba]	0.351	Down	1.81E-05	LOC107415658
XP_024922333.1	phenylalanine ammonia-lyase [Z. jujuba]	1.305	Up	0.040095	LOC107404213;
XP_024926183.1	peroxidase 4-like [Z. jujuba]	0.689	Down	0.000759	LOC107411518
XP_015890285.1	"peroxidase 51, partial [Z. jujuba]"	0.687	Down	2.22E-06	LOC107424909
Alpha-Linolenic acid metabolism					
XP_015889050.1	12-oxophytodienoate reductase 3-like [Z. jujuba]	1.373	Up	0.000138	LOC107423912
XP_015872805.1	"lipoxygenase 3, chloroplastic-like [Z. jujuba]"	0.724	Down	0.005881	LOC107409898
XP_015884706.1	"allene oxide synthase 1, chloroplastic [Z. jujuba]"	1.301	Up	0.004364	LOC107420290
Plant hormone signal transduction					
XP_015899780.1	probable xyloglucan endotransglucosylase/hydrolase protein 23 [Z. jujuba]	1.351	Up	0.000184	LOC107433023
XP_015866400.1	probable indole-3-acetic acid-amido synthetase GH3.1 [Z. jujuba]	1.34	Up	6.73E-08	LOC107403983
Lignin catabolic and metabolic process					
XP_015878553.1	laccase-15-like [Z. jujuba]	0.764	Down	0.000999	LOC107414863
XP_015902437.1	laccase-14-like [Z. jujuba]	0.708	Down	0.000838	LOC107435354
XP_015878552.1	laccase-15-like [Z. jujuba]	0.629	Down	4.11E-05	LOC107414862
Cell wall macromolecule metabolic process					
XP_015899780.1	Probable xyloglucan endotransglucosylase/hydrolase protein 23 [Z. jujuba]	1.351	Up	0.000184	LOC107433023
XP_015887252.1	xyloglucan endotransglucosylase/hydrolase 2-like [Z. jujuba]	1.403	Up	0.003496	LOC107422327

Table 3. Differential accumulation of proteins related to molecular function oxidoreductase activity, acting on peroxide as acceptor, enzyme regulator activity and peroxidase activity in fruit skins of Chinese jujube (*Ziziphus jujuba*)

Protein accession	Protein description	Cracked vs non-cracked	Cracked vs non-cracked	P value	Gene name
Oxidoreductase activity, acting on peroxide as acceptor					
XP_024926183.1	peroxidase 4-like [Z. jujuba]	0.689	Down	0.000759	LOC107411518
XP_015890285.1	"peroxidase 51, partial [Z. jujuba]"	0.687	Down	2.22E-06	LOC107424909
Enzyme regulator activity					
XP_015882735.1	Bowman-Birk type proteinase inhibitor PVI-3(2)-like [Z. jujuba]	0.762	Down	0.000124	LOC107418546
XP_015869388.2	probable pectinesterase/pectinesterase inhibitor 17 [Z. jujuba]	1.54	Up	3.67E-05	LOC107406730
XP_015872399.1	miraculin-like [Z. jujuba]	1.798	Up	5.54E-05	LOC107409476
XP_015867821.1	cell wall/vacuolar inhibitor of fructosidase 1-like [Z. jujuba]	0.752	Down	0.005435	LOC107405305
XP_015877940.1	21 kDa protein-like [Z. jujuba]	0.752	Down	0.000303	LOC107414343
Peroxidase activity					
XP_024926183.1	peroxidase 4-like [Z. jujuba]	0.689	Down	0.000759	LOC107411518
XP_015890285.1	"peroxidase 51, partial [Z. jujuba]"	0.687	Down	2.22E-06	LOC107424909

Mining of anti-cracking genes through correlation analysis of proteomics and transcriptome

In GO and KEGG metabolic pathways, transcripts and proteins regulating CML, peroxidase, plant signal transduction pathway and relevant cellular metabolic enzymes were correlated. In combination with previous studies, the 14 DEGs and DEPs (*EG12*, *PE17*, *POD17*, *POD4*, and *POD51*), phytohormone signal transduction (*ARF*, *JAZ*, *AUX/IAA*, *SAUR*, *GH3.1*) and CML (*CML23* and *CML19*) are associated with fruit cracking. qRT-PCR validation was carried out on these 14 genes and expression level analysis of cracked fruit samples at various developmental stages was carried out. Figure 4 shows the results of qRT-PCR analysis of the selected DEGs. Eight genes identified by transcriptome screening were consistent with the transcript levels in the transcriptome data. This shows that transcriptome data can be used as a basis for screening of relevant DEGs. Meanwhile, the results of proteome analysis showed differential expression levels and qRT-PCR results showed six genes that regulate four proteins. The expression levels of the genes regulating peroxidase (XP_024926183.1 and XP_015890285.1) were consistent with the expression levels in proteome data. However, the expression levels of CML (XP_015881810.1), pectinesterase (XP_015869388.2), and indole-3-acetic acid-amido synthetase (XP_015866400.1) were the reverse of each other. (1.1, 1.2, and 1.3 are the skins of the cracked fruit; 2.1, 2.2, and 2.3 are the skins of non-cracked fruit).

Figure 4. Heat map showing the results of real-time quantitative-polymerase chain reaction (qRT-PCR) validation of selected DEG(P)s

Analysis of expression level changes of the 14 candidate genes in cracked fruit skins at different stages of fruit maturation (Fig. 5) revealed 11 genes (*POD17*, *POD4*, *POD51*, *EG12*, *PE17*, *CML23*, *CML19*, *ARF*, *SAUR*, and *GH3.1*) that were highly expressed in cracked fruit skins. *POD17*, *POD4*, *POD51*, *EG12*, and *PE17* participate in increasing enzymes regulating synthesis and accelerate substrate degradation to change fruit skin structure composition and promote skin structural damage, resulting in skin cracking. *ARF*, *SAUR*, and *GH3.1* genes regulate hormone synthesis and change fruit skin hormone balance to cause fruit skin cracking.

Fig. 5. Heat map showing the results of expression level analysis of candidate genes from cracked and non-cracked fruit skins

PG, Cx, PME, POD and cellulase activities in fruit skins are associated with fruit cracking (Wen and Shi, 2012; Wang *et al.*, 2013; Li *et al.*, 2018; Li *et al.*, 2003; Liu *et al.*, 2018). Chen *et al.* (2015) found that EXP2, EXP3, and PE are closely associated with fruit cracking. The expression level of POD2 in cracking-resistant watermelon varieties is higher than in susceptible ones (Jiang *et al.*, 2019). Six proteins were found associated with fruit cracking in pomelos, of which two proteins are related to Ca^{2+} concentration (Li, 2009). In this study, the combined transcriptome and proteome data analyses identified calcium ion transporter genes in cracked fruit skins. *CML23* gene and *CML19* protein showed high and medium-low expressions, respectively. Both *CML23* and *CML19* genes were highly expressed at different stages of pericarp rupture. However, further experiments are essential to validate the relationship between the *CML* genes (*CML23* and *CML19*) and fruit cracking in jujube. In this study, the results of transcriptome and proteome data analyses revealed differential expression of genes and proteins in hormone signal transduction. Genes showing medium-high expression include *ARF*, *JAZ*, *AUX/IAA*, and *SAUR* while *GH3.1* protein exhibited medium-low expression in cracked fruit skins. Results of qRT-PCR showed 5 genes (*ARF*, *JAZ*, *AUX/IAA*, *SAUR*, and *GH3.1*) with significant differential expression present between cracked fruit and non-cracked fruit skins at different time points. Moreover, *ARF*, *SAUR*, and *GH3.1* showed significantly higher expression in cracked fruit skins at different time points. This showed that fruit skin hormone levels are associated with jujube fruit cracking.

CONCLUSION

Transcriptome sequencing and proteomic analysis, identified 31272 transcripts and 5480 proteins. Of these, 5,425 genes were shared at both transcriptome and proteome levels. Compared with uncracked fruit, 161 differentially expressed genes and 182 differentially expressed proteins were found in cracked fruit. Eleven genes are highly expressed in dehiscent fruit, and these genes may play an important role in the dehiscent process of fruit.

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REFERENCE

1. Chen, H.H., Li, J.G., Zhang, J., and Du, Y. (2012). Research progress in mechanism of Chinese jujube (*Ziziphus jujuba* Mill.) fruit cracking in Xinjiang. *Xinjiang Agric Sci*, 49: 1066-1072.
2. Chen, J., Duan, Y., Mo, Y., Hu, Y., Hu, H. and Xie, J. (2015). Expression analysis of cell wall metabolism gene in pericarp of custard apple cultivars with different fruit cracking characters. *J Fruit Sci*, 32: 769-776.
3. Chen, X., (2019). Comparative proteomics analysis of the difference in fruit size, sugar and acid content between jujube and wild jujube (Master's thesis). Northwest A&F University, Yangling.
4. Chen, Y. (2018). Transcriptome and proteome analysis of early somatic embryogenesis in longans and analysis of expression and functions of genes related to flowering time (Doctoral dissertation). Fujian Agriculture and Forestry University, Fuzhou.
5. Correia, S., Santos, M., Glińska, S., Gapińska, M., Matos, M., Carnide, V., Schouten, R., Silva, A.P. and Gonçalves, B., (2020). Effects of exogenous compound sprays on cherry cracking: skin properties and gene expression. *J Sci Food Agric*, 100: 2911-2921.
6. Du, C., (2016). Analysis of low temperature-induced transcriptome and proteome in *Brassica napus* L. (Doctoral dissertation). Huazhong Agricultural University, Wuhan.
12. Jiang, H., Tian, H., Yan, C., Jia, L., Wang, Y., Wang, M., Jiang, C.j., Li, Y., Jiang, J., Fang, L., Zhang, Q. (2019). RNA-seq analysis of watermelon (*Citrullus lanatus*) to identify genes involved in fruit cracking. *Sci Hortic (Amsterdam)*, 248: 248-255.
14. Jin, Z. (2017). Modification of grape berry quality as affected by the rootstocks and preliminary exploration on the underlying mechanism (Master's thesis). Shandong Agricultural University, Taian.
15. Leng, N., Dawson, J.A., Thomson, J.A., Ruotti, V., Rissman, A.I., Smits, B.M.G., Haag, J.D., Gould, M.N., Stewart, R.M., Kendzierski, C. (2013). EBSseq: an empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics*, 29: 1035-1043.
16. Li, J.G., Huang, X.M. and Huang, H.B., (2003). Comparison of the activities of enzymes related to cell-wall metabolism in pericarp between litchi cultivars susceptible and resistant to fruit cracking. *J Plant Physiol Mol Biol*, 29: 141-146.
17. Li, S., (2009). Study on the differential proteins of fruit dehiscent in different pollinated duweiwendan pomelo (Master's thesis). Fujian Agriculture and Forestry University, Fuzhou.
19. Liu, H., Lin, M.J., Gao, J.S. and Wang, Z.L., (2018). Study on the relationship between the activity of cell wall metabolic enzymes and anti-cracked in jujube peel. *Xinjiang Agric Sci*, 55: 845-854.
20. Liu, M.-J., Zhao, J., Cai, Q.-L., Liu, G.-C., Wang, J.-R., Zhao, Z.-H., Liu, P., Dai, L., Yan, G., Wang, W.-J., Li, *et.al.*, (2014). The complex jujube genome provides insights into fruit tree biology. *Nat Commun*, 5: 5315.
21. Liu, M., Wang, J., Wang, L., Liu, P., Zhao, J., Zhao, Z., Yao, S., Stănică, F., Liu, Z., Wang, L., *et.al.*, (2020). The historical and current research progress on jujube—a superfruit for the future. *Hortic Res*, 7: 119.
22. Pertea, M., Pertea, G.M., Antonescu, C.M., Chang, T.-C., Mendell, J.T., Salzberg, S.L., (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol*, 33: 290-295.
23. Wang, B.M., Ding, G.X., Wang, X.Y., Fu, C.B., Qin, G.J., Yang, J.Q., Cang, G.Y. and Wen, P.F., (2013). Changes of histological structure and water potential of Huping jujube fruit cracking. *Sci Agric Sin*, 46: 4558-4568.
24. Wang, J.Y., Gao, Q.L., Wang, Z.L. and Lin, M.J., (2020). Relationship between cell metabolism enzyme activity, carbohydrate, endogenous hormones and fruit cracking. *Xinjiang Agric Sci*, 57: 1689-1696.
25. Wang, J.Y., Gao, Q.L., Zhang, J.J., Wang, Z.L. and Lin, M.J., (2021). Screening of genes related to date cracking. *Mol Plant Breed*, 19:3196-3207.
26. Wang, J., Gao, X., Ma, Z., Chen, J. and Liu, Y., (2019). Analysis of the molecular basis of fruit cracking susceptibility in Litchi chinensis cv. Baitangying by transcriptome and quantitative proteome profiling. *Journal of plant physiology*, 234-235.