Transcriptome Profiling Reveals Potential Genes Involved in Browning of Fresh-cut Eggplant (Solanum Melongena L.)

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Abstract

Background: Fresh-cut processing promotes enzymatic browning of fresh fruits and vegetables, which negatively affects the appearance of products and impacts their nutrition. We used the RNA-Seq technique to analyze the transcriptomic changes occurring during the browning of fresh-cut eggplant (*Solanum melongena* L.) fruit samples from a browning-sensitive cultivar and a browning-resistant cultivar to investigate the genes and molecular mechanisms involved in browning.

Results: A total of 111.55 GB of high-quality reads were generated, the genomes of each sample were compared, and 83.50%–95.14% of the data was mapped to the eggplant reference genome. Furthermore, a total of 19631 differentially expressed genes were identified, among which 12 genes and two WRKY transcription factors were identified as potentially involved in enzymatic browning in fresh-cut eggplant fruit. Moreover, the 14 differentially expressed genes associated with browning were verified using qRT-PCR.

Conclusions: Several genes associated with phenolic oxidation, phenylpropanoid biosynthesis, and flavonoid biosynthesis were found to be differentially regulated between the eggplant cultivars with different browning sensitivities. This work is of great theoretical significance, as it provides a basis for future molecular studies and improvement of eggplants, and lays a theoretical foundation for the development of browning-resistant fresh-cut fruits and vegetables.

Background

Solanum melongena L., commonly known as eggplant, is an essential vegetable crop. It is a good source of dietary minerals, vitamins, and anthocyanins, with a high oxygen radical absorbance capacity and low caloric value [1]. Eggplant has been shown to have important cardiovascular protecting, anticancer, antiaging effects [1, 2]. However, fresh-cut eggplants brown easily, which can negatively affect their flavor, odor, nutrients, and commercial value. Browning has become one of the most vital limitations in eggplant processing. Therefore, understanding the mechanism of enzymatic browning and controlling the occurrence of browning have become the focus of research in the fresh-cut fruit and vegetable industry, and breeding new eggplant varieties resistant to browning has become an important method to fundamentally solve the browning of eggplant fruit.

Browning, a common postharvest problem for many fruits and vegetables, can be divided into two categories: enzymatic and nonenzymatic browning. Non-enzymatic browning occurs as a result of various chemical reactions such as Maillard reactions, caramelization, oxidation of vitamin C, and polyphenol polymerization in daily life. [3–5]. Enzymatic browning is considered as the main reaction that causes browning in postharvest storage and processing of fruits and vegetables, and has been the focus of postharvest research. It refers to the physiological and biochemical processes in which phenolic substances in plant organs or tissues are oxidized to form quinones under the action of polyphenol oxidase under aerobic conditions. Quinones then polymerize to form brown or black substances, resulting in tissue discoloration. Enzymatic browning occurs only in the presence of phenols, enzymes, and oxygen [6, 7]. As an important substrate for enzymatic browning of fruit and vegetable tissues, phenols are widely distributed

in the roots, stems, leaves, flowers, and fruits of plants; the abundance of phenols in different types of plants varies. Phenolic compounds are important secondary metabolites in plants, which are synthesized mainly through phenylalanine metabolic pathways [8, 9]. Phenylalanine ammonia-lyase (PAL) is the starting enzyme of the phenylpropane metabolic pathway. The phenylalanine amino group is deaminated by PAL to form trans-cinnamic acid, which is hydroxylated by cinnamate-4-hydroxylase (C4H) to form trans-4-coumaric acid. Then, under the actions of coumarate 3-hydroxylase (C3H), 4-coumarate-CoA ligase (4CL), hydroxycinnamoyl-CoA: shikimate/quinate hydroxy cinnamoyl transferase (HCT), chalcone synthase (CHS), cinnamoyl-CoA reductase (CCR), and other enzymes, it enters the metabolic pathways of lignin synthesis, chlorogenic acid synthesis, and flavonoid (anthocyanin, procyanidin, rutin) synthesis [9, 10]. Furthermore, enzymatic browning is mostly a result of transforming phenols into o-quinones, with this process being catalyzed by polyphenol oxidase (PPO) [11, 12]. Other enzymes, such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT), play a role in inducing or inhibiting the enzymatic browning of fresh-cut fruits and vegetables. [13, 14]. Therefore, it is essential to study these genes that encode key enzymes in eggplant enzymatic browning and phenolic compound formation, which may lay the foundation for better understanding the browning mechanism of fresh-cut fruits and vegetables, and provide new ideas and perspectives for understanding fruit and vegetable browning in the future.

With the development of high-throughput sequencing technology, genome, transcriptome, proteomics and metabolomics technologies have been widely used to deeply study specific biological processes and molecular mechanisms of organisms, tissues. [15–17]. In recent years, as a new efficient and fast transcriptome research method, RNA-Seq is changing people's understanding of transcriptome, and it has been increasingly used to explore differences in gene expression in plants, such as licorice (*Glycyrrhiza glabra L.*), *Luffa cylindrica* (L.) Roem., potato (*Solanum tuberosum* L.), strawberry (*Fragaria ananassa Duch*), and pear (*Pyrus betulifolia Bge.*) [18–22]. In Zhu's study on fresh-cut luffa browning, he found that there were 15 genes with significantly different expressions in enzymatic browning of fresh-cut luffa, including *PPO*, *POD*, *PAL*, *CAT*, and four WRKY transcription factors [23]. However, there are few reports on the browning mechanism of fresh-cut eggplant fruit.

We identified two cultivars, F and 36, as resistant and sensitive to browning, respectively, by fresh-cutting 60 eggplant cultivars in the field and recording the browning time of each cultivar. In the current study, we aimed to 1) conduct transcriptomic profiling of the two cultivars, 2) identify the key differentially expressed genes (DEGs) related to enzymatic browning and phenolic compound formation, and 3) provide new insights into the molecular mechanisms of eggplant browning.

Results

Overview of transcriptomic data for cultivars F and 36

The browning-sensitive cultivar (36) and browning-resistant cultivar (F) of eggplant showed different degrees of browning with treatment time (Fig. 1). As shown in Table 1, a total of 111.55 GB of clean data were obtained after 18 samples were analyzed and sequenced using a reference transcriptome. The effective data volume of each sample was 5.73–7.04 GB; 93.61%–94.50% of bases had a quality above

Q30, and the average GC content was 45.56%. Reads were compared to the reference genome to obtain the genome alignment of each sample, with an alignment rate of 83.50%–95.14%. We also analyzed the distribution of gene expression levels in each sample (Fig. 2). We determined the minimum, the first quartile (25%), the median (50%), the third quartile (75%), and the maximum value of gene expression level for each sample. The symmetry and degree of dispersion of the data distribution were observed, with remarkably high gene expression levels.

Table 1
Summary statistics of clean reads in the transcriptomes of eggplant

Sample	raw_reads	raw_bases	clean_reads	clean_bases	valid_bases	Q30	GC
F 15min_1	49.75M	7.46G	48.83M	6.63G	88.89%	94.15%	44.08%
F 15min_2	43.77M	6.57G	42.98M	5.84G	88.89%	93.84%	43.55%
F 15min_3	43.15M	6.47G	42.38M	5.77G	89.21%	94.08%	43.46%
F 2min_1	50.03M	7.50G	49.08M	6.54G	87.16%	93.68%	43.82%
F 2min_2	48.79M	7.32G	47.97M	6.49G	88.66%	94.36%	43.43%
F 2min_3	49.04M	7.36G	48.16M	6.52G	88.62%	94.20%	48.59%
F CK0_1	45.73M	6.86G	44.96M	6.11G	89.06%	94.20%	43.50%
F CK0_2	47.44M	7.12G	46.49M	6.20G	87.17%	93.87%	44.36%
F CK0_3	44.35M	6.65G	43.44M	5.75G	86.47%	93.61%	44.04%
36 15min_1	43.53M	6.53G	42.75M	5.81G	89.00%	94.33%	46.06%
36 15min_2	44.28M	6.64G	43.46M	5.95G	89.60%	94.04%	46.80%
36 15min_3	44.47M	6.67G	43.65M	5.89G	88.30%	94.31%	49.09%
36 2min_1	49.59M	7.44G	48.75M	6.57G	88.30%	94.50%	50.06%
36 2min_2	43.62M	6.54G	42.83M	5.73G	87.57%	94.07%	43.78%
36 2min_3	46.19M	6.93G	45.34M	6.05G	87.39%	94.01%	49.84%
36 CK0_1	48.12M	7.22G	47.28M	6.33G	87.74%	94.03%	44.55%
36 CK0_2	52.72M	7.91G	51.84M	7.04G	89.07%	94.40%	44.25%
36 CK0_3	47.98M	7.20G	47.12M	6.33G	88.01%	94.02%	46.79%

Gene structure annotation optimization

Currently, new structural features of some genes have not been found yet. We used StringTie [24] software to assemble reads, and then compared the assembled gene with the gene annotation information of the reference sequence. It may be found that the assembled gene extends the 5 'or 3' end of gene annotation, optimizing the starting and ending positions of known genes (Table S1).

SNP/INDEL analysis

Single Nucleotide Polymorphisms (SNP) refer to the variation of a single nucleotide on the genome. The polymorphisms shown in SNP involve only the variation of a single base, which can be caused by the transition (TS) or transversion (TV) of a single base as well as the insertion or deletion of bases. However, the SNP generally do not include the latter two cases, which are represented by INDEL, it is means insert and delete.

The differences between the eggplant varieties used in the experiment and the eggplant varieties with the reference genome, and the presence of RNA editing in the samples treated differently, may lead to SNP mutation sites [25]. Tools such as Samtools[26], BedTools [27] were used to predict SNP and INDEL sites in samples. The number of SNP sites found in each sample were 187982 (F 15min), 185271 (F 2min), 177684 (F CK0), 158720 (36 15min), 138662 (36 2min) and 190694 (36 CK0), mainly distributed in exon region, intron region, intergenic region and upstream and downstream regions of genes (Table S2). The number of INDEL sites found in each sample were 11478 (F 15min)，11905 (F 2min)，11503 (F CK0)，158720 (36 15min)，9275 (36 2min)，12472 (36 CK0)，mainly distributed in upstream and downstream regions (Table S2). In addition, we divided the number of SNP/INDEL loci of each gene by the length of the gene to obtain the density value of SNP loci of each gene, counted the density value of SNP/INDEL loci of all genes and made the density distribution map (Fig. S1).

SNP sites can be classified into transition and transversion depending on how the base is replaced. Among the samples in different treatment groups, the highest frequency of transversion was SNP(A/T), and the number of SNP sites was 10438 (F 15min), 10181 (F 2min), 9814 (F CK0), 8489 (36 15min), 7513 (36 2min) and 10192 (36 CK0), respectively (Table S3). The highest frequency of transition was SNP(A/G), and the number of SNP sites was 37700 (F 15min), 37412 (F 2min), 35932 (F CK0), 32396 (36 15min), 28518 (36 2min) and 38527 (36 CK0), respectively. The ratios of Ts/Tv were 2.017(F 15min), 2.042 (F 2min), 2.025 (F CK0), 2.036 (36 15min), 2.039 (36 2min) and 2.040 (36 CK0), respectively (Table S3).

Identification and classification of DEGs

Identification of DEGs between the two eggplant cultivars was one of the aims of the present study. To achieve this goal, we compared the transcriptional profiles of cultivars 36 and F, which were used as a test and a control for screening DEGs, respectively. According to the standard criteria (fold change > 2 and *p*-value < 0.05), we analyzed the difference in the expression of the same gene between treatment pairs. For cultivar 36, we identified 4095 DEGs between treatment 1 and the control (1971 and 2124 up- and down-regulated genes, respectively), 2212 DEGs between treatment 2 and the control (857 and 1355 up- and

down-regulated genes, respectively), and 1957 DEGs between treatment 2 and treatment 1 (996 and 961 up- and down-regulated genes, respectively). For cultivar F, we identified 20 DEGs between treatment 1 and the control (11 and 9 up- and down-regulated genes, respectively), 1313 DEGs between treatment 2 and the control (1188 and 125 up- and down-regulated genes, respectively), and 1087 DEGs between treatment 2 and treatment 1 (1014 and 73 up- and down-regulated genes, respectively). Furthermore, there were 2299 DEGs between the control of cultivar 36 and that of F (1363 and 936 up- and down-regulated genes, respectively), 2810 DEGs between treatment 1 of cultivar 36 and that of F (1633 and 1177 up- and down-regulated genes, respectively), and 3820 DEGs between treatment 2 of cultivar 36 and that of F (1336 and 2484 up- and down-regulated genes, respectively) (Fig. 3). The DEGs were then subjected to GO and KEGG pathway enrichment analyses.

Functional annotation and classification of DEGs

To determine the function of DEGs, GO and KEGG pathway analyses were carried out. For cultivar 36, the treatment pair with the most DEGs was the treatment 1/control pair. In this treatment pair, GO functional enrichment analysis was carried out (Fig. 4a). “Metabolic process,” “cell,” and “binding” were the dominant terms in the “biological process,” “cellular component,” and “molecular function” categories, respectively. We also identified a relatively large number of genes associated with “catalytic activity,” “antioxidant activity,” “biological regulation,” “response to stimulus,” “single-organism process,” and “cellular process,” with only a few genes related to “cell killing,” “nucleoid,” and “protein binding transcription factor activity.” As shown in Fig. 4b and c, GO functional enrichment analysis of the treatment pairs F treatment 2/F control and 36 treatment 2/F treatment 2 showed similar results as the 36 treatment 1/36 control treatment pair.

We used KEGG pathway analysis to determine the biological functions of DEGs. Pathway entries with a number of corresponding DEGs greater than 2 were screened and sorted by the $-\log_{10}p$ value of each entry. The top 20 pathways were retained. In the 36 treatment 1/36 control pair (Fig. 5a), the DEGs were mostly involved in phenylpropanoid biosynthesis, flavonoid biosynthesis, tyrosine metabolism, pentose and glucuronate interconversions, and starch and sucrose metabolism. In the F treatment 2/F control pair (Fig. 5b), the DEGs were mostly involved in the AMPK signaling pathway, metabolism of xenobiotics by cytochrome P450, drug metabolism - cytochrome P450, plant hormone signal transduction, and starch and sucrose metabolism. However, in the 36 treatment 2/F treatment 2 pair (Fig. 5c), the DEGs were mostly involved in phenylpropanoid biosynthesis, flavonoid biosynthesis, glyoxylate and dicarboxylate metabolism, stilbenoid, diarylheptanoid and gingerol biosynthesis, and tyrosine metabolism.

Identification of potential genes associated with enzymatic browning

Enzymatic browning is one of the leading causes of tissue browning in fresh-cut fruits and vegetables. We analyzed the expression of the key enzymatic browning enzymes, and found that four PPO-encoding genes (Sme2.5_25992.1_g00001.1、Sme2.5_01441.1_g00006.1、Sme2.5_06843.1_g00001.1、

Sme2.5_33651.1_g00001.1), two POD-encoding genes (Sme2.5_00423.1_g00003.1, Sme2.5_00745.1_g00004.1), one CAT-encoding gene (Sme2.5_01411.1_g00008.1), one PAL-encoding gene (Sme2.5_16832.1_g00001.1), and one CHS1B-encoding gene (Sme2.5_13923.1_g00001.1) had higher transcript levels in cultivar 36 than in cultivar F. Three genes had higher transcription levels in cultivar F compared with cultivar 36, one COMT-encoding gene (Sme2.5_29908.1_g00001.1), one GST-encoding gene (Sme2.5_09086.1_g00002.1), and one L-ascorbate oxidase-encoding gene (Sme2.5_05133.1_g00002.1). In addition, we identified two transcription factors (Sme2.5_00574.1_g00007.1, Sme2.5_07339.1_g00001.1) with differential expression in the two species; these proteins belong to the WRKY transcription factor gene superfamily, which affects different physiological responses and metabolic pathways (Fig. 6, Table S4).

Validation of differentially expressed genes with quantitative real-time PCR

Heat maps for the 12 browning-related genes and two WRKY transcription factor indicated that the expression levels varied with both cultivar and treatment (Fig. 6). In order to confirm the expression level of the 12 genes and two WRKY transcription factors, qRT-PCR was carried out. The design list of primers is shown in Table S5. The quantitative results are shown in Fig. 7. The results of qRT-PCR were consistent with the analysis of the transcriptome, confirming the reliability of the RNA-seq data.

Discussion

Fresh-cut fruits and vegetables are ready-to-eat products made from fresh fruits and vegetables, which are cleaned, peeled, cut, dressed, packaged, and kept refrigerated. They are becoming more popular due to their convenience [28]. However, browning not only affects sensory quality, nutritional quality and food quality of fruits and vegetables, and reduces commodity value, but also hinders product circulation, shortens the shelf life, and even seriously affects people's visual perception and appetite, making consumers unable to accept it. It has become one of the main obstacles restricting the development of fruits and vegetables storage, transportation and processing industry. High-throughput sequencing analyses have revealed many genes related to browning of vegetables and fruits [18, 23], but a more comprehensive understanding of gene expression during fresh-cut eggplant browning and strategies for browning control are still needed. The present study investigated the causes of browning and antioxidant capacity in a fresh-cut browning-sensitive cultivar (36) by comparing it with a browning-resistant cultivar (F).

Enzymatic browning is considered the main reason for browning of fresh-cut fruits and vegetables. Enzymes related to phenolic oxidation (i.e., PPO, POD, CAT, and SOD) play a crucial role in the browning of fresh-cut fruits and vegetables. It is widely accepted that the enzymes encoded by the *PPO* and *POD* genes function together to induce browning [29]. Ali et al. (2020) found that the enzymatic browning of fresh-cut lotus root can be repressed by applying oxalic acid to decrease PPO and POD activity [30]. Similarly, an ascorbic acid and *Aloe vera* gel combined treatment suppressed POD and PPO activities, providing indirect evidence that PPO and POD function together to influence the browning of fresh-cut lotus root [30, 31]. Zheng et al. (2019) showed that the 0.1 mM melatonin treatment significantly reduced the expression of

genes related to enzymatic browning and phenolic synthesis pathways, to decrease POD and PPO activity and thus reduce the surface browning of fresh-cut pear [32]. Inhibition of PPO-encoding genes by artificial microRNAs in potato has been found to lead to low PPO protein levels and low browning in potato tubers [33]. Gonzalez et al. found that specific editing of a single member of the *StPPO* gene family through the CRISPR/Cas9 system reduced PPO activity by 69% and enzymatic browning by 73% in potato tubers [34], whereas evidence indicated that overexpression of *PPO* in transgenic sugarcane led to higher PPO activity and darker juice color [35]. In the present study, four PPO-encoding genes and two POD-encoding genes were differentially regulated in the browning-sensitive cultivar (36). The expression level of the PPO-encoding genes in 36 was significantly higher than that in F, and the expression level of PPO (Sme2.5_25992.1_g00001.1) was approximately 550-fold higher in cultivar 36 than in cultivar F (Fig. 6). It is generally accepted that the browning mechanism involves the interaction between the polyphenol substrate and PPO in the presence of oxygen (Fig. S2), including two reaction processes: (1) hydroxylation of monophenols to diphenols, and (2) oxidation of diphenols to quinones. The V_{max} of the hydroxylation reaction is 1.8 mM/min, and results in colorless products, while the V_{max} of the oxidation reaction is 24.5 mM/min, and the resultant quinones are colored. The subsequent reaction of quinones leads to the accumulation of melanin, which leads to browning of plant tissue. The specific structure of the polyphenol substrate determines the specific reaction time of browning of plant tissue [36]. Additionally, the SOD and CAT enzymes play a pivotal role in the browning of fresh-cut fruits and vegetables. As the most important enzyme for scavenging free radicals, CAT can enhance the antioxidant properties of plants by catalyzing the degradation of H_2O_2 [37]. UV-C treatment has been found to inhibit surface browning of fresh-cut apple by improving reactive oxygen species (ROS) metabolism and enhancing the activities of SOD and CAT [38]. Meanwhile, Zhou et al. (2019) reported that a methyl jasmonate treatment induced enzymatic browning of fresh-cut potato tubers and enhanced PPO, POD, and CAT activities [39]. In our study, one CAT-encoding gene (Sme2.5_01411.1_g00008.1) was identified. It was significantly up-regulated in the browning-sensitive cultivar (36) (Fig. 6). Therefore, we postulate that CAT enzyme activity increased rapidly when the plant tissue was cut and damaged, ROS were removed, and the oxidation tolerance was enhanced.

Phenols are vital substrates of enzymatic browning in fresh-cut fruits and vegetables.

Total phenolic content plays a major role in the browning of stored fruits. Phenolic levels have been found to increase in eight cultivars of eggplant during storage, and phenolic concentration varied significantly among fresh-cut samples of eggplant cultivars [40]. Biosynthesis of phenol compounds is involved in the pentose phosphate, shikimate, flavonoid, and phenylpropanoid pathways in plants [8, 41], which involve a variety of key enzymes, such as PAL, 4CL, CHS, F3'H, and COMT. PAL is an important enzyme in the phenylpropane metabolic pathway, catalyzing L-phenylalanine ammonia hydrolysis to generate trans-cinnamic acid, and then through a series of transformations to form various phenols, lignin, anthocyanins, alkaloids, and other compounds. The phenols formed provide a substrate for the browning reaction [42]. In a study of PAL and HSPs expression in fresh-cut banana fruit, it was observed that mechanical injury resulted in the accumulation of PAL mRNA in fruits, which led to a rapid increase in PAL activity. Mechanical injury also increased the synthesis rate of ethylene, which accelerated the decomposition of phenylalanine and the synthesis of a large number of phenolic substances. At this point, phenolic compounds combined with PPO

activated by mechanical injury to induce browning. Through linear regression analysis, it was found that the increase in PAL activity in fruit induced by mechanical injury (fresh-cutting) was highly correlated with the accumulation of phenolic substances [43]. CHS is the key enzyme in anthocyanin biosynthesis [44]. Melatonin treatment at 0.1 mM increased the expression of PAL and CHS, enhanced PAL and CHS activities, and reduced the browning of fresh-cut pear fruit [32]. Studies of fresh-cut sweet potatoes treated with ultrasound have found that the induction of PAL was positively correlated with higher total phenolic content, thereby enhancing antioxidant capacity of the fresh-cut sweet potatoes against browning [45]. An 80% oxygen treatment was found to increase the activities of PAL and POD and the total phenolic content of fresh-cut potato, which effectively enhanced its antioxidant capacity [46]. Conversely, Gong et al. reported that browning inhibition has no observable correlation with either total phenol or POD activity [47]. Zhang et al. identified one PAL-encoding gene, one CHS-encoding gene, and two 4CL-encoding genes that were significantly up-regulated in Chinese walnut husks that browned, but another PAL-encoding gene was significantly up-regulated in anti-browning husks [48].. In the present study, many genes connected with phenylalanine metabolism were differentially regulated in the browning-resistant cultivar compared with the browning-sensitive cultivar. One PAL-encoding gene (Sme2.5_16832.1_g00001.1) and one CHS-encoding gene (Sme2.5_13923.1_g00001.1) were up-regulated in the browning-sensitive cultivar. However, one COMT-encoding gene (Sme2.5_29908.1_g00001.1) was down-regulated in the browning-sensitive cultivar at 2 min compared with the browning-resistant cultivar at 2 min, and also compared with the browning-sensitive cultivar control (Fig. 6). These results suggest that fresh cutting treatment induced the expression of the key enzyme gene in phenylalanine metabolism and flavonoid biosynthesis, and promoted the synthesis of polyphenols in plant cell tissues to improve their antioxidant capacity.

Ascorbic acid (AsA) and glutathione (GSH) also play important roles in maintaining the stability of proteins, structural integrity of the biofilm system, and defense against membrane lipid peroxidation. Together with antioxidant enzymes such as glutathione S-transferases (GST), ascorbate peroxidase (APX), dehydroascorbic acid reductase (DAR), monodehydroascorbic acid reductase (MDAR),and glutathione reductase (GR), the AsA-GSH circulatory system can effectively eliminate free radicals [49]. The coordinated operation of each component in the AsA-GSH cycle enables the accumulation of ROS in plants to be removed, which plays an important role in the enzymatic ROS removal mechanism of fresh-cut fruits and vegetables. Under normal conditions, the production and clearance of ROS in plants are in dynamic balance. When plants are subjected to mechanical damage stress, the content of ROS increases significantly. Excessive ROS will destroy biological molecules such as proteins and destroy the integrity and function of membranes. In the enzymatic defense system, GST catalyzes the reaction of GSH with membrane lipid peroxides, thus reducing the damage to the membrane structure caused by mechanical stress [50]. GST participates in the AsA-GSH cycle [51], catalyzing H_2O_2 to generate oxidized glutathione (GSSG) and H_2O, and decompose organic hydroperoxides into alcohols, H_2O, and GSSG, which are then reduced by GR to GSH [52]. With AsA as the electron donor, APX catalyzes the reduction of H_2O_2 to MDHA and H_2O. MDHA has two reduction pathways: (1) it is reduced to AsA by MDHAR and (2) it is reduced to dehydroascorbic acid (DHA) and then reduced to AsA through DHAR with reduced GSH as an electron donor [53]. AsA is involved in the oxidation protection system against free radicals; it is easily soluble in water and has sensitive antioxidant properties. Therefore, a change in its content level is considered an important indicator

for monitoring fruit and vegetable quality [54]. L-ascorbate oxidase plays a catalytic role in physiological changes, which can oxidize AsA to DHA and reduce H_2O_2, thus eliminating free radicals in cells and delaying senescence [55]. Browning and PPO activities of fresh-cut artichoke bottoms were significantly inhibited by Cg supplemented with AsA [56]. Salminen et al. found that 3% AsA and 0.1% green tea extract prevented the browning of fresh-cut apple slices for up to 14 days [57]. GST is present in almost all plants; there are up to 90 genes encoding GST in plants. Most of these genes are differentially expressed under stress induction and play an important role in the mechanism of enzymatic ROS scavenging [58]. We found that the expression level of an L-ascorbate oxidase-encoding gene (Sme2.5_05133.1_g00002.1) and a glutathione S-transferase-encoding gene (Sme2.5_09086.1_g00002.1) were significantly higher in the browning-resistant cultivar compared with the browning-sensitive cultivar under all treatments, indicating that the browning-resistant cultivar had a stronger antioxidant capacity. This could explain why the fruit did not easily brown after cutting (Fig. 6).

The browning of fruits and vegetables is closely related to reactive oxygen metabolism in plants [59–61]. WRKY transcription factors play an vital role in regulating various physiological processes in plants, including plant growth and development, as well as responses to biological, abiotic and oxidative stresses [62–64]. Furthermore, Wang et al. revealed that WRKY transcription factor plays an important induction role in ROS metabolic pathway [65]. Overexpression of ATWRKY30 increased the activity of antioxidant enzymes (i.e., CAT, SOD, POX, and APX) in wheat plants [66]. Zhang et al. reported that one WRKY transcription factor was differentially regulated in the fresh-cut browning and white husks of the Chinese walnut [48]. There were four WRKY transcription factors Significantly differentially expressed in fresh-cut luffa fruit [23]. These findings provide direct evidence that WRKY transcription factors play a crucial role in fresh-cut vegetables and fruits. In the current study, two WRKY transcription factors (Sme2.5_00574.1_g00007.1, Sme2.5_07339.1_g00001.1) was observed. It was significantly up-regulated in the browning-resistant cultivar compared with the browning-sensitive cultivar (Fig. 6). The relationship between WRKY transcription factors and fresh-cut eggplant browning was revealed in our study. However, further studies on their structural and functional properties are needed to determine their potential influence on the browning of fresh-cut eggplant fruits.

Conclusions

In this study, the transcriptomes of two eggplant varieties with different browning sensitivities after fresh cutting were studied using a high-throughput sequencing platform. We found 12 genes and two WRKY transcription factors that were differentially regulated in fresh-cut eggplant fruit, and hence are potentially involved in enzymatic browning. Furthermore, GO and KEGG pathway analyses showed that most of these genes were involved in tyrosine metabolism and the phenylalanine metabolism pathway. The identification of these potential candidate genes and metabolic pathways related to eggplant responses to browning are of great significance to the study of the molecular characteristics of eggplant and the improvement of eggplant varieties.

Methods

Plant materials and fresh-cut treatment

The eggplant cultivars 36 (browning sensitive) and F (browning resistant) are from a breeding line produced by our lab at the Shanghai Key Laboratory of Protected Horticultural Technology at the Shanghai Academy of Agricultural Sciences, Shanghai, China. The fruit samples were carefully peeled with a stainless steel knife. Three treatments were established based on time since cutting: control, fresh-cut for 0 min; treatment 1, fresh-cut for 2 min; and treatment 2, fresh-cut for 15 min. After samples were left for the prescribed amount of time, they were frozen in liquid nitrogen and kept at -80 °C until RNA extraction.

Total RNA extraction, library construction, and RNA sequencing

Total RNA was isolated using a mirVana™ miRNA ISOlation Kit (Ambion-1561) according to the manufacturer's protocol. The concentrations and quality of the RNA samples were assessed using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, CA, USA). The A260/280 ratios of individual samples were all above 2. The 28S/18S ratio and RIN values were determined using an Agilent 2100 system (Agilent, California, USA). A TruSeq Stranded mRNA LTSample Prep Kit (Illumina, CA, USA) was used to construct the sequencing library, and then samples were sequenced using the HiSeq™ 2500 system (Illumina Inc., San Diego, CA, USA) after quality control analysis using an Agilent 2100 Bioanalyzer (Santa Clara, CA, USA).

RNA sequence analysis and DEG identification

Raw data (raw reads) were processed using Trimmomatic [67]. The reads containing ploy-N and low-quality reads were removed to obtain clean reads. Then, the clean reads were mapped to the reference genome using HISAT2 [68]. The reads were reassembled using StringTie [69]. Then, gene structure extension and novel transcript identification were performed by comparing the reference genome and the known annotated genes using cuffcompare software [70].

The gene expression level was indicated by the FPKM value [71]. To calculate the different expression levels of genes among the samples, the htseq-count [72] was used to acquire the number of reads in each sample. Two functions (estimatSizeFactors and nbinomTest) in the DESeq [73] R package were used to normalize the data and calculate the *p*-value and fold-change. A p-value < 0.05 and fold-change > 2 or < 0.5 were set as thresholds for significantly differential expression.

Functional annotation and classification of DEGs

The sequences of the DEGs were aligned to the KEGG database using BLASTX, retrieving proteins with the highest sequence similarity with the given sequences along with functional annotations for their proteins [74-76]. Gene ontology (GO) annotations of the DEGs were obtained using the Blast2GO program [74, 77].

The Web Gene Ontology Annotation Plot software was used to perform GO functional classification with a Pearson chi square test [74, 78]. The DEGs were mapped to GO terms according to the analyses, and the number of DEGs in each term was calculated [74].

Quantitative real-time PCR (qRT-PCR) validation

qRT-PCR was performed to verify the accuracy of the gene expression profile obtained from the RNA-Seq data. Total RNA was extracted as described above. First-strand cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) and Oligo (dT)18 in a 25-μL reaction. Real-time PCR was performed with SYBR Green PCR mix (Takara, Shiga, Japan). SmPKG was used as an endogenous control gene for qRT-PCR analyses. Relative expression levels of the target genes were calculated using the 2-ΔΔCt method. The primers used are listed in additional file5: Table S5.

List Of Abbreviations

4CL, 4-coumarate-CoA ligase; C3H, coumarate 3-hydroxylase; CAT, catalase; CHS, chalcone synthase; CCR, cinnamoyl-CoA reductase; DEG, differentially expressed genes; GO, gene ontology; HCT, hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyl transferase; POD, peroxidase; PPO, polyphenol oxidase; ROS, reactive oxygen species; SOD superoxide dismutase

Declarations

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Authors' contributions

DZ and XW conceived the study idea; LX and SJ performed the preparation and treatments of test materials. XW and AZ designed and performed the experiments. LX analyzed the data and wrote the manuscript. AZ revised the article. All authors read and approved the final manuscript.

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Availability of data and materials

The data charts supporting the results and conclusions are included in the article and additional files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

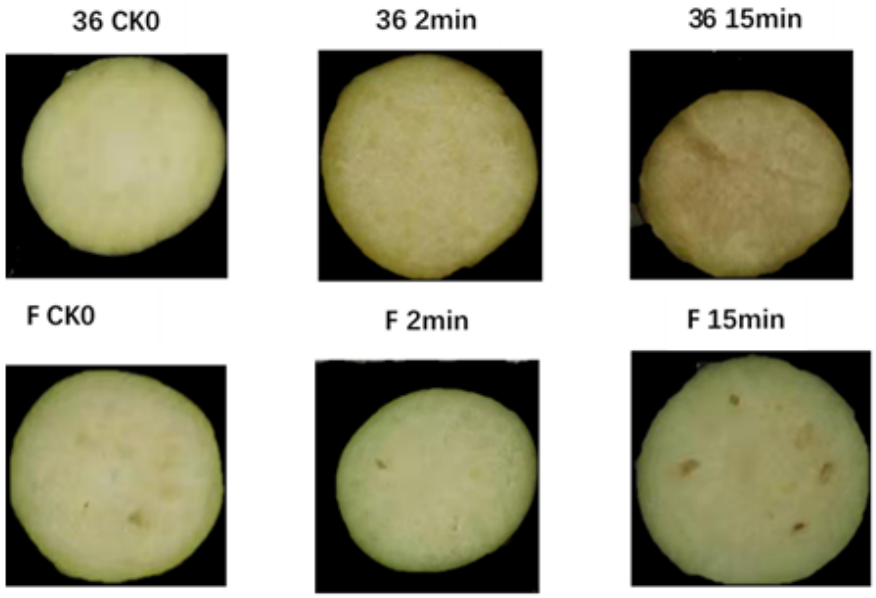

Figure 1

The browning degree of browning-sensitive cultivar (36) and browning-resistant cultivar (F)

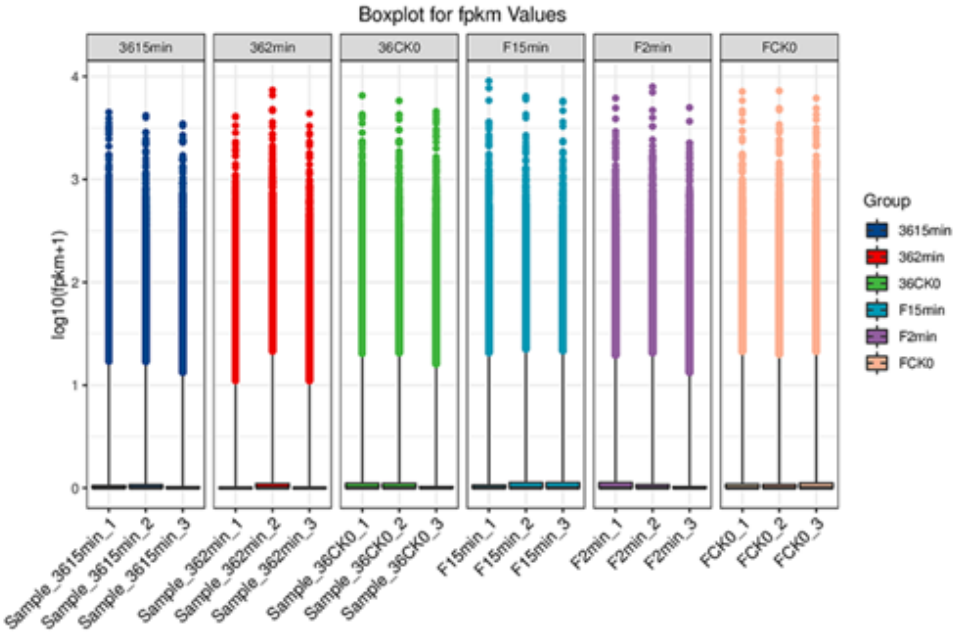

Figure 2

Distribution of gene expression levels in each sample

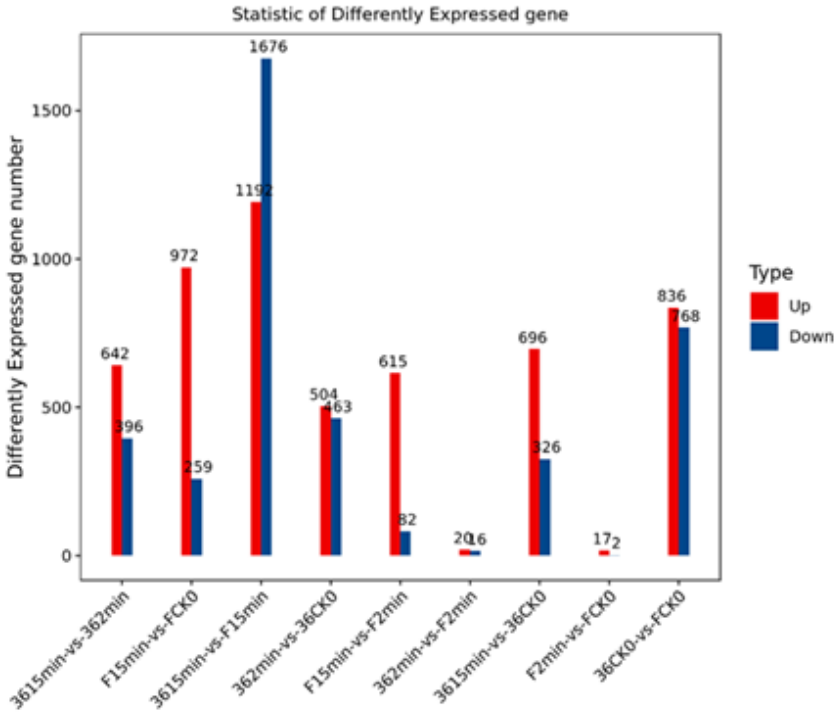

Figure 3

Differentially Expressed Genes (DEGs) in each comparison group

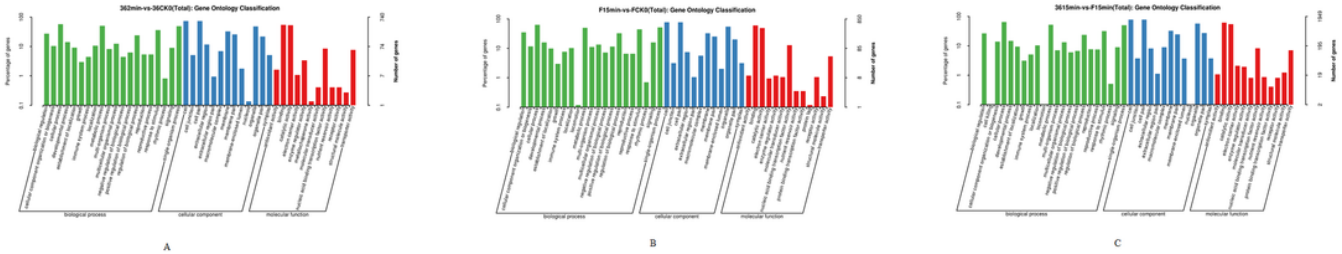

Figure 4

a GO functional enrichment of the 36 2min vs 36 CK0 groups b GO functional enrichment of the F 15min vs F CK0 groups c GO functional enrichment of the 36 15min vs F 15min groups

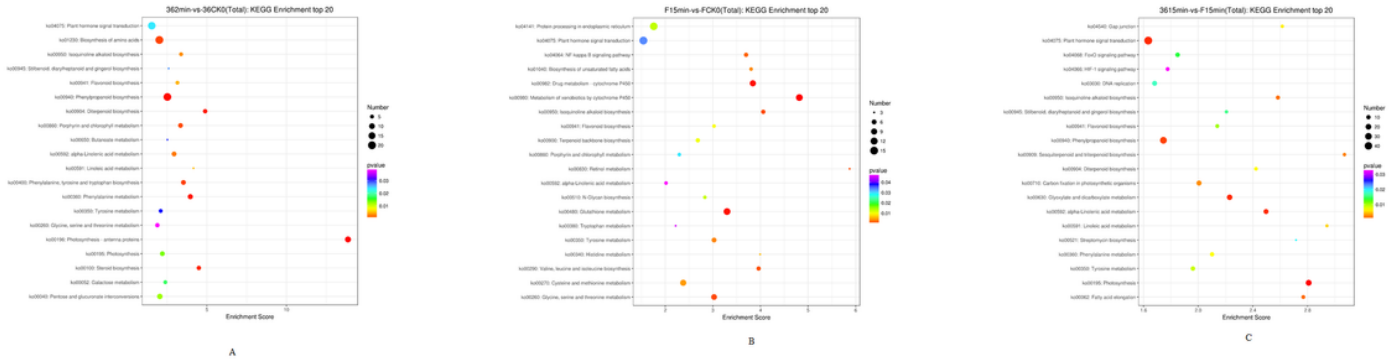

Figure 5

a KEGG enrichment of the 36 2min vs 36 CK0 groups b KEGG enrichment of the F 15min vs F CK0 groups c KEGG enrichment of the 36 15min vs F 15min groups

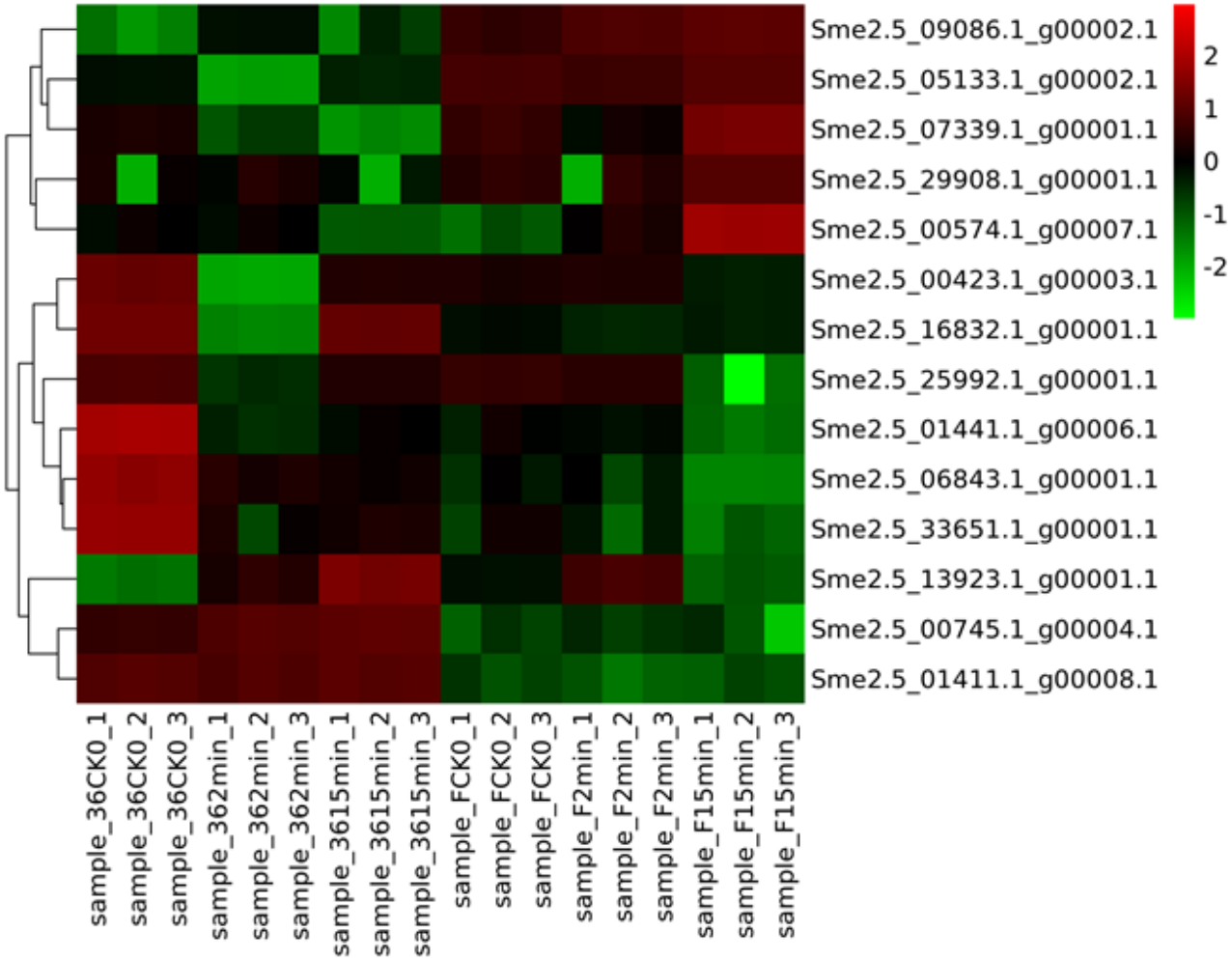

Figure 6

The heat maps for the 14 browning-related genes. The red color in the picture indicates high expression genes, while the green color indicates low expression genes.

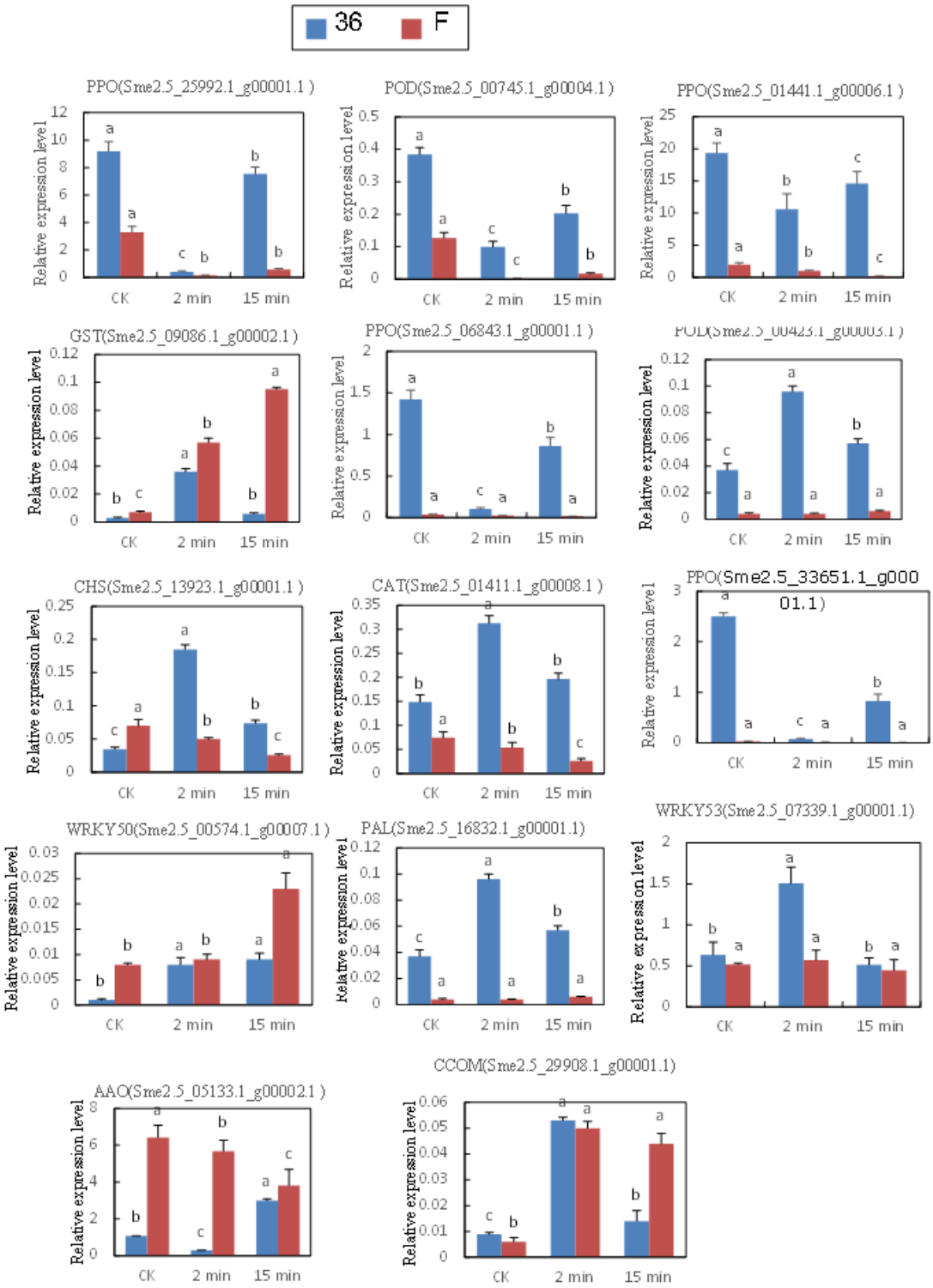

Figure 7

Quantitative real-time polymerase chain reaction analyses of the expression levels of 14 browning-related genes.

Supplementary Files

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