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Transcriptomic Analysis of Late-Ripening Sweet Orange Fruits (*Citrus sinensis*) after Foliar Application of Glomalin-Related Soil Proteins

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Abstract: Glomalin, one of the glycoproteins generated in the spores and hyphae of arbuscular mycorrhizal (AM) fungi, has multiple functions in plants and soil, while the role of foliar spray of easily extractable glomalin-related soil proteins (EE-GRSP) in citrus fruits is not well defined. Our study aimed to use referenced transcriptome sequencing to uncover the mechanism and the role of exogenous EE-GRSP in two late-ripening varieties of sweet orange (*Citrus sinensis*) fruits including Navel Lane Late (LW) and Rohde Red Valencia (XC). The 1804 and 1861 differentially expressed genes were identified in fruits of LW and XC, respectively, following foliar spray of EE-GRSP. Photosynthesis ranked second in the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolism in the LW variety, and carbon fixation in photosynthetic organizations ranked first in KEGG metabolism in the XC variety. The response to foliar spray of EE-GRSP affected the fruit starch and sucrose metabolism of KEGG, with 15 (10 up-regulated and 5 down-regulated) and 13 (2 up-regulated and 11 down-regulated) differentially expressed genes identified in the LW and XC variety, respectively. *Cs5g19060* (sucrose phosphate synthase 4) was activated and reduced by EE-GRSP on XC and LW, respectively. *Cs1g18220* (*β-fructofuranosidase*) and *Cs2g12180* (glycosyl hydrolase family 9) genes were up-regulated and down-regulated in LW and XC, respectively. These results established the involvement of molecular signaling in response to foliar spray of EE-GRSP activating fruit sugar metabolism is dependent on citrus varieties.

Keywords: citrus; fruit quality; gene expression; glomalin; hexose; mycorrhiza

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1. Introduction

Citrus is an extensively grown fruit tree [1], besides being significantly trade and tariff involved [2]. Among them, the fresh fruits of sweet oranges (*Citrus sinensis* (L.) Osbeck) mature almost throughout the year. However, in China, most of the sweet orange fruits mature in October–December, causing market glut and adversely affecting the sale price of fresh fruits [3]. China, as one of leading citrus producing countries, is working on developing late-ripening sweet oranges, expected to be available in the market from February to July [4]. However, one of the disadvantages of late-ripening sweet oranges is the persistence of granulation upon fruit maturity, with relatively low soluble solids content [5]. Improving fruit quality is therefore urgently needed for harnessing the maximum market advantage of late-ripening sweet oranges.

A beneficial class of soil microorganisms that exists in plant rhizospheres, called arbuscular mycorrhizal (AM) fungi, enter the cortical cells of plant roots to form AM structures and, thus, help the host absorb nutrients and water by means of extraradical hyphae, in exchange for plant carbohydrates and lipids [6]. Most studies on citrus indicated that AM fungi stimulated plant growth, improved nutrient acquisition, and enhanced their resistance to drought, low temperature, flooding, salinity, etc. [7–9]. AM fungi produce hydrophobic glycoproteins [10] and glomalin on spore walls and the surface of mycorrhizal mycelium, which reacts with immunofluorescence to monoclonal antibodies (MAb32B11) [11]. Glomalin is released into the rhizosphere soil after mycelium and spore die of AM fungi. The protein extracted from the soil by the current technique contains extra components of non-AM fungal origin, thus defined as glomalin-related soil protein (GRSP) in the soil [12,13]. GRSP has a strong ability to glue small soil particles into bigger aggregates [14], playing an important role in soil organic carbon fixation, water retention, and nutrient cycling [7,15–18], apart from one of the indicators of evaluating the degree of soil erosion and structural development [17].

GRSP consists of easily extractable GRSP (EE-GRSP) and difficult-to-extract GRSP (DE-GRSP) [19]. Amongst them, EE-GRSP is relatively newly generated by AM fungi and assumes some of the essential functions of AM fungi, while DE-GRSP is a turnover from EE-GRSP, a relatively less active component of GRSP, which is inert in nature and has higher nutrient storage capacity than EE-GRSP [15,19]. Exogenous EE-GRSP, as a biostimulater, improved plant growth, promoted nutrient uptake, and enhanced drought tolerance [20–22]. Since purified GRSP contains many elements such as K, Mg, Si, etc., and is thus beneficial to the development of better fruit quality [23,24], we suspected that exogenous EE-GRSP might have an improved effect on fruit quality, although the direct evidence was lacking.

Our recent study revealed that foliar application of EE-GRSP improved the fruit quality of two late-ripening sweet oranges, Navel Lane Late (LW) and Rohde Red Valencia (XC), with a more conspicuous influence on the LW variety [23]. However, the underlying mechanisms explaining the effect on soluble solid content and fruit sucrose, glucose, and fructose largely remain unexplored. It is hypothesized that foliar spraying of EE-GRSP could induce molecular changes in improved fruits of citrus. The objective of the present study was to unravel the internal mechanism regarding the improved effect of exogenous EE-GRSP on late-ripening sweet orange fruits according to transcriptome technology, with a focus of sucrose metabolism.

2. Materials and Methods

2.1. Experimental Design and Application of EE-GRSP

The EE-GRSP was extracted in the laboratory following the procedural steps outlined by Wu et al. [19]. Soil samples from 28-year-old Satsuma orange (trifoliate orange as the rootstock), located within the campus of Yangtze University, were collected, air-dried, sieved, mixed with 20 mM citrate buffer (pH 7.0) (soil: citrate buffer, 1/8, g/v), extracted after autoclaving for 30 min, and then centrifuged at 10,000× *g* for 5 min. The supernatant was collected as the stock solution, and was stored at 4 °C. The protein concentration of EE-GRSP in the stock solution was 16 mg/L as per the protocol of Bradford [25]. The stock solution of EE-GRSP was diluted twice with 20 mM citrate buffer as the solution for foliar spray of EE-GRSP.

Trees of LW (*C. sinensis* (L.) Osbeck cv. Navel Lane Late) and XC (*C. sinensis* (L.) Osbeck cv. Rohde Red Valencia), uniform in size (8-year-old and a planting distance of 3 m apart), were identified in an orchard (31°80 N, 110°460 E, 200 m) in Xingshan, Hubei, China. EE-GRSP was foliar sprayed on LW and XC on 8 August 2018 using 1 L of EE-GRSP solution, and the control treatment was sprayed with an equal volume of 20 mM citrate buffer (pH 7.0). A total of four treatments were arranged, namely (i) the LW variety sprayed with EE-GRSP (LW_EG), (ii) the LW variety not sprayed with EE-GRSP (LW_Non_EG), (iii) the XC variety sprayed with EE-GRSP (XC_EG), and (iv) the XC variety not sprayed

with EE-GRSP (XC_Non_EG). Each treatment, containing 3 trees, was replicated five times, covering a total of 60 trees, including 30 LW and 30 XC trees. The treatment by foliar spray with EE-GRSP was carried out three times with an interval of one week during fruit set growth stage onwards, with cultural practices followed uniformly as routine management. Fruit harvesting was considered at commercial maturity coinciding with April (LW) and May (XC) months of the following year. Twenty fruits per tree were harvested and brought back to the laboratory for further analysis on different fruit quality parameters.

2.2. Analysis of Fruit Quality

Fifteen fruits per tree were selected for the analysis of fruit quality. The total soluble solids content of fruits was determined by a portable refractometer (WYT-4, Quanzhou Zhongyou Optical Instrument Co., Ltd., Quanzhou, China). Titratable acidity of fruits was assayed with the indicator (0.1 M NaOH-methylene blue)-based titration method [26], and vitamin C content in fruits was measured with 2,6-dichloroindophenol titration method [27]. Sugar (fructose, glucose, and sucrose) contents of fruits were measured according to the protocol described by Wu et al. [28]. These obtained experimental data were statistically analyzed with the analysis of variance by SAS, and significant ($p < 0.05$) differences among treatments were conducted by the Duncan's multiple range test.

2.3. Construction of Fruit Transcriptomics

In the field, we directly picked fresh fruits from trees, and the pulps of some fruits were frozen with liquid nitrogen, brought back to the laboratory, and stored at −80 °C for transcriptome analysis of fruits. The total RNA of fruit pulps was extracted using conventional methods followed by detection of RNA purity by RNA specific agarose electrophoresis. The mRNA with Polya-A tails was further enriched by oligo magnetic beads, and a RNA fragment with a length of about 300 bp was obtained by ion interruption. The first strand of cDNA was synthesized with 6-base random primers and reverse transcriptase; the second strand of cDNA was synthesized with the first strand of cDNA as a template. After the construction of the cDNA library, the fragments were enriched by PCR amplification, and then the library size of 450 bp was chosen according to fragment size. The library was analysed by Agilent 2100 Bioanalyzer for the total and effective concentration. Then, the libraries containing different index sequences were uniformly diluted to 2 nM concentration. The single chain library was formed through alkali denaturation. The samples were sequenced based on Illumina sequencing platform.

2.4. Reference Transcriptome Analysis

The genome information (Genome:csi.gene.fa, http://citrus.hzau.edu.cn/, accessed on 7 August 2019; Base Pairs: 655,710,203) was referenced for the transcriptome analysis. The raw data were initially filtered, then the filtered high-quality sequences (Clean Data) were compared with the reference genome of the sweet orange species. Based on the results of the comparisons, the expression level of each gene was calculated. We used the Fragments Per Kilo bases per Million fragments to standardize the gene expression level. The identification of difference of gene expression level was based on the DEGseq (2010) R package. If the $|\log2(\text{Fold Change})| > 1$ and the p value < 0.05, the gene was assumed to be differentially expressed. The samples were further analyzed for gene expression difference analysis, enrichment analysis (GO enrichment analysis and KEGG pathway analysis), and clustering analysis. The transcript sequence was reduced by splicing the reads on the comparison pairs.

3. Results

3.1. Improvement in Fruit Quality

Two sweet orange varieties (LW and XC) responded significantly to foliar application of EE-GRSP (Figures 1 and 2). The fruits of LW with applied exogenous EE-GRSP recorded significantly higher Vitamin C content than non-applied fruits; the fruits of XC with EE-

GRSP displayed significantly higher titrable acidity and lowering of Vitamin C content than that of non-EE-GRSP (Figure 1). Foliar spraying of EE-GRSP significantly increased fruit sucrose, glucose, and fructose concentrations compared to the non-EE-GRSP control, regardless of LW and XC varieties (Figure 2).

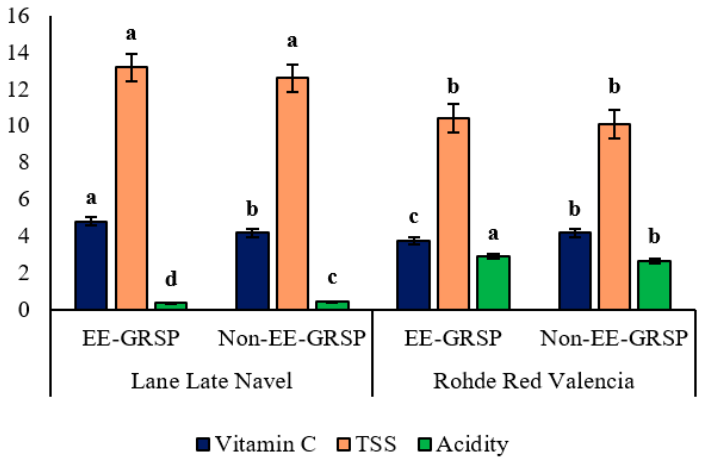

Figure 1. Effects of exogenous easily extractable glomalin-related soil protein (EE-GRSP) on Vitamin C (mg/g), total soluble solid (%), and titrable acidity (%) of fruits of Lane Late Navel (LW) and Rohde Red Valencia (XC) varieties of sweet orange. Data (means ± SD, n = 5) followed by different letters at the bar indicate significant differences ($p < 0.05$) among treatments.

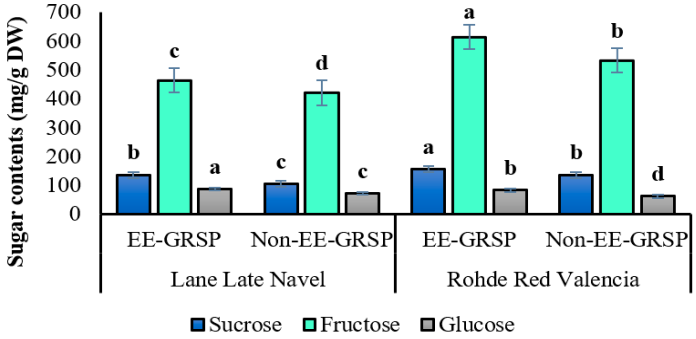

Figure 2. Effects of exogenous, easily extractable glomalin-related soil protein (EE-GRSP) on sucrose, fructose, and glucose concentrations in fruits of Lane Late Navel (LW) and Rohde Red Valencia (XC) varieties of sweet orange. Data (means ± SD, n = 5) followed by different letters at the bar indicate significant differences ($p < 0.05$) among treatments.

3.2. Data Filtering and Comparison Analysis

To remove some spliced, low-quality reads of the sequencing data, we further filtered the results. The criteria of data filtering mainly included: (i) removal of sequences with connectors at the 3′ end using Cutadapt; (ii) removal of reads with average mass fraction below Q20. The results showed that high quality sequence reads accounted for more than 92.5% of sequencing reads (Table 1).

Filtered reads were aligned to the reference genome (Genome:csi.gene.fa; http://citrus.hzau.edu.cn/, accessed on 7 August 2019; Base Pairs:655,710,203) using the HISAT2 software, an upgraded version of TopHat2. The HISAT2 was used to improve the BWT algorithm for faster and fewer resource intensive leads. The mapping proportion of sequencing reads was around 93%, much higher than 70% of the standard, indicating that the mapping proportion was very close to the reference genome.

Table 1. Data filtering and comparison analysis of raw reads.

Samples	Clean Reads No.	Clean Data (Bp)	Clean Reads (%)	Total Mapped	Multiple Mapped	Uniquely Mapped
LW_Non_EG_1	42168768	6367483968	92.79	39396132(93.42%)	1948127(4.94%)	37448005(95.06%)
LW_Non_EG_2	35949522	5428377822	92.76	33544020(93.31%)	1687584(5.03%)	31856436(94.97%)
LW_Non_EG_3	38009648	5739456848	92.54	35475281(93.33%)	1710341(4.82%)	33764940(95.18%)
LW_EG_1	38173108	5764139308	92.84	35648187(93.39%)	1647770(4.62%)	34000417(95.38%)
LW_EG_2	39379550	5946312050	92.69	36801866(93.45%)	1710902(4.62%)	35090964(95.35%)
LW_EG_3	39201784	5919469384	92.75	36588621(93.33%)	1682057(4.60%)	34906564(95.40%)
XC_Non_EG_1	38468020	5808671020	92.95	35969372(93.50%)	1691810(4.70%)	34277562(95.30%)
XC_Non_EG_2	37943920	5729531920	92.85	35444261(93.41%)	1740822(4.91%)	33703439(95.09%)
XC_Non_EG_3	36631568	5531366768	92.80	34254699(93.51%)	1694032(4.95%)	32560667(95.05%)
XC_ EG_1	37449412	5654861212	92.96	35088625(93.70%)	1707696(4.87%)	33380929(95.13%)
XC_ EG_2	40065458	6049884158	92.82	37285676(93.06%)	1977558(5.30%)	35308118(94.70%)
XC_ EG_3	41695874	6296076974	92.54	38705480(92.83%)	1862787(4.81%)	36842693(95.19%)

3.3. Principal Component Analysis

We used the DESeq software package in R language to conduct a principal component analysis (PCA) for each sample according to the expression quantity. The PCA was divided into two principal components (PC1 and PC2) (Figure 3). Among them, PC1 effectively distinguished the response to foliar spray of EE-GRSP between sweet orange varieties, with 66% variance, while PC2 showed a response to foliar spray of EE-GRSP with only 23% variance determination.

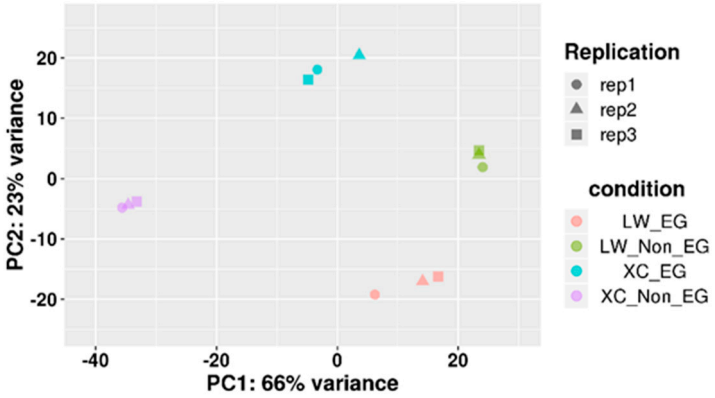

Figure 3. Principal component (PC) analysis of four treatments. Abbreviation: LW_EG, the LW variety sprayed with EE-GRSP; LW_Non_EG, the LW variety not sprayed with EE-GRSP; XC_EG, the XC variety sprayed with EE-GRSP; XC_Non_EG, the XC variety not sprayed with EE-GRSP.

3.4. Analysis of Gene Expression

We observed that, in the LW variety, there were a total of 1804 differentially expressed genes triggered by foliar spray of EE-GRSP, of which 1223 genes were induced and the other 581 genes were inhibited (Figure 4a). While in the XC variety, there were 1861 differentially expressed genes in response to foliar spray of EE-GRSP, of which 403 genes were up-regulated and another 1458 genes were down-regulated (Figure 4b). Such a differential response in gene expression was an indication of more responsiveness of the LW variety to EE-GRSP, since a higher number of genes was up-regulated in the LW variety and down-regulated in the XC variety.

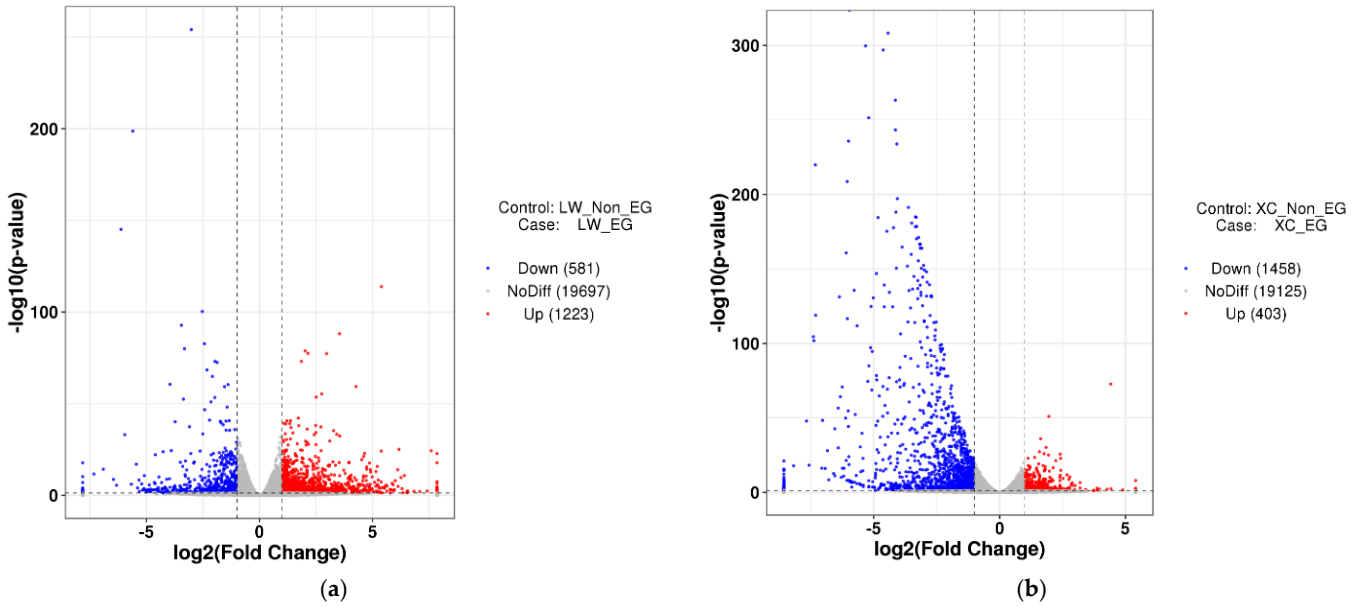

Figure 4. Changes in gene expression in fruits of two late-ripening sweet oranges, Lane Late Navel (LW) (**a**) and Rohde Red Valencia (XC) (**b**) after foliar spray of easily extractable glomalin-related soil protein (EE-GRSP). Abbreviation: LW_EG, the LW variety sprayed with EE-GRSP; LW_Non_EG, the LW variety not sprayed with EE-GRSP; XC_EG, the XC variety sprayed with EE-GRSP; XC_Non_EG, the XC variety not sprayed with EE-GRSP.

3.5. Gene Ontology (GO) Enrichment Analysis

The GO enrichment analysis of differentially expressed genes was classified according to biological process (BP), cell component (CC), and molecular function (MF), besides the selection of the top 20 GO term items with the lowest false discovery rate value, showing significant enrichment (Figure 5a–d). In the LW variety, differentially expressed genes were listed following the trend of MF > CC > BP (Figure 5a). The top 20 GO items were presented in detail in Figure 5b, of which catalytic activity displayed the most GO items. In the XC variety, the GO enrichment analysis showed the decreasing order BP > MF > CC (Figure 5c). The top 20 GO items were shown in Figure 5d, of which membrane and relevant components were most enriched in the XC variety.

3.6. Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment

All enriched KEGG pathways were screened, and a total of 20 significantly enriched KEGG pathways were obtained (Figure 6a–d). In the LW variety, the KEGG enrichment pathway was divided into cellular processes, organismal systems, metabolism, and environmental information processing, of which the metabolism pathway dominated (Figure 6a). The top 20 enriched KEGG pathways had been presented in Figure 6b, of which phenylpropanoid biosynthesis was associated with the highest number of KEGG pathways.

In the XC variety, the KEGG enrichment pathway was divided into human diseases, organismal systems, metabolism, and environmental information processing, of which the metabolism pathway dominated (Figure 6c). The top 20 enrichment KEGG pathways were shown in Figure 6d.

3.7. Starch and Sucrose Metabolism

The starch and sucrose metabolism pathways were affected by foliar spray of EE-GRSP between two sweet orange varieties (Supplementary Figure S1a,b). In the LW variety, a total of 15 differential genes were involved, of which ten genes were up-regulated and five genes were down-regulated (Table 2). In the XC variety, 13 genes showed differential changes, of which only 2 genes were up-regulated, whereas 11 genes were down-regulated.

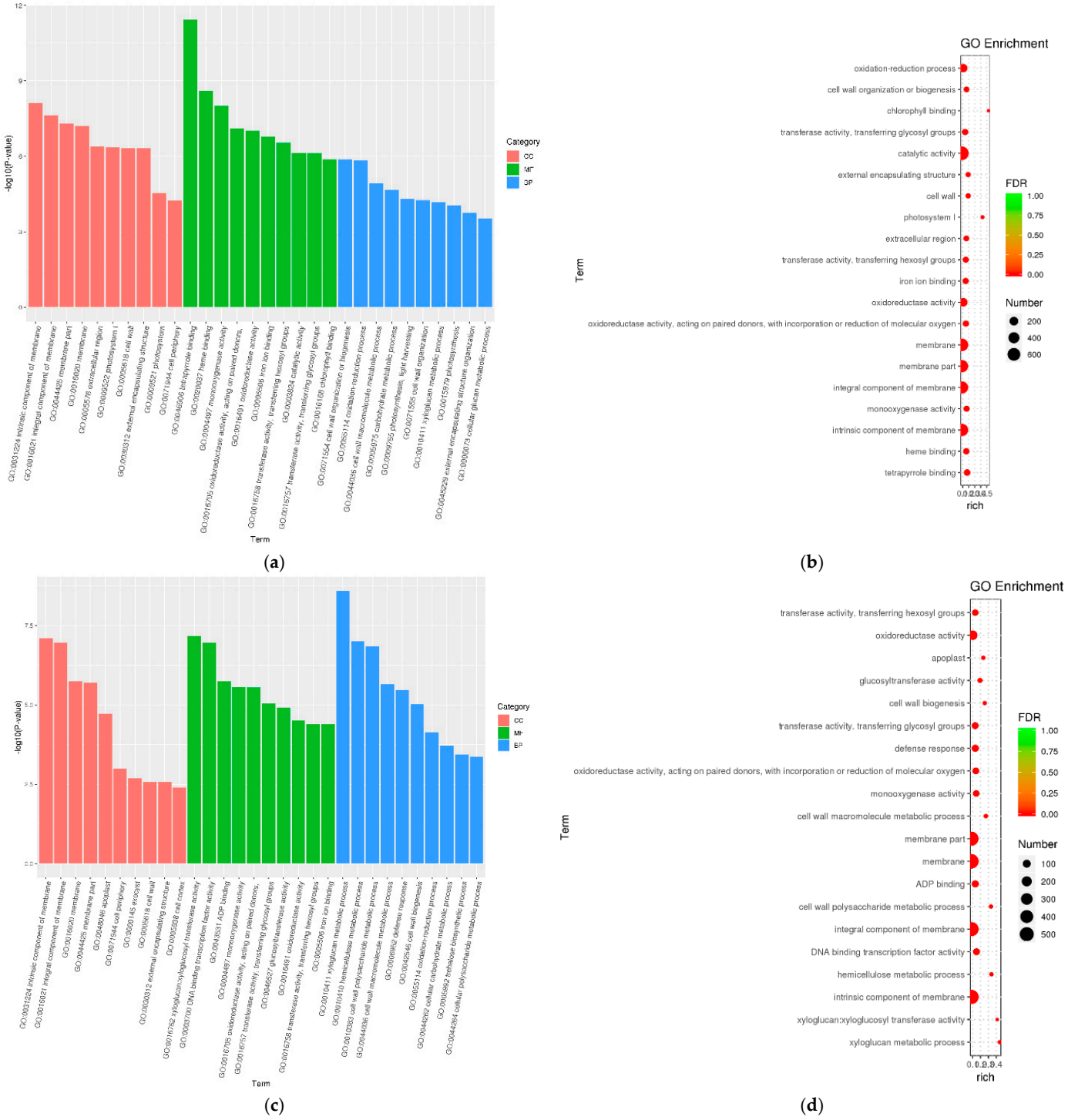

Figure 5. Changes in gene ontology (GO) enrichment analysis in fruits of two late-ripening sweet oranges, Lane Late Navel (LW) (**a**,**b**) and Rohde Red Valencia (XC) (**c**,**d**) after foliar spray of easily extractable glomalin-related soil protein (EE-GRSP). Abbreviation: LW_EG, the LW variety sprayed with EE-GRSP; LW_Non_EG, the LW variety not sprayed with EE-GRSP; XC_EG, the XC variety sprayed with EE-GRSP; XC_Non_EG, the XC variety not sprayed with EE-GRSP.

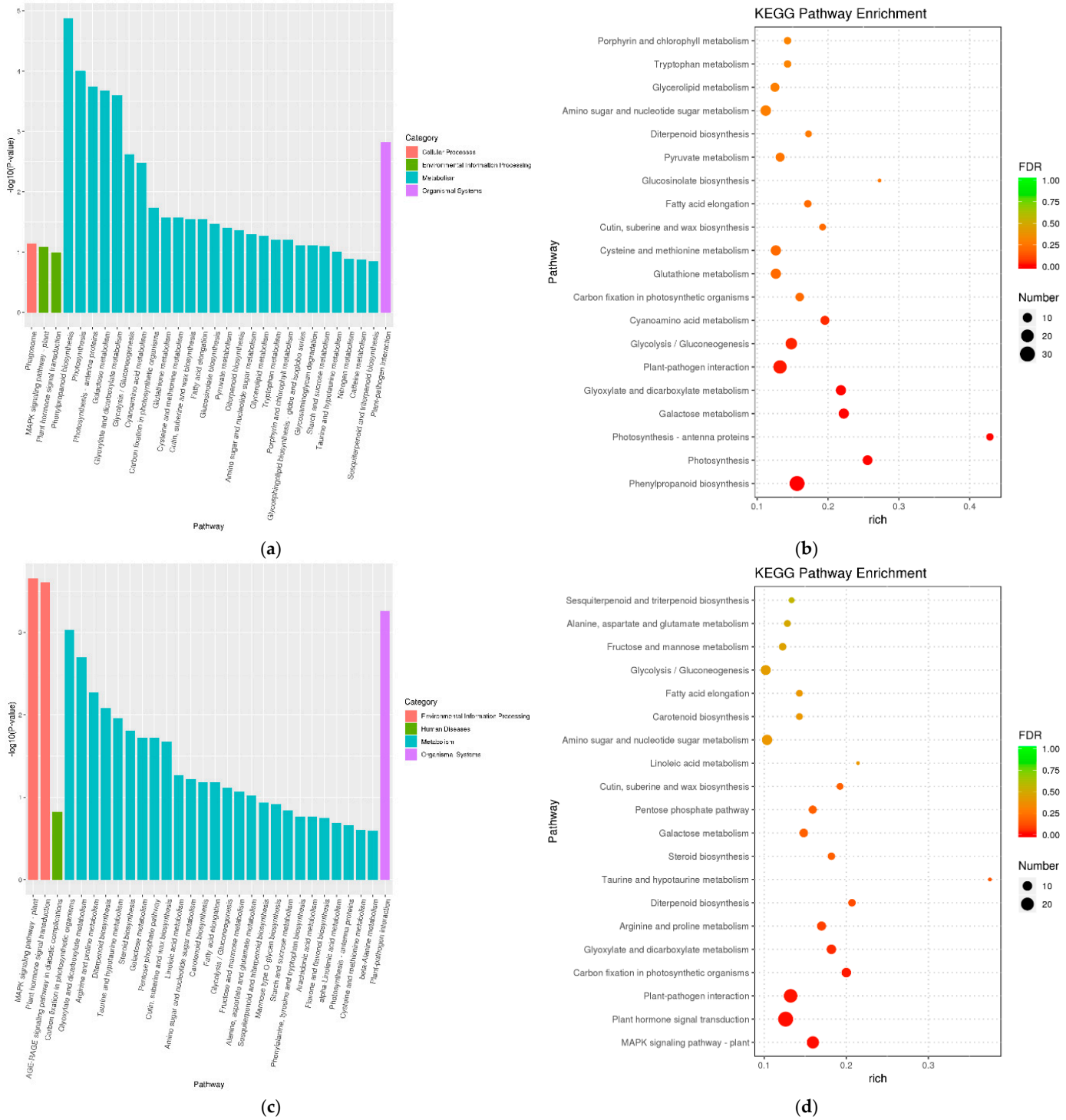

Figure 6. Changes in Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway in fruits of two late-ripening sweet oranges, LW (**a**,**b**) and XC (**c**,**d**) after foliar spraying of easily extractable glomalin-related soil protein (EE-GRSP). Abbreviation: LW_EG, the LW variety sprayed with EE-GRSP; LW_Non_EG, the LW variety not sprayed with EE-GRSP; XC_EG, the XC variety sprayed with EE-GRSP; XC_Non_EG, the XC variety not sprayed with EE-GRSP.

Table 2. Gene species of starch and sucrose metabolism.

Sweet Oranges	Up-Regulated Genes	Up-Regulated Gene Number	Down-Regulated Genes	Down-Regulated Gene Number
LW	Cs3g23560 (α-amylase), Cs1g18220 (β-fructofuranosidase), Cs8g13800 (glycoside hydrolase family 3), orange1.1t00611 (ADP-glucose pyrophosphorylase signature 2), Cs5g33760 (glycosyl hydrolase family 9), Cs9g18290 (trehalose 6-phosphate phosphatase), Cs3g26050 (hexokinase family signature), Cs5g01280 (granule-bound starch synthase 1), Cs2g12180 (glycosyl hydrolase family 9), and Cs4g11970 (glycosyl hydrolase family 1 signature)	10	Cs7g01380 (glycoside hydrolase family 1), Cs4g06900 (sucrose synthase), Cs5g19060 (sucrose-phosphate synthase 4), Cs2g07740 (glycosyl hydrolase family 1 signature), and Cs1g01330 (trehalose 6-phosphate phosphatase)	5
XC	Cs5g19060 and Cs7g08390 (α-trehalose-phosphate synthase [UDP-forming] 9)	2	Cs9g06360 (ADP-glucose pyrophosphorylase signature 1), Cs7g11210 (glycosyl hydrolase family 1), Cs9g14600 (trehalose-phosphate phosphatase A), Cs1g18220, orange1.1t03668 (sucrose-phosphate synthase 3), Cs4g02730 (α-trehalose-phosphate synthase [UDP-forming] 11), Cs5g09200 (trehalose-phosphate phosphatase F), Cs3g18450 (trehalose-phosphate phosphatase C), orange1.1t00483 (trehalose 6-phosphate phosphatase), Cs2g12180, and orange1.1t03470 (plant β-amylase signature)	11

3.8. Transcription Factor Family Analysis

We also analyzed the transcription factor family. A total of 2946 transcription factors were involved, belonging to 30 transcription factor families, which represented the families of more than 100 transcription factors such as C3H, C2H2, bHLH, NAC, far1, MYB related, ERF, MYB, bZIP, and ARF (Figure 7). These responses indicated that a large number of transcription factors were triggered by the foliar application of EE-GRSP.

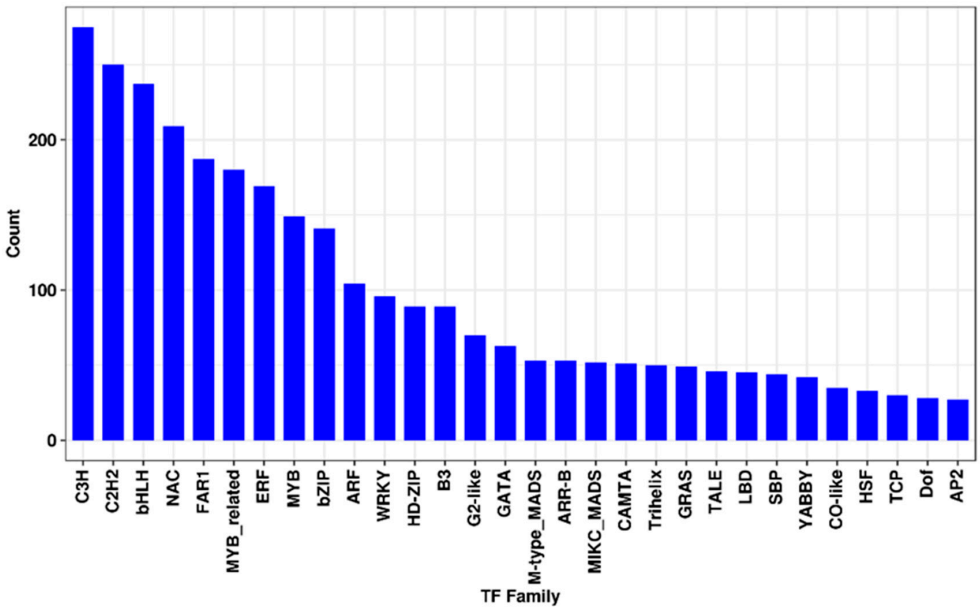

Figure 7. Changes in transcription factor families in two sweet orange varieties under the condition of foliar spray of easily extractable glomalin-related soil protein (EE-GRSP).

4. Discussion

Our previous studies established the improvement in different fruit quality parameters of late-ripening sweet oranges (especially LW variety) in response to foliar spray of EE-GRSP. However, the underlying mechanisms of such a response are yet to be understood using molecular principles. Therefore, we used transcriptome sequencing to study the differences in gene expression in the fruits induced by foliar spraying of EE-GRSP. Through transcriptome sequencing, we observed that the mapping ratio of sequencing reads was 93%, far higher than the standard of 70%, highly similar to the reference genome, indicating a high quality of the data of this transcriptome sequencing.

In the GO analysis in the LW variety, the differentially expressed genes were classified into MF, followed by CC and BP, and the highest GO item was tetrapyrrole binding in the MF type (GO: 0046906). In the XC variety, the GO classification of differentially expressed genes was BP > MF > CC, and the highest GO item was xyloglucan metabolic process (GO: 0010411) in BP. Earlier studies also displayed a similar result in *Amorpha fruticosa* plants inoculated with *Funneliformis mosseae* [29] and *Poncirus trifoliata* plants colonized by *F. mosseae* or *Clariodoglomus etunicatum* [30], coupled with an induced enrichment of BP and MF.

Further analysis of KEGG pathway enrichment showed that, in the LW variety, photosynthesis ranked second in the KEGG metabolism, while, in the XC variety, carbon fixation in photosynthetic organizations ranked first in KEGG metabolism, suggesting that foliar spray of EE-GRSP was preferentially involved in fruit photosynthesis and carbon fixation. Chi et al. [22] studied the treatment of EE-GRSP into rhizosphere of trifoliate orange, which showed a significant increase in chlorophyll content and photosynthetic rate. As reported by Liu et al. [24], the purified EE-GRSP contains a certain amount of C (33.2 mg/g), Mg (4214.83 mg/kg), and Fe (906.37 mg/kg), which are likely to contribute additionally to promoting plant photosynthesis in treated plants and carbon fixation. EE-GRSP is a molecular chaperone which stabilizes the PSII complex and thylakoid membrane [31], thus potentially improving the photosynthetic efficiency of plants [22], although this was not confirmed in our study.

In this study, EE-GRSP application caused an increase in Vitamin C content of the LW variety; however, it had an inhibitory effect on the XC variety. Sucrose, glucose, and fructose contents in fruits were also enhanced by foliar spraying of EE-GRSP, independent of sweet orange varieties [23]. Therefore, we focused on the analysis of the starch and sucrose metabolism pathway. In starch and sucrose metabolism pathways, foliar spraying of EE-GRSP caused different number of differentially expressed genes, thus displaying a strong dependence on late-ripening sweet orange varieties. Interestingly, we observed that *sucrose phosphate synthase* 4 (SPS4, *Cs5g19060*, an enzyme associated with the manipulation of sucrose synthesis) was elevated in response to foliar spray of EE-GRSP in the XC variety and lowered in the LW variety, indicating that foliar spraying of EE-GRSP mainly promoted sucrose production by regulating SPS [32]. On the contrary, Cs2g12180 (glycosyl hydrolase family 9) is an insoluble acid invertase located on the cell wall, which irreversibly decomposes sucrose into glucose and fructose [33]. In addition, glycosyl hydrolases hydrolyze the glycosidic bond between two or more carbohydrates [34]. This gene was up-regulated in the LW and lowered in the XC variety, indicating an important role of EE-GRSP in hexose production and carbohydrate decomposition. Another important sucrose cleavage enzyme, *β-fructofuranosidase* (*Cs1g18220*), was activated by foliar spray of EE-GRSP in the LW variety and lowered in the XC variety, showing a promotion in glucose and fructose production by EE-GRSP through a variety of enzymatic reactions. However, the present study did not use qRT-PCR to verify the expression level of these differentially expressed genes associated with starch and sucrose metabolism pathways, and, thus, further studies are required.

5. Conclusions

In short, through transcriptomic sequencing, we revealed the potential molecular mechanism involved in, and explaining, the response to foliar spray of EE-GRSP in the fruit sucrose metabolism of two late-ripening sweet oranges. More gene expression was up-regulated in the LW than in the XC varieties, especially with regard to sucrose metabolism. Various physiological and metabolic pathways were involved in response to EE-GRSP treatment, and were strongly dependent on the varietal behavior of sweet oranges. These results showed a significant role of EE-GRSP in developing fruit quality, and that it could be effectively used in ensuring sustainable citriculture.

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