Mol Genet Genomics (2016) 291:621–633
DOI 10.1007/s00438-015-1132-5

ORIGINAL ARTICLE

Genome-wide identification and comparative analysis of grafting-responsive mRNA in watermelon grafted onto bottle gourd and squash rootstocks by high-throughput sequencing

Na Liu[1,2] · Jinghua Yang[1] · Xinxing Fu[1] · Li Zhang[1] · Kai Tang[3] · Kateta Malangisha Guy[1] · Zhongyuan Hu[1] · Shaogui Guo[4] · Yong Xu[4] · Mingfang Zhang[1]

Received: 14 May 2015 / Accepted: 9 October 2015 / Published online: 26 October 2015

Abstract Grafting is an important agricultural technique widely used to improve plant growth, yield, and adaptation to either biotic or abiotic stresses. However, the molecular mechanisms underlying grafting-induced physiological processes remain unclear. Watermelon (*Citrullus lanatus* L.) is an important horticultural crop worldwide. Grafting technique is commonly used in watermelon production for improving its tolerance to stresses, especially to the soil-borne fusarium wilt disease. In the present study, we used high-throughput sequencing to perform a genome-wide transcript analysis of scions from watermelon grafted onto bottle gourd and squash rootstocks. Our transcriptome and digital gene expression (DGE) profiling data provided insights into the molecular aspects of gene regulation in grafted watermelon. Compared with self-grafted watermelon, there were 787 and 3485 genes differentially expressed in watermelon grafted onto bottle gourd and squash rootstocks, respectively. These genes were associated with primary and secondary metabolism, hormone signaling, transcription factors, transporters, and response to stimuli. Grafting led to changes in expression of these genes, suggesting that they may play important roles in mediating the physiological processes of grafted seedlings. The potential roles of the grafting-responsive mRNAs in diverse biological and metabolic processes were discussed. Obviously, the data obtained in this study provide an excellent resource for unraveling the mechanisms of candidate genes function in diverse biological processes and in environmental adaptation in a graft system.

Keywords Grafting · Watermelon · Rootstock · RNA-Seq · Gene regulation

Communicated by B. Yang.

Electronic supplementary material The online version of this article (doi:10.1007/s00438-015-1132-5) contains supplementary material, which is available to authorized users.

✉ Yong Xu
xuyong@nercv.org

✉ Mingfang Zhang
mfzhang@zju.edu.cn

[1] Laboratory of Genetic Resources and Functional Improvement for Horticultural Plants, Department of Horticulture, Zhejiang University, Hangzhou 310058, People's Republic of China

[2] Institute of Vegetables, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, People's Republic of China

[3] Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA

[4] Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), National Engineering Research Center for Vegetables, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China

Introduction

Due to the limited availability of arable land and the high market demand for off-season vegetables, crops are continually produced under unsuitable conditions, including extreme temperatures, drought, high soil salinity, and an increased incidence of pests and soil-borne diseases such as fusarium wilt (Lee et al. 2010). These conditions cause various physiological and pathological disorders, leading to severe crop losses. Grafting is an effective and environmentally friendly technique that is widely used in continuous cropping systems around the world (Colla et al. 2010; Davis et al. 2008). Grafting was first used in vegetable crops in

Japan during the late 1920s to limit the effects of soil pathogens by grafting watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] onto pumpkin (*Cucurbita moschata* Duchesne ex. Poir) rootstock. Watermelon was subsequently grafted onto bottle gourd [*Lagenaria siceraria* (Molina) Standl.] (Davis et al. 2008). Grafting is a useful technique to enhance plant vigor through the avoidance of soil pathogens and an increased tolerance of low temperatures, high salinity, and drought. Rootstocks can also enhance nutrient uptake, improve alkalinity tolerance, and limit the negative effects of boron, copper, cadmium, and manganese toxicity (Rouphael et al. 2008; Savvas et al. 2009). It also has a major impact on plant growth and development (Aloni et al. 2010), producing positive effects on vegetable quality, such as improved flavor and increased quantities of health-related compounds (Rouphael et al. 2010). Grafting is consequently increasing in popularity worldwide.

Although the physiological processes responsible for improved tolerance to abiotic and biotic stresses and increased yields and quality of grafted plants have been studied extensively, few studies have investigated the underlying molecular processes. We, therefore, have an incomplete understanding of the mechanism that drives the effects of grafting on plant growth and adaptation to stresses. Grafting may regulate plant growth and tolerance to stresses at transcriptional level. Gene expression is a key event determining plant growth and responses to environmental stimuli (Zahaf et al. 2012). Grafting likely involves a complex signaling system. The genetic mechanisms regulating the effects of grafting on vegetable plants are unclear, and further studies are necessary to identify genes regulated by grafting and to better investigate the signaling events underlying the global plant response to grafting.

Studying the whole genome at the transcriptional level may facilitate the elucidation of the specific mechanisms of grafting-dependent physiological processes. The transcriptome is the overall set of transcribed regions of the genome. Next-generation sequencing technologies such as Roche 454, ABI SOLiD, and Illumina Solexa are powerful strategies for identifying and quantifying gene expression at genome-wide level in unprecedented perspective (Mardis 2008; Morozova and Marra 2008). The extremely high throughput and relatively low cost of these sequencing technologies offer unique opportunities to study genomics and functional genomics in non-model organisms. RNA sequencing (RNA-Seq) based on next-generation deep sequencing is the most powerful tool available for comparative transcriptome profiling (Nookaew et al. 2012; Wang et al. 2009). It is highly reproducible, with few systematic discrepancies among technical replicates (Marioni et al. 2008). The analysis of transcriptional changes of grafted plants may reveal specific genes involved in the regulation of grafting-induced physiological events.

Watermelon (*Citrullus lanatus* L.) belongs to the Cucurbitaceae family, which includes several other important vegetable crops such as melon, cucumber, and pumpkin. The watermelon plant produces large edible fruits containing vitamins, minerals, antioxidants, and fiber, which are important components of the human diet (Collins et al. 2007; Perkins-Veazie et al. 2006). The annual worldwide production of watermelon is approximately 90 million tons, making it among the 20 most important food and agricultural commodities worldwide. Watermelon accounts for approximately 7 % of the worldwide agricultural area devoted to vegetable crops (http://faostat.fao.org). It is an economically and nutritionally important cucurbit crop grown throughout the world. China produces more watermelon than any other country, accounting for more than half of the world's production, of which approximately 20 % of plants are grafted. In a previous study, we evaluated the molecular aspects of miRNA-mediated regulation in grafted watermelon. Using high-throughput sequencing, we discovered 20 and 47 miRNAs were expressed significantly different in watermelon grafted onto bottle gourd and squash rootstocks, respectively, compared with self-grafted watermelon under normal condition (Liu et al. 2013). However, very little information is available regarding the effects of grafting on watermelon at the transcriptional level, especially under conditions with no stress treatment. The transcriptome analysis of grafted watermelon is essential to gain insights into the role of grafting in molecular processes. The recent completion of the watermelon genome sequence (Guo et al. 2013) provides a powerful resource for unraveling such genetic mechanisms.

To explore the genes and gene networks that play regulatory roles in grafted watermelon without any treatments, we used Illumina deep-sequencing technology to study the transcriptome profiles of grafted watermelon (watermelon was grafted onto bottle gourd and squash rootstocks, and self-grafted watermelon was used as control). We explored these data with informatics tools to investigate the transcriptional network and major metabolic activities involved in rootstock-mediated effects on plant growth, development, and acclimation to environmental stresses. Additionally, the differential expression of mRNA was quantified by quantitative real-time RT-PCR (RT-qPCR). Our results constitute a preliminary network in support of future efforts to study the function of candidate genes in the mediation of growth, development, and stress adaptation in grafted watermelon.

Materials and methods

Plant material and growth conditions

Plant materials were selected as described in our previous work (Liu et al. 2013). Watermelon (*Citrullus lanatus* L.

cv. IVSM9) was grafted onto two rootstocks: bottle gourd 'Yongzhen' (Wm/BG) and squash 'Shintozwa' (Wm/Sq). These rootstock varieties are widely used in watermelon production in China and both have high graft compatibility with watermelon (Davis et al. 2008). Watermelon plants grafted onto watermelon (Wm/Wm) were used as the control. For the rootstocks, bottle gourd and watermelon (used for self-graft) were sown directly into trays filled with a mixture of peat/vermiculite (3/1, v/v), while squash was sown 3 days later; watermelon scion seeds were sown 7 days later. When the cotyledon of the watermelon sown for scion had expanded (approximately 1 week), 'top approach grafting' was performed (Davis et al. 2008). The grafted seedlings were maintained at a constant humidity of 95–100 %, a photosynthetic photon flux density (PPFD) of 50 μmol m^{-2} s^{-1} and a temperature of 28–30 °C for 6 days. They were then cultivated in growth chambers at a PPFD of 600 μmol m^{-2} s^{-1} with a photoperiod of 12 h, temperatures of 25 °C (day) and 17 °C (night), and a humidity between 50 % and 85 %. At the two true-leaf stage, the part above the cotyledons of the scion was collected and stored at −80 °C until further use.

To determine the scion biomass, scion height, and leaf area, 12 plants from three replicates were harvested after being grafted for 3 weeks. The scion (the part above the graft union) was separated from the plant, and its height was measured by a ruler. The total leaf area was measured using a leaf area meter (LiCOR 3100, Li-cor, Lincoln, NE, USA). The scion was dried in a forced air oven at 105 °C for 15 min, and then at 72 °C for 72 h to determine the scion biomass.

cDNA preparation and sequencing

Total RNA was extracted from Wm/Wm, Wm/BG, and Wm/Sq separately using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and DNase I was used to remove DNA. The transcriptome assembly library was pooled by mixing equal quantities of RNA from the three grafting combinations, Wm/Wm, Wm/BG, and Wm/Sq. The three digital gene expression (DGE) profiling libraries consisted of separate RNA extracts from scions of different grafting combinations. The experiment was performed on three biological replicates from each of the three grafting combinations. RNA was checked on 1 % agarose gel to avoid possible degradation and contamination and was then examined by a Nano photometer spectrophotometer for the RNA purity. The RNA concentration and integrity were measured by Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Only RNA that passed the quality tests was used for RNA-Seq analysis. The mRNA was isolated using oligo (dT)-attached magnetic beads and cleaved into short fragments (approximately 200 bp), which were used as templates for the synthesis of first-strand and second-strand cDNAs. Sequencing adapters were then ligated to the fragments. The required fragments were purified by agarose gel electrophoresis and enriched by PCR amplification. Finally, all libraries were sequenced using an Illumina HiSeq™ 2000.

cDNA sequence assembly and functional annotation

The raw reads in fastq format were first filtered by removing the adapter sequences and low quality sequences, which included the reads with *N* percentage (i.e., the percentage of nucleotides in reads which could not be sequenced) over 5 % and ones containing more than 20 % nucleotides in read with *Q* value ≤10. The *Q* value represents the sequencing quality of related nucleotides. Only clean reads were used in the subsequent de novo assembly of the transcriptome. The Trinity method was used for the de novo assembly of the clean reads to generate UniGenes (Grabherr et al. 2011).

Gene functional annotation was performed based on the watermelon genome database (Guo et al. 2013). Based on the results of protein database annotation, Blast2Go (Conesa et al. 2005) was employed to obtain Gene Ontology (GO) annotation according to molecular function, biological process, and cellular component ontologies. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to annotate the pathways of these UniGenes with an *E* value threshold of 10^{-5} (Kanehisa et al. 2008).

Identification of differentially expressed genes (DEGs)

Clean reads were mapped to our transcript reference database using the short oligonucleotide analysis package SOAPaligner/soap2 (Li et al. 2009). The unique mapped reads were used in subsequent analyses. For gene expression analysis, the number of unique reads was calculated and then normalized to RPKM (reads per kb per million reads) (Mortazavi et al. 2008). The RPKM method eliminates the influence of different gene lengths and sequencing discrepancies on the calculation of gene expression, allowing the calculated gene expressions to be directly compared among samples. *P* value was used to identify genes differentially expressed between samples. The FDR (false discovery rate) was applied to identify the threshold of the *P* value in multiple tests and analyses. UniGenes differentially expressed between two samples were screened with a threshold of FDR ≤0.05 and the absolute value of log2Ratio ≥1. Furthermore, GO and KEGG pathway enrichment analysis was used to identify significantly enriched functional classifications or pathways in differentially up- and down-regulated UniGenes, as previously described (Shen et al. 2013). Briefly, enrichment analysis for

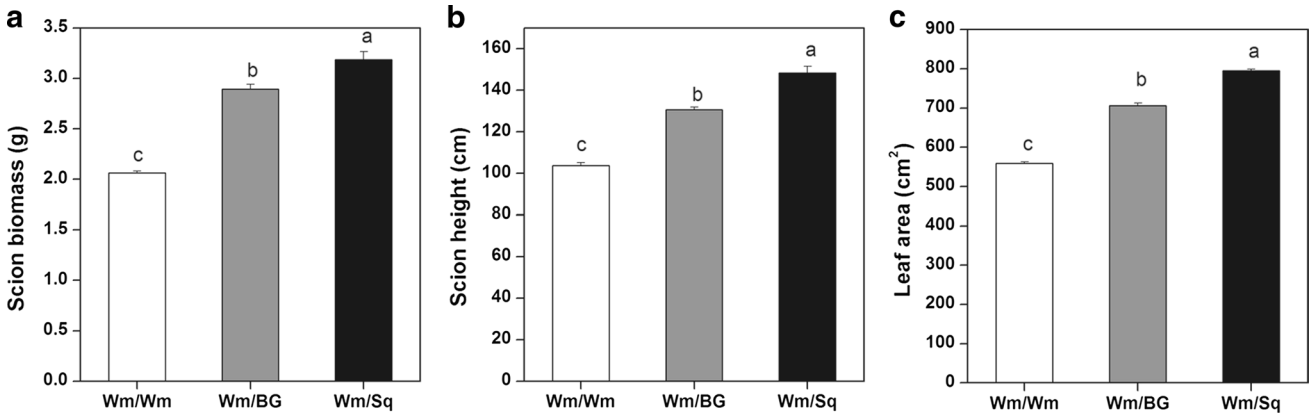

Fig. 1 Effects of graft combination on shoot biomass (**a**), shoot height (**b**), and leaf area (**c**) of watermelon plants. *Different letters* indicate that the parameters are significantly different according to Duncan's test ($P < 0.05$)

GO and KEGG pathways of differentially expressed UniGenes was performed using the hypergeometric test. We first calculated the significance of GO and pathway enrichment, then obtained the gene lists for each significant GO and KEGG pathways, and plotted these as figures.

Quantitative real-time RT-PCR (RT-qPCR) analysis

Total RNA used for RT-qPCR analysis was extracted from scions of Wm/Wm, Wm/BG, and Wm/Sq plants with three biological replicates. Total RNA was extracted as described above, genomic DNA was removed with DNaseI, and the total RNA concentration was measured. First-strand cDNA was synthesized from 3 μg of DNA-free RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. The cDNA was then used as the template for RT-qPCR. Primer sequences are listed in Supplementary Table S1. The reactions were performed on an ABI StepPlus™ cycler (Applied Biosystems, Foster City, CA) with the Fast Start Universal SYBR Green Master Mix (Roche, Basel, Switzerland). The two-step RT-qPCR program began at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. Fluorescent signals were collected at each polymerization step. Expression was calculated as $2^{-\Delta\Delta Ct}$ and normalized to that of the control gene *18S rRNA* (Livak and Schmittgen 2001).

Results

Illumina sequencing, assembly, and functional classification of the grafted watermelon transcriptome

To explore the genes and gene networks that play regulatory roles in grafted watermelon under normal condition, we performed transcriptome and expression profiles analysis of watermelon grafted onto the bottle gourd rootstock 'Yongzhen' (Wm/BG) and the squash rootstock 'Shintozwa' (Wm/Sq), with self-grafted watermelon (Wm/Wm) as the control. For 3-week-old seedlings, the scion biomass, scion height, and total leaf area were significantly larger in watermelon grafted onto bottle gourd and squash rootstocks compared with self-grafted watermelon (Fig. 1). Notably, the squash rootstock had a much greater effect on watermelon growth than the bottle gourd rootstock. The scion biomass increased by 40 and 54 % in Wm/BG and Wm/Sq compared with Wm/Wm, respectively. Given that gene expressions usually display significant differences prior to morphologic changes, we chose early stage seedlings for RNA-Seq.

To obtain an overview of the grafted watermelon transcriptome, a cDNA library was generated from equal amounts of RNA isolated from Wm/Wm, Wm/BG, and Wm/Sq, and the library was then paired-end sequenced using the Illumina platform. In total, we obtained 155,309,206 raw reads with a single read length of approximately 200 bp. After cleaning and quality checking, approximately 144,234,876 high-quality reads were assembled into 99,270 contigs with a minimum length of 200 bp. Using paired-end joining and gap-filling, these contigs were further assembled into 27,733 unique sequences with length greater than 1000 bp and N50 length of 1849 bp (half of the assembled bases were incorporated into UniGenes with length of at least 1849 bp) (Supplementary Table S2). Then, we used GO and KEGG assignments to classify the functions of the predicted UniGenes. Approximately 11,623 and 10,069 UniGenes were categorized into three gene ontologies and 145 KEGG pathways, respectively (Supplementary Table S3 and S4). The functionally assigned UniGenes encompassed a comprehensive range of GO categories and biological pathways.

Table 1 Summary for the DGEs of grafted watermelon

Samples	Total number of reads	Reads after QC1	Alignment with transcriptome	Percentage (%)
Wm/Wm	31,185,852	25,825,034	25,027,246	96.91
Wm/BG	26,293,104	22,180,957	21,498,268	96.92
Wm/Sq	24,054,479	20,711,145	19,868,996	95.93

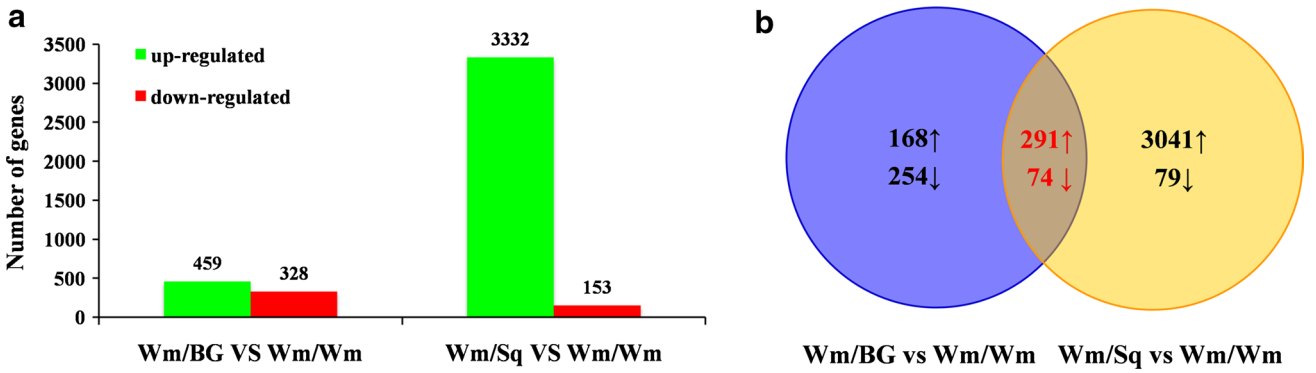

Fig. 2 Changes in gene expression profiles of Wm/BG and Wm/Sq compared with Wm/Wm. **a** The number of up- and down-regulated genes in the comparisons of Wm/Wm vs Wm/BG and Wm/Wm vs Wm/Sq. **b** Venn diagram of up- and down-regulated genes in both Wm/BG and Wm/Sq compared with Wm/Wm. Significance of gene expression differences was judged by FDR ≤ 0.05 and an absolute value of $\log_2$ Ratio ≥ 1

Identification of DEGs

RNA-seq is a robust, sensitive tool for the measurement of gene expression compared to traditional hybridization-based microarray technologies (Wang et al. 2009; Wilhelm and Landry 2009). We analyzed changes in gene expression in Wm/BG and Wm/Sq compared with Wm/Wm. Three DGE libraries (from scion samples of Wm/BG, Wm/Sq, and Wm/Wm) were sequenced to generate between 21 million and 26 million clean reads per library after filtering the raw reads. The total number of mapped reads in each library ranged from 20 to 25 million, and the percentages of these reads ranged from 95.9 to 96.9 % (Table 1). We identified 787 genes that were significantly differentially expressed in Wm/BG compared with Wm/Wm. Of these, 459 were up-regulated and 328 were down-regulated (Fig. 2a; Supplementary Table S5). Notably, many more genes were differentially expressed in the comparison of Wm/Sq and Wm/Wm. A total of 3485 genes were differentially expressed in Wm/Sq compared with Wm/Wm, with 3332 up-regulated and 153 down-regulated (Fig. 2a; Supplementary Table S5). Among these differentially expressed genes, 291 and 74 genes were up- and down-regulated in both Wm/BG and Wm/Sq compared with Wm/Wm, respectively (Fig. 2b; Supplementary Table S6). These results suggested that some genes responded in the same way in both hetero-grafts compared with the homo-graft control.

Validation of RNA-Seq data by RT-qPCR

We randomly selected 20 genes with various degrees of expression levels to validate the RNA-Seq data by RT-qPCR. RNA from the scions of Wm/Wm, Wm/BG, and Wm/Sq was used as the template for RT-qPCR validation. The RT-qPCR data for these genes were generally consistent with the RNA-Seq results (Fig. 3a). Linear regression [(RT-PCR value) = a (RNA − Seq value) + b] analysis showed a correlation coefficient of 0.7935, indicating a positive correlation between the RNA-Seq data and the RT-qPCR data (Fig. 3b). Although the observed fold changes differed slightly between RT-qPCR and RNA-Seq data, this may reflect differences in the sensitivity and specificity of RT-qPCR and high-throughput sequencing technology. These results indicated that the RNA-Seq data were highly reliable.

Functional classification of the DEGs of grafted watermelon

We used GO and KEGG assignments to classify the functions of DEGs of Wm/BG and Wm/Sq compared with Wm/Wm. Comparisons of Wm/BG and Wm/Sq with Wm/Wm revealed significant enrichment in the cellular component category for “plasmodesma”, “cytosol”, and “plasma membrane” terms. In the molecular function category, “protein binding” and “sequence-specific DNA binding

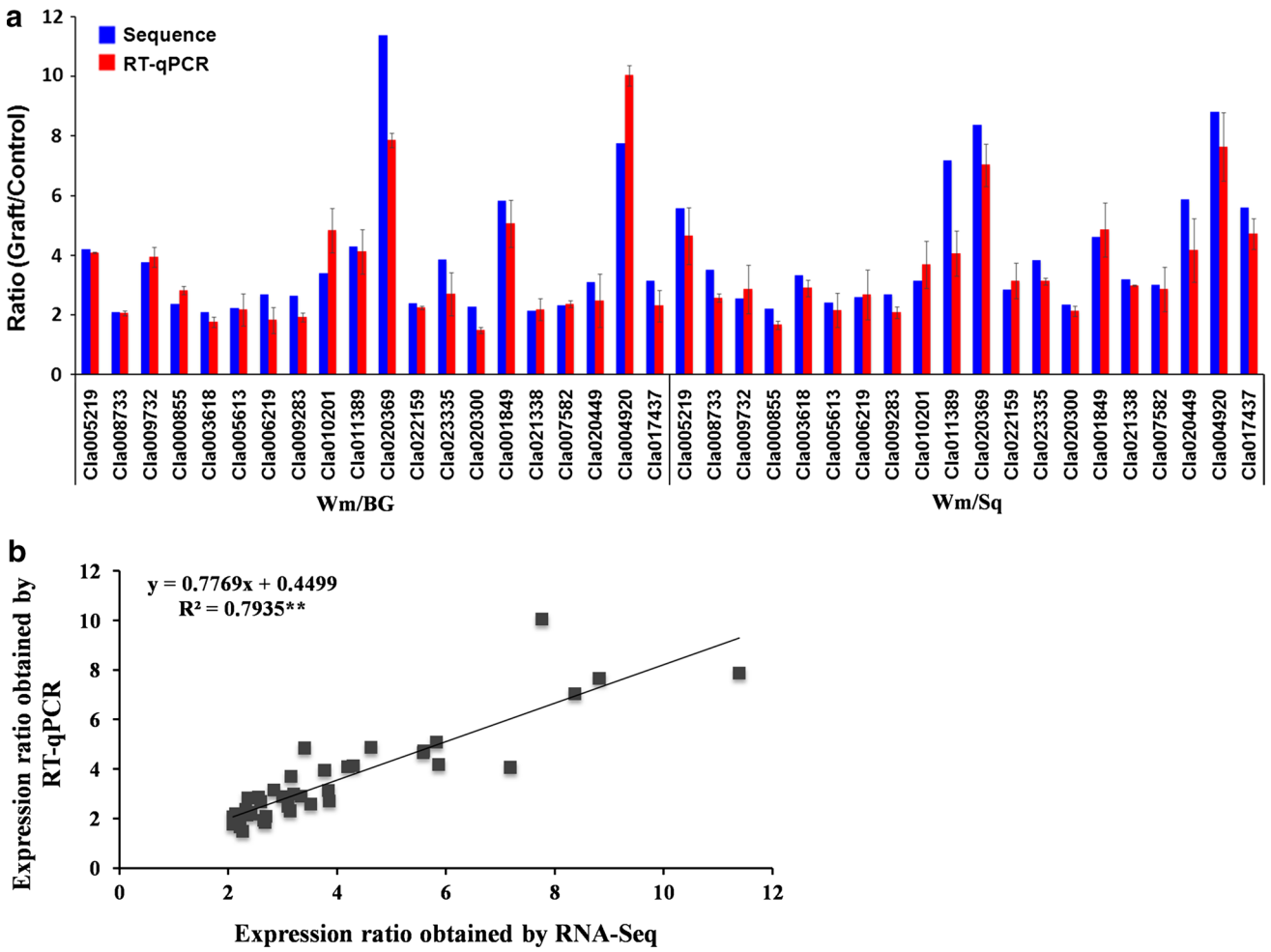

Fig. 3 RT-qPCR validation of differential gene expression of watermelon grafted onto bottle gourd and squash rootstocks (**a**) and coefficient analysis between gene expression ratios obtained from RNA-seq and RT-qPCR data (**b**). ** indicates a significant difference at $P < 0.05$

transcription factor activity" were significantly enriched. In the biological process category, 'response to salt stress', 'defense response to fungus', 'response to abscisic acid stimulus', 'response to cold', 'response to karrikin', 'regulation of transcription, DNA-dependent', 'response to auxin stimulus', and 'serine family amino acid metabolic process' were significantly enriched (Fig. 4). GO analysis of the DEGs revealed that they were functionally enriched in diverse biological processes. Significant enrichment in stress-related biological processes was observed in both Wm/BG and Wm/Sq, strongly indicating that grafting affects the ability of watermelon to adapt to stresses.

We also identified the KEGG pathways in which the DEGs are involved. In Wm/BG vs Wm/Wm and Wm/Sq vs Wm/Wm comparisons, most DEGs in both hetero-graft combinations were mapped to "metabolic pathways", "biosynthesis of secondary metabolites", "starch and sucrose metabolism", and "biosynthesis of phenylpropanoids" (Fig. 5; Supplementary Table S7).

Pathway categories of global up- or down-regulation of gene expression in grafted watermelon

Metabolic processes

A large proportion of the genes that were differentially regulated in watermelon after grafting onto bottle gourd or squash are related to metabolic activity, including starch and sucrose metabolism, galactose metabolism, phenyl metabolism, nitrogen metabolism, purine metabolism, amino sugar and nucleotide sugar metabolism, and the biosynthesis of secondary metabolites, phenylpropanoids, monoterpenoids, and plant hormones (Fig. 4). For example, UDP-glucose 4-epimerase (Cla011477), beta-amylase 6 (Cla004462), galactinol synthase 3 (Cla010955), beta-galactosidase (Cla011212), glutathione S-transferase (Cla021388), glutamine synthetase I (Cla08338), asparagine synthetase (Cla013371), 1-aminocyclopropane-1-carboxylate oxidase-2 (Cla007573), gibberellin

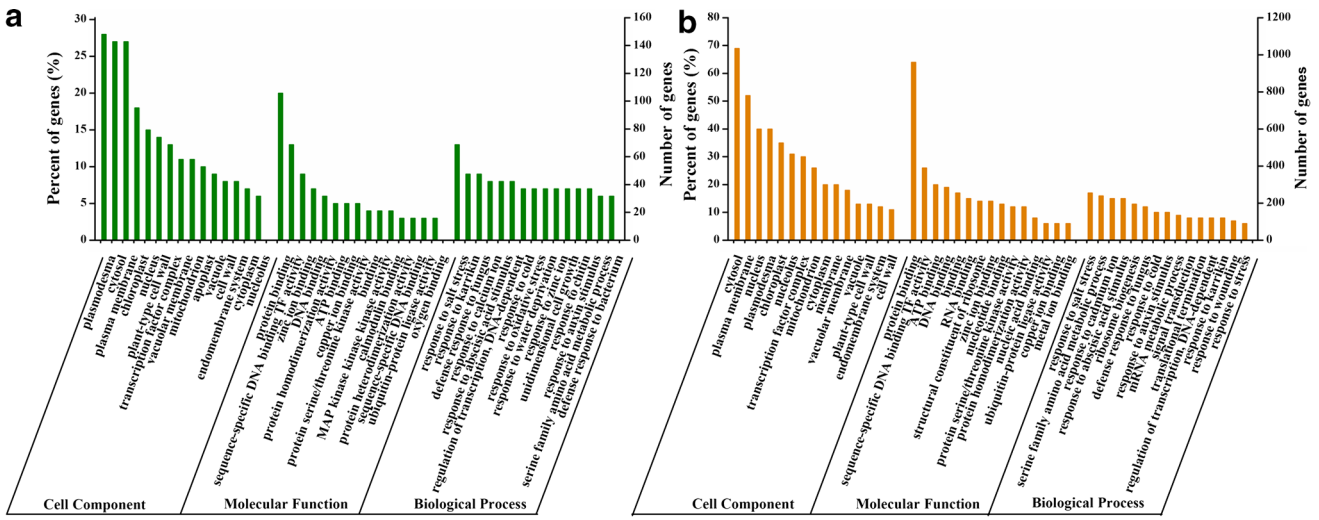

Fig. 4 Functional categories of differentially expressed genes in the Gene Ontology. GO categories that were significantly enriched ($P < 0.05$) were analyzed in pairwise comparisons Wm/BG vs Wm/Wm (**a**) and Wm/Sq vs Wm/Wm (**b**). The abscissa represents GO categories; the ordinate on the *left* represents the percentage of genes within each category, whereas the ordinate on the *right* represents the gene number within each category

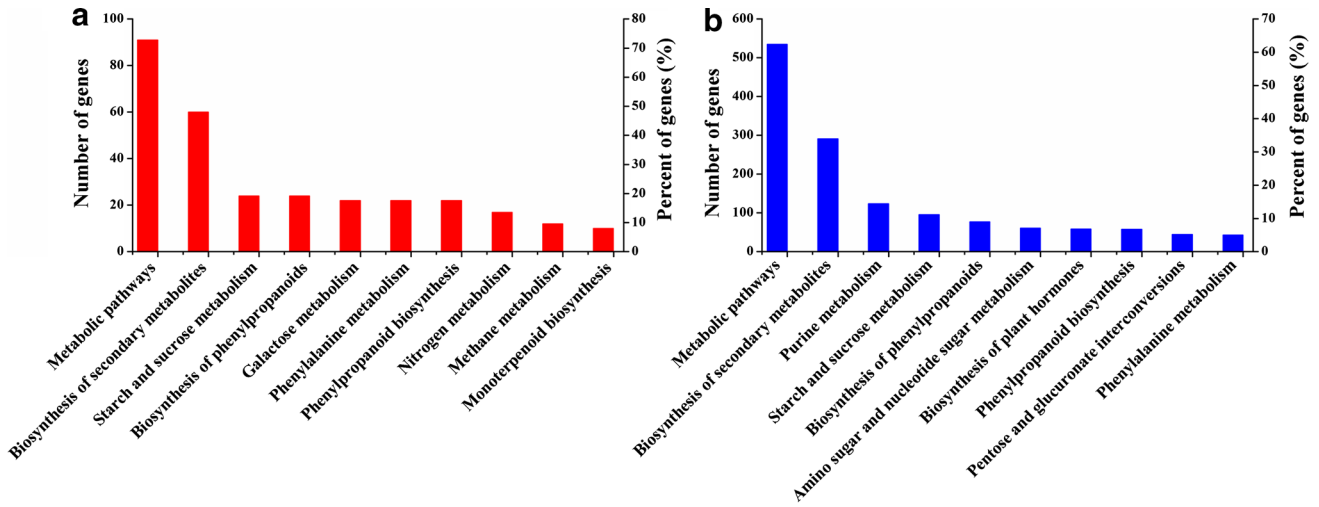

Fig. 5 Top 10 significantly enriched KEGG pathways between Wm/Wm and Wm/BG (**a**) as well as Wm/Wm and Wm/Sq (**b**). The ordinate on the *left* represents the number of genes within each pathway, whereas the ordinate on the *right* represents the percentage of genes within each pathway

3-beta-hydroxylase (Cla015407), and cytochrome P450-related genes, which are involved in primary and secondary metabolic pathways, exhibited differential expression in Wm/BG and Wm/Sq compared with Wm/Wm (Supplementary Table S5).

Signaling

Post-translational modifications of pre-existing proteins are believed to be part of a rapid pathway and to play important roles in plant growth and development (Zhao et al. 2012). In this study, many genes whose products are involved in protein phosphorylation were over-represented in graft-regulated DEGs (Table 2 and Supplementary Table S8). The most abundant protein kinases in graft-regulated DEGs encoded leucine-rich repeat kinases and receptor-like cytoplasmic kinase VII. Other protein kinases, such as SNF1-related protein kinase (SnRK), the GmPK6/AtMPK1 family, MAPKKK, the MAPK family, the CDC2-like kinase family, and calcium-dependent protein kinase, were also

Table 2 DGE data for selected genes related to protein kinase in Wm/BG and Wm/Sq compared with Wm/Wm

UniGene	Self-graft	Hetero-graft	log2 fold change	Gene annotation	Gene description	Kinase classification
Wm/BG vs Wm/Wm						
comp762_c0_seq3	3.91726	10.516	1.42467	Cla019556	CBL-interacting protein kinase 04	PPC:4.2.4 SNF1 Related Protein Kinase (SnRK)
comp16711_c0_seq1	1.91005	5.06496	1.40694	Cla016117	Receptor-like protein kinase At3g21340	PPC:1.2.2 Receptor-Like Cytoplasmic Kinase VII
comp7229_c0_seq1	5.1452	13.06	1.34386	Cla010719	Receptor-like kinase	PPC:1.Other Other Protein Kinase
comp21933_c0_seq1	9.90591	3.57337	−1.471	Cla019541	Protein kinase	PPC:4.2.6 IRE/NPH/PI dependent/S6 Kinase
comp14863_c0_seq1	8.50891	2.81113	−1.59783	Cla013656	Receptor-like protein kinase	PPC:1.12.4 Leucine-Rich Repeat Kinase XI & XII
comp23391_c0_seq1	4.04	1.09183	−1.8876	Cla014506	Receptor-like kinase	PPC:1.12.2 Leucine-Rich Repeat Kinase II & X
comp3941_c0_seq1	90.2087	10.1322	−3.15431	Cla004248	OBP3-responsive gene 1	PPC:4.4.1 Unknown Function Kinase
Wm/Sq vs Wm/Wm						
comp5844_c0_seq3	2.92879	11.4497	1.96693	Cla021931	Receptor-like protein kinase At3g21340	PPC:1.2.2 Receptor-Like Cytoplasmic Kinase VII
comp28496_c0_seq1	2.48578	8.76558	1.81815	Cla006279	Receptor protein kinase-like protein	PPC:1.12.4 Leucine-Rich Repeat Kinase XI & XII
comp13894_c0_seq1	1.75088	5.54601	1.66337	Cla015608	Receptor-like protein kinase	PPC:1.8.1 Leucine-Rich Repeat Kinase I
comp28229_c0_seq1	2.97734	9.37409	1.65465	Cla004964	Receptor-like kinase	PPC:5.1.2 Other Kinase
comp45306_c0_seq1	4.82801	14.7666	1.61283	Cla007542	CBL-interacting protein kinase 20	PPC:4.2.4 SNF1 Related Protein Kinase (SnRK)
comp4733_c0_seq2	3.90623	11.3763	1.54218	Cla008928	Kinase family protein	PPC:2.1.4 GmPK6/ AtMRK1 Family
comp5892_c0_seq1	4.80486	13.6608	1.50748	Cla014790	Serine/threonine kinase	PPC:4.1.6 Unknown Function Kinase
comp10902_c0_seq1	2.62511	7.05839	1.42696	Cla017130	Protein-tyrosine kinase 6	PPC:2.1.5 ATN1 Like Family
comp3941_c0_seq1	90.2087	11.3098	−2.9957	Cla004248	OBP3-responsive gene 1	PPC:4.4.1 Unknown Function Kinase

graft-regulated (Supplementary Table S8). A receptor-like kinase (Cla010719) and a CBL-interacting protein kinase (Cla017212) were shown to be up-regulated in both Wm/BG and Wm/Sq compared with Wm/Wm (Supplementary Table S9).

Plant signal transduction pathways are connected through many levels and by diverse mechanisms, while plant growth is regulated by a set of plant hormones. The majority of genes involved in the regulation of diverse hormones were differentially expressed in Wm/BG and Wm/Sq (Table 3 and Supplementary Table S10). For example, abscisic acid (ABA) receptor PYR1, PYR1 interaction gene protein phosphatase 2C (PP2C), auxin-induced SAUR-like protein, auxin efflux carrier, auxin transporter-like proteins, auxin responsive proteins, dormancy/auxin associated family protein, cytokinin oxidase/dehydrogenase, cytokinin riboside 5 and apos-monophosphate phosphoribohydrolase LOG3, 1-aminocyclopropane-1-carboxylate oxidase, gibberellin-regulated family proteins, gibberellin 3-beta-hydroxylase, and jasmonate-induced protein exhibited different expression patterns in Wm/BG and/or Wm/Sq compared with Wm/Wm. Furthermore, we identified 12 genes involved in ABA, auxin, cytokinin, and ethylene signaling pathways that were significantly up-regulated in both hetero-graft combinations compared with the homograft control (Supplementary Table S9).

Transcription factors

Analogous to the molecular function networks of DEGs, 'sequence-specific DNA binding transcription factor activity' in grafted watermelon (Fig. 6) indicated that 41 and 141 transcription factors (TFs) were differentially expressed in Wm/BG and Wm/Sq, respectively (Supplementary Table S11). Diverse biological activities were determined to be directly or indirectly regulated by these TF genes. AP2/EREBP was one of the most abundantly represented families, with 9 and 19 genes found to change

Table 3 DGE data for selected genes related to hormones in Wm/BG and Wm/Sq compared with Wm/Wm

UniGene	Self-graft		Hetero-graft	log2 fold change	Gene annotation	Gene description
Wm/BG vs Wm/Wm						
comp11384_c0_seq2	0.618491	6.96032	3.49233	Cla007573	1-aminocyclopropane-1-carboxylate oxidase-2	
comp46894_c0_seq1	2.29013	17.7741	2.95628	Cla006604	Abscisic acid receptor PYR1	
comp3209_c0_seq1	4.23837	17.7788	2.06858	Cla005219	Dormancy/auxin associated protein	
comp21471_c0_seq1	1.62323	5.30576	1.7087	Cla002234	Protein phosphatase 2C	
comp118_c0_seq1	41.2595	127.473	1.6274	Cla009633	Auxin-repressed protein	
comp13330_c0_seq1	2.5496	7.56889	1.56981	Cla016627	Auxin-induced SAUR-like protein	
comp5510_c0_seq1	16.0118	4.94337	−1.69557	Cla020839	Gibberellin-regulated family protein	
comp20803_c0_seq1	4.4477	1.25525	−1.82508	Cla022055	Gibberellin receptor GID1-like protein	
comp18747_c0_seq1	16.0778	3.69725	−2.12054	Cla015407	Gibberellin 3-beta-hydroxylase	
Wm/Sq vs Wm/Wm						
comp38473_c0_seq1	0.641302	9.69178	3.91769	Cla002932	Cytokinin oxidase/dehydrogenase 1	
comp21378_c0_seq1	0.509796	5.45727	3.42019	Cla013931	IAA-amino acid hydrolase 9	
comp46894_c0_seq1	2.29013	13.4594	2.55512	Cla006604	Abscisic acid receptor PYR1	
comp3209_c0_seq1	4.23837	23.6386	2.47957	Cla005219	Dormancy/auxin associated protein	
comp1509_c0_seq3	3.50387	15.3848	2.13449	Cla008733	Cytokinin riboside 5&apos-monophosphate phosphoribohydrolase LOG3	
comp21156_c0_seq1	1.84371	7.22382	1.97015	Cla022463	Cytokinin oxidase/dehydrogenase	
comp8529_c0_seq6	1.70441	5.98219	1.8114	Cla015867	Auxin-induced SAUR-like protein	
comp118_c0_seq1	41.2595	143.698	1.80024	Cla009633	Auxin-repressed protein	
comp13002_c0_seq2	1.96381	6.3314	1.68887	Cla007621	Auxin responsive protein	
comp4195_c0_seq1	5.06204	16.2413	1.68187	Cla016932	Jasmonate-induced protein	
comp2372_c0_seq8	2.79744	8.41556	1.58895	Cla003909	Auxin efflux carrier	
comp18747_c0_seq1	16.0778	5.24952	−1.61481	Cla015407	Gibberellin 3-beta-hydroxylase	

expression in Wm/BG and Wm/Sq, respectively. The classification results revealed that bHLH, C2H2, NAC, WRKY, and MYB family members were abundant TFs with significantly differential expression in Wm/BG and Wm/Sq compared with Wm/Wm (Fig. 6). Notably, 23 TFs were significantly up-regulated in both Wm/BG and Wm/Sq compared with Wm/Wm (Supplementary Table S9).

Response to stimulus

Genes that regulate responses to stimuli include those related to abiotic and biotic factors, stress and defense responses, and reactions to endogenous stimuli. These genes are involved in morphological, biochemical, and physiological changes activated by grafting watermelon onto rootstocks. Among genes identified in this category, we focused on pathogen-induced proteins, calcium signaling, and reactive oxygen species (ROS) scavenging. Pathogenesis-related and pathogen-induced proteins participate in plant oxidative stress tolerance by inhibiting hydrogen peroxide production or facilitating its removal (Karpinski et al. 1999). A number of pathogenesis-related proteins (Cla001628, Cla005378, Cla001628, Cla020173, and Cla019802) and pathogen-induced proteins (Cla007582) were up-regulated in Wm/BG and Wm/Sq compared with Wm/Wm (Supplementary Table S12), emphasizing the importance of these proteins in the graft system.

Similarly, the induction of several genes related to 'calcium' and 'calmodulin', such as calcium-binding protein (Cla020681, Cla001741), calmodulin-binding protein (Cla001741 and Cla020869), calcium-responsive transactivator (Cla006556), and a putative mitochondrial calcium uniporter, LETM1-like (Cla004386), demonstrates that calcium and calmodulin play graft-mediated roles in watermelon (Supplementary Table S12). The high expression of several candidate genes related to ROS scavenging, such as glutathione S-transferases (Cla018159, Cla021388, Cla018150, Cla018163, Cla005410, and Cla014676), ascorbate oxidase (Cla004750, Cla004752, and Cla004755) and peroxidase (Cla003194, Cla018276, Cla002251, and Cla003191), supports the differential regulation of oxidative stress mechanisms in grafted watermelon. Moreover, most of the identified ROS scavenging-related genes were up-regulated in both hetero-grafts compared with the homo-graft control (Supplementary Table S9).

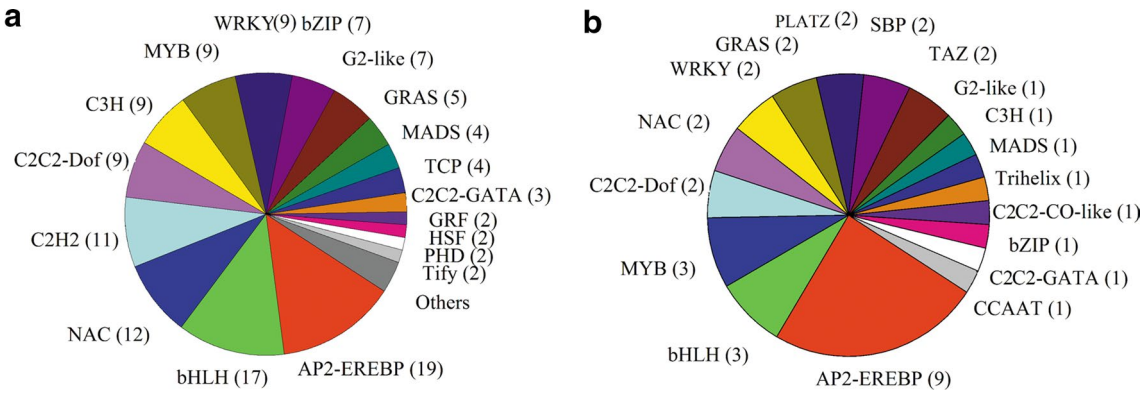

Fig. 6 Classification of different expressed TFs in Wm/BG (**a**) and Wm/Sq (**b**) compared with Wm/Wm

Transport

Genes related to transport composed a large proportion of the identified DEGs (Supplementary Table S13). This category includes genes encoding membrane-transport proteins. For example, aquaporins (Cla009718, Cla018286, Cla022216, Cla020563, and Cla013772), membrane proteins that are responsible for transport, particularly of water (Baiges et al. 2002), were significantly differentially expressed in Wm/BG and Wm/Sq. The differential expression of genes related to the transport of ATP-binding cassettes, amino acids, cations/H^+, nitrate, phosphate, sulfate, and vacuolar iron, zinc, and copper was also detected.

Discussion

Grafting is an important technique that is widely used in commercial horticultural crop production to improve plant growth, stress resistance, and productivity (Lee et al. 2010). However, the molecular mechanisms involved in these processes remain largely unknown. As an important food and agricultural commodity, watermelons are widely cultivated worldwide, with most being grafted. This study used RNA-Seq technology to map in detail the transcriptional differences between Wm/BG or Wm/Sq and self-grafted watermelon. To our knowledge, this is the first study to use RNA-Seq to identify large numbers of genes involved in various pathways in watermelon grafts.

A total of 787 and 3485 genes were significantly differentially expressed between Wm/BG vs Wm/Wm and Wm/Sq vs Wm/Wm, respectively. Of these, 365 genes were shared by both Wm/BG and Wm/Sq compared with Wm/Wm. The differentially expressed genes in grafted watermelon should be related to the regulatory role of grafting in plant growth, development, and adaptive responses to stresses. Rootstocks are known to have a wide range of effects on scion development (Gregory et al. 2013; Lee et al. 2010). In our present work, the plant biomass, scion height, and leaf area were significantly increased in Wm/BG and Wm/Sq compared with Wm/Wm. Previous studies on grafted apple and grapevine suggested that the shoot apex of grafted trees was a more effective carbon sink that promoted more vigorous growth (Cookson and Ollat 2013; Jensen et al. 2003). Similarly, in our study, grafted watermelon with increased scion vigor resulted in the up-regulation of many genes from the functional categories of carbohydrate metabolism and sugar transporters, suggesting that a similar mechanism may exist in watermelon. Additionally, we observed that several homeobox-leucine zipper proteins (Cla000198, Cla015276, and Cla014061) had higher expression level in hetero-grafted watermelon compared with homo-grafted watermelon. Consistent with our result, Jensen et al. (2003) confirmed that the expression level of homeobox-leucine zipper protein correlated with plant size, implying that the protein is involved in the grafting trigger of a more vigorous scion.

In plant cells, regulatory networks are activated in response to different developmental stages and various abiotic and biotic stimuli. These networks are often conserved between different abiotic stresses and between different plant species (Bressan et al. 2009). Many of the DEGs identified in the present study are orthologous to genes that have previously been described to play central roles in adaptation to environmental stresses and the regulation of plant growth. Consistent with these findings, DEGs in the shoot tip of apple trees grafted on to different rootstocks were shown to be enriched in response to abiotic and biotic stress, as well as other biological processes (Jensen et al. 2003, 2012). Our data provide evidence that some hormones are involved in the regulatory role of grafting. Based on the GO enrichment analysis, 'response to abscisic acid stimulus' was significantly enriched in both pairwise comparisons of the present study. Parallel, the expression of ABA signaling pathway related genes including the ABA receptor PYR1 (Cla006604, Cla018017, Cla018075, and

Cla007250), protein phosphatase 2C (PP2C) (Cla018947 and Cla002234), and SNF1-related protein kinase (SnRKs) (Cla019556, Cla003149, Cla017212, Cla007542, and Cla012230) were induced by graft. In agreement with this, ABA signaling has been proposed as a mechanism of rootstock control of scion growth (Gregory et al. 2013). ABA is known to regulate the expression of many genes in plants and has critical functions in stress resistance and growth and development (Cutler et al. 2010; Fujii et al. 2009; Miyazono et al. 2009; Zhu 2002). How these ABA pathway genes are regulated by grafting requires further investigation. Additionally, the up-regulation of drought responsive genes could be related to the graft union not yet being fully functional at the time of scion harvest (Kumari et al. 2015).

Recently, increasing evidences have revealed that miRNA-mediated gene regulation play important roles in plant growth and the response to environmental stresses at both transcriptional and post-transcriptional levels (Dugas and Bartel 2004; Khraiwesh et al. 2012; Mallory and Vaucheret 2006). We previously identified a number of differentially expressed miRNAs and their corresponding targets in watermelon grafted onto bottle gourd and squash rootstocks (Liu et al. 2013). Among these, miR172 was shown to be less abundant in Wm/Sq than in Wm/Wm. Consistent with this observation, the yellow stripe-like transporter (YSL), that was predicted to be one of the targets of miR172, was significantly up-regulated in Wm/Sq compared with Wm/Wm. Members of the YSL family play important roles in plant iron homeostasis (Lee et al. 2012), and a great deal of evidence indicates the importance of YSLs in the long distance transport of metals such as copper, cadmium, and nickel (Conte and Walker 2012; Zheng et al. 2012). Grafting has also been shown to improve the plant tolerance to metal stress (Savvas et al. 2010), so we hypothesize that the miR172-YSL regulation network may be involved in the graft-mediated effects on metal stress tolerance in watermelon, although the specific mechanism of this involvement requires further investigation.

It is notable that more genes were differentially expressed in Wm/Sq than in Wm/BG compared with Wm/Wm (Supplementary Table S5). Consistent with these observations, we also observed increases in plant biomass of 40 and 54 % in Wm/BG and Wm/Sq, respectively (Fig. 1). These results suggest that the squash rootstock has a much greater effect on the watermelon plant then the bottle gourd rootstock. It is, therefore, possible that some DEGs are specific to squash rootstock-mediated grafting. In the present study, 36 genes encoding pentatricopeptide repeat (PPR) proteins and 13 genes encoding WD40 repeat-containing proteins were up-regulated in Wm/Sq compared with Wm/Wm, but not in Wm/BG (Supplementary Table S14). PPR proteins are involved in the processing of mitochondrial RNAs and related to ATP synthesis and ROS generation (He et al. 2012; Liu et al. 2010). WD40 domain proteins function in several cellular, metabolic, and molecular pathways, and play important roles in plant development and stress signaling (Mishra et al. 2012). We speculate that PPR and WD40 proteins may act as hubs in cellular networks and play important roles in specific developmental events and stress adaptation in watermelon grafted onto squash rootstock.

In conclusion, grafting is particularly important for the cultivation of cucurbit crops such as watermelon. High-throughput sequencing revealed that 787 and 3485 genes were significantly differentially expressed in watermelon grafted onto bottle gourd and squash rootstocks, respectively, compared with self-grafted watermelon. Many of these genes are associated with primary and secondary metabolism, hormone signaling, transcription factor regulation, transport, and responses to stimuli. The expression patterns of these genes indicate that the related biological pathways may be responsible for the improved performance. Although the gene-to-phenotype relationship of these genes was not examined in the current study, detailed functional analyses in the future should provide further insights into mechanisms underlying the improved performance of watermelon grafts. Taken together, the identification of entire sets of gene expression patterns obtained in this study provides a broad overview of the molecular mechanisms of the complex regulatory system of grafted watermelon, as well as a rich list of candidate genes for further functional analysis.

Acknowledgments We thank Dr. Huiming Zhang of Shanghai Center for Plant Stress Biology, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences for critical comments on this paper. This work was supported by a grant from the National Natural Science Foundation of China (NSFC31372077), the earmarked fund for Modern Agro-industry Technology Research System (CARS-26-17) of China, the National Key Technology R&D Program of China (2011BAD12B04), Science and Technology Program of Zhejiang province (2011C12001), and the Science and Technology Program for Agricultural Breeding of Zhejiang province (2012C129031-2-11).

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical standard This article does not contain any studies with human participants or animals performed by any of the authors.

References

Aloni B, Cohen R, Karni L, Aktas H, Edelstein M (2010) Hormonal signaling in rootstock-scion interactions. Sci Hortic-Amsterdam 127:119–126

Baiges I, Schaffner AR, Affenzeller MJ, Mas A (2002) Plant aquaporins. Physiol Plantarum 115:175–182

Bressan R, Bohnert H, Zhu JK (2009) Abiotic stress tolerance: from gene discovery in model organisms to crop improvement. Mol Plant 2:1–2

Colla G, Rouphael Y, Leonardi C, Bie ZL (2010) Role of grafting in vegetable crops grown under saline conditions. Sci Hortic-Amsterdam 127:147–155

Collins JK, Wu GY, Perkins-Veazie P, Spears K, Claypool PL, Baker RA, Clevidence BA (2007) Watermelon consumption increases plasma arginine concentrations in adults. Nutrition 23:261–266

Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21:3674–3676

Conte SS, Walker EL (2012) Genetic and biochemical approaches for studying the yellow stripe-like transporter family in plants. Curr Top Membr 69:295–322

Cookson SJ, Ollat N (2013) Grafting with rootstocks induces extensive transcriptional re-programming in the shoot apical meristem of grapevine. BMC Plant Biol 13:147

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Davis AR, Perkins-Veazie P, Sakata Y, Lopez-Galarza S, Maroto JV, Lee SG, Huh YC, Sun ZY, Miguel A, King SR, Cohen R, Lee JM (2008) Cucurbit grafting. Crit Rev. Plant Sci 27:50–74

Dugas DV, Bartel B (2004) MicroRNA regulation of gene expression in plants. Curr Opin Plant Biol 7:512–520

Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutler SR, Sheen J, Rodriguez PL, Zhu JK (2009) In vitro reconstitution of an abscisic acid signalling pathway. Nature 462:660–664

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29:644–652

Gregory PJ, Atkinson CJ, Bengough AG, Else MA, Fernandez-Fernandez F, Harrison RJ, Schmidt S (2013) Contributions of roots and rootstocks to sustainable, intensified crop production. J Exp Bot 64:1209–1222

Guo SG, Zhang JG, Sun HH, Salse J, Lucas WJ, Zhang HY, Zheng Y, Mao LY, Ren Y, Wang ZW, Min JM, Guo XS, Murat F, Ham BK, Zhang ZL, Gao S, Huang MY, Xu YM, Zhong SL, Bombarely A, Mueller LA, Zhao H, He HJ, Zhang Y, Zhang ZH, Huang SW, Tan T, Pang EL, Lin K, Hu Q, Kuang HH, Ni PX, Wang B, Liu JA, Kou QH, Hou WJ, Zou XH, Jiang J, Gong GY, Klee K, Schoof H, Huang Y, Hu XS, Dong SS, Liang DQ, Wang J, Wu K, Xia Y, Zhao X, Zheng ZQ, Xing M, Liang XM, Huang BQ, Lv T, Wang JY, Yin Y, Yi HP, Li RQ, Wu MZ, Levi A, Zhang XP, Giovannoni JJ, Wang J, Li YF, Fei ZJ, Xu Y (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51–58

He JN, Duan Y, Hua DP, Fan GJ, Wang L, Liu Y, Chen ZZ, Han LH, Qu LJ, Gong ZZ (2012) DEXH box RNA helicase-mediated mitochondrial reactive oxygen species production in arabidopsis mediates crosstalk between abscisic acid and auxin signaling. Plant Cell 24:1815–1833

Jensen PJ, Rytter J, Detwiler EA, Travis JW, McNellis TW (2003) Rootstock effects on gene expression patterns in apple tree scions. Plant Mol Biol 53:493–511

Jensen PJ, Halbrendt N, Fazio G, Makalowska I, Altman N, Praul C, Maximova SN, Ngugi HK, Crassweller RM, Travis JW, McNellis TW (2012) Rootstock-regulated gene expression patterns associated with fire blight resistance in apple. BMC Genom 13:9

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T, Yamanishi Y (2008) KEGG for linking genomes to life and the environment. Nucleic Acids Res 36:D480–D484

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Science 284:654–657

Khraiwesh B, Zhu JK, Zhu JH (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Bba-Gene Regul Mech 1819:137–148

Kumari A, Kumar J, Kumar A, Chaudhury A, Singh SP (2015) Grafting triggers differential responses between scion and rootstock. PLoS One 10:e0124438

Lee JM, Kubota C, Tsao SJ, Bie Z, Echevarria PH, Morra L, Oda M (2010) Current status of vegetable grafting: diffusion, grafting techniques, automation. Sci Hortic-Amsterdam 127:93–105

Lee S, Ryoo N, Jeon JS, Guerinot ML, An G (2012) Activation of rice yellow stripe1-Like 16 (OsYSL16) enhances iron efficiency. Mol Cells 33:117–126

Li RQ, Yu C, Li YR, Lam TW, Yiu SM, Kristiansen K, Wang J (2009) SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25:1966–1967

Liu Y, He JN, Chen ZZ, Ren XZ, Hong XH, Gong ZZ (2010) ABA overly-sensitive 5 (ABO5), encoding a pentatricopeptide repeat protein required for cis-splicing of mitochondrial nad2 intron 3, is involved in the abscisic acid response in Arabidopsis. Plant J 63:749–765

Liu N, Yang JH, Guo SG, Xu Y, Zhang MF (2013) Genome-Wide identification and comparative analysis of conserved and novel micrornas in grafted watermelon by high-throughput sequencing. PLoS ONE 8:e57359

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2(T)(-\text{Delta Delta C})$ method. Methods 25:402–408

Mallory AC, Vaucheret H (2006) Functions of microRNAs and related small RNAs in plants. Nat Genet 38:S31–S36

Mardis ER (2008) The impact of next-generation sequencing technology on genetics. Trends Genet 24:133–141

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res 18:1509–1517

Mishra AK, Puranik S, Prasad M (2012) Structure and regulatory networks of WD40 protein in plants. J Plant Biochem Biot 21:S32–S39

Miyazono K, Miyakawa T, Sawano Y, Kubota K, Kang HJ, Asano A, Miyauchi Y, Takahashi M, Zhi YH, Fujita Y, Yoshida T, Kodaira KS, Yamaguchi-Shinozaki K, Tanokura M (2009) Structural basis of abscisic acid signalling. Nature 462:609–614

Morozova O, Marra MA (2008) Applications of next-generation sequencing technologies in functional genomics. Genomics 92:255–264

Mortazavi A, Williams BA, Mccue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5:621–628

Nookaew I, Papini M, Pornputtapong N, Scalcinati G, Fagerberg L, Uhlen M, Nielsen J (2012) A comprehensive comparison of RNA-Seq-based transcriptome analysis from reads to differential gene expression and cross-comparison with microarrays: a case study in Saccharomyces cerevisiae. Nucleic Acids Res 40:10084–10097

Perkins-Veazie P, Collins JK, Davis AR, Roberts W (2006) Carotenoid content of 50 watermelon cultivars. J Agr Food Chem 54:2593–2597

Rouphael Y, Cardarelli M, Colla G, Rea E (2008) Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. HortScience 43:730–736

Rouphael Y, Schwarz D, Krumbein A, Colla G (2010) Impact of grafting on product quality of fruit vegetables. Sci Hortic-Amsterdam 127:172–179

Savvas D, Papastavrou D, Ntatsi G, Ropokis A, Olympios C, Hartmann H, Schwarz D (2009) Interactive effects of grafting and manganese supply on growth, yield, and nutrient uptake by tomato. HortScience 44:1978–1982

Savvas D, Colla G, Rouphael Y, Schwarz D (2010) Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. Sci Hortic-Amsterdam 127:156–161

Shen Y, Zhang YZ, Chen J, Lin HJ, Zhao MJ, Peng HW, Liu L, Yuan GS, Zhang SZ, Zhang ZM, Pan GT (2013) Genome expression profile analysis reveals important transcripts in maize roots responding to the stress of heavy metal Pb. Physiol Plantarum 147:270–282

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57–63

Wilhelm BT, Landry JR (2009) RNA-Seq-quantitative measurement of expression through massively parallel RNA-sequencing. Methods 48:249–257

Zahaf O, Blanchet S, de Zelicourt A, Alunni B, Plet J, Laffont C, de Lorenzo L, Imbeaud S, Ichante JL, Diet A, Badri M, Zabalza A, Gonzalez EM, Delacroix H, Gruber V, Frugier F, Crespi M (2012) Comparative transcriptomic analysis of salt adaptation in roots of contrasting medicago truncatula genotypes. Mol Plant 5:1068–1081

Zhao ZG, Tan LL, Dang CY, Zhang H, Wu QB, An LZ (2012) Deep-sequencing transcriptome analysis of chilling tolerance mechanisms of a subnival alpine plant. Chorispora bungeana. BMC Plant Biol 12:222

Zheng LQ, Yamaji N, Yokosho K, Ma JF (2012) YSL16 Is a phloem-localized transporter of the copper-nicotianamine complex that is responsible for copper distribution in rice. Plant Cell 24:3767–3782

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273