Transcriptome Analysis, CmMYB1 Gene Clone From Cucumis Melo L. And Its Functional Roles Under Salt Stress

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Research Article

Abstract

MYB TFs plays an important role in plant adaptation to abiotic stress, especially in response to salt stress. Preliminary research found that the stress resistance of the two melon varieties 'M15' and 'baogua' are quite different. To this end, we compared the transcriptomes of 'M15' and 'baogua'. Transcriptome analysis found that the expression levels of 12 MYB transcription factors were significantly different between the two varieties.*CmMYB1* gene was cloned from muskmelon (*Cucumis melo* L.), and the subcellular localization results showed that *CmMYB1* was expressed in the cytoplasm and nucleus. Transform *CmMYB1* gene into Arabidopsis thaliana, observe T3 phenotypes during the vegetative and reproductive growth periods.The results showed that the transgenic *A. thaliana* phenotype did not change significantly compared with the wild type . After treatment with 200 mM sodium chloride solution for 1 h and 3 h, the *CmMYB1* expression increased significantly and began to decline after 6 h of salt stress, indicating that it functions in the early response to salt stress. These results provide a theoretical basis for the further study of the biological function of *CmMYB1*.

1. Introduction

MYB transcription factors (MYB TFs) are a large and diverse family, expressed in all eukaryotes[1].The N-terminal amino acid residue has a conserved DNA binding domain, which binds to the promoter region of genes and regulates gene expression.According to the repeats number of the conserved DNA binding domain at the N-terminus of the MYB transcription factor, it is divided into 4 categories: 1R-MYB (including MYB-related), R2R3-MYB, 3R-MYB and 4R-MYB[2]. Currently, dicotyledonous plants such as Arabidopsis[2], Populus trichocarpa[3], grapes[4] and monocotyledonous plants such as corn[5]and rice [6], we have a more comprehensive understanding of the classification and function of MYB TFs.

Among the 4 types of MYB TFs, R2R3-MYB has the largest number and is mainly involved in regulating plant-specific growth and metabolic pathways.R2R3-MYB can regulate the life cycle of cells; regulate plant growth and development; regulate primary and secondary metabolites, such as flavonoids[7] and flavonols [8,9], anthocyanins and procyanidins[10,11], theanine[12]; participate in the regulation of secondary cell wall synthesis[13,14]; regulation of hormone signaling[15–17]; resistant biological and abiotic stresses[18–21].

In recent years, the planted melon cultivation area in my country has expanded rapidly, but soil salinization will harm its growth and development, and reduce the yield and quality of melon.At present, the research on genes related to salt stress in melon is mainly focused on vacuolar membrane Na+/H + antiporter gene (*CmNHX1*)[22], *CmLOX08* promoter[23], *CmRAV1* gene [24], *CmCAD* gene[25], *CmNAC gene* [26]. There is no research on the role of *MYB* gene in salt stress of melon.

Here, we cloned a *CmMYB1* gene. *CmMYB1* gene overexpression did not change the phenotype of transgenic Arabidopsis. However, the expression of *CmMYB1* gene increased significantly in the first and

third hours of 200 mM NaCl treatment, and the expression of *CmMYB1* gene was induced by salt stress, indicating that it played a crucial role in the salt tolerance mechanism of melon.

2. Results

2.1. Transcriptome analyses

We obtained 28 days fruits of 'M15' and 'Baogua' after pollination (Fig. 1), and compared their gene expression levels using strand-specifific mRNA sequencing. The results showed that there were a total of 6817 differently expressed genes. Compared to 'M15', there were 4370 genes up-regulated and 2377 genes down-regulated in 'Baogua' (Fig. 2). We found that the expression levels of 12 MYB transcription factors were significantly different between the two varieties, including MELO3C013071.2, MELO3C012039.2, MELO3C012926.2, MELO3C009678.2, MELO3C010833.2, MELO3C015228.2, MELO3C035029.2, MELO3C017134.2, MELO3C021555.2, MELO3C028590.2, MELO3C020701.2, MELO3C006728.2.

The transcriptome sequencing results of 'M15' and 'Baogua' were analyzed for significant enrichment in the GO (gene ontology) database, including biological process, molecular function, and cellular component.The results show (Fig. 3) that the differently expressed genes of the two involve multiple metabolic pathways. The proportion of differently expressed genes involved in cell components is highest in the cell periphery, followed by the membrane, component of membrane, extracellular region, external encapsulating stracture, and cell wall also accounts for a higher proportion. Oxidoreductase activity accounts for the highest proportion in molecular functional classification, followed by catalytic activity and protein kinase activity. Differently expressed genes involved in biological processes are mainly concentrated in oxidation-reduction process, followed by response to stimulus, and also involved in aminoglycan catabolic process, and chitin metabolism Chitin metabolic process, chitin catabolic process, cell wall macromolecule catabolic process, amino sugar catabolic process, compound containing glucosamine Glucosamine-containing compound metabolic process, glucosamine-containing compound catabolic process, protein phosphorylation, and so on.

KEGG pathway enrichment analysis showed that differentially expressed genes are mainly involved in starch and sucrose metabolism, MAPK signaling pathway-Plant, amino sugar and nucleotide sugar metabolism (Amino sugar and nucleotide sugar metabolism) and phenylpropanoid biosynthesis(Fig. 4).

2.2. CmMYB1 protein subcellular localization analysis

Throughlaser confocal scanning microscopy, it was observed that CmMYB1 protein is distributed in the cytoplasm and nucleus . However, the green fluorescent signal of *CmMYB1-GFP* was mainly concentrated on the plasma membrane(Fig. 5). These results indicate that the CmMYB1 protein may be a transcription factor that plays a role in the nucleus and cytoplasm.

2.3. Phylogenetic analysis of CmMYB1

Phylogenetic relationships between *CmMYB1* and *MYB* genes in other plants were explored by constructing an evolutionary tree of amino acid sequence using MEGA 5.1 software. The relationship between *CmMYB1* and a gene from the *Cucumis sativus* (XP004147900.1) was the closest, with 98.19% homology. However, homology with *Morella rubra* was only 57.68%(Fig.6). This suggests that the *MYB* gene may have evolutionary differences.

2.4. Phenotypic observation of transgenic A. thaliana lines

The phenotypes of wild-type and transgenic *A. thaliana* showed no significant difference between the vegetative and reproductive growth phases (Fig. 7).

2.5. RT-PCR to detect the expression of CmMYB1 under salt stress

RT-PCR results showed that, compared with the control, 200 mM NaCl solution treated melon seedlings at 1 h and 3 h showed a significant increase in *CmMYB1* expression and began to decline after 6 h. After 24 h, *CmMYB1* expression was increased (Fig. 8), indicating that it functions in the early response to salt stress.

3. Discussion

Soil salinization is one of the important abiotic stress factors affecting agricultural production and ecological environment. Salt stress destroys ion imbalance and osmotic balance in plants, causing oxidative damage, inhibiting growth and metabolism, and even causing plant death [27,28].

Transcription regulation is a key step for plants to respond to stress, and transcription factors play an important role in the response of plants to stress. Members of the transcription factor family such as AP2/EREBP, MYB, WRKY, NAC, and bZIP/HD-ZIP have been identified to participate in plant response to salt stress. Different transcription factors respond differently under salt stress, indicating that they play different roles in the salt stress response pathway [29-31].

A large number of MYB TFs in plants respond under salt stress. In common wheat, 42 *MYB* genes respond to salt stress, and most of the *MYB* genes are transiently up-regulated and down-regulated[30].Under 150 mM NaCl stress, the induced levels of *MYB15* in the roots of Arabidopsis thaliana at three detection time points (6 h, 24 h, and 48 h) increased by an average of about 16 times [2].Arabidopsis *AtMYBL2* was significantly induced under 300 mM NaCl treatment [6].*MYB119*, *MYB634* and *MYB636* were highly induced in the roots of Medicago truncatula; MYB119 reached a maximum at the transcription level after 30 minutes of stress, and began to decline after 6 hours of stress[31]. It shows that it functions in the early response to salt stress.*OsMYB91* and *OsMYB2* genes were overexpressed in rice, and the results showed that MYB transcription factors have a positive regulation effect on salt stress [32,33].In our experiment, after treatment with 200mM NaCl solution for 1h and 3h, the *CmMYB1* gene expression increased significantly, and began to decline after 6h of stress, indicating that it functions in the early response to salt stress.

4. Materials And Methods

4.1. Experimental materials

'M15' and 'Baogua' were planted in 28 days fruit. 'M15'(male and female) cDNA, 'M15' seedlings, wild-type *A. thaliana* Col-0.

4.2. Transcriptome analysis of 'M15' and 'Baogua'

Total RNA was extracted from fruit using Trizol method (3 biological replicates per sample). RNA integrity was evaluated on 1% agarose gels stained with ethidium bromide (EB). RNA concentration and purity were measured using an Agilent 2100 Bioanalyzer. Total RNA was enriched using Oligo (dT)-attached magnetic beads. First-strand cDNA was synthesized using random hexamer primers and M-MuLV reverse transcriptase. Second-strand cDNA synthesis was subsequently performed using DNA polymerase I and RNase H. After the cDNA library was constructed, PCR amplification was used to enrich the library fragments. The quality of the cDNA library was checked by Agilent 2100 Bioanalyzer, and then the total concentration and the effective concentration of the cDNA library were tested.Mix cDNA libraries with different Index sequences in proportion. Next-Generation Sequencing (NGS) was used to perform paired-end (PE) sequencing of these libraries based on the Illumina sequencing platform.

Raw Data uses Cutadapt to remove sequences with adapters at the 3 'end and removes reads with an average mass fraction below Q20. Use the upgraded version of TopHat2 HISAT2 (http://ccb.jhu.edu/software/hisat2/index.shtml) software to match the filtered Reads to the reference genome (CM3.6.1_pseudomol.fa). DESeq was used for differential analysis of gene expression, and the conditions for screening differentially expressed genes were: the expression multiple | log2 Fold Change |> 1 and the significance P-value <0.05.

4.3. Gene Enrichment and Pathway Analysis

Top GO was used to perform Gene Ontology(GO)enrichment analysis, and differentially expressed genes were analyzed for metabolic pathways of Kyoto Encyclopedia of Genes and Genomes (KEGG).

4.4. Instantaneous transformation of Nicotiana tabacum L.

Instantaneous transformation of *Nicotiana tabacum* L. refers to the method of Zhang *et al.*[34].After 72 h, the tobacco leaves were scanned with a laser confocal scanning microscope (Olympus FV1000).

4.5. A. thaliana transformation and Transgenic A. thaliana phenotype observation

Arabidopsis thaliana transformation refers to the method of Zhang *et al.* [34].Observe the phenotypic differences of transgenic and wild-type *A. thaliana* during the vegetative and reproductive growth periods

4.6. Melon seedling salt treatment

Melon seeds were germinated via hydroponics to the three-leaf stage and treated with 200 mM sodium chloride for 0-24 h. The culture medium was used as a control.

4.7. RNA extraction and reverse transcription cDNA

RNA was extracted from 200 mM NaCl treated and control melon seedlings using a CWBIO company RNA extraction kit (CW2598S). cDNA was obtained by reverse transcription using total RNA as a template. Reverse transcription reactions were performed with a TaKaRa company PrimeScriptTM RT Reagent Kit.

4.8. RT-PCR

For RT-PCR, a TaKaRa company SYBR® Premix Ex Taq Kit was used. PCR amplification system refers to the method of Wang *et al.*[35]. RT-PCR amplification primer sequences were:

CmMYB F1:TCCATGGATGGAAAGGACACT, *CmMYB* R1:TCAACATCTGGTTTTGACACTTGAA; Actin MELO3C023264 F: TGCCCAGAAGTTCTATTCCAGC, Actin MELO3C023264 R: CATAGTTGAACCACCACTGAGGAC.

5. Conclusions

We compared the transcriptomes of 'M15' and 'Baogua'. Transcriptome analysis found that the expression levels of 12 MYB transcription factors were significantly different between the two varieties. We cloned the melon *CmMYB1* gene, and subcellular localization revealed that the CmMYB1 protein is distributed in the cytoplasm and nucleus. The transgenic *CmMYB1A. thaliana* was not significantly different from wild type. With 200 mM NaCl solution treatment on melon seedlings, the expression of *CmMYB1* increased significantly at 1 h and 3 h. This shows that *CmMYB1* plays a role in the salt resistance of melon.

Declarations

Author Contributions: Z.H.J. and Y.Z. conceptualized the project. Formal analysis of results were conducted by Y.S.Y., Z.Y.D. and S.Q.W. Funding for the project was acquired by H.J.Z. Methodology was designed by H.J.Z. ,Y.Z. and Y.S.Y., and all experiments were supervised by Z.Y.D. and S.Q.W. Y.Z. wrote the original draft of the manuscript, and H.J.Z., Y.S.Y. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: *The authors declare no conflict of interest.*

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Figures

Figure 1

28 days fruit phenotype of melons afte pollination.(a) 'M15'; (b)'Baogua'.

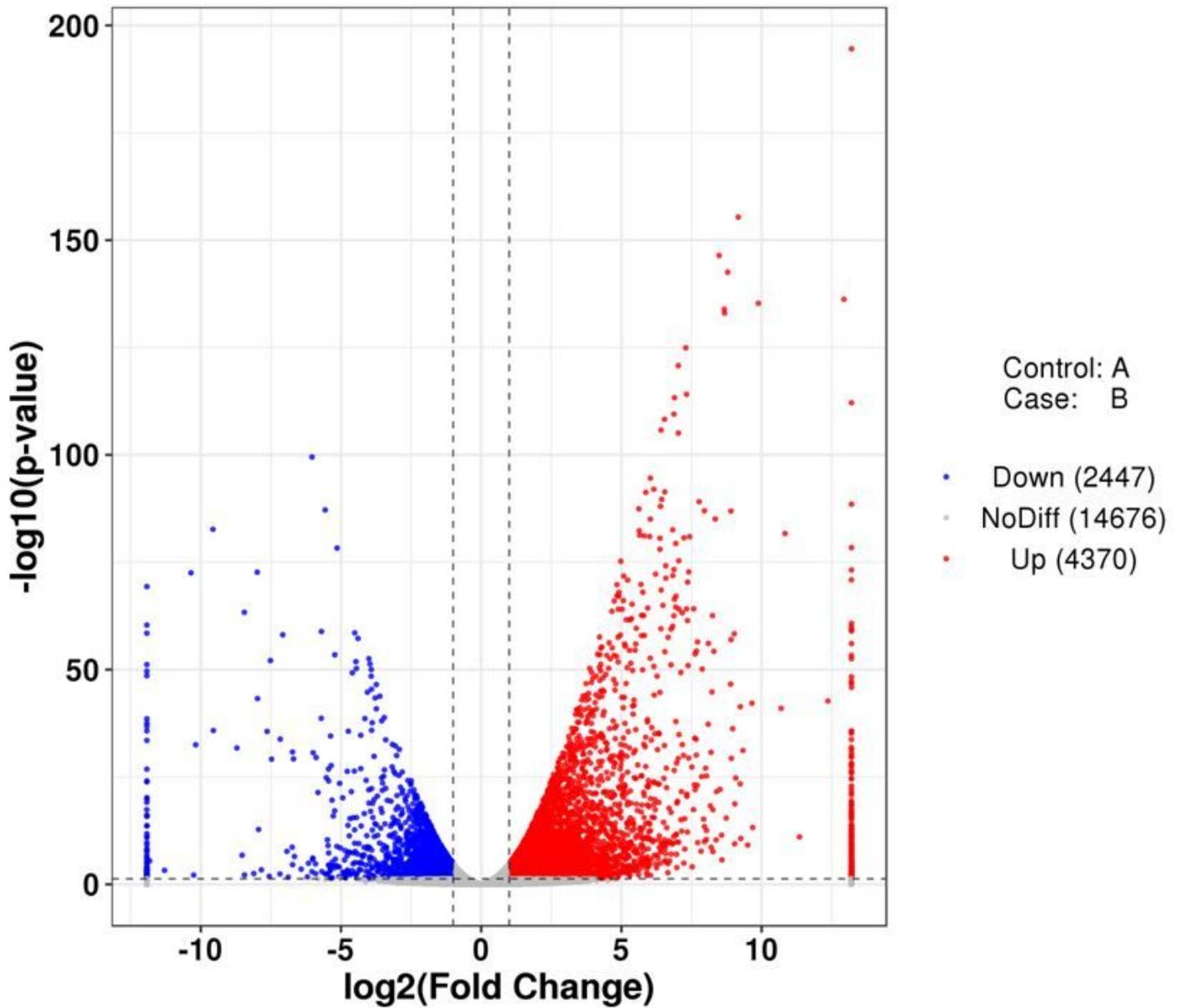Figure 2

Volcano plot of DEGs.Spots above the threshold line (Q-value = 0.05) indicate signifificant DEGs. Genes for which expression in Baogua fruit was less than half that in M15 fruit, with a P-value < 0.05, are shown in green, while those for which expression in Baogua fruit was more than two fold that in M15 group are shown in red. Genes in gray were neither up- nor downregulated.A: 'M15';B: 'Baogua'.
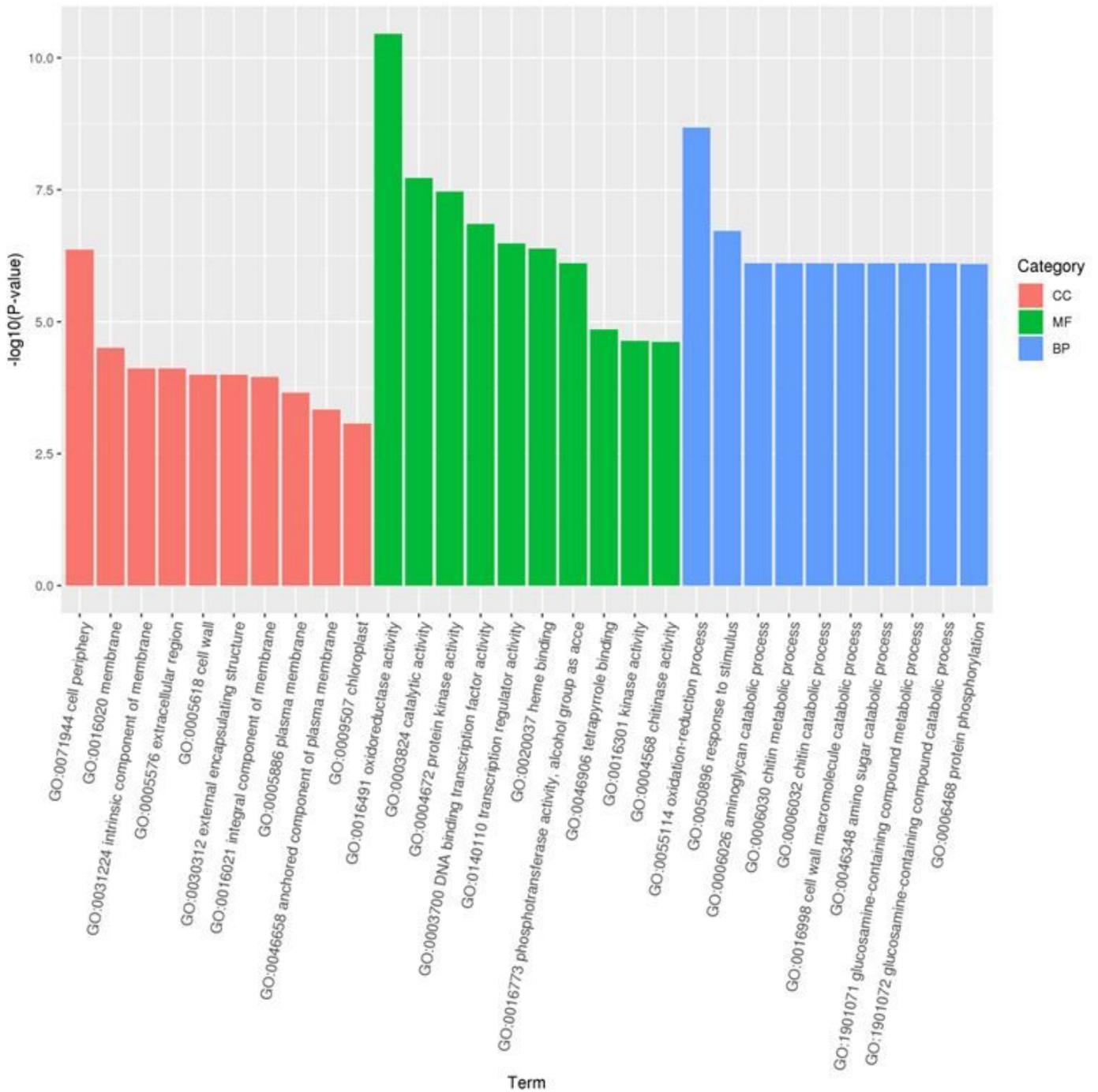
Figure 3

GO analysis of DEGs between 'M15' and 'Baogua' fruit. The abscissa is the term of Go level2, and the ordinate is -log10 (p-value) enriched for each term.

KEGG Pathway Enrichment
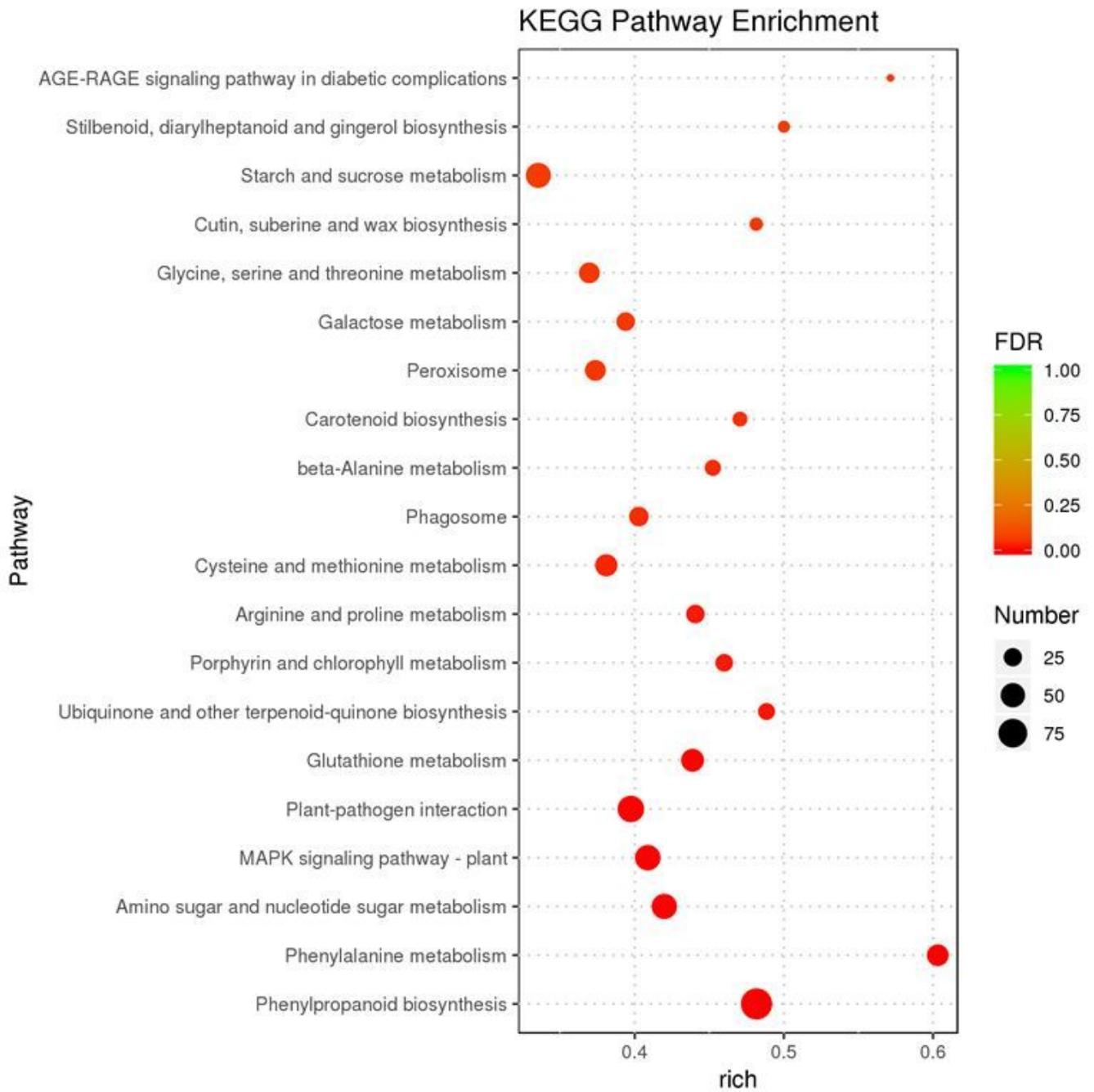
Figure 4

Pathway enrichment analysis of DEGs between 'M15' and 'Baogua' fruit.
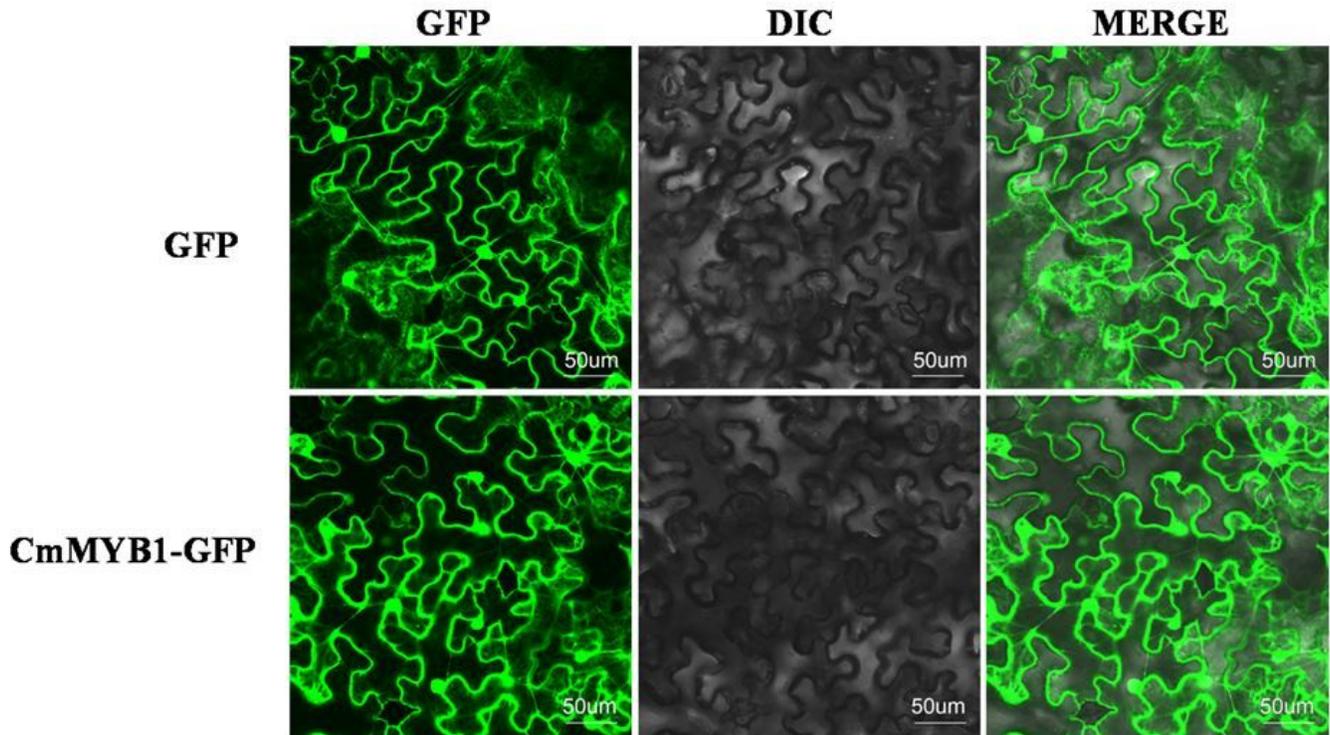
Figure 5

Subcellular localization of CmMYB1 protein in N. benthamiana leaves. CmMYB1 fused to the N-terminal of GFP under the control of the 35S promoter. Bars=50 μm.
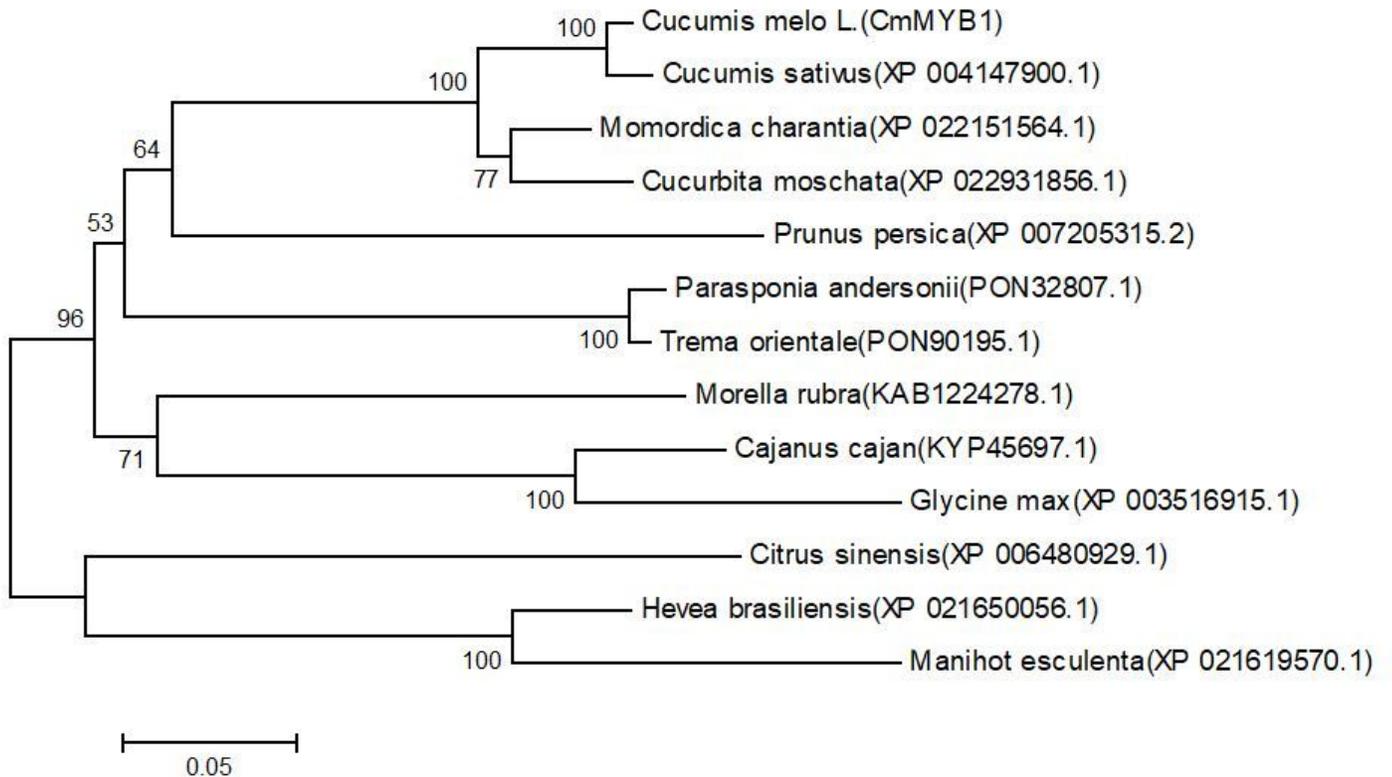
Figure 6

Phylogenetic tree of CmMYB1.The amino sequences were subjected to phylogenetic analysis using the neighbor-joining method in MEGA (version 5.1) software.
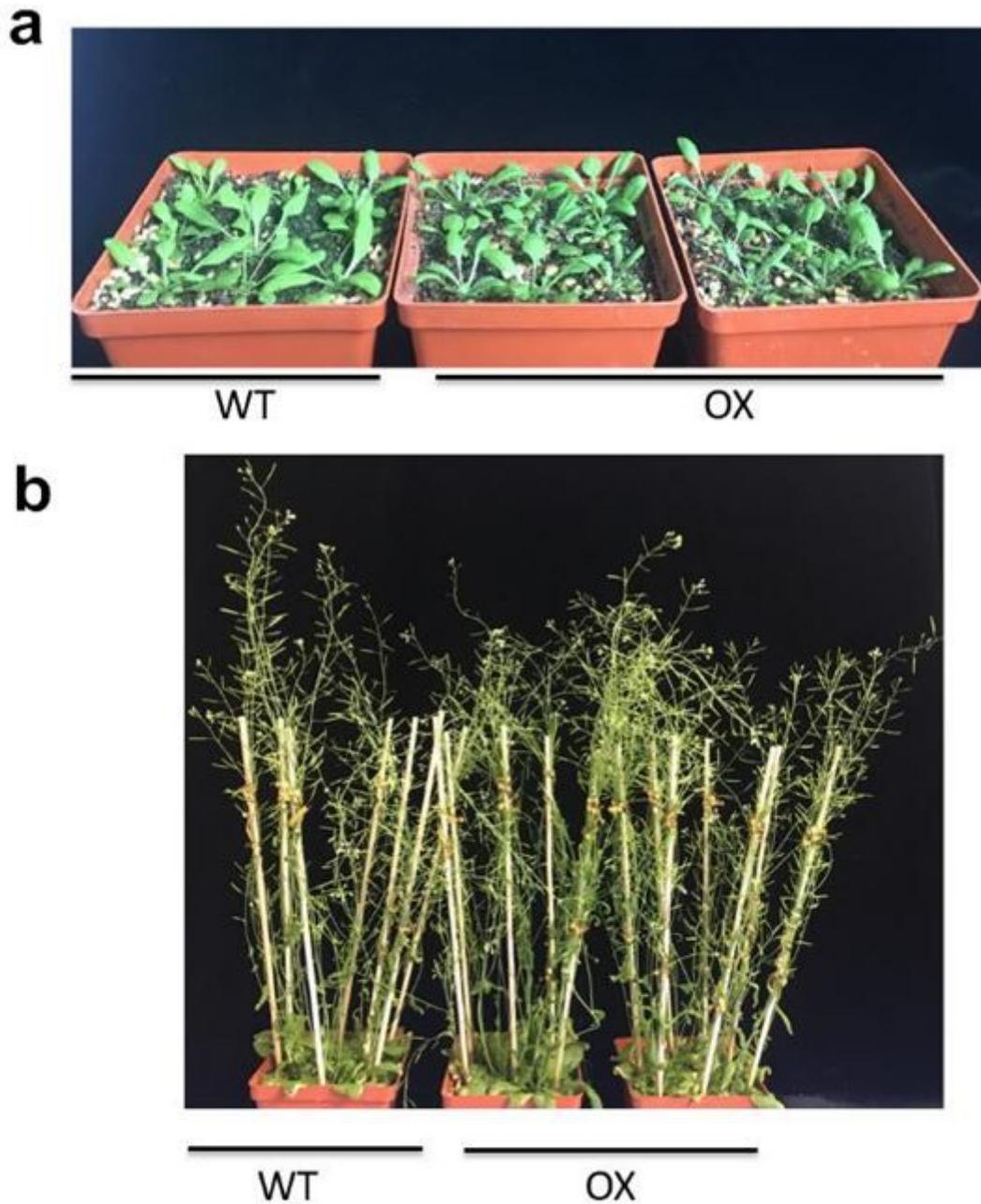

Figure 7

Phenotype analysis of transgenic A. thaliana. WT: wild-type Col-0; OX: CmMYB1 overexpression transgenic A. thaliana T1 different ines. (a)Wild-type and overexpression A. thaliana vegetative growth conditions;(b)Growth status of wild-type and overexpressing A. thaliana during reproductive growth period.
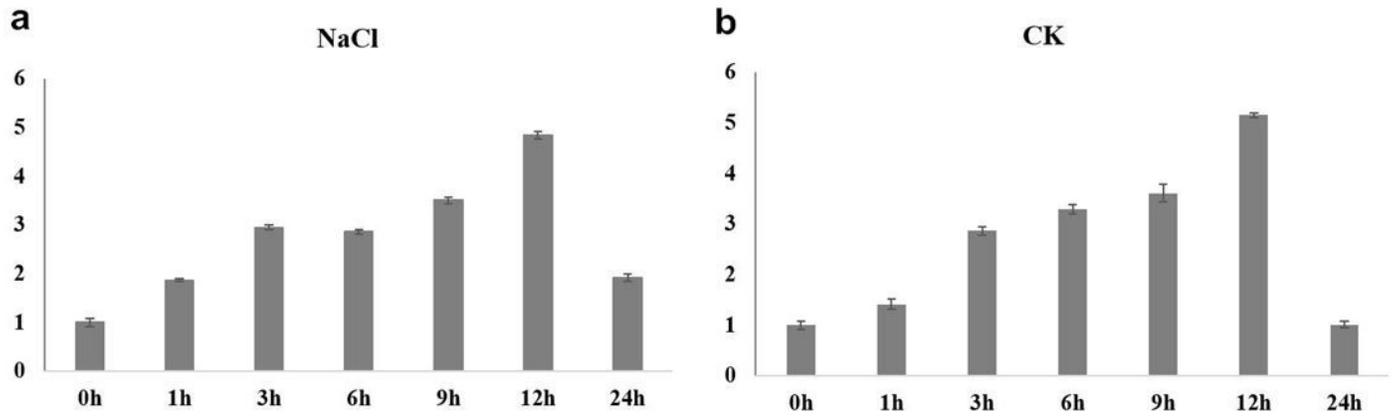
Figure 8

RT-PCR to detect the expression of CmMYB1 under salt stress.(a)sample treated with 200 mM NaCl solution; (b)culture treated sample.