RNA-Seq analysis differentially expressed genes in purple pakchoi (Brassica campestris ssp. chinensis Makino.) under low temperature

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Introduction

Anthocyanins, are a group of colorful and bioactive natural pigments with numerous important physiological and ecological functions in plants.

Anthocyanins play an important role in protecting plants against abiotic and biotic stressors and bring beneficial effects to human health.

Purple pakchoi contains high levels of anthocyanin in its leaves. This plant is very popular in China, Japan and surrounding countries.

Low temperature is the main environmental factor to effect the anthocyanin biosynthesis and accumulation in purple pakchoi,

Materials and Methods

- ***Plants Culture Conditions and treatment*** The experiment was carried out in in the growth chamber, Horticultural Research Institute of Shanghai Academy of Agricultural Sciences. The purple pakchoi 'ziyi' seeds were sown in plastic plates containing peat:vermiculite (2:1,v/v) in a green house The low temperature treatment was performed four weeks after germination. Before the temperature treatments, the plants were divided into two groups: normal temperature (NT) group, which was maintained at 20° C (day/night), and the low temperature (LT) group (5° C), with fouty plants in each group. The growth chamber was set to an average of 60% relative humidity, and the photoperiod was maintained for 12 h using a photosynthetic photon flux density (PPFD) of 800～1,000μmol $m^{-2}s^{-1}$. The plants were watered and fertilized daily with half-strength Hoagland nutrient solution.
- ***RNA-seq*** RNA-seq was used the Illumina NextSeq 500 instrument at the Shanghai Personal Biotechnology Co., Ltd.
- ***The qRT-PCR) analysis*** The qRT-PCR was conducted using a power SYBR Green PCR Kit (ABI7300) in a MicroAmp™ 96-well plate on a StepOnePlus™ Real-Time PCR System (ABI). The 2 $^{-\Delta\Delta}$ Ct method was used to calculate therelative expression levels of the target genes using Microsoft Excel software.

Acknowledgement

This work was supported by Shanghai Municipal Agricultural Commission: 7-8-1 (2014) and 03-2 (2019)..





Results

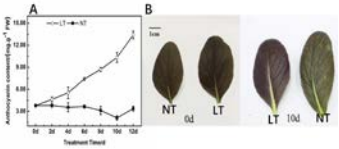


Fig. 1 Anthocyanin content and the color of purple pakchoi at 5° C and 20° C.Anthocyanin content changes in purple pakchoi treated with low temperaturesfrom 0 d to 12 d (A); comparison of color in purple pakchoi under low temperature conditionsat 0 d and 10 d.

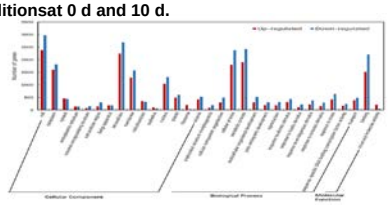


Fig. 2 Four major pathways likely implicated in the effects of low temperatures on purple pakchoi during DNC identified by a KEGG enrichment analysis. Thered line representsa p-value of 0.05

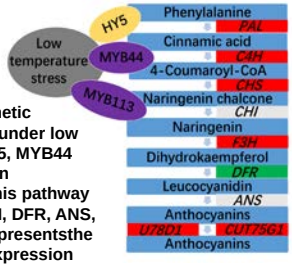


Fig. 3 Anthocyanin biosynthetic pathwaysin purple pakchoi under low temperature conditions. HY5, MYB44 and MYB113are transcription factors.Genes involved in this pathway include PAL, C4H, CHS, F3H, DFR, ANS, U78D1andCUT75G1. Red representsthe up-regulation of the gene expression level, while green denotesdown-regulation.Gray indicates a non-DEG..

Conclusions

- After the de novo assembly and quantitative assessment of the obtained reads, 114,043 unigenes were obtained, and 76,369 sequences were annotated by aligning the sequences against five public databases.
- Of all the differentially expressed genes (DEGs), 4,172 and 3,918 up-regulated genes were identified after the 5° C and 20° C treatments, respectively.
- Twelve major anthocyanin accumulation- and biosynthesis-related genes, including transcription factors (TFs), were identified, and their expression levels were estimated. The structural genes PAL, C4H, F3H, CHS, and CUT75G1 and the TFs HY5, MYB44, and MYB113 were induced by the low temperature conditions.