果 树 学 报 2017,34(5):541-551
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20160379

葡萄*ZTL*、*COP1*基因的鉴定及其在不同光质和转光条件下的表达分析

马彦妮,陈佰鸿*,毛 娟,左存武,吴金红,杨始锦,胡紫璟,李婷婷

(甘肃农业大学园艺学院,兰州 730070)

摘 要:【目的】在葡萄试管苗离体培养下,研究不同波长对基因*ZTL*、*COP1*表达的影响。【方法】以'红地球'葡萄(*Vitis vinifera* 'Red Globe')试管苗为材料,经RT-PCR法克隆获得葡萄*ZTL*、*COP1*基因全长cDNA,并利用生物信息学分析了以上基因的理化性质、蛋白质二级结构、亚细胞定位以及系统进化树。利用qRT-PCR技术分析了以上基因在不同光质处理下表达量的变化情况。利用荧光参数系统分析不同光质对葡萄试管苗生长的影响。【结果】*ZTL*的分子式为$C_{1\,972}H_{3\,107}N_{553}O_{576}S_{20}$,是亲水性稳定蛋白,脂溶性较差;*COP1*的分子式为$C_{832}H_{1\,291}N_{209}O_{225}S_9$,是疏水性稳定蛋白,脂溶性较好。*ZTL*和*COP1*的二级结构均由α-螺旋、β-转角、无规则卷曲和延伸链结构组成;亚细胞定位分析结果显示,*ZTL*、*COP1*均位于细胞质中;系统进化分析表明,*ZTL*与桃的亲缘关系最近,*COP1*与其他物种的亲缘关系均较远,单独聚为一个亚族。在不同光质处理后*ZTL*均为上调表达,其中白光转蓝光、红光、红光转白光、红光转蓝光和蓝光转红光处理后分别上调达11.2、22.3、12.0、32.9和19.6倍。不同光质处理后*COP1*基因的表达呈现不同的变化趋势:红光转蓝光处理后,*COP1*上调1.97倍,红光处理后表达量无显著变化,其他光质处理后均为下调表达,其中蓝光转白光和蓝光转红光处理后下调达18倍。荧光参数分析表明,qP、qN、NPQ和F_v/F_m在转光培养中表达活跃,均在红光转蓝光的转光培养下表达量最高。【结论】*ZTL*在葡萄转光培养中表达活跃,均呈显著上调趋势,而*COP1*总体表现为下调趋势。

关键词:葡萄试管苗;*ZTL*基因;*COP1*基因;克隆;表达分析

中图分类号:S663.1 **文献标志码**:A **文章编号**:1009-9980(2017)05-0541-11

Identification of the *ZTL* and *COP1* gene in *Vitis vinifera* and expression analysis of different light qualities and light transfers

MA Yanni, CHEN Baihong*, MAO Juan, ZUO Cunwu, WU Jinhong, YANG Shijin, HU Zijing, LI Tingting

(*College of Horticulture, Gansu Agriculture University, Lanzhou 730070, Gansu, China*)

Abstract:【Objective】The *ZTL* and *COP1* genes in *Vitis vinifera* were identified. Furthermore, the expression levels were detected after the plantlets were treated with different light conditions.【Methods】Full-length cDNA sequences of *ZTL* and *COP1* were successfully cloned from tissue cultural plantlets of the 'Red Globe' grape (*Vitis vinifera*). To understand their structure and function, physical and chemical characterization, protein secondary structure, sub-cellular localization, secondary structure and phylogenetic relationships of the two genes above were determined by using bioinformatic analysis. The expressions of the two genes were analyzed by using qRT-PCR and applying different treatments. In addition, the fluorescence expressions of the grape test-tube plantlets were analyzed by using a fluorescence parameter and applying different treatments.【Results】The sequence analysis revealed that the open reading frame of *ZTL* was 1 239 bp in size, encoding 412 amino acids with ATG as the initiation codon and TAA astermination codon. *COP1* showed that the open reading frame was 486 bp, encoding 160 amino acids with ATG as the initiation codon and TGA and TAA astermination codons. The results showed that *ZTL* exhibited the molecular formula of $C_{1972}H_{3107}N_{553}O_{576}S_{20}$, which is a hydrophilic stable protein and also dis-

收稿日期:2016-11-11 接受日期:2017-01-31

基金项目:国家自然科学基金(31401500)

作者简介:马彦妮,女,在读硕士研究生,研究方向:果树生理与生物技术。Tel: 18298333016,E-mail: 1554932816@qq.com

*通信作者 Author for correspondence. Tel: 13893210816,E-mail:bhch@gsau.edu.cn

played poor fat solubility. However, *COP1* showed a molecular formula of $C_{832}H_{1291}N_{209}O_{225}S_9$, which is a hydrophobic stable protein and showed favorable fat solublility. The protein of *ZTL* and *COP1* showed the same secondary structure, which is comprised of alpha helix, bela turn, random coil and an extended strand. However, the *ZTL* showed random coil > extended strand > alpha helix > bela turn, the *COP1* showed that the extended strand > random coil > alpha helix > bela turn. Transmembrane domain and signal peptide showed that ZTL was the membrane protein and COP1 was the transmembrane protein. Subcellular localization analysis showed that the protein of the two genes was located in the cytoplasm. The functional domain showed that *ZTL* contains a highly conserved domain of F-box between the first to sixtieth amino acids. *COP1* contains a highly conserved domain of WD40/YVTN between the 20th to 123rd amino acids. Homology analysis showed that the total similarity between ZTL and other plant homologues exhibited a 74.59% identity relationship in amino acid sequences. While the total similarity between COP1 and other plant homologues exhibited a 61.24% identity relationship in amino acid sequences. The phosphorylation site showed that ZTL consisted of 19 phosphorylation sites, including 9 serine phosphorylation sites, 9 threonine phosphorylation sites and 1 tyrosine phosphorylation site. COP1 consisted of 5 phosphorylation sites, including 2 serine phosphorylation sites, 2 threonine phosphorylation sites and 1 tyrosine phosphorylation site. Phylogenetic analysis showed the most closely identified relationships between *ZTL* and the orthologous genes were in *Prunus persica*. While *COP1* was much further from orthologous in the other species, which could be clustered into an individual subgroup. Compared with the control (white light), the up-regulation of the *ZTL* gene were discovered within all treatments. Within them, the higher expressions were detected from white to red light, red light, red to white light and red to blue light treatments, which up-regulated 11.2, 22.3, 12.0, 32.9 and 19.6 folds, respectively. The different changes in *COP1* expression were discovered from distinguishable light treatments. The expression was up-regulated 1.97 folds and treated by blue light with no significant changes in the red light treatment. Down-regulation was detected within other treatments. Within them, the expressions were down-regulated 18 folds from blue to white light and blue to red light treatments. The fluorescence parameter showed that the different changes in the qP expression were discovered from distinguishable light treatments. After red to blue, red, blue to red and red to white were significantly higher than the control (white light), the expressions were up-regulated 1.97 folds treated by red to blue light. There was no significant difference between the expression level of the white light treatment and control (white). The expression level was significantly lower than that in the control (white) after the blue treatment. The different changes in qN and NPQ expressions were discovered from distinguishable light treatments. After red to blue, red and blue to red were significantly higher than the control (white), the expressions of qN and NPQ were up-regulated 1.41 and 1.79 folds and treated by red to blue light. The expression level was significantly lower than that in the control (white) after the blue to white treatment. The different changes in F_v/F_m expression were discovered from distinguishable light treatments. After red to blue and blue to red were significantly higher than the control (white), the expression of F_v/F_m was up-regulated 1.08 folds treated by red to blue light. There was no significant difference between the expression level of red, red to white, white to blue, blue and blue to white light treatment and the control (white). The expression level was significantly lower than that in the control (white) after the white to red treatment.【Conclusion】Comprehensive analysis showed that the *ZTL* expression was active in the light of the grapes, and showed a significant upward trend, while *COP1* showed a downward trend. Fluorescence parameter analysis showed that qP, qN, NPQ and F_v/F_m expressions were active in the red to blue light of the grapes, and showed a significant upward trend.

Key words: Grape plantlets; *ZTL* gene; *COP1* gene; Cloning; Expression analysis