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苹果*MdRCD1*基因的表达分析以及功能的初步鉴定

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摘 要:【目的】分离克隆苹果*MdRCD1*基因,分析其蛋白结构和逆境响应,并初步鉴定*MdRCD1*在苹果愈伤中的功能。【方法】同源克隆*MdRCD1*并测序,用DNAman和MEGA5相关软件分析MdRCD1氨基酸序列以及进化关系,不同逆境处理'嘎拉'苹果组培苗,qRT-PCR分析*MdRCD1*在逆境条件下的表达量。农杆菌介导的遗传转化方法获得过量表达MdRCD1的转基因愈伤,不同盐浓度处理野生型和转基因愈伤,观察愈伤的长势,检测愈伤的鲜质量、脯氨酸和丙二醛含量,鉴定MdRCD1的初步功能。【结果】从'嘎拉'苹果中克隆了*MdRCD1*(MDP0000234325)基因。该基因ORF为1 803 bp。通过进化树和蛋白同源性分析,表明苹果MdRCD1和中国白梨PbRCD1进化亲缘关系最近。在MdRCD1的N端有1个保守的WWE结构域,1个PARP催化中心,在C端有1个RST结构域。qRT-PCR实验表明*MdRCD1*在苹果各个组织器官中都有表达,在茎中的表达量高于其他组织;同时*MdRCD1*的表达受NaCl、ABA、渗透胁迫等逆境胁迫的诱导。通过农杆菌侵染获得过量表达*MdRCD1*转基因苹果愈伤。盐胁迫处理条件下,过量表达*MdRCD1*的抗性明显提高。【结论】MdRCD1在进化过程中比较保守,苹果不同组织中都有表达,过量表达MdRCD1苹果愈伤的抗盐性得到提高。

关键词:苹果;*MdRCD1*;基因克隆;表达分析;盐胁迫

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Expression analysis and preliminary functional characterization of the apple *MdRCD1*

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Abstract:【Objective】Plants are constantly exposed to a broad spectrum of biotic and abiotic stresses during growth and development in nature, such as salt, drought, cold and oxidation. In order to adapt to the environment, plants have developed versatile stress-response mechanisms. Recent studies have shown that SROs (SIMILAR-TO-RCD-ONE) play important roles in response to stresses in diverse species. SROs have been identified as positive regulators to protect plants from oxidation and salt damage. However, the function of SROs in woody plants remains largely unknown, particularly in apples. In this study, we isolated and cloned a SRO gene, named as *MdRCD1*. Furthermore, we analyzed the protein structure and expression patterns of *MdRCD1*, and researched the functions of *MdRCD1* in apple callus. 【Methods】The phylogenetic tree of SROs proteins were constructed using a neighbor-joining method associated with the MEGA5 program (http://www.megasoftware.net/). The image of the phylogenetic tree of SROs proteins was drawn in MEGA5. Homology researches of the NCBI (National Center for Biotechnology Information) databases were performed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) with default parameters. The full-length cDNA of *MdRCD1* was determined by PCR. Tissue culture seedlings of 'Gala' were used to clone *MdRCD1* and analyze its expression levels in various stress conditions. Five

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years of strong 'Gala' trees were used to study the tissue expression pattern of *MdRCD1* in different tissues. The tissue culture seedlings were grown on an Murashige and Skoog (MS) medium with 0.1 mg·L^{-1} of gibberellins, 0.5 mg·L^{-1} of 6-Benzylaminopurine (6-BA) and 0.2 mg·L^{-1} of 1-Naphthaleneacetic acid (NAA) at 25 ℃, the tissue cultures were subcultured at monthly intervals under the long-day photoperiod (16-h-light/8-h-dark). The 'Orin' callus was grown on an MS medium containing 1.5 mg·L^{-1} 2, 4-dichlorophenoxy (2, 4-D) and 0.5 mg·L^{-1} of 6-BA in the dark at 25 ℃, and subcultured at 15-20 d intervals. To mimic salt-, osmosis-, ABA-, high temperature-induced stress, the tissue cultures were treated with an MS medium containing 100 mmol·L^{-1} NaCl, 200 mmol mannitol, 100 μmol ABA, 40 ℃, respectively, then samples were harvested at denoted times and stored at -80 ℃ for further analysis. Gene expression analysis was performed using qRT-PCR. In order to construct the *MdRCD1* overexpression vector, its full-length cDNA was first cloned into the PMD18-T vector. After sequencing, the plasmid was digested by *Eco*R Ⅰ and *Sal* Ⅰ, and then the recovered fragment was introduced into the PRI-101 expression vector. The recombinant plasmid was transferred into an *Agrobacterium tumefaciens* strain LBA4404, and then introduced into the apple callus with an *Agrobacterium*-mediated method. To test the function of *MdRCD1* on salt resistance, the wild-type callus and *MdRCD1* transgenic callus at the same growth state were treated with different concentrations of salt for 15 d. Then the growth state of the wild-type and *MdRCD1* callus were observed. Meanwhile, we measured the fresh weight and analyzed the content of proline and malonaldehyde (MDA) in them. 【Results】*MdRCD1* (MDP0000234325) was cloned from 'Gala' apple (*Malus*×*domestica* 'Gala'). Sequence analysis indicated that the Open Reading Frame (ORF) of *MdRCD1* contained 1 803 bp, encoding a protein of 600 amino acid residues. Its molecular mass was predicated to be 67.39 ku, and pI was 7.06. The results exhibited the homology of the amino acids sequence of MdRCD1 with RCD1s from other species. Multiple sequence alignment and phylogenetic analysis showed that MdRCD1 had the highest evolutionary relationship with PbRCD1 from the pear, and had a classic WWE domain in the N-terminal. The results of the protein sequence alignment indicated that MdRCD1 was highly conserved among SROs family in diverse species. The qRT-PCR results demonstrated that *MdRCD1* extensively existed in various apple tissues, and the expression level in the stem was highest, suggesting that *MdRCD*1 is a constitutive expression gene. In addition, *MdRCD1* was induced by multiple stresses including ABA, NaCl and osmotic. The open reading frames (ORFs) of *MRCD1* were introduced into the PRI-101 plant transformation vector downstream of the cauliflower mosaic virus (CaMV) 35S promoter, and then the recombinant structure was transformed into *Agrobacterium tumefaciens* LBA4404. Transgenic apple callus was generated by *Agrobacterium*-mediated genetic transformation. Under normal conditions, the growth of MdRCD1 and wild-type apple callus showed no differences. However, in the condition of salt stress, overexpression of *MdRCD1* in apple callus significantly enhanced resistance compared to wild-type callus. In addition, the fresh weight and proline content of *MdRCD1* callus were higher than wild-type callus, and the MDA content was lower. These results revealed that *MdRCD1* may play an important role in apple response to salt stress. 【Conclusion】Taken together, MdRCD1 is a member of the SROs family and the protein sequences of the RCD1 are conserved among different species. *MdRCD1* is widely expressed in all tissues tested in apples, including root, stem, flower, leaf and fruit, however its expression levels are different. *MdRCD1* is induced by multiple stresses such as NaCl, ABA and osmotic stresses. Overexpressing *MdRCD1* in apple callus can significantly improve salinity tolerance. Therefore, we speculate that *MdRCD1* may play an important role in salt stress. *MdRCD1* also can be used as a resistance gene to screen stock, resulting in improving the production of apples.

Key words: Apple; *MdRCD1*; Gene clone; Expression analysis; Salt stress