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H<sub>2</sub>O<sub>2</sub>诱导拟南芥*NIT*2基因甲基化特征与转录水平改变

DNA methylation alterations and gene transcription changes of NIT2 in Arabidopsis plants induced by hydrogen peroxide

关键词:  $\underline{H_2O_2}$  拟南芥  $\underline{NIT2}$ 基因  $\underline{DNA}$ 甲基化 基因转录

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摘要:用外源H<sub>2</sub>O<sub>2</sub>处理拟南芥植株,亚硫酸氢盐修饰后测序法分析胁迫生理中*NIT*2基因启动子区甲基化特征变化,RT-PCR检测该基因的转录水平.结果显示:用100 ul • L<sup>-1</sup>的 H<sub>2</sub>O<sub>2</sub>处理3 h后,*NIT*2启动子区胞嘧啶总甲基化水平与对照差异不大,但对照组CHG(H为C、A或T)和CG位点甲基化水平分别为35.0%和93.3%,H<sub>2</sub>O<sub>2</sub>处理组CHG和CG位点甲基化水平分别为50.0%和96.9%;H<sub>2</sub>O<sub>2</sub>处理组CHH位点则表现为甲基化水平升高、降低或去甲基化;H<sub>2</sub>O<sub>2</sub>胁迫组拟南芥地上组织中*NIT*2基因转录水平提高.研究结果表明:*NIT*2基因的转录应答和DNA甲基化修饰参与了植株的逆境生理过程:氧化胁迫与DNA甲基化改变、基因转录上调同时发生.说明胁迫诱发的活性氧增高可能参与胞嘧啶甲基化修饰和基因转录的调节

Abstract: DNA methylation is one of the most important heritable epigenetic modifications of the genome and could be changed in plant responses to environmental stresses. Environmental stimuli can induce DNA methylation alterations and some important consequences for plant adaptations. In this study, DNA methylation alterations and transcriptional changes of a gene encoding nitrilase 2(NIT2) protein in Arabidopsis were investigated under oxidative stress. Arabidopsis shoots, which were exposed to 100 u • L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> for 3 h and showed increased intracellular reactive oxygen species (ROS) levels, were selected as the materials. DNA methylation analysis by bisulfite sequencing showed that the total level of methylation in the promoter region of NIT2 gene did not change much after plants were exposed to 100 u • L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> for 3 h, but for the different DNA sequence contexts in the promoter region of Arabidopsis NIT2 gene, cytosine residues could have different methylation status in H<sub>2</sub>O<sub>2</sub>-exposed samples. In control plants, the methylation levels of CHG (where H is C, A or T) and CG contexts were 35.0% and 93.3% respectively, while in H<sub>2</sub>O<sub>2</sub>-exposed samples, the methylation levels of CHG and CG contexts were 50.0% and 96.9% respectively. For CHH context, cytosine residues could be hypomethylated, hypermethylated or demethylated in H<sub>2</sub>O<sub>2</sub>-exposed samples. Exposure to H<sub>2</sub>O<sub>2</sub> also induced transcriptional activation of NIT2 gene in Arabidopsis shoots. The present results indicated that H<sub>2</sub>O<sub>2</sub> caused the changes of transcription and DNA cytosine methylation of NIT2 gene, which can affect plant physiology contributing to plant adaptation. Our results also suggested that increased ROS may play a key role in triggering cytosine methylation and gene expression in plant responses to environmental stresses.

**Key words:**  $\underline{H}_2\underline{O}_2$  <u>Arabidopsis</u> <u>NIT2gene</u> <u>DNA methylation</u> <u>gene expression</u>

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