

Analysis of DNA Cytosine Methylation on Cotton under Salt Stress

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DNA methylation, especially methylation of cytosine in eukaryotic organisms, has been implicated in gene regulation, genomic imprinting, the timing of DNA replication, and determination of chromatin structure. It was reported that 6.5% of the whole cytosine residues in the nuclear DNA in higher plants were methylated. The methylation of cytosine in plant nuclear DNA occurs usually in both CpG and CpNG sequences, and the methylation state can be maintained through the cycles of DNA replication and is likely to play an integral role in regulating gene expression. We investigated the differences in methylation level and the changes of cytosine methylation patterns under NaCl stress in two different salt-tolerant cotton varieties by using the methylation-sensitive amplified polymorphism (MSAP) method. We also analyzed the changes in methylation level by cotton seedling from the cotyledon unfurling period to the three-leaf period. The results showed that there existed obviously DNA methylation polymorphism in the cotyledons between the salt-tolerant cotton variety (CCRI 35) and the salt-sensitive cotton variety (CCRI 12). The number of the methylation of CCGG sites in the salt-tolerant cotton variety was less than that in the salt-sensitive cotton variety. Under salt (NaCl) stress, we found that the methylation of the cytosines in CCGG sites did change in the salt-treated cotton varieties compared to the corresponding controls, and our results showed that extensive cytosine methylation alterations including hyper- and demethylation as well as the potential conversion of methylation types (from external cytosine to internal cytosine) occurred in the salt-treated cotton varieties compared to the corresponding controls. Interestingly, we found that salt stress-induced demethylation loci that occurred in the salt-tolerant cotton variety were greater than those in salt-sensitive cotton variety, and salt stress-induced hyper-methylation loci that occurred in the salt-tolerant cotton variety were less than those in salt-sensitive cotton variety. It seemed that the demethylation owed a positive contribution to salt tolerance and the hyper-methylation did a negative effect on salt tolerance on cotton. Another finding was that the DNA methylation level was variable during seedling stage and on the basis of the whole analysis of methylation status at 5'CCGG sites in the two cotton varieties we found that more loci were methylated at the three-leaf period growing fast than that at the cotyledon unfurling period. In the two cotton varieties we found that more loci were methylated at the three-leaf period growing fast than that at the cotyledon unfurling period.