

Abstract

Algae, as well as plants, are continuously exposed to a variety of abiotic and biotic stresses which could potentially reduce their growth, development, productivity and survival. Therefore, they have developed tolerance, avoidance and resistance mechanisms to cope with these challenging conditions. These mechanisms are very complex and involve the activation of many genes and the interconnection among various signalling pathways regulating numerous metabolic networks. Upon a permanent stress, plants respond with long-term strategies including transgenerational changes involving development of heritable gene expression. In this scenario, it appears evident the relevance of epigenetic mechanisms in assuring plant growth plasticity and withstanding stresses through a rapid and extensive modification of gene expression in a manner that overcomes the restrictions of a highly stable DNA sequence.

Epigenome landscape is largely related to DNA methylation process, which is one of the most significant players in the control of plant responses to environmental changes and stressors. Literature data indicate that DNA methylation systems are mosaics of conserved and derived features and that DNA methylation has likely played an important role in plant evolution. In this scenario, algae, represent an important study object, since as plant progenitors, likely evolved ancestral mechanisms of epigenetic regulation. Several algal genomes have been recently sequenced, providing insights into the great complexity of these organisms, nevertheless the complex mechanisms by which DNA methylation modulates stress in algae are yet largely unresolved, mainly with respect to heavy metal stress, for which in plants, metal- and species- specific responses were instead evidenced. For these reasons, in this work, we focused our attention on two strains of the green alga *Scenedesmus acutus*: the wild type (wt) and a chromium-tolerant strain (Cr-t), the latter selected from the wt population after prolonged culture in Cr(VI) supplemented medium. Since previous studies, conducted by immuno-localization of methylcytosine (5-mC) in CG context, evidenced a different DNA methylation level between the two strains, *S. acutus* represent a good model to define the reprogramming of epigenetic events leading to the tolerance to external stimuli. In order to gain further insight into relationship between DNA methylation pattern and chromium-tolerance, we performed a comparative whole-genome bisulfite sequencing (WGBS-seq) in Cr-t strain vs wt. The pattern of distribution of 5-mC showed significant differences in Cr-t strain vs wt, concerning both differentially methylated contexts (CG vs CHG, CHH) and differentially methylated regions (DMRs) as well. The results suggest that DNA methylation may be of particular importance in defining signal specificity associated with Cr-tolerance and in establishing new epigenetic marks which can not only contribute to a better adaptation to metal stress, but also transmit the epigenomic traits to the progeny. The analysis showed a differential methylation between strains in different pathways common with

those involved in Cr(VI) stress response in other plants, among these genes involved in sulfur metabolism.

Given the relationship between chromium stress and sulfur reductive assimilation pathway and even the sulfur requirement in the synthesis of molecules scavengers of heavy metals (e.g. phytochelatins, metallothioneins), we paid a particular attention to some genes for sulfur uptake/assimilation. Our data shows that DNA methylation plays an important role in modulating the differential expression level of the analysed genes in the two *S. acutus* strains. These results strengthen the hypothesis of a link between the enhanced sulfate uptake/assimilation pathway shown by Cr-t strain and its Cr(VI)-tolerance and suggest that epigenetic mechanisms can have been at the basis of the onset of metal tolerance mechanism.