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CPG METHYLATION OF THE PROXIMAL PROMOTER REGION REGULATES THE EXPRESSION OF NAC6D GENE IN RESPONSE TO HIGH TEMPERATURE IN WHEAT (*TRITICUM AESTIVUM*)

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Methylation of DNA promoter sequences at the CpG islands has become a molecular tool for gene regulation. NAC6D gene is induced by different biotic and abiotic stimuli. The proximal promoter sequence of NAC6D was investigated for the impact of CpG methylation on its expression in response to high temperature in wheat. Gene expression was estimated by real time PCR and methylation of NAC6D promoter sequence was investigated by bisulfite sequencing. Results showed that NAC6D was highly induced by high temperature, whereas DNA methylatransferase 3 (Met3) was highly reduced by high temperature. Close investigation of NAC6D promoter methylation revealed that high temperature caused hypomehtylation of the proximal promoter sequence. Twelve CpGsites showed low difference in methylation compared to the control (normal temperature, 25 °C), while 3 CpGs(−59, −169, −204) were extremely hypomethylated in response to high temperature compared to their methylation status under the normal condition. The induction of NAC6D was negatively correlated with Met3 suppression and methylation level at the CpG sites in the promoter region. Results prove that methylation greatly contribute to the regulation of NAC6D in response to high temperature. This will improve our current understanding of how plants respond to abiotic stresses at the molecular level and the integration of DNA methylation and epigenetics in the next generation plant breeding.

Key words: *NAC6D, promoter, methylation, CpG, Wheat, epigenetic, DNA methyltransferases, epialleles.*

РЕГУЛЯЦІЯ ЕКСПРЕСІЇ ГЕНУ NAC6D У ПШЕНИЦІ (*TRITICUM AESTIVUM*) ШЛЯХОМ МЕТИЛЮВАННЯ CPG ДІЛЯНКИ ПРОКСИМАЛЬНОГО ПРОМОТЕРА ЯК РЕАКЦІЯ НА ВИСОКІ ТЕМПЕРАТУРИ

Метилювання промоторних послідовностей ДНК у CpG острівцях стало молекулярним інструментом генної регуляції. Ген NAC6D зазнає впливу різних біотичних та абіотичних факторів. Послідовність проксимального промотера NAC6D вивчали для встановлення впливу метилювання CpG на його експресію як реакцію пшениці на високі температури. Експресію генів оцінювали за допомогою ПЛР у реальному часі, а метилювання послідовності промотера NAC6D досліджували за використання бісульфітного секвенування. Результати продемонстрували, що за впливу високої температури відбувалась значна індукція NAC6D та суттєве зменшення ДНК-метилтрансферази 3 (Met3). Детальне вивчення метилювання промотера NAC6D показало, що висока температура спричиняла гіпометилювання послідовності проксимального промотера. Дванадцять ділянок CpG продемонстрували меншу різницю щодо метилювання порівняно з контролем (нормальна температура, 25 °C), а 3 CpG (−59, −169, −204) були суттєво гіпометильованими у відповідь на високі температури порівняно з їхнім статусом метилювання за нормальних умов. Індукція NAC6D мала негативну кореляцію з рівнем пригнічення та метилювання Met3 в CpG острівцях у ділянці промотера. Результати доводять, що метилювання здійснює значний вплив на регуляцію NAC6D у відповідь на високі температури. Ці дані поглиблять наші поточні знання про те, як рослини реагують на абіотичні стреси на молекулярному рівні та на інтеграцію метилювання ДНК і епігенетики у селекцію рослин наступного покоління.

Ключові слова: NAC6D, промотер, метилювання, CpG, пшениця, епігенетичний, метилтрансферази ДНК, епіалелі.

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