

## Cold acclimation alters DNA methylation patterns in *Brassica rapa* and confers tolerance to heat and high growth rate

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Epigenetic modifications are implicated in plant adaptations to abiotic stresses. Exposure of plants to one stress induces resistance to other stresses, which is called cross-adaptation and is not well understood. In this study, we aimed to unravel the epigenetic basis of elevated heat-tolerance in cold-acclimated *Brassica rapa* by genome-wide DNA methylation analysis of leaves from Control (CK) and Cold-Acclimated (CA) plants. We found both methylation and demethylation occurred during cold acclimation. Moreover, two significantly altered pathways, malate dehydrogenase activity and carbon fixation and 1,562 differentially methylated genes, including *BramMDH1*, *BraKAT2*, *BraSHM4* and *Bra4CL2* were identified in CA. Genetic validation and treatment of *B. rapa* with 5-aza-2-deoxycytidine (Aza) suggested promoter demethylation of four

candidate genes increased their transcriptional activities. Physiological analysis suggested elevated heat-tolerance and high growth rate were closely related to increases in organic acids and photosynthesis, respectively. Functional analyses demonstrated that the candidate gene *BramMDH1* (mMDH-mitochondrial Malate Dehydrogenase) directly enhances organic acids and photosynthesis to increase heat-tolerance and growth rate in *Arabidopsis*. However, Aza-treated *B. rapa*, which also has elevated *BramMDH1* levels, did not exhibit enhanced heat-tolerance. We therefore suggest DNA demethylation alone is not sufficient to increase heat-tolerance. This study demonstrates that altered DNA methylation contributes to cross-adaptation.