H_2O_2 and Ca^{2+} signaling mediates melatonin-induced cold tolerance in watermelon

Hao Li[1]*, Jingjing Chang[1], Yanliang Guo[1], Zixing Zhang[1], Jiayue Li[1], Xian Zhang[1]

[1] College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China

*Corresponding author. Email: lhyy@nwafu.edu.cn

Abstract

Cold is a major environmental factor that detrimentally affects plant growth and development. Melatonin is a pleiotropic signaling molecule that regulates plant response to cold; however, the underlying signal transduction mechanisms remain unclear. In this study, we cloned the first melatonin biosynthetic gene (caffeic acid *O*-methyltransferase, *ClCOMT1*) from a species in the Cucurbitaceae and clarified that H_2O_2 and Ca^{2+} signal mediated the melatonin-inducing cold tolerance in watermelon. The main results are as follows:

1. Watermelon *O-methyltransferase 3* (*ClOMT*) was considered as a potential *COMT* gene (renamed *ClCOMT1*) based on bioinformatics and qRT-PCR analysis. Overexpression of *ClCOMT1* significantly increased melatonin contents, while *ClCOMT1* knockout using the CRISPR/Cas-9 system decreased melatonin contents in watermelon. Overexpression of *ClCOMT1* enhanced plant tolerance against cold, drought, and salt stress. These results indicate that *ClCOMT1* plays an essential role in melatonin biosynthesis and plant tolerance to abiotic stresses.

2. Exogenous melatonin and Ca^{2+} conferred watermelon tolerance against cold stress. Melatonin promoted Ca^{2+} influx and the accumulation of cytoplasmic free Ca^{2+} ($[Ca^{2+}]_{cyt}$). Cyclic nucleotide-gated ion channels (CNGCs) are important Ca^{2+}-permeable channels. The expression of *ClCNGC2*, *ClCNGC10*, *ClCNGC17*, and *ClCNGC20* was significantly upregulated by melatonin and cold; however, only *ClCNGC20* knockout significantly compromised melatonin-induced Ca^{2+} influx under both normal and cold conditions. Furthermore, we confirmed the interactions between ClCNGC20 and ClCaM2, ClCaM5, or ClCaM7 using yeast two hybrid screen, bimolecular fluorescence complementation, and luciferase complementation test. These findings suggest that melatonin stimulates the Ca^{2+} influx by regulating the activity of ClCNGC20, which may be negatively regulated by CaM7.

3. Melatonin induced H_2O_2 accumulation and upregulated the expression of *Respiratory Burst Oxidase Homolog D* (*ClRBOHD*) during the early response to cold stress in watermelon. Both melatonin and H_2O_2 induced $[Ca^{2+}]_{cyt}$ accumulation and upregulation of *ClCNGC2* in watermelon response to cold. However, blocking of Ca^{2+} influx abolished melatonin- or H_2O_2-induced CBF pathway and cold tolerance. Ca^{2+} also induced *ClRBOHD* expression and H_2O_2 accumulation, whereas inhibition of H_2O_2 production by editing *RBOHD* or using RBOH inhibitor compromised melatonin- or Ca^{2+}-induced CBF pathway and cold tolerance. These findings indicate that positive interaction between H_2O_2 and Ca^{2+} mediates melatonin-induced CBF pathway and cold tolerance in watermelon.

Funding

National Key Research and Development Program of China (2018YFD1000800), National Natural Science Foundation of China (31801884, 31972479), Earmarked Fund for Modern Agroindustry Technology Research System of China (CARS-25), and Tang Scholar of Northwest A&F University.