Early Cold-induced Peroxidases and Aquaporins Are Associated with the High Cold Tolerance in Dajiao (Musa spp. ‘Dajiao’)

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INTRODUCTION

Banana (Musa spp.) is an important tropical fruit with high economic value. It originates in the tropics, and its growth is completely arrested and injured at 8 °C. One of the main species (cultivar Cavendish) is susceptible to low temperature, while another close relative specie (Dajiao), has considerably higher cold tolerance. Besides, we previously reported that some membrane proteins appear to be involved in the cold tolerance of Dajiao. Therefore, Dajiao and Cavendish could be used as the excellent test case for investigation of cold-tolerance mechanisms in banana by membrane proteomics.

AIM

To investigate early cold stress response of Dajiao, here we applied comparative membrane proteomics analysis for both cold-sensitive Cavendish and cold-tolerant Dajiao, and provided new insights into the cold stress tolerance mechanism in banana at the membrane protein level, toward potential applications for ultimate genetic improvement of cold tolerances in banana.

MATERIAL AND METHODS

A two-step method was used for extraction of membrane proteins in Cavendish and Dajiao seedlings in response to cold stress (10 °C for 0, 3, and 6 h), and three sets of biological replicate samples were analyzed by an iTRAQ-based 2D-LC/MS/MS workflow for examining proteome changes. Phobius, Scampi-single, and TMHMM programs were used for filtering and predicting membrane proteins. Some important candidate proteins identified were evaluated and verified by RT-PCR, MRM, recombinant green fluorescent protein (GFP)-based subcellular localization, and enzyme activity analyses.

RESULTS

Physiological and biochemical responses to cold stress

After cold treatment for 3 and 6 h at 10 °C, Cavendish leaves displayed slight wilt that developed gradually into observed necrotic spots, while Dajiao leaves remained unchanged. We also found that high levels of ROS accumulated, significantly increased MDA content and cell membrane permeability in the Cavendish, while Dajiao remained unchanged.

Differentially abundant membrane proteins

Subsequent bioinformatics analyses showed that 692 membrane proteins and 524 Dajiao proteins were predicted to be membrane proteins, of which 82 and 137 differentially abundant membrane proteins (DAMPs) were found in Cavendish and Dajiao, respectively. Interestingly, the number of DAMPs with increased abundance following 3 h of cold treatment in Dajiao (80) was seven times more than that in Cavendish (11). Gene ontology molecular function analysis of DAMPs for Cavendish and Dajiao indicated that they belong to eight categories including hydrolyase activity, binding, transporter activity, antioxidant activity, etc., but the number in Dajiao is twice that in Cavendish. Strikingly, we found peroxidases and aquaporins among the protein groups whose abundance was significantly increased after 3 h of cold treatment in Dajiao.

Validation of the DAMPs

The result of RT-PCR, MRM and GFP-based subcellular localization were consistently in line with the proteomics data, indicating the reliability of our membrane proteomics datasets and peroxidases and aquaporins appear to be involved in the cold tolerance of Dajiao.

Peroxidases in response to cold stress

When treated with NaCl, the seedlings of Dajiao showed more intense DAB staining compared with the treatment with water (Figure 5). The soluble peroxidase activity revealed a sustained increased at 3 and 6 h in Cavendish, whereas it remained stable in Dajiao, but CWP activity in Dajiao increased earlier (Figure 6). Taken together, we hypothesize that effectively regulating the balance of the oxidative burst and detoxification of H2O2 by a rapid increase in the abundance of membrane-bound Peroxidase 52 and Peroxidase P7 may at least in part explain the greater tolerance of Dajiao than Cavendish to cold stress.

CONCLUSION

In combining with the physiologic and biochemical data, we found that membrane-bound Peroxidase 52 and P7, and aquaporins (PIP1:1, PIP2:4, PIP2:6, TIP1:3, PIP1:2), are mainly involved in decreased lipid peroxidation, and maintaining leaf cell water potential, which appear to be the key cellular adaptation contributing to the cold tolerance of Dajiao.