Screening for the Oxidative Stress Signaling Factor in Arabidopsis by Functional Complementation of a Yeast Mutant

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ABSTRACT

In this study, we constructed an Arabidopsis cDNA library to search for the factors mediating oxidative signals by complemenyation of a Yap1-deficient strain. In order to improve the screening efficiency, We developed a high throughput system based on the co-cultivation of hundreds of transformants on a 96-well microplate. To evaluate the reasonable number of co-cultivated transformations, different ratios of the wild-type strains to the mutants were prepared to mimic the real situation. It is revealed that the H2O2 tolerance of the wild-type strains (or trasformants) were not sheltered when the number of pooled population was below 200. According to this system, we isolated a colony showing somewhat of a resistance to hydrogen peroxide. However, further examination indicated that the H2O2 tolerance may be due to the cell mutation instead of aquiring the Arabidopsis cDNA. Moreover, we also verified that freezing tolerance of Arabidopsis induced by hydrogen peroxide was also explored physiologically in this study. Seedling pretreated with hydrogen peroxide obtained freezing tolerance which is comparable to that induced by cold acclimation. However application of EGTA (a calcium chelator), La3+ (a calcium channel inhibitor) or W7 (a protein kinase inhibitor) prior to the pretreatment of hydrogen peroxide will reverse the freezing tolerance induced by hydrogen peroxide. Taken together, seedlings pretreated with hydrogen peroxide a series of signaling cascade that leads to the anti-freezed gene expression. Furthermore, calcium and calcium dependent protein kinase may be involved in the signaling pathways.

Keywords : Arabidopsis ; hydrogen peroxide ; Yap1 ; freezing tolerance

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