Title Effects of superoxide radicals on ACC synthase activity in chilling-stressed etiolated mungbean seedlings

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Abstract

The activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase, ACS) and the concentrations of superoxide radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) were measured in etiolated mungbean seedlings following their transfer to a growth chamber at 25°C after a 5-h-chilling treatment at 5°C. All of these variables increased dramatically after the transfer, and strong correlations were found between ACS activity and the concentrations of superoxide and H_2O_2. Exogenous applications of two generators of superoxide radicals, methylviologen (MV) and xanthine–xanthine oxidase (X–XOD), enhanced ACS activity in seedlings, but their effects were inhibited by exogenous applications of specific scavengers of $O_2^{\cdot -}$. However, applications of H_2O_2 or specific H_2O_2-scavengers had no significant effects on seedlings ACS activity. The results indicate that $O_2^{\cdot -}$ was involved in the chilling-induced increases in ACS activity, but not H_2O_2. ACS activity peaked ca. 8 h after the transfer, and then declined, but the decline could be counteracted by exogenous applications of specific $O_2^{\cdot -}$ scavengers, this suggests that damage was caused by superoxide radicals influencing ACS activity in etiolated mungbean seedlings. Further analysis of changes in two key kinetic parameters of ACS activity—V_{max} (maximum velocity) and K_m (the Michaelis constant)—in the seedlings indicated that the presence of $O_2^{\cdot -}$ may reduce K_m, i.e. increase substrate (S-adenosyl methionine, SAM) affinity. That would be the main mechanism responsible for the observed chilling-induced increases in ACS activity in etiolated mungbean seedlings.