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The Presence of FeSOD in the *Aceraceae* Family

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Summary

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Isoenzyme analysis of *Acer pseudoplatanus* and *Acer campestre* leaves revealed the presence of one form of iron superoxide dismutase (FeSOD). FeSOD was also found in the green line of *Acer pseudoplatanus* callus, but not in the white line (without chloroplasts). It seems that the expression of this metalloenzyme is a characteristic of photosynthetic competent tissue.

Introduction

Iron containing SODs (FeSOD) are mainly present in procaryotic organisms and some eucaryotic algae, but have also been found in the plant families: *Gingkoaceae, Nymphaceae* and *Cruciferae* (BRIDGES & SALIN 1981), *Papilionaceae* and *Solanaceae* (KWIATOWSKI & KANIUGA 1984). The occurrence of FeSOD has also been demonstrated in some species of the plant families *Rutaceae*, *Rubiaceae* and *Caryophyllaceae*. This type of SOD appears to be located in chloroplasts and in peroxisomes from carnation petals (ALMANSA & al. 1994).

In this paper we provided evidence for the presence of FeSOD in two species belonging to the *Aceraceae* family. The second aim of this paper was the comparison of the SOD isoenzyme pattern of *Acer pseudoplatanus* leaves with in vitro cultured *Acer pseudoplatanus* calli differing in plastid status and metabolic activity.

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Material and Methods

Leaves of *Acer pseudoplatanus* L. and *A. campestre* L. were taken from the trees grown in the Botanical Garden in Cracow (Poland). The white and green callus lines of *A. pseudoplatanus* were cultured in vitro. The culture was performed at the temperature of 25°C, using 16 h photoperiod of 150 μmol m^{-2} s^{-1} PPFD. White wild line was originally derived from nonphotosynthetic cambium cells grown heterotropically on the agar medium as previously described by BLIGNY 1977. Green mutant cells, which were originally selected by LESCURE 1969 by mutagenic treatment of the white wild type, were maintained in the CNRS laboratories of Marseille (France), in 1982 kindly provided by Prof.K.STRZALKA (Jagiellonian University, Cracow, Poland), and since that time cultivated in our lab.

Leaves or callus were ground in 50 mM phosphate buffer pH 7.8, containing 0.1 % BSA, 0.1% L-ascorbate and 10 mM DTT. SOD activity of the crude extracts was visualized by native electrophoresis on 11.5 % polyacrylamide gels according to BEAUCHAMP & FRIDOVICH 1971. Five experiments were performed.

The three metallo-forms of the enzyme were distinguished using 1 mM KCN and 5mM H_2O_2 added to the staining solution.

Results and Discussion

In extracts from leaves of *A. pseudoplatanus* three SODs has been detected by conventional polyacrylamide gel electrophoresis : Mn- Fe- and CuZn- type. Though this method is not quantitative, a much more narrow band of FeSOD isoform compared with that of MnSOD and of CuZnSOD suggests its lower activity. The electrophoresis of the white callus revealed the occurrence of only one band of MnSOD (cyanide and H_2O_2 insensitive) while the green callus showed the presence of two superoxide dismutases - MnSOD and FeSOD (H_2O_2 insensitive), as shown in Fig.1.

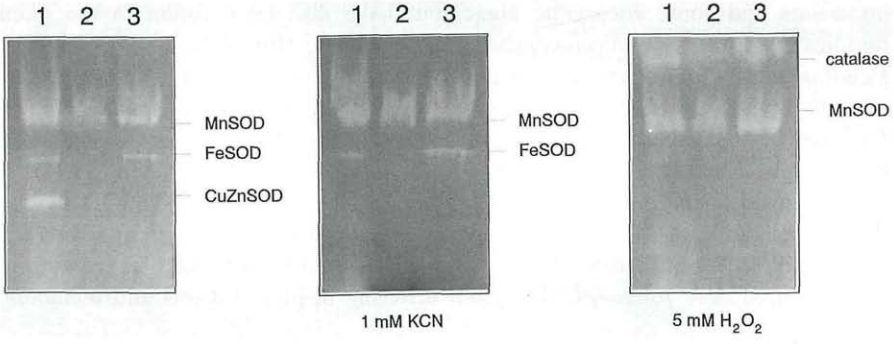

Fig.1. SOD isoenzymatic pattern determined in extracts of *Acer pseudoplatanus*: leaves (1), white line of callus (2), green line of callus (3).

The white line of callus has, according to NGERNPRASIRTSIRI & al. 1988, only one type of plastids - amyloplasts. Mutagenic treatment of the white line caused amyloplasts to develop into functionally competent chloroplasts, which exhibit photosynthetic O_2 evolution. As might be expected, the formation of $O_2^{\cdot-}$ in plastids is correlated with their photosynthetic activity and hence a much higher SOD scavenging activity is needed in chloroplasts in comparison with photosynthetically-inactive amyloplasts. The presence of FeSOD in the cells of *A. pseudoplatanus* is correlated with the presence of chloroplasts. This result is in agreement with literature data (BRIDGES & SALIN 1981, KWIATOWSKI & al. 1985, ALMANSA & al. 1994). The presence of FeSOD has also been revealed in the leaves of *A. campestre* L. (Fig. 2).

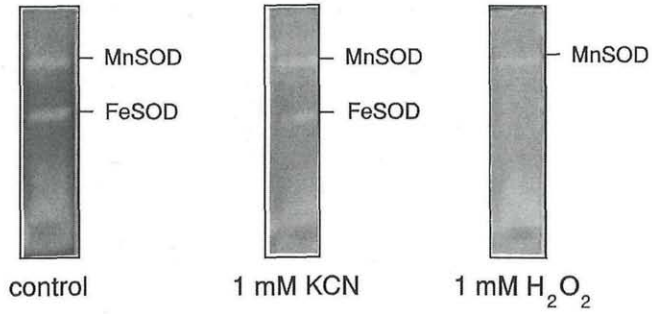

Fig. 2. SOD isoenzymatic pattern determined in extracts of *Acer campestre* leaves: control (without inhibitors), with 1 mM KCN or with 5 mM H_2O_2.

The experiments of NGERNPRASIRTSIRI & al. 1988 strongly suggest the homology between DNAs from amyloplasts and chloroplasts of *A. pseudoplatanus,* but the status of their expression is different. On the other hand, as pointed by KWIATOWSKI & al. 1985, the expression of FeSOD in plants might be dependent on the ability of the plants to develop adaptive protection against different types of stress conditions. The expression of this metalloenzyme is characteristic for photosynthetic competent tissue.

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