Detection of Superoxide in Arabidopsis roots by an Electron Spin Resonance Spin-Probe method

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Abstract

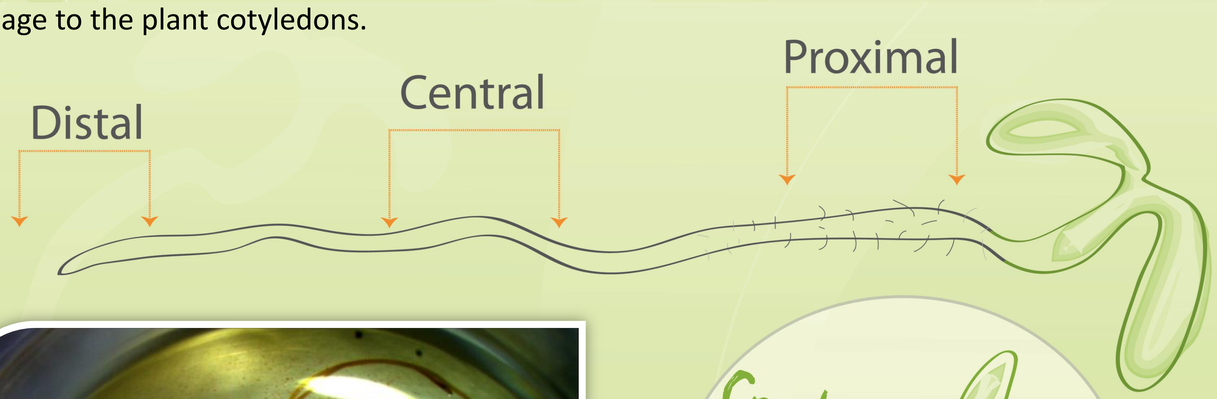
Plants are subjected to a wide range of severe environmental conditions that influence their growth, metabolism and may even lead to cell death. Reactive Oxygen Species (ROS) are part of the plant response to many of these stress situations. In recent years it is becoming evident that ROS levels, determined by the balance between producing and scavenging systems, is an important component in directing the plant to acclimation or, in extreme cases, to programmed cell death. Moreover, ROS have been implicated in development and in diseases both in plants and animals.

As a consequence, many efforts have been made to develop methods for detection and imaging of these species *in-vivo*. However, most these methods use optical approaches, which have many pitfalls, provide inconclusive results and are controversial in the literature. Superoxide (O_2^-) is an important reactive oxygen species thought to participate in signal transduction in diverse stress conditions by the regulated activity of NADPH oxidases. In plants, NADPH oxidase D (also known as RBOHD) is thought to be the most prominent. Here we made use of Electron Spin Resonance (ESR)- based method for accurate detection of superoxide in *Arabidopsis thaliana* plants, generated due to leaf injury.

Our approach is based on the use of a novel paramagnetic spin probe; perchlorotriphenylmethyl radical-tricarboxylic acid (PTM-TC), which reacts specifically with superoxide and becomes diamagnetic. Hence, loss of signal is indicative of O_2^-.

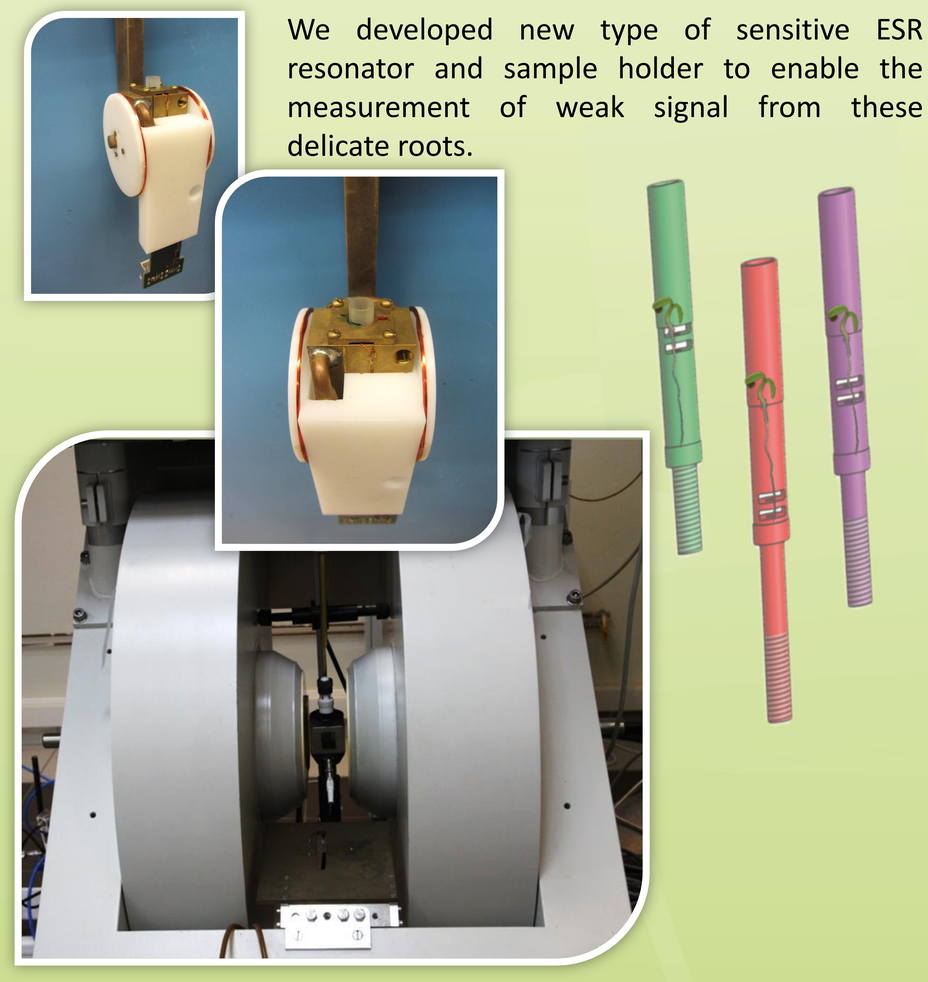
Methodology

Arabidopsis roots are incubated in PTM-TC solution for 30 min and then measured by ESR before and after leaf injury. ESR measurements are made in 3 types of resonators in order to observe the superoxide levels generated in different regions of the systemic root as a result of applying mechanical damage to the plant cotyledons.

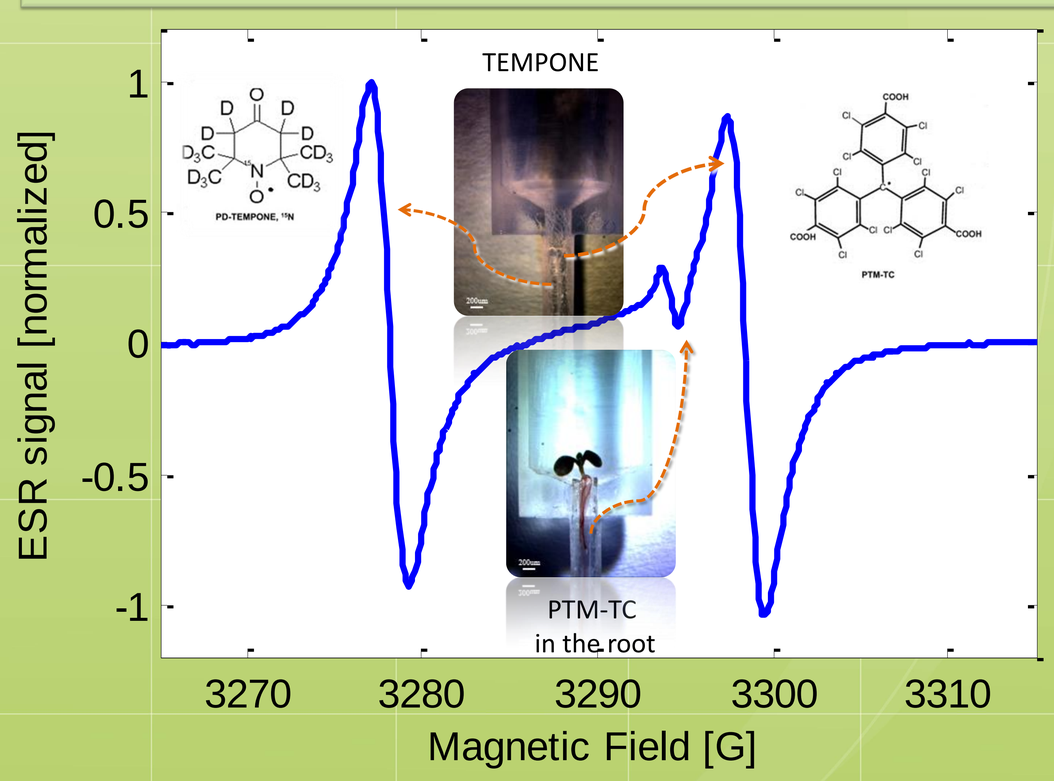


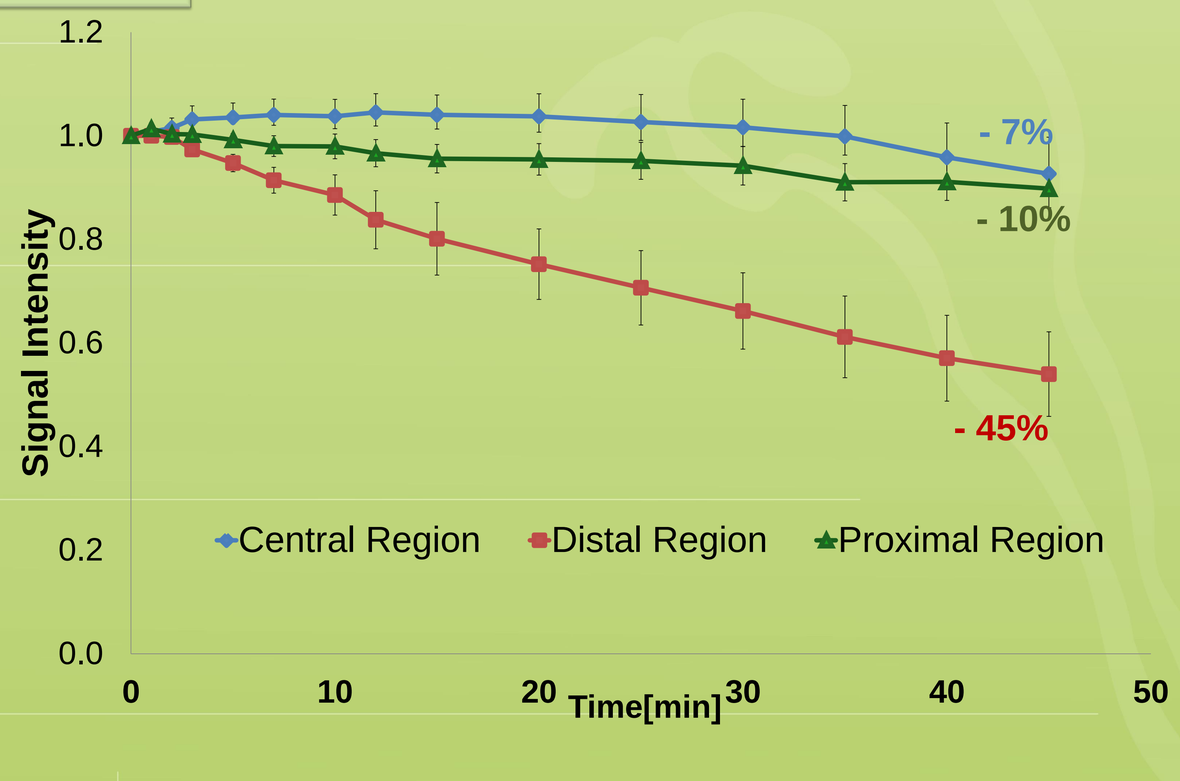
Experimental System

We developed new type of sensitive ESR resonator and sample holder to enable the measurement of weak signal from these delicate roots.



Experimental Data





Conclusions

Preliminary results show that superoxide is rapidly induced in root tips but not in other parts of the root. The data suggest that superoxide does not autopropagate along the root of the wounded seedlings, but rather occurs in the root tip. The results give evidence for the existence of alternative signal to superoxide. Additionally, we show that tip localized superoxide signal is partially dependent on RBOHD.