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Meeting abstract

Effect of nitric oxide on concentration of intracellular free Ca^{2+} in transgenic *Arabidopsis thaliana* plants during oxidative stress

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Background

In recent years exogenous NO was shown to (i) influence plant growth and development, (ii) take part in the response of plants to pathogens, light and ABA-induced stomatal closure, and (iii) reduce consequences of oxidative stress generated by treatment with herbicides [1-4]. It is also known that a number of chemical and physical stimuli, including oxidative stress, mediates their effects *via* transient increases in the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_{cyt}$). Therefore, the aim of our work was to study the influence of exogenous NO on the increase of $[Ca^{2+}]_{cyt}$ induced by oxidative stress in plant cells with changes in $[Ca^{2+}]_{cyt}$ during oxidative stress being measured in *Arabidopsis thaliana* seedlings transformed to express apoaequorin.

Materials and methods

8-day old seedlings of *A. thaliana* were incubated in coelenterazine (5 μM) diluted in methanol for 6 h to reconstitute the aequorin. Chemiluminescence measurements were performed with a digital chemiluminometer. The peak-value of the stimuli-induced $[Ca^{2+}]_{cyt}$ transient was calculated as a described by Cobbold and Rink [5] with some modifications [6]. Oxidative stress induced by hydrogen peroxide (10 mM) [7]. To evaluate a possible effect of NO on the increase $[Ca^{2+}]_{cyt}$ during the oxidative stress seedlings were pre-treated with NO donor NOR-1 (25×10^{-6} M).

Oxidative stress resulted in increased $[Ca^{2+}]_{cyt}$ (See Figure 1). The single spike of chemiluminescence was observed after a lag-phase of 20–40 sec. After $[Ca^{2+}]_{cyt}$ was increased from 0.1 μM to 1.3 μM and in 1.5–2 min was returned to a basal level. Our data are in accordance with data obtained by Price et al [7].

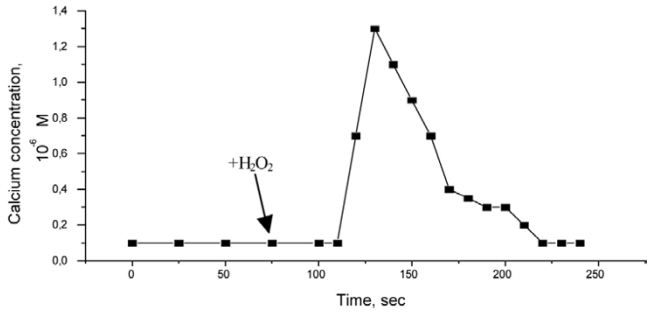

Figure 1
Cytosolic calcium response of *Arabidopsis* seedlings under action of oxidative stress induced by H_2O_2

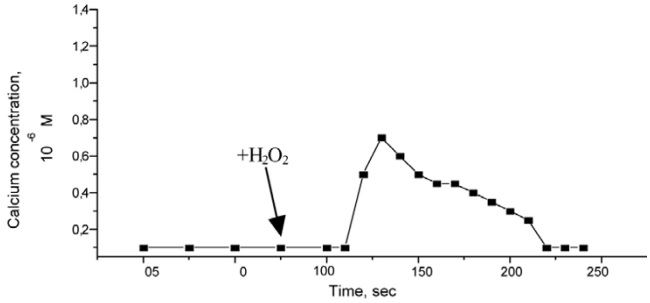

Figure 2
Effect of NOR-1 pretreatment on $[Ca^{2+}]_{cyt}$ response induced by oxidative stress

Results

Pre-treatment of seedlings with NOR-1 led to a single Ca^{2+}-spike (see Figure 2) similar to that observed during oxidative stress. However, the level of $[Ca^{2+}]_{cyt}$ was increased only to 0.7 MM. In this way, the increase in $[Ca^{2+}]_{cyt}$ was by 50 % lower as compared with control ones. These data testify that NOR-1 interferes with $[Ca^{2+}]_{cyt}$ elevations caused by the oxidative stress in seedlings and that a potential communication point for cross-talk between signal transduction pathways (NO signal transduction and signal transduction during oxidative stress) is the $[Ca^{2+}]_{cyt}$.

Conclusion

These data suggest that NO plays an important role in the activation of plant defense responses after oxidative stress. It may partly explain that NO is able to inactivate directly the reactive oxygen species (ROS) [8]. The presence of an unpaired electrons within the NO molecule gives it its reactive species properties and is also the origin of its duality. At physiological concentrations NO may play a protective role acting as a chain inhibitor to limit the damage.

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