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Nitric oxide effect on heat production and oxygen consumption of wheat root cells

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Abstract The effect of nitric oxide (NO) on heat production and oxygen consumption was studied in excised roots of 5-day-old wheat seedlings grown in $CaCl_2$ solution (2.5×10^{-4} mol/L). Sodium nitroprusside (SNP), $NaNO_3$, $NaNO_2$ were used as NO donors. Incubation of the cut roots (wound stress) in the presence of NO donors led to the decrease of heat production and suppressed oxygen consumption. The increase of potassium (K^+) ions exit was observed, pointing to the increase of the plasma membrane permeability and to the disruption of the adaptive processes development in roots in the $NaNO_2$ presence.

Keywords Nitric oxide · Heat production · Microcalorimetry

Introduction

In the past decade, the nitric oxide (NO) effect on plants metabolism has been given much consideration [1, 2]. NO is a short-lived free radical that diffuses through membranes. NO is found in different plant organs, cells, and cell compartments [3–5]. This small molecule plays a multifunction role in plant growth, development, and regulation of such fundamental processes as programmed cell death, stressful responses, and adaptation [2, 6, 7].

It is shown that NO can be an effective regulator of respiration as it possesses great affinity for a number of glycolysis and mitochondrial oxidation enzymes [8]. However, the NO influence on an energy metabolism (respiration, heat production) of plant tissues under stress conditions is practically not known.

Nitric oxide was first characterized as a biological product of nitrite reduction by denitrifying bacteria. Plant roots live in the environment where NO concentration sometimes might be higher than above ground [5]. In this connection, the role of NO in root cells energy metabolism at stress conditions is of particular interest.

Microcalorimetry has been extensively applied as a useful method for rapid bioassay of chemical compounds on living organisms [9–11]. Earlier we used this technique for research of response of the wheat cut roots to the substances influencing the electron transport chain in plant cells mitochondria [12].

The main task of this study was to compare the influence of different NO donors (SNP, $NaNO_3$, $NaNO_2$) on heat production, as the plants heat production correlates with the changes of all anabolic and catabolic processes in a live organism being an indicator of efficiency of energy use [13, 14] and oxygen consumption of wheat roots in wound stress.

Materials and methods

The excised roots of 5-day-old wheat (*Triticum aestivum* L.) seedlings grown hydroponically in a solution of $CaCl_2$ (0.25 mM) were used as the subject of the investigation. In all incubation solutions, $CaCl_2$ as a membrane-stabilizing compound was present. The pH of all solutions was adjusted to 5.5. Roots incubated in 0.25 mM $CaCl_2$ only were used as a control. Concentration of SNP, $NaNO_3$, and $NaNO_2$ was 5×10^{-3}M.

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The heat production by roots was measured using an LKB differential microcalorimeter (LKB-2277 Bio Activity Monitor, Sweden) at 30 °C. The excised roots in 3 mL of the various solutions were sealed in the glass vials and incubated for 5 h.

The oxygen consumption was measured using Warburg's manometric method. Twenty milliliter glass vessels containing 150 mg of the excised roots and 3 mL of incubation solution were shaken at 110 oscillations per min at 30 °C for 5 h.

K^+-ion release from cells was determined from the change of its content in the solution after incubation of roots and measured with flame photometer, Phlapho 41 (Carl Zeiss, Jena, Germany).

The calorimetric experiments were repeated five times. The experiments on determining the oxygen consumption and potassium (K^+) ion release were performed three times with three replicates. The standard error was calculated using the program Microcal Origin™ V.5.0.

Results

The data on oxygen consumption in the presence of various donors NO are shown in Fig. 1. It can been seen that in the presence of SNP and $NANO_3$, there was no significant decrease in oxygen consumption during the first hours of incubation which then was replaced by a little stimulation. In the presence of $NaNO_2$, oxygen consumption was considerably inhibited. These results correlate with the data by change in heat production in the presence of these NO donors (Figs. 2, 3, 4). The heat production was not considerably changed in comparison with the control in the presence of SNP and nitrate (Figs. 2, 3), and was strongly inhibited in the presence of $NaNO_2$ (Fig. 4) Definition of the K^+-ion quantity in a solution of cut roots incubation has shown that there is an exit of potassium ions in comparison with the control increased in the presence of SNP and to a large extent in the presence of $NaNO_2$ (Fig. 5).

Discussion

It is known that there are various ways of NO formation in plants. NO can be generated chemically and from enzyme activity [15]. As NO itself is a reactive gas with a short half-life in air experiments on research of its action were made as a rule with the application of NO donors. SNP as chemical donor of NO is transition metal-NO complex. The mechanism of NO release from SNP is not clear, although it used in clinical therapy for over 70 years [16]. For plants the use such NO donors as nitrates and nitrites seems to be more physiologic as they are available in soil.

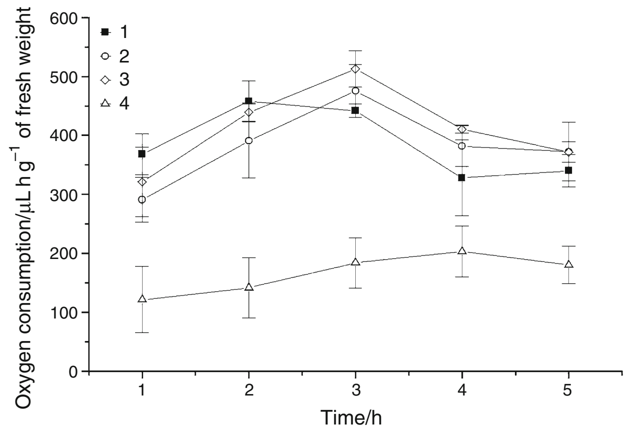

Fig. 1 Oxygen consumption by wheat roots in the presence of SNP, $NaNO_3$, and $NaNO_2$ (5×10^{-3} M): *1* control, *2* SNP, *3* $NaNO_3$, *4* $NaNO_2$

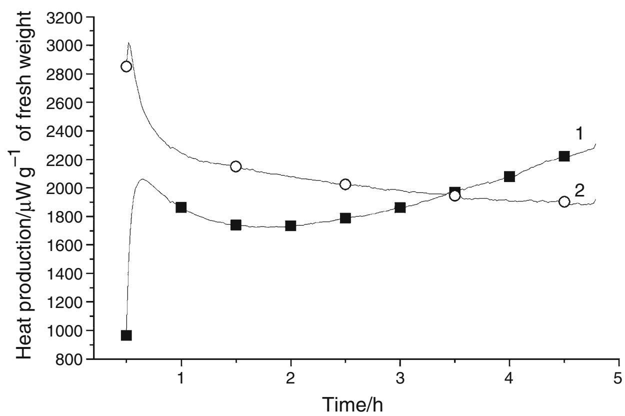

Fig. 2 Heat production by wheat roots in the presence of SNP (5×10^{-3} M): *1* control, *2* SNP

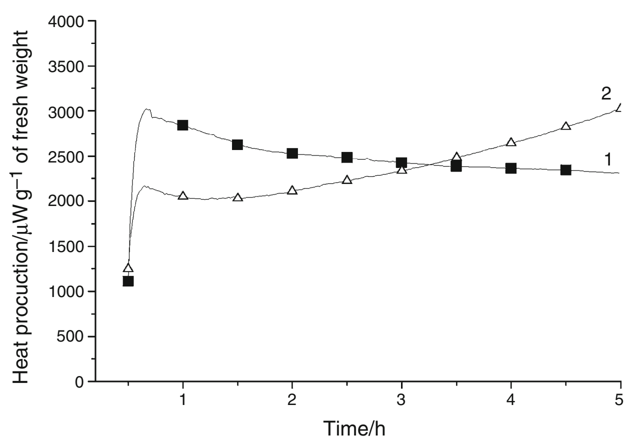

Fig. 3 Heat production by wheat roots in the presence of $NaNO_3$ (5×10^{-3} M): *1* control, *2* $NaNO_3$

One of enzymatic source of NO is nitrite reductase (NR). The primary function of the NR family of enzymes in plants is one of nitrogen assimilation by converting

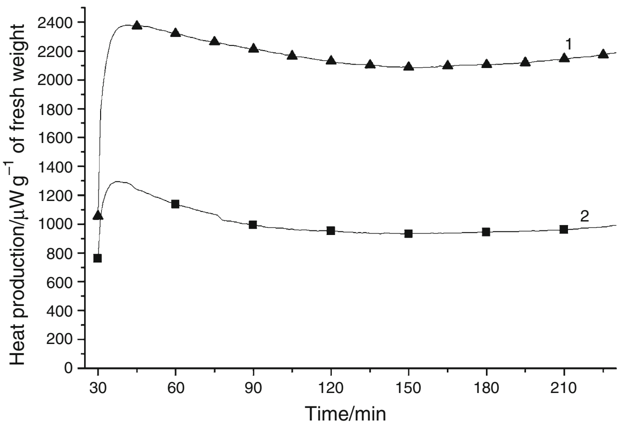

Fig. 4 Heat production by wheat roots in the presence of $NaNO_2$ (5×10^{-3} M): *1* control, *2* $NaNO_2$

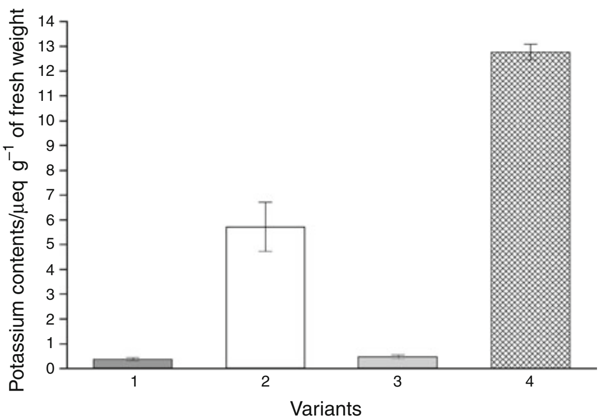

Fig. 5 Effect NO donors on the K^+ efflux from excised wheat roots *1* control, *2* SNP, *3* $NaNO_3$, *4* $NaNO_2$

nitrate to nitrite. NR can also convert nitrite to NO via a NAD(P)H-dependent reaction.

Nitrite as a substrate is used by cytosolic nitrate reductase (cNR) and by the root-specific plasma membrane-bound nitrite: NO reductase (Ni-NOR).

In the plant apoplast, a non-enzymatic production of NO from nitrite [17] depending of the acidic apoplastic pH and reducing agents was shown.

Possibly, the decrease in oxygen consumption at formation NO in our experiments is caused by its influence on cytochrome oxidase, and also on other complexes mitochondrial electron transport chain in plant cells. [7].

The inhibition of root respiration by NO was accompanied by a decrease in heat production in the presence of nitrate and nitrite. We previously demonstrated that blockage of the first and third complexes of the mitochondrial electron transport chain also led to a decrease in heat production by wheat roots [12].

It has been shown by us earlier that the excision of roots from wheat seedlings was accompanied by K^+-efflux from roots into incubation medium, this is a response reaction on the mechanical damage. But during incubation, the roots of control variant reabsorb all the K^+-ions and transform into the state of "relative dormancy." This state is characterized by the recovery of ion homeostasis, the decrease of plasma membrane permeability for ions as a result of alteration in the structural lipid content and reduction of energetic expense of root cell for ion transport [18]. Loss of potassium ions by root cells in variants with SNP and to a large degree with nitrate suggests that damage of a plasmatic membrane and its inability to restore an ionic gradient take place.

It is possible to assume that quantity of NO, formed with the use of nitrate, does not exert a considerable damage action on root cells. In the presence of the SNP, the cells adaptation to wound stress is worse, though heat production and oxygen consumption slightly differ from the control, but cells cannot reabsorb K^+-ions. The heat production and respiration in the presence of nitrite strongly decreases, root cells lose a significant amount of K^+-ions, probably NO formed in that case exert a toxic effect on root cells.

Roots have two separate membrane-bound systems to reduce nitrite to NO. Nitrite-dependent NO formation in roots serves for specific and important purpose. As a secondary messenger, NO appears to trigger cell death [19, 20].

Thus, our experiments with the use of SNP and sodium nitrate and sodium nitrite have shown that the cut roots did not equally react to different sources of NO. If at the presence of SNP and nitrate heat production, respiration of roots were slightly changed in comparison with the control variant and in the presence of nitrite there was a strong decrease in these indicators. Generation of NO from nitrite, apparently, exceeds that at action only of enzymes. Surplus formed NO brakes mitochondrial electron transport chain that leads to decrease heat production. Besides the energy lack leads to damage of work of ionic pumps of plasmatic membranes that is expressed in leak of potassium ions by the cells of wheat roots.

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