filled with sod-podzol soil with medium macroelement availability, pH 5.2–5.6 (in different test years). The cultivars were grown in 6l plots. The procedure included a control (without aluminum), Al1 (6 mg/kg soil), Al2 (13 mg/kg soil), Al1+K (100 mg K/kg soil), and Al2 + K (100 mg K/kg soil).

**Results and Discussion.** We determined that productivity gains in Al1 and Al2, in the presence of aluminum ions, exceeded the control and the variants with potassium. These data do not confirm nor disprove the hypothesis that potassium ions in a soil solution would diminish aluminum toxicity; aluminum is toxic as it is. However, detailed analysis of the situation helped unveil the reason of such results. Initially, the test in distilled water was conducted to exclude irrelevant effects. Wheat seedlings were grown in distilled water with using a control, 1 mg/l Al, 3 mg/l Al, 12 mg/l Al, and 40 mg/l Al. The results did not show a strict dependence in root length in the test variants, but we explicitly established activate growth in the above-ground parts of the plant at very low concentrations of aluminum in the medium (1 and 3 mg/l). These plants were called aluminum sensitive (Table 1). Less sensitive cultivars would have above-ground growth at higher aluminum concentrations.

**Table 1.** Length of wheat lseedlings at the 10 day (cm) in solution containing aluminum ions.

| Aluminum concentration | Cultivar | | | | |
|---|---|---|---|---|---|
| | Voronegskaya | Irgina | Priokskaya | Omskaya | Kerba |
| Control | 9.5 | 13.5 | 12.1 | 12.1 | 14.3 |
| 1 mg/l Al | 11.0 | 14.8 | 12.1 | 8.7 | 14.2 |
| 3 mg/l Al | 10.7 | 13.2 | 13.9 | 10.5 | 14.0 |
| 12 mg/l Al | 10.5 | 13.5 | 12.7 | 12.5 | 10.7 |
| 40 mg/l Al | 10.6 | 13.3 | 11.3 | 9.2 | 13.4 |

Exactly which cultivars are capable of active growth in the presence of aluminum at the lowest possible concentrations have yield ability exceeding that of the control (Fig. 2). These results show that those cultivars capable of steady growth on soils containing aluminum ions are also highly sensitivity to aluminum ions at the lowest possible concentrations at the earliest stages of development. The capability for sensing aluminum ions enabled plants to activate the mechanism to adapt and gain steady growth.

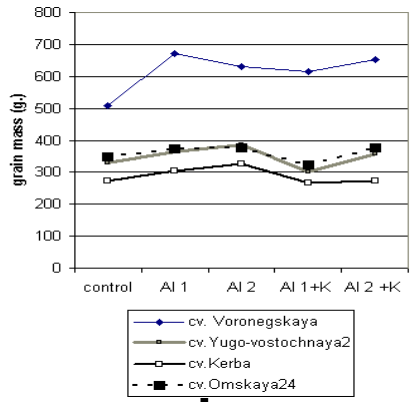


**Fig 2.** Grain yield (g/plant) for the cultivar Lada in the control (Ca) and in a solution of Ca + Al.

We know that potassium ions diminish the effects of aluminum toxicity and increase the general competition of ions in solution. However, in vegetative tests, the variants with added potassium ions showed low fertility, below that of control plants. Based on the activation of adaptation to aluminum ions by the reduction in the effects of aluminum toxicity by potassium ions, we believe that potassium ions are the reason for a plants inability to develop full tolerance against aluminum. The early growth phases, i.e., the period of adaptation against edaphic stress, on basis of aluminum ion sensitivity is vitally important for wheat cultivars capable of steady growth and adaptation to aluminum ions.

**SIBERIAN INSTITUTE OF PLANT PHYSIOLOGY AND BIOCHEMISTRY**
**Siberian Division of the Russian Academy of Sciences, Lermontov str., 132, Irkutsk-33, P.O Box 317, Irkutsk, Russian Federation, 664033.**

## *The oxidation of saturated free fatty acids by winter wheat mitochondria.*

N.Yu. Pivovarova, O.I. Grabelnych, T.P. Pobezhimova, N.A. Koroleva, and V.K. Voinikov.

The most important catabolic pathway of free fatty acids (FFA) is β-oxidation with acyl-CoA formation, which is further fully oxidized in the Krebs cycle to $CO_2$ and $H_2O$. Animal β-oxidation is known to take place in mitochondria and peroxisomes (Schulz H 1991). Questions about the localization of plant mitochondrial β-oxidation was under discussion

for a long time (Masterson and Wood 2000a). Using FFA as a mitochondrial oxidation substrate was discovered only at early stages of germination of oil-containing seeds such as sunflower and lettuce (Raymond et al. 1992; Salon et al. 1988). In experiments with pea mitochondria, differences in contribution of peroxisomal and mitochondrial β-oxidation at different stages of growth and different organs were shown (Masterson and Wood 2000b, 2001). We suppose that mitochondrial β-oxidation in plants plays a key role in the response of lipid metabolism on changes in plant organism development. Data exists on the possibility of highly purified mitochondria from glucose-starved root tips of maize to oxidize octanoate and palmitate (Dieuaide et al. 1993). At the same time, no data exists about the possibility that the mitochondria of winter wheat shoots use saturated fatty acids as oxidation substrate.

Previously, we showed that unsaturated (linoleic, oleic, petrozelinic, and erucic) and saturated (lauric, palmitic, stearic and begenic) fatty acids cause uncoupling of oxidative phosphorylation in the mitochondria of winter wheat shoots (Grabelnych et al. 2003, 2004, 2005). We found that unsaturated FFA could be used as the sole oxidation substrate for winter wheat shoots mitochondria (Grabelnych et al. 2003, 2004). The present investigation studied the possibility of saturated fatty acids as the sole oxidation substrate for winter wheat shoots.

**Materials and Methods.** Three-day-old etiolated seedlings of the winter wheat cultivar Irkutskaya ozimaya were germinated on moist paper at 26°C. Mitochondria were extracted from winter wheat shoots by differential centrifugation and purified on Percoll gradient as describes previously (Pobezhimova et al. 2001). The isolated mitochondria were resuspended in the following medium: 40 mM MOPS-KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA, 1 mM $MgCl_2$. Mitochondrial activity was recorded polarographically at 26°C using a closed-type platinum electrode in a 1.4-ml cell (Estabrook 1967). The reaction mixture contained 125 mM KCl, 18 mM $KH_2PO_4$, 1 mM $MgCl_2$, and 5 mM EDTA, pH 7.4. Mitochondrial β-oxidation was initiated by addition to mitochondrial incubation medium of 0.5 mM L-carnitine, 0.2 mM ATP, 10 mkM CoA, 0.1 mM $MgCl_2$, and 10 mM malate. In our work, we used such saturated fatty acids as lauric (C12:0), palmitic (C16:0), stearic (C16:0), arachidic (C20:0), and lignoceric (C24:0) in concentrations from 1 mkM to 500 mkM. The concentrations of respiratory chain inhibitors used were 0.4 mM KCN and 1 mM benzhydroxamic acid (BHAM). The concentration of etomoxir (carnitine *O*-palmitoyltransferase I inhibitor) was 37.5 mkM. The concentration of mitochondrial protein was analysed by Lowry method (Lowry et al. 1951). All experiments were performed on 3–6 separate mitochondrial preparations. The data obtained were analyzed statistically and arithmetic means and standard deviations are presented.

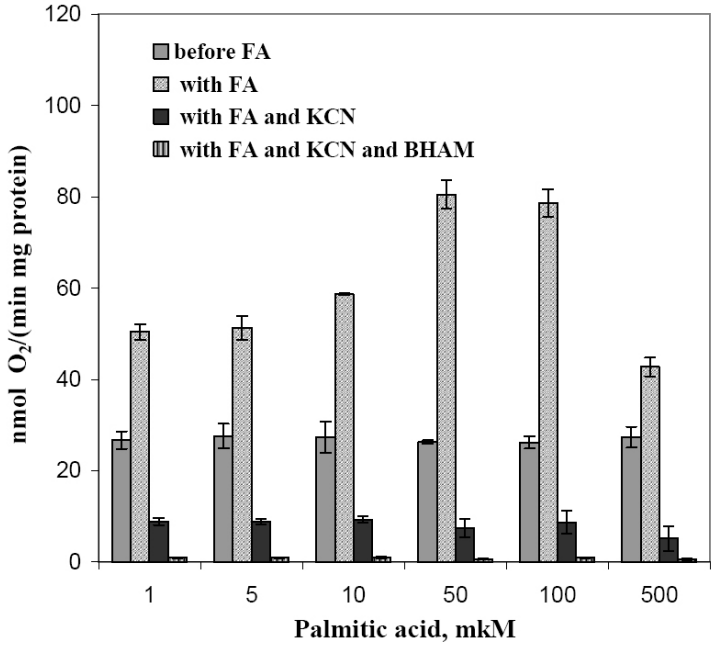


**Fig. 1.** The influence of palmitic acid on the consumption of oxygen in the mitochondria of winter wheat shoots. Winter wheat mitochondria (0.25 mg/ml) were suspended in reaction medium and respiration was initiated by fatty acid addition. Before FA, oxygen consumption rate of winter wheat mitochondria before fatty acid addition in presence of carnitine cycle and mitochondrial β-oxidation activators; with FA, oxygen consumption rate after fatty acid addition; with FA and KCN, oxygen consumption rate after addition KCN to mitochondria oxidizing fatty acid; and with FA and KCN and BHAM, oxygen consumption rate after subsequent BHAM addition. M±SD, *n=3-6*.

**Results and Discussion.** Previously, we found that the greatest uncoupling activity among studied saturated fatty acids had C12 and C16 acids. So it was interesting to study others roles of saturated acids in winter wheat mitochondria. We also found that unsaturated fatty acids, especially linoleic (18:2, n-9, 12) and α-linolenic (18:3, n-3) acids, could be used as a sole oxidation substrate for winter wheat shoots mitochondria. Here we show that saturated fatty acids could be used as a sole oxidation substrate for winter wheat mitochondria too.

All studied saturated fatty acids could not be used as oxidation substrate for winter wheat mitochondria without the addition of incubation medium substrates, which were necessary for mitochondrial β-oxidation and the carnitine cycle (Master-

son and Wood 2000a) and which was shown in the experiments when we did not add these substrates to the mitochondria incubation medium. In these experiments, no stimulation of respiration by winter wheat mitochondria occurred. But in experiments with the addition of L-carnitine, CoA, ATP, $MgCl_2$ and malate, mitochondria of the winter wheat shoots could use saturated acids as a sole oxidation substrate.

The possibility of using palmitic acid as an oxidation substrate for winter wheat mitochondria was shown in the presence of mitochondrial β-oxidation and carnitine cycle activators (Fig. 1. p. 124). The increasing rate of respiration depended on the palmitic acid concentration. The most significant increase in oxygen consumption by winter wheat mitochondria was observed after the addition of 50 and 100 mkM palmitic acid. The increase was 3-fold for both these concentrations. Higher concentrations of palmitic acid (500 mkM) did not cause significant increase in respiration rate. To determine how the cytochrome and alternative electron transport pathways participate during oxidation of saturated fatty acids, we studied the sensitivity of oxygen consumption induced by saturated fatty acid to such inhibitors of these pathways as KCN (that blocks electron transport through complex IV) and BHAM (that blocks electron transport through alternative CN-resistant oxidase). The palmitate-induced mitochondria respiration was sensitive to KCN and BHAM addition (Fig. 1, p. 124).

The use of stearic acid as an oxidation substrate for winter wheat mitochondria in presence of mitochondrial β-oxidation and carnitine cycle activators depended on concentration of this acid also (Fig. 2). The most significant increase in oxygen consumption by winter wheat mitochondria was observed after the addition of 50 and 100 mkM stearic acid. This increase was 3-fold and 3.6-fold for 50 and 100 mkM concentrations, respectively. Addition of KCN to winter wheat mitochondria oxidizing stearic acid caused full inhibition of respiration (Fig. 2).

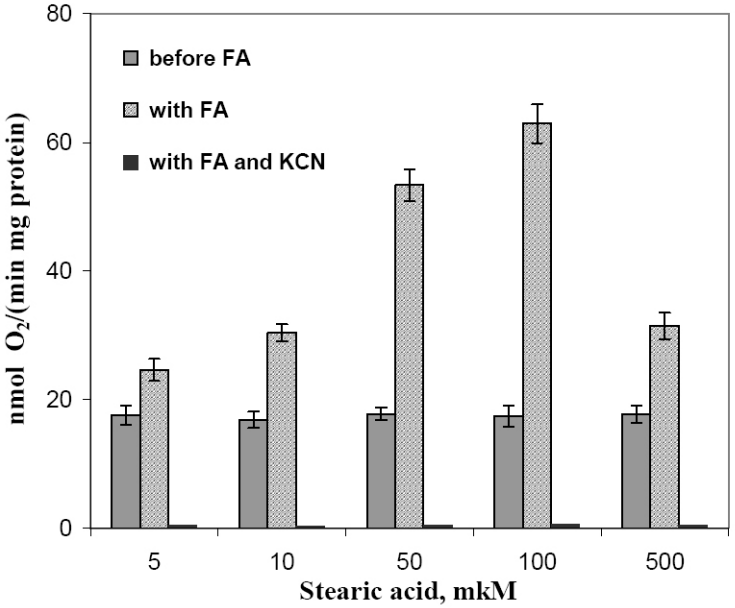


**Fig. 2.** Influence of stearic acid on the oxygen consumption of winter wheat shoots mitochondria. Winter wheat mitochondria (0.25 mg/ml) suspended in reaction medium and respiration was initiated by fatty acid addition. Before FA, oxygen consumption rate of winter wheat mitochondria before fatty acid addition in presence of carnitine cycle and mitochondrial β-oxidation activators; with FA, oxygen consumption rate after fatty acid addition; and with FA and KCN, oxygen consumption rate after addition KCN to mitochondria oxidizing fatty acid. M±SD, *n=3-6*.

We used etomoxir, which is irreversible inhibitor of carnitine *O*-palmitoyltransferase I (EC 2.3.1.21; CPT-I), the rate-limiting enzyme in the transport of long-chain fatty acids into mitochondrion, to prove that oxidation of saturated fatty acids associated with mitochondrial β-oxidation. The addition of etomoxir to winter wheat mitochondria oxidizing palmitic or stearic acid decreased the rate of oxidation caused by fatty acid addition (Fig. 3, p. 126). Sensitivity of mitochondrial respiration induced by palmitic or stearic acid oxidation to inhibitor of carnitine *O*-palmitoyltransferase I showed that oxidation of these acids was the result of classical β-oxidation process with carnitine cycle involvement. These data allow us to conclude that transport of palmitic and stearic acids into mitochondrial matrix involve carnitine shuttle system. Sensitivity of mitochondria respiration induced by palmitic or stearic acid oxidation to KCN and BHAM showed that during oxidation of these acids electrons could pass through cytochrome and cyanide-resistant pathways. We found that lauric (C12:0), arachidic (C20:0), and lignoceric (C24:0) acids could not be used as a sole oxidation substrate for winter wheat mitochondria even in presence of activators mitochondrial β-oxidation and carnitine cycle.

The data obtained allowed us to conclude that the mitochondria of winter wheat shoots could use saturated fatty acids as a sole oxidation substrate only in presence of mitochondrial β-oxidation and carnitine cycle activators. This means that oxidation of saturated fatty acids in winter wheat mitochondria is the result of classical mitochondrial β-oxidation with participation of carnitine shuttle systems. During mitochondrial β-oxidation of palmitic and stearic

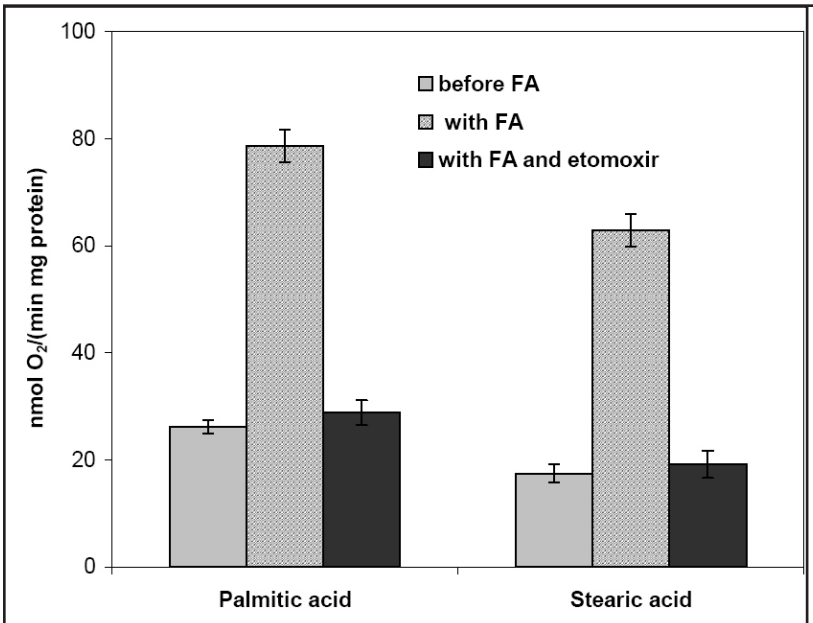


**Fig. 3.** Sensitivity of winter wheat mitochondria to oxygen consumption induced by palmitic acid or stearic acid oxidation to etomoxir. With FA, oxygen consumption rate after fatty acid addition; with FA and etomoxir, oxygen consumption rate after 37.5 mkM etomoxir addition to mitochondria oxidizing fatty acid. M±SD, *n=3-6*.

acids, electrons passed through the cytochrome and alternative pathways of mitochondrial electron transport chain.

**Acknowledgements.** This work was performed, in part, with the support of President Russian Federation grants for state support of leading scientific schools of Russian Federation (SS-4812.2006.4) and a young candidate of science of Russian Federation (YC-1876.2007.4), an interdisciplinary integration project of the Russian Academy of Sciences №47, and the Siberian Division of Russian Academy of Sciences Youth Grant (project 115).

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## *The sensitivity of winter wheat mitochondria swelling to inhibitors of ADP/ATP-antiporter and uncoupling proteins under stress conditions.*

N.S. Pavlovskaya, O.I. Grabelnych, T.P. Pobezhimova, N.A. Koroleva, and V.K. Voinikov.

The ADP/ATP-antiporter and uncoupling proteins (UCP) are integral proteins of inner mitochondrial membrane of plants and participate in induced by free fatty acid uncoupling of oxidative phosphorylation (Skulachev 1999; Bouillaud et al. 2001). Fatty acid-dependent uncoupling of oxidative phosphorylation plays an adaptive role during hypothermia and oxidative stress in the plant mitochondria (Casolo et al. 2000; Pastore et al. 2000). Moreover, the ADP/ATP-antiporter participates in formation of permeability transition pore (PTP) and in apoptotic processes of cell (Tsujimoto et al. 2006). PTP is opened by two modes: on the one hand, PTP is activated by $Ca^{2+}$ ions and inhibited by cyclosporine A (CsA) and $Mg^{2+}$ ions; on the other hand, PTP is a $Ca^{2+}$ - independent and insensitive to CsA and $Mg^{2+}$ ions (He and Lemasters 2002).

Our previous study showed that CsA-sensitive $Ca^{2+}$/palmitate-dependent mitochondrial PTP exists in mitochondria of the winter wheat seedlings (Pavlovskaya et al. 2007). Under conditions of cold stress and hardening, the mitochondrial pore functions as CsA-insensitive, whereas the oxidative stress followed short-term cold stress and cold hardening cause the appearance of mitochondria sensitivity to CsA (Pavlovskaya et al. 2007). Thus, different mechanisms seem to be responsible for the PTP function.

The aim of the present investigation was to study of swelling sensitivity from cold-stressed and cold-hardened winter wheat seedlings mitochondria to inhibitors of ADP/ATP-antiporter and uncoupling proteins and influence of oxidative stress on change of this sensitivity.

**Materials and Methods.** Three-day-old etiolated seedlings of the cold-resistant, winter wheat cultivar Zalarinka germinated on moist paper at 26°C were used. Seedlings were subjected to short-term (-1°C, 1 h) cold stress, cold hardening for 7 days at 4°C, oxidative stress, short-term (-4°C, 1 h) cold stress with subsequent oxidative stress or cold hardening for 7 days at 4°C with subsequent oxidative one. Oxidative stress was induced by immersing root tips of intact three days-old etiolated seedlings in 0.5 mM solution of $H_2O_2$ in the dark at 26°C for 4 h. The mitochondria were isolated from seedlings shoots by differential centrifugation (Pobezhimova et al. 2001), and their swelling was studied. The isolated mitochondria were resuspended in the following medium: 40 mM MOPS-KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA and 1 mM $MgCl_2$. Mitochondrial swelling was followed spectrophotometrically by the decrease in optical density (OD) of the mitochondrial suspension (0.25 mg/ml) under de-energized conditions at 26°C at 540 nm. We used the incubation medium including 200 mM KCl and 20 mM MOPS (pH 7.4). The following concentrations of test reagents were used: 0.1% bovine serum albumin (BSA) clear free fatty acids; 1 mkM carboxyatractyloside (Catr), an inhibitor of ADP/ATP-antiporter; and 1 мM GDP, an inhibitor of plant uncoupling mitochondrial proteins. The concentration of mitochondrial protein was analysed by Lowry method (Lowry et al. 1951). Results are represented as the mean of at least three determinations per experiment. The data obtained were analyzed statistically and arithmetic means and standard deviations are presented.

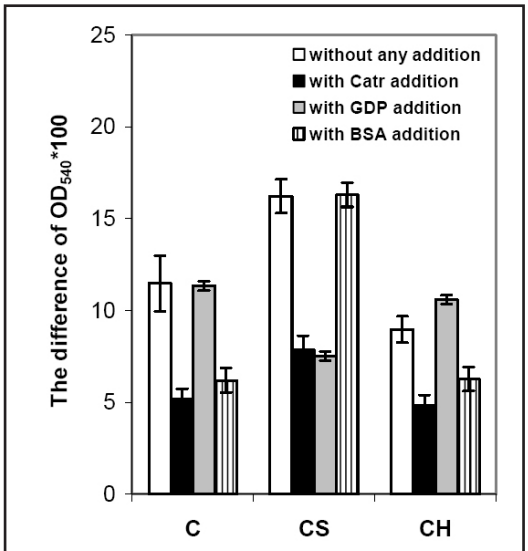


**Fig. 4.** The influence of inhibitors of ADP/ATP-antiporter and uncoupling proteins on the swelling of mitochondria from control (C), cold-stressed (-4°C, 1 h) (CS) and cold-hardened (4°C, 7 days) (CH) winter wheat shoots. The concentrations used were 1 mkM Catr, 1 mM GDP, and 0.1% BSA. The difference in optical density was calculated by formula $dOD=(OD_{t0} - OD_{t1})*100$, where $OD_{t0}$ is the initial optical density and $OD_{t1}$ the optical density after 5 min of incubation. M±SD, *n=3-6*.

**Results and Discussion.** In experiments with incubation of mitochondria isolated from control winter wheat seedlings with Catr, we detected the decrease in optical density of mitochondrial suspension in 5 min of incubation about 46.0% whereas GDP did not influence (Fig. 4). After BSA addition we observed the decrease of swelling extent mitochondria about 46.0% (Fig. 4).

The short-term cold treatment of seedlings was accompanied by increase of mitochondrial swelling extent and change of its sensitivity to studying inhibitors (Fig. 4, p. 127). The decrease of swelling after 5 min of incubation with Catr and GDP was 51.7% and 53.6%, respectively. After BSA addition to mitochondria incubation medium swelling extent of these organelles did not change (Fig. 4, p. 127). The extend of the decrease of mitochondrial swelling under the action of uncoupling proteins and ADP/ATP-antiporter inhibitors pointed to contribution of these proteins in response to short-term cold stress. If in mitochondria of control seedlings the action of GDP on swelling was absent, then its influence on mitochondrial swelling from seedlings subjected to cold stress pointed to important role of uncoupling proteins under conditions of cold stress. The absence of the influence of BSA on mitochondrial swelling was explained by increase of free fatty acid content during cold stress. We believe that used BSA concentration (0.1%) is not sufficient for binding these free fatty acids.

The cold hardening of seedlings led to a decrease in swelling isolated from mitochondria, however, the action of Catr, GDP, and BSA was similar to their actions in control mitochondria (Fig. 4, p. 127). These data indicated that only ADP/ATP-antiporter participated in swelling of winter wheat mitochondria caused by actions of free fatty acids during cold hardening as well as non-stressed conditions.

The oxidative stress caused by treatment of seedlings with a 0.5 mM solution of $H_2O_2$ did not accompany changes in mitochondrial volume. After the addition Catr to mitochondria incubation medium, seedlings subjected to oxidative stress we observed significant decrease of swelling (81.5%), whereas BSA caused only a 43.9% decrease in swelling; GDP did not influence (Fig. 5). These data indicated important role of ADP/ATP-antiporter during oxidative stress. Short-term cold stress and cold hardening of seedlings after subsequent oxidative stress was not accompanied by significant changes of volume isolated of them mitochondria (Fig. 5). The action of used inhibitors on mitochondrial swelling was similar to the action of these inhibitors on mitochondrial swelling from seedlings subjected to oxidative stress only. In both cases, the additions of Catr and BSA led to decrease of mitochondrial swelling extent whereas GDP did not have such influence (Fig. 5). These data prove that, under combined actions of two stress factors, the ADP/ATP-antiporter and uncoupling proteins contribution to mitochondrial swelling differed from one that of an individual stress factor.

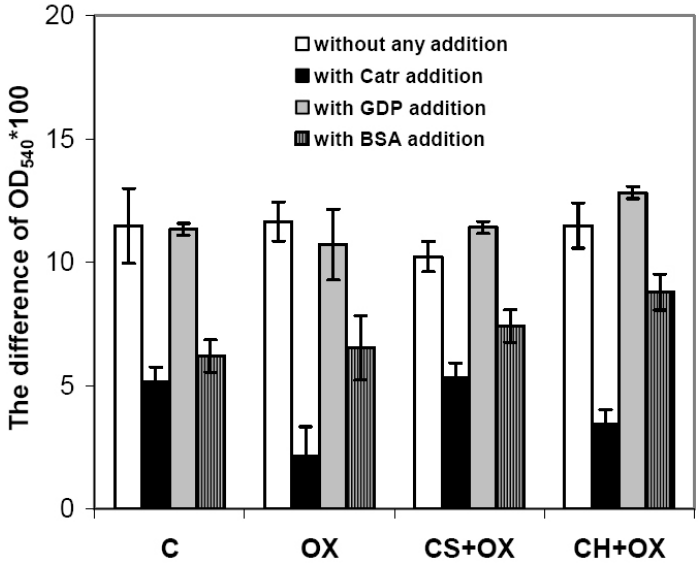


**Fig. 5.** The influence of inhibitors of ADP/ATP-antiporter and uncoupling proteins on the swelling of mitochondria from control (C), oxidative-stressed (0.5 mM $H_2O_2$, 4 h) (OX), cold-stressed (-4°C, 1 h) with subsequent oxidative stress (0.5 mM $H_2O_2$, 4 h) (CS+OX), and cold-hardened (4°C, 7 days) with subsequent oxidative stress (0.5 mM $H_2O_2$, 4 h) (CH+OX) winter wheat shoots. The concentrations used were 1 mkM Catr, 1 mM GDP, and 0.1% BSA. The difference in optical density was calculated by formula $dOD=(OD_{t0} - OD_{t1})*100$, where $OD_{t0}$ is the initial optical density and $OD_{t1}$ the optical density after 5 min of incubation. M±SD, *n=3-6*.

Our data indicate that ADP/ATP-antiporter participated in stimulated by free fatty acids swelling both in mitochondria from control winter wheat seedlings and in mitochondria from seedlings subjected to cold and oxidative stresses. The uncoupling proteins participated in mitochondria swelling process only during short-term cold stress when increase of free fatty acids occurred (Vojnikov et al. 1983). Because the CsA-sensitive pore functions in mitochondria of the winter wheat seedlings (Pavlovskaya et al. 2007), then ADP/ATP-antiporter contribution to mitochondrial swelling can suppose that this carrier can be involved to this pore formation.

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**VAVILOV INSTITUTE OF GENERAL GENETICS, RUSSIAN ACADEMY OF SCIENCES**
**Gubkin str. 3, 119991 Moscow, Russian Federation.**

**SHEMYAKIN AND OVCHINNIKOV INSTITUTE OF BIOORGANIC CHEMISTRY, RUSSIAN ACADEMY OF SCIENCES**
**Ul. Miklukho-Maklaya 16/10, Moscow, Russian Federation.**

## *Defensins of Triticum urartu and T. monococcum subsp. aegilopoides seed.*

T.I. Odintsova, T.V. Korostyleva, G.V. Kozlovskaya, and V.A. Pukhalskiy (Vavilov Institute of General Genetics) and A.K. Musolyamov and T. A. Egorov (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry).

Plants have evolved diverse mechanisms to combat fungal and bacterial infections. The most important among them are the reinforcement of plant cell walls and the release of different components with antimicrobial properties. They comprise the reactive oxygen species, phytoalexins, and PR-proteins including antimicrobial peptides (AMPs) (Selitrennikoff 2001; Garcia-Olmedo et al. 2001).

Defensins are the most conserved cysteine-rich AMPs which were found in nearly all taxa of living organisms: invertebrates, vertebrates, plants and fungi (Thomma et al. 2002). Plant defensins are small (45–54 amino acid residues), basic peptides with four disulphide bridges. Despite a conserved scaffold, the amino acid sequences of defensins vary considerably with only eight cysteine residues being conserved. Variation in amino acid sequences most likely accounts for diverse biological functions displayed by different members of the family. By *in vitro* studies, defensins were shown to exhibit antifungal/antibacterial and insecticidal activities, some of them inhibit enzymes, others act as ion channel