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ORIGINAL ARTICLE

The role of mitochondria in response of wild grass *Elymus sibiricus* L. seedlings to temperature stress, water deficiency and hydrogen peroxide exposure

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The relationship between energetic parameters and content of stress proteins (alternative oxidase, uncoupling protein and HSP70) in wild grass *Elymus sibiricus* mitochondria during different stress exposure (cold hardening, cold shock, high-temperature stress, water deficiency and oxidative stress) has been studied. It has been shown that influence of mild stress factors (cold hardening and exogenous 0.5 mM hydrogen peroxide treatment) result in adaptive changes related to the increase of activity alternative oxidase and the increase of content such proteins as alternative oxidase, uncoupling protein and HSP70. The comparative analysis of the function of the cultured and wild plants mitochondria in stress conditions has been produced.

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Key words: alternative oxidase / Elymus sibiricus / mitochondria / stress / stress proteins.

The plants undergo different stress effects (low and high temperatures, drought, salinization and other) in natural conditions of habitation. Subsequently external unfavorable factors often activate of similar signalling pathways and cell responses such as formation of stress proteins, regulators and antioxidations. In addition the abiotic stress is reason of oxidative stress (Wang *et al.,* 2003). Formation of reactive oxygen species (ROS) represents danger for cell and may lead to programmed cell death (Jabs, 1998). The effective protection against oxidative stress on cellular and subcellular levels shall provide high resistance of organism to external unfavorable factors. The mitochondria and their electron transport chain (ETC) are essential sources of ROS generation in

the majority of cell types, including plant cells (Navrot *et al.,* 2007; Kowaltowski *et al.,* 2009; Blokhina and Fagerstedt, 2010). In plants the avoidance of ROS formation as first line of protection against oxidative stress (Moller and Kristensen, 2004) may be achieved by functioning systems of uncoupling and noncoupling respiration such as alternative oxidase (AOX) and uncoupling proteins (Vanlerberghe and McIntosh, 1997; Sluse and Jarmuszkiewicz, 2002; Juszczuk and Rychter, 2003; Smith *et al.,* 2004; Tourchaninova *et al.,* 2005; Vercesi *et al.,* 2006; Jarmuszkiewicz *et al.,* 2010). AOX is known to activate by low temperatures (Vanlerberghe and McIntosh, 1992; Gonzalez-Meler *et al.,* 1999; Atkin and Tjoelker, 2003; Calegario *et al.,* 2003; Grabelnych *et al.,* 2004; Fiorani *et al.,* 2005; Sugie *et al.,* 2006; Armstrong *et al.,* 2008; Szal *et al.,* 2009; Grabel'nykh *et al.,* 2011), conditions that induced oxidative stress (Maxwell *et al.,* 1999; Szal *et al.,* 2003; Polidoros *et al.,* 2005), deficiency of water (Bartoli *et al.,* 2005; Ribas-Carbo *et al.,* 2005; Pastore *et al.,* 2007) and others. Induction of plant uncoupling mitochondrial proteins also occurs under cold treatments (Laloi *et al.,* 1997; Ito, 1999; Nantes *et al.,* 1999; Calegario *et al.,* 2003), oxidative and hyperosmotic stresses (Considine *et al.,* 2003; Trono *et al.,* 2004). AOX and uncoupling proteins may be called «stress proteins» because their syntheses are activated under stress conditions. It is known that AOX and uncoupling proteins act as regulators of ROS level in a cell (Popov *et al.,* 1997; Purvis, 1997; Kowaltowski *et al.,* 1999; Maxwell *et al.,* 1999; Grabel'nykh *et al.,* 2011) therefore activation and/or biosynthesis their proteins in stress conditions allow to prevent formation of ROS. In spite of a large number investigations devoted functioning of the nonphosphorylating systems in cultivated plants information about operation these systems in mitochondria of wild plants are few. Information about valuable agricultures of Baikal region is absent.

Thereby the aim of this work was the investigation of connection between energetic characteristics and some stress mitochondrial proteins composition and content in wild grass *Elymus sibiricus* L. under different stress conditions.

MATERIALS AND METHODS

Shoots of 7-day-old etiolated seedlings of *Elymus sibiricus* L. were used. Seeds have been gathered in Polovina station area (Circum-Baikal railway). Seedlings were grown on wet filter paper in thermostat at 26°C with preliminary cooling seeds at 4°C during 4 days. The assessment of temperature influence was done by exposure of control (C) seedlings in thermostat with different temperature conditions: the short-term treatment with subzero temperatures (cold shock, CS) was made by moving cuvette with seedlings in thermostat with -4°C for 2 h; the long-term low-temperature treatment (cold hardening, CH) was made under 4°C for 7 days; the high-temperature treatment (heat shock, HS) was made under 42°C for 4 h. Oxidative stress (OS) was made by moving seedlings on 0,5 mM H_2O_2 solution for 4 h at 26 ºC. In order to model condition of water deficiency (WD) seedlings were leaved without watering for 48, 72 and 96 h at 26°C. Water content of seedling shoots were estimated by their drying till constant weight in thermostat at 80°C.

Mitochondria were isolated from the seedling shoots by differential centrifugation as described previously (Pobezhimova *et al.,* 2001), but homogenization of plant tissue was made in mortar. The energy activity of mitochondria was measured by polarographically at 26ºC with the use of a closed platinum electrode in a 1.4-ml cell. The reaction medium contained 18 mM KH_2PO_4, 125 mM KCl, 5

mM EDTA, 1 mM $MgCl_2$ (pH 7.4). The oxidation substrates were: 10 mM malate + 10 mM glutamate, 8 mM succinate + 5 mM glutamate and 1 mM NADH. The incubation medium contained 3 mM rotenone (inhibitor of complex I ETC) during the oxidation of succinate and NADH. 1 mM benzhydroxamic acid (BHAM; inhibitor of AOX) and 0.4 mM KCN (inhibitor of complex IV ETC) were used. Inhibitors were added to state 4 mitochondria in polarograph cell. 5 mM ditiotreitol (DTT) and 1 mM pyruvate were used for AOX activation. Polarograms were used to calculate the rates of phosphorylative (state 3) and nonphosphorylative (state 4) respiration, respiration control by Chance-Williams (RC) and the ADP:O ratio (Chance and Williams, 1956). The concentration of mitochondrial protein was determined with Lowry method (Lowry *et al.,* 1951).

Mitochondrial samples were prepared with 5% β- mercaptoethanol as reductant in the sample buffer (62.5 mM Tris-HCl- buffer, pH 6.8, 1 mM EDTA, 1% SDS, 20% glycerol, and 0.001% bromphenol blue), boiled for 5 min at 92ºC and centrifugatied at 10000 rpm for 15 min. Supernatant was used for electrophoresis. For determination of content AOX the mitochondrial suspension was preliminary incubated in the presence of 1 mM DTT (30 min at 4ºC). The concentration SDS in sample buffer for determination of content AOX was 5%. The concentration of EDTA was 10 mM in sample buffer for determination of content PUMP. Determination of mitochondrial protein composition and content was carried out method of SDS-electrophoresis in 12.5% polyacrylamide gel (Laemmli, 1970) with following transfer of proteins to nitrocellulose according to the method of Towbin et al. (Towbin *et al.,* 1979) and Western-blotting with antibodies to HSP70 (HSP70/HSC70, SPA-820, «StressGen», USA), HSP60 («StressGen», USA), cytochrome *c* ("Biosan", Russia), AOX (antibodies were generously supplied by Dr. T.E. Elthon, Lincoln), uncoupling protein (antibodies were generously supplied by Dr. A. Vercesi, Campinas, SP, Brazil).

All the experiments were performed using 3-8 independent mitochondrial preparations. The data obtained were analysed statistically, i.e. arithmetic means and standard deviations were determined.

RESULTS

The influence of different stress factors on the energetic parameters of *E. sibiricus* mitochondria

Mitochondria isolated from control shoots of seedlings *E. sibiricus* had coupling of oxidation and phosphorylation processes during oxidation of all used substrates (malate, succinate and NADH).

On Fig. 1 and Fig. 2 data about influence of different stress factors on rate of state 3 respiration (phosphorylative respiration) and changes of RC coefficient of *E. sibiricus* mitochondria are showed. The cold shock (short-term treatment of subzero temperature) caused significant decrease of state 3 respiration during oxidation of all used substrates (Fig. 1A). At the same time decrease of RC coefficient was observed (Fig. 1B). The cold hardening of seedlings did not cause any decrease of state 3 respiration during oxidation of succinate and NADH (Fig. 1A). But in this conditions the increase of state 4 respiration and the decrease of RC coefficient occurred (about 32% and 26% during oxidation of succinate and NADH, respectively) (Fig. 1B).

The high temperature caused the significant decrease of state 3 respiration in mitochondria of *E. sibiricus* seedlings (about 38% during oxidation of malate and about 47% during oxidation of NADH) (Fig. 1A). It should be noted that decrease of RC

coefficient was observed during oxidation of succinate and NADH (about 21% and 14%, respectively) whereas any decrease of RC coefficient was not observed during oxidation of malate (Fig. 1B) that is related to significant decrease of state 4 respiration in this conditions.

The decrease of state 3 respiration was observed in mitochondria isolated from *E. sibiricus* seedlings after exogenous hydrogen peroxide treatment too. The most significant decrease was exhibited during oxidation of NADH (about 46%) (Fig. 1A). The increase of nonphosphorylative respiration rate (state 4) was feature of succinate oxidation by mitochondria (about 29%) that resulted in decrease of RC coefficient (about 25%) (Fig. 1B) at unchanging state 3 respiration rate (Fig. 1A).

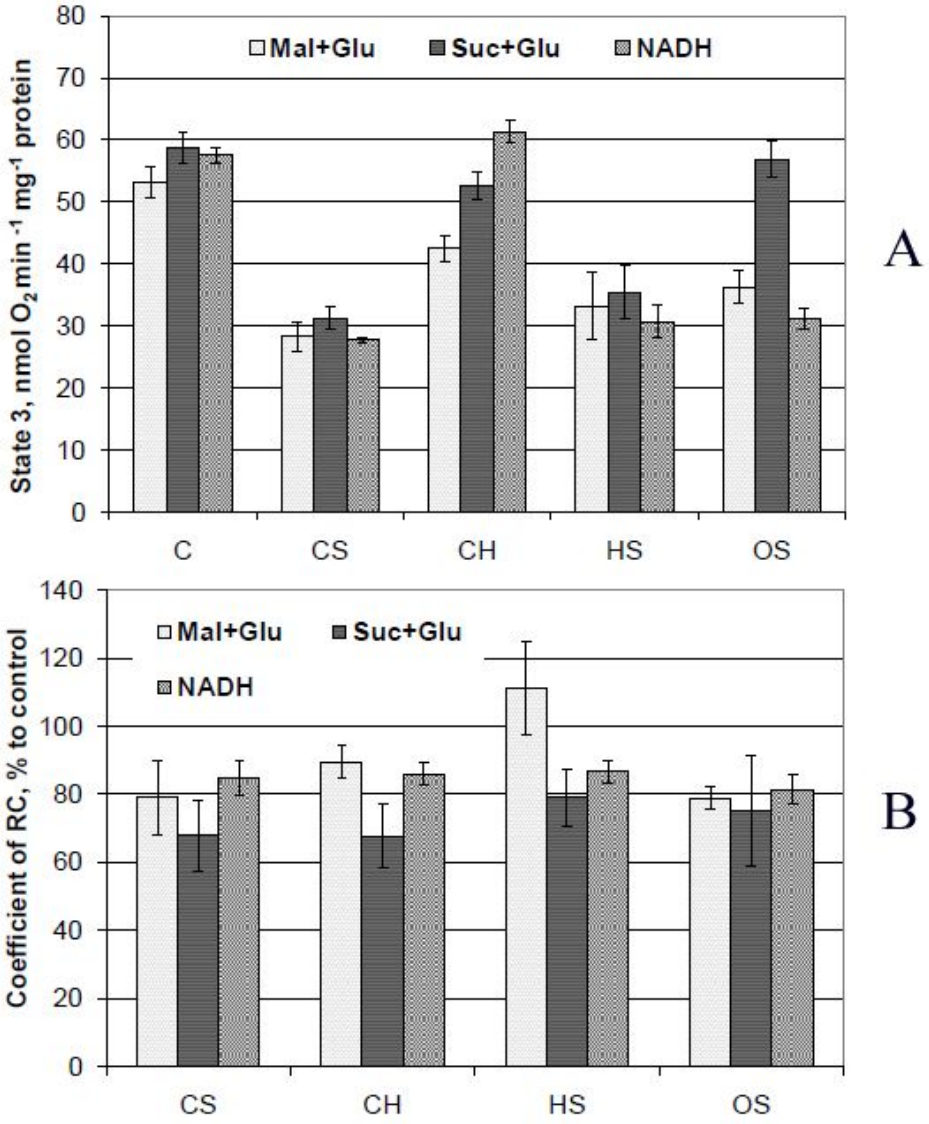

Fig. 1. An influence of low-temperature, high-temperature and oxidative stresses on rate of phosphorylative respiration (state 3) (A) and changes of RC coefficient (B) in mitochondria of *Elymus sibiricus*. 100% is RC coefficient of mitochondria isolated from seedlings without any stress factor.
C – control (without any stress factor); CS – cold shock (2 h at -4ºC,); CH – cold hardening (7 days at 4ºC); HS – heat shock (4 h at 42ºC); OS – oxidative stress (4 h with 0.5 mM H_2O_2). Mal+Glu - 10 mM malate + 10 mM glutamate, Suc+Glu - 8 mM succinate + 5 mM glutamate, NADH - 1 mM NADH. M±S.D., n=3-8.

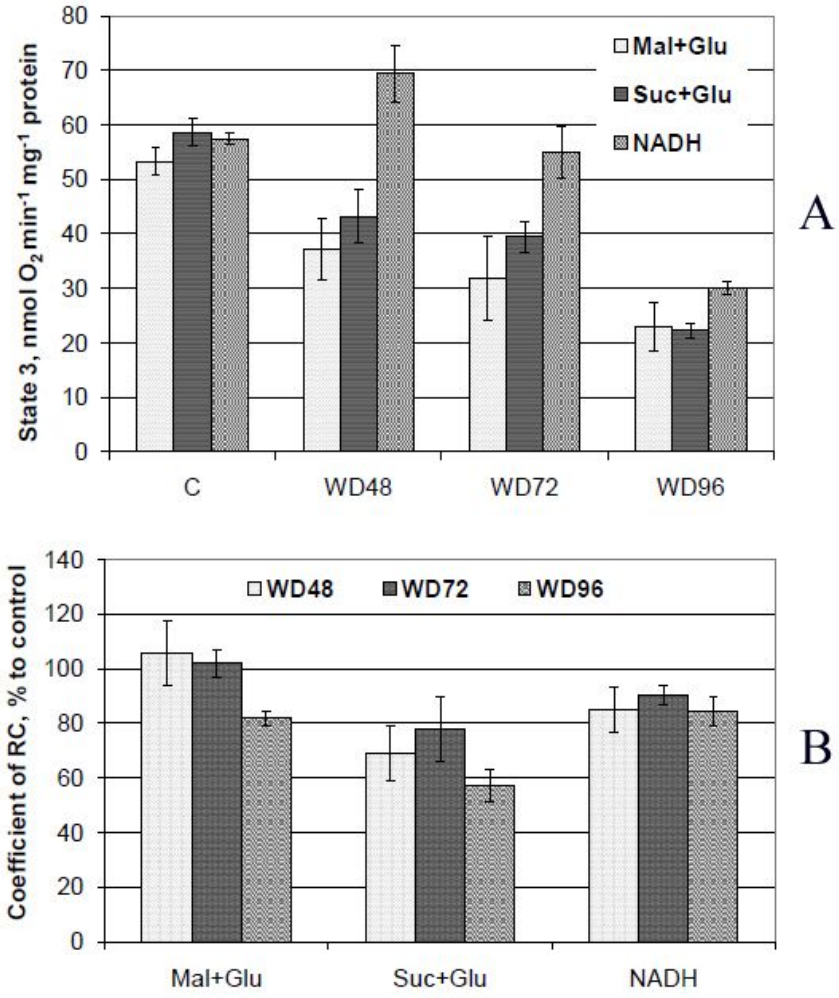

Fig. 2. The influence of water deficiency on rate of phosphorylative respiration (state 3) (A) and changes of RC coefficient (B) in mitochondria of *Elymus sibiricus*. 100% is RC coefficient of mitochondria isolated from seedlings before water deficiency.
C – control (without any stress factor); WD48 – water deficiency for 48 h; WD72 – water deficiency for 72 h; WD96 – water deficiency for 96 h. Mal+Glu - 10 mM malate + 10 mM glutamate, Suc+Glu - 8 mM succinate + 5 mM glutamate, NADH - 1 mM NADH. M□S.D., n=3-8.

The study of water deficiency on *E. sibiricus* mitochondrial activity indicated that absence of seedlings watering during 48 h was accompanied 20-30% decrease of state 3 respiration in mitochondria during oxidation of malate and succinate (Fig. 2A). At the same time capacity of mitochondria to oxidize exogenous NADH was increased and was on level of control in subsequent 72 h of WD (Fig. 2A). RC coefficient in 48 h of WD was not changed to comparison with control during oxidation of malate while during oxidation of succinate and NADH it was decreased about 31% and 15%, respectively (Fig. 2B). Expressive decrease of state 3 respiration and decrease of coupling extent of oxidative phosphorylation in mitochondria of *E. sibiricus* during oxidation of all used substrates was observed after 96 h of WD. Decrease of state 3 respiration rate was about 50-

60% (Fig. 2A). Analysis of relative water content of the seedlings *E. sibiricus* indicated that reliable decrease of watering tissue level by seedlings exposed to WD for 48 h was not occurred unlike seedlings exposed to dewatering for 72 and 96 h. In this case the significant decrease of relative water content was observed, about 42% and 53%, respectively. But relative content of water in tissues of seedlings was repaired on 98-99% through 3 days of exposure in conditions of normal wetting and survival of seedlings was 100%.

The contribution of alternative pathway in respiration of *E. sibiricus* mitochondria isolated from seedlings subjected to different stress factors

Method of inhibitory analysis with using of BHAM (inhibitor of alternative pathway of mitochondrial electron transport) (Schonbaum *et al.,* 1971) and KCN (inhibitor of ETC complex IV) was used for study of AOX capacity in mitochondria isolated from seedlings of *E. sibiricus* exposed to different types of stress. Since inhibitors of cytochrome pathway may cause the alternative pathway activation that we estimated the real AOX contribution, i.e. the rate that was sensitive to BHAM alone. Inhibitors was added to mitochondria in state 4 respiration. The study of AOX contribution in process of respiration carried out in presence of 5 mM DTT and 1 mM pyruvate (activators of this enzyme) (Umbach and Siedow, 1993) and 0.15% bovine serum albumin (BSA), inhibitor of uncoupling protein PUMP (Almeida *et al.,* 1999).

Inhibitory analysis showed significant contribution of alternative pathway in respiration of *E. sibiricus* control shoots seedlings mitochondria during oxidation of malate (about 36%) (Fig. 3). Contribution of alternative pathway in state 4 respiration during oxidation of succinate and NADH was 28% and 27%, respectively (Fig. 3).

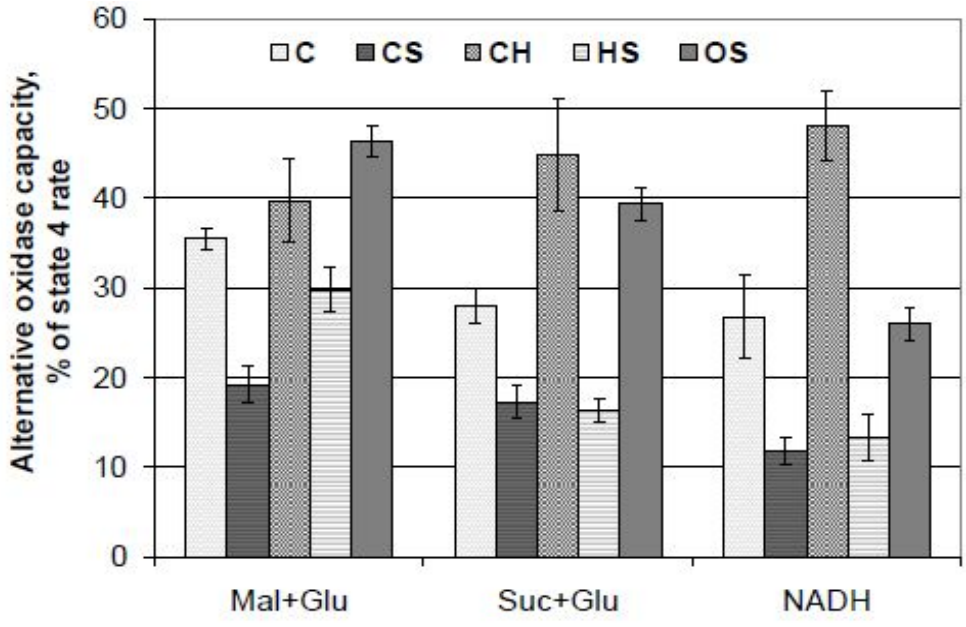

Fig. 3. Alternative oxidase capacity in mitochondria of *Elymus sibiricus* isolated from the seedling shoots subjected to influence of different stress factors. 100% is state 4 respiration of mitochondria before inhibitors addition.
C – control (without any stress factor); CS – cold shock (2 h at -4єC,); CH – cold hardening (7 days at 4ºC); HS – heat shock (4 h at 42ºC); OS – oxidative stress (4 h with 0.5 mM H_2O_2). Mal+Glu - 10 mM malate + 10 mM glutamate, Suc+Glu - 8 mM succinate + 5 mM glutamate, NADH - 1 mM NADH. M±S.D., n=3-6.

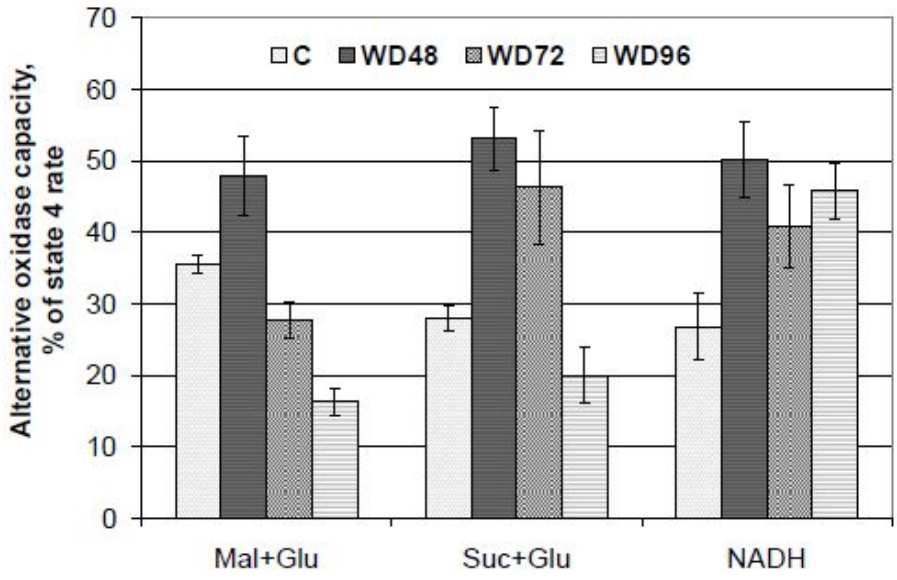

Fig. 4. The influence of water deficiency on alternative oxidase capacity in mitochondria of *Elymus sibiricus*. 100% is state 4 respiration of mitochondria before inhibitors addition.
C – control (without any stress factor); WD48 – water deficiency for 48 h; WD72 – water deficiency for 72 h; WD96 – water deficiency for 96 h. Mal+Glu - 10 mM malate + 10 mM glutamate, Suc+Glu - 8 mM succinate + 5 mM glutamate, NADH - 1 mM NADH. M±S.D., n=3-6.

Cold shock caused decrease of AOX contribution during oxidation of all used substrates (Fig. 3), that was related to increase contribution of cytochrome pathway. AOX capacity was decreased during oxidation of malate about 46%, succinate about 38%, NADH about 56% (Fig. 3). Cold hardening of *E. sibiricus* seedlings led to significant increase of AOX capacity in mitochondria during oxidation of succinate and NADH in contrast to action of subzero temperature. This increase was 60% for succinate and 80% for NADH, respectively (Fig. 3).

The influence of high temperature on AOX capacity in mitochondria of *E. sibiricus* seedlings was similar to influence of cold shock. The decrease of alternative pathway was observed during oxidation of all used substrates, especially NADH (about 50%) (Fig. 3). This significant decrease of AOX capacity in mitochondrial respiration was related to increase of cytochrome pathway.

Hydrogen peroxide treatment caused increase of contribution of alternative pathway in respiration during oxidation of malate and succinate by mitochondria. This increase was about 31% for malate and about 41% for succinate (Fig. 3).

Duration of water deficiency had an influence on AOX capacity in mitochondria of *E. sibiricus* seedlings too. The increase of alternative pathway during oxidation of all used substrates, especially succinate and NADH, for mitochondria from seedlings exposed for 48 h WD, was typical (Fig. 4). If the increase of AOX capacity observed during oxidation of malate was about 35% in comparison with mitochondria from control seedlings then this increase during oxidation of succinate and NADH was about 90% and 87%, respectively (Fig. 4). The contribution of alternative pathway in mitochondria of seedlings subjected to WD for 72 h during oxidation of succinate and NADH remained on only high level while during oxidation of malate it was decreased on 22% (Fig. 4). WD for 96 h caused

subsequent decrease of AOX capacity during malate oxidation (about 54%) (Fig. 4). Decrease of AOX contribution in mitochondrial respiration observed during oxidation of succinate was less expressed in comparison with such during oxidation of malate (Fig. 4). Contribution of alternative pathway respiration in mitochondria of *E. sibiricus* during oxidation of NADH was higher than in control mitochondria and the increase was about 71% (Fig. 4).

The study of changes in content of AOX, PUMP and HSP70 in *E. sibiricus* mitochondria under stress factors

Functioning of *E. sibiricus* mitochondria in stress conditions may be protected the composition of different stress proteins. Fig. 5 indicates the content of heat shock proteins (HSP70), uncoupling proteins (PUMP) and AOX in mitochondria of this wild grass. Immunoblotting with antibodies against HSP70 showed that all stress exposure caused increase of staining intensity of this protein band, but especially considerably heat shock (Fig. 5). Changes of PUMP content in of *E. sibiricus* mitochondria were detected too. While a low presence this protein (in form of dimer with molecular weight 64 kDa) in mitochondria from control of seedlings was observed then all stress factors caused increase its content but in a different degree (Fig. 5). The most significant increase of PUMP content was observed under cold hardening and hydrogen peroxide exposure (Fig. 5). Immunoblotting with antibodies against AOX allowed detecting in mitochondria of *E. sibiricus* seedlings two bands with molecular weight 31.5 and 34.5 kDa (Fig. 5). The increase of AOX content occurred after cold hardening and hydrogen peroxide exposure as well as PUMP content (Fig. 5). On Fig. 5 immunoblotting of *E. sibiricus* mitochondrial proteins with antibodies against cytochrome *c* and HSP60 has been presented too. The similar content of cytochrome *c* and HSP60 in all mitochondrial preparations is evidence of identical loading of protein and integrity of mitochondria isolated from *E. sibiricus* seedlings exposed to different stresses, too.

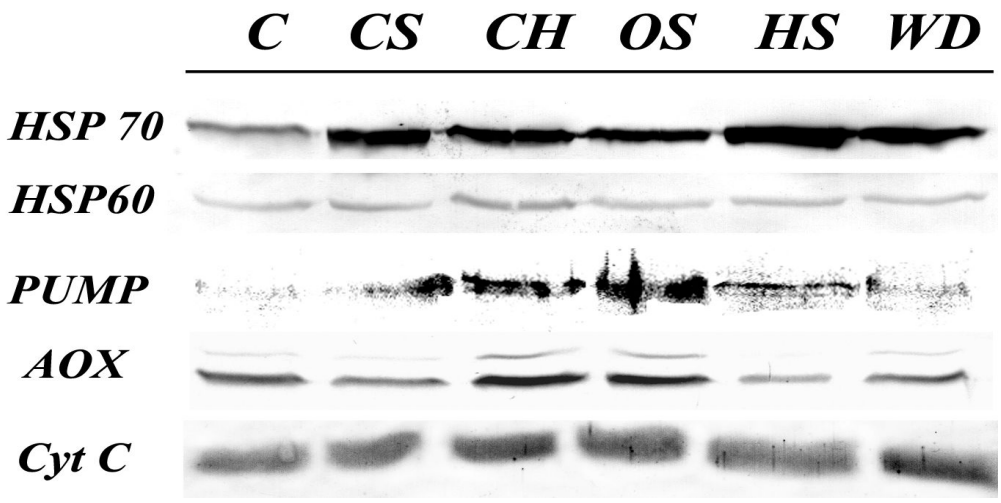

Fig. 5. Western blotting of total proteins of the mitochondria isolated from *Elymus sibiricus* seedling shoots subjected to influence of different stress factors.
C – control (without any stress factor); CS – cold shock (2 h at -4ºC,); CH – cold hardening (7 days at 4ºC); OS – oxidative stress (4 h with 0.5 mM H_2O_2); HS – heat shock (4 h at 42ºC); WD – water deficiency for 48 h.

DISCUSSION

The ability to defense against damage and unfavorable environment is obligatory property of any organism including plant. Plant mitochondria are one of regulating centers of energetic metabolism and play a crucial role in plant response to stress factors. In plant cell, especially nonphotosynthetic, mitochondrion is the main source of ROS production (Navrot *et al.,* 2007; Blokhina and Fagerstedt, 2010). At the same time mitochondria are able to defense themselves (and cell) against excess ROS production, they display three different strategies of defense. The first level of defense consists in avoidance of ROS production owing to preservation ETC components in sufficiently oxidized state, at the same time the second and the third defense levels are detoxication of ROS and reparation of damages induced ROS, respectively (Moller and Kristensen, 2004). It seems that the first level of defense is more advantageous for plant mitochondria and it is related to functioning of nonphosphorilating electron transfer systems. Alternative pathway of electron transport, related to functioning of CN-resistance AOX (Vanlerberghe and McIntosh, 1997; Juszczuk and Rychter, 2003), is branched from the main (cytochrome) ETC on the CoQ level, omitting two of three point of energy storage, which is released as a heat. Uncoupling proteins are supposed to be phylogenetic specialized proteins, which help to realize regulated uncoupling of oxidation and phosphorilation processes (Vercesi *et al.,* 2006). Results of our investigation have shown that adapting changes take place in mitochondria during treatment of seedlings of mild stress factors (hardening temperature, mild oxidative stress). These changes are related with activating of AOX capacity and increase of AOX, PUMP and HSP70 contents.

Cold shock, heat shock and long water deficiency (for 96 h) caused significant decrease of the respiratory rate. These stress factors also produced the most degree of inhibition of seedling growth that can be evidence of their damaging nature. Though *E. sibiricus* is defined as a wild grass with high cold resistance, the data about inhibition of the growth and respiration activity after the treatment with subzero temperature (-4°C, 2 h) at the laboratory conditions may be related with mild climate in the vegetation area of this grass. Seeds of *E. sibiricus* have been gathered on the coast of the Lake Baykal in Polovina station area (Circum-Baikal railway). The climate of this region is acutely continental with subzero average annual atmospheric temperatures (-0.6°C). The average temperature of July is +14-+16°C, of January is -16-18°C, but the climate of the shore is approximated to coast climate: here winter is more mild, a summer is cool. The atmospheric temperature of this region is five degrees above then in the Prebaikalia. This fact may explain the inhibition of the growth and respiration activity after the seedlings treatment with high temperature (42°C, 4 h). It is significant that subzero and high temperatures caused inhibition AOX capacity (Fig. 3) and some decrease of this protein content in mitochondria (Fig 5).

In contrast to action of short-term treatment with subzero temperature the long-term treatment of seedling with cold hardening temperature (4°C, 7 days) caused transfer of mitochondria to low-energetic state, that may be evidence of respiration reorganization directed to increase of tissue resistance. The increase of the rate of state 4 respiration was related to activating of AOX capacity. The AOX content in mitochondria has been observed in these conditions, too (Fig. 5). The data about increase of AOX activity in mitochondria

of *E. sibiricus* during cold hardening temperature are agreed with data obtained for cultured plants (Vanlerberghe and McIntosh, 1992; Gonzalez-Meler *et al.,* 1999; Atkin and Tjoelker, 2003; Calegario *et al.,* 2003; Grabelnych *et al.,* 2004; Fiorani *et al.,* 2005; Sugie *et al.,* 2006; Armstrong *et al.,* 2008; Szal *et al.,* 2009; Grabel'nykh *et al.,* 2011). Earlier research of the AOX activity in the mitochondria of different plant cultured species has been carried out by us. These species differed on the cold resistance level: winter wheat, maize and pea. The differences of alternative pathway functioning between cereals and dicotyledons have been discovered (Grabelnych *et al.,* 2004). The constitutive AOX activity has been showed to be higher in a pea, whereas the cold hardening decreases activity of this enzyme. At that time the AOX activity in mitochondria from wheat with high cold resistance was increased after the cold hardening of seedling independently of oxidation substrate. AOX activity in maize after cold hardening was increased too, but only during oxidation of succinate. Studied in this work wild grass *E. sibiricus* is response to cold hardening in a similar manner as winter wheat. The increase of AOX capacity to low temperature response is possible to be one of number genetically fixed features.

The increase of nonphosphorilative respiration and AOX activation also has been observed during mild oxidative stress. Western blotting has displayed increase of AOX content in mitochondria in these conditions (Fig. 5). These data point to the fact that increase of electron transport during cold hardening and oxidative stress through AOX may be related to the increase of protein activity and its content.

The influence of water deficiency on energetic activity of *E. sibiricus* mitochondria depended on its duration and the type of oxidation substrates. Some increase of the rate of nonphosphorilating respiration has been observed during water deficiency for 48 h, whereas after 72 – 96 h dehydration the decrease of the respiration rates has been occurred. The data about AOX activating during water deficiency are of interest. In mitochondria isolated from seedlings exposed to 48 h of WD the increase of AOX capacity has occurred during oxidation of the all investigated substrates: malate (electron transfer begins from the complex I of ETC), succinate (electron transfer begins from the complex II of ETC) and NADH (electron transfer begins from the "outer" NADH-dehydrogenase) (Fig. 4). At the more prolonged water deficiency to 72 h AOX activation has been observed during the succinate and NADH oxidation. Water deficiency during 96 h has caused activation of AOX capacity only at the NADH oxidation (Fig. 4). It is likely this fact is evidence of more functional stability of the part of ETC: "outside" NADH-dehydrogenases – ubiquinone – AOX, under water stress conditions. There are few in number data assuming defense role of AOX under water deficiency (Bartoli *et al.,* 2005; Ribas-Carbo *et al.,* 2005; Pastore *et al.,* 2007). It is supposed that in green tissues during water stress AOX acts as antioxidant defense system together with photorespiration cycle, whereas in etiolated tissues its role is open to question.

The increase of the PUMP content after cold hardening and hydrogen peroxide exposure points to the fact that the increase of nonphosphorilating respiration observed in our experiments during these treatments may be related with the contribution of uncoupling proteins (Fig. 5). The increase of PUMP expression during long-term cold treatment and oxidative stress has been observed by other authors (Brandalise *et al.,* 2003; Calegario *et al.,* 2003). It has been shown that high expression of PUMP in the transgenic tobacco cells lead to significant (in comparison with control plants) increase of the

resistance against oxidative stress caused the treatment with hydrogen peroxide (Brandalise *et al.,* 2003).

Sluse and Jarmuszkiewicz have been showed that increase of the PUMP content strongly inhibited CN-resistance respiration mediated by AOX activity (Sluse and Jarmuszkiewicz, 2000). Furthermore, investigation of the AOX and PUMP expression and their activities during maturing of tomato fruits let to establish that these proteins works sequentially (Sluse and Jarmuszkiewicz, 2000). This fact is agreed to data by Sluse and co-authors about that AOX and PUMP don't work simultaneously (Sluse *et al.,* 1998). However in our work cold hardening and oxidative stress induced simultaneous increase of AOX activity and content as well as PUMP content in the mitochondria of *E. sibiricus* (Fig. 5). It has been displayed in the work by Calegario and co-authors (Calegario *et al.,* 2003) that treatment of the potato tubers with cold temperature induces simultaneous increase of AOX and PUMP activity.

Increasing the content of the one of a number heat shock proteins HSP70 in mitochondria of *E. sibiricus* has been showed at our work (Fig. 5). Heat shock proteins (HSP) are ones of crucial elements of stress defense system in an organism. The level of their induction depends on the ability of organisms to withstand influence of different stress factors. Many HSPs is also present in the cell without high temperatures because they realize some functions different from defense the cell against stress. Constitutive forms of the HSP70 family functioning without stress promote folding of the synthesized proteins, protein transport into cell organelles and defective protein degradation. Stress-induced forms of HSP70 prevent from aggregation of the denaturated proteins; promote refolding and recovering their biological functions (Mayer and Bukau, 2005). Activation of the HSP70 synthesis under stress conditions need defense proteins against irreversible damage.

AOX, uncoupling proteins and HSP70 investigated by us are the proteins of nuclear coding. Mitochondrial regulation of the nuclear genes expression, named as "retrograde regulation" (Rhoads and Subbaiah, 2007), has been investigated not enough. ROS may play a role inducing AOX gene expression, as proposed by Wagner and Krab (1995). It has been showed that ROS are ones of the factors underling transfers of the signals from mitochondria to nuclei necessary for *AOX1* expression (Gray *et al.,* 2004). Szal with co-authors (2009) suggested that the intensity of the signal from the mitochondria leading to the induction of nuclear genes depends not only on an overall increase in ROS production by the respiratory chain but also on its amount generated specifically on the outer face of the inner mitochondrial membrane. The central role in mediating mitochondrial retrograde signals to induce the expression of *AOX1a* plays ABI4, an ABA responsive transcription factor (Giraud *et al.,* 2009).

Thus the involvement of AOX into cell response to stress let to suggest that this protein realizes one of the important adaptive strategies in plant cell and all its functions are aimed to maintenance cell homeostasis under mobile environment conditions. However plants may have different strategies at the mitochondrial level to survive under unfavorable stress factors, in order to understand these strategies and their functional significance, both short and long-term experiments with cultured and grass plants under a variety of stresses (field and laboratory conditions) are needed.

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