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Alternative Engagement Investigation of Electrical Transport Circuit of Plant Respiration in Extreme Salinity Condition

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Abstract

Alternative pathways of electrical transport circuit of plant respiration has been investigated in extreme salinity condition. It was revealed that in extreme salinity condition the of the respiration circuit pathway concerning to oxidation-phosphorate (I) activates at low partial pressures of oxygen. However at high salt density or salts long-term effect due to oxidizing-phosphorate separation the II alternative respiration circuit engagement/pathway, id.est. cionide-resistant free oxidation pathway starts to activate and forms water here as it occurred in the I engagement. The III engagements/pathways, id. est. a two-electronic free alternative oxidizing tract of the respiration circuit at strong salt stress condition starts to activate; on the result much amount of H_2O_2 is accumulated in seeds. Peroxidase and catalase systems play an important role at decomposition of the accumulated H_2O_2 in free oxidation pathway.

Keywords: *Mitochondrion, Poliarography, Inhibitor, Electrical transport circuit of respiration, Alternative engagements/pathways.*

Two billion ha of productive lands have lost their significance on the Earth during a short enough period of historical time. It is more than 1.5billion ha of used for sowing all around the world currently. So, mankind loses 15billion ha of biologically productive land every year. One of the principal causes of decrease of productive lands is increase of saline soil areas in the Earth (Dobrovolskiy, 2004).

According to the recent calculations 1/15 part of the land area of the Earth including the lands used in agriculture are saline lands. In addition approximately 20% of the irrigated area are saline lands (Kafi et.al., 2003; Kouznetsov, Dmitriyeva, 2006) .

According to the available information approximately 50% of the lands that should be sown will be sustained to salination in 2050 (Ashraf, 1994). Thereby land salination is one of the most serious problems that negatively effects onto productivity of agricultural crops in all over the world. (Greenway, Munns, 1980; But et.al., 1998; Gasymov, 2004; Abdiyev, Gasymov, 2012).

According to the result of the carrying out researches negative effect of the salts onto plants more sharply shows itself in the first periods of the ontogenesis; the plants are fewer salt-resistant (Weiping Ch. Zenan Hou, 2010).

That is why to identify mechanizm of salt effect onto plant organizm study of physiological and biochemical processes are of great significance at development phases of sprouts.

One of the central and global processes is respiration at plant organisms. And the respiration process itself is directly a component of the biological oxidation that is considered as more general one (Polevoi, 1989; Kouznetsov, Dmitriyeva, 2006; Gasymov, 2008).

Besides biological oxidation at the same time biological reduction also has widely spread (photosynthesis) at green plants and it has number of alternative pathways. It is impossible to investigate all the alternative engagements/pathways in the both systems. That is why the investigations that we carried out have been devoted only to study of the alternative engagements of the biological oxidation.

Though respiration intensity, gas exchange and other indices at plants have been studied in detail all the alternative engagements/pathways of biological oxidation weren't enough investigated yet. That is why definition of extreme salt effect and energetic efficiency onto respiration intensity at plants, as well determination of alternative oxidation engagements/pathways are of extremely important problems at plants.

Object and methods of the research

As an object of the research 5-days sprouts of *Bərəkətli* (*Triticum aestivum* L.) wheat variety have been used.

The sprouts etiolated in the dark have been cultivated in the condition of a normal aeration in a

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Knop solution at (0.04 mg O_2 (min) 25°C temperature thermostat.

At first the seeds have been wetted in distilled water or salt solutions in Petri cups within 20 hours. Then the swelling seeds were placed on the single-layered filter paper on the glass board fastened to edges of special cell 300 ml volume with water or salt solutions in it. Edges of the filter paper are put into water so to let it always be humid. Then placing the 2nd filter paper onto the sprouts one edge of it is put into water so that let the water be absorbed. Two days later the sprouts are shifted into big glass vessel of 1,5 liter volume placing them between the glass plates which are located parallel to each other. This time the growing goes normally and plants are not being subjected to mechanical effects. Every day the solution with roots of the sprouts are saturated with air by means of electromagnet micro-compressor (MK-2) within 2-3 hours.

Oxigen absorbtion at roots of the sprouts has been researched by polyarographic method using platin electrode (Gasymov, 2012).

A platinum wire (0.5 mm diameter) placed to a glass capillary is used as a cathode in the circle. This electrode associates with copper wire by mercury. Platinum electrode tips have been isolated accurately by remaining 2-3 mm length bare.

And chlorine silver electrode associated with (electrode which does not make polarization) KCl small bridge connected by means of the liquid environment is used as an anode. The generated currents are registered by means of a galvanometer (M-95) that possesses $2 \cdot 10^{-9}$ A/division sensibility. Difference of potentials equal to 0.65V is kept (to specify the oxygen) in the electrodes. The device sensibility is $3{,}2 \cdot 10^{-7}$ MO_2/l. Sensibility of the platinum electrode is checked by Winkler method allowing to determine whole quantity of the oxygen available in the water. Zero thickness of the oxygen is generated after filling 1% solution of sodium sulphide into a glass vessel. Block scheme of the polarographic device have been shown (figure 1) to determine oxygen absorption.

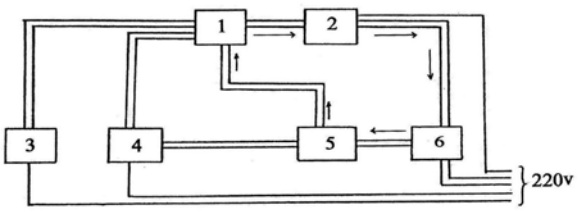

Figure 1. General block scheme of the polarographic device to determine O_2 absorption by plants: 1.electrochemical glass vessel of little volume; 2.lab pH-meter; 3.reflecting galvanometer; 4.ultrathermostat; 5.thermostatic cuvette where the objects in it; 6.micropump.

Isolation method of mitochondria

Homogenate obtained of root of the etiolated 5-days sprouts of '*Bərəkətli*' wheat variety have been used for the investigation.

Mitochondria's isolation has been carried out as follows: (Gavrilenko, Ladygina, Khandobina, 1975).

Density of isolation components, incubation environments, and hydrogen index (pH) all have been exactly specified to obtain mitochondria active fraction.

On the purpose of mitochondria phosphorate activity increase and morphological integrity stabilization – 0,5 M saccharose, 0,005 M EDTA (ethylene diamine tetra acetate) and 0,06 M potassium phosphat buffer (pH-7,4) have been used as an isolating environment. All solutions used for mitochondria isolation have been prepared in bidistilled water.

Purity degree of the mitochondria fraction has been determined visually in the light microscope, later on it was done by means of saccharose density gradient. Moreover, mitochondria integrity has been defined according to respiration controle number ADP/O ratio. Mitochondria's intactness was over 93%. Mitochondria obtained by this method are characterized with high phosphorate and respiratory coefficient. Oxygen absorption at mitochondria has been carried out in cylindrical vessel of $8cm^3$ volume and mitochondria fraction was mixed by means of electromagnet (fig.2).

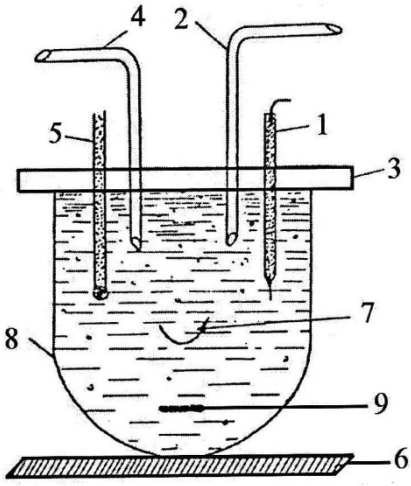

Figure 2. Polarographic glass vessel of a little volume: 1. Platinum electrode; 2. Plastic small tube (output); 3. Rubber stopper; 4. Plastic small tube (input); 5. Electrode for comparison; 6. Frame; 7. Circulating liquid; 8. Magnetic mixer; 9. An iron string in the small glass tube.

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Research of Peroxidase activity

Peroxidase activity in the roots of plant sprouts is based on speed of the oxidation reactions generated up to definite density of benzidine oxidation blue solution. Density of blue oxidation solution is identified by photo-electro-colorimeter (UXL-42) (Yermakov, 1987). Peroxidase activity has been calculated according to the reaction speed.

Definition of Catalase activity

This method is based on quantity of substratum (H_2O_2) decomposed by catalase ferment during certain period of time (Dobrynina, Svetnikova, 1967).

H_2O_2 quantity has also been specified by titration method (Dobrynina, Svetnikova, 1967).

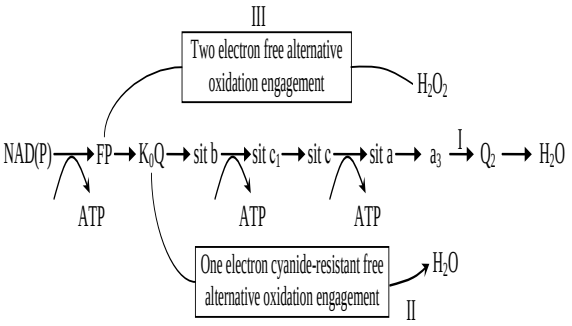
The investigations have been carried out by 3-4 repeats; the obtaining results were statistically developed (Lakin, 1990). Exactness index has been below 5% in the investigations; the obtained results are mathematically reliable.

Salts as: ADP, KCN, 2,4 DNF, rotenone, antimitsin A, NAD·H and NADP·H have been used ("Serva" firm of the Germany Federative Republic).

Results and discussions

Alternative engagements of respiration circle at plants are more than at animals and basically they can be grouped as follows (scheme1):

1. Oxidizing phosphorate engagements/pathways (phosphorating oxidation) connected with ATP synthesis;
2. Cyanide-resistant alternative oxidation engagement/pathways;
3. Alternative oxidation engagement/pathways observed by the H_2O_2 generation.



Scheme 1. Respiration circle alternative engagement.

An English scientist Lenincer (Lehninger, 1951) proved by means of experiments that there were alternative (parallel) engagements/pathways at electron transportation.

One of them is 'free' another one – is connected with phosphorating as above said. Both engagements/pathways are located in a highly ordered subcell structure in mitochondria. But the oxidizing phosphorating is connected with inside membrane of mitochondria, and the free oxidation is connected with the outside membrane (Severin, 2004; Gasymov, 2008).

It is notable that comparative investigation of respiration electro-transport circle components' functional status at intactical plant roots and isolated mitochondria in extreme saltiness condition is one of the important problems that stand in front of the Physiologists.

Even in the 30-th years of the XX Century the fact of oxigen absorbtion acceleration was revealed while adding 6 - carbonate acid, 5 - carbonate acid and 4 - carbonate acid to animal tissures homogenatone in the investigations carried out by A.Sent-Diyerdi and H.Krebs (Leninger, 1985). Afterwaeds H.Krebs determined cyclic character of this process.

Effect of lemon acid to O_2-absorbtion on the organizm level (at plant sprout roots) has been investigated by polarographic method.

On the identification purposes of engagement/pathways normal functioning accompanied by (ETC) ATP synthesis of respiration electronic transport circle of oxygen absorption at wheat sprout roots 100 mcM ADP and 1 mM lemon acid have been added into the system (fig2). As it is seen O_2 absorption becomes very difficult when ADP is added into the root system kept in the water. However the process is more accelerated when 1 mM lemon acid is added. It means normal function of the respiration ETC. In such case by ATP synthesis at tree NAD→FP, sit b→sit c_1 and sit c→sit a areas also on the result of a one-electron transportation H_2O is generated at the end.

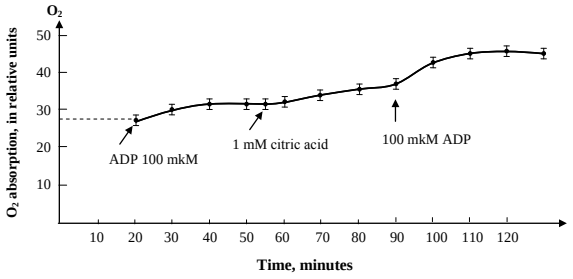


Figure 3. Kinetic curve of oxygen absorption at wheat sprout roots when ADP and lemon acid effect.

While organic acids turn within the Krebs cycle protons and electrons enter into the respiratory

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circle by means of two coferment types. One of them belong to NAD^+, $NADP^+$ anaerobe dehydrogenasea and another one – to (FAD^+) aerob dehydrogenasea. Thus, on the result of the organic acids turn, 6 pair electron (proton) connect into respiratory circle by means of anaerobe dehydrogenasea, with (NAD^+, $NADP^+$) pridine system; and 2 pair connect into respiratory circle with flavan oxidase on the KoQ level; as a result in the 1-st phosphorate point (NAD→FP) 10 molecules, in each of the 2-nd and 3-rd phosphorate point 12 molecules ATP are synthesized. And 34 molecules ATP are synthesized at coferment phosphorate. So, NAD·H and FAD·H participate as a 'substrat' in respiratory circle by glycolise and Krebs cycles. That is why to ensure normality of functional activity of the respiratory END in the experiments we have added 0,5-1 mM NADH and NADPH in an exogenous state into the system. It is clear from the 3rd figure that NADH has increased the O_2 absorption more sharply than NADP·H.

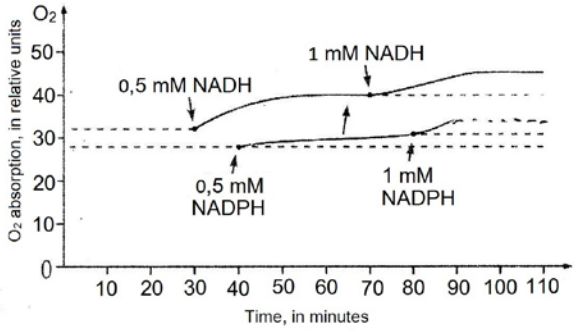

Figure 4. Kinetic curve of oxygen absorption at roots of wheat sprouts when NAD·H and NADP·H effect.

In the next researches wheat seeds (25-50 mM) were wetted in NaCl and continuously kept in the salt solutions for 6 days.

During the experiments plant root moved to water and 1mM of NAD·H was added into the system. It is clear from the fig. 4 that NAD·H accelerates oxygen absorption.

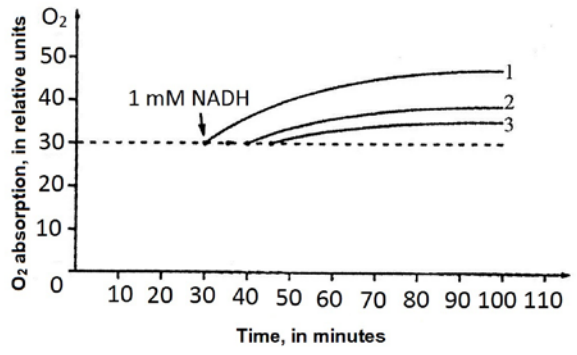

Figure 5. O_2 absorption of wheat sprout roots grown in NaCl solutions of different dense. Effect of NAD·H: 1 – control; 2 – 25 mM NaCl; 3 – 50 mM NaCl.

Basic components of the respiration electron transport circle passes into oxidation state during long-term effect of salts that is why quantity of respiration substrates sharply decreases. As it is seen from the figure 4 oxygen absorption by plant roots increase; however the increase is less than the controle version when 1 mM NADH is added into the system.

It has been ascertained in the previous investigations that oxygen absorption at plant roots reduced on the result of salts long-term effect (Abdiyev, Gasymov, 2012).

Reduction of oxygen absorption at plant roots is connected with a decrease of reduction activity in root system (Gasymov, 2002).

It is notable that the first investigations about mitochondria have been devoted to study of their structure. Only then the investigations concerning to study of their functional activity have been carried out. This time while using apple acid as a substratum and the NADH ATP synthesis has been revealed.

While studying consecutive addition effect of respiratory electron transportation sytochrom engagement/pathway inhibitors it has been revealed that their functions are entirely within the existing present-day imaginations about electron transport circle structure at plant mitochondria (Voynikov et.al, 2006). Indeed, addition of the rotenone – which is the first segment inhibitor of the respiratory electron transportation causes decrease of oxygen absorption by the mitochondria. Then addition of the antimitsin-A which inhibits the transportation of electrons to mitochondria by the main sytochrom engagement/pathway at the III complex accelerates decrease of oxygen use by mitochondria. After 1 mM NADH addition to mitochondria the oxygen absorption acceleration shows that mitochondrial oxidation blockade in the I and III complexes of the electron transportation circles occurs due to start of alternative engagements/pathways of reducing equivalents pass to (figure 5).

It has been ascertained that when adding 1 mM KCN (kalium-cyanide) – inhibitor respiratory circle's fourth segment into the system oxygen absorption by mitochondria decreases. However addition of 1mM NADH not only liquidates the oxygen absorption decrease but increases it (figure 6).

Oxygen absorption reduces on the result of inhibitors effect but increase of the partial pressure of the O_2 causes start of cyanide-resistant engagement/pathways.

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It was ascertained that while adding NADH, ADP və NaCl into the system O_2 absorption in mitochondria is accelerated. This experiment has also been carried out in the opposite direction. By NAD·H and salts effect ADP with 100 mkM density was added to the system after stimulating the oxygen absorption at mitochondria. It was revealed that after of NAD·H and salts effect addition of the ADP into the system inhibits the process (figure 7); and this is connected with the not acceptor function of the ADP. This effect also is considered one of the criteria used to determine oxidation phosphorate separation by the salts effect.

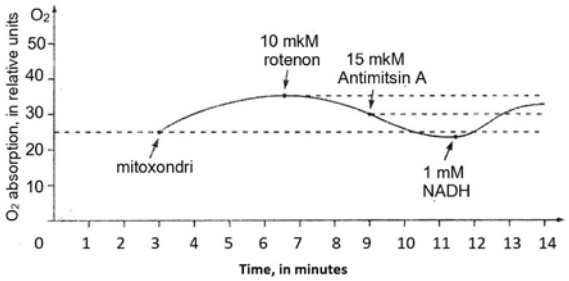

Figure 6. Oxygen absorption kinetics at isolated mitochondria when Rotenon, Antimitsin and NAD·H systematically effect.

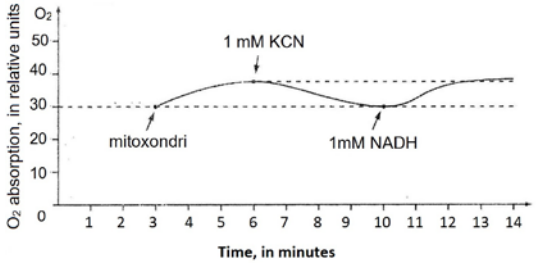

Figure 7. Oxygen absorption kinetics at isolated mitochondria when KCN and NADH systematically effect.

Besides oxygen absorption at roots of 5-days wheat sprouts and isolated mitochondria have been studied when NAD·H, NaCl and 2,4 DNF systematical effect. After stimulating oxygen absorption by mitochondria on the base of NAD·H and NaCl effect 2,4 DNF with dese of $5 \cdot 10^{-5}$ has been added. It was ascertained that after effect of the salts effect stimulating of the respiration by dinitrophenol effect hasn't been observed. At the same time, at first 2,4 DNF, and then salt effect have been studied. It was observed that DNF causes keen acceleration of the oxygen absorption in this case. And addition the salts to the system liquidates its stimulating effect.

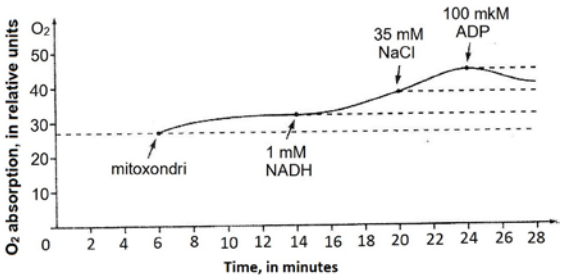

Figure 8. Oxygen absorption kinetics at isolated mitochondria when NAD·H, NaCl and ADP systematically effect.

It was ascertained that there was an analogy between the obtained results concerning to oxygen absorption at the roots of 5-days intact plant sprouts also by mitochondria isolated from the root system during effect of salts and different physical and chemical factors. Then efficiency of oxidizing phosphorate connections at plant roots and isolated mitochondria in normal and salty conditions was investigated.

It is notable that intact sprouts and isolated mitochondria have been used by us. Mutual connection durability of the oxidation and phosphorate, by adding ADP with density of 100 mkM into the system acceleration of respiration has been observed. Thus, recently it is enough convincingly observed that oxidation reaction energetic connection mechanism almost exist in all structures happened membranes in cell (Gasymov, 1983).

As it is seen from the figure 8 after registering the stationary level of the oxygen absorption velocity adding 100 mkM ADP into the system a keen increase of oxygen absorption by sprout roots are observed. Then 1 mM NADH is added to the system that accelerates oxygen absorption by the plant roots much more.

One can come to such a conclusion that the oxidation was connected with the phosphorating. It proves increase of oxygen absorption velocity as well decrease of the additive after its finishing at addition of ADP (acceptor of phosphate) into environment according Chans – existence of respiratory controle.

The abovementioned three systems activity depends on partial pressure quantity of the oxygen in cells, so cytochrome-oxidase has got very high sensitiveness to oxygen like the terminal oxidase in the oxidation-phosphorating engagement/pathways (I engagement/pathway). That is why this engagement / pathway can be activating at low partial pressure of the oxygen. As the low partial pressure of the oxygen increases (at extreme pressures) the oxidation phos-

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phorating gets broken that is why cyanide-resistant engagement/pathway (II engagement/pathway) of the respiratory circle gets activated; and its efficiency approximately equals to 90%. On the result of mono-electron transportation water is obtained, however ATP isn't generated as in the oxidation-phosphorating in this engagement/pathway. The engagement/pathway doesn't form a danger for organism. Thus, 95% of total amount of oxygen in the cells turns to water. However when I and II engagements are broken at high partial pressure of the oxygen III engagement/pathway starts. On the result of two-electrine transportation H_2O_2 is formed in this engagement. Sensivety of this engagement to oxygen is relative to I and II engagements is lower.

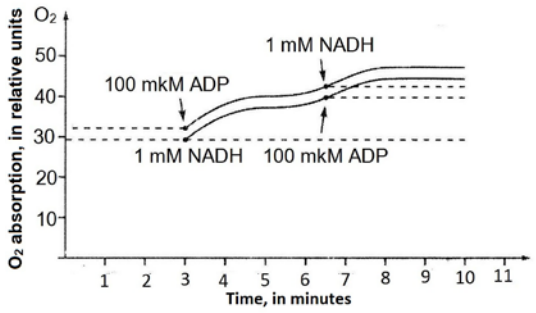

Figure 9. Oxygen absorption kynetics at NADH and ADP systematic effect by wheat roots.

Two electron free oxidation engagement/pathway of the bio-oxidation is more dangerous for organism. It is clear from reference literature information that H_2O_2 accumulates in condition of extreme salinity within plant organisms. (Tan jian-Kang, Anshi-King, 2004; Panda, Upadhyay, 2004; Garifzyanov et.al, 2012).

And III engagement/pathway begins on the flavor-proteid level. It is known that flavor-proteids are of aerobe dehydrogenase group, passes the electrons by means of direct or special cytochromes to oxygen and H_2O_2 forms this time. On the result of H_2O_2 decomposition free radicals including OH* radical is formed; and as its duration is very short ($10^{-8} - 10^{-9}$ *san*) and mobility very high it is capable to oxidize all components including DNA. Like a mechanism of cell protection from this process reduce of intra-mitochondria oxygen quantity or decrease of ubi-semi-xenon duration are possible (Skulachev, 1996).

H_2O_2 accumulated in the organism implements peroxide oxidation of the lipid component in the membranes of plant cell; on the result free radicals accumulate in the cell (Gasymov, 1983).

In extreme condition including two-electron free transportation strengthens as a result of the salts stress and first of all oxidation engagement/pathway is broken (Gasymov, 1975; 1983; 2004).

To specify respiratory intensity character in the plant roots in extreme salty condition - Peroxidase, catalase ferments activity being one of the alternative engagements/pathways, as well identification of the H_2O_2 quantity have a great importance.

It turns clear from the achieved results that as (NaCl, Na_2SO_4) salt density increases, the increase of H_2O_2 quantity in the bleached-out wheat sprout roots have got a linear character. Metal proteids (cytochrome c, a, a_3) soon inactivate under direct effect of chlorine ion, within respiration circle, in extreme salinity condition (Gasymov, 2008; Gasymov, 2012). Its main cause is their easily change the OH group in chlorine compounds, in the center of macromolecule (at porphyry nuclear) which joined with metal (Cu, Fe). So, ion mobility of chlorine in comparison with OH group is lower for several times (3 times). In this case easily oxidizing flavine proteids directly or by special cytochromes pass the electrons to oxygen at alternative oxidation (dew to two-electron transportation) in the respiratory circle. On the result H_2O_2 accumulates in the seeds. Depending on the amount of accumulated H_2O_2 ferment systems decomposed it – peroxidase, catalase play an important role. When amount of H_2O_2 in seeds is less than 10^{-9} M peroxidase ferment activates. When it is more than 10^{-9} M catalase ferment plays a conclusive role.

Out of the results we conclude that as salt density increases (25-100 mM) increase of peroxidase activity in the sprouts root system has got a linear character.

It was ascertained that further increase of salt density (100-300 mM) is observed by encrease of catalase activity.

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