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Study of Superweakbiochemiluminescence and Oxygen Absorption in Plant Roots under Salt Effect

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Abstract

Superweakbiochemiluminescence (SBL) and oxygen absorption in plant roots of 5-6-day sprouts of monocotyledonous and dicotyledonous plants have been studiedin the present work. SBC registration of sprouts was carried out by means of quantometric device and the measurements of O_2 absorption – by polarographic aggregate. The advantage of a SBC method is:it allows obtaining accurate information on intracellular oxidation processes not bringing any fluctuations in development of chemical reactions and their coordination in cells.It was ascertained that inserted sprouts Cu^{2+} ions into root system effectively participate in catalytic decomposition of the hydroperoxide formed in lipid phase of biomembranesas a result of auto-oxidizing reactions. The decomposed hydroperoxides initiate more active radicals of RO and OH types that interacting with KCN they promote sharp increase of a luminescence intensity correlating as O_2 absorption by sprouts increase.

Key words: antioxidants, free (alternative) oxidation, hydro peroxides, intensity, peroxiradicals, polarography, quantometry, superweakbiochemiluminescence

Introduction

As it is known, metabolic processes stationarity in live organisms is observed in certain conditions only and at an environmental constancy of factors. Considerable change of one of factors (temperature, humidity, salinisation, illumination et.al.) results infringement of stationarity of vital processes [Gasymov, 2012].

In spite of the fact that the problem of salt effect onto a vegetative organism is studied for a long time [Gasymov, 1983; Strogonov, 1973], but a specified mechanism of salt resistance of plants is still insufficiently found out.It seems to us one of the reasons of it is the absence of advanced study methods. At present a number of methods are used for the purpose of diagnostics [Gurova, 2000]. But these methods are not deprived of lacks.

That is why the discovered phenomena of superweakbiochemiluminescence (SBC) of vegetative [Colli et al., 1954] and animal tissues [Tarusov et al., 1961] represent certain interest. As the method of SBC registration spontaneously emitted by plants gives the chance to receive accurate information on endocellular oxidative processes in certain degreenot bringing any fluctuations in development of chemical reactions and their coordination in cells as well as it allows to control for kinetics of these reactions [Goncharova, 2011; Vladimirov, 1999; Vladimirov et al., 2009]. SBCmethod advantage is: plant biological integrity during the research completely remains which is especially important at the study of salt resistance of plants. Hence according to B.P.Strogonov, A.M.Smirnov and N.I.Sheviakova, at infringement of integrity of an organism in separate plant bodies' adaptability to salts decreases [Smirnov et al., 1960].

Objects and Research Methods

5-6-day sprouts of monocotyledonous and dicotyledonous plants (barley, bean, cotton, wheat) grown up in thermostat at 25^0C have served as object of the research.To ascertain the connection between quanta generation processes and respiration in the rootsof the investigated plants, we spent a series of experienceswhere various agents like inhibitors and disconnectors (KCN, $CuSO_4$, $ZnSO_4$, 2,4 DNF, NaCl) were used.

The experiences were carried out at 3-5 fold frequencies and calculated per 1gr weight of a crude root.Reagents have been prepared in various concentrations as: (10^{-1}-10^{-3}-10^{-5}M).

SBCRegistration and sprouts was carried out by a quantometre consisting of a photoelectronic multiplier (FEM-42, FEM -85), a broadband amplifier (BA-2), a scaler, (S-100), a recording potentiometre (RPM-4) and a high-voltage rectifier (HVR-2) [Gasymov, 2012].

Oxygen absorption measurement at root system of plant sprouts was carried out on the stationary polarographic device installed by us and consisting of an electrochemical cell, lab pH-metre, reflecting galvanometre (M-95), an ultrathermostat, amicropump with electromagnetic system and a thermostatted cell withan object [Gasymov, 1983].The device sensitivity is $3{,}2 \cdot 10^{-7}$ MO_2/l. The obtained data were statistically processed [Plokhinsky, 1970].

Results of the Research and their Discussion

The obtained results indicated that the change of SBC intensity of radiation under inhibitors effect ($CuSO_4$, $ZnSO_4$, KCN) depends on time of their effect.Besides,inhibition efficiency of each inhibitor also depended on plants biological feature.For example, at KCN effect onto wheat intensity of radiation initially increases within 30 minutes right after the processing,and further it decreases. In the case of processing of bean and cotton sprouts, the intensity of their radiation in comparison with wheat quickly decreases after preliminary intensification.

Zn^{2+} ions at all plants result sharp decrease of radiation intensity within 20-30 minutes after the effect. At the further exposition in experiences with wheat and bean partial restoration of SBC intensity levelis observed and radiation intensity of cotton continues to decrease. Cu^{2+} ions also lead to sharp decrease in radiation intensity of all plants even at long-time effects (fig. 1).

Thus, it was established that separately these substances (Zn^{2+}, Cu^{2+}) inhibit a luminescence in roots of plants and especially a strong inhibition is observed at 10^{-3} M $CuSO_4$ effect. It is notable that the effect of various respiratory inhibitors such as: potassium cyanide, copper and zinc ions have strongly demonstrated specificity on SBC intensity of plants.

The inhibition of intensity of sprout luminescence is observed at (10^{-1} M NaCl) salts effect too (fig. 2).

The acquired data indicated that at keeping the sprout roots in water luminescence intensity is observed on stationary level after certain time.

Itshould be noted thatsuchastationarycontinuousfor a long period and depends on air tension passing through a room filled with water. When changing water with NaCl solution at 10^{-1}M (tension of the passing air remains the same as in the case of water), a luminescence inhibition is observed and approximately in 10 minutes SBC intensity goes to another stationary level (fig.3). On this level, airblowdownsubjects to a sharp fall of luminescence intensity and its further pass restores the initial luminescence level (S_1).

Polarographical measurements carried out at the same time with SBC observation of sprouts showed that luminescence intensity fall correlate with the increase of oxygen absorption by the sprouts under salt effect (fig.4).

Further on, it was ascertained by us that the effect of 10^{-3}MKCN luminescence intensity of sprouts was highly strengthened and then was changed with its sharp fall. However, such a flash was not observed at effect of 10^{-3}M$CuSO_4$, $ZnSO_4$ and NaCl.

Highly strengthening of luminescence intensity at 10^{-3}MKCNeffect was observed by Y.A.Vladimirov together with his collaborators in their researches in mitochondria suspension of animals [Vladimirov, 1966].

Such a flash is connected with antioxidant property of KCN. According to A.I.Zhuravlev [Zhuravlev, 1965], the reaction between oxidizing antioxidant can be followed by flash as follows:

$$Jn^{\cdot} + O_2 \rightarrow JnO_2^{\cdot}$$

$$JnO_2^{\cdot} + JnO_2 \rightarrow [P_1^*] \rightarrow P_1 + h\nu_1$$

$$JnO_2^{\cdot} + RO_2 \rightarrow [P_2^*] \rightarrow P_2 + h\nu_2$$

– where $Jn^{\cdot}$ - is an inhibitor radical (antioxidant)

P_1andP_2 – are reaction products.

RO_2 –is subtractingperoxiradicals.

It is difficult to explain the further sharp luminescence inhibition as the inhibition cannot be connected with repressing of terminal oxidation of plant respiratory system only. At the same time, there is an activity depression of lots of components on both general and free (alternative) ways of oxidation that contain metal [Rubin, 1966]. Inthiscase, itseems antioxidant (KCN) is expended as a result of tying it with cell components of metal content or actively participates at peroxide decomposition accumulated in plant tissues.

To check the above-said items, some experiments have been carried out in presence of Cu^{2+} ions that were oxidation activators of ascorbic acid, air oxigen by hydrogen peroxide generation [Mikhlin, 1960].

Itwasascertainedthat at 10^{-3}M$CuSO_4$ effect sprouts luminescence was sharplyinhibited;$CuSO_4$ solution was changed by NaCl solution in 10^{-1}M concentration after 10 minutes.

At the same time not significant flash was observed and the further luminescencewas rapidly inhibited at once. After a 10-minute action ofNaCl, 10^{-3}KCN was entered into the system instead of NaCl.In this case, exclusively strong flash was observed.10^{-3}KCNintroduction was carried out repeatedly in the same volume in 25 minutes after introduction of the KCN. Moreover, the amplitude of flash was much less than in the first case. The replacement of KCNsolution by a 10^{-3} M $CuSO_4$solution also results in flash appearance. The experiences with Zn^{2+} ions were carried out in the same order. However, the effect of strengthening of the luminescence intensity was not observed at Zn^{2+} action.

These facts can testify that the effects of strengthening of luminescence intensity in the NaCl + KCN + system, the vegetative tissue takes place only at participation of Cu^{2+}ions in this system.

Data obtained in the experiments with nonsaturated fat acids can testify to active participation of Cu^{2+} ions in the peroxidation process.

In his work, Will stated [Wills, 1966] the comparative catalytic activity of various metals of variable valence and ferriheme compounds at peroxidation of nonsaturated fat acids. It has been indicated that hemoglobinisapproximately 100 times more active than salts of bivalent iron and relative activity of Fe^{3+}, Fe^{2+}, Cu^{2+}, V^{2+}, Mn^{2+}, Co^{2+}wasaccordingly expressed in figures: 3; 5,8; 9; 13; 9; 136. Ag^{+}, Cr^{3+}, Mg^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+} et. al. were inactive (relative activity equals to 1). It is interesting that metals are located just on the same row; on their ability to decompose hydroperoxide of organic compounds [Hawkins, 1964].

Thus, one can suppose that the introduced ones intoCu^{2+}system in catalytic decomposition of the hydroperoxide, formed in biomembranes lipid phase, effectively participate on the result of auto-oxidizing reactions. The decomposed hydroperoxidesinitiate more active radicals of $RO^{\cdot}$ and $OH^{\cdot}$types, which interacted with KCNand promote sharp increase of luminescence intensityon the above-stated reaction [Vasilik, 2011].

Thus, the experiments with use ofKCN, $CuSO_4$, $ZnSO_4$and NaCl allow assumingthat the SBC is not connected only with a terminal site of a respiratory chain, but also with cytochrome level in → C_1 cytochrome. According to V.Skulachyov, Zn^{2+}ions inmicromolar concentrations are effective inhibitor in a conjugated chain of oxidation oncytochrome level in → C_1 cytochrome [Skulachev, 1969]. If considering that electrons transportation to molecular oxygen of air onthe conjugated waystops at of Zn^{2+} and CN^{-}ions action, thenthe incomplete inhibition of luminescence testifies to its connection with another way,i.e. free (alternative) oxidation.

As the way of free (alternative) oxidation is less activein physiological conditionsthan the conjugated systems of electrontransport,the free (alternative) way of activity can bedisguised until cytochrome system is not removed or notinhibited.Thus, the competition betweenthe electron transportsystems for oxygen can play importantregulatory role in energy distribution in plants' cell at various functional conditions including action extreme salinization.

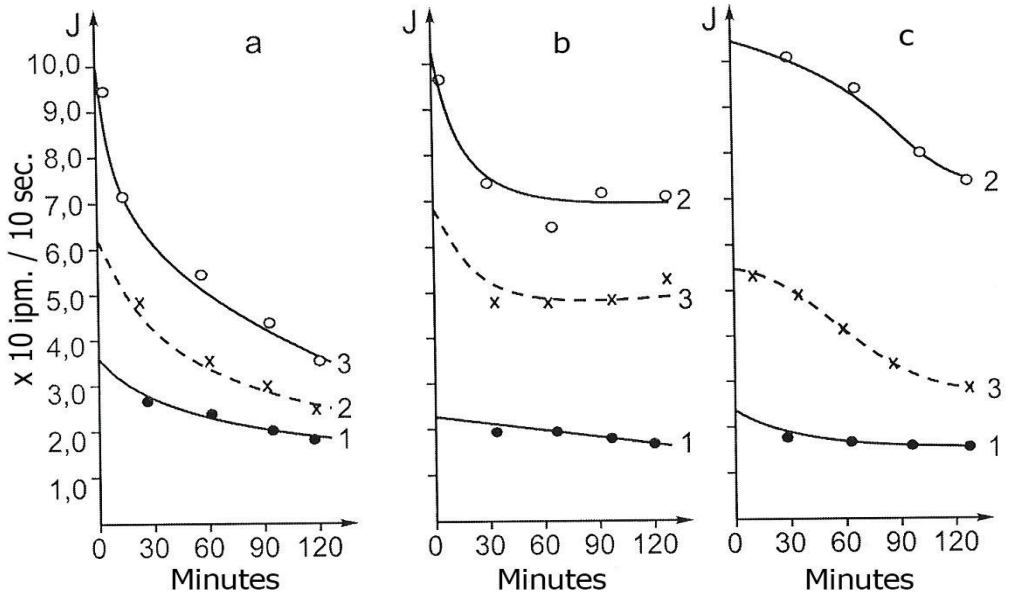

Fig 1.BCL intensity of sprouts at action $CuSO_4$ (a), $ZnSO_4$ (b) andKCN (c)

1. Cotton; 2. Wheat; 3. Bean

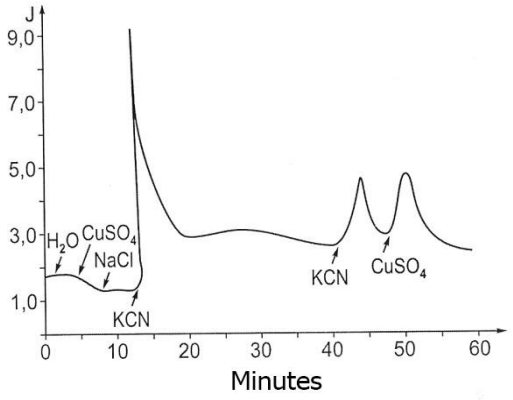

Fig. 2.Cu^{2+} ions effect ontothe intensity of BCL sproutsof barley in the system: a vegetative tissue + NaCl+KCN

J-intensity BCL in relative unit
The arrows indicate the action moment of agents.

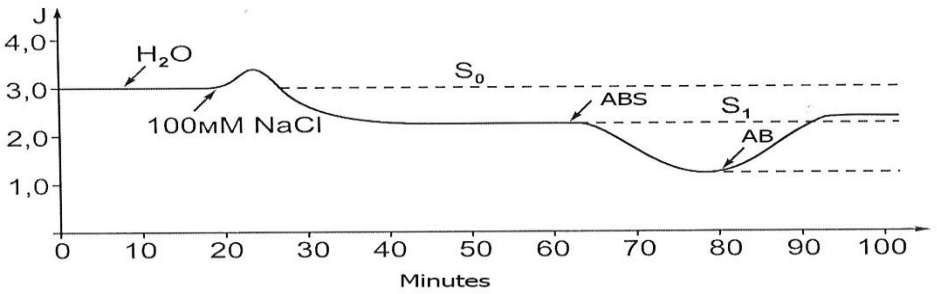

Fig 3. Change of BCL intensity of plantroots duringNaCl effect

ABS– air blowdown was stopped
AB – air was blown down
S_0, S_1-stationary levels
J - BCL intensity in relative units

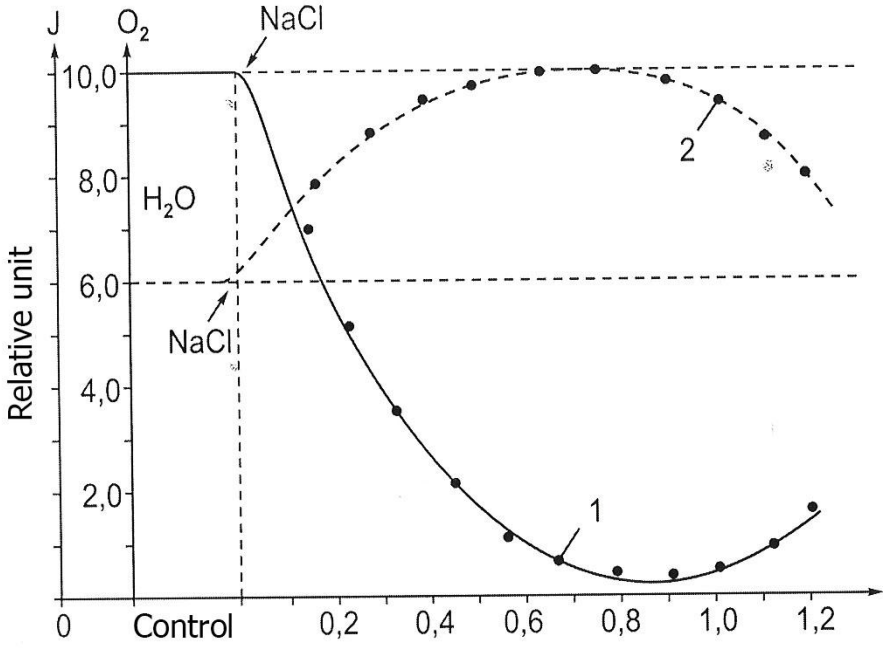

Fig. 4.Dependence of oxygen absorption (1) and BCLintensity(2) in barley roots from NaClconcentration.Each point corresponds to 30 minutes of ageingof sprouts in the specified salt concentration.
J - BCL intensity. O_2 - absorption in relative unit

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