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Kinetics of Cl^- Ions Transport in the Roots of Plants at Temperature Change and pH Environment

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Abstract **– Thermodynamic parameters (temperature coefficient, activation energy, free energy et.al.) of Cl^- absorption by barley *Hordeum vulgare* root system have been studied in the manuscript. It was revealed that Cl^- accession rate in the root linearly increases and reaches a maximum at 30°C while the temperature rises; then it decreases and reaches a minimum at 40ºC.**

It was ascertained that accession of Cl^- ions into root system from *NaCl* solutions is characterized by two kinetic components which differ very much from each other. The first component is connected with diffusion process and the second one is limited by metabolic processes in Cl^- transport into plant cell.

Environmental acidification more considerably accelerate Cl^- absorption by barley cells than neutral and alkaline environment that conforms to H^+/Cl^+ hypothesis of the symport system.

Keywords **– Activation Energy, Free Energy, Kinetic Components, Temperature Coefficient, Transport of Ions.**

I. INTRODUCTION

Study of substances transport through a live cell membrane is one of the major questions of Physiology, Biophysics, Biochemistry and other fields of Biology. More clearly to present importance and difficulty of this problem it's notable that it is being developed over hundred years and interest of researchers to it increases more and more.

Ion transport into root system considerably depends on many autogenic and exogenic factors. One of such exogenic factors of the environment is temperature.

The role of temperature in salt absorption first of all is explained by its effect onto intensity of breath of roots. Metabolic processes rate, also transpirations effecting onto absorption depend on the temperature as a factor coordinating enzymes activity (Yermakov, 2005). Transport fibers in the membrane activate more slowly at low temperatures. While temperature rises 5 to 40ºC rate of absorption increases to the maximum. The further rise of temperature subjects decrease of salt absorption. It is probably connected with inactivation of the proteins that participate in absorption of ions. However, temperature factor of absorption processes of nutritious salts are not equal for different substances (Kuznetsov, Dmitrieva, 2006).

It is notable that ion absorption by roots of plants and also soil features in many respects depend on acidity of environment. At neutral and alkaline reactions cations are faster absorbed and at acidic reactions – anions are absorbed. The salts solubility depends on edaphon acidity. Absorbing features of the plant itself can be changed depending on pH environments (Kuznetsov, Dmitrieva, 2006).

It is notable that depending of salt absorption by plants on temperature is being studied 60 years (Sergeyev, 1953, Satkliff, 1964, Church, 1974; Bowen, Nissen, 1977; Nandi, Pant, 1984; Musayev, 2012). However kinetic regularities of ions acception of chlorine depending on temperature have been studied insufficiently in these works; whereas research of this question in certain degree would let judge the mechanism of transport reactions and the reasons of toxicity of ions of salts at temperature rise.

Aim of the present manuscript is study of kinetic regularities of ion acception of chlorine in roots of plants depending on temperature and pH environments.

II. OBJECT AND METHODS OF THE RESEARCH

As an object of the research have served 5-day sprouts of barley (*Hordeum vulgare*) of Pallidium-596 Variety. Barley sprouts were grown up in the thermostat at 20°C in aerated conditions.

To evaluate transport of substances into the cell they usually apply plasmolytic, hemolytic, plasmometric, chemical, isotopic, photometric and other methods that are not deprived lacks. The most convenient one was isotopic. When using it kinetics of substance accession into plant cell the followings are taken into account: 1) change of specific activity of the object (the cut off roots, leaves et.al.) or 2) decrease of the labeled atoms in the environment where intact object is placed.

Basically the chemokinetic method offered by us corresponds to the second variant of the isotope method (Gasymov, 2012). Along with full intact preservation of biological object the change in time of absolute content of Cl^-in test is considered in it.

To avoid pollution of the investigated volume of NaCl solution by ions of chlorine of flowing water the roots of plant sprouts (60 pieces) sank into distilled water before measurement and were carefully washed out; next they were dehydrated by filtering paper (to prevention reduction of concentration of investigated salt solution that has stuck by the water to roots); next they were placed into a small pure and dry glass of 50 ml volume. Besides, each of 5ml NaCl solution in the investigated concentration was poured into 10 test tubes and carefully closed by a stopper (to prevent evaporation). The small glass and the test tubes were immersed into a water thermostat and set desirable temperature (20°C). Then 20 ml of NaCl solution of the set concentration was poured into the small glass and fixed the time by means of a stopwatch. Content of the small glass was continuously shook up by means of a specially made mixer with an electromotor during the experiment. Such agitation is especially necessary for narrow time-interval because in free solution (in the small glass) time required for diffusion increases proportionally to the squared distance that needs to pass the ion.

Per 1ml of $NaCl$ solution was poured onto each three test tubes from the taken tests and titrated. Process volumetric solution is a silver-bath in concentration of 0,05N. To define completeness of deposition/ sedimentation i.e. to define the end of the reaction 5% K_2CrO_4 solution was used that gives a deposit with ions Ag^+ of brown colour.

The essence of the sub-micromethod developed by us is: Cl^- transport kinetics in roots of plants is studied in a stationary mode i.e. during all experiment solution volume in the small glass where roots of intactic sprouts were sank/dipped remains invariable; that allows to keep a constancy of absorbing surface of plant roots (6 sm^3) and excludes possibility of some methodical discrepancies.

To create such a stationary mode after taking each sample per 5*ml* out of the small glass then 5*ml* of $NaCl$ solution in initial concentration (control) is added at once. This operation is carried out during 5*ceconds* as the delay worsens diffusion processes at mixing of $NaCl$ solutions that possess various concentration (the control and tests/samples). Thus the volume of $NaCl$ solution in the small glass remains constant in time.

If there aren't any sprout roots in the small glass the concentration gradient Cl^- between "control" (T_2) and "trial" T_1 will not arise, as the content of the Cl^- in both cases are equal. However at immersing of plant roots in the small glass owing to absorption of ions Cl^- by plants difference between concentration of control and trial solutions on chlorine is observed.

Thus concerning to degree of Cl^-transport rate in a cell of plant roots judged according to the decrease of quantity of chlorine ions in test and counted on 1gr crude weight of root system.

As after each addition of a control solution of 5*ml* into the small glass there occurres a change (increase) of concentration of a test solution the increment content Cl^-in test should be calculated separately for each time interval of the experience. For acceleration, speeding-up and simplification of calculations a calculation table is being preliminary made.

Each found value of T_2 serves as a control for the subsequent test. If T_2-T_1=0 – it means absorption Cl^- by plants is absent. Then a schedule of dependence $\frac{dm}{dt} = V_S$ is drawn where *dm* is a change of the chlorine ions content in plant roots; *dt* is time change. Then a dependence of average rate of transport *Cl*-(V_S) into roots from time i.e V_S/t is defined. is defined.

The method allows defining the absolute Cl^-content accessed into roots of intactic plants in a wide time interval (5-160*min*) and concentration of $NaCl$ solution. That is why a number of kinetic and thermodynamic parameters of Cl^- absorption by plants are defined. Sensitivity of the method of $2\cdot10^{-2}$ *mg/ml* on chlorine.

The experiences were carried out 4-6 fold replication and the results counted per 1*gr* crude weight of root. The obtained data were exposed to statistical processing according to G.F.Lakin (1990).

The error of average arithmetic size under the formula $S_{\bar{x}} = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n(n-1)}}$ is defined, and the index of accuracy: $c_S = \frac{S_{\bar{x}}}{\bar{x}} \cdot 100$. The index of accuracy does not exceed three percent.

After curve decomposition into kinetic components their speed and life time constants were calculated under the formula $V_s = V_0 e^{-kt}$. Hence $\lg V_s = \lg V_0 - kt \lg e$, if $V_s = V_0$, meanwhile $\frac{\lg V_s}{t} = \mathrm{tg}\,\alpha$, then $k \lg e = \mathrm{tg}\,\alpha$ and $k = \frac{\mathrm{tg}\,\alpha}{\lg e}$ or $k = \frac{\mathrm{tg}\,\alpha}{2{,}303}$. Life time (duration) ($\tau$) of each component was defined by return value of the velocity constant, i.e. $\tau = 1/k$. The temperature coefficient was calculated under the formula $Q_{10} = R_{t+10}/R_t$. Free energy has been calculated under formula $\Delta G = -RT \ln \frac{c_2}{c_1}$. A dependence curve of the transport rate of Cl^- ions in Arrenius co-ordinates that allowed calculation of activation energy ($E_{акт}$) $E = tg\alpha \cdot 2{,}303R$ had been drawn up by us.

III. RESULTS AND DISCUSSION

It was ascertained that Cl^- transport into vegetative cell is a superposition of some mechanisms (Gasymov, 2012). It's indicated that average velocity ($V_s = dm/dt$) of Cl^- transport into sprout roots of barley is expressed by the exponential law ($V_s = V_0 e^{-kt}$) as the fast recession of the average velocity is replaced by slower one that specifies onto process staging (fig. 1).

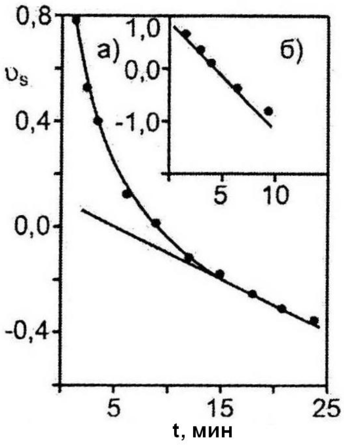

Fig.1. Average velocity of ion transport of chlorine into barley roots in half-logarithmic co-ordinate: a) for the

whole process; b) for the first component $\tau_{k_1} = 2,44$;
$\tau_{k_2} = 24,4\ min$, τ - life time of a corresponding
component $\tau = 1/k$.

Thus, curve changes of the average velocity of Cl^- transport into roots can be presented by the sum of two exponents: $V_s = V_1 e^{-k_1 t} + V_2 e^{-k_2 t}$, specifying to simultaneous percolation in the system of two processes which velocity constants are various. For decomposition of components the kinetic curve of average velocity of Cl^- penetration into plant roots in half-logarithmic co-ordinate is drawn up drawn up. Thus calculated velocity constant for the first stage of the process is $k_1 = 4,1 \cdot 10^{-1}$ $мин^{-1}$, and for the second stage equals $k_2 = 4,1 \cdot 10^{-1}$ $мин^{-1}$.

The specified components (I and II) differ not only under kinetic characteristics but related to temperature too. Change of temperature at the interval of 1-40°C considerably effects onto the first and second kinetic components value. Apparently from fig. 2, at low temperatures (1-10°C) absorption of Cl^- ions of plant roots from solution *NaCI* in concentration of 100*mM* decrease to 70-80% in comparison with the control.

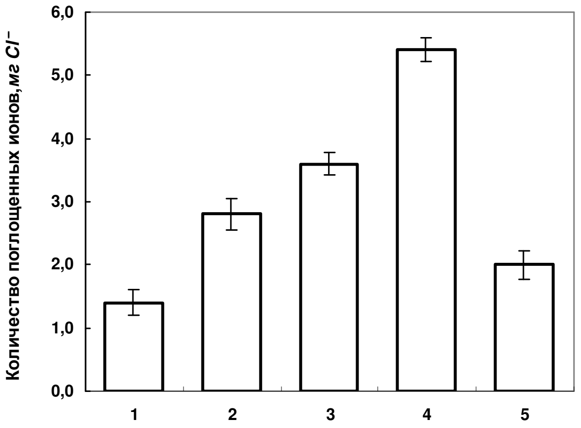

Fig.2. Quantity of the absorbed Cl^- ions out of solutions 100*mm* (a) and 50*mm* (b) *NaCl* by roots of barley depending on temperature within 160minutes.
1 – 1°C, 2 – 10°C, 3 – 20°C (control), 4 – 30°C, 5 – 40°C.

The temperature maximum of Cl^- transport in cells was at 30ºC in our experiences.

Apparent activation energy ($E_{акт}$) of Cl^- transport into barley roots calculated by us has appeared equal to 3,10*kcal/mole* at 10-30°C interval. Temperature coefficient Q_{10} of Cl^- ions transport out of a 100*mm NaCl* solution during 160 min has been defined. This coefficient has got a significance at an interval of: 1-10°C – 2,0; 10-20°C – 1, 28; 20-30°C – 1,5; 30-40°C – 0,37.

Besides, as one of thermodynamic indices of transport process the free energy (ΔG) of Cl^- acception in roots depending on temperature (1-40°C) has been defined..

It was ascertained that as the temperature rises at an interval (1-30ºC) the free energy value increases and reaches a maximum at 30°C, then it decreases (fig. 3). It can be one of arguments in favour of metabolic character of Cl^- transport into root (Leninger, 2005).

Along with thermodynamic indices we also had been defined effect of pH onto absorption of Cl^- by plant roots.

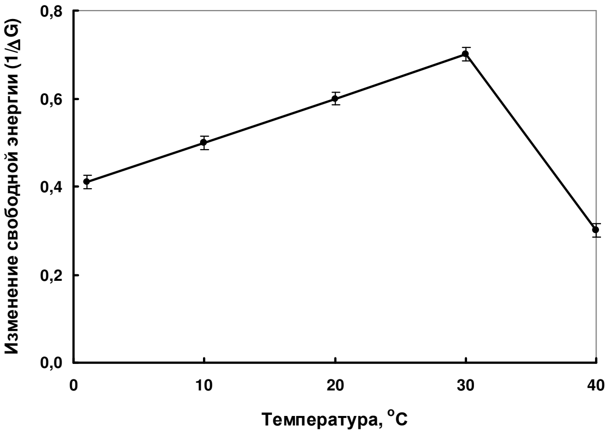

Fig.3. Change of free energy ($1/\Delta G$) at absorption of chlorine ions (mg) out of a solution of 100*mm NaCl* by barley roots depending on temperature.

To define absorption of chlorine ions by plant roots depending on pH a phodphatic-citratic buffer (McIlvain) pH equals to 3; 5 and 8 was used by us. At pH=3 absorption of Cl^- ions by barley roots has appeared much more above than at pH 5 and 8.

Environmental acidation is accelerated considerably Cl^- absorption by barley cells in comparison with the neutral and alkaline environment that is reconciled with the hypothesis H^+/Cl^- of the Simport System.

It is notable that the above-mentioned thermodynamic indices testify that ions acception into a vegetative organism is not completely limited by passive diffusion as some passive processes in which course overcoming of power barriers is not required, i.e. such for which $E_{акт}$ is equal to zero it is characterized Q_{10} components approximately 1. However one should not forget that in distinction of diffusion process the biological membrane in a free solution often forms a considerable power barrier to diffusion of the charged dissolved substances and size $E_{акт}$ for passive move of ions through it can reach 11,5 *kcal/mol* (0,50V).

As it is known, depending on temperature coefficient substances transport can be divided into 2 principal types. If temperature dependence of substances transport is close to temperature coefficient of diffusion in a free solution (Q_{10} =1,2-1,4) – the accession is called as simple: propyl alcohol diffusion like an intermediate polarity substances (between polar and not polar) defines principal value of power barriers μ_e close to power barriers of molecular skipping at diffusion in pure water. Substance transport processes going along with a concentration gradient but with temperature coefficient (Q_{10} >2), are called activation diffusion (i.e. demanding overcoming considerable activation barriers over 8-10 *kcal/mol*); they should not be mixed/confused with the processes of active transport going against the concentration gradient.

According to Sutcliff (1964) at temperature rise absorption rate/velocity of salts increases and beginning from 40°C – it decreases. The author explains this reaction by component inactivation present at absorption. Reduction of ion absorption by plants at low temperature (about 0°C) explains attenuation of chemical reactions and reduction of permeability of cell membranes. At temperature decrease 30 to 0,5 ° or in the presence of 2,4 DNP absorption rate Mg^{2+} and especially Cl^- sharply falls (Maas, Ogata, 1971).

R.Church (1974) underlined that at young plants of wheat ‘Bezostaya-1’ (meaning bald ear) as the temperature raised 2 to 30 ° absorption intensity of different forms of nitrogen sharply raised.

Musaev N.A. (2012) specified that at temperature rise to 30°C there was a sharp increase of membrane potential (E_m). Maximum E_m was reached at 30°C and at the subsequent temperature increase there occurred progressive reduction E_m with temperature coefficient. Change E_m was reversible at temperature rise to 38-40°C.

It is notable that diffusion process is passive, goes on a concentration gradient and doesn’t require any expenditure of energy. In spite of the fact that in the external solution (in the small glass) the content of Cl^- ions is several times more (71*mg*) than in the part which penetrated into plant roots (~3*mg*); process rate of transport of Cl^- ions sharply decreased. It seems over certain time after the beginning of transport of Cl^- ions the process is limited by any factor possessing a biochemical nature and not by stictly diffusive one.

Data on change of distribution coefficient of chlorine ions (k_p) also testify at change of chloride sodium concentration in the environment.

As *NaCl* concentration increases in the environment the distribution coefficient of Cl^- ions decreases. Such decrease of the distribution coefficient of Cl^- ions can be interpreted that absorption by a substance cell due to its adsorption and chemical linkage is limited as the both processes have a certain limit (Gasymov, 2012).

Researchers (Petterson, Jensen, 1981) specify that addition DNP into medium/environment or its cooling to 0°C suppresses systems of active transport but doesn’t effect onto diffusive flux ^{86}Rb to root tissue.

One of the factors effecting onto absorption process of substances and their distribution in a cell is acidity of environment (pH) (Rubinstein, 1974). Rubinstein (Rubinstein, 1974) compared acidification effect medium/environment pH 6,5 to 3,0 and effects of indoleacetic acid (30 *micro/M*) onto Cl^- absorption by coleoptiles segments of 1-5*mm* length, cut off on the distance of 3*mm* from the tip. Decrease of pH – medium/ environment within 5,0 - 3,0 strengthened absorption and braked the exit.

On the basis of considerable Cl^- accumulation in barley and maize roots at pH 4,0 in comparison with pH 6,5 in B. Jacoby’s work, (B.Rudich, 1979) it is come to a conclusion about existence of H^+/Cl^- of Simport system in roots.

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