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# Kinetics of chloride ions absorption by plant sprouts in the presence of $NaNO_3$, $Ca(NO_3)_2$ and $Na_2SO_4$

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**Abstract**

In the present research we studied the absorption of chloride ions from 100mM NaCl solution by the roots of barley in the presence of $NaNO_3$, $Ca(NO_3)_2$ и $Na_2SO_4$. The data obtained shows that $SO_4^{2-}$ ions don't affect antagonistically on the absorption of chloride ions in barley roots, but high concentrations of $NO_3$ ions have an inhibitory effect. It was determined that the transporters for $Cl^-$ и $NO_3^-$ are probably the same and it can be assumed that the ions of $Cl^-$ and $NO_3^-$ are transported into the plant cells by the same mechanisms

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**Introduction**

The major part of the plant kingdom carries out its life cycle under conditions of high salt concentrations. Therefore, the problem of the salt resistance is both of great practical and educational significance in the evolution of the plant life on the Earth. This problem is closely connected with the study of mechanisms of absorption and ion transport in plants (Gasumov, 1983). The most commonly used ions in the researches of this area are $Na^+$, $K^+$, $Cl^-$. It is believed that $Cl^-$ ions negatively affect onto the growth and survival of plants, but $Na^+$ ions do not (Luo Quing-yun, Yu Bing-yun, Lui You-liang, 2003). However, we suppose, that the effect of $Li^+$, $Na^+$, $K^+$, $Ca^{2+}$ and other active cations on the plants should not be neglected.

A significant amount of researches were dedicated to the study of ion transport into the plant cells, where the absorption of ions by the separate roots, isolated mitochondria, slices of leaves, chloroplasts, biomembranes and artificial membranes was studied at their physiologically harmless low concentrations ($10^{-2}$ - $10^{-4}$ M). However the features of $Cl^-$ ions transport by the roots of intact sprouts at high concentrations of polyionic salt solutions (NaCl, $Na_2SO_4$, $Ca(NO_3)_2$, $NaNO_3$ etc.) is still poorly known. In this case the interactions of ions could be antagonistic, additive or synergistic. Therefore, the detailed investigation in this area is an important issue in physiology, biophysics and other fields of biological sciences.

**Materials and methods**

The object of investigation was 5 days old (without counting the day of seed- soaking) green sprouts of barley variety 'Karabakh-21'. Sprouts were grown in knop solution at 25°C in the thermostat and in aerated conditions.

We used chemokinetic semimikromethod (Gasumov, 1979). This method allows to study the transport of $Cl^-$ in the root in the steady state: during the experiment, the volume of solution in the beak, where the roots of intact seedlings are submerged, remains unchanged and it allows preserving the constancy of the root surface of plants (6 $cm^3$) and excludes the possibility of a number of methodological inaccuracies of changes in the absorbing surface of the plant root system.

The method used is easy to install and is quick to perform, allows determining the absolute content of $Cl^-$, absorbed by the roots of intact plants in a wide range of time and concentration of NaCl solution and other compounds as well.

In our experiments the solutions of $Na_2SO_4$, $Ca(NO_3)_2$ and $NaNO_3$ at different concentrations (50-200 m) were also added to 100mM NaCl. The rate of $Cl^-$ transport into the root cells was determined according to the decrease of chloride ions in the investigated volume of NaCl solution. The sensitivity of the method was $2 \cdot 10^{-2}$ mg/mL to chloride.

Experiments were carried out with 4-6 repetitions and the results were calculated per 1g wet weight of the root. The statistic analysis of obtained data was performed according to G.Ph. Lakhin (1990). The indicator of accuracy was below 5%.

**Results and discussions**

One of the most important issues of physiology, biophysics and many other fields of biological sciences is the study of transport of substances across the cell membrane, interest in which, despite the almost century-long history of development, is still growing nowadays.

Thus, first of all we were interested in comparative studies of kinetics of chloride transport into the plant cells from the concentrated solutions of NaCl in the presence of $Na_2SO_4$, $Ca(NO_3)_2$ , $NaNO_3$ at different concentrations.

It should be noted, that different salts and even anions and cations of the same salt enter into the plants in different speeds (Liutte, Highinbottam, 1984). For example, in the case of sodium nitrate or calcium nitrate the nitrate anion ($NO_3$) is absorbed more rapidly and intensively than $Na^+$ or $Ca^{2+}$ cations. In this case alkalinisation of the media occurs as a result of $Na^+$ or $Ca^{2+}$ cations accumulation. Such salt is named physiological alkaline. In the case of sodium sulphate, cations and anions are absorbed with the same intensity and such salt is named physiological neutral one.

From the Table 1 we can see that the absorption of $Cl^-$ ions by the root system of barley in the presence of 50 mM $NaNO_3$ was reduced for about 22-23%, and in the presence of 200 mM $NaNO_3$ the absorption of $Cl^-$ ions from the solution of 100 mM NaCl was reduced three times and it was linear.

**Table 1.** The decrease of $Cl^-$ ions (mg) from the solution of 100 mM NaCl as a result of their absorption by the roots of barley during 20 min ( t - 20°C).

| Concentration of $NaNO_3$ (mM) | 100 mM NaCl |
|---|---|
| control | 3.1 ± 0.1 |
| 50 | 2.4 ± 0.09 |
| 100 | 2.0 ± 0.07 |
| 150 | 1.5 ± 0.06 |
| 200 | 1.1 ± 0.04 |

Moreover, we studied the absorption of chloride ions by the roots of barley in the presence of $Ca(NO_3)_2$ at different concentrations. From the data in the Table 2 we can see, that the absorption of chloride ions by the roots of barley was dramatically decreased in the presence of $Ca(NO_3)_2$ compared to $NaNO_3$. In the presence of 200 mM $Ca(NO_3)_2$ the absorption of chloride ions from 100 mM NaCl solution was decreased about five times and it was linear.

The obtained results conform to the previously published literature data. Some authors already reported that $Ca^{2+}$ ions reduce the absorption of chloride ions by plant roots (Liutte, Highinbottam, 1984). Moreover, the influence of 1-10 mM $CaCl_2$ on the salt tolerance of plants, which were grown in nutrient media with the introduction of 50-250 mM NaCl was also determined. The authors have concluded that $Ca^{2+}$ ions can enhance the adaptation and salt tolerance of the plants by increasing the resistance to osmotic and oxidative stress (Ma Shu-Ying, Zhao Ming, 2006).

**Table 2.** The decrease of $Cl^-$ ions (mg) from the solution of 100 mM NaCl as a result of their absorption by the roots of barley during 20 min ( t - 20°C).

| concentration $Ca(NO_3)_2$ (mM) | 100 mM NaCl |
|---|---|
| control | 3.1 ± 0.1 |
| 50 | 2.3 ± 0.08 |
| 100 | 1.7 ± 0.07 |
| 150 | 1.1 ± 0.05 |
| 200 | 0.6 ± 0.04 |

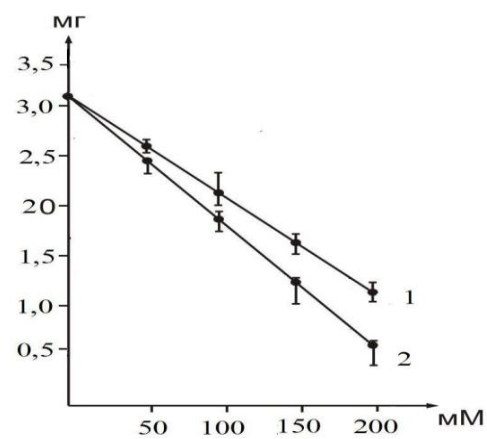


**Fig.1.** Absorption of chloride ions by the roots of barley from the solution of 100 mM NaCl in the presence of $NaNO_3$ (1) and $Ca(NO_3)_2$ (2) at different concentrations during 20 minutes (t-20°C)

The absorption of chloride ions by the roots of barley in the presence of $Na_2SO_4$ at different concentrations has also been studied. It was found, that in the presence of 50 mM $Na_2SO_4$ the amount of $Cl^-$ ions in the apoplast vessels and the metabolic rate of absorption were almost unchanged. With the increase of $Na_2SO_4$ concentration the amount of $Cl^-$ ions in the apoplast vessels was somewhat decreased, but the metabolic rate of absorption was not changed.

In the previous studies procedure and molecular mechanism of the $Cl^-$ ions transport reaction into the cell was determined by using the kinetic analysis (Gasumov, 1983; Abdiyev, 1987). It was ascertained, that the periods of half changes of the average speed for the first and second components are linked by the following relation: $T_{2\,1/2} : T_{1\,1/2} = 3; T_{2\,1/2} = 3T_{1\,1/2}$ (fig.2).

This kind of relation between the kinetics components is typical for the second type reactions. Thus, we can say, that these components are most probably associated with two convenient processes of $Cl^-$ transport and it seems that separately they are characterised by the kinetics of the first type reactions. The evidence of this is proved by fact that $T_{1\,1/2}$ is not dependent on initial concentration of NaCl. One of the methods for determination of reaction type is based on this independence of $T_{1\,1/2}$ from initial concentration of NaCl.

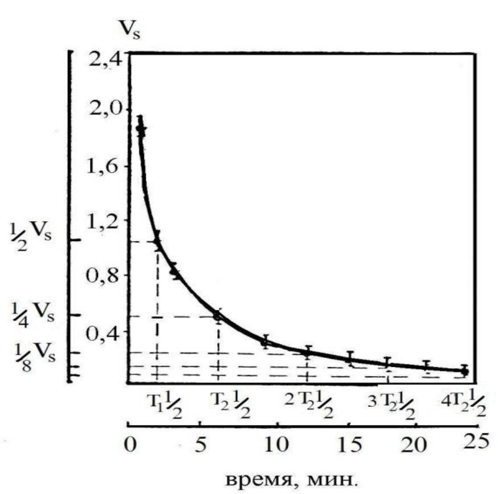


**Fig.2.** The time dependence of average rate of $Cl^-$ absorption by the roots of 5-day old barley sprouts (100 mM NaCl; t-20$^o$C)

On the basis of the formula $T_{2\,1/2} = 3T_{1\,1/2}$ we presume, that the reaction of primary assimilation of $Cl^-$ in roots is a bimolecular (in the case of taking into account the presence of transporter in the membrane), as the diffusion is not important for the monomolecular reactions and the three molecular reactions are hardly possible (Yeremin, 1976; Bayramov, 2003).

It is important to say that the absorption of chloride ions by plant tissues has got kinetic properties, which should be considered as a result of transporters mechanisms action, at least the action of mobile transporters and this has been clearly shown (Liutte, Highinbottam, 1984).

The term «antagonism» has been used for many years in pharmacology and has got several meanings (Mashcovskiy, 2008). Generally it is believed that antagonism occurs when the two substances exert lower effect than it is expected according to individual action of each of them. Before making any conclusions about antagonism an objective method for determination of expected results of summation for common cases should be developed. In fact, such approach, when the effect of combined action of two inhibitors is compared with the effect of their individual action is not very convenient. It is more reasonable to estimate the effect of one of the inhibitors in the absence and in the presence of another one (Webb, 1966).

It is known, that the antagonism of ions is observed during the simultaneous adsorption of various ions from external solution by cells: when some ions enter the cell it causes the driving out of other ions as a result of substances competition, which enter the cell. The antagonistic anions are $Cl^-$, $Br^-$, $J^-$ and etc.; and the cations are $K^+$, $Rb^+$, $Cs^+$, $Na^+$.

Thus, the results obtained in the present study indicate, that during the absorption of chloride ions by the roots of barley the $SO_4^2$ ions do not have antagonistic effect, however the $NO_3^-$ ions have an inhibitory effect when their concentration increase. These results, together with the previously reported data, clearly show that $Cl^-$ and $NO_3^-$ ions have most probably the same transporters and we can presume that they are transported into the plant cells by the same mechanisms.

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