



UDC: 582.32.575.17

MECHANISMS OF MAINTENANCE OF CYTOPLASMIC OSMOTIC HOMEOSTASIS IN BRYOPHYTES CELLS UNDER SALINITY STRESS

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Kyyak V.H., Kyyak N.Ya. Mechanisms of maintenance of cytoplasmic osmotic homeostasis in bryophytes cells under salinity stress. **Studia Biologica**, 2019: 13(2); 55–66 • DOI: <https://doi.org/10.30970/sbi.1302.605>

The mechanisms of stabilization of cytoplasmic osmotic homeostasis in the bryophytes cells the participation of H⁺-ATPase and the intracellular ratio K⁺/Na⁺ were investigated as one of the effective means of control over absorption and compartmentation of excess sodium ions in plant cells under salinity at the territory of the tailings storage facility of Stebnyk Mining and Chemical Enterprise "Polymineral". Samples of *Barbula unguiculata* Hedw., *Didymodon rigidulus* Hedw. and *Brachythecium campestre* (Müll. Hal.) Schimp. mosses were taken from 2 experimental plots in the territory of the tailings storage facility, which significantly differed in substrate salinity levels. The highest activity of ATPase was detected in *B. unguiculata* and *D. rigidulus* plants, which grew on the substrate with very high salinity level. This indicates an important role of the plasma membrane H⁺-pump in the adaptation of bryophytes to salt stress. In *B. campestre* shoots growing on the outskirts of the tailings storage with less salinity of the substrate, the enzymatic activity was found to be lower. It has been shown that the H⁺-ATPase activity depends on changes of the structural state of membranes induced by lipoperoxidation processes under stress conditions. The salt-tolerant *B. unguiculata* and *D. rigidulus* moss species have a lower content of TBA-active products, which positively affects on the functioning of membrane enzymes and leads to the development of general protective stress reaction. It has been shown that increasing of the H⁺-ATPase activity in moss cells under salinity conditions provides a removal of excess Na⁺ ions to outside, that was confirmed by the results of our investigations of cellular localization of sodium. The sequential elution technique for determining cellular localization of cations developed for bryophytes by D. Brown and J. Walls (Brown, Wells, 1995) was used. It made possible to determine the ratio between the amount of sodium and potassium

cations localized on the extracellular sites and in the intracellular matrix. In shoots of the studied moss species, a correlation between ATPase activity and the localization of sodium ions on the extracellular sites was found. This indicates an important role of H⁺-ATPase in eliminating of the excess cytotoxic sodium ions from moss cells under salt stress conditions. It was showed that potassium ions are important osmolytes in the bryophytes cells since under saline stress, an increase in content of potassium and K⁺/Na⁺ correlation was observed in the intracellular matrix, which improves the plants tolerance to the influence of excess Na⁺ ions.

Keywords: moss, salinity, H⁺-ATPase, sodium and potassium content

INTRODUCTION

Today, nearly 6 % of the Earth's surface is affected by salinization, so investigation of the processes of plant growth and development under the influence of increased salt concentrations is important for identifying salt-tolerant species that may be useful for further selection of salt-tolerant plants and soil phytoremediation. Under salinity conditions, high concentrations of Na⁺ and Cl⁻ ions lead to hyperosmotic shock and ion imbalance. One of the main mechanisms regulating the concentration of sodium ions in cytosol is functioning of membrane pumps – H⁺-ATPases, which create the electrochemical gradient required to remove excess monovalent Na⁺ ions outside the cells or into the vacuole [31]. It is also known that Na⁺ is a major competitor of K⁺ in the metabolic processes of cell. So, high content of K⁺ ions in plant cells significantly increases the tolerance of plants to the influence of high concentrations of Na⁺ ions and, thus, increases salt resistance [8].

The mechanisms of bryophytes resistance to salinity need further study. Most terrestrial bryophytes adapted to water deficit, which obviously caused their tolerance to various osmotic stresses. First of all, this is ensured by the presence of high concentrations of osmolytes in moss cells [9; 12; 31]. There is still insufficient information about the mechanisms that ensure the maintenance of ion homeostasis in the bryophyte cells [4]. It is known that a significant difference between the genomes of vascular plants and bryophytes is the presence of genes encoding Na⁺-ATPase in the bryophytes [1; 18]. In higher vascular plants, these genes were lost during the evolution process. It has been shown that under salinization conditions ion homeostasis in bryophytes cells was significantly dependent on the activity of H⁺-ATPase (*PpSHP1* and *PpSHP2*) and Na⁺-ATPase (*PpENA1* and *PpENA2*) [31]. It was experimentally proved that at *Physcomitrella patens* (Hedw.) Bruch & Schimp the gene ENA1, which encodes Na⁺-ATPase (*PpENA1*), moss salt tolerance is increased in conditions of salt stress caused by 300 mM NaCl solution [18]. D. Bates found in *Schistidium maritimum* (Turner ex Scott, Robert) Bruch & Schimp. Na⁺-ATPase which prevents an excessive accumulation of sodium ions in cells [1]. It has been shown that the bryophyte genes *PpSHP1* and *PpSHP2* encoding H⁺-ATPase have a high degree of homology to the yeast PMP3 gene and the RCI gene from *Arabidopsis thaliana* (L.) Heynh. [18].

The aim of this study was to investigate the mechanisms of maintenance of the cytoplasmic osmotic homeostasis in bryophyte cells with the participation of H⁺-ATPase and to evaluate the intracellular ratio of K⁺/Na⁺ as one of the effective instrumentality of controlling of the absorption and compartmentalization of excess sodium ions in plant

cells. The study of conditions of salinization was carried out on the territory of tailing storage for the extraction of potassium and magnesium salts of Stebnyk Mining and "Polyminerall" Chemical Enterprise.

MATERIALS AND METHODS

Samples of *Barbula unguiculata* Hedw., *Didymodon rigidulus* Hedw. and *Brachythecium campestre* (Müll. Hal.) Schimp. mosses were collected for investigations during April–May 2018 from 2 experimental plots on the territory of tailing storage of Mining and "Polyminerall" Chemical Enterprise, which differed significantly in the level of substrate salinity. *Barbula unguiculata* plants growing outside the tailing storage in the outskirts of Stebnyk were used as controls in investigations. Fresh plant material was used in the investigations.

Determination of content of the water-soluble ions in the upper layer of the tailings substrate (0–3 cm) was carried out by a complexometric method [24]. Chemical ionic composition of the aqueous extracts filtrate prepared from the tested substrate samples was determined by standard methods: HCO_3^- [24], Cl^- [25], SO_4^{2-} [26], Ca^{2+} and Mg^{2+} [27]. The number of cations (Na^+ and K^+) was determined by the difference between the sum of anions (HCO_3^- ; Cl^- ; SO_4^{2-}) and the sum of cations (Ca^{2+} ; Mg^{2+}) in mg-eq/100 g of soil.

The hydrolytic activity of H^+ -ATPase was measured spectrophotometrically by the increase of inorganic phosphate in the incubation medium during the ATPase reaction [20]. The aliquot of plant homogenate was added to the incubation medium containing 3 mM MgCl_2 and 50 mM KCl in 0.025 M Tris-acetate buffer (pH 6.5). The hydrolase reaction was initiated by the addition of 3 mM ATP and incubation for 30 min at 38 °C. The reaction was stopped by the addition of 10% trichloroacetic acid. The precipitate was separated by centrifugation, and the supernatant was used for spectrophotometric determination of the inorganic phosphate amount (P_i) on a Specord 210 Plus spectrophotometer at the wavelength of 720 nm by the method of O. Lowry and D. Lopez [5].

For determining the concentration of TBA-active products (thiobarbituric acid-active products), plant material was homogenized in a 20% trichloroacetic acid solution and incubated with 0.5% thiobarbituric acid solution. The content of TBA-active products was determined spectrophotometrically at the wavelength of 532 nm and expressed in nm/1g of the fresh weight [19].

The localization of sodium and potassium ions in cells was evaluated by the method of sequential elution of cations from the extracellular and intracellular sites of mosses [3]. Intact apexes of leaf-stem shoots, without dead parts, were used in this experiment. For washing away the cations, absorbed by the cell walls, a portion of the plant material (1 g) was submerged in 10 ml of 20 mM nickel chloride solution (NiCl_2) and washed twice successively for 30 min each, continuously shaking the samples. After washing, the plants were dried in an oven at 80 °C for 16 h to determine the dry weight of the washed plants. For exudation of Na^+ and K^+ ions from the cytosol cells, the dried samples were submerged in 10 ml of 1 M HNO_3 solution and washed twice in succession. The solutions were not filtered to prevent the loss of the elements. Plants were re-dried in the same way as described above to determine the dry weight. The content of sodium and potassium in solutions and plants was determined by atomic adsorption method on a Spectrophotometer "C-115M1" (Ukraine, "Selmi"). All analyses were performed on a four-fold basis and analysed statistically.

RESULTS AND DISCUSSION

The peculiarity of the overgrown of the tailing storage territory of Mining and “Poly-mineral” Chemical Enterprise is the formation of pioneer stages from plants of halophytic and salt-resistant ecological groups: *Salicornia europaea* L., *Tripolium vulgare* Nees, *Sagina nodosa* Fenzl. and *Puccinella distans* Parl. Mosses are also pioneers of the overgrowth on saline tailings substrates, forming bryophytes communities together with vascular halophyte species [14].

At the plot No 1 the content of SO_4^{2-} ions was 23.2 mg-eq/100 g of soil, the content of Cl^- ions was 13.1 mg-eq/100 g of soil (Table 1) which indicates a very high degree of substrate salinity according to the classification of saline soils by N.I. Basylevych and E.I. Pankova [2]. At this plot mainly halophytes, salt-tolerant species of vascular plants and bryophytes grew. Bryophytes communities are presented by pioneer short-turf xeromesophytic moss species (*Funaria hygrometrica* Hedw., *Didymodon rigidulus* Hedw., *D. fallax* (Hedw.) Zander., *Bryum caespiticium* Hedw., *Aloina ambigua* (Bruch et Schimp.) Limpr., *Barbula unguiculata* Hedw. and *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey & Schreb.). They are more adapted to dehydration native in salinization conditions and are characteristic for the different devastated areas [12; 21]. The overgrowth of the tailing storage substrate on these areas occurs by the “islands”, mostly up to 3–4 m². Samples of *Barbula unguiculata* and *Didymodon rigidulus* were collected on this plot.

Table 1. Characterization of substrates of experimental plots on the territory of tailings storage facility

Таблиця 1. Характеристика субстратів дослідних ділянок на території хвостосховища

Place of substrate sampling	The content of water-soluble ions, mg-eq/100 g of soil							
	Na ⁺ + K ⁺	Ca ²⁺	Mg ²⁺	sum of cations	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	sum of anions
Plot 1	8.3±0.4	17.5±0.9	14.2±1.3	40.0	3.3±0.6	13.1±0.9	23.2±1.1	39.6
Plot 2	3.9±0.1	9.6±0.8	7.8±0.4	21.3	2.4±0.2	7.9±0.5	9.8±0.8	20.1

The content of the SO_4^{2-} and Cl^- anions and the sum of Na^+ + K^+ cations were fixed twice less at the plot No 2 on the outskirts of the tailings storage among the resistant herbaceous communities (Table 1), where mainly *Ceratodon purpureus* (Hedw.) Brid., *Bryum intermedium* (Brid.) Blandow., *Leptobryum pyriforme* (Hedw.) Wilson, *Dicranella varia* (Hedw.) Schimp. occurred. Somewhere, species of the *Brachythecium* genus with the life strategy of perennial stayers began to form the loose weft. At this plot samples of *Brachythecium campestre* were selected.

The physiological effect of salt stress is associated with dehydration that was caused by a decrease of water potential in soil and complicates the absorption of water and nutrients.

A salinity induces the ionic stress that is manifested by an increasing concentration of cytotoxic Na^+ and Cl^- ions and changing the ratio of Na^+/K^+ concentrations. Furthermore, an increase in the extracellular sodium content adversely affects the potassium entrance. In general, ion absorption and compartmentation are crucial for plant growth under salinization conditions. An increase in the intracellular concentration of sodium ions is toxic for cells of all plant organisms, since it affects the ions redistribution in the

cytoplasm, disruption of the cell cycle and membrane structure, slow down the photosynthesis process and stimulating the formation of the reactive oxygen species which leads to inhibition of plant growth [6]. One of the main mechanisms regulating the concentration of sodium ions in the cytosol is the functioning of membrane pumps – H⁺-ATPases which create the electrochemical gradient required to remove excess monovalent Na⁺ ions outside the cells or into the vacuole.

The hydrolytic activity of H⁺-ATPase was investigated in mosses that grew under different salinity conditions at the territory of tailing storage. It was found that ATPase activity depended on the intensity of salt stress and the species-specific peculiarities of mosses. Under conditions of very high salinity, enzyme activity was in the range of 0.824–0.911 mM P_i/hour/mg of protein (Table 2).

The highest ATPase activity was detected in *Barbula unguiculata* plants, simultaneously hydrolytic activity of the enzyme was lower in shoots of this species from the background territory (outskirts of Stebnyk), indicating the participation of the plasma membrane H⁺-pump in adaptation of bryophytes to salt stress.

Table 2. Influence of salinity on the H⁺-ATPase hydrolytic activity and content of TBA-active products in mosses shoots from the territory of tailings storage and the outskirts of Stebnyk

Таблиця 2. Вплив засолення на гідролітичну активність H⁺-АТФ-ази і вміст ТБК-активних продуктів у пагонах мохів із території хвостосховища й околиці м. Стебник

Moss species	H ⁺ -ATPase activity, mM P _i /hour/mg of protein	TBA-active products content, nmol/g of fresh weight
Tailings storage		
<i>plot 1</i>		
<i>Didymodon rigidulus</i>	0.824±0.075	96.2±6.8
<i>Barbula unguiculata</i>	0.911±0.068	115.4±9.7
<i>plot 2</i>		
<i>Brachythecium campestre</i>	0.693±0.025	143.5±11.6
Outskirts of Stebnyk		
<i>Barbula unguiculata</i>	0.640±0.051	84.9±6.3

In *Didymodon rigidulus* plants, the enzymatic activity was quite high that indicates stabilization of the membrane potential with the participation of H⁺-ATPase under salinity conditions. In the *Brachythecium campestre* shoots, the enzymatic activity was the lowest, apparently as a result of the greater sensitivity of membrane enzymes in plants of this species to osmotic stress.

Investigations on salt-tolerant plant species indicate an important role of proton pumps in the adaptation of plants to salinity. It was marked by an increase in ATPase activity under the influence of salt stress [11]. It was also shown that cultivation plants on medium with different concentrations of NaCl activate the proton plasma membrane pumps in both halophytes and glycophytes [10; 23]. Thus, these investigations indicate a significant role of H⁺-ATPase in maintaining of ionic homeostasis in plant cells and in enhancing of salt tolerance.

The activity of H⁺-ATPase depends on the structural state of membranes, so the enzyme is sensitive to changes in the composition and properties of the lipid matrix, induced

by the processes of lipid peroxidation under the stress [31]. In the studied plants, the level of lipid peroxidation was tested by the TBA-active products content, a marker of development of destructive processes in the lipid component of biological membranes.

The TBA-active products concentration was in the range of 96.2–143.5 nmol/g of fresh weight. In the *Didymodon rigidulus* and *Barbula unguiculata* species, the lipid peroxidation index was lower which could have contributed to stabilization of the hydrolytic activity of the H⁺-ATPase since in the plants of these species recorded the highest enzymatic activity. In *Brachythecium campestre* plants, the TBA-active products content increased to 143.5 nmol/g of fresh weight that was consistent with a significant decrease of the ATPase activity (Table 2). Thus, salt-tolerant moss species are characterized by a decreased level of oxidation processes in the intracellular membranes, which positively influences functionis of the membrane enzymes and leads to a development of the protective stress response.

Na⁺ ions are the direct competitor of K⁺ ions in plant absorption systems. It should be noted that plant absorption of sodium by the plants under salinity conditions is much more effective than potassium. The physical and chemical similarity between the Na⁺ and K⁺ ions also leads to the competition for catalytic sites, which under normal conditions attach the required K⁺ cation. It has been confirmed that maintaining of high proportion of K⁺ ions to Na⁺ in the cytosol significantly increases plants' tolerance to high concentrations of Na⁺ and, thus, increases salt resistance of plants [31]. The use of a sequential elution technique for determining cellular location of the cations developed by D. Brown and J. Wells for bryophytes [3], that allowed to determine the relationship between the cations localized on the extracellular sites and in the intracellular matrix. Sodium content in the studied mosses species was in the range of 98.35–151.57 μg/g of fresh weight (Table 3).

Table 3. Content of sodium and potassium in mosses shoots from the territory of tailings storage and the outskirts of Stebnyk

Таблиця 3. Вміст натрію та калію у пагонах мохів із території хвостосховища й околиці м. Стебник

Moss species	Sodium content, μg/g of fresh weight	Potassium content, μg/g of fresh weight
Tailings storage		
<i>plot 1</i>		
<i>Didymodon rigidulus</i>	133.09±14.23	2905.94±198.73
<i>Barbula unguiculata</i>	151.57±16.52	3277.12±483.26
<i>plot 2</i>		
<i>Brachythecium campestre</i>	138.21±14.11	4091.67±521.64
Outskirts of Stebnyk		
<i>Barbula unguiculata</i>	98.35±10.12	1248.41±144.37

In *Didymodon rigidulus* and *Barbula unguiculata* plants 133.09–151.57 μg/g of fresh weight of sodium was found, and almost 75% of ions were localized in the cell wall (Fig. 1). Similar results were obtained in the experiments with *Funaria hygrometrica* and *Fontinalis antipyretica* Hedw. mosses under the osmotic stress [3; 8].

It should be noted that in *Barbula unguiculata* shoots from the outskirts of Stebnyk, the sodium content was by 35.2% less than in the plants from the tailing storage area, and its quantity in the extracellular sites and intracellular matrix was similar. Thus, to prevent the negative effects of salt stress in salt-tolerant mosses species sites of the cell wall are able to “condense” an excess of sodium ions and prevent passing them inside the cell.

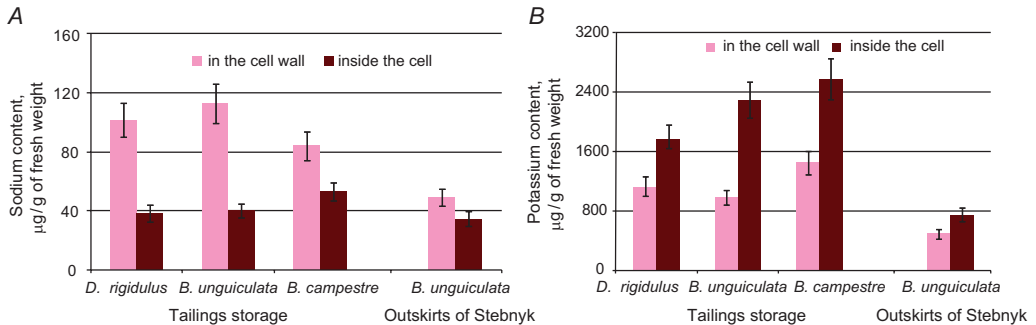


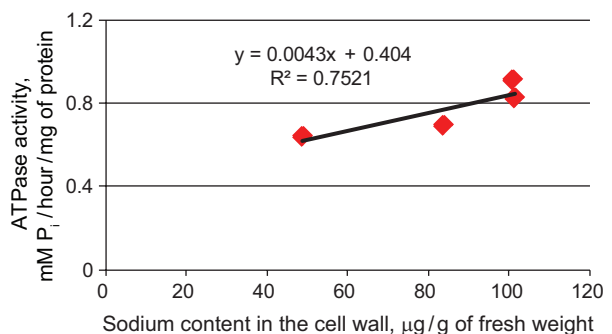
Fig. 1. Localization of sodium (A) and potassium (B) in moss cells from the territory of the tailings storage and the outskirts of Stebnyk

Рис. 1. Локалізація натрію (A) та калію (B) у клітинах мохів із території хвостосховища та околиці м. Стебник

Correlation-regression analysis of the relationship between H^+ -ATPase activity and accumulation of Na^+ ions in the extracellular sites of shoots of the investigated moss species showed that the obtained dependence is described by a linear equation and has a sufficiently high correlation coefficient (0.87). It is known that the trend line is the most reliable if its probability of approximation (R^2) is equal or close to 1. In our study, the approximation level (R^2) was 0.75, thus, the removal of excess sodium ions from the cell under the influence of salinization clearly correlates with an increase of the ATPase activity (Fig. 2). In general, the obtained results indicate an important role of H^+ -ATPase in maintaining of the ion homeostasis and eliminating excess cytotoxic sodium ions from moss cells at the salinization conditions.

Fig. 2. Dependence of sodium accumulation in the cell wall on the activity of H^+ -ATPase in mosses' cells

Рис. 2. Залежність акумуляції натрію у клітинній стінці від активності H^+ -АТФ-ази у клітинах мохів



An increased concentration of the intracellular Na^+ is toxic for plants. It was considered that the violation of protein synthesis in the conditions of excess Na^+ ions can be caused by Na^+/K^+ imbalance [28]. It is known that more than 50 cell enzymes are activated in

the presence of potassium, and its high concentration (100–200 mM) in the cytoplasm compared to sodium (10–30 mM) provides optimal ion homeostasis for the functioning of enzyme systems and protein synthesis [31].

Since Na^+ and K^+ ions compete for intracellular catalytic sites in transport systems of plant, the maintenance of high proportion of K^+/Na^+ in the cytosol can increase plant salt resistance [8].

In the investigated mosses species from the tailing storage area, the content of potassium in shoots was an order of magnitude higher than sodium (Table 3). Moreover, the main pool of these ions was localized in the intracellular matrix (~ 62.3–68.9 %) (Fig. 1). In all moss species, the intracellular K/Na ratio was quite high regardless of the degree of substrate salinity. For example, in *Barbula unguiculata* and *Didymodon rigidulus* plants, this index was 46.6–57.3, whereas in *Barbula unguiculata* shoots from the outskirts of Stebnyk – 22.1. This indicates the presence in bryophyte cells of an effective control of the absorption and compartmentalization of excess sodium ions. This is confirmed by high correlation between potassium and sodium content in the intracellular matrix (0.86) and a probability of the approximation (0.74) (Fig. 3).

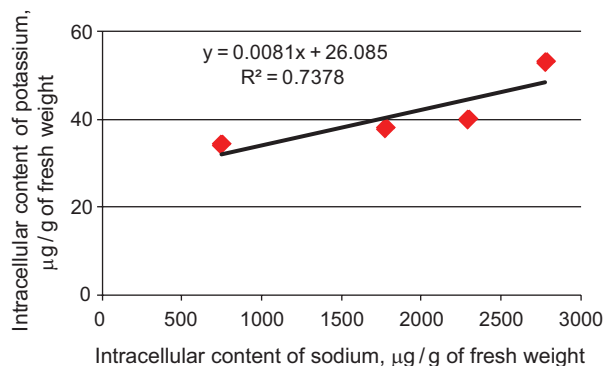


Fig. 3. Relationship between sodium and potassium content in the intracellular matrix of mosses cells

Рис. 3. Залежність між вмістом натрію та калію у внутрішньоклітинному матриксі клітин мохів

Therefore, on the area of tailing storage of extraction of the potassium and magnesium salt, mosses are pioneers of overgrowth and their distribution is associated with the initial stages of plant successions. An important role in the formation of moss cover on the initial stage of overgrowth of very heavily salted tailing substrates most often belongs to short-turf xeromesophyte species (*Didymodon rigidulus*, *Barbula unguiculata*, *Funaria hygrometrica*, *Bryum argenteum*, *B. caespiticium*) that forms pioneer groups together with halophytic and salt-resistant species of vascular plants. With a decreasing degree of the substrate salinization species of the genus *Brachytecium* with life strategy of the perennial stayer join to the bryophyte communities which indicates a certain improvement in conditions of edaphotopes.

It should be noted that in natural ecosystems mosses rarely grow under salinization conditions. For example, in Canada, in the oil sands region, natural salt marshes with high concentration of Na salts form groups of salt-tolerant plants, among which *Bryum pseudotriquetrum*, *Campylium stellatum* (Hedw.) C.E.O. Jensen and *Drepanocladus aduncus* (Hedw.) Warnst. were found [29]. The species composition of the bryophytes in the territory of natural salt steppes in Austria has been investigated [30]. *Entosthodon hungaricus* (Boros) Loeske, *Pottia heimii* (Hedw.) Hampe, *Drepanocladus aduncus*, *Phascum cuspidatum* var. *mitriforme* Limpr., *P. cuspidatum* Hedw., *Funaria hygrometrica*

and *Bryum algovicum* Sendtn. ex Müll. Hal. were found. Some mosses, such as *Fontinalis dalecarlica* Bruch & Schimp., *Campylium stellatum* and *Schistidium maritimum* have a high ability to survive under permanent irrigation by the seawater on the coastal cliffs, although bryophytes rarely grow in seawater [31].

Therefore, despite the distribution of bryophytes under salinization conditions, they lack evolutionarily formed specific mechanisms of salt resistance, such as halophytes, which have adapted to salinity through the formation of specialized subcellular, biochemical and molecular mechanisms. Whereas bryophytes adaptation to salt stress bound with effective mechanisms of desiccation resistance, the important criteria of which (as well as salt resistance) are the ability to osmotic regulation, the stability of water, and the osmotic potentials of plant cells.

That was confirmed by our results with mosses that differ both in ecological and biological properties, and in tolerance to water deficit. *Didymodon rigidulus* and *Barbula unguiculata* are short-turf xeromesophytic mosses species which confined to dry open areas with the unfavorable hydrothermal conditions. A survival of plants under such conditions was ensured by a considerable plasticity of the components of the osmoregulatory system which is obviously caused by the presence of powerful systems of endogenous regulation that counterbalance the unfavorable influence of the environmental factors [13; 15–17]. The plants of these species are characterized by high levels of H⁺-ATPase functioning and, consequently, stabilization of ionic homeostasis under the salinization conditions. *Brachythecium campestre* plants are less tolerant to the osmotic stress and confined to habitats with relatively favorable environmental conditions. They grow on the outskirts of tailing storage area, among the resistant herbaceous community with much lower substrate salinity. In our investigations, the plants of this species were more sensitive to a salinity influence, as they exhibited a higher level of oxidation processes in the intracellular membranes and, as a consequence, a significant decrease in the ATPase activity.

At increase of the H⁺-ATPase activity in plant cells under salinization conditions creates an electrochemical gradient on the plasma membrane, required for Na⁺/H⁺ antiporters to remove an excess Na⁺ ions externally. The results of our investigations evidenced cellular localization of sodium. A correlation between ATPase activity and localization of sodium ions in the extracellular sites was found in the shoots of the studied moss species. It indicates an important role of H⁺-ATPase in the elimination of excess cytotoxic sodium ions from moss cells in the conditions of salt stress. It was also found that under salinization conditions the potassium content in the intracellular matrix and the intracellular K⁺/Na⁺ ratio increased in the studied moss species that provided optimal ion homeostasis during the osmotic stress.

CONCLUSIONS

The bryophytes are the pioneer of saline substrates overgrowing and colonizing areas with a very strong of salinization at the territory of tailing storage of Mining and “Polyminerall” Chemical Enterprise, unsuitable for other plants.

An increase of tolerance of bryophytes to salinization was achieved through the mechanisms of maintaining of cytoplasmic osmotic homeostasis in cell by elevation of the activity of H⁺-ATPases, that provides energy support for the activity of secondary active Na⁺/H⁺-pumps and by immobilization of excess of Na⁺ ions in the cation exchange sites of the cell wall.

The potassium ions are the important osmolytes in the bryophyte cells. An increase of potassium content and K^+/Na^+ ratio in the intracellular matrix was observed under the conditions of salt stress that increased the tolerance of plants to an excess of Na^+ ions. As a result, it enhanced salt resistance of plants.

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МЕХАНІЗМИ ПІДТРИМАННЯ ОСМОТИЧНОГО ГОМЕОСТАЗУ У ЦИТОПЛАЗМІ КЛІТИН БРІОФІТІВ ЗА УМОВ ЗАСОЛЕННЯ

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Досліджено механізми підтримання цитоплазматичного осмотичного гомеостазу у клітинах бріофітів за участю H^+ -АТФ-ази та оцінено внутрішньоклітинне співвідношення K^+/Na^+ як один із ефективних засобів контролю за поглинанням і компартментацією надлишку йонів натрію у клітинах рослин в умовах засолення на території хвостосховища видобутку калієвих і магнієвих солей Стебницького гірничо-хімічного підприємства “Полімінерал”. Для досліджень відбирали зразки мохів *Barbula unguiculata* Hedw., *Didymodon rigidulus* Hedw. і *Brachythecium campestre* (Müll. Hal.) Schimp. із двох дослідних ділянок на території хвостосховища, які відрізнялися за рівнем засолення субстрату. Найвищу активність АТФ-ази визначено у рослинах *B. unguiculata* та *D. rigidulus*, які росли в умовах дуже сильного засолення субстрату, що свідчить про участь H^+ -помп плазматичної мембрани в адаптації бріофітів до сольового стресу. У пагонах *B. campestre*, які росли на околиці хвостосховища в умовах меншого засолення субстрату, ферментативна активність була нижчою. З’ясовано, що активність H^+ -АТФ-ази залежала від змін структурного стану мембран, які індукувалися процесами ліпопероксидації в умовах стресу. Солетолерантні види мохів *B. unguiculata* і *D. rigidulus* мали нижчий вміст ТБК-активних продуктів, що позитивно впливало на функціонування мембранних ферментів і призводило до розвитку загальної захисної стрес-реакції. Виявлено, що підвищення активності H^+ -АТФ-ази у клітинах мохів в умовах засолення забезпечує видалення надлишку йонів Na^+ назовні, що підтверджується результатами наших досліджень клітинної локалізації натрію. У пагонах досліджуваних видів мохів встановлено кореляцію між АТФ-азною активністю та локалізацією йонів натрію на зовнішньоклітинних сайтах, що свідчить про важливу роль H^+ -АТФ-ази у виведенні надлишку цитотоксичних йонів натрію з клітин мохів в умовах сольового стресу. Встановлено, що важливими осмолітами у клітинах бріофітів є йони калію, оскільки в умовах сольового стресу зафіксовано збільшення вмісту калію та співвідношення K^+/Na^+ у внутрішньоклітинному матриксі, що підвищує толерантність рослин до впливу надлишку йонів Na^+ і тим самим збільшує їхню солестійкість.

Ключові слова: мохи, засолення, H^+ -АТФ-аза, вміст натрію та калію

Одержано: 23.10.2019