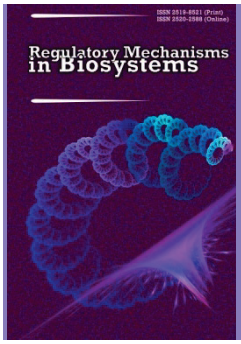

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2022, 13(2), 189–196
doi: 10.15421/022224

Metabolism of carbohydrates and activity of the antioxidant system in mosses on a post-technogenic salinized territory

N. Y. Kyyak

Institute of Ecology of the Carpathians of National Academy of Sciences of Ukraine, Lviv, Ukraine

Article info

Received 06.04.2022
Received in revised form 03.05.2022
Accepted 04.05.2022

Institute of Ecology of the Carpathians NAS of Ukraine, Kozelnytska st., 4, Lviv, 79026, Ukraine. Tel.: +38-032-270-74-30. E-mail: kyyak_n@i.ua

Kyyak, N. Y. (2022). Metabolism of carbohydrates and activity of the antioxidant system in mosses on a post-technogenic salinized territory. Regulatory Mechanisms in Biosystems, 13(2), 189–196. doi:10.15421/022224

Adaptive physiological and biochemical reactions of mosses *Didymodon rigidulus* Hedw., *Barbula unguiculata* Hedw. and *Brachythecium campestre* (Müll. Hal.) Schimp. to salt stress have been investigated from the territory of the tailings storage of the Stebnyk Mining and Chemical Enterprise "Polymineral" (Lviv region, Ukraine). The peculiarities of carbohydrate metabolism in mosses under salinity conditions have been studied. The content of soluble carbohydrates and proline, the antioxidant activity, the content of ascorbate and reduced glutathione as well as the activity of enzymes of their metabolism – ascorbate peroxidase and glutathione reductase at the initial stages of the stress (salt shock) and prolonged stress exposure (salt stress) have been evaluated. It has been found that the increase of α-amylase activity, enhancement of the hydrolysis of starch and the increase of the concentration of soluble carbohydrates under salt stress were the reactions of the studied species of mosses. It has been established that there was an increase in the concentration of soluble carbohydrates by 1.2–1.5 times in moss shoots under salinity conditions, compared with plants from the background area (vicinity of Stebnyk). Experimental studies have shown that under salinity conditions sucrose dominates in the pool of soluble carbohydrates (59.0–79.5% of the total sugars content). The sucrose content was 1.5–2.0 times higher in the plants *B. unguiculata* and *D. rigidulus* from the highly saline area of the tailings storage. It has been indicated that under stress conditions constitutive adaptive mechanisms are more expressed in resistant moss species, and plants with a lower level of resistance adapt to the stressor, mainly due to induced protective systems. Experimental studies have shown that plants *B. unguiculata* and *D. rigidulus*, which are resistant to abiotic stressors, have a high constitutive pool of soluble carbohydrates both at the beginning of the experiment and under prolonged exposure of the salt stress. In the shoots of the sensitive moss *B. campestre* the stress-induced character of the sugars accumulation has been revealed. The accumulation of proline in mosses cells under salt stress depended on their species characteristics. The stress-induced accumulation of proline can be considered as a part of the bryophytes' protective system, but this osmolyte does not play a key role in the formation of the mosses' resistance to salt stress. Obviously, soluble carbohydrates are the main osmolytes in the moss cells. It has been found that resistant moss species have a high constitutive antioxidant status, while in the sensitive moss *B. campestre* the increase in the antioxidant activity occurred during prolonged salt stress, which may indicate its induced nature. It has been shown that the resistant mosses *B. unguiculata* and *D. rigidulus* have 3–4 times higher levels of glutathione and ascorbate content and 1.6–2.5 times higher activity of enzymes of their metabolism – glutathione reductase and ascorbate peroxidase, compared to plants of the less tolerant moss species *B. campestre*, which provided reduction of the lipid peroxidation process in plasma membranes and decreased the content of TBA-active products under stress.

Keywords: salt stress; bryophytes; α-amylase; starch; sucrose; monosaccharides; proline; antioxidant activity; ascorbate; glutathione; glutathione reductase; ascorbate peroxidase.

Introduction

Soil salinization is one of the abiotic factors that cause significant unfavourable influence on plants. According to the FAO (Food and Agriculture Organization of the United Nations), about 30% of land may become unfit for agricultural use due to salinization by 2030. Therefore, the study of growth and development of plants in salinity conditions is relevant for the detection of salt-tolerant species and improvement of methods of increasing salt resistance of plants (Boychuk, 2021).

Two components can be defined in the effect of salinity on the plants' organisms: osmotic, which is associated with the decline in water potential of the soil solution and decrease of water availability for plants, as well as the toxic component, which is caused by the penetration of Na^+ salts into the cells' cytoplasm (Flowers et al., 2014; Gao et al., 2017; Ketehouli et al., 2019). In other words, the physiological effects of salt stress are connected with dehydration as high concentrations of salt ions lead to hyperosmotic shock and ionic imbalance (Liu et al., 2016; Karpets et al., 2017; Stark, 2017; González-Orenga et al., 2021). The main stress factor that inhibits the formation of plant cover at the tailings storage of the Stebnyk Mining and Chemical Enterprise "Polymineral" (Lviv Region) is the salinity of the substrate due to the accumulation of waste flotation enrichment of potassium and magnesium ores. Mosses are pioneers of the overgrowth of the tailings storage saline substrates, as well as other post-technogenic territories (Rabyk et al., 2018; Kyyak & Kyyak, 2019, 2020; Lavrenko et al., 2019; Lobachevska et al., 2019; Grishutkin et al., 2020; Kyyak et al., 2020). As of today, there is little information on both the effects of salt stress on bryophytes and the mechanisms that ensure their survival under conditions of salinity (Garbary et al., 2008; Wang et al., 2008; Pouliot et al., 2012). Specialized mechanisms of resistance to salt, as in vascular plants, have not been detected in bryophytes, so it is important to know the peculiarities of their adaptation to salinity (Cosić et al., 2018; Sabovljević & Sabovljević, 2007, 2020).

The resistance of plants to stressors is determined by the functioning of constitutive and induced protective systems (Lobachevska et al., 2005; Liu et al., 2019; Hasanuzzaman et al., 2020; Oke et al., 2020).Their fundamental difference is that constitutive systems are constantly in a functionally active state and are detected immediately under short-term stress, and induced – are not normally present and are detected only in response

to prolonged stress (Boots & Best, 2018; He et al., 2018; Venegas-Molina et al., 2020).

In this regard, the aim of the work was focused on an experimental study of adaptive physiological and biochemical reactions of mosses to salt stress on the territory of the tailings storages of the Stebnyk Mining and Chemical Enterprise "Polymineral". The content of osmoprotectors – soluble carbohydrates and proline, antioxidant activity of plants, content of ascorbate and reduced glutathione and activity of enzymes of their metabolism – ascorbate peroxidase and glutathione reductase, in the initial stages of stress response (salt shock) and with the prolonged influence of stress (salt stress) will be determined.

Material and methods

Mosses from the territory of the tailings storage of the Stebnyk Mining and Chemical Enterprise "Polymineral" were used for the experimental study of the constitutive and induced protective systems. Samples of resistant moss species *Didymodon rigidulus* and *Barbula unguiculata* were collected from the heavily saline area, and samples of the less resistant moss species *Brachythecium campestre* were collected from the area with lowest level of salinity. Plants from the vicinity of Stebnyk (background area) were used as controls in investigations. The ions' content in the substrate of experimental plots at the tailings storage territory was described in a previous publication (Kyyak & Kyyak, 2019).

Plant material for experiments was obtained by regeneration of shoots in sand culture. Fragments of shoots were washed in water, sterilized for 1 min with 20% sodium hypochlorite solution and washed three times with sterile distilled water. Cultures were grown in pots on sterile sand for 2 months in a phytotron under controlled conditions of light 70 μmol•m^2/s, temperature +20…+22 °C and relative humidity 85–90% and sprayed twice on the week with diluted 1:2 Knop-II solution. For modeling salt stress, plants were sprayed with 100 mM NaCl solution for 7 days. The activity of protective systems was evaluated after 1 day exposure with NaCl solution (salt shock) and after 7 days of the experiment (salt stress).

The total carbohydrate content was determined by the phenol-sulfate method after the acid hydrolysis of samples (Sadasivam & Manickam, 2007). The content of soluble carbohydrates was determined by the phenol-sulfate method of DuBois et al. (2002). The optical density of solutions was measured on a Specord 210 Plus spectrophotometer at a wavelength of 490 nm.

The content of monosaccharides and starch was evaluated spectrophotometrically using picric acid (Aversi-Ferreira et al., 2004). The plant material was extracted in distilled water at 40–50 °C for 1 h, cooled and centrifuged (4000 g, 5 min). The supernatant was used to determine monosaccharides. To determine the starch, the precipitate, formed after centrifugation of the samples, was hydrolyzed in 2% HCl for 2 h in a boiling water bath. The reaction mixture contained saturated picric acid solution and 20% Na_2CO_3 solution. Samples were measured on a Specord 210 Plus spectrophotometer at a wavelength 490 nm. The sucrose content was determined by the resorcinol method (Monsigny et al., 1988). The optical density of the solutions was measured spectrophotometrically at a wavelength of 400 nm. The amount of the carbohydrates was expressed in μg/g dry weight.

Activity of α-amylase was determined by amyloclastic method (Mac Gregor et al., 1984). The plant material was homogenized in 1% NaCl solution (1:5 ratio) and centrifuged for 15 min (6000 g, +4 °C). The incubation medium for the determination of enzyme activity contained 0.1 M acetate buffer (pH 5.5), 2% starch solution and plant extract. After incubation (+40 °C, 60 min), 1 N HCl solution was added to stop the amylase activity. For detection of starch that did not react with amylase, 3 mL of distilled water, 0.1 mL of 0.1 N HCl, 5 drops of 0.3% iodine solution and 1.5 mL of investigated solution were added to the test tubes. The enzyme activity was determined spectrophotometrically on a Specord 210 Plus spectrophotometer at a wavelength of 595 nm and expressed in μg of hydrolyzed starch/min/mg protein.

The content of thiobarbituric acid-active (TBA-active) products was determined spectrophotometrically at a wavelength of 532 nm using 0.5% solution of 2-thiobarbituric acid (TBA) and expressed in nM/g dry weight (Lushchak et al., 2004). Proline was extracted and determined using the ninhydrin reagent by the method of Bates et al. (1973). Samples were measured spectrophotometrically at 520 nm. Content of the free proline was expressed in μmol/g dry weight.

Antioxidant activity was evaluated in the reaction of the plant extract with a solution of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by the method of Brand-Williams et al. (1995). The optical density of the solutions was determined on a Specord 210 Plus spectrophotometer (λ = 517 nm). Antioxidant activity was expressed as the concentration of plant extract that caused 50% inhibition of free radical coloration (EC_{50}).

To determine the content of reduced glutathione, plant material was homogenized in solution of 5% trichloroacetic acid and centrifuged for 15 min at 4000 g. The reaction mixture contained an enzyme preparation, 15 mM EDTA, 0.02% egg albumin protein, 0.3 mM 5.5-dithiobis(2-nitrobenzoic) acid, 50 mM imidazole and 0.48 units of the glutathione reductase. The reaction was initiated by the addition of 0.9 mM $NADPH_2$. Samples were measured on a Specord 210 Plus spectrophotometer at a wavelength 412 nm. The content of reduced glutathione was expressed in μM $NADPH_2$/g dry weight (Yenne & Hatzios, 1990).

The ascorbic acid content was determined spectrophotometrically at a wavelength of 530 nm using 0.025% solution of 2.6-dichlorophenolindophenol and expressed in mg/g dry weight (Mehta et al., 2018).

To determine the activity of ascorbate peroxidase (APO), the plant material was homogenized in 0.1 M phosphate buffer (pH 7.6) and centrifuged for 10 min (12000 g, +4 °C). The incubation medium for the determination of the enzyme activity contained: 0.1 mL of 0.1 mM EDTA, 0.1 mL of 0.25 mM ascorbic acid, 2.25 mL of 0.1 M phosphate buffer (pH 7.6), 0.6 mL of plant extract and 0.1 mL of 0.5 mM hydrogen peroxide solution. Ascorbate peroxidase activity was determined spectrophotometrically on a Specord 210 Plus spectrophotometer at a wavelength of 290 nm. The activity of the enzyme was expressed in μM ascorbate/mg protein/min (E = 2.8 mM/cm) (Nakano & Asada, 1981).

To determine glutathione reductase (GR) activity, the plant material was homogenized in 0.05 mM potassium phosphate buffer (pH 7.0) and centrifuged for 20 min (5000 g, +4 °C). The incubation medium for the determination of the enzyme activity contained: 0.05 mL of plant extract, 1 mL of 0.2 M K-phosphate buffer (pH 7.5) with 1 mM EDTA, 0.5 mL of 3 mM 5,5'-dithiobis-(2-nitrobenzoic) acid and 0.1 mL of 2 mM $NADPH_2$. The reaction was initiated by the addition of 0.1 mL of 5 mM reduced glutathione. Enzyme activity was determined on a Specord 210 Plus spectrophotometer at a wavelength of 412 nm and expressed in μM $NADPH_2$/g protein/min (Smith et al., 1988).

The results were statistically analyzed, determining the mean value, median, standard deviation (SD), and the first and the third quartiles for each characteristic in all the variants of the experiment. The selections were compared using single-factor dispersion analysis (ANOVA) with Bonferroni correction, considering differences between the selections reliable at the level of $P < 0.05$. All calculations and developments of diagrams were made in Statistica 8.0 software (StatSoft, USA, 2012).

Results

On the territory of the tailings storage, the studied species of mosses differed in the total content of carbohydrates. The highest concentrations were found in the samples of *D. rigidulus* and *B. unguiculata*. 2.5–2.8 times lower carbohydrates content was found in *B. campestre* shoots both from the tailings storage area and from the background territory (Table 1). Therefore, the accumulation of carbohydrates depends on both the level of salt stress and the species characteristics of mosses. The higher content of these compounds was determined in moss resistant to osmotic stress (*D. rigidulus* and *B. unguiculata*), which in natural conditions are confined to habitats with moisture deficiency.

Analysis of the starch content showed the lowest concentration of this polysaccharide in mosses from the experimental plot of the tailings storage with a high level of salinity (4.3–4.9% of the total carbohydrate content in plants). In particular, the lowest quotient of starch in the carbohydrate pool was found in the shoots of mosses *D. rigidulus* and *B. unguiculata* (Table 1).

Table 1
The content of carbohydrates in shoots of mosses *Didymodon rigidulus*, *Barbula unguiculata* and *Brachythecium campestre* from the tailings storage territory of the Stebnyk Mining and Chemical Enterprise "Polymineral" and background area (μg/g dry weight, x ± SD, n = 12)

Moss species	Total content of carbohydrates	Content of starch	Content of soluble carbohydrates	Content of monosaccharides	Content of the sucrose
Background area (vicinity of Stebnyk)					
D. rigidulus	1049 ± 61	129.8 ± 13.2	157 ± 16	18.3 ± 1.6	112.2 ± 11.7
B. unguiculata	1378 ± 82	98.7 ± 10.2	349 ± 34	61.9 ± 6.1	121.3 ± 11.2
B. campestre	426 ± 39	63.7 ± 5.1	144 ± 13	25.1 ± 2.3	71.6 ± 8.3
Tailings storage area					
D. rigidulus	1318 ± 71*	54.6 ± 5.2*	219 ± 12*	38.6 ± 1.3*	178.2 ± 19.6*
B. unguiculata	1455 ± 62	70.2 ± 8.5*	379 ± 15	73.8 ± 5.1	253.5 ± 24.8*
B. campestre	523 ± 47*	44.9 ± 4.3*	161 ± 12	33.6 ± 1.9*	113.8 ± 13.1*

Note: * – difference between moss samples from the tailings storage and background area (control) for one moss species is statistically reliable at $P < 0.05$.

Under conditions of lower salinity in the moss *B. campestre*, a larger share of starch in the total pool of moss carbohydrates was determined (8.1–8.8% of the total carbohydrate content). Therefore, under conditions of salinity a low concentration of starch was revealed in bryophyte cells, and the intensity of the polysaccharide hydrolysis process was directly dependent on the level of salinity of the substrate. Comparing the results obtained for each species from the tailings storage and background area, the higher starch content was established in the shoots of all plants from the vicinity of Stebnyk. The polysaccharide content was almost twice as high and amounted to 7.9–17.1% in the total pool of carbohydrates. Therefore, the hydrolysis of starch is a nonspecific response of all investigated mosses to salinity, but changes in the direction of carbohydrate metabolism are more pronounced in mosses resistant to water deficit, which had grown in conditions of the very high salinity.

The activity of α-amylase, which catalyzes the starch hydrolysis, has been analyzed. Plants from the tailings storage area responded to osmotic stress by increasing the enzyme activity by 1.5–1.8 times, compared to plants from the background area, which indicated activation hydrolysis of polysaccharides under salt stress (Fig. 1). Higher enzyme activity was found in shoots of *B. unguiculata* and *D. rigidulus*, and lower amylase activity was detected in *B. campestre* shoots from the locality with lower substrate salinity.

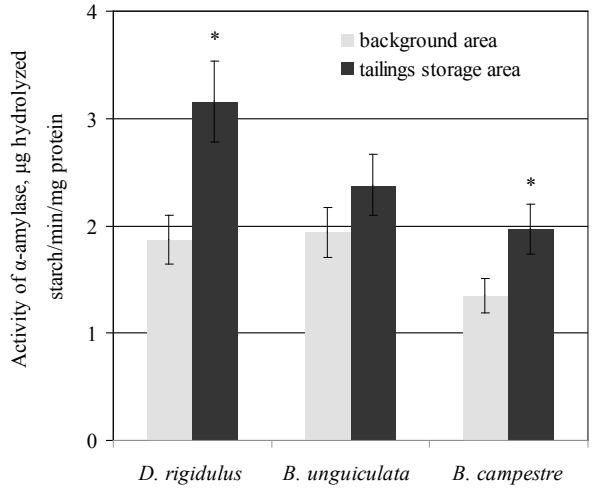

Fig. 1. Activity of α-amylase in shoots of mosses *Didymodon rigidulus*, *Barbula unguiculata* and *Brachythecium campestre* from the tailings storage territory of Stebnyk Mining and Chemical Enterprise "Polymineral" and the background area (vicinities of Stebnyk) (x ± SD, n = 12); * – the difference between moss samples from the tailings storage and the background area (control) for one moss species is statistically reliable at $P < 0.05$

The most important osmoprotectors in moss cells are soluble carbohydrates. The content of soluble carbohydrates and their component composition (monosaccharides and sucrose content) in moss samples from the tailings storage area were studied. It was found that carbohydrate metabolism in mosses under the salt stress changes towards the accumulation of sugars, which function as osmotic regulators that increase the water holding capacity of plants and also as osmoprotectors that stabilize macromolecules and membrane structure of moss cells under stressful conditions. Higher accumulation of sugars was recorded in moss shoots under conditions of high salinity: their content was the highest (379.3 μg/g dry weight) in *B. unguiculata* while in *D. rigidulus* shoots it was 219.4 μg/g dry weight (Table 1). The lowest amount of osmolytes was determined on the substrate with a lower level of salinity in *B. campestre* plants. On the contrary, their quantity was 1.1–1.5 times less in plants from the background area. It should be noted that no significant difference was found in the shoots of *B. unguiculata* regarding the content of sugars in the samples from the tailings storage and the vicinity of Stebnyk. Plants of this species are confined to open dry habitats in nature and are often negatively affected by high levels of insolation, temperature, moisture deficiency, so the presence of high content of osmolytes in cells is a necessary condition for their existence. Therefore, the increase of the concentration of soluble carbohydrates under salt stress, compared with plants from the background area, was the general reaction of the studied species of mosses.

Correlation analysis between content of soluble carbohydrate and α-amylase activity in moss shoots has shown that the relationship, described by a linear equation, has a fairly high correlation coefficient (0.65), confirming that the increase in soluble carbohydrate content in moss shoots under salt stress significantly depends on amylase activity (Fig. 2).

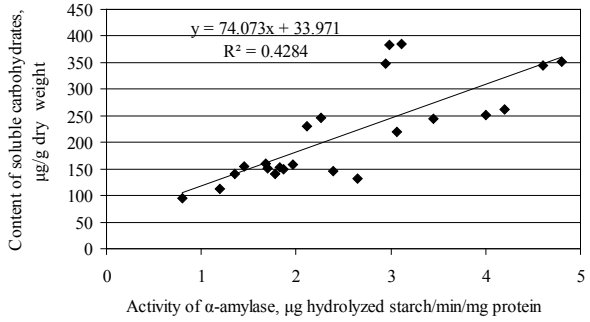

Fig. 2. Relationship between soluble carbohydrate content and α-amylase activity in moss shoots from the tailings storage territory of Stebnyk Mining and Chemical Enterprise "Polymineral" and background area

A similar trend was found for the content of monosaccharides in moss shoots. The content of monosaccharides also depended on the level of salt stress and species characteristics of mosses, although the share of monosaccharides in the total pool of soluble carbohydrates was less than 20% (Table 1). Comparing the obtained data with the content of monosaccharides in plants from the background area, it should be taken into account that the general response of all studied species to salt stress has been detected as the increase of their concentration. The most significant increase of the content of monosaccharides was discovered in the shoots of *D. rigidulus* – almost double. By contrast, their concentration was found to be 1.3–1.4 times higher in the plants of *B. campestre*. The number of monosaccharides in the shoots of *B. unguiculata* was high in the samples from the tailings as well as from the background area. The obtained results give the opportunity to draw the conclusion that monosaccharides participate in the protection of bryophyte cells under conditions of

osmotic stress. Our research has shown that disaccharide sucrose dominates in the composition of soluble carbohydrates of mosses under conditions of salt stress (Table 1). Considering the shoots of *B. unguiculata* and *D. rigidulus*, which had grown under conditions of strong salinity of the tailings storage substrate, the concentration of sucrose was 66.1–79.5% in the total pool of soluble carbohydrates. Regarding *B. campestre* plants from less saline areas, the disaccharide content was 1.5–2.0 times lower, however its share in the total amount of soluble sugars was also high – 59.0–71.4%. In addition, it was observed that sucrose concentrations in plants from the background area were 1.6–2.2 times lower. Therefore, the accumulation of soluble carbohydrates in the cells was the response of all species of mosses to salinization. In addition, sucrose comprised the highest share in the total pool of sugars.

The damaging effect of sodium chloride in experimental conditions was assessed by the content of TBA-active products and it was found that the rate of lipid peroxidation in shoots of *D. rigidulus* and *B. unguiculata* was increasing by an average of 20–25% 24 hours after the start of plants treatment with 100 mM NaCl solution compared to the beginning of the experiment (control), and on the 7th day of salt stress, the total content of TBA-active products approached the initial values (Fig. 3).

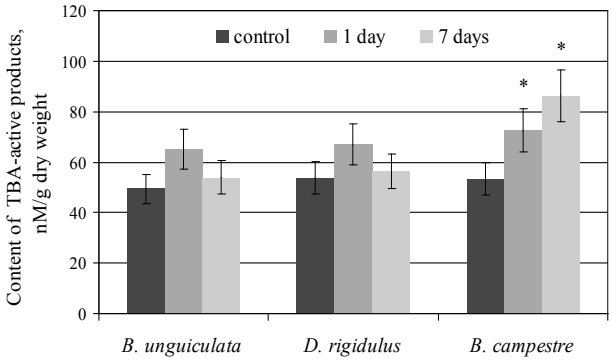

Fig. 3. The effect of 100 mM NaCl solution on the content of TBA-active products in shoots of mosses *Barbula unguiculata*, *Didymodon rigidulus* and *Brachythecium campestre*: control – without treatment of plants with NaCl solution; 1 day and 7 days – duration of plants' treatment with 100 mM NaCl solution (x ± SD, n = 12); * – the difference between the experimental sample and control for one moss species is statistically reliable at $P < 0.05$

In *B. campestre* plants with a lower level of tolerance to abiotic stress, the content of TBA-active products was gradually increasing during the experiment and reached a maximum value, compared to the control, on the 7th day of NaCl treatment. Under conditions of the experimental research on the influence of salt stress, simulated by spraying plants with 100 mM NaCl solution, it has been shown that mosses more resistant to salinization have high constitutive levels of soluble carbohydrates. For example, considering the shoots of *D. rigidulus*, their number was consistently high both at the beginning of the experiment and during prolonged exposure to salt stress (Table 2). High levels of soluble carbohydrates were also recorded in *B. unguiculata* shoots along with the exposure to salt stress (1.38–1.61 μg/g dry weight). Regarding *B. campestre*, the stress-induced nature of sugar accumulation was found because on the first day of exposure to salt shock their concentration in moss cells decreased almost twice compared to the beginning of the experiment, which indicated the use of the pool of soluble carbohydrates under conditions of the salt shock. On the other hand, on the 7th day of the experiment their significant increase was recorded, which was twice as much as it was at the start. Therefore, it was apparently caused by restructuring of carbohydrate metabolism in the direction of increasing the hydrolysis of polysaccharides (Table 2). As the result, it indicates the constitutive nature of the accumulation of soluble carbohydrates in the shoots of moss species which are resistant to osmotic stress.

Accumulation of proline in moss shoots occurred in different ways under conditions of salt stress (Table 2). For example, samples of *D. rigidulus* showed a 1.6-fold increase in the content of this osmoprotector 1 day after the start of the experiment, compared with the control, and the maximum concentration of amino acid in plants (three times higher than the control) was determined on day 7 of salt stress impact, which may indicate the stress-induced nature of the proline accumulation in the shoots of *D. rigidulus*. In contrast, there was no increase in the concentration of proline under the influence of NaCl in the plants of *B. unguiculata* and *B. campestre*, but on the contrary, there was a gradual decrease in the content of this osmoprotector from the first day of the experiment. The minimal, almost 2.5 times lower concentration of proline was detected on the 7th day of exposure to salt stress for both *B. unguiculata* and *B. campestre* (Table 2). It means that the accumulation of proline under conditions of salt stress depends on the species characteristics of mosses.

The development of oxidative stress under influence of salinity is associated with the generation of reactive oxygen species in plant cells. The antioxidant activity of moss samples was studied by comparison of the effective concentrations of plant extracts (EC_{50}), which caused 50% inhibition of the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The lower EC_{50}, the higher the antioxidant activity of plants. It was found that plants *D. rigidulus* and *B. unguiculata* in control were characterized by a high level of the antioxidant activity. Under influence of the salt stress, antioxidant activity remained at a high level, indicating that species of mosses more resistant to abiotic stressors have a significant constitutive antioxidant status (Table 2). It was found that levels of antioxidant activity in shoots of *B. campestre* were 1.6–1.8 times lower at the beginning of the experiment, which decreased slightly under short-term salt shock (1 day) and increased 1.2 times during prolonged salt stress (7 days), indicating the induced character of antioxidant activity in *B. campestre*.

Table 2

The effect of 100 mM NaCl solution on the content of soluble carbohydrates, proline and antioxidant activity in shoots of mosses *Didymodon rigidulus*, *Barbula unguiculata* and *Brachythecium campestre* (x ± SD, n = 12)

Moss species	Duration of the experiment, days	Content of soluble carbohydrates, μg/g dry weight	Content of proline, μM/g dry weight	Antioxidant activity, EC_{50}, mg/mL
D. rigidulus	control	315 ± 36	0.581 ± 0.073	1.94 ± 0.21
	1	303 ± 31	0.944 ± 0.091	1.36 ± 0.09*
	7	348 ± 35	1.873 ± 0.212*	1.42 ± 0.11
B. unguiculata	control	224 ± 25	0.635 ± 0.062	1.64 ± 0.21
	1	268 ± 31	0.536 ± 0.064	1.23 ± 0.17
	7	277 ± 30	0.261 ± 0.032*	1.45 ± 0.20
B. campestre	control	148 ± 99	0.531 ± 0.257	3.06 ± 0.17
	1	89 ± 9*	0.422 ± 0.053	3.67 ± 0.15*
	7	185 ± 25*	0.214 ± 0.023*	2.45 ± 0.09*

Note: * – the difference between the experimental sample and control for one moss species is statistically reliable at $P < 0.05$.

The content of ascorbate and reduced glutathione, as important components of the antioxidant system, and enzymes of their metabolism – ascorbate peroxidase and glutathione reductase, were studied. In all investigated moss species an increase in the amount of glutathione during exposure to salt stress was shown (Table 3). Higher concentrations of this antioxidant were found in shoots of *D. rigidulus* and *B. unguiculata* compared to *B. campestre* plants, both in control plants and plants under the influence of NaCl. On the 7th day of exposure to salt stress, the glutathione content in these plants increased 1.8–2.4 times, which indicates both a significant constitutive pool of reduced glutathione in cells of resis-

tant moss species and its effective synthesis under prolonged exposure to salt stress. The increase in the content of reduced glutathione could be conditioned by its participation in maintenance of proteins thiol groups in a functional state and in the process of reactive oxygen species detoxification.

These results correlate with the activity of glutathione reductase (GR), which catalyzes the reduction reaction of oxidized glutathione. In *D. rigidulus* and *B. unguiculata* salt stress induced a 2–3 times increase in enzyme activity. In shoots of *B. campestre* lower levels of glutathione in cells were found at the beginning of the experiment, compared to other species, and a gradual increase in its amount almost by 2.0 times on the 7th day of the experiment. GR activity increased in proportion to the increase in glutathione content, but was lower than in *D. rigidulus* and *B. unguiculata*.

The results of analysis of the ascorbic acid showed that mosses *B. unguiculata* and *D. rigidulus* have a higher ascorbate content, and short-term salt shock increased its concentration by 2.0–2.5 times, which indicates a high constitutive pool of ascorbate in more resistant mosses. Under conditions of the prolonged salt stress its amount partially decreased (Table 3).

Table 3

The effect of 100 mM NaCl solution on the content of components of the glutathione-ascorbate cycle in shoots of mosses *Didymodon rigidulus*, *Barbula unguiculata* and *Brachythecium campestre* (x ± SD, n = 12)

Moss species	Duration of the experiment, days	Content of reduced glutathione, μM $NADPH_2$/g dry weight	Glutathione reductase activity, μM $NADPH_2$/g proteins/min	Content of ascorbate, mg/g dry weight	Ascorbate peroxidase activity μM/min/g dry weight
D. rigidulus	control	48.3 ± 5.1	0.413 ± 0.054	0.671 ± 0.084	0.323 ± 0.041
	1	78.4 ± 8.9	0.892 ± 0.122*	1.546 ± 0.181*	0.452 ± 0.064
	7	121.4 ± 11.2**	1.264 ± 0.141*	0.782 ± 0.093	0.284 ± 0.041
B. unguiculata	control	64.7 ± 8.1	0.632 ± 0.074	0.910 ± 0.112	0.294 ± 0.052
	1	92.2 ± 8.5	1.205 ± 0.143*	1.924 ± 0.231*	0.401 ± 0.062
	7	119.6 ± 9.5**	1.534 ± 0.192*	1.064 ± 0.122	0.314 ± 0.041
B. campestre	control	28.4 ± 2.3	0.131 ± 0.025	0.653 ± 0.082	0.226 ± 0.032
	1	26.5 ± 2.8	0.226 ± 0.032	0.395 ± 0.052	0.162 ± 0.024
	7	52.7 ± 6.5*	0.674 ± 0.091*	0.424 ± 0.053	0.170 ± 0.032

Note: * – the difference between the experimental sample and control for one moss species is statistically reliable at $P < 0.05$; ** – $P < 0.01$.

It was shown that in *B. unguiculata* and *D. rigidulus* cells the 24-hour salt shock led to an increased activity of ascorbate peroxidase by 1.3–1.4 times compared to the control, which was obviously due to mobilization of the enzyme available in the cells. The following decrease in APO activity was clearly associated with a decrease in the amount of ascorbate, which was actively used in reactions of H_2O_2 reduction involving APO or directly interacting with ROS. Correlation analysis of the relationship between ascorbate content and ascorbate peroxidase activity in moss shoots under salt stress has shown a high correlation coefficient (0.88) between the indexes (Fig. 4).

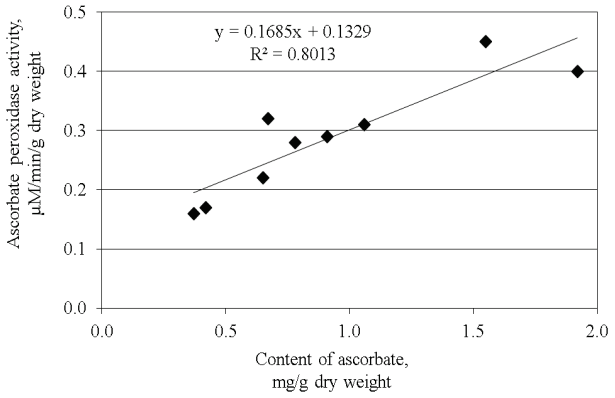

Fig. 4. Relationship between ascorbate content and ascorbate peroxidase activity in shoots of mosses *Barbula unguiculata*, *Didymodon rigidulus* and *Brachythecium campestre* under the influence of 100 mM NaCl

The less resistant plant *B. campestre* responded to salt stress by reducing the concentration of ascorbate by almost 50% from the first day of the experiment, which remained at this level during the exposure to salt stress. Ascorbate peroxidase activity depended on the concentration of substrate in cells, so a gradual decrease in its activity was found under stress conditions. In general, our studies have shown that resistant species of mosses have significantly higher levels of non-enzymatic components of the glutathione-ascorbate cycle and the activity of enzymes of their metabolism, which provided a significant reduction of the oxidative processes and rapid stabilization of lipid peroxidation, compared to the sensitive moss *B. campestre*, which was confirmed by the results of the TBA-active products analysis. Thus, in bryophytes which are resistant to abiotic stressors the constitutive adaptive mechanisms are more expressed (constitutive pool of soluble carbohydrates and non-enzymatic components of the glutathione-ascorbate cycle, high antioxidant status), and plants with a lower level of resistance adapted to the stressor mainly due to induced protective reactions.

Discussion

The ability to accumulate osmotically-active substances mostly determines plants' resistance to stress, and the most stable organisms simultaneously accumulate compounds of different types – sucrose, monosaccharides, organic acids and amino acids (Glime, 2007). Among the biochemical indicators of salt resistance, osmolytes are the most important, especially sugars (Isaenkov, 2012; Kolupaev et al., 2017). Accumulation of soluble carbohydrates provides intracellular regulation of water potential and promotes active water absorption by the plant organism, which is important under salinity conditions (Munns & Tester, 2008; Li et al., 2014; Wu et al., 2014). Sugars also have an anti-denaturation effect on the protein-lipid complex of membranes, intercept active forms of oxygen and inhibit the free radical oxidation of biological molecules in the process of oxidative stress (Kolupaev & Karpets, 2009). From the literature data it is also known that sugar accumulation occurs under conditions of water deficit. In particular, in shoots of *Syntrichia caninervis* Mitt. and *Plagiomnium acutum* (Lindb.) T. J. Cop. a significant increase in the total content of soluble carbohydrates was established in response to moisture deficit (Li et al., 2014; Wu et al., 2014). In the shoots of the desiccation-tolerant moss *Polytrichum formosum* Hedw. at the initial stages of the stress reaction a significant decrease in the concentration of starch and its rapid resynthesis during rehydration was shown, which is an important adaptation to the changing hydrothermal regime (Pressel et al., 2006).

Since one of the most important mechanisms of adaptation of mosses to osmotic stress is the augmentation of the soluble carbohydrates' concentration, which increases the osmotic potential of the cell, peculiarities of carbohydrate metabolism in mosses from the tailings' storage territory have been analyzed. Basic patterns of its direction under salinity conditions have been established. It was shown that salt stress enhances the hydrolysis of polymeric forms of carbohydrates, especially starch, and increases the activity of α-amylase. Therefore, studied mosses in salinity conditions are characterized by high concentrations of mono- and disaccharides and low starch content. It was established that there was an increase in the concentration of soluble carbohydrates by 1.2–1.5 times in moss shoots under salinity conditions, compared with plants from the background area. In plants *B. unguiculata* and *D. rigidulus* from the tailings storage area with a high degree of salinity 1.4–2.3 times higher soluble carbohydrate concentrations compared to plants of the less resistant species *B. campestre* was determined. Therefore, the results have shown that changes in the

direction of carbohydrate metabolism were similar in all studied species of mosses, which may indicate the non-specificity of protective reactions, but the reactions of plants more resistant to water deficit (*D. rigidulus* and *B. unguiculata*) were more expressed, which in natural conditions are mainly confined to localities with unstable water regime and high level of insolation. They have higher levels of soluble carbohydrates. Thus, the adaptation of bryophytes to salt stress was based on the mechanisms of resistance to moisture deficit, an important criterion of which (as well as salt resistance) is the ability to maintain the osmotic regulation and stability of the intracellular environment, which is provided by the high concentrations of osmotic protectors – soluble carbohydrates in moss cells.

Sucrose plays a key osmoprotective role in many moss species, because mosses which are resistant to water deficit are characterized by the high sucrose concentrations and low starch content (Zhu, 2002). For example, in the desiccation-tolerant moss *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr, the sucrose content in the cells during drying can reach 10% of the plants' dry weight (Nagao et al., 2005; Proctor et al., 2007; Hatanaka & Sugawara, 2010). In plants *Dicranum majus* Turner, *Hookeria lucens* (Hedw.) Sm., *Polytrichum commune* Hedw., *Racomitrium lanuginosum* (Hedw.) Brid., *Thuidium tamariscinum* (Hedw.) Schimp. and *Tortula ruraliformis* (Besch.) W. Ingham under conditions of osmotic stress sucrose concentration reached 40% of the total content of soluble carbohydrates (Smirnoff, 2005). It is known that disaccharides in moss cells stabilize phospholipid bilayers in plasma membrane by forming hydrogen bonds with polar groups. This maintains the distance between the phospholipid bilayers and prevents damage to the phase transitions (Proctor & Tuba, 2002).

Experimental studies have shown that under conditions of salinity sucrose predominates in the pool of soluble carbohydrates (59.0–79.5% of the total sugars content). In the plants *B. unguiculata* and *D. rigidulus* from the highly saline area of the tailings storage, the sucrose content was 1.5–2.0 times higher. It was found that under the influence of 100 mM NaCl solution mosses more resistant to osmotic stress have a constitutive character of soluble carbohydrates' accumulation, whereas in the shoots of *D. rigidulus* and *B. unguiculata* their amount was high both at the beginning of the experiment and during prolonged exposure to salt stress. For *B. campestre* the stress-induced character of sugars accumulation was revealed, as their significant increase by almost two times was found on the 7th day of the experiment, which, obviously, was caused by induced restructuring of carbohydrate metabolism.

The amino acid proline is a polyfunctional compound. In addition to osmolytic, proline performs other interrelated functions: membrane-protective, chaperone and antioxidant (Kovács et al., 2012; Kolupaev et al., 2014; Yan et al., 2021; Westbrook et al., 2022). There are few studies on the involvement of proline in moss stress reactions. For example, in the moss *Marshantia polymorpha* L. a significant increase of the proline content under conditions of osmotic stress was found. In the shoots of mosses *Hylocomium splendens* (Hedw.) Schimp., *Pleurozium schreberi* (Willd. ex Brid.) Mitt and *Rhytidiadelphus squarrosus* (Hedw.) Warnst. under the influence of heavy metals the level of proline accumulation differed significantly, depending on the peculiarities of the moss species and its adaptive capacity (Lobachevska, 2008). The increase of proline synthesis under the influence of drying in mosses *Hypnum plumaeforme* Wilson and *Pogonatum cirratum* (Sw.) Brid. as well as its participation in cross-adaptation to low temperatures was revealed (Liu et al., 2019). Thus, analysis of the current literature data has shown that there are significant differences in the ability to accumulate proline in different moss species in the case of the same stress influence and in the same species under various types of stress, which requires further investigation.

Proline accumulation in moss cells under salt stress depended on its species peculiarities. In *D. rigidulus* shoots, a gradual increase in amino acid content by 3.0 times was observed under the influence of salt stress, which may indicate the induced nature of proline accumulation, while in *B. unguiculata* and *B. campestre* samples the osmoprotector content was reduced by 2.5 times. Thus, stress-induced proline accumulation under salinity conditions can be considered as a part of the protective system of bryophytes, however, obviously, this amino acid does not play a key role in the formation of resistance of mosses to various osmotic stresses. Proline accumulation under stress is more characteristic for vascular plants, and for mosses soluble carbohydrates are the main osmolyte in cells. Components of the ascorbate-glutathione cycle play an important role in neutralizing reactive oxygen species and maintaining high antioxidant activity in moss cells under stress (Noctor et al., 2016; Durand et al., 2019; Kyyak et al., 2021). Ascorbic acid and glutathione directly interact with reactive oxygen species and are involved in the reduction of other low molecular weight antioxidants in non-enzymatic and enzymatic reactions (Paciolla & Tomassi, 2003; Sofo et al., 2015; Kyyak & Khorkavtsiv, 2016; Onele et al., 2018). Due to the importance of these antioxidants, many current studies are aimed at determining ascorbate and glutathione as biomarkers of the physiological state of plants under stress (Smirnoff, 2005; Kolupaev et al., 2021).

It has been shown that more resistant moss species *D. rigidulus* and *B. unguiculata* have 2.3–4.0 times higher levels of glutathione and ascorbate and 1.6–2.5 times higher activity of enzymes of their metabolism – glutathione reductase and ascorbate peroxidase, compared to plants of the species more sensitive to water deficit *B. campestre*. In all studied species of mosses a gradual decrease in the ascorbate concentration in the shoots under conditions of salt stress was found. As this occurred simultaneously with the increase of glutathione content and high glutathione reductase activity, it can be assumed that the decrease in the ascorbate amount is not associated with insufficient activity of the regeneration system of oxidized ascorbate and cannot be an indicator of the low antioxidant status of moss cells, but is rather conditioned by the active use of ascorbate in antioxidant reactions. Similar results were obtained in experiments with water stress-tolerant moss *Tortula ruraliformis* (Besch.) Ingham. In this plant drying led to a decrease in the ascorbate pool, while the concentration of total glutathione in moss cells remained unchanged. Simultaneously, the combined effect of high levels of insolation and water deficit led to the significant reduction in the content of both antioxidants, which indicated their effective use under oxidative stress (Seel et al., 1992).

Thus, constitutive protective mechanisms are more expressed in resistant moss species under salt stress, and plants with a lower level of resistance adapt to the stressor, mainly, due to induced protective systems.

Conclusions

Adaptation of bryophytes to salt stress is based on the mechanisms of resistance to water deficit, an important criterion of which (as well as salt resistance) is the ability to maintain osmotic regulation and stability of the intracellular environment provided by the accumulation of soluble carbohydrates. Sucrose predominates in the pool of soluble carbohydrates (59.0–79.5% of the total sugar content) as an important osmotic regulator and osmotic protector in moss cells.

In moss species resistant to osmotic stress *D. rigidulus* and *B. unguiculata* the constitutive mechanisms of salt resistance were more expressed (high antioxidant status and constitutive pool of soluble carbohydrates), while the moss *B. campestre* with lower level of resistance adapted to the stressor mainly due to induced protective reactions.

Under conditions of salt stress in shoots of resistant moss species 3–4 times higher content of low molecular weight antioxidants – glutathione and ascorbate – was determined, and higher activity of enzymes of their metabolism – glutathione reductase and ascorbate peroxidase – was found, compared to plants of the more sensitive species *B. campestre*, which provided suppression of the lipid peroxidation process in plasma membranes and reduction of the content of TBA-active products.

The article was prepared according to the plans of Scientific-Research Work of the Institute of Ecology of the Carpathians of the National Academy of Sciences of Ukraine "Resistance and adaptive structural and functional changes of mosses under the influence of abiotic stressors in the conditions of anthropogenically transformed environment" (state registration No. 0118U002646).

Author declares no conflict of interest.

References

Aversi-Ferreira, T., Penha-Silva, N., Fonseca, A., Brito, A., & Penha, M. (2004). Rapid determination of reducing sugars with picric acid for biotechnological use. Bioscience Journal, 20, 183–188.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205–207.
Boots, M., & Best, A. (2018). The evolution of constitutive and induced defences to infectious disease. Proceedings of the Royal Society, Biological Series, 285, 20180658.
Boychuk, M. A. (2021). Mosses (Bryophyta) of the Kostomuksha State Nature Reserve, Russia. Nature Conservation Research, 6, 89–97.
Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Food Science and Technology, 28, 25–30.
Ćosić, M., Vujićić, M., Sabovljević, M., & Sabovljević, A. (2018). What do we know about salt stress in bryophytes? Plant Biosystems, 9(4), 51–60.
DuBois, M., Hamilton, J. K., Rebers, P., & Smith, F. (2002). Calorimetric Dubois method for determination of sugar and related substances. Analytical Chemistry, 28, 350–356.
Durand, E., Zhao, Y., Ruesgas-Ramón, M., Figueroa-Espinoza, C. M., Lamy, S., Coupland, J. N., Elias, R. J., & Villeneuve, P. (2019). Evaluation of antioxidant activity and interaction with radical species using the vesicle conjugated autoxidizable triene (vesicat) assay. European Journal of Lipid Science and Technology, 121(5), 126–142.
Flowers, T. J., Munns, R., & Colmer, T. D. (2014). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Annals of Botany, 115, 419–431.
Gao, B., Li, X., Zhang, D., Liang, Y., Yang, H., Chen, M., Zhang, Y., Zhang, J., & Wood, A. J. (2017). Desiccation tolerance in bryophytes: The dehydration and rehydration transcriptomes in the desiccation-tolerant bryophyte *Bryum argenteum*. Scientific Reports, 7, 117–126.
Garbary, D. J., Miller, A. G., Scrosati, R., Kim, K., & Schofield, W. B. (2008). Distribution and salinity tolerance of intertidal mosses from Nova Scotia. The Bryologist, 111, 282–291.
Glime, G. M. (2007). Bryophyte ecology. Biological Sciences, Michigan Technological University.
González-Orenga, S., Marius-Nicusor, G., Boscaiu, M., & Vicente, O. (2021). Constitutive and induced salt tolerance mechanisms and potential uses of *Limonium* Mill. species. Agronomy, 11(3), 413–422.
Grishutkin, O. G., Boychuk, M. A., Grishutkina, G. A., & Rukavishnikova, V. V. (2020). Check-list and ecology of *Sphagnum* mosses (Sphagnaceae) in the Republic of Mordovia (Russia). Nature Conservation Research, 5(3), 114–133.
Hasanuzzaman, M., Borhannuddin Bhuyan, M. H. M., Zulfiqar, F., Raza, A., Mohsin, M. S., Mahmud, J. A., Fujita, M., & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defence regulator. Antioxidants, 9(8), 2–52.
Hatanaka, R., & Sugawara, Y. (2010). Development of desiccation tolerance and vitrification by preculture treatment in suspension-cultured cells of the liverwort *Marchantia polymorpha*. Planta, 231(4), 965–976.
He, M., He, C.-Q., & Ding, N.-Z. (2018). Abiotic stresses: General defences of land plants and chances for engineering multistress tolerance. Frontiers in Plant Sciences, 9, 1771.
Isayenkov, S. V. (2012). Physiological and molecular aspects of salt stress in plants. Cytology and Genetics, 46(5), 302–318.
Karpets, Y. V., Kolupaev, Y. E., Yastreb, T. O., & Lugova, G. A. (2017). Activity of antioxidant enzymes in leaves of barley plants of various genotypes under influence of soil drought and sodium nitroprusside. Plant Physiology and Genetics, 49(1), 71–81.
Ketehouli, T., Idrice Carther, K. F., Noman, M., Wang, F.-W., Li, X.-W., & Li, H.-Y. (2019). Adaptation of plants to salt stress: Characterization of Na^+ and K^+ transporters and role of CBL gene family in regulating salt stress response. Agronomy, 9(11), 687.
Kolupaev, Y. E., & Karpets, Y. V. (2009). Aktivnye formy kisloroda pri adaptatsii rastenii k stressovym temperaturam [Reactive oxygen species during plants' adaptation to thermal stress]. Fiziologiya i Biokhimiya Kul'turnych Rastenii, 41(2), 95–104 (in Russian).
Kolupaev, Y. E., Karpets, Y. V., & Yastreb, T. O. (2017). Funktsionirovanie antioksidantnoj sistemy rastenij pri solevom stresse [Functioning of the antioxidant system of plants under salt stress]. Bulletin of Kharkiv National Agrarian University, Series: Biology, 3, 23–45 (in Russian).
Kolupaev, Y. E., Shklyarevskiy, M. A., Karpets, Y. V., Shvidenko, N. V., & Lugovaya, A. A. (2021). AFK-zavisimoe indutsirovanie geminom antioksidantnoy sistemy i teploustoychivosti prorostkov pshenitsy [ROS-dependent hemin induction of the antioxidant system and heat tolerance in wheat seedlings]. Fiziologiya Rasteniy, 68(2), 177–186 (in Russian).
Kolupaev, Y. E., Vayner, A. A., & Yastreb, T. O. (2014). Prolin: Fiziologicheskie funktsii i regulyatsiya soderzhaniya v rasteniyakh v stressovykh usloviyakh [Proline: Physiological functions and regulation of the content in plants under stress conditions]. Bulletin of Kharkiv National Agrarian University, Series: Biology, 32, 6–22 (in Russian).
Kovács, Z., Simon-Sarkadi, L., Vashegyi, I., & Kocsy, G. (2012). Different accumulation of free amino acids during short- and long-term osmotic stress in wheat. The Scientific Word Journal, 8(3). 243–258.
Kyyak, N. Y., & Khorkavtsiv, Y. D. (2016). Otsinka okysniuvalnoho stresu mokhu *Pohlia nutans* (Hedw.) Lindb. zalezhno vid vplyvu hravitatsiyi [Estimation of the oxidative stress in moss *Pohlia nutans* (Hedw.) Lindb. depending on the influence of gravity]. Space Science and Technology, 22(4), 58–66 (in Ukrainian).
Kyyak, N. Y., Lobachevska, O. V., & Khorkavtsiv, Y. D. (2021). Morfo-fiziolohichni reaktsii hravichutlyvosti ta adaptatsiyi do UF-vyprominiuvannia mokhu *Bryum caespiticium* Hedw. iz Antarktyky [Morpho-physiological reactions of gravisensitivity and adaptation to UV irradiation of the moss *Bryum caespiticium* Hedw. from Antarctica]. Space Science and Technology, 27(5), 47–59 (in Ukrainian).
Kyyak, N. Y., Lobachevska, O. V., Rabyk, I. V., & Kyyak, V. H. (2020). Role of the bryophytes in substrate revitalization on a post-technogenic salinized territory. Biosystems Diversity, 28(4), 419–425.
Kyyak, V. H., & Kyyak, N. Y. (2019). Mechanisms of maintenance of cytoplasmic osmotic homeostasis in bryophytes cells under salinity stress. Studia Biologica, 13(2), 55–66.
Lavrenko, S. O., Lavrenko, N. M., & Lykhovyd, P. V. (2019). Effect of degree of salinity on seed germination and initial growth of chickpea (*Cicer arietinum*). Biosystems Diversity, 27(2), 101–105.
Li, J., Li, X., & Chen, C. (2014). Degradation and reorganization of thylakoid protein complexes of *Bryum argenteum* in response to dehydration and rehydration. The Bryologist, 117(2), 110–118.
Liu, B.-Y., Lei, C.-Y., Jin, J.-H., Guan, Y.-Y., Li, S., Zhang, Y.-S., & Liu, W.-Q. (2016). Physiological responses of two moss species to the combined stress of water deficit and elevated N deposition (II): Carbon and nitrogen metabolism. Ecology and Evolution, 6, 7596–7609.
Liu, W., Xu, J., Fu, W., Wang, X., Lei, C., & Chen, Y. (2019). Evidence of stress imprinting with population-level differences in two moss species. Ecology and Evolution, 9(11), 6329–6341.
Lobachevska, O. V. (2008). Vmist vilnoho prolinu ta aktyvnist antyoksydantnoho zakhystu u mokhopodibnykh za stresovykh umov [Content of free proline and antioxidant protection activity in mosses under stress conditions]. Chornomorski Botanical Journal, 4(2), 230–236 (in Ukrainian).
Lobachevska, O. V., Kyyak, N. Y., & Rabyk, I. V. (2019). Ecological and physiological peculiarities of bryophytes on a post-technogenic salinized territory. Biosystems Diversity, 27(4), 342–348.
Lobachevska, O., Kyjak, N., Khorkavtsiv, O., Dovgalyuk, A., Kit, N., Klyuchivska, O., Stoika, R., Ripetsky, R., & Cove, D. (2005). Influence of metabolic stress on the inheritance of cell determination in the moss, *Pottia intermedia*. Cell Biology International, 29(3), 181–186.
Lushchak, V. I., Bahniukova, T. V., & Lushchak, O. V. (2004). Tiobarbiturataktyvni produkty i karbonilni hrupy bilkiv [Thiobarbituric acid-active products and carbonyl groups of proteins]. The Ukrainian Biochemical Journal, 76(3), 136–141 (in Ukrainian).
MacGregor, A. W., MacDougall, F. H., Mayer, C., & Daussant, J. (1984). Changes in levels of a-amylase components in barley tissues during germination and early seedling growth. Plant Physiology, 75, 203–206.
Mehta, N., Patani, P., & Singhvi, I. (2018). Colorimetric estimation of ascorbic acid from different varities of tomatoes cultivated in Gujarat. World Journal of Pharmaceutical Research, 7(4), 1376–1384.
Monsigny, M., Petit, C., & Roche, A. C. (1988). Colorimetric determination of neutral sugars by a resorcinol sulfuric acid micromethod. Analytical Biochemistry, 175, 525–530.
Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651–681.
Nagao, M., Minami, A., Arakawa, K., Fujikawa, S., & Takezawa, D. (2005). Rapid degradation of starch in chloroplasts and concomitant accumulation of soluble sugars associated with ABA-induced freezing tolerance in the moss *Physcomitrella patens*. Journal of Plant Physiology, 162(2), 169–180.
Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, 22(5), 867–880.
Noctor, G., Mhamdi, A., & Foyer, C. H. (2016). Oxidative stress and antioxidative systems: Recipes for successful data collection and interpretation. Plant, Cell and Environment, 39, 1140–1160.
Oke, T. A., Turetsky, M. R., Weston, D. J., & Shaw, J. A. (2020). Tradeoffs between phenotypic plasticity and local adaptation influence the ecophysiology of the moss, *Sphagnum magellanicum*. Oecologia, 193(4), 867–877.
Onele, A. O., Chasov, A., Viktorova, L., Beckettc, R. P., Trifonova, T., & Minibayeva, F. (2018). Biochemical characterization of peroxidases from the moss *Dicranum scoparium*. South African Journal of Botany, 119, 132–141.
Paciolla, C., & Tomassi, F. (2003/2004). The ascorbate system in two bryophytes: *Brachytecium velutinum* and *Marchantia polymorpha*. Biologia Plantarum, 47(3), 387–393.
Pouliot, R., Rochefort, L., & Graf, M. (2012). Impacts of oil sands process water on fen plants: Implication for plant selection in required reclamation projects. Environmental Pollution, 167, 132–137.

Pressel, S., Ligrone, R., & Duckett, J. (2006). Effects of de- and rehydration on food-conducting cells in the moss *Polytrichum formosum*: A cytological study. Annals of Botany, 98, 67–76.
Proctor, M. C. F., & Tuba, Z. (2002). Poikilohydry and homiohydry: Antithesis or spectrum of possibilities? New Phytologist, 156, 327–349.
Proctor, M. C. F., Oliver, M. J., Wood, A. J., Alpert, P. R., Stark, L., Cleavitt, N. L., & Mishler, B. D. (2007). Desiccations-tolerance in bryophytes: A review. Bryologist, 110(4), 595–621.
Rabyk, I. V., Lobachevska, O. V., Kyyak, N. Y., & Shcherbachenko, O. I. (2018). Bryophytes on the devastated territories of sulphur deposits and their role in restoration of dump substrate. Biosystems Diversity, 26(4), 339–353.
Sabovljević, M. S., & Sabovljević, A. D. (2020). Bryophytes. IntechOpen, London.
Sabovljević, M., & Sabovljević, A. (2007). Contribution to the coastal bryophytes of the Northern Mediterranean: Are there halophytes among bryophytes? Phytologia Balcanica, 13(2), 131–135.
Sadasivam, S., & Manickam, A. (2007). Biochemical methods. New Age International, New Delhi.
Seel, W. E., Hendry, G. A. F., & Lee, J. A. (1992). The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. The Journal of Experimental Botany, 43, 1023–1030.
Smirnoff, N. (2005). Antioxidants and reactive oxygen species in plants. Blackwell Publishing Ltd., Oxford.
Smith, I. K., Vierheller, T. L., & Thorne, C. A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Analytical Biochemistry, 175, 408–413.
Sofo, A., Scopa, A., Nuzzaci, M., & Vitti, A. (2015). Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. International Journal of Molecular Sciences, 16, 13561–13578.
Stark, L. R. (2017). Ecology of desiccation tolerance in bryophytes: A conceptual framework and methodology. The Bryologist, 120(2), 129–164.
Venegas-Molina, J., Proietti, S., Pollier, J. Orozco-Freire, W., Ramirez-Villacis, D., & Leon-Reyes, A. (2020). Induced tolerance to abiotic and biotic stresses of broccoli and *Arabidopsis* after treatment with elicitor molecules. Scientific Reports, 10, 10319.
Wang, X., Liu, Z., & He, Y. (2008). Responses and tolerance to salt stress in bryophytes. Plant Signaling and Behavior, 3(8), 516–518.
Westbrook, R. L., Bridges, E., Roberts, J., Escribano-Gonzalez, C., Eales, K. L., Vettore, L. A., Walker, P. D., Vera-Siguenza, E., Rana, H., Cuozzo, F., Eskla, K.-L., Vellama, H., Shaaban, A., Nixon, C., Luuk, H., Lavery, G. G., Hodson, D. J., Harris, A. L., & Tennant, D. A. (2022). Proline synthesis through PYCR1 is required to support cancer cell proliferation and survival in oxygen-limiting conditions. Cell Reports, 38(5), 110320.
Wu, N., Zhang, Y. M., Downing, A., Zachary, T. Aanderud, C., Ye Tao, A., & Williams, S. (2014). Rapid adjustment of leaf angle explains how the desert moss, *Syntrichia caninervis*, copes with multiple resource limitations during rehydration. Functional Plant Biology, 41, 168–177.
Yan, L., Li, S., Riaz, M., & Jiang, C. (2021). Proline metabolism and biosynthesis behave differently in response to boron-deficiency and toxicity in *Brassica napus*. Plant Physiology and Biochemistry, 167, 529–540.
Yenne, S. P., & Hatzios, K. (1990). Influence of oxime ether on glutathione content and glutathione-related enzyme activity in seeds and seedlings of grain sorghum. Zeitschrift für Naturforschung, 45, 96–106.
Zhu, J.-K. (2002). Salt and drought stress signal transduction in plants. Annual Review of Plant Biology, 53, 247–273.