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Research Article

Biochemical Specifics of Seedlings Adaptation to Short-Term Carbonate Salinization

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2. Keywords

Seedlings; Carbonate salinization; Adaptation; Stress indicators; Antioxidant system; Principal component analysis; Cluster analysis

1. Abstract

The paper presents the results of a study on the effect of short-term carbonate (120 mM) salinity on triticale seedlings (*Triticosecale*). The alterations in the accumulation of hydrogen peroxide, Malonic Dialdehyde (MDA), the activity of catalase, ascorbate peroxidase and guaiacol peroxidase, as well as the content of ascorbic acid and glutathione were studied. It is established that the development of oxidative stress in shoots and roots occurred in different ways. The results obtained and their analysis with the Principal Component Analysis (PCA) method and cluster analysis allowed us to discover new relationships between the studied biochemical characteristics, which could not be detected by determining the correlation coefficients between them. At the same time, the specificity of interrelations between the studied characteristics for shoots and roots was found. As markers of stress in carbonate salinity, catalase activity can be distinguished for shoots and roots and glutathione content for roots. This may reflect the specificity of the adaptation of triticale seedlings to this type of salinization. Therefore, the biochemical strategy for the adaptation of triticale seedlings under such conditions may include maintaining a relatively stable (least varying) concentration of glutathione in the roots and catalase activity (both in the roots and shoots).

3. Introduction

Saline soils are wide spread in many countries of the world. They occupy about 25% of the land surface, including half of all irrigated lands, and the areas of saline areas gradually increase [1]. It occurs in the conditions of arid climate with close bedding of hard groundwater, when calcareous, dolomitized carbonate accumulations occur in the soil profile, causing soil compaction. Today the carbonate salinization is the most poorly studied type of salinization, in contrast to chloride and sulphate. Some authors [2,3] showed that carbonate ion has a stronger effect on plants than chloride and sulphate. This can be explained by the fact that upon the hydrolysis of carbonates, even with weak acidification of the medium, they are converted into carbonic acid, with subsequent release of CO_2, which ultimately leads to a significant change in pH of the medium in the alkaline direction. Increased pH of the solution is an additional stress factor affecting the development of plants.

Oxidative stress induced by carbonate salinization activates the generation of Reactive Oxygen Species (ROS) in plants [4,5]. All these forms are highly reactive and cause disruption of cell metabolism as a result of lipid peroxidation of cell membranes, as well as damage to proteins and nucleic acids. An increase in ROS synthesis in plants can be caused by osmotic stress during salinization, which also causes the development of water deficiency and inhibition of the most important physiological processes: photosynthesis and respiration. This may occur due to the toxic effects of an excess of inorganic ions for cellular metabolism [2, 4].

One of the agricultural crops of great practical importance is triticale, a hybrid of rye and wheat. This culture is capable of surpassing both parents in many agricultural areas of Russia and some European countries, and by resistance to adverse soil and climatic conditions and to the most dangerous diseases, triticale is superior to wheat and is not inferior to rye [6]. Of particular interest is the study of plant responses in the early stages of development, since today this issue has been studied very little, especially in conditions of carbonate salinity [5].

Numerous early studies on the effect of various types of salinization of the environment on plant growth and development have

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made it possible to accumulate significant experimental material [4,7]. However, a statement of the facts of the negative impact of salinization often does not allow detecting statistically substantiated relationships between indicators, except for correlation coefficients, which may not reflect at all the true connections (including cause-effect ones) between the studied indicators. At the same time, researchers, as a rule, only state the presence of many alterations in biochemical and physiological properties of plants without attempts to reach a more general level of generalization, based on statistical approaches [8-16].

On the other hand, the study of salt tolerance genes showed that their number exceeds several tens [17]. Moreover, the regulation of the activity of such genes can be so complicated that it may be impossible to select the main (key) genes from them.

Overcoming this state and further developing this area of research can be achieved through the application of more complex and specific methods of statistical analysis, which have found applications in other areas of science and would have made progress in understanding the negative impact of salinity on plants and the detection of the specificity of such an action.

Between such methods, for example, we can applied the Principal Component method (PCA) and cluster analysis, which make it possible to detect closer relationships between individual physiological and biochemical characteristics. This allows you to take a fresh look at the state of plants in the new environment or experiment and progress in understanding the effects of stress, as well as the specifics of such an effect [18]. It should be noted that such attempts to assess the results of the research in the described direction were carried out very little and in different aspects [19, 20]. At the same time, it was possible to discover some features of the relationship between the physiological and biochemical parameters of plants, which are important for understanding the plant resistance in different environmental conditions. Such an analysis makes it possible to discover new relationships that cannot be obtained either with the application of correlation coefficients or with the analysis of knowledge about known biochemical pathways of metabolism.

Therefore, the aim of the work was to study a number of biochemical stress indicators for triticale plants in the early stages of onto genesis under stress conditions caused by the presence of an excess of carbonate ions in the medium with further analysis of the results with statistical methods: Principal Component Analysis (PCA) and cluster analysis.

4. Materials and Methods

The object of the study was the seedlings of winter triticale (*xTriticosecale*) variety Tribune. Seeds were sterilized in a 2.5% solution of potassium permanganate, washed with running water and three times with distilled water. Plants were grown on a Knop nutrient medium with the addition of trace elements according to Hoagland at a 12-hour light day, an air temperature of 23/20° C (day/night) and a humidity of 65%. Illumination was 1900 lux.

Upon reaching the tillering stage, the nutrient medium was replaced with a similar one with the addition of sodium carbonate at a concentration of 120 mM. Samples were taken for analysis after 12, 24, 48, 72 and 96 hours of exposure [21].

The determination of the hydrogen peroxide content was carried out according to the method of [22], based on the formation of a complex of titanium peroxide. Spectrophotometric measurements were performed at 415 nm. The content of hydrogen peroxide was calculated by the value of the absorption of samples of the calibration dependence, built for standard concentrations of H_2O_2 (from 0.1 mm to 1 mm) in 100% acetone.

The intensity of lipid peroxidation was determined by the accumulation of the oxidation Product – Malonic Dialdehyde (MDA) according to the method of [23]. To do this, the plant material was homogenized in a medium of 10% TCA, centrifuged and reacted with 0.5% thiobarbituric acid. The optical density of the final solution was determined at a wavelength of 532 nm.

The activity of ascorbate peroxidase was determined as described previously [24]. The reaction mixture contained 50 mM K/Na-phosphate buffer (pH 7.8), as well as 1 mM EDTA and 1 μM H_2O_2. The enzyme activity was determined by the kinetics of consumption of ascorbate, registering changes in optical density at 290 nm.

The activity of guaiacol peroxidase was also determined by the spectrophotometric method, based on the rate of pyrogallol oxidation. The absorption was measured at a wavelength of 430 nm. For the calculations, the millimolar extinction coefficient of purpurogallin formed during the reaction, equal to 2.47, was used.

Catalase activity was determined spectro photometrically with a modified method [25]. The reaction mixture contained: K, Na-phosphate buffer (pH 7.0), extract. The reaction was started by adding 0.6 M hydrogen peroxide to the reaction mixture. The control cuvette contained the same reagents, but hydrogen peroxide was not added. Catalase activity was determined by the change in the optical density of the reaction mixture at 240 nm.

The activity of all enzymes was expressed in μmol of the transformed substrate per 1 mg of protein per 1 minute.

Determination of glutathione content was carried out according to the method, based on its ability to restore free iodine [26].

The determination of the content of ascorbic acid was performed by the method of titration with a 0.001 N solution of 2,6-dichlorophenolindophenol (2,6-DCPIF) [27].

The content of glutathione and ascorbic acid was expressed in mg per 1g wet weight.

The experiments were carried out in four biological replicates. The results of the experiments are statistically processed. ANOVA at 5% level of significance ($p \geq 0.5$) and the separation of averages worth less significant difference LSD. The tables show the arithmetic mean values. A pairwise comparison of indicators was performed with the Duncan criterion ($P<0.05$). On the basis of such data, in the tables, different letters indicate the values statistically different from each other. For the processing of the results, the Principal Component Analysis (PCA) method and cluster analysis were also applied.

5. Results

The study of the content of the main markers of oxidative stress – hydrogen peroxide and Malonic Dialdehyde (MDA) showed that with a 12-hour exposure, more than three fold increase in the concentration of hydrogen peroxide occurred in the shoots (Table 1).

Then there was a decrease in the concentration of this ROS, but even by the end of the experiment, it was twice the initial value. In the roots, by 12 o'clock in the experiment, there was also an increase in the concentration of this indicator, but to a lesser extent – by 1.5 times. Further it was also observed the maintenance of elevated levels of hydrogen peroxide in the roots.

The content of another marker of oxidative stress – Malonic Dialdehyde (MDA) in the shoots also increased during the first day of the experiment (Table 1). But then its content decreased sharply and was lower than the initial values until the end of the experiment. In the roots, during the first 3 days, a two-fold increase in the concentration of MDA was observed, and then its concentration altered.

Under carbonate salinization conditions, the peak of ascorbate peroxidase activity in shoots occurred on the first day of the experiment, after which it sharply decreased by 48 hours (Table 2). Further, this indicator increased again and remained close to the initial value. In the roots, the maximum value, twice the initial one, was already reached by 12 hours of the experiment. After that,

Table 1: Dynamics of accumulation of hydrogen peroxide and MDA in shoots and roots of triticale in the presence of 120 mM Na_2CO_3 in the medium (All values are means, the letters indicate the values statistically different from each other, Duncan, $P<0.050$)

Exposure time, hours	Content of hydrogen peroxide, µmol / g wet weight		Content of MDA, µmol / g wet weight	
	Shoots	Roots	Shoots	Roots
0	5,8 d	5,2 e	0,077 b	0,013 c
12	17,8 a	7,8 a b	0,123 a	0,020 b
24	18,0 a	6,5 d	0,116 a	0,026 a
48	15,1 b	7,6 b c	0,052 c	0,019 b
72	14,2 b	7,4 c	0,045 c,d	0,026 a
96	12,6 c	8,1 a	0,039 d	0,019 b

Table 2: Dynamics of enzyme activity in shoots and roots of the triticale at 120 mM Na_2CO_3 salinization (All values are means, the letters indicate the values statistically different from each other, Duncan, $P<0.050$)

Exposure time, hours	APO activity, µmol / g wet weight * min		Catalase activity, µmol / g wet weight * min		Activity of guaiacol peroxidase, µmol / g wet weight * min	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
0	0,36 c	0,15 c	67,2 d	17,2 c	10,0 a	3,5 d
12	0,41 b	0,29 a	74,4 c	18,4 b	4,5 c	3,0 d
24	0,51 a	0,24 b	89,1 b	19,5 a,b	9,0 a	10,7 b
48	0,21 e	0,16 c	96,7 a	20,1 a	5,2 c	6,9 c
72	0,32 d	0,15 c	71,2 c,d	10,2 d	7,8 b	12,8 a
96	0,32 d	0,11 d	86,5 b	9,4 e	7,6 b	10,6 b

Table 3: Dynamics of the content of ascorbic acid and glutathione in the shoots and roots of triticale in the presence of 120 mM Na_2CO_3 in the medium (All values are means, the letters indicate the values statistically different from each other, Duncan, $P<0.050$)

Exposure time, hours	Content of ascorbic acid, mg / g wet weight		Content of glutathione content, mg / g wet weight	
	Shoots	Roots	Shoots	Roots
0	0,73 b	0,12 b	0,33 c	0,45 b
12	0,75 a,b	0,09 c	1,08 a	0,36 c
24	0,64 c	0,10 c	0,69 b	0,45 b
48	0,82 a	0,16 a	0,08 e	0,55 a
72	0,32 d	0,11 b,c	0,37 c	0,39 b,c
96	0,22 e	0,11 b,c	0,24 d	0,16 d

a gradual decrease in the activity of the enzyme to values close to the initial was observed.

Catalase activity in shoots increased with a peak of activity at 48 hours exposure of seedlings under saline conditions (Table 2). Then there was a slight decrease in the activity of the enzyme. In the roots, a slight increase in the activity of the enzyme was noted by 48 hours of exposure, after which it decreased to values below the initial values.

The alteration in the activity of guaiacol peroxidase under the conditions of carbonate salinization occurred more sharply in both shoots and roots (Table 2). At the same time, we observed values both above and below the initial values.

The study of the content of ascorbic acid showed that its content in the shoots significantly decreased after 48 hours of exposure (Table 3).

Such dramatic changes in this indicator was not observed for the roots.

The content of reduced glutathione in shoots varied more significantly (Table 3). The maximum content was noted by 12 hours of the experiment, and with further exposure, this indicator decreased to 48 hours of the experiment. In the roots, on the contrary, by this time the maximum content of glutathione was noted, which in further decreased.

The investigation of obtained results with statistical methods show next picture.

5.1. Shoots

The application of the principal component analysis method for processing the experimental results obtained under carbonate salinization conditions for winter triticale shoots showed the picture presented in (Figure 1a).

The projection of the data on the plane of the two main components revealed that the greatest contribution to the total dispersion of the first main component was made by the content of glutathione (0.99), MDA (0.89), hydrogen peroxide (0.83) and ascorbate peroxidase activity (0.94) . Ascorbic acid (0.79) and catalase activity (0.55) made the largest contribution to the total dispersion of the second main component.

The highest correlation coefficients are observed between the content of hydrogen peroxide and glutathione (0.85), hydrogen peroxide and the activity of ascorbate peroxidase (0.79), the content of MDA and glutathione (0.89), MDA and ascorbate peroxidase

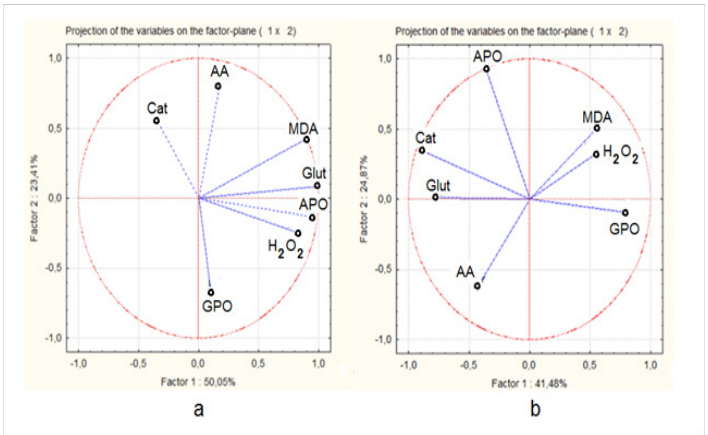

Figure 1: Projection of data on changes in the content of hydrogen peroxide, MDA, ascorbic acid (AA), glutathione (Glut), catalase activity (Cat), ascorbate peroxidase (APO) and guaiacol peroxidase (GPO) on the plane of the two principal components (a – shoots, b – roots)

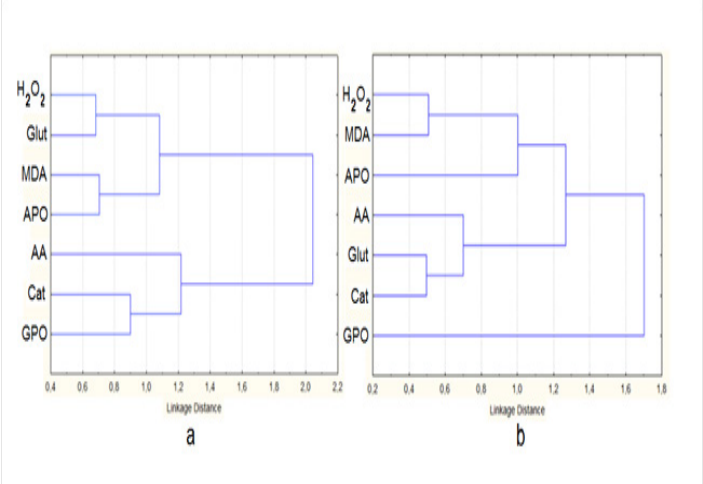

Figure 2: Dendrograms of the studied stress indicators and the antioxidant system of triticale seedlings under conditions of high concentration of sodium carbonate in the medium (a – shoots, b – roots)

(0.80), glutathione and ascorbate peroxidase (0.89).

To discover possible hierarchical relationships between the studied indicators, the cluster analysis method was applied. (Figure 2a) shows that the closest relationships were found between the content of hydrogen peroxide and glutathione, the content of malonic dialdehyde and the activity of ascorbate peroxidase, the activity of catalase and guaiacol peroxidase.

5.2. Roots

The application of the principal component analysis method to assess the characteristics of oxidative stress and the state of the antioxidant system in triticale roots with an excessive content of carbonate ions in the medium gave a picture that was completely different than for the shoots (Figure 1b). For the roots, the largest contribution to the dispersion of the first principal component

was made by guaiacol peroxidase activity (0.78), MDA content (0.55) and hydrogen peroxide (0.54). The dispersion of the second principal component was maximally affected by the activity of ascorbate peroxidase (0.93) and the value of MDA (0.50).

High correlation coefficients were found only between indicators such as MDA content and guaiacol peroxidase activity (0.78), glutathione content and catalase activity (0.77), catalase and ascorbate peroxidase activities (0.62).

The application of the cluster analysis method to identify the hierarchical coordination of triticale root indicators under carbonate salinization conditions gave a dendrogram of the following type (Figure 2b). Based on the picture obtained, it can be noted that the closest relationships are found between the content of hydrogen peroxide and the product of lipid peroxidation – MDA, glutathione and catalase activity.

6. Discussion

Comparison of the obtained results with the data on the study of chloride salinity [20,7] suggests that carbonate salinization is more affected in the content of hydrogen peroxide in the shoots, where more intensive accumulation of this metabolite occurs than in the roots (Table 1). At the same time, sulfate salinization, on the contrary, led to more intensive accumulation of hydrogen peroxide in the roots [2,21]. These results indicate the specificity of the formation of hydrogen peroxide under conditions of various types salinization of medium.

The intensity of lipid peroxidation in triticale tissues was assessed by the content of malonic dialdehyde (Table 1). During the first two days of the experiment, a two-fold increase in the concentration of MDA was noted in the roots and shoots of triticale, which can be explained by an increase in the concentration of hydrogen peroxide during this period, and as a result, damage to plant cell membranes. Similar results for carbonate and bicarbonate types of salinization were obtained by other authors [3,5]. This picture is similar to the results obtained under conditions of NaCl-salinization [28,20,7]. Based on the results, it is possible to speak about the similarity of the accumulation of MDA at carbonate and chloride salinization.

The activity of ascorbate peroxidase in shoots and roots of triticale increased during the first days of salinization (Table 2). By 48 hours of the experiment, a sharp decrease (below the initial) of the enzyme activity was noted, followed by its increase. Under the conditions of chloride salinity [20,7], a similar picture was observed in triticale shoots, as well as with an excessive concentration of sulfate ions in the medium [21]. Other authors noted the inhibition of the activity of ascorbate peroxidase, but with a significantly longer – 30-day exposure to stress factors [29].

The dynamics of catalase activity (Table 2) was similar to the pictures shown by other authors under chloride and sulfate types of stress [30,31,32]. At the same time, under conditions of sulfate salinization, the maximum activity of catalase was observed at a shorter exposure – 12 hours of the experiment [21]. An increase in catalase activity has also been described for *Morus alba* L. plants under stress caused by the application of sodium carbonate [5].

Under conditions of carbonate salinization, the activity of guaiacol peroxidase (Table 2) significantly increased in the roots, while it sharply changed in the shoots. Two peaks of enzyme activity were noted in the roots: at 24 and 72 hours of exposure. At the point of 24 hours, there is also a peak in the activity of guaiacol peroxidase in the shoots. The picture obtained differs from the results obtained earlier for the chloride and sulfate types of salinization [10, 7]. Thus, under sodium chloride stress in triticale seedlings, a different picture was observed, namely, the enzyme activity decreased in the shoots and in the roots as early as 12 hours of the experiment [7]. It is possible that this picture is related to the specifics of stress caused by carbonate salinization.

The content of non-enzymatic components of the antioxidant protection of triticale seedlings altered during the experiment as follows: for 12 hours, the content of ascorbic acid remained at the same level, and by 24 hours a decrease in this indicator was observed (Table 3). A similar pattern was noted for chloride salinization [20, 7]. The change in the content of reduced glutathione in the shoots (Table 3) also turned out to be similar to the data obtained for chloride salinity [7]. A decrease in the content of reduced glutathione was found in cucumber plants (*Cucumis sativus* L.) with a 10-day exposure to sodium chloride [33].

Thus, the obtained results are somewhat similar to the response of triticale seedlings with other types of salinization of the medium [29, 20, 7]. The specifics of carbonate salinization include: (1) more intense accumulation of hydrogen peroxide in shoots than in roots, (2) a decrease in MDA content in triticale shoots by 48 hours of exposure, (3) an earlier manifestation of the maximum activity of ascorbate peroxidase – after 12 hours of the experiment, (4) the peak of catalase activity was at 48 hours versus 12 hours at sulfate salinization, (5) the activity of guaiacol peroxidase was characterized by significant differences in the roots and shoots, (6) the content of ascorbic acid in shoots decreased by the end of the experiment.

The application of statistical methods (PCA an cluster analysis) allow us to detect a specifics of relations between studied biochemical indicators.

6.1. Shoots

PCA method allow us showed that catalase was a part of different groups of studied indicators (interpretation of Figure 1 data). It probably shows its key role in plant adaptation, for example, to salt stress [14].

The obtained high correlation coefficient between ascorbate peroxidase and hydrogen peroxide is agreed with the concepts of biochemistry. Or, the value of MDA in this aspect may to some extent depend on the activity of ascorbate peroxidase, the family of which is characterized by both cytosolic localization and association with membranes [34]. The components of the other pairs have no direct connections. Therefore, it should be remembered that such correlations may not at all show direct causal relations between biochemical characteristics.

The investigation of hierarchical relationships between the studied indicators with cluster analysis show that the physiological and biochemical interpretation of such results is not always obvious. Thus, the close relationship between catalase activity and guaiacol peroxidase can be explained by the fact that both enzymes use hydrogen peroxide as a substrate in their respective reactions. At the same time, the correlation coefficient between these characteristics was negative (-0.40), which, apparently, may reflect their competitive interactions with respect to the indicated substrate of the reaction, or the features of functioning depending on the concentration of peroxide in the medium (due to different affinity to this substrate).

The relationship between MDA and ascorbate peroxidase activity can be explained by the role of this enzyme in membrane protection. In favor of such an interpretation, one can cite data on the existence of four forms of this enzyme, include – membrane-bound, and one of them is located in the cytosol, and the rest – in chloroplasts, glyoxisomes, peroxisomes [34].

At first glance, it is difficult to explain the close relationship between the content of hydrogen peroxide and glutathione in this aspect, apart from the chemically obvious its (H_2O_2) potential possibility for oxidation of the sulfhydryl group of reduced glutathione, although the rate of this reaction is small [35]. At the same time, in paper [36] it was shown that the interaction of these substances (as positive and negative controllers of the redox state of the system) can provide signal transmission(s) to enable adaptive reactions in the Arabidopsis plants (*Arabidopsis thaliana*).

The close connection (the formation of a cluster of the first-order) between guaiacol peroxidase and catalase can be explained by the use of the same substrate in both reactions catalyzed by these enzymes.

The formation of second-order clusters can be explained by the well-known relationship between hydrogen peroxide content, MDA value and ascobrate peroxidase activity, and the role of glutathione in this group appears to be determined by the control of the redox system state [36]. The inclusion of ascorbic acid in the cluster of the second order with the activity of catalase and guaiacol peroxidase from a biochemical point of view remains unclear.

The cluster of the third order reflects the significance of all these indicators to save the life of triticale shoots under the experimental conditions.

6.2. Roots

The application of PCA method allow us that one indicator can be included in different groups, which does not bring clarity to the picture of the relationship between the studied biochemical parameters (Figure 1b). Moreover, it turns out to be more complex than for shoots (Figure 1a). At the same time, a distinctive feature of the response of the root system of triticale seedlings is that a positive contribution to the dispersion of the first principal component was made by indicators such as hydrogen peroxide content, MDA, and guaiacol peroxidase activity, while all other studied indicators made a negative contribution (Figure 1b). That is, we see here the differences in the reactions of shoots and roots to the action of carbonate stress.

The application of the cluster analysis method to identify the hierarchical coordination of triticale root indicators under carbonate salinization conditions showed that the closest relationships are found between the content of hydrogen peroxide and the product of lipid peroxidation – MDA, which corresponds to physiological and biochemical concepts in this field of science (Figure 2b).

However, the close relationship between the content of glutathione and catalase activity remains unclear. Nevertheless, it was shown that the application of a number of herbicides – catalase inhibitors led to an increase in the content of glutathione in the leaves of barley (*Hordeum vulgare* L.), tobacco (*Nicotiana tabacum* L.), soybean (*Glycine max* [L.] Merr.) And corn (*Zea mays* L.) [37]. It can be assumed that an excess amount of hydrogen peroxide

provides signal reactions to enhance the synthesis of glutathione in triticale roots under experimental conditions.

The inclusion of ascorbic acid in the cluster of the second order with these characteristics may reflect the well-known ideas about the ascorbate-glutathione cycle, whose work may be physiologically more important in triticale roots under this type of stress.

The inclusion of ascorbate peroxidase with a primary cluster between hydrogen peroxide and MDA in the second-order cluster may reflect the significance of this enzyme activity for neutralization of hydrogen peroxide in membrane protection. Above it was noted the presence of at least four different forms of this enzyme in plant cells, including forms associated with membrane structures [34], which fully explains the observed picture.

The inclusion of guaiacol peroxidase in the common cluster at the final stage reflects the least significant value of this enzyme in the defense reactions of triticale roots under carbonate salinization conditions. This seems strange in view of the fact that, for example, two forms of the enzyme associated with plasma membranes were found in the roots of maize cells [38]. At the same time, it is known that flavonoids (as potential substrates for this enzyme) are synthesized, firstly, in the leaves and, secondly, in the later stages of plant development, which apparently reflects the situation under consideration.

7. Conclusion

Considering the results of the study in general, it can be noted that the traditional analysis of alterations in the biochemical parameters of triticale seedlings in adaptation to the conditions of short-term salinity gives information about alterations in the intensity of hydrogen peroxide accumulation, MDA content, low-molecular antioxidants and activity of the enzymes studied. However, such information does not contribute to advancement in the assessment of the specifics of responses and relationships between the studied parameters in the experimental conditions.

The application of the principal component analysis method made it possible to divide the studied indicators into separate groups, despite the fact that their substantive part may overlap. At the same time, the picture for shoots turned out to be more definite (clear) than for roots. Thus, the division of the studied biochemical characteristics into groups depending on their contribution to the dispersion of the principal components of the system turned out to be more stringent for the shoots than for the roots. At the same time, the only negative contribution of catalase to the dispersion of the first principal component in comparison with other indicators for shoots (some what unexpectedly) found agreement with the notion that catalase probably plays a key role in plant adaptation to salt stress [14]. A similar largest negative contribution to the dispersion of the first principal component is also shown for root catalase (-0.88), although a similar but slightly smaller contribution of glutathione (-0.77) was observed here.

Determination of the correlation coefficients between the studied characteristics showed their high values for both shoots and roots. At the same time for the shoots, they were more numerous and had higher values. It was noted that the high values of the correlation coefficients between the individual indicators do not reflect direct cause-effect relationships (and do not have biochemical rationales) between them. Among the interesting features, it is possible to note the high correlation coefficient between catalase and glutathione (0.77), which, in particular, has no direct biochemical explanation.

The application of cluster analysis method to evaluate the results of experiments showed that if the primary cluster between hydrogen peroxide and MDA is biochemically justified for root indicators, it is difficult to explain the primary cluster between hydrogen peroxide and glutathione for shoots, despite the known evidence that the use of a number of herbicides - inhibitors catalases led to an increase in the content of glutathione in the leaves of some plants [37]. Similarly, it is difficult to find an explanation in terms of biochemistry and plant physiology for the formation of a primary cluster between catalase and glutathione for roots. Also note worthy is the inclusion of ascorbic acid in secondary clusters for shoots (association with catalase and guaiacol peroxidase) and for roots (with catalase and glutathione).

Thus, the results obtained and their analysis with the Principal Component Analysis (PCA) method and cluster analysis allowed us to discover new relationships between the studied biochemical characteristics, which could not be detected either by determining the correlation coefficients between them, or by explaining with the known relationships between biochemical processes and reactions. At the same time, the specificity of interrelations between the studied characteristics for shoots and roots was found. As markers of stress in carbonate salinity, catalase activity can be distinguished for shoots and roots and glutathione content for roots. This may reflect the specificity of the adaptation of triticale seedlings to this type of salinization. Therefore, the biochemical strategy for the adaptation of triticale seedlings under such conditions may include maintaining a relatively stable (least varying) concentration of glutathione in the roots and catalase activity (both in the roots and shoots).

In conclusion, PCA method allow us to form four groups of studied characteristics.

Another characteristic of specifics of adaptive reactions to carbonate salinization for shoots and roots include a positive correlation coefficients between different parameters. So, for shoots they were obtained for the content of hydrogen peroxide and glutathione (0.85), hydrogen peroxide and the activity of ascorbate peroxidase (0.79), the content of MDA and glutathione (0.89), MDA and ascorbate peroxidase (0.80), glutathione and ascorbate peroxidase (0.89). For roots we have observed a positive correlations between MDA content and guaiacol peroxidase activity (0.78), glutathione content and catalase activity (0.77), catalase and ascorbate peroxidase activities (0.62).

The third characteristic of specifics of adaptive reactions to carbonate salinization for shoots and roots include the results of cluster analysis method application to the obtained data. It was established that hierarchical relationships between the studied indicators were different for shoots and roots. The first-order clusters were formed between the content of hydrogen peroxide and glutathione, the content of malonic dialdehyde and the activity of ascorbate peroxidase, the activity of catalase and guaiacol peroxidase for shoot characteristics. However for the same root characteristics the first-order clusters were formed between the content of hydrogen peroxide and malonic dialdehyde, glutathione and catalase activity followed by another picture with other-order clusters formation.

Thus, the application of the principal component analysis method and cluster analysis to the results of studying the biochemical indicators of stress development in triticale seedlings under the short-term effect of sodium carbonate allowed us to discover new relationships between the indicators, which gives grounds for detecting the specific relationships between them and clarifying the biochemical strategy of seedling adaptation to the studied conditions an experiment.

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