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Research Article

Effects of Salt Stress of the Respiratory Components of Some Plants

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Abstract: Oxygen absorption, kinetic regularities of free radical oxidation and anti-oxidation activity were studied in roots and isolated mitochondria for various groups of plants under salt stress of NaCl, Na_2SO_4 and Na_2CO_3.

The results show that spearation of oxidation and phosphorylation obviously stimulates the oxygen absorption by plants under salt strees. Therefore, the anti-oxidation activity of roots decreases abruptly, resulting in an uncontrolled acceleration of free radical processes. The obtained data is a definite contribution to the concept concerning the toxic effect of salt on plants.

Key Words: Salt stress, oxidation, antioxidants, luminescence.

Tuz Stresinin Bazı Bitkilerin Solunum Komponentlerine Etkisi

Özet: Çeşitli bitki gruplarının köklerinde ve izole mitokondrilerinde oksijen absorbsiyonu, serbest radikal oksidasyonu ve anti-oksidant etkenliğini kinetik düzenlemeleri, NaCl, Na_2SO_4 ve Na_2CO_3 stresi altında araştırılmıştır.

Mevcut bulgular, oksidasyonu ve fosforilasyonun ayrımının, tuz stresi altında bitkilerin oksijen absorbsiyonunu açık bir biçimde teşvik ettiğini göstermektedir. Bu nedenle, köklerin anti - oksidasyon etkenliği, serbest darikal süreçlerinde kontrolsüz bir hızlanmaya neden olacak biçimde hızla azalmaktadır. Elde edilen bulgular, tuzun bitkiler üzerindeki toksik etkileri konusunda mevcut literatüre destekleyici bir katkı sağlamaktadır.

Anahtar Sözcükler: Tuz sitresi, oksidasyon, antioksidantlar, lüminesens.

Introduction

According to recent estimates one third of the total area under irrigated agriculture, approximately 400 x 10^6 acres, is saline (1). The problems of soil salinity and improving the salt tolerance of cultivated plants are of particular urgency in semi-arid zones where soils have been already either been partially salinized or can become saline because of irrigation.

Thus, different aspects of the plant metabolism under saline conditions, arouses the interest of researchers. Such attention to the problem has been stipulated not only by general biological significance but also by an exceptional practical current interest.

We are confident of studying the primary physical and chemical processes developing in a plant cell just after salt stress.

This is of great importance nowadays to reveal the mechanism of salt stress and the nature of salt tolerance in plants. The recent introduction of biophysics and biochemistry into the study of salt tolerance in plants has promoted the formation of a synthetic approach to an essence recognition of vital functions of plants under salt stress.

The aim of this study was to determine the influence of salt stress on the respiratory components of some plants.

Materials and Methods

5-day-old seedlings of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) kidney bean (*Phaseolus vulgaris* L.), cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) grown at a constant 25°C as well as isolated mitochondria of barley root system were the subjects of this research.

Salts were applied to the medium directly and their concentrations are shown in the figures.

The isolation of mitochondria was based on the methods used in previous research (2, 3).

A polarographic unit was used to measure rates of oxygen absorption by seedling roots and isolated mitochondria (4). The oxygen absorption rate was determined by the reduction of oxygen in the electrochemical cell.

A quantum meter was used to record a spontaneous generation and it was induced by chemiluminescence or their homogenates (5).

A long duration of H_2O_2 effect inducing chemiluminescence was a criterion for the elaborated method in determining the anti-oxidation activity of an extract (aqueous or alcohol) (5).

The experiments were repeated 3-5 times and the data was analyzed statistically.

Results and Discussion

Our research has revealed a significant influence of ions (Na^+, Cl^-, SO_4^{-2}, etc.) on the functioning of cells when they enter even at low concentrations (<0.01 M). A high rate of oxygen absorption by seedlings and isolated mitochondria is a reaction of cellular organelles to the salt stress (Fig. 1)●.

Lundergardh (6) also observed such an effect on plants and called it "anion respiration". According to Lundergardh's hypothesis the "anion or salt respiration" is revealed only under salt stress. Setting forth the hypothesis Lundergardh proceeded from the entire suppression of salt absorbing and "anion respiration" in the presence of the corresponding cyanide concentration (10^{-3} - 10^{-4} M), whereas the "basic respiration" remained fixed.

However, at present, a stimulation of the plant oxygen absorption under salt stress is explained by the effects of specific K^+, Na^+, adenosine triphosphases (ATP-ases)

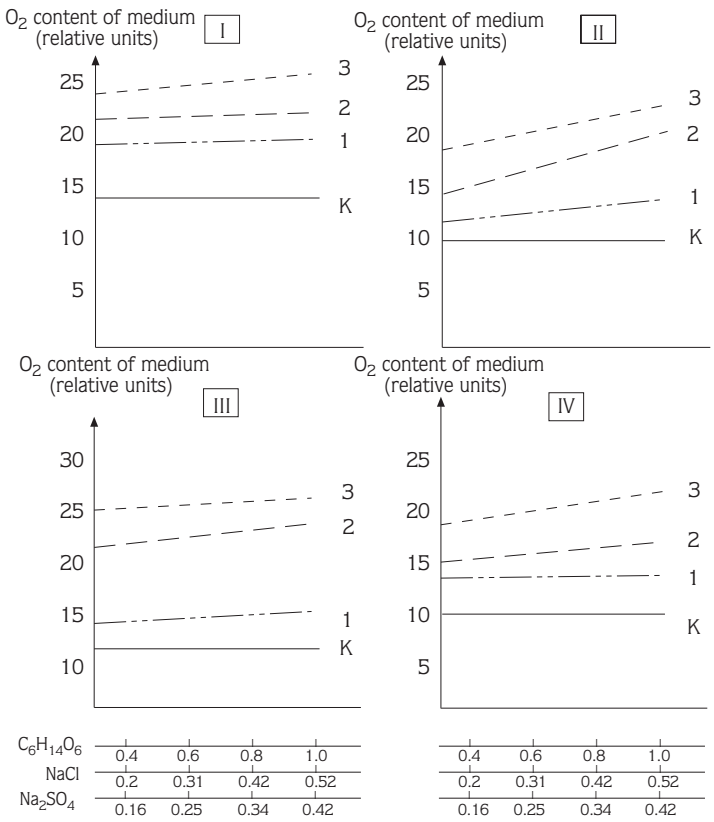

Figure 1. Effect of salts and mannitol in iso-osmotic concentrations (M) on respiratory activity of seedling root mitochondria: I- Barley (*H. vulgare*); II- Wheat (*T. aestivum*); III- Kidney Bean (*P. vulgaris*) IV- Maize (*Z. mays*). K- control; 1- mannitol; 2- NaCl; 3- Na_2SO_4

contrary to Lundergardh (7, 8, 9). This point of view presumes that the sodium and potassium promote the adenosine triphosphate (ATP) to split into inorganic phosphate (P). The formed adenosine diphosphate (ADP) if gets into the respiration chain promotes the regulation of plant respiration.

However, our data and those published elsewhere, show no evidence for the "anion respiration" of Lundergardh. The respiration stimulation effect is observed on plants not only under salt stress but also under the effect of other substances such as dinitrophenol, dicoumarol, gramicidin, etc. (4, 6).

Having been confident of salt stimulating the respiration of seedlings and isolated mitochondria, we attempted to find out the stimulation origin. We presume three systems to be effected by salt stress: 1) electron transfer chain; 2) energy transfer chain; 3) points of conjugation for electron and energy transfer.

Using a hightly sensitive and practically intertialess polarographic unit for successively testing enzyme and metabolism inhibitors, we could determine the stimulation origin as follows: the oxidation (electron transfer) separates from the phosphorylation (energy transfer) (Fig. 2).

We considered an increase in respiration intensity of roots of the seedlings and mitochondria under salt stress the first criterion revealing the oxidation and phosphorylation splitting.

There is no difficulty in observing some equivalent actions of salts (10^{-1} M NaCl) and 2, 4- Dinitrophenol (2.4-DNP) (10^{-3} - 10^{-4} M) (Fig. 2) 0. However, such similarity cannot be certain proof for identifying their effective mechanims.

The effect of 10-1 mM 2.4-DNP was successively tested. It was introduced into the system after the salt stress to reveal a separation action. That was also tested in reverse order, i.e. first, 2.4-DNP effect was checked and then the NaCl one. According to the results, 2.4-DNP caused an abrupt increase in the oxygen absorption rate but NaCl adding reduced to some extent its stimulating effect.

The potassium cyanide (KCN) inhibition of the so-called "salt respiration" was considered the second criterion. Thus, along with the NaCl-KCN testing on the oxygen absorptivity of seedlings and mitochondria, the KCN effect was checked after the application of 2.4-DNP. The kinetic curves obtained in 2.4-DNP-KCN and NaCl-KCN versions were quite similar (Fig. 3)■.

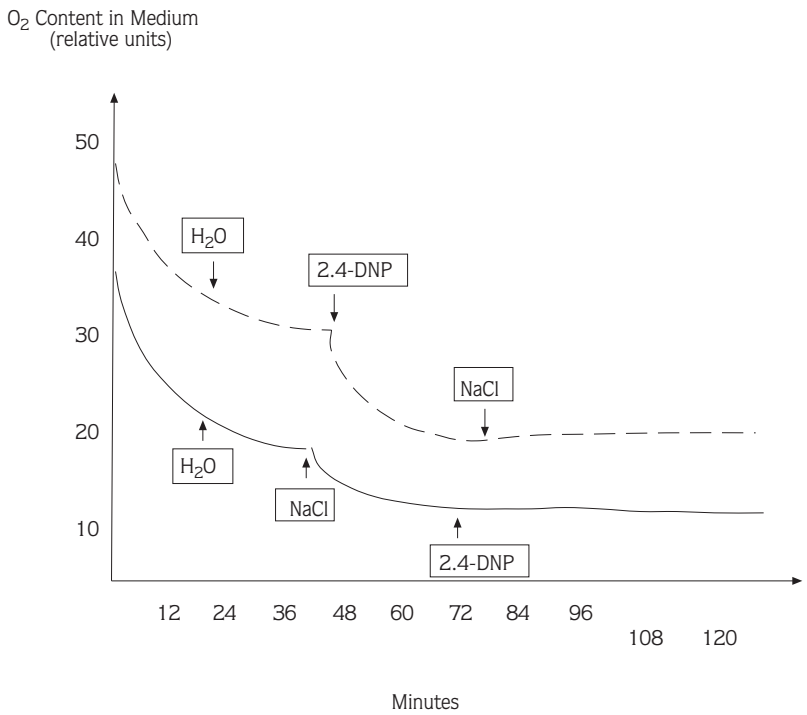

Figure 2. Polargraphic record for oxygen absorbtion by seedlings under NaCl alterating effect and 2.4-DNP, t=18°C.

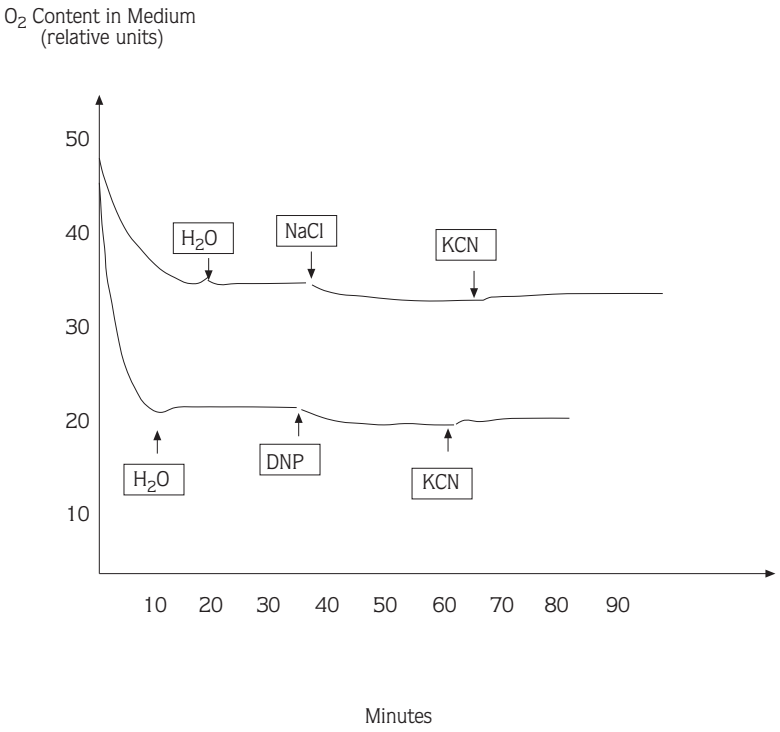

Figure 3. Comparison of kinetic curves for oxygen absorption by barley seedlingb under the effect of NaCl-KCN and 2.4-DNP-KCN, t=18°C.

We know that the experiments with 2.4-DNP are not condusive proof of the resultant oxidative phosphorylation. In this case, we can speak about the conjugation loosening for these processes but we cannot interpret it as ineffective oxidative phosphorylation (10). Therefore, we measured a stoichiometric ratio of ADP/O and respiration control (RC) to study conjugation efficiency between oxidation and phosphorylation which we considered the third criterion. According to obtained data RC was absent in seedling roots after the effect of 100 mM NaCl. The ADP/O stoichiometric ratio was negative indicating a marked disturbance of the conjugation between oxidation and phosphorylation (4).

As known, in compelety separated states respiration does not form ATP but proceeds at the maximum rate without ADP and inorganic phospate (11, 12, 13).

ADP introduced into the system after salt stress, evidently does not stimulate oxygen absorption by plants but even slightly inhibits this process. Of interest is the fact that such regularity was obtained by a number of researchers on different subjects and it was called the "non-acceptor effect of ADP". This effect is the fourth criterion to reveal a separation of oxidation and phosphorylation during salt stress on seedlings. In this case the ATP as activity rockets and the ATP molecule splits into ADP and P. Adams and Ronnan (14) referred to the increased content of ADP in the medium after salt stress on a plant. The increase of the ATP activity has been shown elsewhere (15, 16).

First, a plant cell loses its principle mechanism of energy accumulation as a result of the ATP splitting. Second, a separation of oxidation and phosphorylation promotes peroxidation of a biologic membrane in the phospholipid phase. A peroxidation initiation forms a free radical of fatty acid. The addition of oxygen produces a peroxide radical. According to Lehninger (17) an excessive amount of the peroxide separates oxidation and phosphorylation and causes a swelling of mitochondria. The mitochondria swelling in cells has been revealed elsewhere (18). Our research on isolated mitochondria confirmed the actual swelling of mitochondria under substrate salination (Fig. 4)❑. As a result of the separation, the accumulated peroxide triggered a free radical oxidation. A marked intensification of the free radical oxidation after salt stress of relatively high concentrations can be detected easily by chemiluminescence. We revealed such an effect first in 1996 on differnt plants using a highly sensitive photoelectron unit (19).

The marked intensification of free radical reactions is a consequence of an over-peroxidation of components of a biomenbrane.

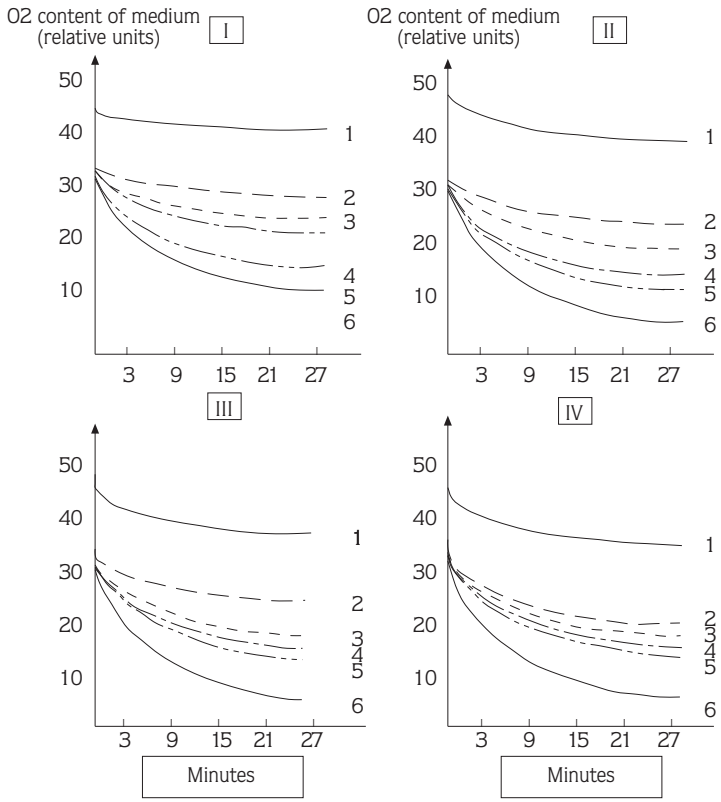

Figure 4. Synergistic effect of NaCN and salts on the variation in the respiratory activity of seedling root mitochondria:
I-Barley (*H. vulgare*); II- Wheat (*T. aestivum*); III- Kidney Bean (*P. vulgaris*); IV- Maize (*Z. mays*).
1- medium; 2- NaCN 10^{-3} M; 3- Na_2SO_4+NaCN; 4- NaCl+NaCN;
5- $C_6H_{14}O_6$+NaCN; 6- control (NaCl, Na_2SO_4, $C_6H_{14}O_6$-0,1 M)

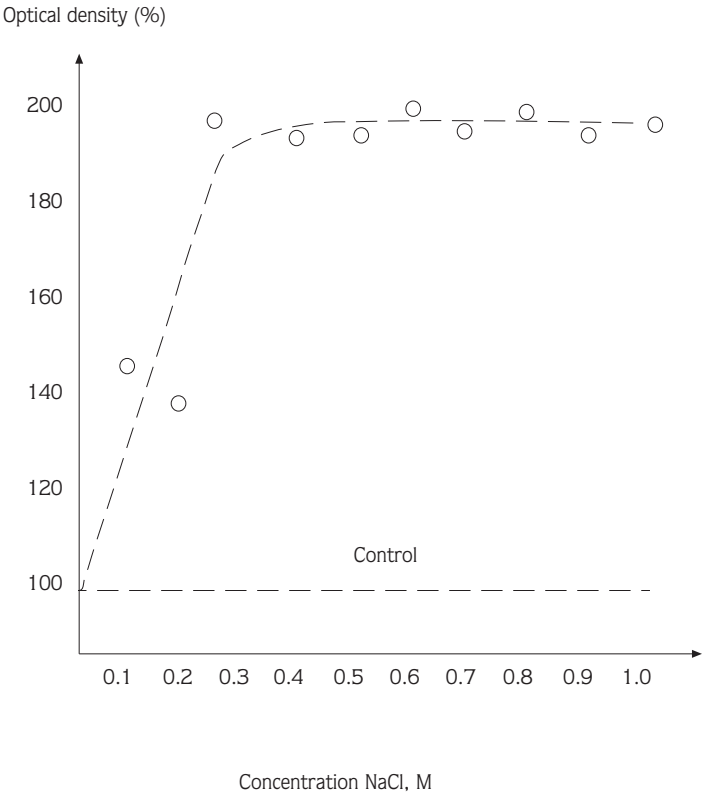

Figure 5. Variation of optical density for salt solutions with ninhydrin seedlings depeding on NaCl concentration.
5-day-old seedlings of barley were used in the experiments. The optical density was measured with a red light filter FEK-56M.

Because of peroxidized phospholipid components, a membrane structure deteriorates and its permeability changes. We obtained experimental proof in the process of the ninhydrin color reaction. The ninhydrin reaction is known as one of the most sensitive for detecting a-aminoacids. Thus, an abrupt increase in a-aminoacids in the outher salt solution with placed seedlings indicates a higher permeability of a cell membrane and a loss of its selectivity (Fig. 5).

The accumulation of both inorganic (lipid, epoxides) peroxides and their subssequent conversion promotes an intensive oxidation of reduced products in a cell. In this case, various biologic antioxidants are natural protectors.

According to experimental data the antioxidant system of a plant organism is damaged by salt stress. The number of antioxidants is sharply reduced when salt concentrations become higher in a medium. When the level of a cell antioxidant activity falls it results in a marked and uncontrolled development of the chain oxidation. As known, antioxidants reduce active concentrations and hence, the reaction rate.

It should be noted, however, that a free radical reaction does not stop completely though it proceeds slowly even with the presence of a strong antioxidant in the system. It has been proved by a rather intensive superweak luminescence of intact plants. A sufficient amount of antioxidants in the roots maintains the rate of chain oxidation at a fixed level. But when the antioxidant stock runs out, there is, for instant, a fairly rapid fixed-to-variable level transition, with the reaction self-acceleration Apparently, the marked consumption of natural antioxidants under salt stress is connected with the oxidating rocketing after the leakage of hydrolytic enzymes. The intensification of free radical reactions under salt strees is clearly observed by following superweak luminescence (Fig. 6; third phase) ¥

Reduced forms of non-hermine ferrum (Fe+2), a-tocoferol (Vitamin E), sulfohydryl groups (-SH) cmopounds and some others are refferred to as natural antioxidants to run out. As for Wills (20) peroxides can inactivate tiol enzymes and oxidize SH groups of aminoacids and proteins.

Thus, the intensive peroxidation of membrane lipids and ATP molecule splitting under salt stress result in degradation of a sophisticated plant system, breakdown of a ribosome-membrane interaction and consequently, disturbance and distortion of the protein synthesis. An obvious activation of aminoacids is required for protein synthesis of a ribosome. Aminoacids do not join the protein biosynthesis without preactivation. ATP activates these aminoacids directly.

References ta o reduction in the protein synthesis rate as well as an increased content of free aminoacids under salt stress can be found in many publications (21, 22). A significant reduction in plant cell energy output under salt stress adversely effects the total metabolism. It can be

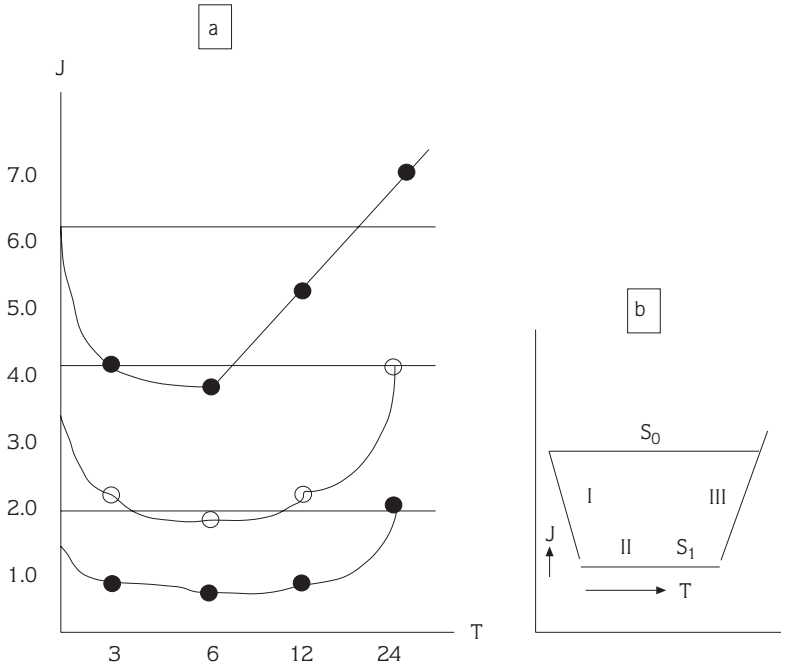

Figure 6. Kinetics of superweak luminescence of plants under NaCl effect (a) and three phases of its development (b).
1- seedlings of kidney beans
2- seedlings of wheat
3- seedlings of cotton
S_o, S_1 - stationary states for luminescence of plants in standard conditions and under salt stress.
I, II, III - phases of luminescence development
T - time (hours)
J - luminescence intensity in relative units

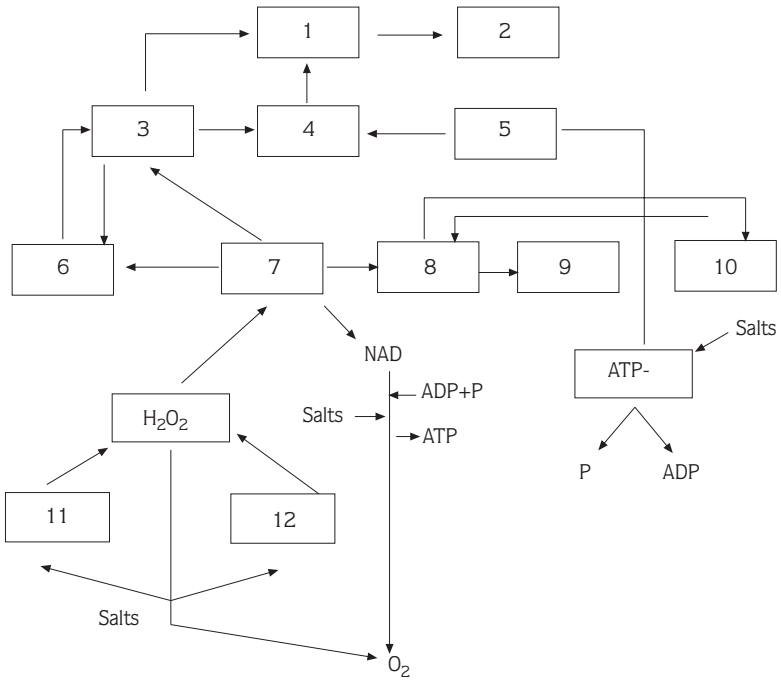

Figure 7. Assumed primary biophysical mechanism of salt stress on metabolism in a plant cell. 1- decomposition of proteins 2- growth processes; 3- hydrolytic ferments; 4- synthesis of proteins; 5- activation of amino acids; 6- membrane permeability; 7- peroxydation of biomembrane; 8- free radical reactions; 9- chemiluminescence; 10- antioxydative system; 11- catalase; 12- peroxydase.

shown schematically as in Fig. 7◆. According to Tappel, approximately $4x10^6$ - $6x10^6$ free radicals are required for lysosome hydrolyses to enter the cytoplasm.

According to Tappel (23), Green (24), and others, peroxides accumulating in lysosome membranes destroy them, release the organoid or hydrolytic enzymes causing an abrupt intensification of hydrolytic processes and autolysis in tissues.

Thus, an abnormal excess of the standard level of peroxide content in plant cells under stress causes an accumulation of derivatives such as lipid-aldehydes, ketones and epoxides. These derivatives together with toxins of a non-lipid nature such as highly reactive quinones cause the general intoxication of a cell.

The correlation of data obtained with halophytes and glycophtes has shown a lower total reduction activity of halophytes against glycophytes.

The same feature can observed on glycophytes growing on salinity. From this point of view, a relatively high reducing activity is particularly more dangerous on glycophytes.

Therefore, salt tolerance shows an inversion with reduction activity. If we explain the nature of salt tolerance in plants from this point of view, the reason of the low reducing activity will be celar as well as the low respiration intensity, low membrane permeability of root cells and low antioxidation activity. Some kinds of terrestrial plants (halophytes) apparently, developed salt tolerance as a protection during long periods of evolution.

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References

1. Joseph, A., Barry, G., John, s., Photosynthesis, MSSR, 2, 338, 1987 (in Russain).
2. Andreeva, I.N., Cell and cells structure, Moscow, Nauka, 1968 (in Russian).
3. Andreeva, I.N., Kurkova, E.B., Plant Physiology, 12, 4, 584-587, 1965 (in Russian).
4. Kasumov, N.A., Effect of chloride salination on respiratory energy for seedlings. Sci. Proc., Az. St. Univer., 1, 1973 (in Russian).
5. Kasumov, N.A., Physiological and biophysical aspects of salt strees mechanism on phytoorganism, Baku, 144, 1983 (in Russian).

6. Lundergardh, H., Ann. Rev. Plant Physiol., 6, 1955.
7. Laties, G.G., Survey Biol. Progr., 3, 215, 1957.
8. Einstein, E., Biochemistry of Plants, M., Mir., 254, 1968 (in Russian).
9. Gordon, L. Kh., Respiration and water-salf exchange of phytotissues, M., Nauka, 112-130, 1967 (in Russian).
10. Semikhatova, O.A., Estimation methods of energy efficiency for plant respiration, Leningrad, Nauka, 1967 (in Russian).
11. Borst, P., Slater, E. C., Need in inorganic phosphate in oxidation stimulated by dinitrophenol, Nature, 184, 1959.
12. Gaitskhoki, B. S., Structuring of biological oxidation and conjugated processes, Leningrad, Nauka, 103, 1967 (in Russian).
13. Skulachev, V.P., Energy accumulation in a cell, Moscow, Nauka, 1969 (in Russian).
14. Adams, P. B., Ronan, K.S., Plant Physiol., 50, 6, 682-686, 1972.
15. Zhukovskaya, N. B., Matukhin, G. R., Diversity of soil salinity effect on content of phosphoorganic compounds in barley., J. Plant Physiology (Soviet Union), 350-356, 1967.
16. Hasson-Porth E., Poljakoff-Mayber, Content of adenosine phospate compounds in pea roots grown in saleni media, Plant Physiology, 47, I, 109-113, 1971.
17. Lehninger, A.L., The mitochondria molecular basis of structure and function, 4, 1964.
18. Hecht-buchholz Pflueger R., Marchener, H., Pflanzen Physiol., 65, 5, 410-417, 1971.
19. Kasumov, N. A., The study of different toxic salt stresses on plants by biocnemiluminescence, Sci. Proc., Az. St. Univer., 2, 1966 (in Russian).
20. Wills, E. O., Mechanism of Lipid peroxide formation in animals tissues, Biochem. J., 99, 667, 1966.
21. Kahane, J., Poljakoff-Mayber, Effect of substrate salinity on the ability for protein synthesis in pea roots, Plant Physiol., 43, 7, 115-117, 1968.
22. Udovenko, G. V., Possible inhibition of protein synthesis on plants under salination, 2nd All-Union Biochem. Congr., Tashkent, 28-29, 1972 (in Russian).
23. Tappel, A. L., Free-radical lipid peroxidation damage and its inhibition by vitamin E and selenium, Feeder Proc., 24, 1964.
24. Green, F. A., To lipid metabolism - a fundamental feature in tummer - hostü relationship, J. Theor. Biol., II, 213, 1966.