SALT STRESS AT PLANTS AND ITS INVESTIGATION METHODS

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Abstract

The article is devoted to study of the stress condition at plant tissues by means of NaCl, Na2SO4. On this purpose methods like: microscopy, quantometry, stationary polarography, photo-colorimetry and conductometry have been used.

It was ascertained metabolic processes at plants in extreme salt condition happens on the direction of *norm* ⇄ *stress* →*loss* as well as only stress factor is reversible here.

Key words: Stress, Plasmolyse, Free radicals, Exoosmose

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Introduction

It is known that life activities are closely connected with external environmental factors (as: light, temperature, humidity et.al.) of plants. There are definite change limits of condition (low, high and/or extremums) in the environment of plants that is possible to regulate biological processes run within these limits. So resistance of plant organism to: low/high temperature, draught, salt, diseases et.al.is identified [1].

Attitude of plants have been changed to external environmental factors in the evolution process. That is why certain ecological forms (xerophytes, hydrophytes, halophytes et.al.)that always exist in the environment and according to the factor that it is more than others have appeared [9].

As standard condition in tissues and sells of alive organisms (25^0C, 1atm, 1M, pH 7,0 et.al.) seldom happens major part of the reactions run in non-standard condition that cause stress factors occurrence sometimes [6]. Structure and functions of the organisms are closely connected with successive steps like: *norm* ⇄ *stress* →*loss.* Some specific properties of stress factor of these steps attract ones attention.

The English word ‘stress’ that means tension, load, influence et.al. has been used first in 1936 by G.Selye [12].When stress factors (*eustres və ya disstres*) are very strong or of a long-period they may soon pass into a loss phase. Such a pass are different for different stressors. Fir instance: the temperature at a human body normally is 36-36,6^{0}C; under stress it gets to 37-42^{0}C; at loss it gets up to 42-45^{0}C.

The main goal at the article is salt stress at plants ascertained by different methodsand some parameters characteristic to it especially providing with information about its reversibility.

Objects and Methods of the Research

Root and leaves of monocotyledonousand dicotyledons (barley, wheat, pea, bean) have been used as an investigation object. The plants have been grown in a thermostate at 25^0C temperature within Knop fluid in a normal aerasional condition.

Various methods have been used to study salt stress at plants. One of these methods is a *microscopy*. as a matter of fact microscopic investigations are based on changes in tissues and cellsstructure. Depending on investigations character cuttings have been prepared of roots and leavs of the plants; visual observations done under the light microscope on painting them by special coloring means like red methile, blue of methilen et.al. Stress factor in the sells have been created in different thicknesses (0,05; 0,1; 0,2; 0,4; 0,8 M) by salt liquids (NaCl, Na_2SO_4 et.al.).

Only root system of 5-day-sproutsof plants in entire state were used in other experiments. On this purpose *methods of quantometry and stasionar polarography* could by considered as opportune ones [11]. Aggregate block-schemes of *quantometry and stasionar polarography* were indicated on the figures number 1 and 2 [4] .

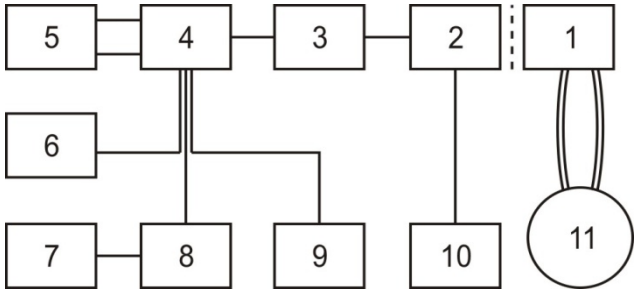

Fig.1. General block-scheme of *quantometry* aggregate. 1. thermostatic room; 2.photo-electronic Photo-Electronic Multiplier-85; 3.Initial amplifier; 4.broad-stripped impulsed amplifier – BSIA-2; 5.feeding block of the amplifier; 6.electronic ossilographer; 7.self-writing potenciometer – (KSP-4); 8.integrator of impulces; 9.calculating device – PST-100; 10. High-voltage stabiled amplifier; 11.ultrathermostat – U-10.

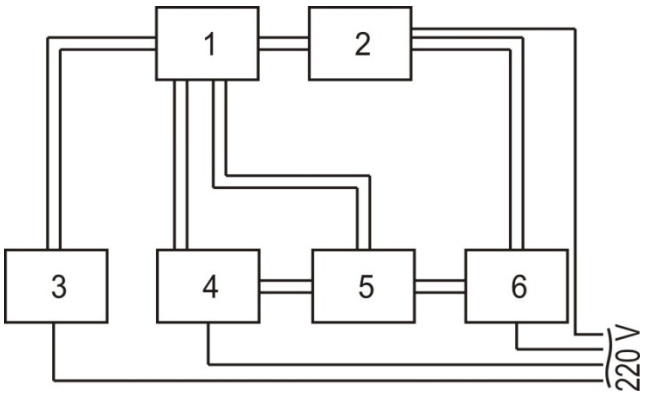

Fig.2.General block scheme of the polarographic aggregate identifying absorption of oxigen by plants. 1) electrochemical chamber; 2) lab pH-meter; 3) reflecting galvanometer; 4) ultrathermostat; 5)thermostatic cuvette with the object in it; 6)micropump.

Conductometry and photocolorimetry also can be used in study of the stress factors at plants. Both of these methods are based on exoosmos process by the root system of the plant sprouts. At conductometry identification of the electric conductivity value (of definite volume of root system 15-20 ml) due to the exoosmos of inorganic substances – ions [8]; and at photocolorimetry

identification of the organic combinations especially α-amino-acids amount (that appeared on the surface of the water in the same volume) according to ninhydrin test is taken into account [3].

Experiments have been carried out 10-times repeatedly and the results statistically calculated [7]. Rms (root mean square) deviations were not more than 5%.

Results of the investigation

The Microscopy method (in a light microscope only) is based on plasmolyse and deplasmolyse events under effect of the stressors (for instance: NaCl, Na_2SO_4 and other salts) in different seeds (roots, leaves et.al.) of plants. So, water goes out of the cells under osmotic pressure (π – fluid $>\pi$ vacuole; 'π' osmotic pressure) at hypertonic fluids of different salts as: NaCl, Na_2SO_4 et.al. ($C \geq$ 0,2 M; 'C' is density of fluids). 'Plasmolyse' occurs on the result of the move next to the vacuole that dilates and contracts from the rigid cell coat of the deformed cytoplasm which lasts first to cell coat's completely releasing (turgor pressure – P=0)and then up to contraction. At this time the hypertonic fluid (plasmolitic) fills in the space between the cell coat that is conductive for it and semiconductive plasmolemma (Fig.3).

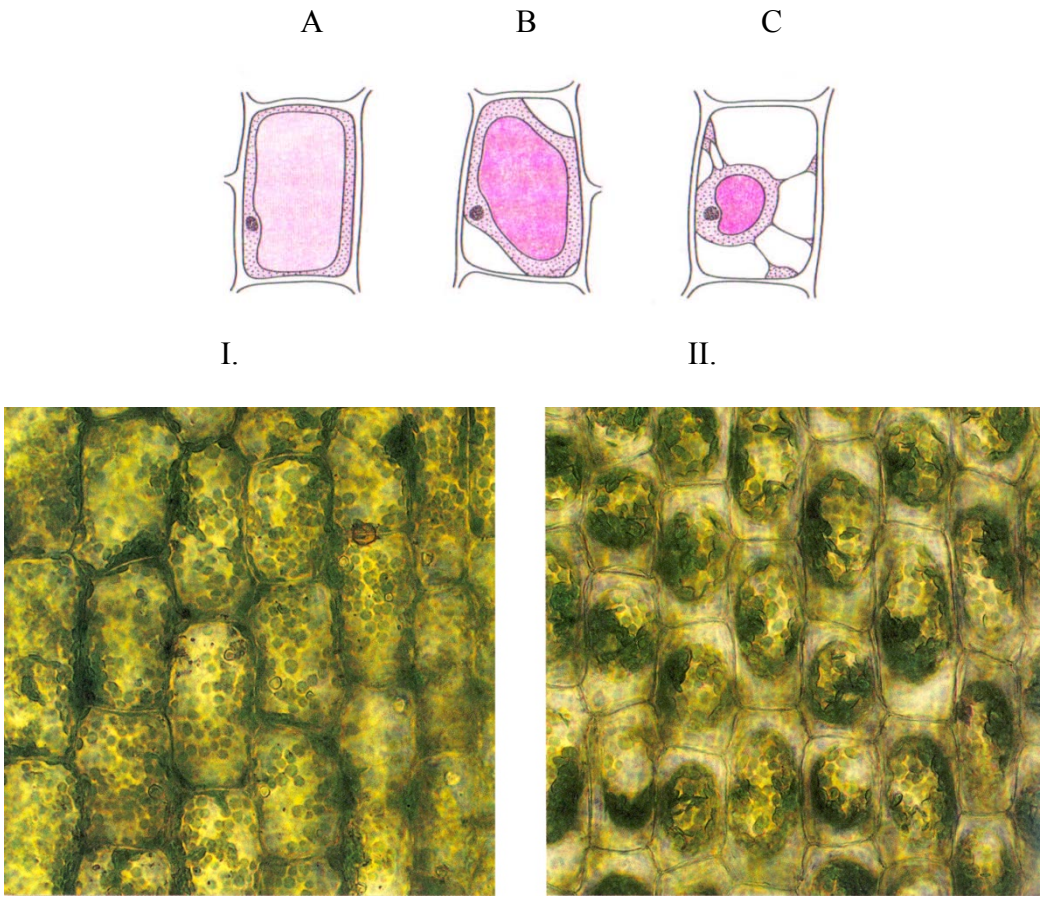

Fig.3. I – plasmolyse in the epidermis cells of a leaf. A – At a normal condition the protoplasm fills in the space surrounded by the cell wall; B – Water goes out of the cell in the dense fluid of saccharose and the plasmolyse occurres; C – the cell loses more water in more dense fluid and a convex plasmolyse occurres; Hext threads are observed. **II – cells of elodeya leaves.** A – the cells are in the turgor state; B – plasmolyse placed into dense fluid of saccharose.

The cytoplasm doesn't lose its connection with the cell coat for few or much period in separate fields of the cell and origins Hext threads [10]. Plasmolyse event may be in different forms, eg.: in the case of few cytoplasm viscosity; and hollow plasmolyse event with the presence of substances of high viscosity and swelling reduce ($CaCl_2$) are observed. At the hollow plasmolyse event the turgor pressure – (P) is negative; and absorption strength – (S) is more than the osmotic pressure – ($S>\pi$). When osmotic pressures of the substance originated vacuole and plasmolyse are equal plasmolyse process comes to finite limit.

It is realized from the obtained results that0,2-0,3 M density of the salts (NaCl, KCl, Na_2SO_4) subject to plasmolyse in cells of root system of sprouts within approximately 15-20 minutes. When passing the plasmolysed seeds into hypotonic fluid (for instance, water) plasmolyse stops and inverse process – deplasmolyse occurres. If the plasmolyse process lasts for a long period or it occurres under very dense plasmolyse pressure it has a non-changeable character, i.e. it is not in the state of deplasmolyse and the seeds begin to lose soon [2].The main reason of it is – existence of a lot of damages (as: plasmadesma break, deformation of chloroplasts and mitochondrils in leaf cells et.al.).at plasmolyse and deplasmolyse. However although such damages are under stress effect the cell doesn't lose aliveness feature and the lost ones in deplasmolyse form again. And this is one of the appearance forms of dynamic state of membrane.

Thus, Physical & chemical changes occur at structure of plants at salt stress: on the result the normal physiological state is violated.

When studying salt resistance of plants one of the most actual problems is use of the methods giving opportunity to obtaining of required data not violating stationary state of the metabolic reactions at a plant organism. From this point of view quantometry method that notes spontaneous bio-chemical-luminescence event (very weak radiation) ascertained in organs and tissues of plant and animal is very opportune. In the investigations carried out by this method biological entirety of the object completely remains and study of kinetics of free radical reactions is possible. Sprouts of plants as: 4-5-day barley, wheat, cotton et.al. have been used at such as experiments. Density of salt fluids was taken between 0,1-1,0 Mat the experiments. The obtained results have been shown in the Figure 4 [5].

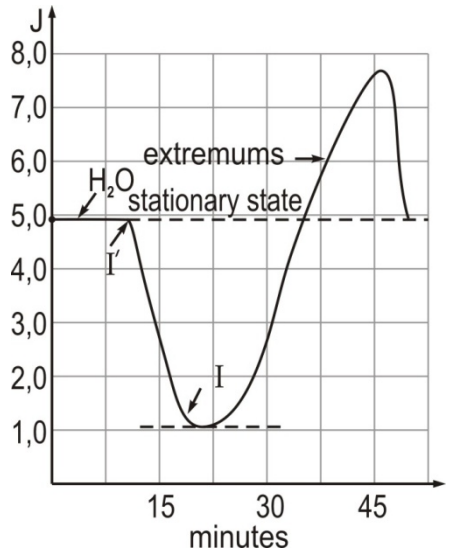

Fig.4. Changeability of very weak radiated intensiveness at bean sprouts after stopping NaCl effect. 1– level of radiation at the sprouts in normal state (in water); 2 – level of radiation at the sprouts at salt effect; I – start of the salt (NaCl) effect; I/– after washing the sprouts by water; j – intensity of the radiation in relative values.

As it's indicated in the figure intensity of very weak radiation is sharply reduces during 10-15 minutes at plant sprouts of bean, barley et.al. It passes to a new stationary level and this case lasts for several hours. In such case at liquidation of the salt fluid stress in the system (0,1 M) intensity of the radiation returns back to its initial stationary state. The extremums at the passage clearly seems here.

Thus three phases ascertain at kinetics of very weak radiation at the sprouts maintained in salt fluids. At the first phase (I) intensity of the radiation returns to its initial state after washing the root system by water that indicates reversibility of the process. The reversible character of the free radical reactions lasts up to the mid of the II phase; however at the third phase (III) sharp strengthening of the very weak radiation occurres and sharp increase of both α-amine acids and electric conductivity of the fluid is observed at the samples taken out of the salt fluids where the sprouts were put when testing by ninhydrin [8]. And it proves degradation of proteins/albumens; also violation of the conductivity and selectivity of bio-membranes (Fig-s 5, 6).

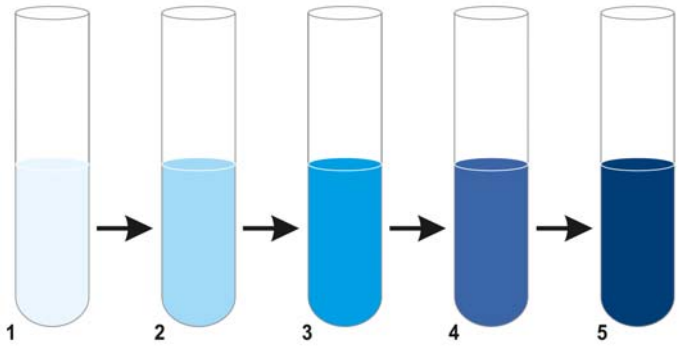

Figure 5. Ninhydrin reaction of the α-amine acids

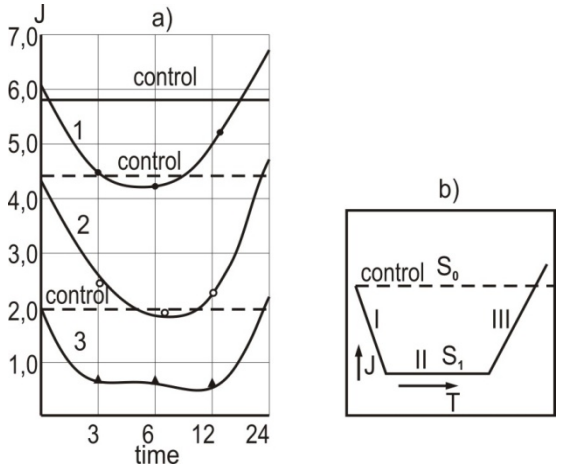

Figure 6. Kinetics of the very weak radiation at plants when NaCl effects (a) and 3 phases of its development (b). 1– bean sprouts; 2– wheat sprouts; 3 – cotton sprouts; S_0; S_1– stationary state of radiation at plants normally and when the salts effect; I,II,III – development phases of the radiation; T – time in hours; j – intensity of radiation in relative values.

The intensity of the very weak radiation at this phase rises in the linear character and the process is not reversible in the lump. Sharp increase of the radiation intensity degradation (of proteins) at the sprouts appears at different times depending on plant species; this can be used as an index of

the plants salt-resistance. After the degradation radiation (III phase) the sprouts die soon if they are planted in soil or passed into a fertilized environment (Knop fluid et.al.).The degradation process results in violation of the membrane conductivity and release of hydrolytic ferments as well as seeds autolysis by means of the peroxides accumulated in the cell membrane [13].Tappel notes that [14] existence of an approximate free radical $4 \cdot 10^6$-$6 \cdot 10^6$ is enough to appearance of cytosome (lysosome) hydrolases in cytoplasm. The free radical reactions can be expressed as follows:

$$P^* + O_2 \rightarrow P + O_2 + h\nu_1$$
$$RO_2 + RO_2$$
$$R^{\cdot} + (O_2)_2^* \rightarrow R + O_2 + h\nu_2$$

Here: P– is product of the reaction; R –organic compound radical; RO_2 – peroxiradical.

Changeable character of a stress factor at plants is also possible to study according to absorption kinetics of O_2-at sprout roots by the *stationary polarography method*[4].For this purpose 100мkM the fluid of ADP has been used (Fig.7).

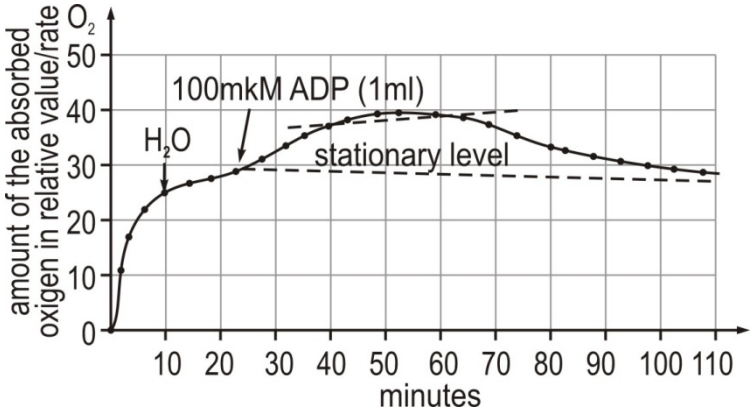

Fig.7. Changeability of O_2 absorption after ADP effect at the plant sprouts.

As it is seen in the figure stationary state of the O_2 absorption in the root system of the 5-day barley (*Hordeum*) sprouts put into the water passes onto the new stationary level after adding 100 мkM ADP into the system; in some 50 minutes velocity of the O_2 absorption reduces and returns to its initial level. Such a passage is perhaps connected with the turn of ADP into ATP at the period of breath.

Thus it is possible studying changeability of salt stress at plants by the methods of: microscopy (on the base of plasmolyse $\rightleftharpoons$ deplasmolyse events), quantometry (according to the very weak radiation intensity characteristics), stationary polarography (according to the O_2 absorption velocity), photocolorimetry (according to the reaction of α-amine acids appeared in the environment with the ninhydrin) and conductometry (according to the change of electric conductivity of fluid).

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