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Study of Breath of Monocotyledonous and Dicotyledonous Plants Seeds in their Turgescence Phases at Salt Stress

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Abstract: *The article describes the respiration intensity in germinating seeds of monocotyledonous and dicotyledonous plants in salt stress conditions. The solutions of NaCl and Na_2SO_4, and, in some experiments, also of mannitol have been used as stress factor within the range of 0.1 to 1.0 M concentrations.*

It has been established that oxygen absorption by seeds, regardless of their biological features, is characterized by a three-stage curve. A similar pattern develops during the swelling of seeds. The first stage lasts about 60 minutes, and has a lag period. Apparently, this is directly related to the water uptake and hydration of seeds. Oxygen absorption by seeds increases within the following 6 to 8 hours (the second stage), and their respiration sharply increases afterwards (the third stage).

This research aims to determine the nature of the respiratory rate changes of monocotyledonous and dicotyledonous plants during salt stress.

Keywords: *intensity, staging, osmotic pressure, swelling, germination of seeds, respiration, stress*

1. Introduction

Respiration is one of the essential and labile processes of plant cells, being hypersensitive to external factors, which makes it an important indicator of the organism while experimentally exposed to the environment, likewise under the influence of salinity.

During the first swelling stage, as established, the respiration intensity of seeds increases in isoosmotic solutions of the salts (NaCl, Na_2SO_4), and in solution of mannitol at low osmotic pressure (~ 6 atm). This, however, decreases at high osmotic pressure (~ 16 atm). At low osmotic pressure, evidently, salts affect the respiration of seeds basically not in the osmotic, but in a specific (ionic) way, and this starts with the second swelling stage as a rule. At high osmotic pressure, however, this also develops under the influence of osmotic factor. Thus, salts stimulate respiration intensity at low concentrations or under short-term influence, although high concentrations inhibit the respiration of seeds.

As a global and central process within metabolism of a cell, respiration comprises a set of reactions, which lead to oxidation of organic substances ($C_6H_{12}O_6$) into simpler compounds

(6 CO_2 u 6 H_2O) [Gasimov, 2008].

The experimental data provide basis to determine that the germination of seeds starts at a humidity of not less than 40 to 50 per cent. Water does not only participate in swelling of colloids within germination, but it also helps activate enzymes, dissolve reserve nutrients, and remove inhibitors. During swelling and germination, seeds lose solubles (such as sugar, amino acids, organic acids, phenolic compounds etc.) to the environment, and such loss sometimes constitutes up to 10 per cent of their initial weight [Grodzinskiy et all, 19733]. Leached substances promote development of the concomitant microflora and, occasionally, of pathogenic stimulants.

The respiration intensity of germinating seeds strongly depends on water content; as the seed respiration very sharply increases at a humidity of 33 per cent [Polevoy, 1989].

2. Methods

The seeds of monocotyledonous and dicotyledonous plants (wheat, bean, cotton, barley etc.) have been researched by this study. The solutions of NaCl and Na_2SO_4 and of mannitol, in some experiments, have been used within the wide range of 0.1 to 1.0 M concentrations as stress factor. It is worth noting that cation (Na^+) and anion (Cl^-) appear as positive and negative hydrating ions in the NaCl solution respectively, while both (Na^+) and (SO_4^{2-}) are positive hydrating ions in the Na_2SO_4 solution. The control seeds have been treated with distilled water.

The concentration of salts, causing stress conditions, has been predetermined by several methods: plasmolytic, polarographic, quantometric [Abduyeva-Ismayilova et all, 2013].

The cryoscopic method has been applied for determining the osmotic pressure of mannitol and salt solutions, by using the TLM semiconductor micro-refrigerator with the VSP-33 power supply [Gasimov, 1983].

Presently, the following methods, such as gasometry (as to the amount of CO_2 and O_2), polarography (as to the recovery of O_2 on platinum electrode) and infrared spectroscopy (as to the absorption of CO_2 radiation) are being successfully applied for studying the respiration intensity in plants and for determining the respiratory coefficient (RC) in plants. Effectiveness of this or another method substantially depends on a plan of the experiment and on a subject of the study. As such, the respiration intensity in greenhouse conditions can be determined by infrared spectrometry. As to seedling plants, respiration is mostly studied by polarographic method. The method of gasometry is usually used for determining the respiratory coefficient of seeds. This method allows to determine the absolute amount of CO_2 and O_2, which is necessary for the respiratory coefficient ($\frac{CO_2}{O_2}$) of germinating seeds.

In 1951, Warburg manometric method was used to determine the respiration intensity in seeds. A batch of the seeds was placed on the surface of the vessel containing the test solution. The experiments were carried out throughout 12 hours at 25 ºC. The reports were filmed after every 10 minutes during the first hour and then every hour. The obtained data underwent statistical processing [Plokhinskiy, 1978]. The experiments were repeated 5 to 10 times.

3. RESULTS

The obtained data indicate that oxygen absorption by the cotton seeds increases insignificantly in the early steeping (~ 60 minutes). Even a time lag can herewith be seen easily. (Fig.1) Apparently, this is directly related to water uptake and hydration of seeds. During the following six hours, however, oxygen uptake by seeds increases, after which the respiration of the seeds sharply grows. Thus, a three-stage change of respiration clearly appears during seed swelling. Such staging is also typical for the process of oxygen absorption by the seeds of barley and beans. Generally, however, the seeds of beans take up less amount of oxygen in swelling than cotton and barley seeds. This is apparently due to the fact that proteins in the bean seeds act as main oxidation substrates during respiration. It is known that proteins disintegrate to amino acids (e.g. fumaric acid), in which the respiratory coefficient is greater than unity.

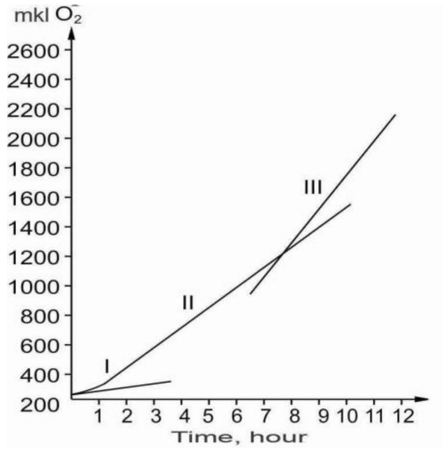

Fig1. *The kinetics of oxygen absorption by cotton seeds in normal conditions (in water)*

We have established that relatively low concentrations of NaCl (0.01 to 0.05 M) cause an increase in the respiration intensity of barley seeds during the three stages of swelling (Fig.2). Many researchers have found out that respiration is stimulated under the influence of low concentrations of salts [Boyko, 1971; Gordon, 1976; Epstein, 1954; Lundegardh, 1955; Lundegardh, 1960]. The mechanism of this effect has been studied by N.A.Gasimov in detail [Gasimov, 1975; 1983; 2012].

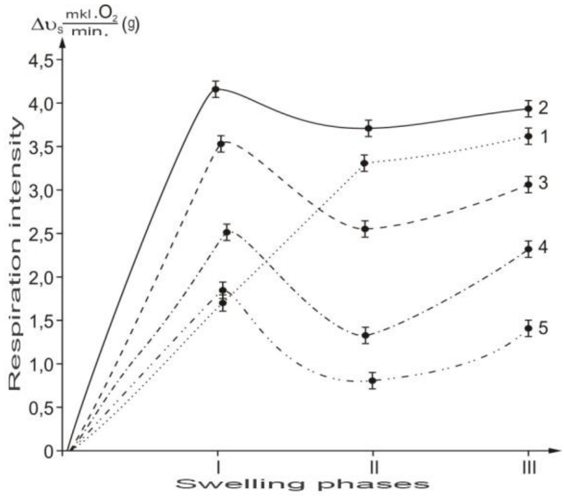

Fig2. *Oxygen absorption rate by barley seeds in normal conditions and under the influence of NaCl.*

1. Control (distilled H_2O)

2. 0.05 M NaCl

3. 0.1M NaCl

4. 0.5M NaCl

5. 0.8M NaCl

Δ Vs - the increment of the average rate of oxygen uptake

It is noteworthy that higher concentrations of NaCl (0.1 to 0.8 M) increase respiration during the first stage of swelling, but respiration decreases in relation to the control seeds during the second and third stages of swelling.

Sodium sulphate (0.05 to 0.8 M) increases the respiration intensity of barley seeds in the first stage; the respiration intensity remains higher in the second and third stages than in the control seeds under the action of 0.05 to 0.1 M solution of Na_2SO_4, while it decreases under the influence of 0.5 to 0.8 M solution (Fig.3).

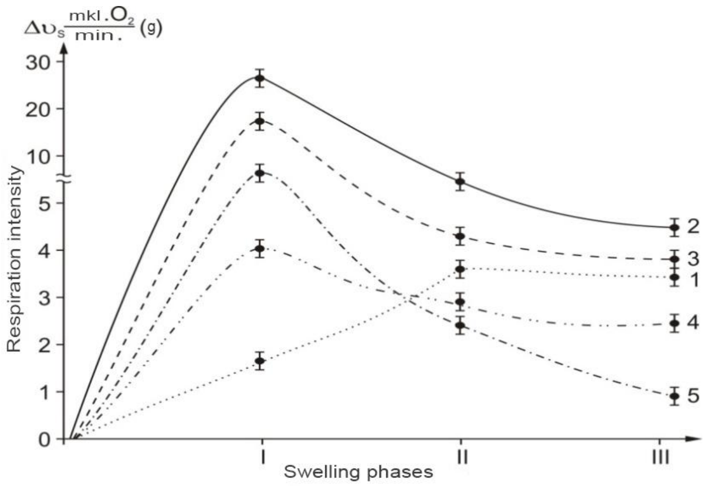

Fig3. *Oxygen absorption rate by barley seeds in normal conditions and under the influence of Na2SO4.*

1. Control (distilled H_2O)

2. 0.05 M Na_2SO_4

3. 0.1M Na_2SO_4

4. 0.5 M Na_2SO_4

5. 0.8 M Na_2SO_4

Δ Vs - the increment of the average rate of oxygen uptake

4. DISCUSSION

In chloride salinity, the cotton seeds exhibit downward trend in the respiratory process with increasing concentration of salts similar to the barley seeds. In sulphate salinity, the concentrations that we have tested, including high ones (0.8 M Na_2SO_4), increase the respiration intensity of the cotton seeds during the first stage. In the second stage of swelling, respiration is slightly decreased under the action of 0.1 M solution of Na_2SO_4, but it remains higher than the control seeds. At the same time, 0.5 to 0.8 M solution of Na_2SO_4 decreases respiration in relation to the control seeds. The third stage of swelling is characterized by the increase of the respiration intensity under the action of 0.1 M solution of Na_2SO_4. The respiration intensity in seeds that are under sulphate salinity (0.5 to 0.8 M) in the third stage remains lower than the control seeds. It is noteworthy that sulphate salinity also inhibits the oxybiotic process, although in lesser degree in comparison with chloride salinity.

The identification of changes in the respiration intensity under the action of inert substances and saline solutions should be reckoned as one of the important problems in the field of salt tolerance of plants. Such a comparative study would allow to recognize the essence of the mechanism of osmotic and toxic (ionic) action of salts to a certain extent. We have carried out a series of experiments on the subject where we have taken mannitol as an osmotically active substance, and besides, the solutions of mannitol, NaCl, and Na_2SO_4 have been isoosmotic. The magnitude of the osmotic pressure of these solutions has been determined by cryoscopic method (Fig.4), using the TLM semiconductor micro-refrigerator with the VSP-33 power supply.

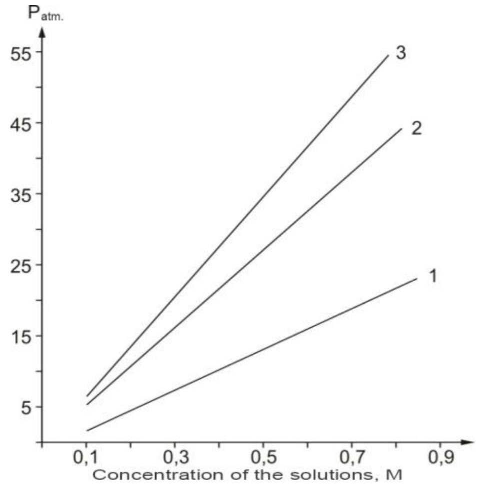

Fig4. *Correlation between the osmotic pressure and the concentrations of the solutions of mannitol (1), NaCl (2), Na2SO4 (3)*

The obtained results show that the respiration intensity of the cotton seeds increases in the first stage during swelling in the solutions of salts and mannitol at low osmotic pressure (~ 6 atm). The respiration intensity decreases in the second and third stages in chloride salinity. This process increases in sulphate salinity. However, mannitol reduces respiration compared to salts in these stages. The respiration also decreases at high osmotic pressure (~ 16 atm) in the cases with salts. At low osmotic pressure, evidently, salts affect the respiration of seeds basically not in the osmotic, but in a specific (ionic) way, and this starts with the second swelling stage as a rule. At high osmotic pressure, however, this also develops under the influence of osmotic factor. (Fig.5).

It is easy to notice that ions Cl^- and SO_4^{2-} act specifically in the process of O_2 absorption of the germinating seeds, i.e. if Na_2SO_4 sharply increases the respiration intensity at the same osmotic pressure (e.g., 4.5 atm), then NaCl stimulates this process slightly, and mannitol somewhat decreases it on the contrary. Change in the respiration intensity of plants under the influence of mannitol is observed in a number of studies. As such, studies on leaves of *Pelargonium Zonale L* were conducted at the Paris University in 1967, which revealed that shifting mature leaves of pelargonium into the mannitol solution (0.3 and 0.5 M) brought the respiration intensity to the maximum, and then, this decreased to the minimum followed by 3 to 5 hours, and the respiration intensity slowly increased afterwards. In 0.5 M solution of mannitol (11.38 atm), however, the respiration intensity decreased for 2 to 5 hours without preliminary growth, and then slowly increased.

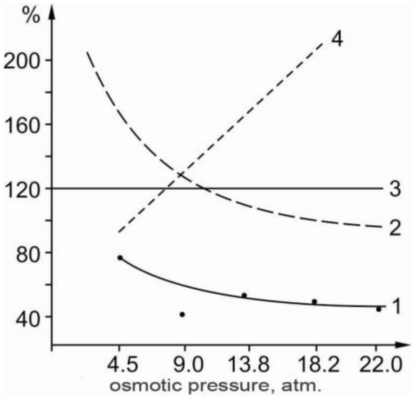

Fig5. *Oxygen absorption by barley seedlings under the influence of isoosmotic solutions of mannitol (1), NaCl (4) and Na2SO4 (2). 3-control (water). For all the cases, the batch is 1 g. The absorption of O2 within 30 minutes is expressed in %.*

Thus, a comparative study of the oxygen absorption rate of the germinating seeds under the action of isoosmotic solutions of mannitol, NaCl and Na_2SO_4 indicates that a more serious hazard for the plant body is substantially built up not under the osmotic action of salts, but by their toxic effects.

To sum up, it should be noted that each swelling stage of seeds, regardless of their biological specificity, is limited by a certain factor. As such, the first stage of seed swelling is characterized by high osmotic pressure during water uptake and high suction force of seeds. In this stage, the respiration intensity is still at a low level. Overall, the process of water uptake is limited by the size of water potential gradient between the environment and seeds. Duration of the first stage of swelling is approximately 40 to 60 minutes.

The second stage, which lasts about 10 to 12 hours, is characterized by a sharp acceleration of hydrolytic processes that result in disintegrating carbohydrates, proteins, fats, etc., into relevant constituents. Herewith, respiration synchronously increases along with biochemical processes. The third stage has a longer duration than the first and second stages of swelling, which is about 40 to 60 hours. The rate of physiological processes, such as respiration, growth etc., side by side with the biochemical process, also sharply increases in this stage.

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