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Determination of the Respiratory Coefficient in Germinating Seeds of Plants under Salt Stress Conditions

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Abstract – **It was determined that while studying the plant seeds (wheat, barley, pea, bean, cotton-plant, sunflower), the magnitude of the respiratory coefficient (RC) varies considerably. As a rule, the seeds rich in fats are characterized by low values of respiratory coefficient. In such seeds, this indicator is equal to 0.57-0.60 in normal conditions (in water). The similar results were obtained for the emission of CO_2 by the seeds mentioned above.**

It was found that in normal conditions (in water), the speed of the emission of CO_2 lags behind unity. This may indicate that the main substrates in the process of respiration in seeds are carbohydrates, proteins and fats than different amino acids having lots of oxygen-containing components.

Keywords – **Respiratory Coefficient, CO_2 Wheat, Barley, Pea, Bean, Cotton-Plant, Sunflower.**

I. Introduction

It is easy to see that under the influence of salt stress, as a result of the acceleration of oxygen absorption in germinating seeds, the value of RC slightly decreases and this is particularly noticeable in seeds rich in fats. The reason for this decline of RC is that a very substantial proportion of absorbed oxygen is used during this period for the transformation of spare fats into carbohydrates. After this process is completed, RC increases again and may averagely reach 0.7-0.8.

Numerous experiments have shown that complete oxidation of the molecules of Hexose absorbs 6 molecules of oxygen and evolves 6 molecules of carbon dioxide. This process results in the formation of 6 molecules of water. Thus, the ratio of the volume of the evolved carbon dioxide to the volume of the absorbed oxygen is equal to unity. This ratio is known as the respiratory coefficient (RC) [Rubin,1976].

The value of RC depends on the degree of regeneration of the organic substance utilized during respiration, on the respiring cell's oxygen provision level and, finally, on the cell's ability to utilize oxygen, which in turn is defined by the characteristics of the enzyme systems responsible for the regulation of respiration processes [Gasimov, 2008].
We know that respiration incorporates and interrelates metabolism of proteins, nucleic acids, carbohydrates, fats and other components of protoplasm. However, it should be noted that the transformation from the primary energy resources to the generation of adenosine triphosphate during respiration is much more complex than during photosynthesis [Skulachev, 1969].

Respiration's central position in metabolism explains its key meaning for a range of biologically and economically valuable characteristics of plants [Grodzynskiy, 1973]. The rate and intensity of respiration as well as the value of RC depend on a various endogenous and exogenous factors. Substrate salinity is among such factors.

Professional literature contains rather inconsistent data on the effects of various salts (NaCl, Na_2SO_4, Na_2CO_3 and others) on the intensity of respiration. For instance, some researchers have witnessed an increase in the plants' respiration intensity under the effect of salts [Gasimov, 1973; 2012; Boyko, 1971; Lapina 1969] in some cases, while others have noted a decrease in the respiration intensity [Razmaev et all, 1969; Stroganov, 1962; Tur,1969; Lundegardh, 1955; Lundergardh, 1960].

This article aims to research the subject of respiration intensity of germinating seeds of various plants under the conditions of salt stress and to measure their RC values.

II. Research Subject and Methods

The subject of this research was the seeds of different types of plants with varying degree of salt-endurance: wheat, barley, pea, bean, cotton-plant and sunflower. In the research work, the salts of NaCl and Na_2SO_4 were used in a wide range of concentrations (0,05;0,1; 0,2; 0,4; 0,8 M). The control seed samples were treated with distilled water.

Now, to study the intensity of respiration in plants and the determination of the respiratory coefficient in plants, the following methods are successfully applied: gasometry (according to the amount of CO_2 and O_2), polarography (according to the recovery of O_2 on platinum electrode) and infrared spectroscopy (according to the absorption study of CO_2). The effectiveness of one or another method greatly depends on the setting of the experiment and the research object. So, in greenhouse conditions, the determination of the intensity of respiration can be carried out by infrared spectrometry. The seedling plant respiration is mainly studied by polarographic method. The method of gasometry is usually used for the determination of 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, germinating seeds $\left(\frac{CO_2}{O_2}\right)$.

The determination of respiration intensity of seeds was carried out by Warburg manometric method (1951). The weighed sample of the seeds was placed in the main space of the vessel on the testing solution. The experiments were

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carried out during 12-24 hours at 25 ºC. The temperature inside the Warburg device (AG) was regulated with the help of a contact thermometer. The oscillation frequency of the device was set at 80 times per minute. The reports were filmed every 10 minutes during the first hour and then every hour.

The obtained data were processed statistically [Littge et all, 1979]. The experiences were repeated 3-10 times.

III. Research Results and Their Discussion

It should be noted that the objects we studied differ not only in their salt-endurance, but also in their chemical composition. For instance, spare substances of wheat and barley seeds are mainly represented by carbohydrates, spare substances of peas and beans are mainly by proteins and the ones of cotton-plant and sunflower seeds by fats. Therefore, there is a great variability in the value of RC for the studied seeds. As we see from the table, the rate of oxygen uptake per 1 gram of dry substance during 24 hours is 87.6 ± 2 for wheat, 105.0 ± 4.2 – for barley seeds, whereas their RC is equal to 0.95-0.96. The value of RC of the seeds, whose spare substances are mainly by proteins, varies from 0.71 to 0.84. The seeds rich in fats are characterized by low values of respiratory coefficient. In such seeds, this indicator is equal to 0.57-0.60 in normal conditions (in water). The similar results were obtained for the emission of CO_2 by the seeds mentioned above. The table obviously shows that in normal conditions (in water), the speed of the emission of CO_2 lags behind from the absorption speed of O_2. Therefore, in all the options, the value of RC does not reach unity. This may indicate that the main substrates in the process of respiration in seeds are carbohydrates, proteins and fats than different amino acids having lots of oxygen-containing components.

In addition, we have attempted to define the value of the RC in the test seeds under the influence of different concentrations of NaCl and Na_2SO_4. The obtained results are summarized in the table.

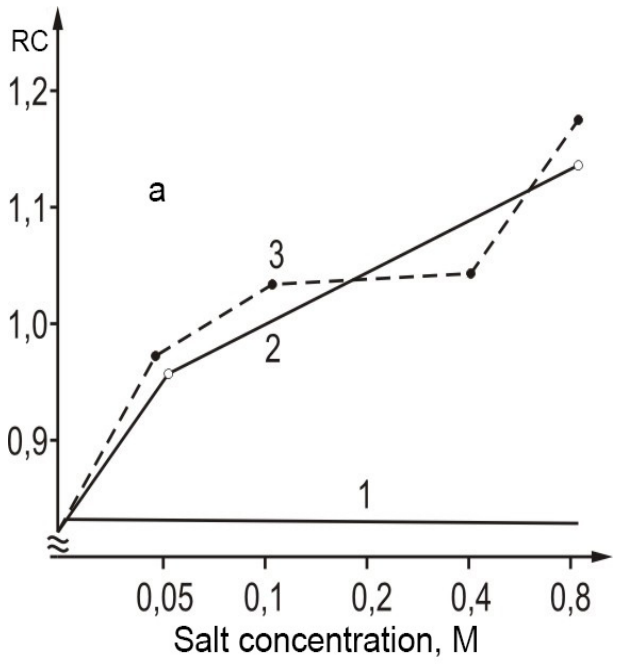
The table shows that the concentration of NaCl at first leads to a considerable increase (0.05 M) of O_2 absorption by seeds of all types of plants, which is then followed by a gradual drop in the respiration intensity. However, the CO_2 emission by seeds also reaches maximum levels. Thus, the increased emission of CO_2 in relation to the control samples is also manifested in 0.05-0.1M NaCl concentrations. Inconsistency between the maximum values of O_2 uptake and CO_2 emission by the seeds can be derived from the fact that plant tissues are capable of respiration not only under the conditions of high oxygen content in atmosphere, but also when the atmospheric oxygen is low or even non-existent.

It follows that the increase of the concentration of NaCl eventually leads to the increase of the seeds' RC values. This may be related to the fact that during the early phases of the germination process of seeds, the RC for all seeds is about 1, because during these phases their inherent small amounts of carbohydrates are utilized for respiration.

It is easy to see that under the conditions of salt stress, the magnitude of RC of germinating seeds decreases as a result of the enhanced oxygen consumption and that it is especially evident for seeds which are rich in fats (cotton-plant and sunflower). This decrease in RC is explained by the fact that during this stage a rather large portion of the absorbed oxygen is used for the transformation of spare fats into carbohydrates. Upon completion of this process, the RC begins to increase again and may averagely reach 0.7-0.8.

Thus, under the conditions of salt stress (0.1-0.2 M NaCl, Na_2SO_4), the degree of regeneration of organic substance utilized by seeds considerably decreases in the respiration processes. The stimulation of oxygen consumption by seeds in the germination process is most likely related to the disconnection of the oxidation process (the energy transfer).

In summary, our research data allow us to confirm that the influence of salts results in the violation of the conjugation of oxidation with phosphorylation during the early stages of the swelling of seeds, which deprives the cell of its primary mechanism of energy accumulation. This may become the main reason for the development of various pathological reactions and incoordination of metabolic processes.



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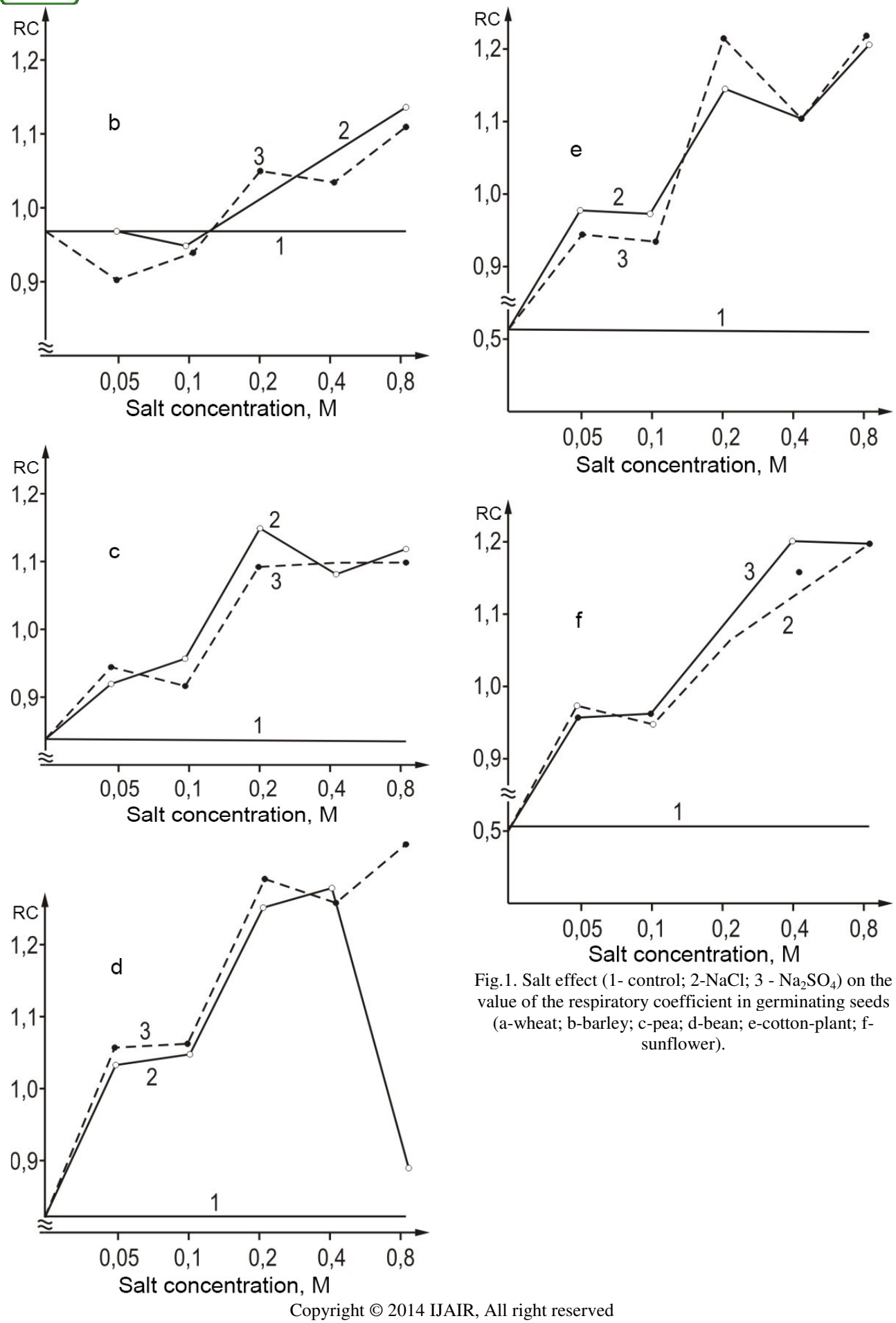

Fig.1. Salt effect (1- control; 2-NaCl; 3 - Na_2SO_4) on the value of the respiratory coefficient in germinating seeds (a-wheat; b-barley; c-pea; d-bean; e-cotton-plant; f-sunflower).

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Table 1: Salt effect on the intensity of the respiration in germinating seeds of plants

No.	Plants	Absorption of O_2 (mkL O_2 for 1 g dry weight per 24 hours)						Emission of CO_2 (mkL CO_2 for 1 g dry weight per 24 hours)					
		Control (H_2O)	0,05 M NaCl / 0,05 M Na_2SO_4	0,1 M NaCl / 0,1 M Na_2SO_4	0,2 M NaCl / 0,2M Na_2SO_4	0,4 M NaCl / 0,4 M Na_2SO_4	0,8 M NaCl / 0,8 M Na_2SO_4	Control (H_2O)	0,05 M NaCl / 0,05 M Na_2SO_4	0,1 M NaCl / 0,1 M Na_2SO_4	0,2 M NaCl / 0,2M Na_2SO_4	0,4 M NaCl / 0,4M Na_2SO_4	0,8 M NaCl / 0,8 M Na_2SO_4
1.	Wheat	87,6± 2,6	89,2±2,6 / 88,4 ±3,5	92,1±3,7 / 88,7±2,7	90,4±3,6 / 91 ± 2,7	84,2±3,4 / 79 ± 2,4	66,4±2 / 63 ± 1,9	73,3 ± 2,2	86,3±3,4 / 87,1 ± 2,6	93 ± 3,7 / 92,6 ± 3,7	94,1±3,7 / 95,3 ± 2,8	93±4,6 / 83,1 ± 3,3	76 ±2,3 / 74,2± 3,7
2.	Barley	105 ± 4,2	105±4,2 / 103 ±4,1	107,4±4,3 / 109 ±5,4	97,6±3,9 / 100,2 ± 4	87,5±3,5 / 93,7 ±3,7	70,1±2,1 / 71,8 ±2,8	102,5 ± 4,1	102,5 / 94,3 ±2,8	103,1±3 / 102,7 ± 4	104,3±4,2 / 106,5 ±4,3	95,1±2,8 / 97,6 ±2,9	78,6 ± 2,3 / 80 ±3,2
3.	Pea	85,7± 3,4	87,1±4,3 / 92,3 ±3,7	88,7±2,6 / 85,6 ±3,4	84,6±2,5 / 86,1 ±3,4	78,1 ± 3,1 / 77 ±3,8	67,6±2 / 69 ±2,7	72 ± 2,9	83,4±3,3 / 86,5 ±2,6	84,4± 4,2 / 79,3 ±3,2	98,7±3,9 / 94,8 ±3,8	93,2±3,7 / 85,4 ±3,4	78 ± 3,1 / 76,1 ±3,8
4.	Bean	82 ± 3,3	79,7±2,4 / 81,2 ±4,1	68,7±2 / 69,2 ±2,7	62,3±1,8 / 64,1 ±1,9	56,1±1,7 / 57 ±1,7	52,4±1,6 / 54,6 ±1,6	58 ± 2,1	75,3 ± 3 / 78,3 ±3,1	65,3±1,9 / 66,3 ±3,3	72,6±2,9 / 78,1 ±2,3	66,3±2,6 / 67 ±2,7	63,2±3,2 / 68,1 ±2,7
5.	Cotton-plant	78 ± 3,8	74,5±2,2 / 76,4 ±3	64,1±3,2 / 63,8 ±2,5	59,1±1,7 / 57 ±2,3	55,3±1,1 / 52,4 ±1,8	50,6± 1,5 / 51,3 ± 2	46 ± 1,8	73,2±2,9 / 72,8 ±2,2	62,7±1,9 / 59,2 ±2,4	68,2±2,7 / 70,3 ±2,1	61 ± 3 / 58,1 ±2,3	60,4 ± 2,4 / 62 ± 1,8
6.	Sunflower	73 ± 2,2	74,6±2,2 / 73,2 ±3,6	60,3± 1,8 / 62,1 ±1,9	56,2±2,2 / 58,5 ±2,3	52,1±2 / 53 ±2,1	48,3±1,4 / 47 ±1,9	42 ± 0,8	72,5±2,9 / 70,6 ±2,8	57,4±2,8 / 58,6±1,7	61,1±2,4 / 62 ±2,5	63,2±1,9 / 59,4 ±2,9	58 ±2,3 / 55,9 ±2,2

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