

# Dynamics of Changes in the Amount of Photosynthetic Pigments in Bean Seedlings Under NaCl and Na<sub>2</sub>SO<sub>4</sub> Salinity Conditions

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## ***Abstract***

One of the most important problems facing humanity currently is soil salinization. Excessive salt concentration in the soil is considered one of the main environmental factors limiting plant growth and productivity. The effect of different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> in water (10-30mM) and soil (0.2-0.6%) cultures on the morphometric parameters of bean seedlings sensitive to salinity and the dynamics of changes in the amount of photosynthetic pigments (chl a, chl b, and carotenoids) in the initial stage of ontogenesis have been studied.

The amounts of photosynthetic pigments were found to decrease sharply in 7- and 14-day-old bean seedlings with enhancing NaCl and Na<sub>2</sub>SO<sub>4</sub> concentrations (10-30mM). Compared to carotenoids, the decrease in the amount of chlorophylls (chl a, chl b) was sharper. It should be noted that under the influence of both salts, the decrease in the water culture was twice as much as in the soil culture.

**Keywords:** salinity, culture, photosynthetic pigments, chlorophyll, carotenoid, morphological traits, germination percentage

## **Introduction**

Soil salinization is one of the most important problems facing humanity in modern times. Excess salt in the soil is considered one of the main environmental factors limiting plant growth and productivity (Flowers, 2008, 2004). According to various estimates, 15-23% of the total area of Earth, including agricultural land, is saline soils (Khitrov, 2009, Gamalero, 2009).

Saline soils have become a growing problem of irrigated agricultural lands (Kovda, 2008). According to available data, 50% of soils may be subject to salinization by 2050 (Ashraf, 1994).

From a chemical point of view, the type of salinity is determined mainly by the amount of anions in the soil, and therefore, it is divided into chloride, sulfate, chloride-sulfate, and carbonate salinization (Pilipenko, 2005). Chloride-sulfate salinization type is accounted for more than 50% of the area of saline soils (Khitrov, 2009). Since all the listed salts are well soluble in water, they are usually washed out of the soil by atmospheric precipitation in humid climates and remained in very small quantities. In a dry or hot climate, however, washing with rainwater does not occur, on the contrary, salt solutions accumulate in the upper layers of the soil with the rising flow of soil water. Water evaporates and salts remain in the upper layer of the soil (Ivanishev, 2020).

Salinity, which is one of the signs of soil degradation, significantly reduces its fertility (Isanova, 2017), as well as makes it difficult for plants to absorb macro and micronutrients and causes mineral starvation. As a result, the sensitivity of plants to pathogens and pests increases and it ultimately leads to a decrease in their ability to survive. This determines the relevance and importance of studying the problem of salinity and the effect of this factor on the growth and development of plants (Ivanishev, 2020).

Currently, there is a general opinion on the toxicity of the  $\text{Na}^+$  ion and its negative effect on plants (Flowers, 2008).

To clarify the mechanism of action of Na salts on the plant organism, it is important to study the physiological processes in the first stages of the development of seedlings. The initial physical and chemical processes occurring in plant cells in the first stages of ontogenesis have a serious effect on the subsequent course of intracellular metabolism.

Thus, salinity has a negative effect on the formation of morphological traits of bean seedlings and reduces seed germination.

It is necessary to choose more tolerant to negative factors varieties at the initial stages of development, at the stage of sprout formation. According to some authors, it is possible to combine characteristics such as salt tolerance and productivity in one plant, that is, to grow high-yielding varieties (Jhuchenko, 1994; Kumakov, 1995).

Therefore, the effect of different concentrations of Na salts on the seed germination

percentage, growth indicators, and the dynamics of changes in the amount of photosynthetic pigments was studied.

The data presented on the importance and urgency of solving the global soil salinity problem and its impact on plant growth, development, and defense mechanisms are directly related to providing food products to the world population.

## Materials and methods

The bean plant (local Piyada variety), which is widely used in agriculture in our Republic, was chosen as the research object. Bean is a protein-rich dicotyledonous plant belonging to the leguminous family (lat. Phaseolus). It occupies an important place among vegetable crops, both in terms of its nutritional value and its use extent. Technically ripe pods of beans contain 14.0% dry matter, including 6% protein, 4% nitrogen substances, 4-6% carbohydrates, 2.9% sugar, 1% cellulose, and 0.7% mineral substances. Dry seeds contain about 30% proteins.

Seed germination was carried out in the water (Knop's solution) and soil cultures under conditions of 20°C and normal aeration in a special chamber with 420 lx of illumination. In experimental variants, 10, 20, and 30 mM NaCl and Na<sub>2</sub>SO<sub>4</sub> were added to Knop's solution in the water culture. In the soil culture, 0.2% (1 g of salt per 500 g of soil), 0.4% (1 g of salt per 500 g of soil), and 0.6 % (1 g of salt per 500 g of soil) saline environments were created.

The germination percentage is determined based on the number of 5-day-old seedlings.

The amount of pigments was determined spectrophotometrically at wavelengths of 440.5, 644, and 662 nm after crushing leaves in the acetone solution. The amounts of chlorophyll a (Chl a), chlorophyll b (chl b), and carotenoids (Car) were estimated using the formula of HolmWettstein (Tretyakova, 1990):

$$\begin{aligned} \text{Chl a} &= 9.784 \times D_{662} - 0.990 \times D_{644} \text{ (ml/l)} \\ \text{Chl b} &= 21.426 \times D_{644} - 4.650 \times D_{662} \text{ (ml/l)} \\ \text{Ccar} &= 4.695 \times D_{440.5} - 0.268(a+b) \text{ (ml/l)} \\ &\text{and} \\ C_0 &= C \times V \times V_2 / (m \times V_1 \times 10) \text{ (mg/100g)}. \end{aligned}$$

Where, C is the concentration of pigments, mg/l; V - volume of initial extract, ml; V<sub>1</sub> - volume of extract used for dilution, ml; V<sub>2</sub> - volume of the extract added to the

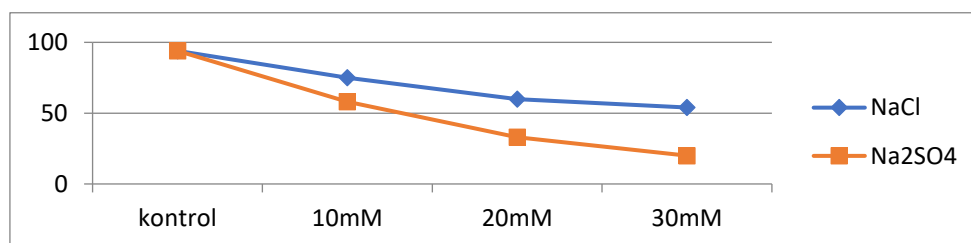
spectrophotometric cuvette, ml; m is the weight of the leaf used for the determination.

The studies were carried out with 3-4 replications. The obtained results were processed statistically (Lakin, 1990 ), and the error did not exceed 5%.

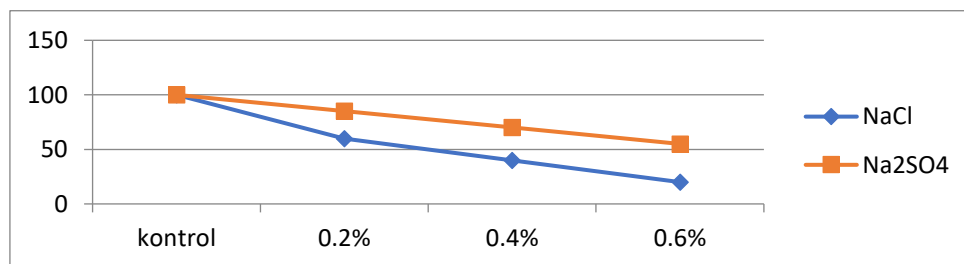
## Results and Discussion

The negative effect of high salinity on plants is manifested starting from the first stages of development. Thus, the first stages of ontogenesis are more sensitive to extreme effects due to high metabolic activity.

To study the effect of NaCl and Na<sub>2</sub>SO<sub>4</sub> on the germination energy of seeds, the germination percentage of the seeds used in the study was determined. For this purpose, the number of germinated seeds in 5 days was found. According to the obtained results, the isocation salts of Na affect seed germination negatively. It is clear from the curves reflecting the effect of different concentrations of these salts on the germination percentage of bean seeds (Figures 1 and 2).



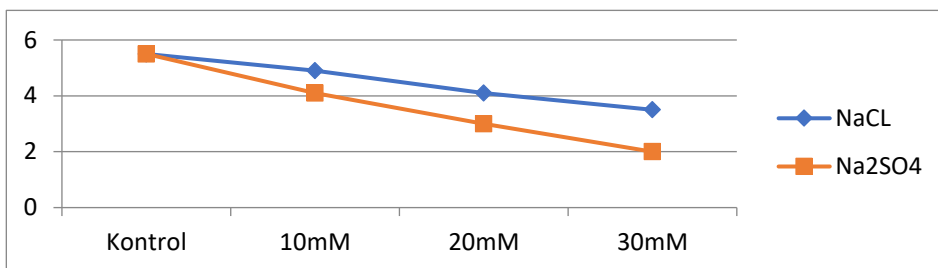
**Figure 1. Germination percentage of bean seeds at different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> in water culture**



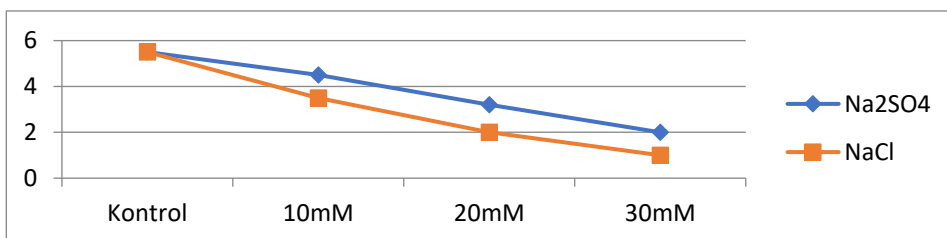
**Figure 2. Germination percentage of bean seeds at different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> in soil culture**

As can be seen, the germination percentage of seeds decreased in both water (10-30mM) and soil cultures (0.2-0.6%) with the enhancing concentrations of sodium salts.

Although Na<sub>2</sub>SO<sub>4</sub> has a stronger effect on the germination percentage of bean seeds than NaCl in water culture, the opposite picture is observed in the soil culture. Thus, the germination percentage decreased more with the increasing NaCl concentrations.



**Figure 3.** The height (cm) of 5-day-old bean seedlings germinated in water culture at different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub>



**Figure 4.** The height (cm) of 5-day-old bean seedlings germinated in soil culture at different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub>

Salt stress also affects the growth parameters of 5-day-old seedlings of bean plants cultivated in water and soil cultures. Thus, under the effect of both salts, a significant reduction in the studied morphological parameters occurred in both water and soil cultures. According to literature data, salinity has a negative effect on the formation of morphological indicators of the plant and reduces seed germination (Besaliyev, 2021).

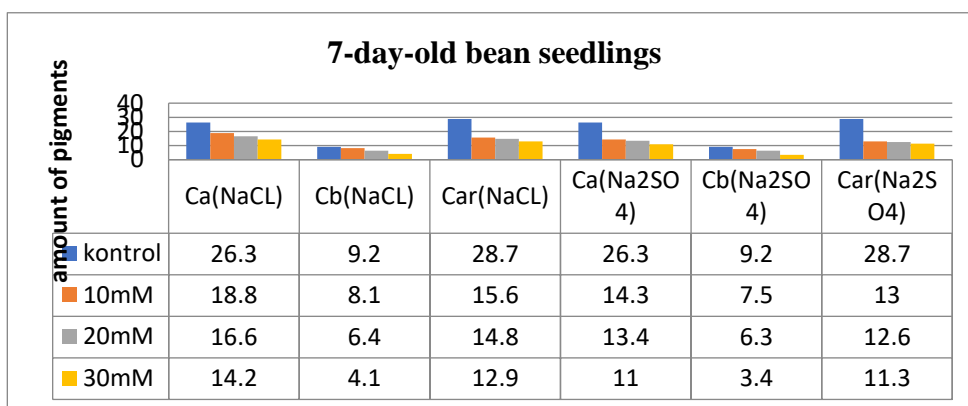
It should be noted that Na<sub>2</sub>SO<sub>4</sub> salinity in water culture especially reduces the growth indicators. As seen in Figure 3, in water culture, SO<sub>4</sub><sup>4-</sup> ion affects the growth indicators of the bean plant more than Cl<sup>-</sup>, while in soil culture, the opposite picture is observed (Figure 4).

It is known from the literature that stress factors, including salt stress, reduce the intensity of photosynthesis in plants (Li, 2010). The excess amount of Na<sup>+</sup>, SO<sub>4</sub><sup>4-</sup>, and Cl<sup>-</sup> ions in the cell affects the photosynthesis pigments, causing the disintegration of

the granules, the disruption of carbon metabolism, and the photophosphorylation processes.

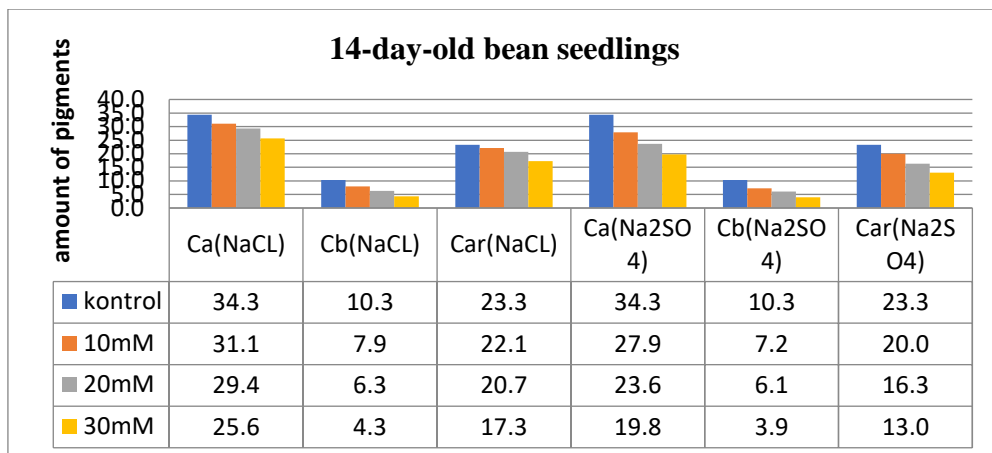
Since the ability of plants to tolerate salt depends on the intensity of photosynthesis, the determination of photosynthesis pigments in plants affected by salt stress is considered one of the important criteria. It should be noted that there are conflicting opinions in the literature about the salt-induced changes in the amount of photosynthetic pigments in plants (Li, 2010). Thus, according to some scientists, the amount of photosynthetic pigments in plants decreases under salinity conditions (Garifzyanov, 2006; Strogonov, 1970), while others stated that it increases or is not changed (Kusakina, 2003; Udovenko, 1977).

Our research revealed that the amount of pigments decreased sharply in 7-day-old bean seedlings grown in water culture with the enhancing (10-30 mM) concentrations of both salts (NaCl, Na<sub>2</sub>SO<sub>4</sub>). Thus, under the influence of 30 mM NaCl, the amount of chl a decreased approximately 1.8-fold, the amounts of chl b and carotenoids decreased 2.2 fold, while 30 mM Na<sub>2</sub>SO<sub>4</sub> caused 2.3, 2.7, and 2.5-fold decreases in the amount of chl a, chl b, and carotenoids, respectively (Figure 5).



**Figure 5. Amounts of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) in 7-day-old bean seedlings grown in water culture**

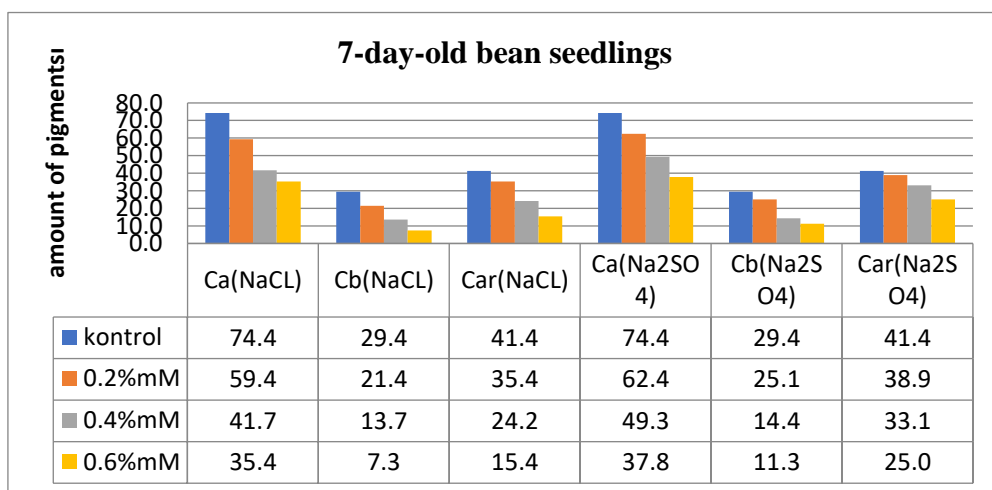
In 14-day-old bean seedlings, the amount of pigments decreased with increasing salt concentrations (10-30 mM) (Figure 6). Thus, 30 mM NaCl caused approximately 1.3, 2.3, and 1.3-fold decreases in Chl a, Chl b, and carotenoids, respectively. Whereas, at 30 mM Na<sub>2</sub>SO<sub>4</sub>, the same parameters decreased 1.7, 2.6, and 1.7-fold, respectively.



**Figure 6. Amounts of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) in 14-day-old bean seedlings grown in water culture**

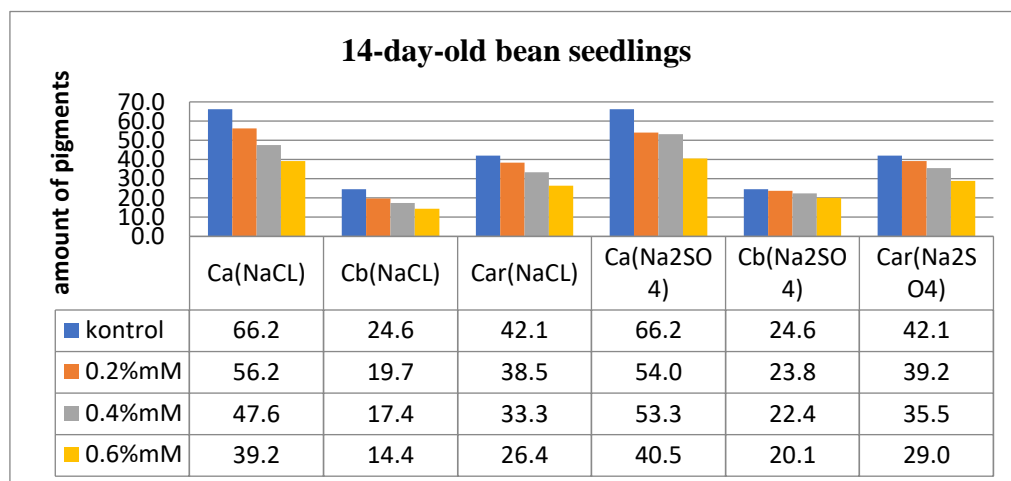
It should be noted that although both salts reduced the amount of pigments, the negative effect of Na<sub>2</sub>SO<sub>4</sub> was higher: SO<sub>4</sub><sup>2-</sup> > Cl<sup>-</sup>

A similar situation is manifested in soil culture. The amount of pigments decreased sharply with increasing concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub>(10-30mM). At 30 mM NaCl, Chl a, Chl b, and carotenoids decreased approximately 2.1, 4.0, and 2.6-fold, respectively, in 7-day-old bean seedlings. While at 30 mM Na<sub>2</sub>SO<sub>4</sub>, the respective parameters decreased 1.9, 2.6, and 1.6-fold (Figure 7).



**Figure7. Amounts of chlorophyll a (Chl a), b (Chl b), and carotenoids (Car) in 7-day-old bean seedlings grown in soil culture.**

In 14-day-old seedlings, 30 mM NaCl caused approximately 1.6, 1.7. and 1.5-fold decrease in the amounts of Chl a, Chl b, and carotenoids, respectively. While at 30mM Na<sub>2</sub>SO<sub>4</sub>, these parameters decreased 1.6, 1.2, and 1.4-fold, respectively (Figure 8).



**Figure8. Amounts of chlorophyll a (Cl a), b (Cl b), and carotenoids (Car) in 14-day-old bean seedlings grown in soil culture.**

According to the obtained results, the inhibitory effect of different concentrations of the isocation salts (NaCl and Na<sub>2</sub>SO<sub>4</sub>) on the amount of photosynthetic pigments in 7- and 14-day-old bean seedlings was high in sulfate-type salinity in soil culture and chlorine-type salinity in water culture.

The amount of Chl a and Chl b decreased more in both water and soil cultures, under the effect of both salts.

High concentrations of Na<sup>+</sup> and Cl<sup>-</sup> ions in soil culture cause osmotic stress in plants due to a sharp decrease in the water potential of the root environment, and their excessive entry into cells changes the ion balance and leads to the disruption of many physiological and biochemical processes (Munns, 2008).

It should be noted that a shortage of water does not occur in plants grown in water culture.

In general, the water potential in saline soil solutions is lower than the water potential of cells in plant roots. Water absorption by plants decreases sharply in saline soils (Kuznetsov, 2017). It should be noted that NaCl combines with soil particles and



accelerates water absorption in the soil. Thus, the potential of water in the soil solution is sharply reduced and a water deficit occurs in plants, as a result of which plant growth, development, photosynthesis, and other physiological processes weaken sharply.

## References

- Ashraf, M.** (1994). Breeding for salinity tolerance in plants. *Crit.Rev. Plant Sci.*, V.13,pp. 17-42
- Besaliev, I.N., Panfilov, A.L., & Reger, N.S.** (2021). Peculiarities of the formation of morphological traits of seedlings and the activity of spring common wheat enzymes against the background of induced salt stress. *Animal Husbandry and Fodder Production*. 104(3).
- Flowers T.** 2004. Improving crop salt tolerance. *Journal of Experimental Botany* Vol. 55. pp. 307-319.
- Flowers, T.J., & Colmer T.D.** (2008). Salinity tolerance in halophytes. *New Phytol.* 179. pp. 945–963.
- Gamalero, E., Berta, G., & Bernard, R.G.** (2009). The use of Microorganisms to Facilitate the Growth of Plants in Saline Soils. *Microbial Strategies for Crop Improvement*. Berlin Heidelberg. Springer. 1-22.
- Garifzyanov, A.R., & Gorelova, S.V.** (2006). Oxidative stress and plant tolerance. *Tula Ecological Bulletin*. Issue 2, 364–368.
- Isanova, G.T. et. al.** (2017). Saline soils and determination of the province of salt accumulation on the territory of Kazakhstan. *Arid ecosystems*. Vol. 23. No. 4(73). 35-43.
- Ivanishev, V.V., Evgrashkina, T.N., Boykova, O.I., & Zhukov, N.N.** (2020). Soil salinization and its effect on plants. No. 3, 28-42.
- Khitrov, N.B., Rukhovich, D.I., Kalinina, N.V., Novikova, A.F., Pankova, E.I., & Chernousenko, G.I.** (2009). Estimation of areas of saline soils on the territory of the European part of Russia (according to the electronic version of the map of soil salinity at a scale of 1:2.5 million) . *Soil science*. 6, p. 627.
- Kovda, V.A.** (2008). Problems of desertification and salinization of soils in arid areas of the world. M.: Nauka. p.415.
- Kumakov, V.A.** (1995). Physiology of Spring Wheat Yield Formation and Breeding Problems. *Agricultural biology*. 30(5), p. 56.
- Kusakina, M.G., Yeremchenko, O.Z., & Chetina, O.A.** (2013). Influence of technogenic salts on the pigment system of plants growing in the zone of influence of salt tailings piles. *Protection of the natural environment and ecological and biological education: materials of the III All-Russian scientific-practical conference with international participation, Yelabuga*, pp. 60–63.

- Kuznetsov, V. V., & Dmitriyeva, G.A.** (2017). Physiology of plants. Yurayt Publishing House. p. 437.
- Lakin, G. F.** (1990). Biometrics, M.: Visshaya shkola, p. 293.
- Li, Z., Baldwin, C.M., Hu, Q., Liu, H., & Luo, H.** (2010). Heterologous expression of Arabidopsis H<sup>+</sup>-pyrophosphatase enhances salt tolerance in transgenic creeping bentgrass (*Agrostis stolonifera* L) plant. Cell Environ. 33, 272-289
- Munns, R., & Tester M.** (2008). Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59, 651–681.
- Pilipenko, V.N., Yakovleva, L.V., & Fedotova, A.V.** (2005). Current state of saline soils in the Volga delta . Fundamental research. No. 8, 145-149.
- Strogonov, B.P. et al.** (1970). Structure and functions of plant cells under salinity. M.: Nauka Publishing House, p.318.
- Tretyakova, N.N.** (1990). "Workshop on plant physiology" Moscow, 90 p.
- Udovenko, G.V.** (1977). Salt tolerance of cultivated plants. L.: Kolos. p.215.
- Zhuchenko, A.A.** (1994). Strategy for adaptive intensification of agriculture (concept). Pushchino: ONTI PNTs RAN, 148 p.