# EARLY EFFECT OF NaCl TREATMENT ON THE PROTEIN CONTENT IN THE SEEDLING OF THREE WHEAT CULTIVAR

**LĂCRĂMIOARA OPRICĂ**[1*]

**Keywords:** soluble protein, wheat seedling (Faur, Iasi 2, Fundulea) cultivar
**Abstract:** The wheat (*Triticum aestivum*), is one of the most important crops in the world and plays a special role human nutrition. Salt stress has became a severe global problem in the last decades and salinity is one of the most important abiotic factors limiting plant growth and yield, affecting morphological, anatomical, biochemical and physiological characteristics in plants. One of the biochemical parameters affected by salinity is protein synthesis. Therefore, the aim of this study is to investigate the NaCl treatment effect on protein content of three seedlings wheat cultivars (Faur, Iasi 2, Fundulea). The results evidenced that saline treatment determined modifications of soluble protein content at the early stages of seedling development depending on: the type of cultivar, the intensity of salt exposure (50mM, 100mM, 150mM and 200mM) and the age of seedling.

## INTRODUCTION

The biotic stress (diseases and pests) and abiotic stress (extreme temperatures but, especially salinity and drought), are the significant primary causes producing crop loss worldwide. Tolerance to abiotic stresses is very complex, due to the interactions between harmful factors and various molecular, biochemical and physiological phenomena affecting plant growth and development. These stress conditions can be detrimental to the plants because they are reduced in their yield in comparison to their potential yield in normal plants (Graiphenberg *et al*., 2000; Yokoi *et al*., 2002). Drought and salinity, two oldest enemies of agriculture, are capable to triggering changes in the plant metabolism. Generally, the environmental stress and in particulary the saline stress, involve o number of mecanisms like change of ion homeostasis and osmotic balance, synthesis and accumulation of many organic osmolytes like proline, betaine, poliamines, soluble sugars (presumed to be osmoprotectants) and proteins (Grigore et al., 2011, Matthew and Hasengawa, 2005, Hasegawa et al., 2000, Duca & Bârsan, 2001), an increased respiration rate, ion toxicity (Sudhir and Murthy, 2004), changes in C and N metabolism (Kim et al., 2004), decreased biosynthesis of chlorophyll (Khan, 2003) and inefficiency of photosynthesis (Munns, 2002). In addition, plants employ biochemical and molecular mechanisms to cope with salt stress such as induction of antioxidative enzymes (Zhu, 2002, Parida & Das, 2005).

One of the cellular acting adaptive-protective reactions to salinity stress is protein synthesis (Blehman, 1987; Levit, 1972). Several results highlight notable quantitative modifications in the polypeptide complex of the cells under the stress of long and short duration. Some researchers have identified quantitative changes of protein content in the presence of different types of stress and shown, in the most cases a decrease of this (Al-Aghabary et al., 2004; Alamgir et al., 1999; Gadallah, 1999; Parida and Das, 2005; Parvaiz and Satyavati, 2008; Wang and Nil, 2000). This decrease of protein content in plants is explained by blocking the processes of biosynthesis and activating hydrolytic reactions which under stress conditions dominate the processes of biosynthesis. Other researchers found in the vegetal tissues (cultivars of barley, sunflower, finger millet, and rice) an increase of protein content under the exposure of saline conditions (Ashraf and Harris, 2004). It seems that the growth process is repressed much strongly than the processes of protein synthesis under salinity conditions. Agastian et al. (2000), have reported that soluble protein increases at low salinity and decreases at high salinity in mulberry cultivars.

This aim of the present study was to study the effect of the NaCl treatment on the early stages of four wheat seedling cultivars (Fundulea, Iaşi 2, Faur).

## MATERIAL AND METHODS

The research was performed on seeds of Fundulea, Iasi 2 and Faur wheat cultivars obtained from the Research Station Podul Iloaiei. Firstly, the seeds from each wheat cultivar were sterilized in 3% $H_2O_2$ and then washed thoroughly to remove $H_2O_2$. Approximately 100 seeds were subjected to the treatment with four concentration of NaCl (50mM, 100mM, 150mM and 200mM), for 4 hours. Control seeds were stored for 4 hours in distilled water. The seeds were then transferred into sterile Petri dishes, containing 2 layers of Whatman 1 paper, imbibed with 10 ml distilled water. Each Petri plate contained 100 seeds and three rehearsals for each variant were carried out. The Petri dishes were kept in dark, at $25^0$C to promote the germination. After that, the Petri plates were transferred in a room assuring the normal conditions for seedlings growth. In order to determine the proteins content and to measure the roots length the seedlings were randomly harvested at 96h, 168h and 240h.

The quantitative determination of proteins was made on the seedling of three wheat cultivars (Fundulea, Iași 2, Faur). To realize the extraction of soluble proteins, the biological material was firstly weighed and well homogenized, then treated with 50mM Tris-HCl buffer, pH – 7.0 (5mM dithiothreitol, 10 mM ascorbic acid, 6mM cystein, 1mM EDTA, 0,1% Tritonx100). After centrifugation at 3000 x g, for 15 minutes, the supernatant was used to determine the protein level by protein dye binding method based on Bradford method, using serum albumine (Sigma-Aldrich) as standard (Bradford, 1976). The results are expressed in mg% fresh weight.

## RESULTS AND DISCUSSIONS

Roots play a number of important roles during plant growth and development and are typically the first part of the plant to encounter salinity. In glycophytes, the root is the primary site of salt stress and the ability to maintain ion homeostasis and redox potential is critical for the normal root growth and function under saline stress (Greenway and Munns, 1980; Hasegawa *et al*., 2000).

The effect of NaCl treatment (50mM, 100mM, 150mM and 200mM) on root size of wheat seedlings (Fundulea, Faur, Iasi 2) cultivars is shown in Figure 1. At 96 hours after treatment the roots lengh of seedlings Fundulea cultivar was not significantly affected by NaCl treatment compared with the control. For the following two intervals, 168h and 240h respectively, 200 mM NaCl concentrations is inhibited. The 150 mM concentration caused a stimulation of root lengh at 240 h while the effect was inhibitory at168 h.

For the Iasi 2 cultivar the root lengh of seedlings ranged, generally, identical at the three selected time intervals (96h, 168h and 240h). Thus, 50 mM and 150 mM concentrations lead the higher values of root lengh compared with the control, while 100 mM and 200 mM concentrations showed low values.

The NaCl treatments influenced differently the roots lengh of Faur cultivar. Thus, 50mM concentration cause a stimulation of root lengh compared with control at the three studied time intervals. Unlike the other two cultivars Fundulea and Iasi 2, the 100 mM and 150mM NaCl concentrations lead at Faur cultivar the root lengh compared with the control. It is surprising that compared with the control the 200 mM concentration provides a root lengh stimulating development which was not observed for the other two varieties.

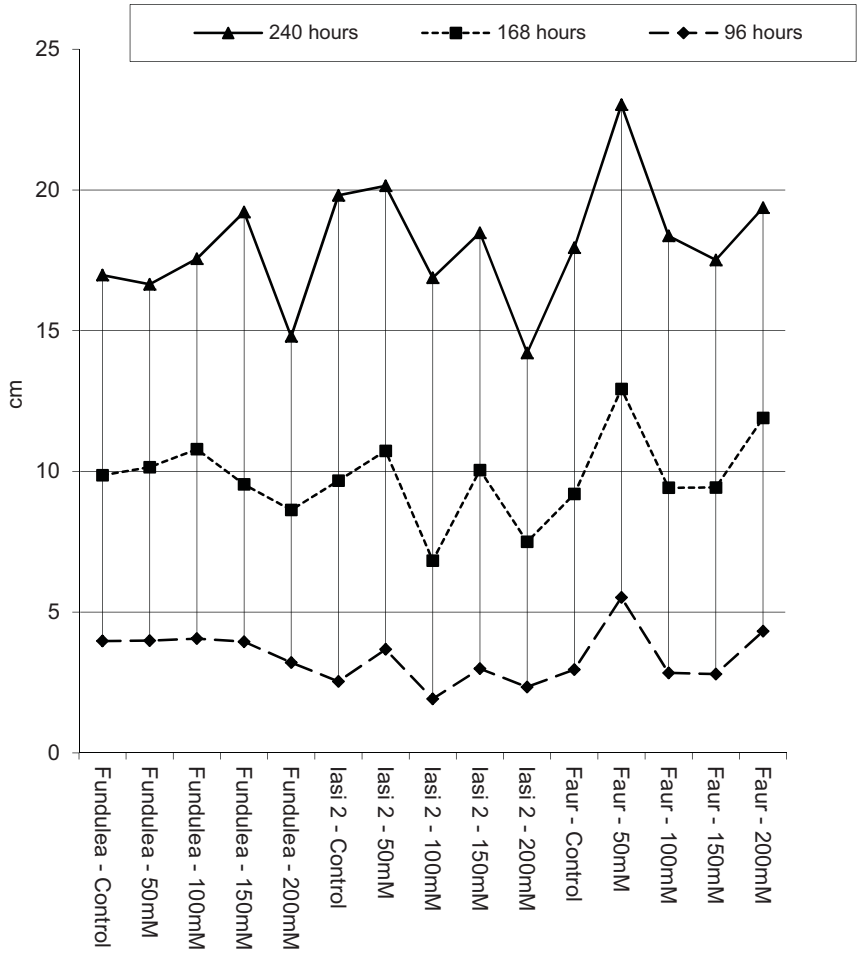


Figure 1. Dynamic of lengh roots wheat seedling cultivar after the NaCl treatments

The protein amount determined at 96 h after NaCl treatment in wheat seedling Fundulea cultivar (Figure 2) is relatively constant compared with control. However the concentration 200 mM induced slightly higher value. At 168 h is noted that all NaCl concentrations used determine an increase in the protein content comparatively with control. The same effect for all NaCl concentrations, observed at 240 h, is the stimulating of protein amount. As the plants have grown, the proteins content increase with the increase of NaCl concentrations. Similar results have been recorded by the other authors who find an increase of protein content in the vegetal tissues especially under the action of high concentration of salts (Duca and Barsan, 2001). It is supposed that this phenomenon is determined by the concentration of proteins in the cells because the processes of growth are repressed much strongly than the processes of protein synthesis under salinity conditions (Duca and Barsan, 2001).

Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later (Singh et al. 1987) and may play a role in osmotic adjustment.

They may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration (Pareek-Singla and Grover 1997). It has been concluded that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells (Hasegawa et al. 2000).

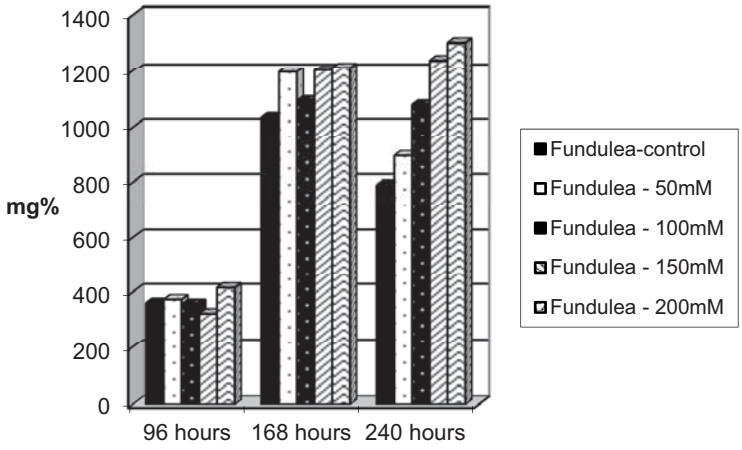


Figura 2. The variation of protein amount in wheat seedling Fundulea cultivars after NaCl treatment

In wheat seedling Iasi 2 cultivar the amount of protein after 96 h treatment, is lower than the control for the variants treated with 50mM, 100 mM and 150 mM NaCl concentration but increased slightly in 200 mM (Figure 3). The protein degradation under saline environment has been attributed to the decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acid and denaturation of enzyme involved in protein synthesis (Levitt 1980).

Compared to control the protein content in wheat seedlings at 168h is higher for all used concentrations. The pattern is an increase of protein biosynthesis with increasing NaCl concentration (excepting the 200 mM treatment); the highest protein content is ascertained at 150mM treatment. At 240 h the amount of protein content was different, with larger or smaller amplitudes. Thus, the amount of protein was relatively constant at concentrations of 50mM, 100mM and 200 mM NaCl, but less than the control.

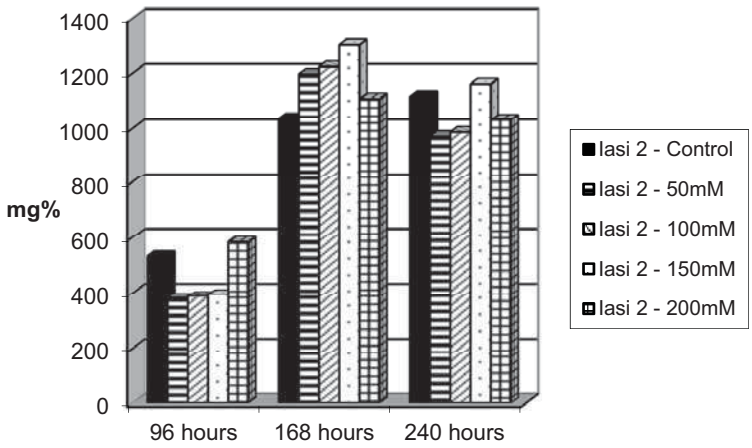


Figura 3. The variation of protein amount at wheat seedling Iasi 2 cultivar after NaCl treatment

Analysis of the results on soluble protein content in wheat seedlings of the Faur cultivar after treatment with NaCl show that at the first interval it is approximately two-fold reduced at 50 mM NaCl, respectively, 100 mM concentrations (Figure 4). For the second interval after treatment there is a tendency in increasing protein content at higher concentrations of NaCl. For the last determination (240 h) it is found that generally, the trend is the decrease of protein content excepting the salt treatment with 100mM.

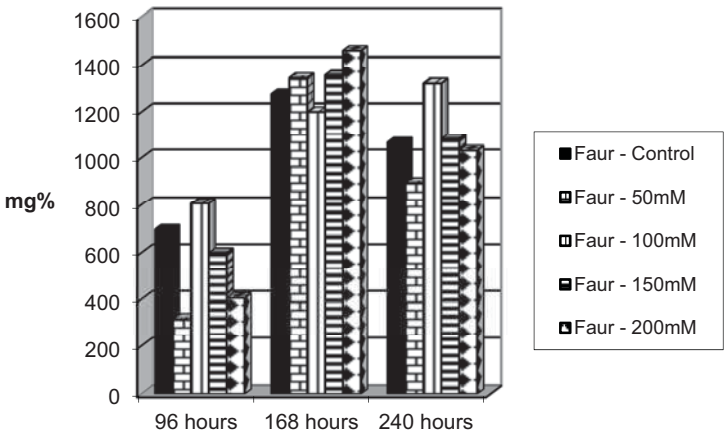


Figura 4. The variation of protein amount at wheat seedling Faur cultivar after NaCl treatment

## CONCLUSIONS

The results of this study suggest that the variation on protein content of seedling wheat (Faur, Iasi 2, Fundulea) cultivars exposed to treatment with NaCl is different, depending on the cultivar, the concentration of NaCl (50mM, 100mM, 150mM and 200mM) and the age of seedlings.

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1 Faculty of Biology, „Al. I. Cuza” Iasi
* lacramioara.oprica@uaic.ro