ABSTRACT

Key words: Lycopersicon esculentum, tolerance, salinity.

The areas affected by salinity in excess are large, being recorded over 200 million ha across the planet and about 400,000 ha in Romania. Soil salinization is a process frequently encountered here and elsewhere affecting crops (Koyro *et al.*, 2009).

The current population of the Earth is about 6.7 billion inhabitants and it is estimated that will grow beyond 8 billion in 2020, which will worsen the current scenario on food insecurity (Athar *et* Ashraf, 2009).

Taking into account the world water crisis, and the fact that from year to year areas with saline soils are more expanding, it is also important to find new ways for the valorification of seawater amazing power and to identify and adapt plant species capable of growth and bring benefits for agriculture in conditions of high salinity. Tomatoes in our country occupies an area of 44,000 ha, of which 40,200 ha in the private sector. Tomatoes have high nutritional value due to the fruit content in vitamins, sugars, minerals, amino acids and organic acids (Munteanu, 2003).

Of environmental stressors, salinity remains the main factor that calls into question the future of agriculture. Salinity and especially the anthropogenic salinity, is a result of mismanagement of irrigation.

The main purpose of this study is a better understanding of the physiological and biochemical mechanisms involved in *Lycopersicum esculentum* species tolerance to salt stress, but the identification of tomato local population salinity tolerant from the N-E Romania. These forms may be used for the improvement of morphological characteristics involved in the production capacity or resistance to diseases, and in the process of grafting of the tomatoes.

The PhD thesis contains seven chapters 238 pages, 40 tables and 106 figures is structured in two parts:

Part I contains bibliographic data and studies about: biological and ecological characteristics of tomatoes, informations about the concept of stress tolerance of plants to unfavorable environmental conditions, effects of salinity on tomatoes and general issues about

soil salinity of Moldavian Plain. This part is written on 49 pages, representing 26.34% and comprises 5 figures and 8 table.

Part II is spread over 137 pages (73.65%) which contains .101 figures and 32 tables. This part contain results of my own research on the subject of the thesis.

Biological material was collected from the following localities: Copălău, Dorohoi, Durneşti, Mileanca from Botoşani County and Balş, Şcheia, Moşna, Osoi from Iaşi County. After a pre-testing in the seedling stage of 30 genotypes originally collected, 10 genotypes were selected as the most resistant to salt stress conditions. Genotypes collected from localities Copalău, Dorohoi, Şcheia and Moşna were remained for research phase.

For the collection was kept in mind that chosen material represent a local population and not a commercial variety.

The methodology for testing the physiological reaction to salinity, which formed the basis of the research contains the following steps: preparing the soil, preparing seed culture, culture substrate preparation, seedling production, earthworks, preparation and application of saline solutions.

Research methods used for testing of saline stressors on tomato genotypes were: determination of growth and development process by means of biometric and gravimetric analysis, of physiological and biochemical processes which addresses issues of water regime, of photosynthesis process, and the accumulation of a metabolic compounds such as proline, ascorbic acid and NaCl.

The research was conducted during 2013 - 2015, in USAMV Iaşi, Faculty of Agriculture Laboratory of Plant Physiology, and experiences were placed in the greenhouse of Phytotron USAMV, Didactic Station ,, Vasile Adamachi "- Farm Adamachi, localized in cadastral territory of Iaşi.

Bifactorial experience was realized in vegetation pots and was organized as randomized blocks with three repetitions. The two factors that influenced the course of the experiment were: factor A, represented by the three concentrations of NaCl (V0 - water, V1-100 mM NaCl - V2-200 mM NaCl) and factor B represented the studied genotypes.

In the first year of experimentation they were studied 396 tomato plants, and next year was made a selection of genotypes resistant to salt stress, studying 252 tomato plants.

In 2013-2014, were studied 11 tomato genotypes, which were subjected to biochemical and physiological tests they watched to determine the intensity of the growth process through direct analysis of aerial and underground organs, photosynthetic pigments concentration analysis, dry substance content and the moisture content at the leaf level. These measurements aimed at highlighting the response to salt stress.

Genotypes identified as resistant to salinity, were utilized for the second year of experimentation 2014-2015.

The determination of saline stress effect on the growth and development process and on the physiological and biochemical processes have been carried out in two stages: at 15 and 30 days after the saline treatment.

Measurements of stem height were realized at 15 or 30 days after saline treatment application, observing that this genetic conditionated character was influenced by salt stress. In the first year of research, after application of saline treatments it was found that genotype *Copălău 4* recorded the best growth rate, the difference between control and variant treated with saline solution beeing 3 cm.

In the second year of research, was recorded the best growth rate in conditions of salinity at *Copălău 4, Copălău 5* and *Moşna 3* genotypes. Leaf area is the major physiological index that characterizes intensity of the growth, perspiration, respiration and photosynthesis processes.

The effect of salinity on growth of foliar system was assessed by determining the foliar area from third of basal, middle and upper stem using portable device Area Meter AM 300 - 0002, ADC Bioscientific Ltd.

In the first year of experimentation leaf area decreased proportionally with increasing of saline concentrations, manifesting differently depending on the insertion place of leaves: on the base, middle or top of stem. Leaf growth at the top of the stem was most affected by saline excess in all cultivars studied compared to the control.

In the second year of experimentation, comparing results after 15 and 30 days of the saline treatment application to salinity tolerant genotype (*Ursula F1*), we note that local populations *Moşna 2*, *Copălău 3* and *Copălău 4* showed greater leaf area values, which shows that the cultivars has good resistance to high concentrations of NaCl.

The best growth rate of root system presented local genotype *Copălău* $_5$, both years of experiementation. Statistical analysis showed that root weight was significantly influenced by genotype and NaCl concentration.

Regarding the length of roots, saline solutions have occured changes compared to control, depending on saline concentration. In the two years of experimentation three genotypes *Copălău* $_3$*, Copălău* $_4$*, Copălău* $_5$*, Moşna* $_3$ have presented higher values of root length compared to control, this fact demonstrating a high degree of adaptability to saline stress.

The comments made on the number of flowers showed that with increasing of salt concentration decreases the number of flowers per plant, both after 15 days and 30 days of saline treatment application. Genotypes *Copălău* $_3$ and *Moşna* $_3$ were least affected by salinity, they recorded similar values in the second year of experience too.

Fruiting evaluation was carried out through the determination of biometrical indices such as the number of fruit/plant, average weight of fruit/plant, the production of fruit/plant.

Number of tomato fruits exposed to saline stress for a period of 30 days decreased compared to control, in all genotypes wetted with 100 mM and 200 mM saline solution.

In the genotypes studied, 100 mM and 200 mM NaCl solutions negatively influenced fruit weight compared to control, it oscillating between 29.8 g/fruit and to 51.7 g/fruit.

On tomato genotypes after 30 days under saline stress it is found that the production of fruits was significant distinct influenced by the genotype and highly significant by the NaCl concentrations. It was noted in particular that tomato yield of *Copălău* local populations was less affected compared to yields of genotype tolerant to salinity.

Water is essential to plant life, having an essential physiological role. At the cellular level water is the dispersion medium of plasmatic colloids and provides cell turgor. Water plays thermoregulatory role, promotes the absorption and transport of mineral elements, controlling the diffusion of CO2 and O2 by opening and closing movements of stomata in the process of photosynthesis.

Liquid water is the major component of cells being present the maximum amount in vacuoles and minimum amount in the cell organelles. Water is in the form of free water and bound water.

Analysis of free water content after 30 days of saline treatment application highlights a lower concentrations compared to control for all genotypes from both 100 mM and 200 mM

concentrations, which suggests that reducing of free water content increases the biological capacity of plants tolerance to abiotic stress conditions.

Under stress conditions when the plant vital activity is reduced, there is an increase of the amount of bound water, which increases the resistance of plants. From this point of view cultivars: *Moşna 3, Copălău 3* and *Moşna 2* shows a higher degree of adaptation because of a large amount of bound water, assimileted under the conditions of a high concentration salin stress (200 mM).

The pace of dehydration of the leaf depends on the morpho-anatomical and physiological characteristics of the foliar limb and may be an indication of the perspiration intensity. Plants with a better resistance to salinity are characterized by a lower cuticular sweating and speed dehydration of leaves and we can say that the studied genotypes shows a good ability to adapt to saline stress conditions over a long period of time because they recorded a lower speed of dehydration compared to control, with the application of 200 mM NaCl saline solution.

Results related to the dry matter content of leaves showed very significant differences in terms of genotype and the concentration of NaCl. These differences show that genotypes under saline stress have begun a series of adaptive mechanisms to saline stress.

Foliar pigment content analysis was performed with the spectrophotometric method, which allows testing of pigments with absorption in the visible spectrum between 400-700 nm and the near UV at 330-400 nm respectively, and using a clorofilometer.

From the results obtained by analyzing the content of chlorophyll pigments resulted that tomato genotypes studied are part of biphasic model proposed by Munns (1993). The duration of the transition from osmotic stress phase to that of ions toxicity is based on the intensity of saline stress, especially depending of cultivated genotype.

The high level of flavonoid pigments in local populations leaves exposed to salin stress demonstrate a good ability to adapt to this type of abiotic stress conditions.

Foliar stomatal conductance of tomatoes was determined using porometer. Both readings after 15 days and after 30 days after application of saline treatment showed values generally lower compared to control, except *Moşna 2* genotype 200 mM variant.

Proline show protective effect against NaCl salinity and exhibit antistress activity in particular by the antioxidant effect. Analysis of proline concentration after 30 days of saline

treatment application show that, with increasing salt concentration increases the concentration of proline in plants too, the values ranging from 0.94 to 12.40 nmol.

Compared to control, the resistant to salinity genotypes selected in the first year of testing (*Moşna $_{2}$, Şcheia $_{1}$, Copălău$_{3}$, Copălău $_{4}$, Copălău $_{5}$, Moşna $_{3}$, Ursula $_{F1}$*) have recorded elevated proline content in the second year of experimentation, which allows us to appreciate that these local populations of tomatoes presents adaptive mechanisms for salinity.

The content of Na^{+} analyzed shows that the increased concentration of salts lead to increases of Na^{+} concentration in leaves and the absence of characteristic symptoms of foliar toxicity shows that plants are able to support these high salt concentrations. The mature leaves have a capacity to accumulate Na^{+} and thus ensure meristematic cells protection.

Cl^{-} content values are significantly higher compared to control on 200 mM saline-stressed genotypes. Maximum value is 5.48 mg for *Copălău 5* genotype and minimum value is 2.68 mg for *Moşna 2* genotype.

Vitamin C or ascorbic acid is an organic acid with antioxidant properties, involved in a number of processes taking place in living cells. In tomato fruits, salt stress favored the synthesis of ascorbic acid based on the strength so that, compared with the control, the concentration of 100 mM NaCl resulted in an increase of 1.36 - 7.34 times the concentration of ascorbic acid, and the 200 mM 4.77 - 12.50 times.

Plants by efforts to adapt to salt stress, are forced to increase antioxidant levels (Ehret *et al*., 2013), mainly by increasing the content of ascorbic acid, which is found in our determinations.

More and more research on the effect of salinity on the tomato demonstrate higher levels of antioxidants in the fruit. Antioxidants have many beneficial effects on the human body of which the most important would be the ability to fight cancer and heart disease. Considering these aspects, the study on the physiological and biochemical salt stress of tomato genotypes is an important step in identifying and using ameliorative purpose not only of this important vegetable species.