# THE INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI ON TOLERANCE TO SALINE STRESS OF TOMATOES

## INFLUENTA FUNGILOR ARBUSCULAR MICORIZALI ASUPRA TOLERANTEI LA STRESUL SALIN LA TOMATE

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***Abstract.*** *A Romanian tomato landrace was tested for tolerance to saline stress in pot culture. To improve the tolerance of plants to osmotic stress, we tested the effect of inoculation with arbuscular mycorrhizal fungi. Plants were assessed before and after application of saline solution for chlorophyll and dry matter content. To test the level of tolerance to salinity, we extracted the proline from plants. Results show that arbuscular mycorrhiza determined a significant reduction of chlorophyll content in normal growing conditions, but after the stress was induced, the differences between mycorrhized and non-mycorrhized plants reduced and differences were not statistically different. Dry matter content was not significantly influenced by any factor. After application of NaCl solution, non-mycorrhized plants had the highest proline content, meanwhile the mycorrhiza alleviated the level of osmotic stress.*
***Key words:*** *tomato, saline stress, tolerance, proline*

***Rezumat.*** *O populație locală de tomate din România a fost testată la toleranța la stresul salin. Pentru a îmbunătăți toleranța la stresul osmotic la plante, am testat efectul inoculării cu fungi arbuscular micorizali. Plantele au fost evaluate înainte și după aplicarea soluției saline, măsurând conținutul de clorofilă și de substanță uscată ale acestora. Pentru a testa nivelul toleranței la salinitate, a fost extrasă și cuantificată prolina din plante. Rezultatele arată că micoriza a determinat o reducere semnificativă a conținutului de clorofilă în condiții normale de creștere, însă după aplicarea stresului, diferențele între plantele micorizate și nemicorizate a scăzut, acestea fiind nesemnificative. Conținutul de substanță uscată din frunze nu a fost influențat semnificativ de niciunul dintre factorii experimentali testați. Conținutul de prolină a crescut datorită aplicării stresului salin. După aplicarea stresului plantele nemicorizate au prezentat cel mai ridicat conținut de prolină, micorizarea ducând la o reducere ale efectelor salinității.*
***Cuvinte cheie:*** *tomate, stres salin, toleranta, prolina*

## INTRODUCTION

Agricultural production is facing several obstacles due to the influences of

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biotic and abiotic stress factors. Drought, floods, lack of nutrients, various pests and diseases can compromise yield and thus conduct to bankruptcy of farmers or even to famine in parts of Earth where food is insufficient. Our century started with frightening statistics: the summers are drier and hotter than ever, extreme temperatures are more frequent, the average global temperatures are increasing and the polar ice already started to melt. The human population is alarmingly increasing and agriculture is constantly looking for ways to assure the massive quantities of food necessary for almost 7 billion people. Intensive agriculture produced soil erosion, salinization of agricultural areas and desertification. Salinization of soils is not common only in coastal areas. Due to intensive agriculture, especially because of irrigation of agricultural lands, a high amount of salts accumulated in the soil. Worldwide, 800 millions of hectares of land are affected by soil salinization, meanwhile in EU 1 million hectares are affected (FAO, 2008).

Most of cultivated species, including tomatoes (*Lycopersicon esculentum* L.) are sensitive to saline stress (Agong *et al.,* 2003; Flowers and Yeo, 1995).

High concentration of salts in soil causes osmotic stress to plants. More, $Na^+$ accumulates in shoots (Pardo and Quintero, 2002) and produces metabolic toxicity by disturbing cytoplasmic enzymatic processes.

Plants use a series of different mechanisms to alleviate the negative effect of osmotic stress: inhibition of growing in above-ground organs, with the growing of radicular system (Creelman *et al.,* 1990), osmotic regulation, modifications of metabolic flux, lignifications of cell walls reduction of growing rate, modifications of biomass phenology, leaf senescence, and finally, plant death (Munns, 2005).

It was also discovered that growth-promoting bacteria confer salt tolerance to plants, including tomatoes too (Mayak *et al.,* 2004). Cortés-Jiménez *et al.* (2014) suggested that microorganisms associated to tomato seedlings growing in saline culture act as osmoprotectant.

Once discovered, scientists are constantly looking for ways to fight the effects of salinity on cultivated plants. Developing tolerant cultivars is a promising but difficult solution, as genes for tolerance to osmotic stress are not naturally present in most of the cultivated species and most of the old cultivars, tolerant to many stress factors, are already forgotten and lost because modern hybrids which are now available on the market proved to be much more productive.

A new approach in agriculture is sustainability through biological solutions. More and more scientists are searching for symbiotic microorganisms which could play a role in protecting their host plants against the adverse conditions of the environment. As naturally plants and edaphic microorganisms evolved together, it proved that these microbes have a more important role in the physiology of plants that we presumed in the past.

Our study is only a step of an extensive research program that aims to collect, assess and preserve local landraces of vegetables in order to discover salt tolerant genotypes.

On the other hand, we aim to isolate salinity tolerant microbes from naturally saline areas and assess them for improving saline tolerance of cultivated plants when inoculated with these microbes.

In the present study we assessed the effect of inoculation with arbuscular mycorrhizal (AM) fungi on salt stress tolerance of a tomato landrace, simulating the cultivation conditions of normal farms. In most studies substrate is sterilized in order to show the effect of AM fungi on the physiology of host plants, but in farms real production conditions are different, as soil and peat are used in natural form, only sometimes fungicides are used in order to control the soil-borne diseases. Thus in this experiment we wanted to simulate farm conditions and to highlight the effects of AM fungi in "out of laboratory" conditions.

## MATERIAL AND METHOD

In the experiment we utilized tomato seeds from local landrace Tarnova 673, selected from our germplasm collection due to its characteristic large fruits. The name of the landrace derives from the village where it originates from.

20 seeds of each landrace were surface sterilized with sodium hypochlorite and germinated in Petri dishes on paper disks impregnated with distilled water. After germination, plantlets were transferred into 5 L pots with 2:1 peat:sandy soil mixture and half of the plants were inoculated with arbuscular mycorrhizal fungi (INOQ, Germany).

Plants were grown until the beginning of generative phenophase then watered with 100mM NaCl solution for 4 weeks. Before and after the saline stress the following parameters were measured: dry matter content of leaves using a KERN electronic moisture analyzer, chlorophyll content of leaves with a SPAD-502 chlorophyll meter (KONICA MINOLTA) and proline content of plants.

## RESULTS AND DISCUSSIONS

It was observed that before saline stress commenced, the mycorrhization produced a considering reduction of chlorophyll content of leaves. Non-mycorrhized plants showed values around 38-39 SPAD units, meanwhile inoculated plants had around 35 SPAD units, representing only 91.65% of the values of non-mycorrhized plants (Fig. 1). Also standard deviation shows that values are uniform, meaning that plants are quite homogenous in means of chlorophyll content of their leaves. A slightly higher diversity of values were recorded in case of mycorrhized plants, meaning that individual plants reacted differently to inoculation and symbiosis had various effects on their physiological processes probably due to the success of infection, the response of the plant to infection and the potential of fungi to behave parasitic (Johnson *et al.,* 1997).

Measurements after the application of stress factor showed that differences became more pronounced after flowering. Even control plants, which did not suffer by osmotic stress, had generally lower values but with a higher variation between individual measurements. The mean chlorophyll content was higher at non-inoculated plants (34.83 SPAD units at control plants and 35.93 SPAD units at plants watered with NaCl solution) than at mycorrhized plants (29.07 SPAD units at control plants and 28.67 SPAD units at stressed variant). In each case, after flowering the chlorophyll content of leaves decreased compared to plants in vegetative stage.

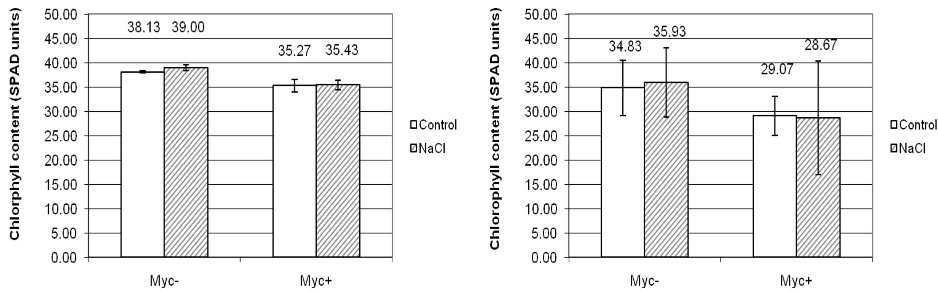


**Fig. 1 -** Chlorophyll conent of leaves before (left) and after (right) saline stress.

Dry matter content of leaves was slightly lower at non-mycorrhized plants before the application of saline stress. I

f compared to chlorophyll content, values show an opposite behaviour. Thus dry matter content is also higher at mycorrhized plants in vegetative stage (an average of 25.85% at non-inoculated plants and 28.23% at mycorrhized plants), meanwhile in generative stage, after stress application, the mycorrhized plants still show slightly higher values (non-mycorrhized plants: 21.40% at control plants and 19.45% at NaCl variant; mycorrhized plants: 21.98% at control plants and 21.63% at NaCl variant) (fig. 2).

As in case of chlorophyll content, in vegetative stage, standard deviation of values recorded for mycorrhized plants were higher, but in case of dry matter content, these deviations were comparable to those in generative stage, meaning they were very high.

If in vegetative stage values for mycorrhized plants were higher, in vegerative stage the mean values are very close, only the non-mycorrhized and NaCl variants had a lower mean value for dry matter content, but also with very high variation of individual values.

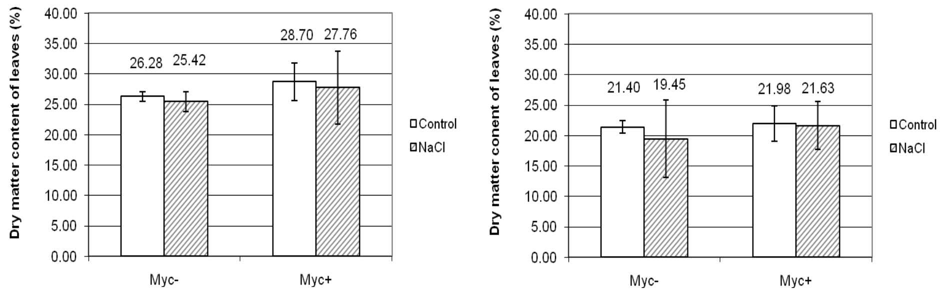


**Fig. 2 -** Dry matter content of leaves before (left) and after (right) saline stress.

Proline content of leaves indicates the level of a stress factor on plant physiology and also the level of plant response to stress (fig. 3). In case of non-mycorrhized plants, the values variated quite as aspected: control plants did not increase the level of proline content meanwhile the application of NaCl in high concentrations for a certain time determined a drastical increase of proline synthesis in plant tissues. In our experiment saline stress determined a 4 fold increase of proline content in leaves of non-inoculated plants. In contrast, an interesting effect of mycorrhization was that in leaves the concentration of proline was significantly lower than in non-mycorrhized plants after salines stress. This proves that mycorrhization alleviates the negative effects of saline stress on plant metabolism by supplemental pathways compared to non-inoculated plants.

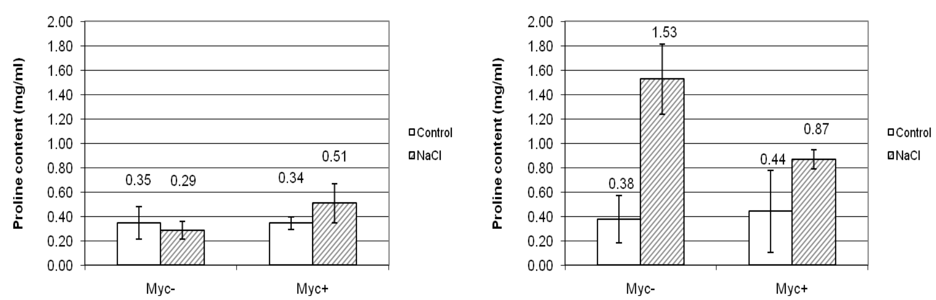


**Fig. 3 -** Proline conent of leaves before (left) and after (right) saline stress.

## CONCLUSIONS

1. Chlorophyll and dry matter content of leaves are two physiological indexes, which were the less affected by experimental factors. Their variations appeared both as a response to inoculation and also as an effect of osmotic stress.

2. A broader insight to the changes produced by mycorrhizal fungi on salt tolerance of host plants are given by the assessment of proline content in plants.

Our results showed that at molecular level the AM fungi produced the reduction of negative effects of saline stress on plant physiology.

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