The Influence of Osmotic Stress on Physiological and Biochemical Indices at Garlic (*Allium sativum* L.) Local Populations

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Abstract

Garlic is an asexually propagated crop with high morphological diversity and uses throughout the world. Although the salt tolerance among the cultivated species is low, there are some genotypes which present an acceptable level of adaptability to moderate saline conditions. In vegetables, these genotypes are represented especially by local varieties breeded by local farmers. It is therefore necessary to achieve a balance between productivity and salinity tolerance, for the obtaining of new genotypes with high productivity. Our experiments aimed to study the main physiological and biochemical indicators for the identification of salinity tolerant genotypes in a collection of local autumn and spring garlic populations from Timis County. The local populations studied show a wide genetic diversity based on synthesis of free proline, by selecting genotypes with good salinity tolerance for five autumn garlic varieties and five for spring garlic. There are major differences both morphologically and physiologically, between studied varieties of halophytes. Identification of these cultivars may represent a resource for breeding programs of cultivated vegetables on saline soils.

Keywords: *Allium sativum,* catalase, tolerant genotypes**,** proline, salinity, stress

Introduction

Garlic (*Allium sativum* L.) is a strongly aromatic bulb and among the oldest cultivated crop commonly used in cooking but also effectively explored for its medicinal use in homeopathy and allopathy (Thomson and Ali, 2003). Recently it has been explored for anti-cancer activity (Lee et al., 2013), antimicrobial activity (Ross et al., 2001), cholesterol reduction (Ackermann et al. 2001), wound healing (Ejaz et al., 2009).

Garlic (*Allium sativum* L.) is an asexually propagated crop with high morphological diversity and is used for its culinary and medicinal uses throughout the world (Wu et al., 2016). Vernalization fulfillment, followed by a exposure temperature and long photoperiod is indispensable for garlic growth and development (Wu et al., 2016).

Growth period and bulb production differ from year to year, planting period and location, because there is a strong genotype-environment interaction. However, some varieties may show different temperature requirements, and the same variety can show differences in different growing areas affecting the commercial production of this crop. This indicates that the length of storage period as well as the temperature used will have different effects on the responses of garlic growth. In addition to their immediate effects, environmental factors also have long-term effects in each of the development stages. Maintaining the bulbs before planting at temperatures from 0 to 10°C for a period of 2 months accelerates the germination process and substitutes the initial climatic requirements of the crop (Dufoo-Hurtado Miguel D et al., 2015).

The importance of soil as a critical resource cannot be neglected. Ecosystem processes e.g. carbon cycle, storage, cleaning, regeneration and biodiversity in natural flora are all supported by soil. The rapacity for intensive farming resulted in over cultivation and over grazing go to degradation and salinization (Alghabari Fahad, 2015).

The salinization may be attributed to the chemical nature of weathered bed rock, soluble organic and inorganic compounds, rainfall accumulated salts and wind transported inorganic salts, overuse of chemical fertilizers and soil amendments, poor quality irrigation and capillary rise of shallow saline water (Rengasamy, 2010). The intrusion of sea water to coast lands and plants' ability to uptake and discharge salts in the soil also contribute to soil salinity development. Munns and Tester (2008) defined a two phase plant response to encounter salinity. A continuous osmotic phase that inhibit water uptake owing to osmotic pressure of saline solution which drop its potential energy and ionic imbalance or toxicity due to accumulation of specific ion over a longer period of time. Interactive effect of soil processes, soil structural stability, solute solubility, nutrient and water movement in root zone are key processes involved in plant growth and ionic uptake under saline conditions. Plant genetic potential for mitigation strategy also plays vital role in adaptation and acclimatization in problematic soils (Alghabari Fahad, 2015).

High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Zhu, 2007). Together, these effects reduce plant growth, development and survival. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are affected (Parida & Das, 2005). During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion.

The osmotic effects of salinity stress can be observed immediately after salt application and are believed to continue for the duration of exposure, resulting in inhibited cell expansion and cell division, as well as stomatal closure (Flowers T J, 2004). During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth (Arun Kumar Shanker and B Venkateswarlu, 2011).

Number of leaves per plant of garlic varied significantly in different stages of growth as those was treated with different levels of salt concentration. At very early stage of growth the number of leaves per plant increased when those were treated with low concentration of salt water *i.e.* 50 mM NaCl and decreased while with highest (200 mM NaCl) dose of salt concentration. The ability of the plant to produce leaves was increased in low concentration of salt water (50 mM NaCl) as those can survive low concentration of salt water but cannot survive salt condition at highest (200 mM NaCl) level because those plants failed to possess different physiological processes at high saline condition (Samiul Alam A K M, 2013).

It has long been noticed that there are competitive biosynthesis processes between chlorophyll and free proline in the leaves of plants subject to osmotic stress.

Proline synthesis is an important physiological indicator to characterize the response of plants to both osmotic stress and other stress types (Shi D C, 1993). Increasing the salinity level leads to the accumulation of proline in tolerant genotypes, which leads to a correlation between the proline synthesis (with osmo-protective role) and the salt stress tolerance.

Proline accumulation is a common metabolic response of superior plants affected by water deficit and saline stress condition. Accumulation of proline may play a role in osmotic adjustment, which is often considered to be involved in the protection of enzymes and cellular structures. Proline acts as a free radical scavenger and stabilizes biological membranes, which results in adjustment of cell metabolism and growth in response to stress conditions and in dehydration tolerance of plants (Verbruggen and Hermans, 2008). Proline accumulation is a stress-inducing tolerance in many plant species (Amini et al., 2014).

Catalase concentration is high in the organelles (plant or animal), known as peroxisome and thus

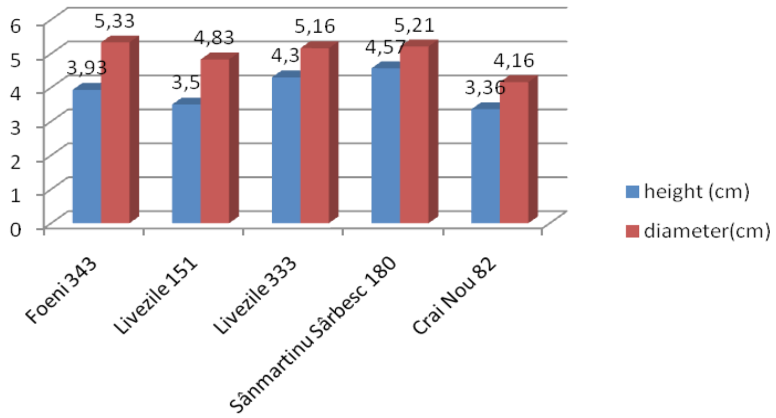

Figure 1. Biometric characteristics in local autumn garlic populations at the harvesting time

protects the cell by hydrogen peroxide (H_2O_2), compound which may be converted into hydroxyl radicals and implicitly to create genetic mutations. Catalase also uses hydrogen peroxide to oxidize a number of toxic compounds, including phenol, formic acid, formaldehyde or alcohols.

Materials and methods

Although salinity tolerance in cultivated species is low, there are genotypes that exhibit acceptable levels of adaptability to moderate salt conditions. In garlic, these genotypes are mainly represented by local populations selected over time by housekeepers in saline soils. The biological material was represented by 10 local garlic populations, including 5 autumn and 5 spring varieties, collected from the Timis county, from the salinized areas. The names of local populations are the locality and the house number from where they come from.

Local populations of autumn garlic: Foeni 343, Crai Nou 82, Sânmartinu Sârbesc 180, Livezile 333 and Livezile 151. Local populations of spring garlic: Valcani 33, Sânmartinu Sârbesc 411, Livezile 333, Toager 29 şi Cruceni 146. The experimental variants were as follows: V_0 (control) – treated with pure water, V_1 - NaCl 100 mM, V_2 - NaCl 200 mM and V_3 - NaCl 300 mM. Local populations of garlic collected are characterized by many age cultivation in the same household, tolerance to environmental factors, growth and development in terms of biological technology (without chemical fertilizers, just manure, they do not use herbicides, they only use irrigation only in extreme dry conditions, the preservation techniques are ancestral and they do not sell the yields. The selection of these populations is conducted every year by choosing the most nice and useful seed material (seeds, bulbs). The bulbs were evaluated for biometric point of view, aiming at height, diameter, and weight at the time of collection.

For the assessment of salinity tolerance we used solution of 100, 200 and 300 mM NaCl, which was applied gradually after each week. The following tests were performed: total chlorophyll content (SPAD units); the accumulation of proline in plants grown in saline conditions (mg /mL); catalase activity (units/mg protein).

The total chlorophyll content (SPAD) of leaves was determined using SPAD-502 portable chlorophyll-meter. The apparatus determines the relative content of chlorophyll, by measuring the absorbance of a leaf in two wavelength ranges. Using this principle chlorophyll-meter calculates a numerical value SPAD (single photon avalanche diode) which is directly proportional to the amount of chlorophyll in the leaf.

The accumulation of proline is based on the fact that this reacts with ninhydrin and ninhydrin acid form a yellow-reddish color compound, which can be extracted in toluene, and the amount of proline can be assessed by spectrophotometric determination of absorbance compound. The concentration of free proline was determined through interpolation by reading on a standard curve developed through determining the absorbance corresponding to the different concentrations of proline.

The capacity to maintain high catalase activity under stress is essential for the balance between formation and removal of H_2O_2 in intracellular medium (Joseph et al., 2011). The increase of catalase activity in the plants under saline stress

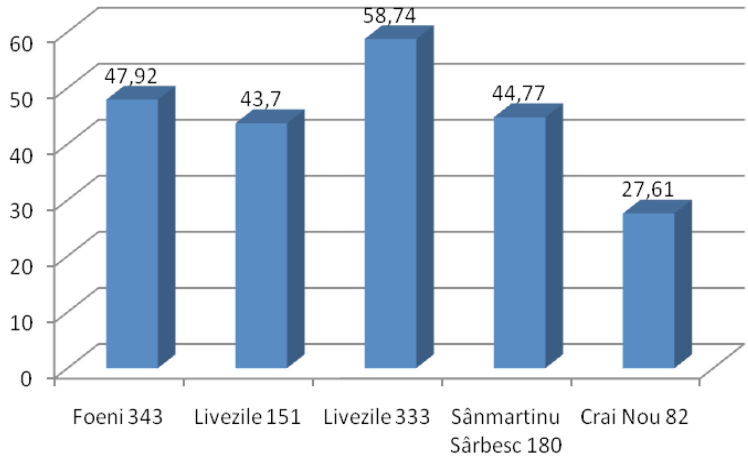

Figure 2. The bulbs weight in local populations of autumn garlic at the harvesting time (g)

Figure.3 Local autumn garlic at the harvesting time

condition should be an adaptation for removal of H_2O_2 (Ben et al., 2009).

Results and discussion

Regarding bulbs' height in autumn garlic, it showed a variation between 3.36 cm at Crai Nou 82 and 4.57 cm at Sânmartinu Sârbesc 180. The diameter ranged between 4.16 cm and 5.33 cm - Figure 1.

The highest weight of autumn garlic bulbs was recorded in Livezile 333 (58.74 g), and the lowest in Crai Nou 82 (27.61 g) - Figure 2, 3.

Regarding bulbs' height in spring garlic, it showed a variation between 2.96 cm at Toager 29 and 3.50 cm at Valcani 33. The smallest diameter was registered at Toager 29 (2.96 cm) and biggest la Cruceni 146 (4.6 cm) - Figure 4.

Regarding the characteristics of spring garlic, the average bulb weight was between 22.66 g at Toager 29 and 40.1 g at Livezile 333 (Figure 5 and 6).

In the case of garlic cultivated in autumn, the high chlorophyll content was recorded in V_0 - control-Foeni 343 varieties with 56.08 SPAD units and the lowest in Livezile 151 (48.33 SPAD units). The most resistant to stress conditions (V_2 - 200 mM NaCl) was Foeni 343 with 52.0 SPAD units, and the least resistant was Livezile 333 with 35.26 SPAD units.

In variant V_1 – 100 mM NaCl the high chlorophyll content was recorded at almost all local varieties compared with control variant V_0, exception recording at Sânmartinu Sârbesc 180. The highest levels of chlorophyll were observed at Crai Nou 82 with 59.9 unităţi SPAD în V_1 (Figure 7).

In the case of spring garlic, Toager 29 recorded the highest levels of chlorophyll in the stressed

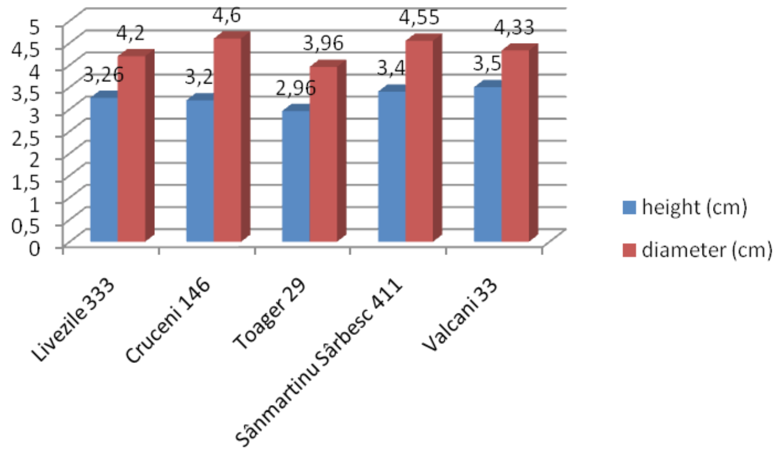

Figure 4. Biometric characteristics of local populations of spring garlic at the harvesting time

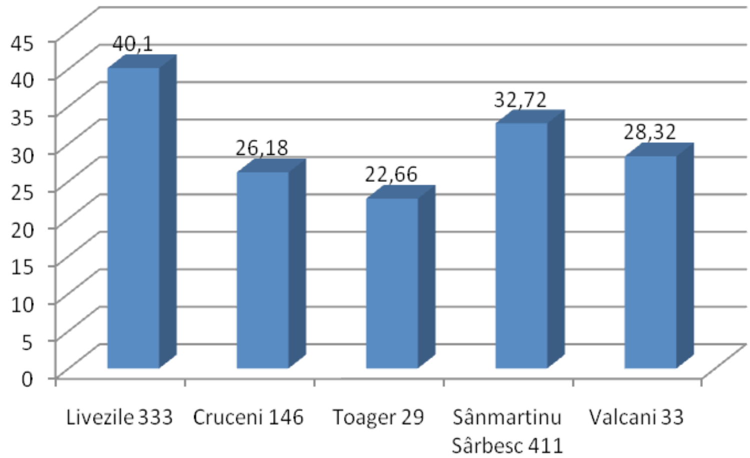

Figure 5. The bulbs weight in local populations of spring garlic at the harvesting time (g)

Figure 6. Local spring garlic at the harvesting time

version with 200 mM NaCl (54.2 SPAD units), and the lowest values were recorded at Sânmartinu Sârbesc 411 with 42.16 units SPAD (Figure 8).

Following the determination of the chlorophyll content in the leaves, it was found that the induction of osmotic stress caused changes in the amount of chlorophyll. It has been observed that a reduced or moderate stress level (V_1 - 100 mM NaCl) cause the increase of chlorophyll amount. However, in V_2 stressed variant (200 mM NaCl), the amount of

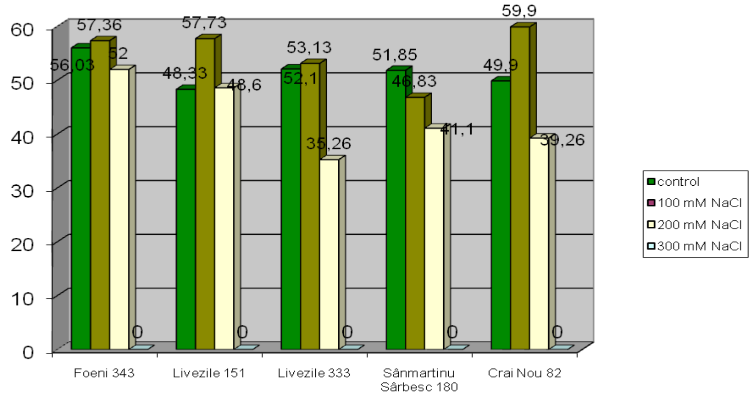

Figure 7. Experimental results on chlorophyll content in local autumn garlic populations (SPAD units)

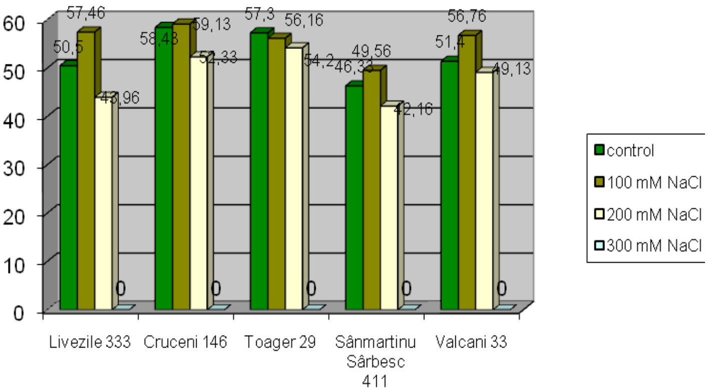

Figure 8. Experimental results on chlorophyll content in local spring garlic populations (SPAD units)

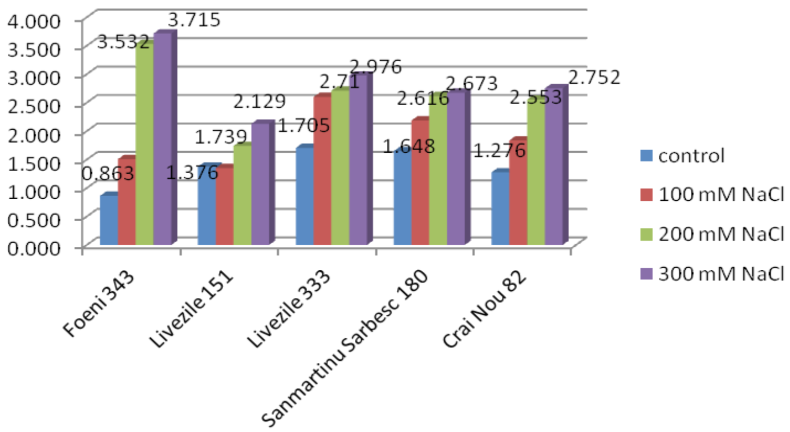

Figure 9. Experimental results on the determination of free proline level in local autumn garlic populations (mg / mL)

chlorophyll was reduced in all genotypes, while in V_3 (300 mM NaCl), chlorophyll could no longer be determined due to lack of leaves.

In autumn garlic, the lowest quantities of free proline were recorded in V_3 Livezile 151 population with 2.129 mg / mL, while Foeni 343 had the highest proline content (3.715 mg / mL) (Fig. 9).

In the case of spring garlic, variant 3, saline stressed/treated with a high concentration of NaCl, had a higher content of free proline (3.830 mg / mL) at the Toager 29 genotype compared with Sânmartinu Sârbesc 411 were proline content was 0 (Figure 10).

The local populations studied show a wide genetic diversity regarding the synthesis of free proline, thus identifying genotypes with good

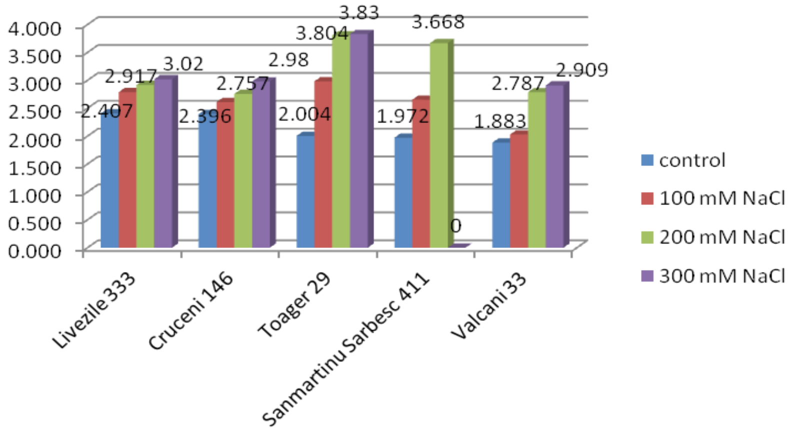

Figure 10. Experimental results on the determination of free proline level in local spring garlic populations (mg / mL)

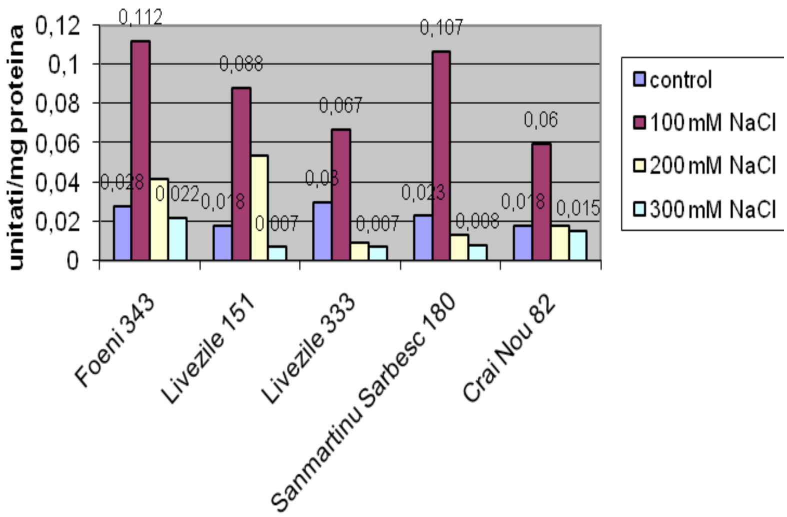

Figure 11. Experimental results on catalase activity in autumn garlic (units / mg protein)

salinity tolerance such as Foeni 343, Livezile 151 and Crai Nou 82 in autumn garlic and Toager 29, Livezile 333, Cruceni 146 and Valcani 33 in the case of spring garlic. In contrast, Sânmartinu Sârbesc 411 is not tolerant to high salinity.

In autumn garlic, Foeni 343 and Crai Nou 82 population have preserved their catalase activity stronger than the other populations, recording 0.022 respectively 0.015 units / mg protein in the 300 mM NaCl variant (Figure 11).

Regarding the catalase activity in spring garlic at Cruceni 146, it increased in V_2 (200 mM NaCl) to 0.304 units/mg protein, from 0.02 units in V_0. At high concentrations of NaCl (300 mM NaCl), all populations had a higher catalase activity, except for Sânmartinu Sârbesc 411, but Toager 29 was shown with 0.05 units / mg of protein (Figure 12).

Conclusion

Saline soils represent an important percentage of cultivated land in Banat area, the production capacity of crops being affected by the level of soil salinity. Qualitative breeding of these crops is an expensive and durable process that often only gives partially satisfactory results. Thus, the majority of researchers support the idea of cultivating these saline soils with salt tolerant crops.

Although salt tolerance among the commonly cultivated crops is reduced, there are genotypes that present acceptable adaptability levels under moderate salinity conditions. In garlic, these genotypes are mainly local populations gathered and selected over time by home growers from areas with saline soils. The identification and morpho-physiological characterization of salt tolerant local populations of garlic from the Banat, an area with

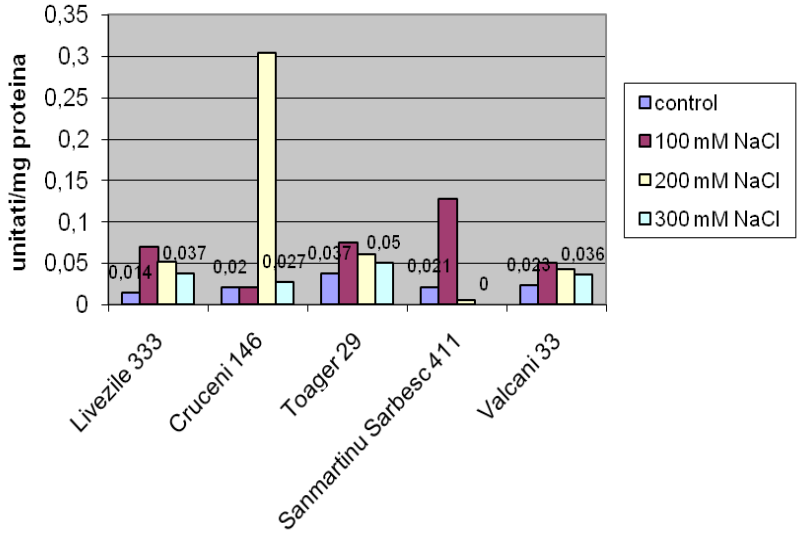

Figure 12. Experimental results on catalase activity in spring garlic (units / mg protein)

a lot of saline soils, represents a primary field in the research activity of USAMVB Timişoara.

Concerning the biometrical characteristics of garlic when collected, these varied among the 10 local populations, for autumn garlic Livezile 333 and Foeni 343 had the highest results, respectively Livezile 333 and Sânmartinu Sârbesc 411 for spring garlic.

On a moderate level of saline stress (V_1) an increase in chlorophyll content was registered in the majority of populations. While all the 200 mM NaCl (V_2) treated local populations showed a decrease in chlorophyll content, at a higher concentration of NaCl (300 mM) the garlic doesn't survives. Regarding the chlorophyll content, the local population most resistant under stress (V_2) conditions was Foeni 343 (autumn garlic) and Toager 29 (spring garlic)

The studied local populations exhibit a wide genetic diversity from the point of view of the free proline synthesis, thus, being able to identify genotypes with good salinity tolerance.

Regarding the content of free proline, it has been observed that it accumulates in large quantities under saline stress conditions. The accumulation of free proline represents a response to the stress manifested by saline excess in the foliar system of species. From the analysis of the presented data we can observe small values in the control variant (V_0) without stress treatment and higher values in stressed variants (V_1, V_2, V_3), thus, confirming the direct correlation between the proline synthesis (with osmo-protective role) and the salt stress tolerance. Inducing saline stress determined increases in the free proline content of all the studied genotypes, especially in Foeni 343 and Toager 29.

Catalase recorded at V_1 (100 mM NaCl) increased activity in all 10 local garlic populations compared to the control. In contrast to higher salt concentrations (200 and 300 mM NaCl), catalase activity decreased compared to V_1 - 100 mM.

For V_3 (300 mM NaCl) a higher level of catalase activity was found in Foeni 343 (autumn garlic) and Toager 29 (spring garlic).

Following these determinations, it was concluded that the local garlic populations tested are moderately tolerant to salinity, while Foeni 343 (autumn garlic) and Toager 29, Cruceni 146 (spring garlic) are tolerant to salinity.

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