ABSTRACT

Key words: Phaseolus vulgaris L., local population, tolerance, resistance, saline stress

Earth is considered a salt planet, since about 71% of the surface is covered by saltwater; about 6% of the total area and 20% of irrigated land are affected by salinity, which is more than 800 million hectares of land affected by salinity worldwide (FAO, 2009).

The United Nations Environment Programme (UNEP) estimates that about 50% of the world's agricultural land is affected by salts (Flowers and Yeo, 1995). Soil salinity is the second major cause of soil degradation after erosion for about 10.000 years. Globally, about 2.000 hectares of arable land lose their productivity every day due to salinization. Salinity causes a 10-25% decrease in yield for many crops and can prevent the productivity of some in its entirety.

At European Union level, there are important saline soils, most of which are located around the Caspian Sea, the Carpathian Basin, the Pannonian Region and the Iberian Peninsula; here the salinity of the soil affects around 1 million ha. In Romania, saline soils were identified in 29 counties of the country, and their total area ended up being 614.000 ha.

In Romania, beans are grown from the 18th century, the most favourable areas being those of the south, south-east and south-west, in the plain area and in the meadows of the most important rivers. According to FAO statistics, *Phaseolus vulgaris* L. is cultivated on about 12.000 to 13.000 ha, with total production of 42.000 to 50.000 tonnes. It is generally adapted to moderate temperatures, precipitations of approximately 400 mM and a growth season of about 60 -120 days. The popularity of culture comes from the fact that it is relatively easy to produce, is tasty, versatile and a good source of nutrition, and with a importance nutritional, agro-technical, economic and social. At the same time, this legume is grown for both dried (field beans) and green pods (garden beans), green-ripe seeds, but also partially dried, which are fully used in human nutrition (Bârcă, 2013).

From an agrotechnical point of view, *Ph. vulgaris* it is a very important crop, because it has the property of fixing atmospheric nitrogen with the help of bacteria *Rhizobium* sp. (Munteanu, 2003).

However, *Phaseolus vulgaris* L. is a glycophyte plant and low soil salinity levels (2 dSm^{-1}) significantly reduce crop productivity (Pessarakli, 1999). At a salinity equivalent to 100 mM NaCI, plant yield is reduced by 85% (Szilaghy, 2003), although some species appear to be much more tolerant of saline stress than others (Kaymakanova and Stoeva, 2008). Bayue-los et al. (2002) reported that the increase in salinity from 0 to 180 mM NaCl decreases the germination process by 50% to species of the genus *Phaseolus*, and Dobrei et al. (2007) at *Phaseolus vulgaris* L. show a high variability in saline stress tolerance, starting from 40-46 mM NaCl up to the values between 196-207 mM NaCl. Therefore, the increase in yields of bean crops could be achieved on the basis of the selection of varieties tolerant to salinity (Fita et al., 2015).

The main aims of the thesis is a better understanding of the physiological and biochemical mechanisms involved in the tolerance to saline stress of the species *Phaseolus vulgaris* L., as well as the identification of local populations of beans tolerant to this abiotic factor, from the region of NE of Romania. Subsequently, these forms can be used to improve morphological self-sufficiency.

The doctoral thesis comprises five chapters with 198 pages, 23 tables and 86 figures, structured in two parts:

Part I includes aspects and bibliographic studies on: the problem of soil salinization and alcanization around the globe, the concept of stress, the tolerance of plants to adverse environmental

conditions, the harmful action of the high concentration of salts to bean plants and other species. This part is edited on 23 pages, representing (11,%) from entire thesis and contains three figure.

Part II-a is written on 101 pages, representing (53,5%) the entire thesis, and comprises 83 figures and 23 tables. This part presents the results of own research, on the subject of the doctoral thesis.

Biological material has been collected from the following locations: Săveni, Truşeşti, Codreni, Copălău şi Iezer, from Botoşani county, Moşna from Iaşi county, and Blăgeşti from Vaslui county. Of the 13 local populations originally collected, 7 local populations have been selected as more resilient at saline stress conditions. Thus, local populations collected from the localities Săveni, Truşeşti, Moşna şi Blăgeşti, remained for research. The collection took into account the fact that the material chosen represents a local population and not a commercial species.

The research methods. During the 2017-2019 period, seven local populations of beans were studied, which were subjected to physiological and biochemical tests which followed: the foliar surface, the water content at the foliar level, foliar dehydration rate, analysis of the content of photosynthetic pigments, stomatal conductance, the content of Na^+ and Cl^- in the leaves, the proline content of the leaves, the raw protein content of the grains, soil pH and elements related to production.These determinations aim to highlight the response of these local populations to saline stress.

Experience was placed in the sera of the ICAM Iaşi, and laboratory analyses were carried out in 2017-2019, in the laboratory "Plant Physiology", from Faculty of Agriculture, USAMV Iaşi, "Institute of Research for Agriculture and the Environment" (ICAM), from Iaşi, and to "Food Quality Control Laboratory" of the USAMV Iaşi.

The bifactorial experience was established in vegetation vessels and organised in the form of randomized blocks with three repetitions. The two factors influencing the conduct of the experiment were: factor A, represented by the three concentrations of NaCl (V1- water, V2- 100 mM NaCl and V3- 200 mM NaCl) and factor B represented by the local populations studied. 372 of plants were studied in the two years of experimentation.

The determinations were carried out 15 and 30 days after the NaCl treatment.

The surface foliar is the physiological index of major importance in terms of characterising the intensity of growth processes, perspiration, respiration and photosynthesis. The effect of salinity on foliar growth was assessed by determining the surface of the leaves from the middle third of the stem using the portable Area Meter AM 300-0002.

In the first and second year of the study, the surface foliar decreased directly proportionally with applied saline concentrations, manifesting a negative effect of the salts on the leaves of the median zone, as these leaves began to grow under the effect of saline stress, as well as the fact that mineral ions have been transported with water to the areas with more intense perspiration where they accumulate, generating toxic phenomena. Higher values of the surface foliar have been recorded in the local populations Blăgeşti 1, Blăgeşti 2, Blăgeşti 3, Blăgeşti 4 and Truşeşti 2, which indicate good resistance to saline stress.

The evaluation of the **fructification process** was carried out on the basis of biometric indicators such as: average number of grains /plant and average weight of grains /pod.

In the first year of study, production (the number of grains per plant) was significantly influenced by the treatments of saline applied at both concentrations. At the variant treated with 200 mM the lowest values were recorded in the furry population Blăgeşti 4, which had a production of 93.40% of that of the witness. On the other hand, the population Săveni has registered a reduced production of 25.00% and Truşeşti 2 of 39.13% of the control variant.

Concentrations of 100 mM and 200 mM NaCl negatively influenced the weight of bean beans, in the case of the seven local populations studied and oscillate between 41.17% (Moşna) and 91.85% (Blăgeşti 4).

In the second year of study, the most productive populations were Blăgeşti 1, Truşesti 2 and Săveni, to both variants. In this case the production has oscillated between 32.18% (Blăgeşti 3) and 78.12% (Săveni). The weight of grains/pods has been reduced much and this time, oscillate between 23.05% and 72.11% to both variants.

The hydraulic regime was assessed by analysing the water content of the leaves and the rate of foliar dehydration.

The analysis of the **water content** of the leaves was investigated by determining the two forms: **free and bound water**.

In the first year of study, 30 days after the application of saline treatments there was a slight increase in **total water** content for all populations, which oscillated between 59.93% and 82.30%. In this case, the population of Blăgeşti 2 and Truşeşti 2 from the 200 mM variant is noted. **Free water** content is lower compared to the control variant for five populations (Blăgeşti 1, Blăgeşti 2, Moşna, Truşesti 2 and Săveni), indicating that the reduction of **free water** content increased the biological capacity of plant tolerance studied at abiotic stress conditions. Instead, there was an increase in the amount of **bound water** that increased the resistance of plants to the high concentration of 200 mM. In the first year of study all local populations (Blăgeşti 1, Blăgeşti 2, Blăgeşti 3, Blăgeşti 4, Săveni, Moşna, Truşeşti 2) presented a higher degree of adaptation due to the large amount of water linked, assimilated under the conditions of a salin stress.

In the second year of study, a high content of **total water** was recorded to all three variants, to the local populations Blăgeşti 2, Săveni, Moşna şi Blăgeşti 3. A lower **free water** content compared to the control variant was recorded for four local populations (Blăgeşti 2, Blăgeşti 4, Săveni, Truşeşti 2), indicating that free water increased the biological capacity of plant tolerance to salin stress. Instead, only three local populations presented a large amount of **water bound**, at the concentration of 200 mM, which suggests that Blăgeşti 2, Săveni şi Blăgeşti 1 presents a higher degree of adaptation.

The **dehydration rate of the leaf** depends on the morpho-anatomical and physiological properties of the leaf tongue and may be an indication of the intensity of perspiration. Plants with better salinity resistance are characterized by cuticular perspiration and a lower leaf dehydration rate. In the first year of the study, have been identified the populations Truşeşti 2 and Blăgeşti 4. For the second year of the study, all populations in the study have a good ability to adapt to saline stress conditions over a long period of time, as they experienced a lower rate of dehydration compared to the control variant, to the variant 200 mM NaCl.

The analysis of the foliar pigments reflecting the intensity of the photosynthesis process was carried out by the spectrophotometric method and with the CCM 200 PLUS.

In the first year of the experiment, after 30 days of saline treatment, in the case of exposure to 100 mM and 200 mM NaCI, results obtained to the populations Moşna, Săveni, Truşeşti 2, Blăgeşti 4, Blăgeşti 2 and Blăgeşti 1, indicates their maintenance in the osmotic stress phase and good adaptation to an intense photosynthetic rhythm.

In the second year of study, the application of the 200 mM saline treatment led to higher levels of chlorophyll pigments for local populations Blăgeşti 4, Blăgeşti 2, Blăgeşti 1 şi Săveni, which allows us to conclude that these populations have a high potential for salt stress.

From the results obtained, we can say that the local populations of the beans studied fit into the biphasic model proposed by Munns (1993). Thus, the transition from the first phase of osmotic stress to the second phase of ion toxicity is based on the intensity of saline stress and the population cultivated.

Stomatal conductance has been determined with the porometer SC-1. Results obtained after 15 days and 30 days treatment showed values indicating that applied saline stress influenced stomatal conductance leading to significant differences between populations. Thus, in both years of study, foliar stomatal conductance, after 30 days of saline treatment was generally reduced to local populations of beans, except the Blăgești 2 from the variant 200 mM.

Prolina is an amino-acid that manifests an antistress activity in particular by the antioxidant effect. In the first year of study, at the end of treatment, it is observed that as saline concentration increases,
the concentration of proline in plants increases in for of the populations (Trușești 2, Blăgești 4, Blăgești 1, Blăgeşti 2), values oscillating between 2.80 nmol·mg FW and 4.70 nmol·mg FW. At the same time, there is a linear increase is observed, meaning that prolina has a role in the mechanism of adaptation of plants to saline stress.

In the second year of study, 30 days after the application of saline treatments were noted local populations Blăgeşti 2, Săveni and Blăgeşti 4; this time, the values oscillate between 9.70 nmol·mg FW and
12.10 nmol·mg FW, and this high prolina content indicates that these local populations are trying through different mechanisms to gain resistance to salinity.

Sodium is a slower absorbed element than other cations, and its level in plants has low values. The physiological role of sodium is mainly found in reducing perspiration. It has the function of maintaining osmotic pressure in cells. Sodium deficiency in halophilic plants is manifested by the open colour of the leaves, almost white and the appearance of necrotic stains. The symptoms of the deficiency are visible on the old leaves. Thus, in the first year of study, at the end of treatment, to the seven bean populations, it is shown that as the saline concentration increases, so does the Na^+ concentration in the leaves, the maximum value recorded for the 200 mM variant was 0.46 mg /100 mg DW to the Trușești 2 and the minimum value to the population Blăgești 1 (0.25 mg /100 mg DW).

On the other hand, **chlorine** is involved in photosynthesis, with the role of removing oxidants harmful to photochemical systems and stimulating the transport of electrons. This has the role of regulating osmotic potential by maintaining the cellular hydraulic level, ensuring the opening of the stomates. Excess of this micro-element adversely affects plant growth by degradation of glucydic metabolism.

The chlorine content were higher to the tratament 200 mM NaCl compared to control variant. Thus, in the first year of the study, the maximum value reached was 12.10 mg /100 mg DW to Blăgești 1, and the minimum value was 5.45 mg /100 mg DW to Trușești 2. In the second year of the study, the chlorine content value are significantly higher than to the control variant, to 200 mM. The maximum value is
18.67 mg /100 mg DW to Blăgești 1 and the minimum value is 4.56 mg /100 mg DW to Blăgești 2.

Some of the local populations of beans studied (Blăgeşti 1, Blăgeşti 3, Săveni) showed signs of toxicity indicating that they are not able to tolerate these concentrations. In addition, mature leaves do not have the ability to accumulate Na^+ and to ensure protection of the meristematic and active growth cells.

Phaseolus vulgaris L., is an important source of raw **protein**, although this crop is strongly affected by soil salinity, information on genes that play a role in salt tolerance is rare. In the first year of

the study, the protein content was adversely affected by the doses administered, indicating a negative effect of saline stress on all local populations studied and causing the accumulation of free anions, nitrates and amino acids, which led to a synthesis of reduced proteins, which eventually led to abnormal plant growth and low yield of populations. However, a high percentage of protein was recorded at Trușești 2, Blăgești 2 and Blăgești 4.