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INFLUENCE OF BIOLOGICAL ACTIVE SUBSTANCES ON THE PROLINE CONTENT OF SOYBEAN SORTS WITH VARIOUS RESISTANCES TO DROUGHT

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Abstract

This paper examines the influence of two types of biologically active substances: humate (LG) and salicylic acid (SA) on soybean cultivars with different response to the action of pedological drought: Licurici, Colina, Horboveanca – sorts with medium resistant to drought, and Zodiac and S-4-04 – sorts with increased resistant to drought. The SBA influence on soybean plantlets was studied at two moisture levels: 1) optimum – 70 % TCW (total capacity of water in soil) and 2) hydric stress – 35 % TCW. Under optimum condition of cultivation the treatment with salicylic acid and humate determined the proline increased content in sorts more sensitive to drought, while resistant sorts reduce its content in comparison with the witness cultivated in the same conditions. On the hydric deficit background proline content increased significantly in the leaves of all sorts studied, and administration of humate and salicylic acid led to an increase even more accentuated of osmoprotective content compared to plants treated exogenously, but assured with water. Under stress conditions, sorts with higher sensitivity to hydric deficit showed superior values of the parameter investigated, while resistant sorts had lower values in comparison with the witness cultivated on the stress background and higher compared to the witness cultivated under optimal moisture conditions. The most pronounced reductions attest for the sorts with high resistance to drought, suggesting possible implications of the substances investigated in modulating cellular metabolism.

Keywords: soybean, proline, hydric stress, foliar treatment, humate, salicylic acid

1. INTRODUCTION

Soybean is an agriculture crop with great economic value. During the vegetation period growth, development and metabolic activity of soybeans plants register important modification depending on a number of endogenous and exogenous factors, agricultural productivity being the resultant of several factor interactions: biochemical, physiological and morpho-anatomical. One of the main causes that reduce the soybean yields is represented by abiotic stress factors, including high temperature and drought which produce in certain cases decreases in yields that can reach 70 per cent level (Allmars et al., 1975; Boyer, 1982; Manavalan et al., 2009; Yancey, 2005).

The drought in Moldova is the most dangerous natural phenomena, presenting specific features of regional climate, conditioned by not homogeneous distribution of atmospheric

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precipitation in time and space on the background of elevated air temperatures. As meteorological phenomenon, drought is characterized in general by the absence of precipitation and increasing potential evapotranspiration. According to several recent assessments, the deficit of precipitation is characteristic on the entire territory of Republic of Moldova, and most of it is characterized by sub humid and semi-arid climate with a high probability of drought occurrence and desertification development.

The probability of drought occurrence is very strong (≤ 50% from normal climate of precipitation) and with catastrophic consequences in some months during vegetation period (Apostol et al., 2012). In the past three decades droughts have been reported more frequently (1982, 1986, 1990, 1992, 1994, 1996, 1999, 2000, 2001, 2003, 2006, 2007, 2011, 2012, 2014). During the period 1990-2014 in the republic have been registered 12 years (1990, 1992, 1994, 1996, 1999, 2000, 2001, 2003, 2006, 2007, 2011, 2012) with different drought intensities and durations. In 1990, 1992, 1994, 2003, 2007, 2012 droughts persisted during the entire vegetation period (IV-IX months). In the remaining years there were droughts in summer and autumn.

Drought deployment is conditioned by a number of factors: rainfall, total soil water reserve and accessible water reserves, humidity and air temperature, evapotranspiration and wind speed. The impact and respectively drought consequences depend on, along with the mentioned, a series of factors which define the land cover (landscape features, soil cover structure and characteristics of soils, ground water depth, vegetation coverage degree), factors that define the physiological particularities of plants (variety, vegetation phase, resistance to dryness) and factors which involve the anthropic impact (fields state in particular of the soil, practiced technologies). In this context we mention that several factors during the last 100-130 years suffered significant changes.

The high frequency of dry years in the last three decades has led to the cumulative effect of drought effects. As a consequence, even in years when rainfalls are close to optimum accessible water reserves and yields remain below the expected ones. This involves the compensation of water reserves in soil, in this context the effects of pedological drought has obtained a progressive trend.

The presence of water in sufficient quantity is an essential factor for the survival, growth and development of plants, while water insufficiency in the soil leads to the appearance of pedological drought that affects physiological and biochemical processes in plants. One of the major consequences of drought is the modification of hydric status of the plants. Recent data demonstrated the importance of preserving water under drought conditions, as primary factor in the coordination and function integration at the organism level, the resistance of plant to drought being correlated with the capacity to maintain the balance of physiological processes, in particular, the adjusting capacity of the water status (Melenciuc, 2009l Ştefîrţă et al., 2013). The disturbance of hydric status in plants involves metabolic changes; processes are adjusted at the genetic level. Thus, at the molecular level, drought causes changes in expression of genes responsible for the synthesis of osmoprotectors, which gradually leads to the activation of the tolerance development to stress factor manifested in the form of acclimatization, or, ideally, activation of these genes resulting in adapted organisms (Ashraf and Foolad, 2007; Ashraf and Harris, 2004). So, as a response to hydric stress, plants accumulate in their body a number of substances with protective functions (Deivanai et al., 2011).

At the same time, a number of studies attest the possibility of regulating metabolic processes through the exogenous application of osmoprotective substances (proline, glycinebetaine, trehalose), phytohormones (abscisic acid, jasmonic acid, brasinosteroids, salicylic acid), which have a decisive role in mitigating the negative effects produced by abiotic stress in plants (Ali et al., 2007; Ashraf and Foolad, 2007; Claussen, 2005; Hanson and Hitz, 1982; Hasegawa et al., 2000).

The data from literature related to the possibility of using compounds by humic and phenolic nature in the process of plant growth (Apel, 1993; Arfan et al., 2007; Bertin et al., 2003; Katkat et al., 2009). The well known antioxidant, antiviral, antibacterial, antifungal, alelochemical proprieties of phenolic compounds suggest their exploitation opportunity in the agricultural sector as biopesticide and plant growth regulators (Macias et al., 2004; Blazej and Shutyi, 1977). Yet, the action mechanism and the role of these compounds in plants are diverse and incompletely understood.

Based on the above, in order to emphasize the role of phenolic and humic nature compounds in adapting plants, we studied the feedback of the soybean genotypes to the foliage treatment with salicylic acid (SA) and humate (LG), through determination of the proline content in the leaves sorts with various resistant to drought.

2. MATERIALS AND METHODS

In order to realize the planned study were installed experiences with soy cultivars which responded differently to the pedological drought: Licurici, Colina, Horboveanca – sorts with average resistant to drought and Zodiac and S-4-04 – sorts with higher resistance to drought. The experiment has been installed in laboratory controlled conditions. The research has examined the influence of two types of biologically active substances: humate (LG) and salicylic acid (SA). The SBA influence on soybean plantlets have been studied in two moisture levels: 1) optimum – 70 % TCW (total capacity of water in soil) and 2) hydric stress – 35 % TCW.

The soybean seeds were planted in growing pots with a capacity of 3 kg. The content of pots was mixed in the ratio of 2:1, two parts carbonated chernozem soil and one part sand. The mass of each pot with soil was of 2.5 kg. Irrigation was performed at an interval of 2 days, bringing the pot mass at 70% moisture. The hydric stress was initiated with the foliage treatment of the plant and maintained for a period of 14 days. In order to induce the hydric stress the plants were wetted every two days, bringing the pot mass to 35 % moisture.

The plantlets treatment was performed for 7 days morning by spraying plants with distilled water, and the experimental versions – humate solution (LG of 0,01 %) and salicylic acid solution (AS of 0,01 %). When they were well developed 2 to 3 three-foliated leaves, each plant was sprayed daily with 25 ml.

Samples for quantitative analysis were taken after 14th day of hydric stress at flowering initiation stage. For the biochemical analysis was collected second trifoliate leaves detached from the petiole.

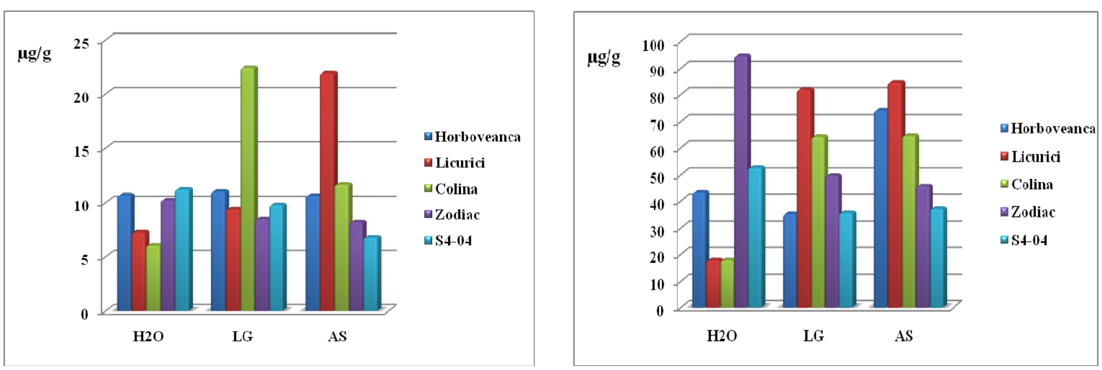
Determination of proline content was achieved through the method proposed by Bates et al. (1973): 500 mg vegetal material was triturated with 10 ml of sulpho-salicylic acid 3 % in the cold and afterwards centrifuged. Aliquots of 1 ml filtrate were mixed with reaction mixture that contains 1 ml of glacial acetic acid and 1 ml of the ninhydrin acid that consist of 1.25 ninhydrin, 20 ml H_3PO_4 (6 M) and 30 ml de glacial acetic acid. The samples were incubated for 60 minutes at t˚ - 96˚ C. Stopping the reaction was performed at ice bath for 3-5 minutes.

The proline extraction was performed by adding 4 ml of toluene to the cooled solution, which was stirred vigorously for 20 seconds. The chromophore phase that contained toluene was collected into another tube. The proline content was determined at the spectrophotometer SF-46 at a wavelength of 520 nm, by using toluene as the reference. The proline content was determined on the basis of the calibration curve constructed using the standard concentration of proline.

3. RESULTS AND DISCUSSION

The plants are adapting to hydric deficiency through different ways and mechanisms. It is known that the potential of the plant resistance to stress factors is determined largely by accumulation capacity of organic compounds, with osmolyte function, osmoprotectors being involved in maintaining cell turgescence and water flow in capturing free radicals, in the protection of macromolecules and membranes, regulation of redox potential, insurance of the cells with additional source of nitrogen, carbon and energy, but also in controlling gene expression (Lehmann et al., 2010; Seyed et al., 2012; Verslues and Hermans, 2008).

Among the compatible osmolyte in adapting plants to water stress, an important role has the amino acid proline which carries out important physiological functions. The data obtained in our experiment demonstrated that under optimal conditions of cultivation (70% moisture level TCW), the Horboveanca, Zodiac and S-4-04 sorts have been characterized through the high content of proline in comparison with Licurici and Colina sorts that have special requirements for water content in the soil (Fig. 1). The lowest values of the parameter investigated were established for the Colina sort, production of which has been compromised in the field under drought conditions (experiment conducted in the dry year 2012). Foliar treatment with humate significantly increased the proline content in the leaves of plants in Licurici sort and especially for the Colina sort and reduced the amino acid content in the case of resistant sorts, Zodiac and S-4-04 cultivated under laboratory controlled conditions, at 70 % moisture level TCW. The reaction of sorts to salicylic acid treatment was similar to that for the humate. At sorts more sensitive to drought was observed the increasing of proline content on the background administration of salicylic acid, while drought resistant sorts – decrease of its content in comparison with the witness cultivated under same conditions.



(A) **(B)**

Fig. 1. Content variation of proline (µg/g) in the leaves of different sorts of soybean, cultivated under optimal conditions (A) and hydric stress (B) on the background of foliar treatment with salycilic acid (SA) and humate (LG)

The proline level of the witness plants cultivated under hydric stress conditions (35 % TCW) raise considerably compared with the cultivated plants under optimal conditions for all sorts studied, but the largest increase was noticed to drought resistant sorts. The proline content increased about 3 times for the Horboveanca sort, approximately 5 times for the S-4-04 and about 10 times in Zodiac sort. Therefore, increased resistance to drought of sort was correlated positively with the proline

content in the leaves. The data obtained in this study are in compliance to those of other authors, who have shown that under drought conditions, in the leaves the increased content in comparison with water provided plants a considerable increase is observing in resistant genotypes (Sheeren et al., 2007).

The increase in proline content in the cell has attested to the action of various types of stress: hydric deficit, salt stress, pathogen attack and serves as an indication of plant stress resistance (Verbruggen and Hermans, 2008; Verslues and Sharma, 2010). Some authors suggest that the proline increases plant resistance to hydric deficit due to the cell osmotic pressure adjustment (Yancey, 2005). It has been demonstrated that the accumulation of osmolyte in the dehydrated plants is caused by enabled biosynthesis of proline, but also due to inactivation of its degradation (Yoshiba et al., 1997), under conditions of hydric deficit amount of proline constituting about 80 % of the total amino acid (Seyed et al., 2012).

On the hydric deficit background administration of humate and salicylic acid resulted in a significant increase of osmoprotective content in comparison with plants treated exogenously, but well assured with water. Note that, in this case, as opposed to witness, sorts with higher sensitivity to hydric deficit (Licurici and Colina) showed higher values of the parameter investigated, while resistant sorts (Zodiac and S-4-04) had lower values in comparison with the witness cultivated on the stress background and higher in comparison with witness cultivated under optimal moisture condition. The most pronounced reductions are attested for the Zodiac sort with high resistance to drought. The data obtained suggest that the substances administered may have an immune-modulatory effect which will be demonstrated in future research. It is assumed that the SA participating in the induction of tolerance mechanism of plants to drought by modulating water absorption, regulation of hydraulic conductivity of the plant, regulation of stomatal resistance and sweat intensity, these adaptive changes being oriented towards maximum efficiency of water use. It is specified that SA reduce the impact of hydric stress caused by the moisture insufficiency being involved in regulating the degree of hydration and water consumption of the plants in the process of sweating (Arfan et al., 2007; Melenciuc, 2009). Effect of humic acids on plant is explained by that these substances influence the cell membrane permeability resulting in increased exchange of necessary minerals under specific metabolic processes (Katkat et al., 2009).

The data suggest that an important contribution in achieving the genetic potential, to the productivity increase and plant resistance to adverse environmental conditions can bring biologically active substances by phenolic nature, application of which would be an effective method to improve the adaptation of plants to hydric deficit.

CONCLUSIONS

The proline content varies depending on the genotype studied; sorts resistant to drought are characterized by high content of proline in the leaves.

Under optimum condition of cultivation foliar treatment with salicylic acid and humate increased the proline content in sorts more sensitive to drought, while resistant sorts reduce its content in comparison with the witness cultivated in the same conditions.

On the hydric deficit background proline content increased significantly in the leaves of all sorts studied, and administration of humate and salicylic acid, led to an increase even more accentuated of osmoprotective content compared to plants treated exogenously, but assured with water.

Under stress conditions sorts with higher sensitivity to hydric deficit showed superior values of the parameter investigated, while resistant sorts had lower values in comparison with the witness

cultivated on the stress background and higher compared to the witness cultivated under optimal moisture conditions, the most pronounced reductions attesting for the sorts with high resistance to drought, these suggesting possible implications of substances investigated in modulating cellular metabolism.

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