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Impact of Drought Conditions and Salt Stress on the Pigment and Lipid Content of Maize During the Vegetative Stage

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***Abstract* – This paper studies the morphological and biochemical mechanisms of plants' resistance to drought and salinity. Impact of soil drought, 200 mM NaCl and Na_2SO_4 salts concentration in artificially regulated systems on sprouting of maize seeds and early phases of growth of sprouts as well as on morphogenesys, certain biometric parameters and biopolymers content is analyzed. The study revealed that there is inverse relationship between the pigment content and stressor's levels, while proportionality between photosynthesis pigments and biopolymer content is impaired by the negative impact of stressors. As a result, the plant's morpho-physiological status and biometric indicators of various plant organs are impaired.**

***Keywords* – Zea Mays, Drought, Salt Stress, Photosynthetic Pigments, Lipids.**

I. INTRODUCTION

Soil solution is one of the key factors having an adverse impact on the plant growth and development. According to Food and Agriculture Organization's (FAO) estimates, as much as up to 22 – 28% of the world's agricultural soils have been damaged by solution. Increasing concentrations of NaCl in soil is deemed as a threat as it impairs nearly all vital functions (Koshkin, 2010; Khassan et al, 2011).

Different biogenic elements act on the plants' moisture status in different ways. As a part of the hydratation process, the free ions of inorganic salts interact with water dipoles by continuously diffusing into the plant cells. As a result, while the ions of one salt may speed up the movement of neighboring water molecules, the ions of others may slow down these molecules. The hydrating properties if ions may change under the influence of serious factors. For example, the hydrating capacity of Na^+ ions decreases at higher temperatures (over 50^o C) and increases with the reduction of temperature. In their free state the ions increase the osmotic pressure of water within the cells (Alekseyev, Abdurakhmanov, 1996).

Solution of lands leads to the onset of unfavorable climatic conditions, diminishes fertility of soil and impairs biological diversity, such negative results in turn leading to significant economic losses (Bayat et al, 2010; Shevyakova et al, 2009).

The available information on the physiological functions fulfilled by lipids, which are considered to be main biological polymers and their importance to the formation of adaptation to the effects of drought conditions, is controversial (Barber et al., 1984). Author points out the fact that adaptive reactions do not lead to any changes in lipid compound, the only changing effect of these being the change in the amount of membrane proteins followed by the change in lipid / protein ratios. In contrary, some authors note significant changes in lipid compound of leaves and chloroplasts occurring due to drought (Jigalov et al, 1989). Reserve lipids of various plants are used in the spout stage for fulfillment of following three functions: (I) direct hydrolysis catalyzed by lipases; (II) acetyl-CoA – DGAT (diacylglycerol acyltransferase) pathway; and (III) lipoxygenase pathway. The paths (I) and (II) do not depend on the path (III). However, the spout stage may be characterized by all three of these. No facts have been established up to now as to: (I) which of these paths play the most significant role in the hydrolysis of reserve proteins; and (II) whether there are any difference at all between these enzymes. Even more, no facts linked directly to the genetic factors of reserve proteins' mobilization during the sprout stage have been established up to the present day (Cheng, Song, 2007).

Being one of the main vital functions of green plants, photosynthesis maintains the required energetic status of plant cells and supplies these with intermediates and plastic substances. The plants' living capacity and sustainability depends in the first instance on course of physiological and biochemical processes accompanying photosynthesis, lability and the amount of reserve substances accumulated by the plant (He, Hou., 2014).

One of the ways that stress factors exert negative impact on the plants is the reduction of the photosynthesis rate to various alterations in photosynthesis pigments. Optical properties and photochemical activity of chlorophylls as well as their ability to receive sunlight energy and use it for the biosynthesis of organic substances are related to and depend on the chemical structure of the pigment molecules (Edwards, Walker, 1983).

Stomata (mouth-like openings) of leaves also actively react to drought and solution conditions. The reduction in the amount of water vaporised through leaves as a result of stress factors occurs due to full or partial closure of stomata cells (Chaves et al., 2002).

The main purpose pursued by this paper is the comparative study of drought and salinity resistance mechanisms of leaves of plants cultivated under normal and drought conditions based on the survey of the changes in pigment and lipid amounts in maize leaves.

II. MATERIAL AND METHODS OF TRIAL

Object of the study. Maize leaves (Zea mays L.) were used in this study. The maize seeds were placed in a 3% H_2O_2 solution for 15 minutes for disinfection purposes, following which the leaves were washed under distilled water for 2 – 3 times for removal of hydrogen peroxide remnants and planted in vegetation vessels filled with soil. Following first sprouts the vegetation vessels were placed in the climatic chamber with the temperature of air ranging 25-28ºC, photoperiod of 14 hours and light intensity of 15 – 20 luxes. Following the sprout and prophyll phase irrigation of control models was continued until the end of vegetation phase, while the irrigation of one of study versions was stopped for formation of artificial drought conditions. The soils of second and third study versions were solunated by adding 200 mM of NaCl and 200 mM of Na_2SO_4 accordingly.

Obtaining leaf extracts. Leaf samples were throughly washed, dried using filter paper and weighed. 5 ml of homogenization solution was added to each of leaves following which they were homogenised at +4ºC in a pounder with the use of quartz sand. The 100 mM (pH 7.8 – 8.0) homogenization solution contained: 5 mM of dithiothreitol, 1 mM of ethylene diamine tetracidic acid, 20 mM of $MgCl_2$, 0.5% solution of polyvinyl pyrrolidone-25 and 0.5% solution of Triton X-100 and tris-HCl as a buffered solution. The homogenate obtained through the homogenization process was passed through 4-layered capron and centrifuged at 300g for 5 minutes and at 1000 g for 10 minutes for sedimentation of whole cells and tissue remnants. The fluid remaining after removal of the sediment was then used for various study purposes.

Quantitave analysis for pigment estimation. The amount of photosynthetic pigments in sample leaves was determined by Sims and Gamon method (Sims and Gamon, 200), i. e. by the spectrometric analysis of the solution acetone / Tris buffer (80:20 volume; pH = 7.8) extract of sample leaves. Amount of various pigments were calculated according to following formulae:

$$\text{Cl a (mkmol/ml)} = 0{,}01373 \cdot A_{663} - 0{,}000897 \cdot A_{537} - 0{,}003046 \cdot A_{647}$$

$$\text{Cl b } (\mu\text{mol / ml}) = 0{,}02405 \cdot A_{647} - 0{,}004305 \cdot A_{537} - 0{,}005507 \cdot A_{663}$$

$$\text{Carotinoid } (\mu\text{mol / ml}) = (A_{470} - (17{,}1 \cdot (\text{Cl a} + \text{Cl b}) - 9{,}479 \cdot \text{Antosian}))/119{,}26$$

$$\text{Antosian } (\mu\text{mol / ml}) = 0{,}08173 \cdot A_{537} - 0{,}00697 \cdot A_{647} - 0{,}002228 \cdot A_{663}$$

Estimation of total lipid content in plant organs. The quantitative determination of fats in plant organs and tissues relies on the solution of these in several organic solvents. There are other substances in plant organs and tissues that may pass to the solution. The ether and petroleum extracts obtained after removal of the solvent are called “liquid fats” and their amount may be expressed in various formats, including even in terms of percentage points. The Soxhlet extraction is one of the most commonly used methods for determination of total lipids in plant organs and tissues and has proven itself as the most accurate method. This is the reason for using the Soxhlet method for determination of total lipid content in maize leaves and seeds (Yermakova, 1972).

Statistic data: the values given in the tables and diagrams below are average mathematical data and reflect mean-square deviation.

III. ACQUIRED RESULTS AND THEIR ANALYSIS

Environmental deterioration observed all over the world, increase of temperature and salinity during summer and spring seasons as well as water scarcity and drought conditions lead to a 30 – 35% deterioration of crop capacity in cultivated plants in comparison to plants supplied with sufficient amount of water (Boyer et al, 1982). At a rough estimate, the world’s population will reach 10 billions by 2025 (Dyson, 1999). Consequently, artificial selection of high-yielding plant varieties with the capability of surviving and growing under adversative environmental conditions may be considered as one of the significant directions (Miflin, 2000). Satisfactory solution of this problem largely depends on the study of physiological and biochemical mechanisms of plant adaptation processes and morpho-physiological symptoms of such adaptation.

If to consider in terms of C_4 - dicarboxylic acid decarboxylation, maize belongs to NADP-malic enzyme – type C_4 plants. It utilizes water, sun light and carbonic acid in a very effective way and is distinguished from C_3 plants by lesser water deficit despite intensive transpiration and higher drought resistance (Tkachuk, 1983).

Abiotic stress factors of environment, including drought and solution lead to certain changes in carbon and nitrogen metabolism of plants through exerting negative impact on the intensity of photosynthesis and water-related parameters (Vogel et al, 1986). Reference literature contains contradictory conclusions as to the impact of soil solution on photosynthesis. While some authors point out that salt stress may reduce the photosynthesis intensity, the others claim that NaCl exerts great impact on the efficiency of the photosynthesis process (Bong, Loreto, 1989).

One of the main active photosynthetic components of plants are chlorophylls. Environmental conditions, including the sun light at the first instance, exercise great influence on the plants’ functional activity, while the pigment apparatus is affected by such influence in the first place. Amount of pigments and the ratios between various pigment contents depends on many internal and external factors. Chlorophyll a/b ratio is considered as an indicator of biosynthesis and cleavage processes within the plant’s photosynthetic apparatus (Dymova, Golovko, 2007). Constancy of this ration is a reflection of the fact that the pigment biosynthesis process is stable.

Figures 1 and 2 reflect the results related to the effect of drought conditions and solution on the green pigment content of maize leaves. Though the content of “a” chlorophyll increases to a certain degree during most active phases of the plant, its eventual reduction rate in different study models varied (see: Figure 1), with the drought model showing the largest and salt soil model showing the smallest reduction rate. Changes in “b” chlorophyll, carotinoid and anthocyanin pigments content show zigzag pattern (see: Figures 1 and 2). If to compare the protein content, it appears that largest amount of proteins are found in the control group, followed by soluted soil and drought groups. Therefore, the a/b chlorophylls ratio assumes a value that is less than unity. Comparison of all study groups' chlorophyll contents revealed that as the plants in the control group got older, their chlorophyll content also increased. However, as the "a" chlorophyll content is larger than "b" chlorophyll at all times, the a/b ratio is greater than unity.

At the same time, though the "a" chlorophyll content of drought models spiraled down in a time-dependent manner, the "b" chlorophyll content increased in contrary, while it decreased as the drought conditions worsened. As the Figure shows, in control group plants the Xl a/b ratio is (in accordance with the growth phases) by 6-9 times greater than unity. In drought models, being by 5 times greater than unity, this ratio decreases down to unity as drought conditions get worse and worse. In salt soil model plants this ratio decreases in parallel to decreases in solution degree. There are many previous authors that obtained similar-to-ours results. So, some authors (Lapina, Popov, 1977) state that solution leads to lesser chlorophyll content in the leaves of cultivated crops. Udovenko established that although the "a" chlorophyll content increases due to the solution, "b" chlorophyll content remains constant (Udovenko, 1977). These authors attribute the change of chlorophyll contents to salt-resistance of plants and eventual soil solution. All these changes root in the changes of solar energy conversation processes occurring within the light-collecting complexes of photosystems (Bassi et al, 1990).

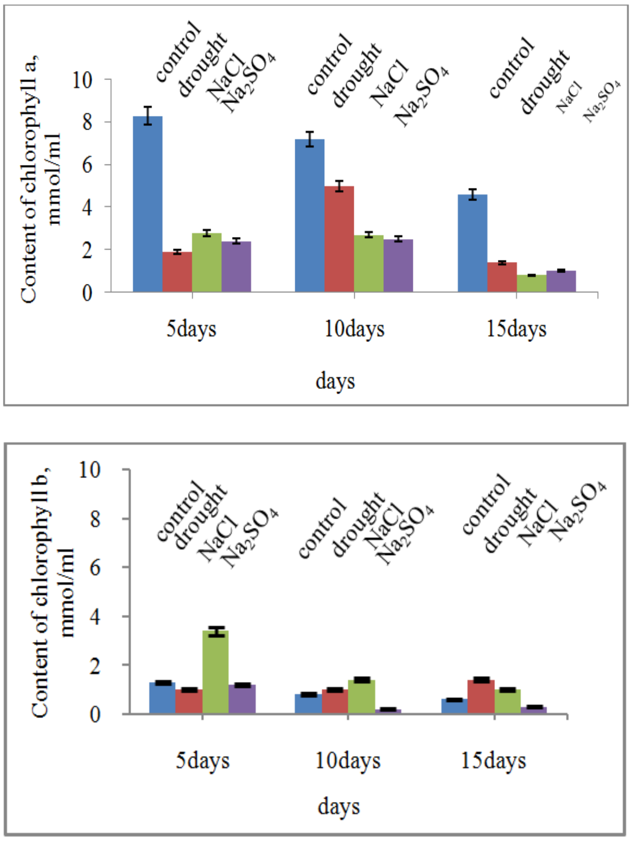

Fig.1. Impact of drought conditions, chlorine and sulfate salts on the chlorophyll content during the early stages of growth

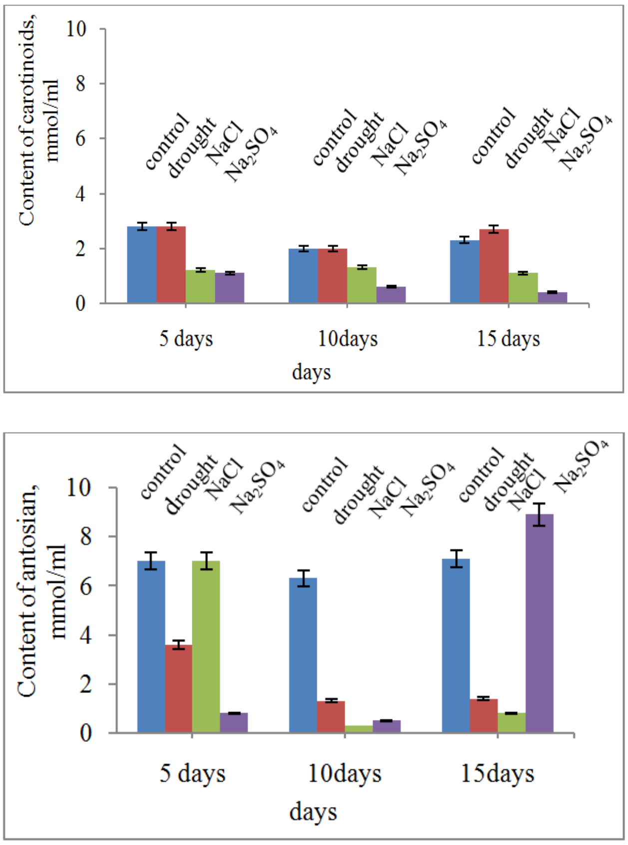

Fig.2. Impact of drought conditions, chlorine and sulfate salts on the carotinoid and anthocian content during the early stages of growth

Chlorophylls in plant leaves bind to chloroplasts by Chlorophyll a-b binding proteins. As these complex compounds break down under the action of NaCl, chlorophyll content of salt-exposed cells reduces over time. This occurs due to the fact that proteins are main protective factors for chlorophylls (Chupakhina, 2000).

In circumstances characterized by solution of soil, toxic and osmotic impacts of salts lead not only to deterioration of photosynthesis but also to negative changes in plants' water intake (Thermat, Munns, 1986; Fricke, 2002). Results of our experiments show that prolonged exposure to salts (200 mM) increases the photosynthesis intensity (longer the exposure time, lesser the intensity), while such negative impact is larger with Na_2SO_4.

In summary, the results of our study together with the analysis of cited literature suggest that soluted environment dramatically reduces the intensity of photosynthesis in plants, such reduction occurring due the reduction of pigment contents and hydrolytic activity of chlorophyllase and degradation of chlorophyll-containing protein complexes.

Under the circumstances characterized by negative impact of stress factors synthesis and accumulation of different biopolymers of various origins - that are the products of various metabolic paths- gains enormous importance in terms of plant protection. Biopolymers with such peculiarities include, inter alia, proteins, fats and hydrocarbons. Plants with high fat content are more resistant to drought due to the fact that fats in plant tissues prevent evaporation. Therefore such plants may grow normally even if they do not intake water for prolonged periods. Stress factors also lead to noticeable changes in the amount of lipids synthesized and accumulated in plant tissues. Results of our study allow us to state that the leaves may produce significantly larger (by 4, even 8-9 times in some cases) amounts of lipids in comparison with other plant organs. As the Table 1 shows, no matter how much dried, the leaves and seeds maintain certain levels of moisture (7-17%). This moisture is required for preserving the germ. In circumstances of full drought the sprouting capacity of seeds deteriorates to the lost of the germ's viability.

Table 1: Study of the impact of drought conditions, chlorine and sulfate salts on the lipid content (lipid content determined by Soxhlet method)

Study models	Moisture, (%)	Fat content, (%)	Fat content, % (absolute dry weight)
Leaves			
Control	16,8	1,6	1,92
Drought	7,2	0,74	0,78
200 mM NaCl	8,9	1,44	0,99
200 mM Na_2SO_4	7,8	1,57	0,95
Seeds			
Total	12,5	6,73	7,69

Weise et al were able to determine the factors that influence the amylolysis of starch in chloroplasts of bean and *Arabidopsis thaliana.* Beans accumulate starch during daytime and break down it at night time of natural light – day cycles. This rhythm may be preserved even under uninterrupted exposure to light, in other words, regulation occurs by means of circadian rythms (circadian clock). 1 hour after replacing the light with darkness maltose content increases by 50%, while the starch and glucose-6-phosphate content remains unchanged. Under conditions that are favorable in terms of photorespiration, the maltose and glucose-6-phosphate content increases by three times after 1 hour, while starch content decreases by 25%. As 100% N_2 completely prevents respiration in bean leaves, the starch, glucose-6-phosphate and maltose content remains unchanged. Introducing oxygen (2%) to N_2 environment stimulates respiration. However, despite slight increase in maltose content, such stimulation doesn't lead to changes in glucose-6-phosphate content. Thus, hydrolytic and phosphorilityc degradation of starch depends on environmental factors (Weise et al., 2006).

Total impact of all above mentioned symptoms affects physiological properties of plants, leading to noticeable anomalities in the plant's morphological and anatomical structure and deteriorating overall crop capacity due to reduction of photosynthesis intensity.

CONCLUSION

In sum, as a conclusion of our results, we can state that adaptive reactions form against effect of stress factors of plants, depending on effect period of stress, on its type. These reactions show themselves, for the first turn, in relative volume of lipid content and density of pigments.

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