

Comparative Analysis of the Effect of Water and Salt to the Quantity of Proteins and Parameters of Gas Exchange, Indices of Photosynthesis in Maize Leaves

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Abstract: Effect of long term ground dryness and high density of NaCl and Na₂SO₄ (200 mM) to the photosynthesis pigments in leaves in different stages of maize development, parameters of gas exchange-to the speed of photosynthesis (P_n), conductivity of mouths (g_s), volume of CO₂ (C_i) in spaces between cells, transpiration intensity (T_r) and quantity of proteins is studied. It is stated that, though development of change of photosynthesis indices in dry and double component systems flow similar and parallel, there are significant differences in further stages. So, enhance of water deficiency from 25% to 35-40%, decline of relative quantity approximately from 89% to 70 caused to decline T_r, P_n and g_s, to the enhance of C_i. Nevertheless quantity of proteins and photosynthesis pigments enhance in initial and middle stress period at the result of the effect of both stresses, gradual reduction is stated in further periods in comparison with controls in both 2 variants.

Keywords: Maize Leaves, Dryness, Saltiness, Photosynthesis Pigments, Protein.

I. INTRODUCTION

Unique adaptation mechanisms of plants, enabling adaptation to abiotic and biotic factors of environment, form during evolution process. Intensive trials have been conducting during last year's regarding with study of respond reactions of plants to the dryness and other possible stresses (saltiness, lower and higher temperature, dryness and so on) (Chaves, Oliveria, 2004; Flexas et al., 2006; Касымов, 2012). 25% of earth become salty in fluctuating degrees in modern times (Hasan and others, 2011). Saltiness of territory make possible non convenient climate change, leads to decline of productivity and weakening of biodiversity and finally, it leads to significant economic loss (Bayat and others, 2010; Shevyakova and others., 2009).

Therefore, formation of new sorts, possessing life possibilities, adapting to the non convenient environment, is one of the important tasks, stand before selector scientists. We understand under term of "adaptation" alterations in structures and functions of organs of bodies in order to preserve the chances to live under effect of extreme factors (Petunkina and others, 2012).

Photosynthesis is one of functions of green plants, provides energetic condition of cell, provides them with mediator and plastic agents. For the first turn, life possibilities and durability of plants depend on the flow of physiologic and biochemical processes, stated during photosynthesis, at the same time, quantity of reserve agents, collected, and lability, corresponded to the condition (He, Hou, 2014).

Study of alteration of total protein quantity and pigments, parameters of gas exchange in leaves depending on water indices during effects to the photosynthesis process of plants consists of basis of some of our researches.

Chlorophyll a, b and carotinoids are included to light collecting pigments in plants and algae. Optical characters and photochemical activity of chlorophylls, character of reception of sun energy and use it for biosynthesis of organic agents regard with chemical structure of pigment molecule (Edwards, Walker, 1983). Stress factors of environment, for the first turn, effect to photosynthesis through pigment apparatus.

Mouth cells of leaves is one of the bodies, responding to effect of dryness and saltiness. Decline of volume of evaporated water through leaves at the result of stress happens at the result of complete and partial closure of mouth cells (Chaves et al., 2002). Second effect is stated in this case, so, closure of mouths leads to the reduction of volume of breathed CO₂ (Chaves, 1991; Cornic, Massacci, 1996; Maroco et al., 1997). Plants with C₄, like maize, amaranth, sugarcane and so on, have relative durability to dryness at the expense of "carbon thickening mechanism). Normal flow of reactions of Calvin cycle is provided regarding with activation of ferments of carbon metabolism and enhance of density of CO₂ in carboxylation centers in closure pile cells (Long, 1999).

Principle aim of work consists of comparative study on the basis of exchange of pigment, protein and gas of dryness durability and saltiness durability mechanisms in leaves of trial plants and maize plants, watering regularly.

II. MATERIAL AND METHODS OF TRIAL

Leaves of maize are used for the purpose of trial (*Zea mays L.*). Seeds of maize are disinfected in hydrogen peroxide solution 3% during 15 minutes in order to remove pathogenic agents. Then, seeds are washed 2-3 times with distilled water in order to remove hydrogen peroxide and they planted in ground in vegetation dishes. After first sprouts, vegetations dishes are placed in artificial climate chamber under temperature 25-28 C, photo period 14 hours, humidity 60-70% and intense of light 15-20 kluks. Watering in control variants is continued up to the end of vegetation after sprout and first leaf period of development, ground dryness is formed stopping watering in one of the experiment variants, artificial saltiness is formed adding 200 mM NaCl in second period, 200 mM Na₂SO₄ in third period. Specimens are taken after lightening of plants for 3-4

hours after each 5 days, at 12:00, and meterings are carried out.

Relative quantity of water (RQW) in leaves is determined through method of Tambussi (Tambussi et al., 2005). Cut leaf fragments are covered with filter paper, placing to the water and completely saturated with water during 24 hours. After saturation, leaves are dried with filter paper and weighed again. The, leaf specimens are dried again in thermostat up to getting absolute dry weight and weighted again. Calculations of water regime are carried out according to the following formulas:

$$SNM=100\% (M_F-M_p)/M_T-M_p)$$

where M_F is weight before dryness; M_T -weight after drying, M_p is dry weight of leaf.

Natural humidity of ground is determined according to Alexandrova-Naydenova (1967) method.

Quantity of chlorophylls in leaf specimen of plants is determined through spectrophotometric method (acetone: Tris-HCl buffer, pH 7.8-volume 80:20) in acetone extract 80% according to Sims and Gamon method (Sims, Gomon, 2002).

Parameters of gas exchange - P_n , g_s , C_i and transpiration speed (T_r) is determined through placing of infrared gas analyser LI-6400 XT (LI-COR 6400 XT, Biosciences, ABŞ), not separating leaves from body.

Quantity of total solved protein is metered with Loury method in 750 nm wave length with spectrophotometric method (Ultrospec 3300 pro, Amersham). Serum albumin of cattle is used for construction of degree curve (Lowry et al., 1951).

Statistics: indices of schedule and graphics are average math indices, reflex average quadratic deviation. Analysis of the results of research average math failures and deviations ($M \pm m$) are taken into consideration.

III. ACQUIRED RESULTS AND THEIR ANALYSIS

Ambiotic stress factors of external environment make definite alterations in carbon and nitrogen metabolism, effecting to photosynthesis intense and water indices, depending anatomic structure of leaf under dryness and saltiness (Vogel et al., 1986). One of the active main photosynthetic components in plant leaves is chlorophyll *a* and *b* with green pigment. First of all light, external environment make strong effect to the functional activity of plants. Quantity of pigments and relativity to each-other are related with several external and internal factors and explained with biosynthesis of pigments-2 processes and activity of distribution processes (Dimova, Golovko, 2007).

From this point of view, results, acquired from implemented experiments show several interesting facts. There is definite conformities between relative volume of water of leaf (RVW) and natural humidity of ground (NHG) as we see from table # 1. Acquired results show that RVW and NHG get highest and non altered value in

all control variants depending on age of plant. Quantity of total protein is also high in these trail specimens (Table #2). Total quantity of protein under dryness reduced 2 times, it keeps relative unchanged index in further meterings, RVW and NHG reduced for 60%, approximately equals to 3 times during last meterings with continuation of the decline.

Table 1. Effect of dryness and saltiness to the quantity of total protein, relative volume of water (RVW) and natural humidity of ground (NHG)

Date	Variant	Protein, mg/g age, mass	RVW,%	NHG, %
19.09.2014	Control	83,5	89,0	83,0
25.09.2014	Control	90,5	88,0	83,0
	Dryness	38,0	68,0	54,0
	200 mMNaCl	51,5	80,0	57,0
	200 mM Na ₂ SO ₄	48,5	83,4	57,0
01.10.2014	Control	88,0	72,6	80,0
	Dryness	39,0	53,1	41,0
	200 mMNaCl	38,0	86,9	62,0
	200 mM Na ₂ SO ₄	35,6	76,0	60,0
06.10.2014	Control	72,4	72,0	87,0
	Dryness	36,0	31,0	30,0
	200 mMNaCl	39,0	50,0	60,0
	200 mM Na ₂ SO ₄	38,5	60,0	66,0

Quantity of total protein in specimens with composition of 200 mM NaCl and 200 mM Na₂SO₄, stated in table 1 reduced up to 50% as in the variants under dryness and this index stay without alteration for the further periods. RVW and NHG is more approximately for 70-80% in comparison with dryness.

Results regarding with effect to the quantity of green pigments in leaves of maize under dryness are stated in the table 2. As you see from table quantity of pigments reduced with participation of salts, quantity of chlorophyll *b* is more than quantity of chlorophyll *a* in these variants in difference from control and dryness variants. Therefore, chlorophyll (*a/b*) ratio is more than a unite. Comparisson of chlorophylls with each-other in experiment variants shows that when age of plant increases quantity of chlorophylls also increases in control variants. However, considering that quantity of chlorophyll *a* is always more than quantity of chlorophyll *b* figure, acquired from their ratio is more than one. Although quantity of chlorophyll *a* gradually reduces, depending on time, under condition of dryness, quantity of chlorophyll *a* gradually increases during initial times in order to resist to stress, it reduces with strengthening of dryness.

Some authors (Lapina, Popov, 1977) state that quantity of chlorophylls reduces in leaves of cultural plants under effect of salt. Udovenko determines that, though quantity of chlorophyll *a* enhances at the result of effect of salt, quantity of chlorophyll *b* stays without change (Udovenko, 1977). They explain change of quantity of chlorophylls with durability of plants to salt and gradual increasing saltiness of substrate.

Table 2. Dryness and effect of salt stress to the pigments of photosynthesis in leaves of maize (mmol/ml)

Date	Variant	Chl a	Chl b	Chl (a/b)
19.09.2014	Control	5,8	5,0	1,2
	Dryness	7,0	6,2	1,1
	200 mM NaCl	3,5	4,2	0,83
25.09.2014	Control	6,4	6,1	1,1
	Dryness	7,0	6,2	1,1
	200 mM Na ₂ SO ₄	2,5	2,9	0,85
01.10.2014	Control	7,6	6,4	1,2
	Dryness	5,3	8,9	0,59
	200 mM NaCl	2,7	4,1	0,65
06.10.2014	Control	7,9	5,9	1,3
	Dryness	1,6	1,4	1,1
	200 mM Na ₂ SO ₄	1,1	2,7	0,4
06.10.2014	Control	7,9	5,9	1,3
	Dryness	1,6	1,4	1,1
	200 mM Na ₂ SO ₄	1,2	2,5	0,48

In our trials under the effect of salts, enhance of chlorophyll b in comparison with chlorophyll a especially, under the effect of sulphate salt is explained with accumulation of chlorophyll b and this mainly regarded with photosystem II (FS). It is known that all of chlorophyll b is included to the composition of II light collecting complex (LCC), transferring of absorbing light energy to the reaction center (RC) FS II (Bassi et al., 1990; Benett, 1983). Few part of chlorophyll b is included to complex, organized FS II RC and I LCC (FS I). Complex, consisting of chlorophyll b and II LCC not only plays important role in regulation of light power flow, entering to RC of FS I and FS II, but also in adaptation of photosynthetic apparatus with changing external condition (Anderson, 1986). Strong effect of chloride saltiness to pigment system of plants is explained with inhibition of chlorophyllase, one of ferments of chlorophyllsynthetasa, responding to synthesis of green pigments. This ferment carries out synthesis from chlorophyll a and phytol to chlorophyll a, that is final stage of synthesis of chlorophyll a molecule (Fang et al., 1998). Minninberg and Zu (1973) state that main reason of decline of chlorophyll quantity under effect of salt is the reduction of velocity of combination of phytol with chlorophyll. The basis of this is formation of better pH condition for the hydrolytic effect of ferment under effect of salt.

As well as, chlorophyll places in combination with proteins in chloroplasts. Quantity of chlorophylls in cell reduces at the result of destruction of this complex combination under the effect of NaCl. It regards that proteins are one of the main factors which protect chlorophylls from negative effects (Chupakhina, 2000).

Photosynthesis activity of plants depends on lightening condition, CO₂ quantity, temperature of environment, water supply, mineral nutrition and other environmental factors. In total, these factors lead to enhance of activity of photosynthetic apparatus, effecting to separate reaction of photosynthesis, finally they determine total productivity of plant.

There are serious alterations in photosynthesis under toxic and osmotic effect in the environment of sand saltiness. There are alterations in water supply beside photosynthesis activity of plants under effect of salts to the plants (Thermat, Munns, 1986; Fricke, 2002).

According to our experiments intensity of photosynthesis gradually reduces while enhance of exposition term of salts in density of salt 200 mM. In this case, effect of Na₂SO₄ is clearer in comparisson with NaCl.

Inhibiting effect of high density of salts to photosyntehsis conditioned with distortion of ultrastructure of chloroplast components and this results to deep and encircling alterations in metabolism. According to Udvoenko, though reduction of photosynthesis activity in salt background, then it combines with control. It may be evaluated as one of the adaptation possibilities in physiologic functions of body under new extreme condition (Udovenko, 1977).

It is know that, there is 2 stages in herbal adaptation reactions to the stress: fast initial respond stage (stress reaction) and relative long term. More reliable and efficient defense reactions form in ontogenesis stage of salts (Pokhomova, 1995). If mutual effect of stress prevails defense possibilities of body destruction will happen, organism may face with danger of death at the result of it (Pokhomova, 1995).

According to the analysis of information in literature, we may conclude that there is acute weakening in intensity of photosynthesis under saltiness condition. It conditioned with decline of green pigments, enhance of hydrolytic activity of chlorophyllase and weakening of durability chlorophyll-protein complex.

It is know that stress factors of environment provide strong effect to gas exchange parameters in plant organisms. From this point of view, the results of our experiments are stated in photo. As you see from photo, intense of photosynthesis in initial days of stress effect insignificantly enhances in comparison with control. Intense of photosynthesis weakens in all variants by increasing stress and significantly reduces in comparison with control under effect of salt density and dryness gradually declining in final stage. Intense of photosynthesis significantly reduces with the effect of Na₂SO₄. Next is NaCl. Effect of dryness to the intensity of photosynthesis is weak in comparison with salts.

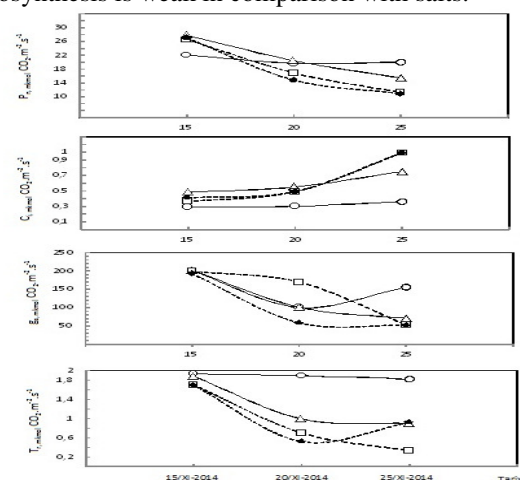


Figure. Dynamicity of effect of dryness, salt stress to the parameters of gas exchange in initial growth of maize leaves (○-control, △-dryness, □ - 200 mM NaCl, ◆-200 mM Na₂SO₄)

According to the acquired results, we can say that conductivity of mouth cells become weak with the effect of stress. We observe significant enhance of CO₂ volume (resistance of mouths) in spaces between cells only and it is seen that it regards with closure of mouth cells with the effect of dryness and salt stress. According to the harmful effect of NaCl to the plants, it is determined with 2 factors: 1) decline of involvement of water to the plant at the result of reduction of water potential in ground solution 2) Weakening of height growth of plants at the result of effect of Na⁺ and Cl⁻ ions to the plants (Termaat, Munns, 1986). Their productivity declines at the result of reduction of herbal biometric and several physiologic and metabolic indices at the result of effect of these factors (Fricke, 2002).

IV. 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 protein and water, density of pigments, as well as intensity of photosynthesis of leaf and indices of gas parameters.

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