PHYSIOLOGICAL EFFECTS OF THIOUREA ON BIOLOGICAL PERFORMANCE OF PLANTS IN DROUGHT CONDITIONS: I. INCREASE OF ANTIOXIDANT PROTECTION

EFECTUL FIZIOLOGIC AL TIOUREEI ASUPRA PERFORMANŢELOR BIOLOGICE A PLANTELOR ÎN CONDIŢII DE SECETĂ: I. MAJORAREA PROTECŢIEI ANTIOXIDANTE

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Abstract. *The greenhouse experiments were conducted to evaluate the effect of grain presoaking and foliar application of cytokinin (CK), thiourea (TU) and combination (TU+C) - thiourea with Composite preparation (pat. MD 813) containing micronutrient, on the changes of antioxidant enzymes activities, photosynthesis and grain yield of maize plants under normal water content and drought stress conditions. Treated with TU and TU+C plants resulted in great increases in the activity of SOD, CAT, APX, GR. The higher antioxidant enzyme activity in pre-treated plants was associated with the lesser MDA. Positive physiological effects of TU and TU+C were confirmed by the higher level of assimilating pigments and photosynthesis. Concentration of carotenoids was affected by drought, but spraying with TU and TU+C alleviated drought effects. It was concluded that combined application of TU+C as seed treatment and foliar spray was more effective than cytokinin in improving the Zea mays performance.*
Key words: plants, drought, thiourea, cytokinin, antioxidant enzymes

Rezumat. *În experienţe de vegetaţie s-a studiat efectul pre-tratării semînţelor pentru semănat şi aparatului foliar al plantelor de porumb cu citokinină (CK), tiouree (TU) şi tiouree + Compozit (TU+C), - preparat, care conţine micronutrienţi, br.MD 813, asupra activităţii enzimelor antioxidante, fotosintezei, creşterii şi productivităţii plantelor în condiţii de umiditate optimă şi de secetă. Pre-tratarea plantelor cu TU şi TU+C condiţionează majorarea activităţii superoxid dismutazei (SOD), catalazei (CAT), ascorbatperoxidazei (APX), glutationreductazei (GR) şi glutationperoxidazei (GPX). Activitatea înaltă a enzimelor antioxidante este asociată cu diminuarea conţinutului di-aldehidei malonice (DAM). Efectul fiziologic pozitiv al TU şi TU+C este confirmat şi de nivelul mai înalt al pigmenţilor de asimilaţie şi fotosintezei. În concluzie: utilizarea combinaţiei tioureei şi compozitului pentru pre-tratarea semînţelor pentru semănat şi aparatului foliar este veridic mai efectivă pentru ameliorarea performanţelor biologice ale plantelor de Zea mays prin majorarea activităţii enzimelor antioxidante, fotosintezei şi productivităţii plantelor.*
Cuvinte cheie: Plante, secetă, tiouree, citokinină, enzime antioxidante

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INTRODUCTION

In connection with climate warming on Earth, the problem of water use by plants and physiological consequences of drought, remains the most important impediment to agriculture and food production on a global scale.

The developing and implementing in practice of innovative methods related to tolerance induction, impact mitigation, and stabilization of plant productivity open big prospects in agriculture country located in the area of climate risk, such as and the Republic of Moldova. Therefore, the development that would increase plant resistance to unfavorable factors is a necessary and current requirement for contemporary agriculture.

A wide variety of physiologically active substances are involved in the regulation of plant growth and development under stress. It is known a positive correlation between plant hormones content, including their exogenous application, and antioxidant protection, and plant drought tolerance formation (Asada, 2006; Parisa Sharifi *et al.,* 2012; Merewitz *et al*., 2015; Ştefîrţă *et al.,* 2015). On the other hand, a series of compounds with thiol groups are involved in maintaining the redox status (SH / -SS-), exhibiting antioxidant properties (Astir *et al.,* 2013; Pandey *et al.,* 2013). It is known the adaptogenic effect of urea derivatives – thiourea, diphenylurea, and difeniltiourea, known as compounds with similar to natural cytokinin action, due to attenuation of oxidative lesions of plant cells (Ламан *et al.,* 2010).

The objective of the researh was the comparative analysis of antioxidant properties of cytokinin and thiourea to develop an innovative method to increase plant tolerance to drought.

MATERIAL AND METHOD

As the study objects served plants *Zea mays* L., cultivar (cv.) P458. The greenhouse experiments were performed on plants grown in Mitcherlih containers with a capacity 30 kg absolutely dry soil under controlled water content conditions. The scheme of experiences provided the following treatments: a) control plants, grown on permanent soil water content - 70% from the total water capacity of soil (TWC); b) plants exposed to the drought (30 % TWC) for 10 days. The parallel treatments included the exogenous pre-treated plants with CK (0.0001%), TU (0004%) and TU (0.0004%)+C (0.0001%) in 1:1 ratio. The analyses were performed after 10 days of water stress at the initial stage of growth and during flowering of plants. Homogenization of the plant material, fixed in liquid nitrogen, and extraction were performed as described (Keshavkant and Naithani, 2010). The values of the total antioxidant status have been evaluated by the degree of modification of the content of malone dialdehyde (MDA) and of the antioxidant enzymes activity.The superoxide dismutase (SOD) activity was measured according to the method (Чевари, Чаба, Секей,1985); the ascorbate peroxidase (APX) activity was assayed by the method (Nakano and Asada, 1981); the catalase activity was determined by the method (Chance B. and Machly A.,1955); glutationreductase (GR) - by reduction of oxidized glutathione in the presence of NADH H2, λ 340 nm (Schadle, Bassham, 1977); Glutation peroxidase (GPX) - by oxidation of reduced glutathione, 260 nm (Полесская,

Каширина, Алехина, 2004).The intensity of CO_2 assimilation, transpiration, stomatal conductivity, and water use efficiency were determined by using a portable LCA-4 gas analyzer in experiments performed under the same conditions of temperature and soil water content. The contents of chlorophyll *a* and *b* and carotenoids were determined, spectrophotometrically in 80% acetone extract. Statistical analysis of results was performed using the computer program "Statistics 7".

RESULTS AND DISCUSSIONS

It has been known, that water stress tolerance was associated with the induction of antioxidant defence systems, including reactive oxygen species (ROS) scavenging enzymes such as SOD, CAT, APX, GPX, GR, and non-enzymatic antioxidants such as ascorbic acid, glutathione, α-tocopherol, and carotenoids. Drought stress induced high production of ROS and caused damages by increasing lipid peroxidation (MDA) of the cells (Sairam and Saxena, 2000; Mittler, 2002, 2006). According to our research results, plants pre-treated with the CK, TU and TU+C reduced the impact of the oxidative stress caused by drought (tab. 1).

Table 1

The influence of pre-treatment of maize seed with PhAS on antioxidant enzyme activity in leaves of plants, exposed to drought in the early stages of ontogenesis

Parameters	Control, optimal	Control, drought	TU, drought	TU+C, drought
	M±m	M±m	M±m	M±m
MDA, мkmol · g^{-1} f.w.	9.5±0.11	14.2±0.2	13.9±0.11	9.6±0.11
SOD, conv.un · g^{-1} f.w.	126.8±2.1	145.6±2.9	197.9±3.1	199.0±2.7
CAT, mmol · g^{-1} f.w.	0.45±0.01	0.38±0.02	0.50±0.001	0.50±0.004
APX, mmol · g^{-1} f.w.	1.9±0.03	2.1±0.05	2.2±0.04	2.8±0.06
GR, mmol · g^{-1} f.w.	55.7±0.77	60.6±1.0	63.5±1.23	73.4±1.12
GPX, mmol · g^{-1} f.w.	26.0±0.54	27.8±0.5	28.9±0.31	31.0±0.28

The effect of TU and TU+C treatments on antioxidant enzymes activity had similar trend under normal soil water content and drought conditions. Namely, it resulted in considerable increases in the activity of SOD and CAT enzymes accompanied by great reduction in MDA content. Maximum growth of SOD and CAT activity was observed by treating plants with TU under drought conditions (56.1% and 11.1%, respectively). Pre-treatment of seeds with the combination of TU+C led to greater increases of APX, GPX and GR activities (43.8%, 19.2%, and 31.7%) compared to the degree of magnification in the activity of these enzymes induced by TU (16.7%, 11.0%, and 14.0%, respectively). Also, greater

antioxidant enzyme activities in pre-treated plants were associated with the lesser MDA content.

In field conditions the drought stress can be aggravated by high temperatures and intense solar radiation, which drastically reduces crop plants. The plants at reproductive growth stage are very sensitive to high temperatures and insufficient soil water content.

According to our previous research findings (Ştefîrta *et al.*, 2015), the exogenous application of phytohormones to some extent neutralized the adverse drought effect. This action could be explained with improving the water status in the cells and activation of antioxidant enzyme system. Major effect occurred following the administration of exogenous CK. Taking into account the close connection between phytohormones and water status it could be assumed that phytohormones were capable to stabilize water homeostasis and to influence on the degree of formation of ROS. These results formed the basis of the idea that applying of substances with cytokinin activity would result in the optimization of the antioxidant systems in moderate drought conditions. Indeed, subsequent studies have demonstrated an activation of enzymatic antioxidant protection system in leaves of plants pre-treated with physiologically active substances such as cytokines (tab. 2).

Table 2

Influence of CK, TU and TU+C on the antioxidant protection in the leaves of *Z. mays* plants under drought conditions

Parameters	Control	CK	TU	TU+C
MDA, мkmol · g^{-1} f.w.	37.35±1.67* 44.30±0.98**	34.43±1.21 35.94±0.57	27.67±1.1 31.44±0.6	25.34±1.2 26.65±0.6
SOD, conv.un · g^{-1} f.w.	50.64±0.88 60.55±0.69	56.95±0.72 63.64±0.88	59.37±0.6 69.89±0.7	75.97±0.8 80.63±1.2
CAT, mmol · g^{-1} f.w.	0.83±0.003 0.76±0.006	0.93±0.002 0.90±0.008	1.17±0.006 1.03±0.008	1.19±0.009 1.08±0.009
APX, mmol · g^{-1} f.w.	2.48±0.03 2.55±0.03	3.81±0.04 5.33±0.07	5.10±0.03 5.70±0.06	4.22±0.05 5.92±0.0
Chlorophyll a+b, mg ·100 g^{-1} f.w.	200.46±2.98 187.91±2.16	239.54±3.5 220.87±2.3	292.12±3.1 242.17±2.2	249.35±3.2 221.13±3.1
Carotenoids, mg ·100 g^{-1} f.w.	33.62±0.98 30.25±0.59	44.87±0.76 39.44±0.45	46.75±0.65 40.21±0.21	44.12±0.45 43.50±0.34

*- in optimal soil water content conditions; **- in drought conditions

The treated plants had a more effective protection system, which ensured the possibility of their functioning under stress. It was evident, the plants pre-

treated with CK, TU, and especially with TU+C, in drought conditions differed from control ones by significantly lower content of MDA, which was indicative of lesser degree of lipid peroxidation and oxidative destructions due to higher protection capacity of antioxidant enzymes SOD, CAT and APX. Positive physiological effect of TU and TU+C was confirmed by the higher level of assimilating pigments. Concentration of carotenoids was affected by drought, but spraying with TU and TU+C alleviated drought effects. Combined application of TU and TU+C as seed treatment and foliar spray was more effective than cytokinin in improving the *Z. mays* performance (tab. 3).

Table 3

The effect of TU and TU+C use on biological performance of *Z. mays* plants in drought conditions

Parameters	Control	CK	TU	TU+C
Height of plant, dm	12.15±0.3 * / 9.85±0.13 **	14.28±0.1 / 11.2±0.12	16.10±0.4 / 10.7±0.17	13.02±0.20 / 12.23±0.21
Leaf area, dm^2	37.82±0.35 / 30.23±0.32	39.04±0.28 / 32.30±0.29	39.19±0.19 / 33.95±0.43	39.34±0.41 / 35.70±0.34
Productivity, g/plant	52.8 ± 0.5 / 34.81±0.5	61.70 ± 0.4 / 38.94 ± 0.3	69.30 ± 0.6 / 37.72 ± 0.6	70.0 ± 0.9 / 39.74±0.9

*- in optimal conditions; **- in drought conditions

CONCLUSIONS

1. Cytokinin (CK), thiourea (TU) and thiourea in combination with Composite preparation, containing micronutrients, (TU+C), all used for *Zea maize* plants treatment through grain presoaking and foliar, provided the increase of the antioxidant capacity of cells, the content of the assimilating pigments, and the reduction of lipid peroxidation.

2. Treating plants with TU and TU+C resulted in considerable increases in the activity of SOD, CAT, APX, GR. The greater antioxidant enzyme activity in pre-treated plants was associated with the lesser MDA content.

3. The TU and TU+C increased the adaptive potential of plants, reduced the negative action of soil water content deficit, and optimized the processes of growth and productivity.

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