EFFECT OF EXOGENOUS POLYAMINE DIETHYLENETRIAMINE ON OXIDATIVE CHANGES AND PHOTOSYNTHESIS IN AS-TREATED MAIZE PLANTS (Zea mays L.)

ЕФЕКТ НА ЕКЗОГЕННИЯ ПОЛИАМИН ДИЕТИЛЕНТРИАМИН ВЪРХУ ОКСИДАТИВНИТЕ ПРОМЕНИ И ФОТОСИНТЕЗАТА В ТРЕТИРАНИ С АРСЕН ЦАРЕВИЧНИ РАСТЕНИЯ (Zea mays L.)

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ABSTRACT

The antioxidant effect of the exogenous polyamine Diethylentriamine (DETA) on the oxidative changes in young maize plants treated with different As concentrations was studied. The plants were grown in a climatic box in a Hogland-Arnon nutrient solution. Arsenic was applied as Na_3As0_4 in concentrations 0, 2 and 5 mg dm^{-3} (pH 5.5). The polyamine DETA (concentration 10^{-4} M) was added to the nutrient environment of some of the plants 24 hours prior to the As treatment. Five days later the lipid peroxidation level, the peroxidase activity, the growth and leaf gas-exchange, and the protein and plastid pigments content were studied.

The physiological analyses proved that DETA had positive effect on the As-treated maize plants by increasing the leaf gas-exchange, the plastid pigments content and soluble protein. The exogenous polyamine DETA, applied 24 hours prior to the As treatment, decreased considerably the lipid peroxidation level and the peroxidase itself in maize plants. DETA had protective effect on the As-induced oxidative stress, but in order to clarify its role as an antioxidant, more detailed and profound studies should be made.

KEY WORDS: Polyamines, maize, oxidative stress, leaf gas-exchange, antioxidants

РЕЗЮМЕ

Проучен беше антиоксидантният ефект на екзогенния полиамин Диетилентриамин (ДЕТА) върху оксидативните промени в млади царевични растения, третирани с различни концентрации арсен. Растенията бяха отгледани в климатичен бокс в хранителна среда на Хогланд-Арнон. Арсенът (As) беше приложен като Na_3As0_4 в концентрации 0, 2 и 5 mg dm^{-3} (pH 5.5). Полиаминът ДЕТА (концентрация 10^{-4} M), беше прибавен към хранителната среда на част от растенията 24 h преди третирането с As. След пет дни беше отчетено нивото на липидната пероксидация, активността на ензима пероксидаза, растежът и листният газообмен, и съдържанието на белтък и пластидни пигменти.

Физиологичните анализи показаха, че полиаминът Дета оказва положителен ефект върху третираните с арсен царевични растения, като повишава листния газообмен, съдържанието на пластидни пигменти и разтворим белтък. Екзогенният полиамин Дета, приложен 24 преди третирането с As намалява съществено нивото на липидната пероксидация и ензима пероксидаза в царевичните растения. Изследванията показват, че ДЕТА изпълнява протекторен ефект срещу As-индуцирания оксидативен стрес, но за изясняване на неговата роля като антиоксидант са необходими по-детайлни и задълбочени изследвания.

КЛЮЧОВИ ДУМИ: Полиамини, Диетилентриамин, царевични растения, оксидативен стрес, листен газообмен, антиоксиданти

INTRODUCTION

Heavy metals are directly implicated in the generation of oxidative stress in the plant-surrounding environment. They function as stress factors causing physiological disorders in plants [8,31,32]. Arsenic is not a heavy metal, but it is related to them. It is strongly phytotoxic at high concentrations. In plants, arsenic is accumulated mainly in the root system, to a lesser degree in the aboveground organs, and causes physiological changes and damages [27,41], and reduction of the crop productivity [35,36]. Arsenic inhibits the growth, fresh and dry biomass accumulation [37]. Arsenic is not a redox metal. Nevertheless, there is significant evidence that exposure of plants to inorganic arsenic does result in the generation of ROS, which is connected with arsenic valance change, a process that readily occurs in plants [10]. Arsenic causes a reduction of the photosynthesis rate [28, 29, 36]. In our previous investigation, we found that the rate of CO_2-fixation in young maize plants treated with arsenic decreased by about 20% and functional activity of PS2 was reduced significantly [36]. Arsenic damaged the chloroplast membrane and disorganized the membrane structure [29]. It has been demonstrated recently that catalase and glutathione-S-transferase in Zea mays were all stimulated upon exposure to arsenic [33]. The increase in lipid peroxidation, superoxide dismutase (SOD) activity [17] and peroxidase (POD) activity [30] were correlated with increasing As stress. According to them, arsenic, accumulated in the plant tissue, stimulates peroxidase synthesis during the early phases of plant development, long before the visible changes take place [39].

Oxidative stress is a phenomenon which has been implicated as one of the main agents causing cellular damage in all aerobic organisms exposed to a wide variety of stress conditions [11,15]. Plant cell can be protected against this oxidative damage by a broad spectrum of radical-scavenger systems, including antioxidant enzymes ascorbate peroxidase, glutatione reductase and superoxide dismutase as well as non-enzymatic compounds such as glutatione, carotenoids and ascorbate [16]. Moreover, plants possess a number of strategies to cope with heavy metals toxicity, e.g. regulation of metal uptake by the root system [23], phytochelatin synthesis [14] and prevention of free radical-induced cellular damage by antioxidants.

Polyamines are compounds widely distributed in living cells and have been implicated in a wide range of regulatory processes as promotion of growth, cell division, DNA replication and cell differentiation [13,26].

In plants polyamines are related to various kind of environmental stresses including osmotic stress, salt stress, acid stress, heavy metals and UV radiation [16]. At physiological pH polyamines are polycations and are fully protonated. It has been suggested that they perform many physiological effects by binding to negative charges of phospholipids and DNA and thereby stabilizing the function of the nucleus and the membranes [16]. During the last years polyamines have been reported as efficient antioxidants in many experimental systems, exerting this effect through the protection of cellular components such as cell membranes, nucleic acids and polyunsaturated fatty acids from oxidative damage516]. However, Bors et al [29] demonstrated that they are poor candidates as radical scavengers.

Polyamines have been also suggested to function as metal chelators and good candidates in protecting plant cell against metal-induced oxidative damage due to their high affinity for biological membranes and because are easily induced in response to stress conditions [24].

Polyamines are a relatively new group of biologically active substances [2, 3]. Recently, the number of investigations on the physiologically effect of these synthetic analogues, and the possibility to apply them as highly efficient protective substances, has increased [34].

The objective of this study was to investigate the protective role of the exogenous polyamine DETA against oxidative changes and photosynthesis in As-treated maize plants.

MATERIAL AND METHODS

Plant and growth conditions: Seeds of maize ((Zea mays L, hivrid Knezha - 613) were germinated on wet paper and six-day-old seedlings were transferred to plastic pots with a capacity of 0.5 dm^3, four seedlings per pot. The plants were grown in a Hoagland-Arnon nutrient solution, replaced twice a week, in a climatic box under irradiance of 200 μmol (PAR) $m^{-2}s^{-1}$, 14-h photoperiod, day/night temperature of 22±2/18±2 ºC, and relative air humidity of 70 %. Fourteen days after the emergence the plants were treated with As in the form of Na_3AsO_4 – 0 (control), 2 and 5 mg dm^{-3} (pH 5.5). The polyamine DETA (concentration 10^{-4} M) was added to the nutrient environment of some of the plants 24 hours prior to the As-treatment.

The polyamine dietylenetriamine (DETA) is a synthetic structural analogue of the polyamine spermidine. For the first time the plant growth regulating activity of DETA was determined in the laboratory for Chemical Phytoeffectors at the Institute of Plant Physiology of Bulgarian Academy of Sciences [1].

Methods

For the measurement of lipid peroxidation in roots, the thiobarbituric acid (TBA) test, which determines malondialdehyde (MDA) content, was applied [18].

The amount of MDA-TBA complex (red pigment) was measured by means of its specific absorbance at 532 nm. Non-specific absorbance at 600 nm was also subtracted [9]. The data were calculated using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Peroxidase (POD) activity (EC 1.11.1.7) was determined according to Herzog and Fahimi [19]. The roots were homogenized in 0.05 M Tris-glycine buffer (pH 8.3) containing 170 g dm^{-3} sucrose. The POD activity was expressed as ΔA_{470} g^{-1} (FM) min^{-1}.

Protein content in the extracts was determined according to Lowry at al. [25]. The plant material was homogenized in a boron buffer (pH 8.7) in a refrigerated centrifuge. The solution absorbency was determined in the presence of Folin reagent at wavelength of 750 nm. The protein amount was determined using a standard curve.

The net photosynthesis rate, transpiration rate and stomatal conductance of the youngest fully developed intact leaves were measured with a portable infrared gas analyzer LCA-4 (Analytical Development Company Ltd,. Hoddesdon, England), equipped with a PLCB-4 chamber. The measurements were made under irradiance of 800 μmol (PAR) m^{-2} s^{-1}, temperature of 26 ± 2 °C, an external CO_2 concentration of 400 μmol mol^{-1}, and relative air humidity of 70 %.

Chlorophyll (Chl) and carotenoids (Car) were extracted with 80 % acetone. The pigments were determined spectrophotometrically, and calculated according to Lichtenthaler and Wellburn [22] formulae.

Three independent experiments, each with 5 repetitions per treatment, were conducted. The results showed similar tendencies. Data from one representative experiment are given in this work. The significance of the differences between control and each treatment was analysed by Student's t-criterion.

RESULTS AND DISCUSSION

In our experiments the accumulation of fresh and dry biomass of As-treated plants (Table 1) was inhibited more significantly at concentration of 5 mg As (52 % and 40 % respectively). The growth of the shoot and root was reduced as well. Increase in growth rate, such as shoot and root length, leaf area, the change of the ratio DM/FM after the pretreatment of maize plants with DETA were observed.

The results indicated, that the shoot biomass changes were in negative correlation with POD activity in roots (Figure 1). This confirms the opinion of Van Assch et al [39] and our previous investigations [37]. The results in Figure 1 show that POD activity was higher at 5 mg dm^3 As – 48 % and at 2 mg dm^3 As – 16% above the control. According to Shaw [42], this induction of POD is a typical reaction of the plants to a presence of oxygen stress, such as heavy metal toxicity [7]. Since peroxidase activity is related to ROS formations, it is evident that As, applied in a soluble form, induced ROS accumulation. The exogenous polyamine DETA reduced the effect of As-treatment, probably as ROS scavenging.

The relationship between metal sensitivity and lipid peroxidation (LP) was clearly illustrated in response to As stress, as well. Arsenic proved to be highly effective in stimulating lipid peroxidation - an increase of MDA accumulation as a result of As stress was observed (40 and 81 % higher than in the control – Figure 1). In accordance with Cakmak et al. [7], we presume that As potentiates or facilitates lipid peroxidation by disorganizing the membrane structure.

Enhanced lipid peroxidation, occurring in response to arsenic, indicate that arsenic toxicity resulted in the increased production of ROS, which, in turn, caused membrane damage.

Lipid peroxidation was considerably increased by heavy metals, which was also reported by Gallego et al. [12]. Kitaga et al [21] were probably the first to suggest that polyamines acted as antioxidants and reported that they were most effective in inhibiting lipid peroxidation. Tadolini et al [38] suggested that polyamines inhibited LP when bound to negative charges on the membranes surfaces, but they also bind cations like Cu (II) or Fe (II) and by this they also acted as cellular protectors [24].

Some authors have tested polyamines as antioxidants by "in vitro" assays checking their capacity as radical scavengers [4], by exogenous addition in the incubation medium. It has been proposed that polyamines scavenge oxygen-derived species, stabilize membranes by reduction LP [5] and could prevent radical formations subsequent to oxygen radical formation by helating iron [24].

The reversion of the metal-induced membrane damage appeared to be in favor of the membrane stabilization allowed by the increasing of the local concentration of the exogenously added amines at the sites of oxidative attack [16].

The soluble protein content in plant cells is an important indicator of their physiological state. The results from the same figure show that as a result of As stress, the soluble protein amount in the maize roots decreased by 14 % at 2 mg dm^{-3} As and 28 % at 5 mg dm^{-3} As. According to Journet et al. [20], the protein degradation is, in fact, an adaptation of the cells to the carbohydrate deficiency. The exogenously applied polyamine DETA reduced the difference from the control, which was 6 and 12% respectively, in the two As concentrations.

Table 1. Protective effect of DETA on maize root immersion in Arsenic (As) solutions of different concentration (0, 2 and 5 mg dm^{-3}) on growh parameters. All parameters were measured 5 d after application. Means ± SE n = 5 * P < 0.1, ** P < 0.01, *** P < 0.001.

Parameters	Control	2 mg As	Deta+2 mg As	5 mg As	Deta+5 mg As
Shoot lenght [cm]	31.86±0.78	25.84±0.54**	29.48±0.66	23.08±0.65***	27.22±1.02**
Root lenght [cm]	28.24±0.52	23.78±0.92*	25.70±0.22**	21.46±0.55***	24.17±0.65**
Dry/Fresh mass ratio	0.076±0.001	0.081±0.001	0.077±0.002	0.096±0.002	0.080±0.001
Leaf area [cm^2]	30.73±1.15	25.77±1.54**	28.77±1.85	20.49±1.78***	24.06±1.0***

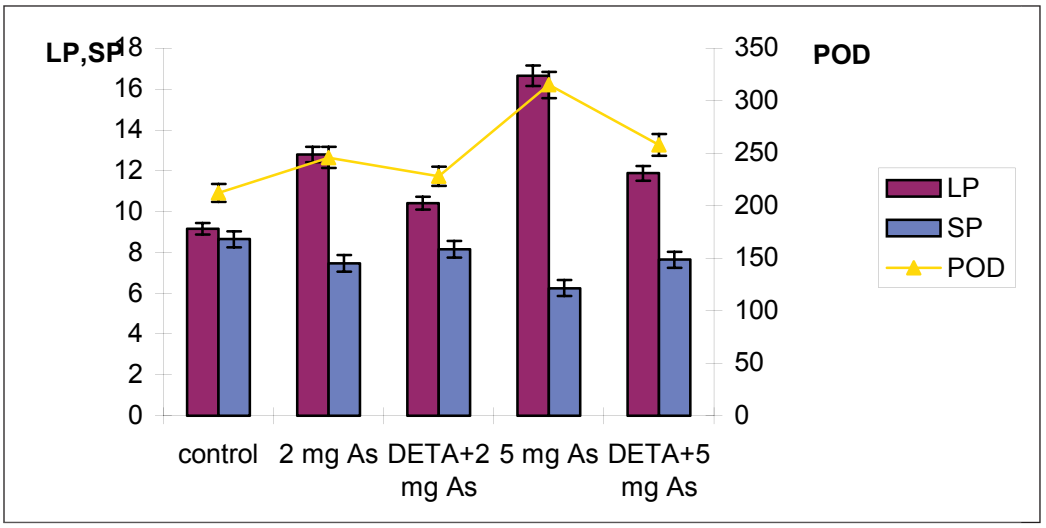

Fig 1. Protective effect of DETA on maize root immersion in Arsenic (As) solutions of different concentration (0, 2 and 5 mg dm^{-3}) on the lipid peroxidation (LP) [nmol (MDA) g^{-1} (FM)], peroxidase activity (POD) [ΔA_{470} g^{-1} (FM) min^{-1}] and soluble protein content (SP) [mg g^{-1} (FM)].

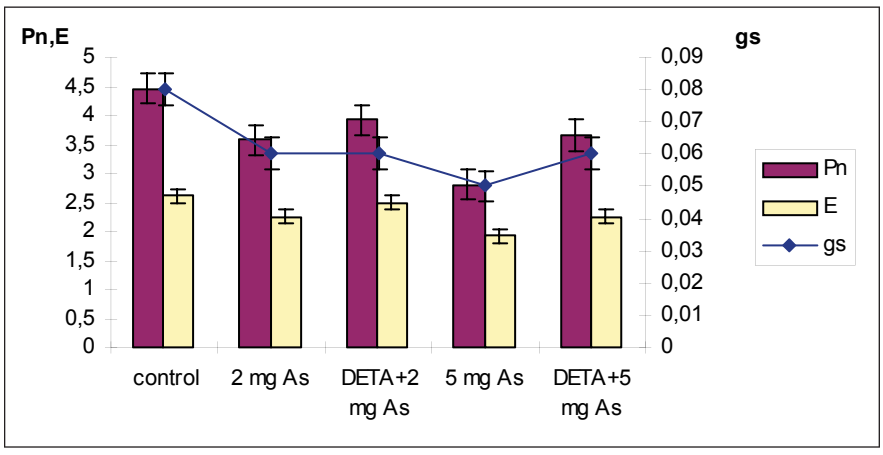

Fig 2. Protective effect of DETA on maize root immersion in Arsenic (As) solutions of different concentration (0, 2 and 5 mg dm^{-3}) on the Net photosynthesis rate (P_N) [µmol (CO_2) m^{-2} s^{-1}], Transpiration intensity(E) [mmol (H_2O) m^{-2} s^{-1}] and stomata conductivity g(s) [mol m^{-2} s^{-1}]

It is well known that photosynthesis is one of the most sensitive processes to the different types of stress, and its resistance is decisive to the plant productivity. The photosynthetic rate (P_N) in leaves decreased in the As treated maize plants (Figute 2). This was more evident at a concentration of 5 mg (As) dm^{-3}, where P_N was 37 % below the control, and at the concentration of 2 mg (As) dm^{-3} - 20 % below the control. The transpiration rate (E) decreased by about 15 % (2 mg dm^{-3}) and 28 % (5 mg dm^{-3}). The exogenous polyamine DETA reduced the arsenic effect as a result of which the photosynthesis was stimulated and reached the level of 15 nad 19% below the control. The same tendency was observed with respect to the transpiration as well, which was 15 % below the control. Probably, the polyamine protective effect on the photosynthetic appratus finds expression mainly in the increase of the lipid bylayer stability and the processes directly connected to it.

The photosynthetic pigments in certain cases are able to limit the photosynthesis rate. It is believed that they are some of the receptor points of the toxic As effect [29]. According to these authors, the limiting step of the heavy metal effect on the plant photosynthesis is a result of the inhibition of Chl synthesis. There was a considerable decrease of Chl and Car contents (24 and 14 % below the control) at the 5 mg (As) dm^{-3} in maize plants. (Figure 3). It was established that Car decreased to a lesser extent than Chl. As e result of the As treatment the correlation Chl/Car was changed in a negative aspect. The exogenously applied polyamine DETA reduced the stress effect and changed in a positive direction the pigments content. The Chl (a+в) content in the case of the higher As concentration was 10 % below the control. The change in the correlation Chl/Car follow the same tendency. It was demonstrated that treatment with polyamines prevented the loss of chlorophyll, stabilized the membranes and delayed senescence [6,40]. Probably, the exogenous polyamines due to their cation characteristics prevent the chlorophyll loss, protecting the tylacoids membrane structure. It is possible that the exogenous polyamines induce physiological effect by means of a non-specific influence on the plasmalema.

The results presented in this paper clearly indicate that single As-treatment (2 and 5 mg dm^{-3}) induced oxidative stress related to membrane damage but did not cause irreversible changes. POD and LP were involved in overcoming of oxidative stress. Leaf gas-exchange and pigment content were suppressed.

Exogenous polyamine DETA applied before As-treatment was suggested to prevent maize plants from oxidative stress situation. For the period (24 h) between DETA and As application, i.e. before As-stress, DETA probably prepared the cell to meet and combat stress by stabilizing membranes and forming a potential of antioxidant capacity [40].

Our results suggest that polyamine DETA was undoubtedly implicated in the protection system of plants and this protection was mainly related to the avoidance of lipid peroxidation reactions and POD activity, preventing the formation of radicals altogether by removing the metal producing the oxidative attack (Arsenic). It has been proposed that polyamines scavenge oxygen-

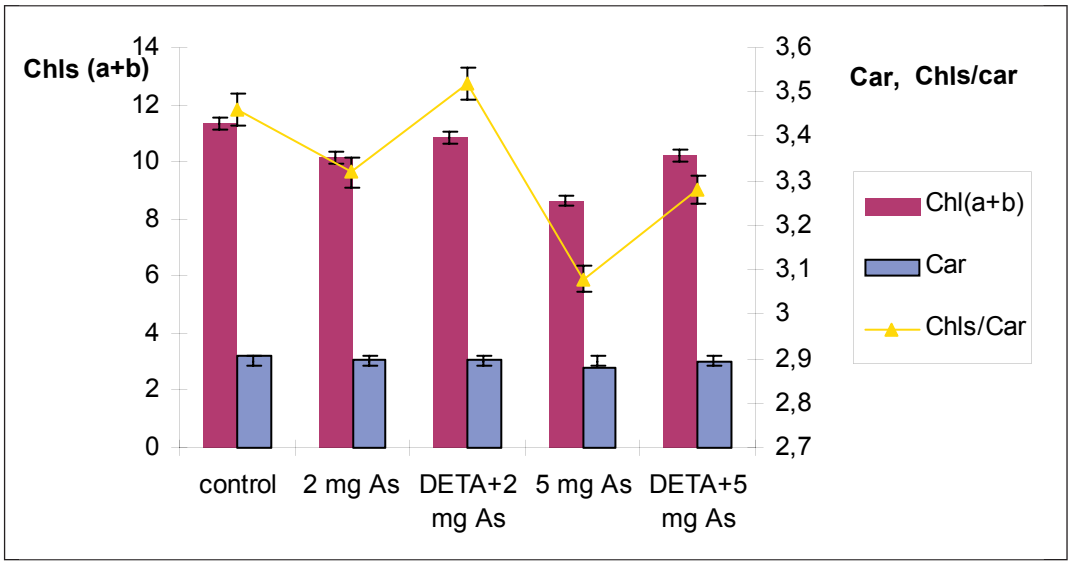

Fig 3. Protective effect of DETA on maize root immersion in Arsenic (As) solutions of different concentration (0, 2 and 5 mg dm^{-3}) on the total Chl and Car levels, and Chl/Car ratio [mg g^{-1} (DM)].

derived species, stabilize membranes by reducing lipid peroxidation in pretreatment plants [16].

In spite of the obvious protective effect of the polyamine DETA, the problem of existence and specificity of the plants' physiological response to the exogenously introduced DETA still has not been fully solved. In order to clarify its role as an antioxidant, more detailed studies should be made.

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