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ARTICLE

Investigation of arsenate phytotoxicity in cucumber plants

Viktória Czech[1]*, Pálma Czövek[1], József Fodor[2], Károly Bóka[3], Ferenc Fodor[1], Edit Cseh[1]

[1]Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary, [2] Protection Institute of Hungarian Academy of Sciences, Budapest, Hungary, [3] Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary

ABSTRACT Arsenate is reduced to As(III) and causes the formation of free radicals in plants. Arsenic induces oxidative stress in the cell wall region and destroys the membrane permeability. Therefore the effect of arsenate on ion efflux, H_2O_2 concentration, ascorbate concentration, ascorbate oxidase (AAO) activity and malondialdehyde formation was investigated in cucumber. The cucumber seedlings have a very sensitive period during their development. Under As(V) treatment of 7-day-old plants the roots, hypocotyls and leaves are flaccid and the fresh weight of the roots decreases because of the efflux of water and solutes. H_2O_2 significantly decreases while ascorbic acid concentration does not change compared to the control roots. In the hypocotyls H_2O_2 does not change but ascorbic acid significantly increases. Ascorbic acid oxidase activity is higher in the roots and smaller in the hypocotyls. As(V) significantly lowers AAO activity in the roots (about 50%) but increases in hypocotyls of 5-day-old plants. Ascorbic acid protects the plants by preventing lipid peroxidation so the hypocotyls remains turgid, and the inhibition of growth is much smaller.
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KEY WORDS

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The presence of arsenic in soils and drinking water and its consequent appearance in the food chain has a potential health damaging effect. The arsenic load of the Hungarian population in the various parts of the country is substantially different; it exceeds 50 µg/l in the Southern part of the Great Plain. The arsenic allowance of drinking water in the European Union is 10 µg/l.Arsenate is reduced to As(III) and causes the formation of free radicals in plants. Free radicals are usually formed during the oxidation-reduction processes taking place in all living organisms. They play a role in the activation of stress responses and defense mechanisms and cell death. The redox homeostasis in plants is maintained by enzymatic and non-enzymatic defense systems. One of the most important units of the non-enzymatic system is ascorbate because it may directly react with hydroxyl radicals, singlet oxygen and superoxide anions and also has a major role in detoxifying H_2O_2.

Arsenic induces oxidative stress in the cell wall region and destroys the membrane permeability. The aim of the present study was to investigate the oxidative stress related effects of arsenate in cucumber.

Materials and Methods

The stress-sensitive vegetable plant, cucumber (Cucumis sativus cv. Joker F1) seeds were grown in modified quarter-strength Hoagland nutrient solution (1.25 mM KNO_3; 1.25 mM $Ca(NO_3)_2$; 0.5 mM $MgSO_4$; 10.0 µM KH_2PO_4; 11.6 µM H_3BO_3; 4.6 µM $MnCl_2$; 0.19 µM $ZnSO_4$; 0.12 µM Na_2MoO_4; 0.08 µM $CuSO_4$) in a climate chamber (light: 80-100 µE with 14/10 hour photoperiod at 26/22°C, relative humidity 65-70%). The iron source was either 10 µM $FeCl_3$ or 10 µM Fe(II)-ascorbate, this latter being prepared by mixing 10 µM $FeCl_3$ and 30 µM ascorbic acid. The treatment solution contained KH_2AsO_4 in 10 µM concentration in order to investigate its phytotoxic effects. Fresh weight, water content, ion efflux (Singh et al. 2006), lipid peroxidation (Sökmen et al. 2001), concentration of H_2O_2 (Gay et al. 1999) and ascorbic acid (Knörzer et al. 1996) and ascorbic acid oxidase activity (Oberbacher et al. 1963) were measured after 48 hours As(V) treatment in the roots and hypocotyls.

Results and Discussion

The growth of cucumber grown in $FeCl_3$ and arsenate containing nutrient solution was inhibited but turgor loss was not observed in the plants treated at transfer to light or 3 days later. However, in case of the plants that were treated with arsenate from the 5th day after transfer to light, the roots became flaccid and their growth rate decreased more than 60%. Plants treated with arsenate on the 7th and 9th day, lost their turgor both in the roots and leaves. The hypocotyl of the plants turned into transparent colour and the plants collapsed. The reduction in the hypocotyl growth rate was 87-90%. Thus, the most sensitive development stage of the plants to the arsenate treatment was between the 5th and 7th day after transfer to light coinciding with the appearance of the first

*Corresponding author. E-mail: ecseh@ludens.elte.hu

leaf. The arsenate treatment of 7-day-old plants caused a significant decrease in the fresh weight of the root after a 48-hours. This is due to the loss of water and solutes.The roots of the arsenate treated plants increased the conductivity of the test solution by 50 %.As(V) is reduced to As (III) in the plants that may lead to the formation of reactive oxygen species. Lipid peroxidation occurs in the hypocotyls in which the malondialdehyde concentration increases due to the oxidative stress. Cucumbers grown on Fe-ascorbate (10 µM $FeCl_3$ + 30 µM ascorbate) had healthy hypocotyls in spite of the As(V) treatment because Fe-ascorbate prevented lipid peroxidation. The H_2O_2 concentration in the roots of non-treated plants doubles during normal growth. In the arsenate treated plants the concentration of H_2O_2 decreases to almost half of the control in the roots, after 48-hours. The H_2O_2 concentration in the hypocotyl was much lower than that of the roots and remained unchanged by the arsenate treatment.

The ascorbic acid concentration in the roots of non-treated plants gradually decreased during the development of the plants while that of the hypocotyls remained the same. Arsenate treatment affected the ascorbic acid concentration of the roots and hypocotyls differently. The ascorbic acid concentration in the hypocotyls significantly increased. The decrease of ascorbic acid concentration in the root is possibly due to the loss of membrane semipermeability.

The copper containing ascorbic acid oxidase (AAO) enzyme that influences the cell expansion via oxidation of ascorbic acid is localised in the cell wall of cucumber plants. Ascorbate in the cell wall inhibits peroxidase dependent oxidation of phenolic compounds, *i.e.* the oxidative polimerization (the formation of cross links during lignification) thus ensures the possibility for cell elongation. There is a significant AAO activity in the roots of cucumber and in the hypocotyls, too. In the control plants AAO activity of hypocotyls near to the cotyledons is significantly higher in every developmental stage compared to its lower parts near to the roots. Arsenate inhibits the AAO activity in the roots but stimulates in the hypocotyls. This depends on development stage of the plants and the parts of the hypocotyls. We were able to measure this stimulation effect of As(V) only in 5-day-old plants treated with As(V) for 48 hour.

During the intensive growth of the cucumber plants a rexigen intercellular space is formed in the hypocotyl. The intercellular space appears during the formation of the second leaf. Arsenate accelerates the formation of this space and the cell walls become heavily lignified.

Aging may be enhanced by cinnamic and ferulic acids. The concentration of cinnamic acid is doubled in the 5-day-old plants treated with As(V) for 48 hour.

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