Investigation of arsenate phytotoxicity in cucumber plants

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Introduction

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.
Cucumber takes up arsenic compounds and the As(V) is reduced to As(III) in the roots. Due to this process free radicals may be formed, too.

Objective

Revealing the physiological changes in cucumber triggered by the reduction of arsenate, As(V).

Background

Arsenate (AsV) is taken up the phosphate uptake system. The transporter protein has a higher affinity to phosphate than arsenate, which in turn competes with phosphate in the cytoplasm. It may substitute phosphate in ATP forming a non-stable ADP-As molecule.
The uptake of **arsenite** (AsIII) is not influenced by phosphate and it does not substitute PO_4^{3-}. It may bind to SH^- groups of protein molecules. Comparing the effects of arsenate and arsenite the possible reduction of arsenate must be taken into account.

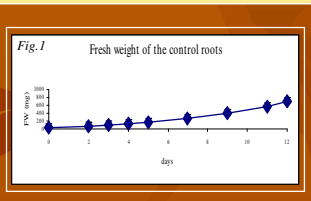
Materials and Methods

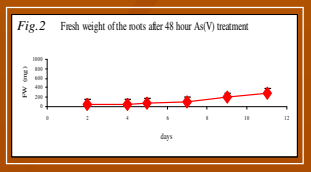
Plant material: Cucumber (*Cucumis sativus cv. Joker F1*) grown in nutrient solution in climate controlled growth chamber.
Nutrient solution: modified Hoagland (1.25 mM KNO_3; 1.25 mM $Ca(NO_3)_2$; 0.5 mM $MgSO_4$; **0.01 mM KH_2PO_4**; 0.01 mM $FeCl_3$; 11.6 µM H_3BO_3; 4.5 µM $MnCl_2.4H_2O$; 0.19 µM $ZnSO_4.7H_2O$; 0.12 µM $Na_2MoO_4.2H_2O$; 0.08 µM $CuSO_4.8H_2O$).
Treatment: 0.01 mM KH_2AsO_4

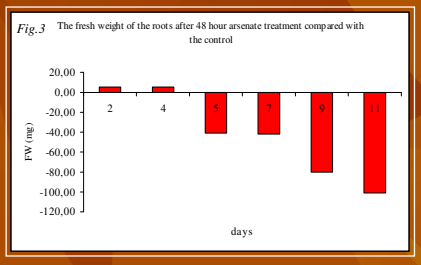
Figs. 1, 2, 3 and 5-8
Plants were grown for 2-12 days, respectively, on modified Hoagland nutrient solution from transfer to light (day 0). 48 hours before harvest plants were treated with 0,01 mM As(V).

Figs. 5-6
Concentration of H_2O_2 was determined after 48 hour As(V) treatment as in Gay et al. (1999).

Figs. 7-8
Concentration of ascorbic acid was determined after 48 hour As(V) treatment as in Knörzer et al.(1996).







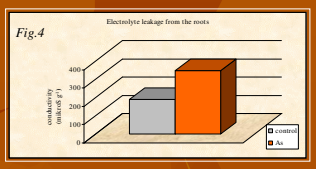
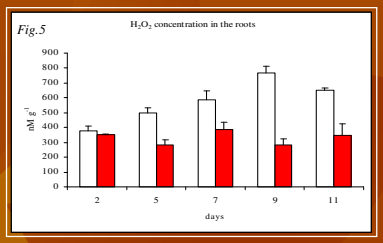
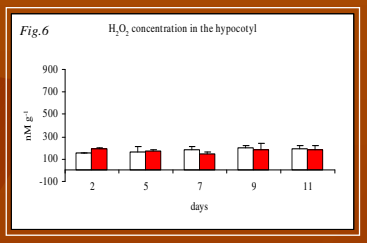


Fig. 4
Ion efflux of the roots of 7-day-old cucumber plants was measured after 48 hour As(V) and 40°C treatment as in Singh et al. (2006).

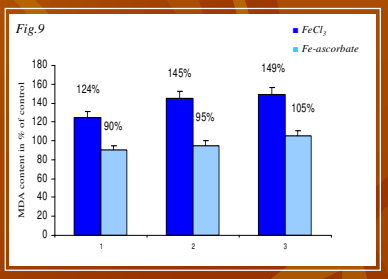
A 48 hour arsenate treatment causes a significant decrease in the fresh weight of the root. This is due to the loss of water and solutes. The roots of the arsenate treated plants increased the conductivity of the test solution by 50 % (Fig. 4).

The growth of the control plant is slowed down till day 2-5 after transfer to light (Fig. 1). In this sensitive period the roots become flaccid and their growth is retarded by As(V) (Fig. 2). The roots of 5-, 7-, 9- and 11-day-old cucumber are faccid as affected by As(V), their fresh weight are much lower as compared to the control due to the water and ion loss (Figs. 3-4).



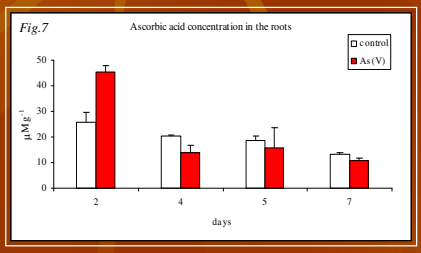


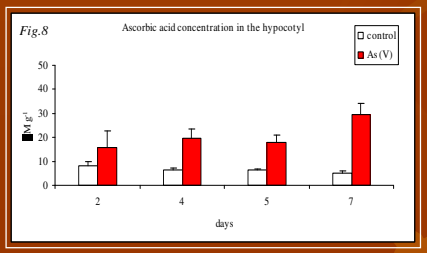
The H_2O_2 concentration in the roots of non-treated plants doubles during normal growth. In the arsenate treated plants the concentration of H_2O_2 in the roots decreases to almost half of the control. This can be explained by the loss of membrane semipermeability (Fig. 5).
The H_2O_2 concentration in the hypocotyl was much lower than that of the roots and remained unchanged by the arsenate treatment (Fig. 6).



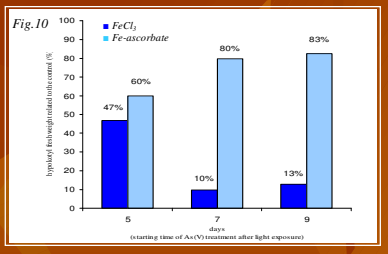
The reduction of As(V) to As(III) causes lipid peroxidation in the hypocotyl of Fe-chloride grown plants while Fe-ascorbate protected the hypocotyl by preventing lipid peroxidation (Fig. 9).

Fig. 9
Changes of the malondialdehyde content in the lower (1), middle (2) and upper (3) section of the hypocotyls as affected by a 3-hour As(V) treatment expressed as the percent of control, in 7-day-old cucumber plants grown on $FeCl_3$ and Fe-ascorbate, respectively. Method after Sökmen et al. (2001).





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 (Fig. 8). Arsenate treatment affected the ascorbic acid concentration of the roots and hypocotyls differently (Fig . 7, 8) The ascorbic acid concentration in the hypocotyls significantly increased (Fig. 8). The decrease in root ascorbic acid concentration is possibly due to the loss of membrane semipermeability, too.



Fe-ascorbate protects the membrane against the damaging effect of As(V) in the hypocotyl and the inhibition of growth is much smaller (Fig. 10).

Fig. 10
Changes in the fresh weight of the hypocotyl in plants treated with Fe-chloride + As(V) and Fe-ascorbate + As(V), respectively. Treatment started on day 5, 7 and 9 after transfer to light and the plants were harvested on day 14.

Conclusions

- Arsenate treatment causes the formation of free radicals in cucumber.
- In the sensitive period of the plants, in the faccid roots the concentration of H_2O_2, ascorbic acid and the ion content decreased due to the loss of membrane semipermeability. The hypocotyls remained turgid because arsenate significantly increased the concentration of ascorbic acid while that of H_2O_2 remained unchanged.
- The application of Fe-ascorbate prevented the toxic effect of arsenate in the hypocotyl, there was no lipid peroxidation.

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