

## EFFECTS OF THE EXOGENOUS GLYCINEBETAINE ON PHOTOSYNTHETIC APPARATUS IN CUCUMBER LEAVES CHALLENGING AL STRESS

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### Introduction

Aluminium (Al) toxicity is one of the most severe environmental factors limiting the productivity of crops, with its bioavailability being mainly restricted to acid environments. Globally, around 30% of all land consists of acid soils, and it has been estimated that over 50% of the potential arable lands are acidic (von Uexküll and Mutert 1995). A considerable effort has been made during the past several decades to understand the fundamental base of the aluminium stress physiology. Studies so far reported have focused on examining the relative importance of symplastic vs. apoplastic lesions of Al toxicity in roots, mechanisms of toxic ions exclusion, the role of organic acid exudates in preventing aluminium ions uptake, and changes in specific genes expression (for review see: Silva 2012). Fewer studies have focused on effects of Al on the leaf physiology and, in particular, on contribution of the processes functioning at the level of chloroplast. Accumulation of compatible solute (e.g. glycine betaine) is a widespread response to various abiotic stresses, including cold, salinity and drought (Chen and Murata 2008). Here, an investigation into the effects of aluminium stress on the regulation of photosynthesis in cucumber was described. In addition, the role of GB and the mechanisms whereby it increases the tolerance to stress provoked by aluminium treatment was examined.

### Materials and Methods

Cucumber seedlings (*Cucumis sativus* L. cv. Wisconsin) grown hydroponically in Hoagland nutrient solution (pH 5.0) under a 16-h photoperiod (PPFD 600  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) were supplemented with: 0 (control plants), 0.1 and 1.0 mM  $\text{AlCl}_3$ . Exogenous, foliar application of glycine betaine (GB) was carried out with 10 and 100 mM GB solution. Gas exchange parameters were monitored using an Infra-Red Gas Analyser and chlorophyll fluorescence measured with a PAM fluorometer. Leaves from control and  $\text{AlCl}_3$ -treated plants were collected and extracted in 80% (v/v) acetone for chlorophyll content. Rubisco activity was determined spectrophotometrically following NADH oxidation at 340 nm.

### Results

In this study, it was found that application of exogenous GB led to an improvement of the multiple physiological attributes in cucumber seedlings challenging aluminium toxicity, thus accounting for the overall level of Al stress tolerance. Accumulation of the toxic ions (e.g.  $\text{Na}^+$ , heavy metals) in leaves may adversely affect chlorophyll content. Indeed, exposure of cucumber to aluminium resulted in a progressive decline in chlorophyll content – there was a loss of both Chl *a* and Chl *b* intensified with increasing  $\text{AlCl}_3$  level. On the other hand, even the lower level of the exogenous GB was sufficient to provoke significant protective effect on chlorophyll content, with this effect being further enhanced when 100 mM glycinebetaine was applied to leaves of the cucumber plants suffering Al stress. Aluminium toxicity in cucumber provoked large differences in gas exchange parameters. Exposure to Al induced rapid stomatal

closure, with this developing further over time. The resulting limitation on CO<sub>2</sub> entry and direct toxic effect of Al accumulation resulted in inhibition of CO<sub>2</sub> assimilation. The harmful effect of aluminium on the CO<sub>2</sub> assimilation rate was greater at the higher AlCl<sub>3</sub> level. The exogenous application of glycinebetaine promoted, however, significant improvement in the determined gas exchange parameters. The decline in stomatal conductance observed in leaves of the cucumber plants suffering aluminium stress was notably prevented by GB at 100 mM level. The assimilation rate measured in the leaves of the Al-stressed cucumber plants was altered in line with stomatal conductance as a consequence of the GB application. The inhibition of CO<sub>2</sub> assimilation in the leaves of Al-stressed cucumber was accompanied by a considerable decrease in the electron transport through PSII, demonstrated by the decline in photochemical efficiency of PSII ( $\Phi$ PSII). Exposure to aluminium, in contrast to  $\Phi$ PSII, did not have any instant effect on maximum efficiency of PSII (Fv/Fm) – throughout the experiment, this parameter declined significantly only in the plants exposed to 1 mM AlCl<sub>3</sub> indicating the episodes of photoinhibition occurring in the PSII reaction centres. Aluminium supplementation in the nutrient solution resulted in a substantial loss of the absorbed light energy in the nonphotochemical processes (NPQ). This was demonstrated, however, that the application of GB to the cucumber leaves affected by aluminium resulted in a well-defined shift in the chlorophyll fluorescence traits –  $\Phi$ PSII and Fv/Fm, reduced in response to Al stress, increased consistent with the increasing CO<sub>2</sub> assimilation. The inhibition of CO<sub>2</sub> assimilation provoked by aluminium in this experiment was clearly accompanied by changes in Rubisco activities – the higher Al concentration in the nutrient solution reduced both the initial and the total Rubisco activity measured in the leaves of cucumber plants, with no significant effects on both parameters being observed when subjected to 0.1 mM AlCl<sub>3</sub>. Importantly, the exogenously applied GB accumulating in chloroplast promoted significant alterations in the Rubisco activities measured under aluminium stress.

## Conclusions

The results obtained clearly demonstrated that foliar application of exogenous glycinebetaine, accumulating at the level of chloroplast, can significantly mitigate effects of Al stress through protection of the photosynthetic apparatus components, resulting in an improved electron transport chain, gas exchange and enzymatic CO<sub>2</sub> fixation.

## References

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