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Impact of atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants

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Summary

Expression and activity of nitrate reductase (NR; EC 1.6.6.1) and glutamine synthetase (GS; EC 6.3.1.2) were analysed in relation to the rate of CO_2 assimilation in cucumber (*Cucumis sativus* L.) leaves. Intact plants were exposed to different atmospheric CO_2 concentrations (100, 400 and 1200 $\mu L\,L^{-1}$) for 14 days. A correlation between the in vivo rates of net CO_2 assimilation and the atmospheric CO_2 concentrations was observed. Transpiration rate and stomatal conductance remained unaffected by CO_2 levels. The exposure of the cucumber plants to rising CO_2 concentrations led to a concomitant increase in the contents of starch and soluble sugars, and a decrease in the nitrate content in leaves. At very low CO_2, NR and GS expression decreased, in spite of high nitrate contents, whereas at normal and elevated CO_2 expression and activity were high although the nitrate content was very low. Thus, in cucumber, NR and GS expression appear to be dominated by sugar levels, rather than by nitrate contents.

Introduction

Human activities have caused the concentration of atmospheric CO_2 to increase continuously from about 280 $\mu L\,L^{-1}$ at the beginning of the 19th century to 369 $\mu L\,L^{-1}$ at the beginning of the 21st century. Future projections of atmospheric CO_2 concentration range between about 450 and 600 $\mu L\,L^{-1}$ by the year 2050, but are strongly dependent on future scenarios of anthropogenic

Abbreviations: DTT, dithiothreitol; DW, dry weight; EDTA, ethylendiaminotetracetic acid; FAD, flavin adenine dinucleotide; FW, fresh weight; GS, glutamine synthetase; NR, nitrate reductase; PMSF, phenylmethylsulphonylfluoride; SLA, specific leaf area

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emissions. CO_2 is a greenhouse gas, and its increasing concentration in the atmosphere causes global warming and climatic change (Woodward, 2002).

Different studies of the effects of atmospheres enriched with CO_2 on the growth and metabolism of plants have been carried out and these studies have shown a great diversity of responses. Most plants respond in the short-term to increased CO_2 concentration rising net photosynthesis and decreasing transpiration (reviewed in Long et al., 2004). In cucumber leaves, short-term experiments indicate that high rates of CO_2 assimilation enhance nitrate reduction by stimulating the synthesis and activity of nitrate reductase (NR; EC 1.6.6.1), and that sugars derived from CO_2 assimilation probably act as positive regulatory metabolites (Larios et al., 2001). Similarly, the expression and activity of glutamine synthetase (GS; EC 6.3.1.2) in sunflower leaves are modulated by the rate of CO_2 assimilation after brief exposure to high atmospheric CO_2, and photosynthesized sugars are presumably involved as regulatory metabolites (Larios et al., 2004). The initial stimulation of photosynthesis and growth of plants exposed to elevated CO_2 concentration diminishes or disappears in the long-term. Long-term experiments have shown that the photosynthesis could be acclimatized in response to CO_2 enrichment (Drake et al., 1997; Woodward, 2002). Acclimation could be explained by a decrease in the photosynthetic capacity which first, affects rubisco amount and activity (Long et al., 2004). Some evidences suggest that the photosynthesis is stimulated in C4 species in response to the increase in atmospheric CO_2 (Watling et al., 2000).

CO_2 and nitrate compete for the reducing power generated during the photosynthetic process, so that photosynthesis can be involved in the regulation of nitrogen assimilation via a rapid modulation of the NR activity (Kaiser and Brendle-Behnisch, 1991). It seems possible that the metabolic signals produced during CO_2 fixation could regulate NR activity, although it is still unknown whether these signals act directly on the NR enzyme itself or if they affect regulatory proteins (Agüera et al., 1999). Therefore, factors affecting the photosynthetic assimilation of the CO_2, such as variation in its atmospheric concentration, would also affect nitrogen assimilation.

NR is a key enzyme in the nitrogen assimilation process, which is subjected to regulation both at enzyme activity level and at de novo protein synthesis and degradation level (Athwal et al., 1998; Campbell, 1999). The ammonium formed in the plant by nitrate reduction is then incorporated into the organic molecules by the GS. GS exists as multiple isoforms that are either cytosolic (GS1) or plastidic (GS2) (McNally et al., 1983).

In recent years, the involvement of sugars in regulation of gene expression has been demonstrated, such that the expression of a large number of genes is altered by changes in the sugar content (Smeekens, 1998). In general, carbohydrate depletion enhances the expression of genes involved in photosynthesis and reserve mobilization. On the contrary, a high sugar level induces the expression of genes involved in processes such as the storage and use of carbon (Jang et al., 1997). Acclimation is also associated with a reduction in the expression of specific photosynthetic genes in response to increased sucrose within mesophyll cells (Long et al., 2004).

The aim of this work was to investigate how the C–N metabolism is affected in cucumber plants exposed to different atmospheric CO_2 concentrations for 14 days. For this purpose several growth parameters, namely CO_2 fixation rates, leaf carbohydrates contents and expression and activity of NR and GS were analysed.

Materials and methods

Plant material and growth

Seeds of cucumber (*Cucumis sativus* L. cv. Ashley) were surface-sterilized in 1% (v/v) hypochlorite solution for 15 min. After rinsing in distilled water, seeds were imbibed for 3 h and then sown in plastic trays containing a 1:1 (v/v) mixture of perlite and vermiculite. Seeds were germinated and plants grown in a growth chamber with 16 h photoperiod (400 μmol m^{-2} s^{-1} of photosynthetically active radiation provided by "cool white" fluorescent lamps supplemented by incandescent bulbs) and a day/night regime of 25/19 °C temperature and 70/80% relative humidity. Plants were irrigated daily with a nutrient solution containing 10 mM KNO_3 (Hewitt, 1966).

Plants were grown under the above conditions for 7 days. Plants were then transferred to different controlled-environment cabinets (Sanyo Gallenkamp Fitotron, Leicester, UK) fitted up with an ADC 2000 CO_2 gas monitor. The plants were maintained for 14 days with different atmospheric CO_2 concentrations (100, 400 and 1200 μL L^{-1}). During this time the photonic flux, temperature and relative humidity (400 μmol m^{-2} s^{-1}, 25/19 °C and 70/80%, respectively) conditions were maintained. CO_2 of a high purity was supplied from a compressed gas cylinder (Air Liquide, Sevilla,