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Effects of prolonged root-zone CO_2 treatment on morphological parameter and nutrient uptake of tomato grown in aeroponic system

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Introduction

Effects of elevated root-zone (RZ) CO_2 on plant morphological parameter and nutrient uptake grown in a greenhouse were investigated over a period of 60 days. The root systems of tomato plants were treated with four different CO_2 concentrations (370 μL L^{-1} air, 2500 μL L^{-1}, 5000 μL L^{-1}, 10000 μL L^{-1}) at first flower anthesis period by areoponics culture by supplying air with CO_2. The result showed that after 60 days of RZ CO_2 treatment, the root mineral uptake and transport and plant growth decreased significantly. During 40 to 60 days of RZ CO_2 treatment, the plant height of all the treatments were significantly lower than that of ambient RZ CO_2. The stem diameter of ambient RZ CO_2 was up to 3.23 cm at the end of 60 days treatment, 1.09-, 1.10-, 1.14-folds respectively of RZ CO_2 2500, 5000, 10000 μL L^{-1} air. From 40 to 60 days, the root length of ambient RZ CO_2 was significantly longer than that of three RZ CO_2 treatments. The results indicated that ambient RZ CO_2 maximized the capability of nutrient uptake compared to RZ CO_2 treatments. At the end of experiment, root H^+-ATPase was significantly reduced for elevated RZ CO_2 treatment. It was predicted that prolonged RZ CO_2 enrichment could reduce plant growth and root nutrient uptake and transport. It may be main reason for aeroponic plant growing stronger than soil cultivation.

Introduction

Elevated atmospheric CO_2 has shown influences on several key physiological processes of plants (NORBY et al., 1999). Elevated root-zone (RZ) CO_2 has been reported to reduce plant growth (CRAMER, 2002), especially with the oxygen deficiency (BORU et al., 2003). According to previous reports, the effects of elevated RZ CO_2 on plant growth depend on a wide range of circumstances including plant species, soil pH, mineral nutrition, abiotic stress, the RZ CO_2 treatment time, the CO_2 concentration applied (CRAMER et al., 1993; 1999; 2001; VIKTOR et al., 2003) and the RZ CO_2 concentration (CRAMER et al., 2005; BORU et al., 2003). From the study on tomato (*L. esculentum* Mill.) seedlings, CRAMER and RICHARDS (1999) found that the biomass of both control and salinity-stressed plants increased by enriched RZ CO_2 grown under high temperature (daily maximum of 37 °C) and an irradiance of 1500 μmol m^{-2} s^{-1}. Two weeks RZ CO_2 enrichment treated plants, the shoot and root productivities of lettuce plants increased and increasing RZ CO_2 could alleviate plant midday depression of photosynthesis (HE et al., 2007).

However, under common conditions for white lupins, the root biomass of RZ CO_2 360 μL L^{-1} treatment was higher than RZ CO_2 6000 μL L^{-1} treatment (CRAMER et al., 2005). It is reported that with 2500 and 5000 μL L^{-1} RZ CO_2 of netted muskmelon, root growth was inhibited compared with the treatment of 350 μL L^{-1} RZ CO_2 (LI et al., 2009). With the concentration of HCO_3^- from 0 to 20 mM, it was found that significant decreases in the growth of barley, sorghum and maize (ALHENDAWI et al., 1997). QI et al. (1994) have demonstrated high soil carbon dioxide concentrations inhibit root respiration of Douglas fir.

Aeroponics is a viable alternative to other soilless culture systems for maintaining plants with a controlled root-zone atmosphere (KRATSCH et al., 2006; WEATHERS et al., 1992). At the same time, aeroponic systems were by their nature well aerated, with free air supply to the roots. A complication of RZ CO_2-enrichment for plants grown in soil and other solid substrates is that plant roots and microflora produce CO_2 through respiration, which accumulates at high concentration. In artificial aeroponic systems, RZ CO_2 is lower than the system in which plants grow in soil and substrate cultivation (HE et al., 2007). This difference could play a key role in plant root metabolism and plant growth characteristics. The aim of this research is to determine the effects of prolonged elevated RZ CO_2 treatment on plant morphological parameters, root N, P, K, Ca, Mg uptake and transport, root H^+-ATPase activity.

Materials and methods

Plant Material

Liaoyuanduoli, an indeterminate and popular tomato (*Lycopersicon esculentum Mill.*) variety in Northeast China, germinated on peat and vermiculite substrate in the greenhouse at Shenyang Agricultural University, China. Seedlings were then transplanted to identical aeroponic containers, using 25 mm thick medium density polystyrene foam. Each container was 120 cm × 75 cm × 40 cm.

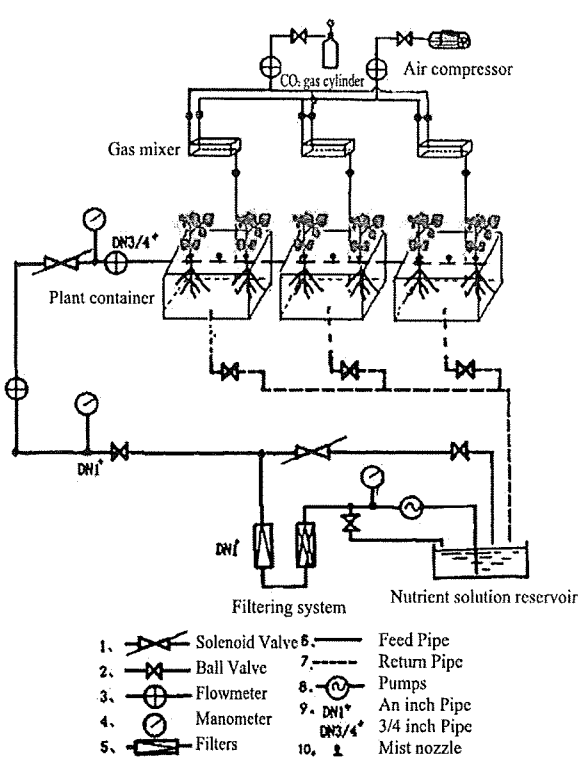

Fig. 1: Diagram of an intermittent aeroponic system for tomato RZ CO_2 treatment.

The day/night temperature in the greenhouse was setted at 33 °C/ 22 °C and photosynthetic photon flux density of sunny days were 430 - 1070 μmol m^{-2} s^{-1}. A removable polystyrene was fitted tightly to the top of each container. A lining of white polythene film ensured that the containers were waterproof. There were misting nozzles on the top of each container. The drainage of the nutrient solution was back to the reservoir. All the treatments received the same recirculated nutrient solution from a common reservoir (Fig. 1). The pH of nutrient solution was daily adjusted to 6.8 and the electronic conductivity was around 1.8 mS, using daily adjustments when necessary. The nutrient solution was renewed every three days. At the first flower anthesis, RZ CO_2 concentration was controlled as 370 (ambient), 2,500, 5,000 and 10,000 μL L^{-1} respectively by mixing CO_2 and air. The RZ CO_2 treatment was applied for 60 days. The gases were mixed by a series of valves, and the gases in the RZ of each container were analysed using Gas Data PCO_2/10 manufactured by Gas Data Ltd. (UK). The experiment was a randomized complete block design with three replicates and four treatments.

Growth measurements

On the days of 0, 20, 40, and 60 after start of treatment, the plant height and root length were measured using a ruler. The stem diameter was determined with a vernier caliper.

Root element concentration determinations

Plant roots were harvested on days 0, 20, 40, 60 after the beginning of RZ treatments. The first cluster fruits were harvested at the mature stages. Then the roots and fruits were put into a forced drought oven at 70 °C. The dried root and fruit materials were ground into a fine powder to pass through a 60-mesh screen for element concentration analysis.

Root and fruit samples of 200 mg were mineralized concentrated sulphuric acid at 250 °C. The total N and P concentrations in the mineralized solutions were analyzed by Segmented Flow Analyzers (Skalar, Netherlands). K, Ca and Mg concentrations were determined with an atomic absorption spectrophotometer (TAS-986, China).

Plasma and tonoplast-enriched membrane isolation and the measurements of plasma membrane H^+-ATPase and vacuolar H^+-ATPase activities

Plasma membrane (PM) and tonoplast-enriched membrane fractions were isolated on sucrose step gradients according to WANG and SZE (1985) and GARBARINO and DUPONT (1988) with some modifications. In brief, 10 g fresh root was homogenized using a mortar and pestle in 20 ml ice-cold buffer containing 30 mM HEPES-Tris (pH 7.4), 250 mM mannite, 2 mM EGTA, 5 mM EDTA, 125 mM KCl, 2 mM PMSF, 1% (w/v) PVPP. Just before use, 1 mM dithiothreitol (DTT), and 0.1% (w/v) BSA were added to the buffer. The homogenate was filtered through four layers of cheesecloth and centrifuged at 480 × g for 10 min. Then the supernatant was centrifuged at 10000 × g for 15 min. After that the supernatant was centrifuged at 60000 × g for another 30 min. The microsomal pellet was first suspended in an ice-cold buffer containing 25 mM HEPES-Tris (pH 7.4) with 250 mM mannite, 125 mM KCl, 1mM EDTA, 1 mM DTT and then lay on a 22% / 36% / 45% (w/w) discontinuous sucrose gradient. The gradient was centrifuged at 70,000 × g for 2 h. The tonoplast-enriched fraction was collected at the 22% to 36% interface and the plasma membrane-enriched fraction was collected at the 36% to 45% interface. The tonoplast-enriched and the plasma membrane-enriched fraction was then stored in -80 °C refrigerator.

Hydrolytic ATPase activities were determined by measuring the release of Pi colorimetrically (FISKE et al., 1925). The reaction was initiated by the addition of 50 μl 20 mM ATP-Tris (pH 7.5). After 20 min of incubation at 37 °C, the reaction was stopped with 1 ml of stopping regents [5 M H_2SO_4, 5% (w/v) $(NH_4)_2MoO_4$] followed immediately by 0.2 ml visualization reagent [0.25 g aminophenol sulfonic acid dissolved in 100 ml 1.5% Na_2SO_3-solution, pH 5.5, then added 0.5 g Na_2SO_4].

After 40 min under room temperature, A_{660} was measured by means of a spectrophotometer. During the measurement process,

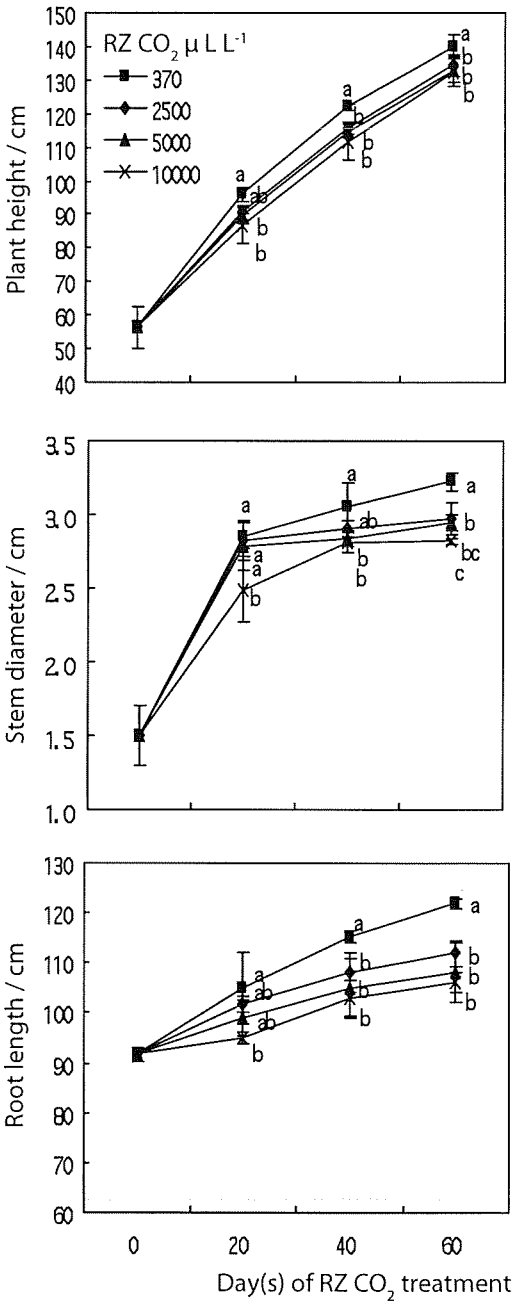

Fig. 2: Effect of different RZ CO_2 treatments on the tomato plant height, stem diameter and root length. Change in plant height, stem diameter, root length for RZ CO_2 370 μL L^{-1}, 2500 μL L^{-1}, 5000 μL L^{-1}, 10000 μL L^{-1}. Data are shown with standard deviations by vertical bars. Each point is the mean of three measurements from three different plants. Different small letters are significantly different between the treatments at 5% level according to Duncan's multiple comparison.

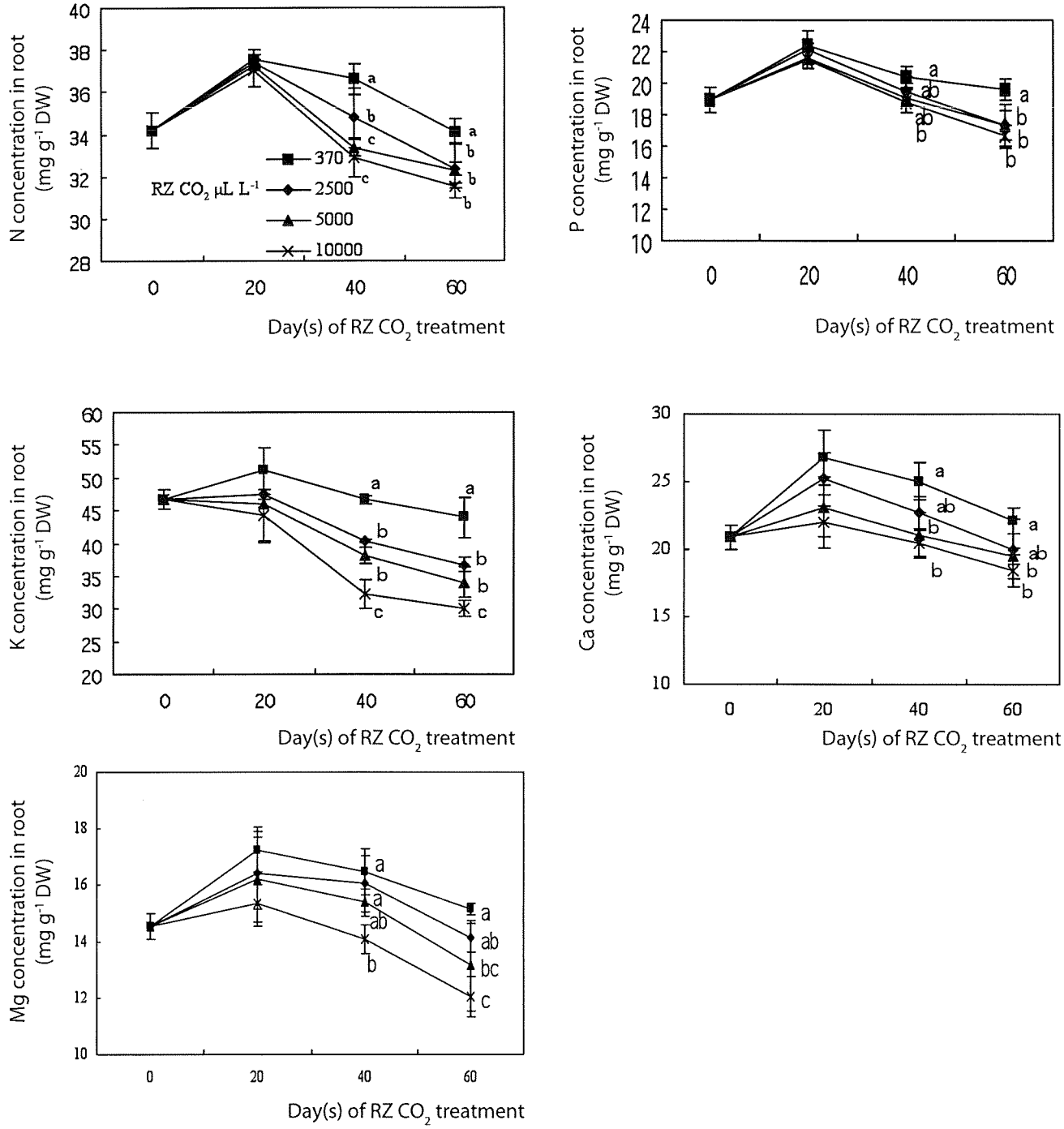

Fig. 3: N, P, K, Ca and Mg concentration in the root of RZ CO_2 treatment. Change in plant root N, P, K, Ca and Mg for RZ CO_2 370 μL L^{-1}, 2500 μL L^{-1}, 5000 μL L^{-1}, 10000 μL L^{-1}. Data are shown with standard deviations by vertical bars. Each point is the mean of three measurements from three different plants. Different small letters are significantly different between the treatments at 5% level according to Duncan's multiple comparison.

plasmamembrane H^+-ATPase (PM-H^+-ATPase) and vacuolar H^+-ATPase (V-H^+-ATPase) activities were measured with and without Na_3VO_4 and KNO_3 respectively because Na_3VO_4 and KNO_3 were the effective inhibitors to PM-H^+-ATPase and V-H^+-ATPase respectively.

Statistical analyses

All treatments were performed randomly using three replicates. The data were analyzed by one-way ANOVA. Differences among means of treatments were tested by Duncan's multiple comparison ($P<0.05$) or liner regression. Statistical analyses were performed by DPS (v7.05).

Results

Effects of prolonged root-zone CO_2 on plant growth characteristics

RZ CO_2 treatment was applied at first flower anthesis. Plant height with RZ ambient CO_2 had no significant difference with RZ

2,500 μL L^{-1} CO_2 at the 20 days of treatment, significantly higher than RZ 5,000 μL L^{-1} and 10,000 μL L^{-1} CO_2 treatments. For the 40 to 60 days of RZ CO_2 treatment, the plant heights of all the treatments were significantly lower than that of ambient RZ CO_2 treatment (Fig. 2).

The plant stem diameter had a great increase during the first 20 days. The stem diameter of the treatment of ambient RZ CO_2, 2,500 μL L^{-1} RZ CO_2, 5,000 μL L^{-1} RZ CO_2 were nearly the same at the first 20 days. During 20 to 60 days of RZ CO_2 treatment, the stem diameter had a gradual increase. At the end of RZ CO_2 treatment, the stem diameter of ambient RZ CO_2 was significantly higher than that of three RZ CO_2 treatments. The stem diameter of ambient RZ CO_2 was up to 3.23 cm for 60 days of treatment and 1.09-, 1.10-, 1.14-folds of that of RZ CO_2 2,500, 5,000, 10,000 μL L^{-1} respectively.

The whole root length of all RZ CO_2 treatments increased over the experimental period. The RZ ambient CO_2 treatment showed the greatest increase rate of the root length, which reached a maximum 122 cm at the end of the experiment period. The increased rate of the whole root length of the ambient RZ CO_2 treatment and the other three treatments were nearly the same during the first 20 days. From 40 to 60 days, total root length under ambient RZ CO_2 treatment was significantly longer than that of the other three RZ CO_2 treatments. The whole root length applied by 10,000 μL L^{-1} RZ CO_2 was the smallest throughout the growth period, which did not exceed 106 cm

Effects of prolonged root-zone CO_2 on nutrient level in roots

The Fig. 3 showed the N, P, K, Ca and Mg concentrations in the roots of tomato plants grown at different RZ CO_2 concentrations from day 0 to day 60 of treatment. Except K concentration, N, P, Ca and Mg had similar change trend. The root N, P, Ca and Mg of all RZ CO_2 treatments reached maximum with the gradual growth rate, then decreased at the end of RZ CO_2 treatment. The root K concentration of 2,500 μL L^{-1}, 5,000 μL L^{-1}, 10,000 μL L^{-1} RZ CO_2 treatment decreased during the experimental period. At the first 20 days, N, P, K, Ca and Mg had no significant difference between the control and treatment plants. The amount of above nutrients of the RZ 10,000 μL L^{-1} treatment was much lower than that of the ambient RZ CO_2 treatment. At the end of the experimental period, the root N, P, K, Ca, Mg concentrations under the ambient RZ CO_2 treatment was 108, 118, 146, 120, 126% higher than that under 10,000 μL L^{-1} RZ CO_2 treatment respectively.

Relationships between PM-H^+-ATPase, vacuolar H^+-ATPase and RZ CO_2 concentration

The root H^+-ATPase was measured for 60 days of RZ CO_2 treatment (Fig. 4). Prolonged high concentration RZ CO_2 treatment reduced both PM-H^+-ATPase and V-H^+ATPase activities. There were linear negative relationships between PM-H^+-ATPase, V-H^+-ATPase activities and RZ CO_2 concentration.

Effects of prolonged root-zone CO_2 on nutrient level in fruits

Five different minerals in the tomato fruits, N, P, K, Ca and Mg were analysed after 60 days of first flower anthesis (start of RZ CO_2 treatment). N, P, K, Ca and Mg of RZ CO_2 10,000 μL L^{-1} treatment were reduced by 22.9%/18.3%/23.3%/14.5% and 28.1% respectively relative to the control plants (Tab. 1). No difference was observed in N, P contents of RZ CO_2 2,500 μL L^{-1} treatment compared to control.

Discussion

The aim of this research work is to determine the effect of RZ CO_2 treatment on the tomato plant growth, and uptake and transport of ions through roots. All the measured growth parameters were higher when plants were grown under ambient RZ CO_2 enrichment conditions than that grown under RZ CO_2 enrichment conditions (Fig. 2). The reduced plant growth caused by prolonged RZ CO_2 treatment has been observed in previous studies on potato and netted muskmelon (SUN et al., 2004; LI et al., 2009) which were also found in our results. The root length, plant height and stem diameter under ambient RZ CO_2 treatment were 115%, 106%, and 114% higher than that under RZ CO_2 10,000 μL L^{-1} treatment at the end of RZ CO_2 application, (Fig. 2). It proved that prolonged high RZ CO_2 had a negative impact on the elongation of tomato roots.

In addition to the effects on the plant growth, the high RZ CO_2 is known to reduce root nutrient uptake. (MARSCHNER 1995; YANG et al., 1993). This was observed in our research as well (Fig. 3). The reduced concentration of investigated elements resulted from RZ

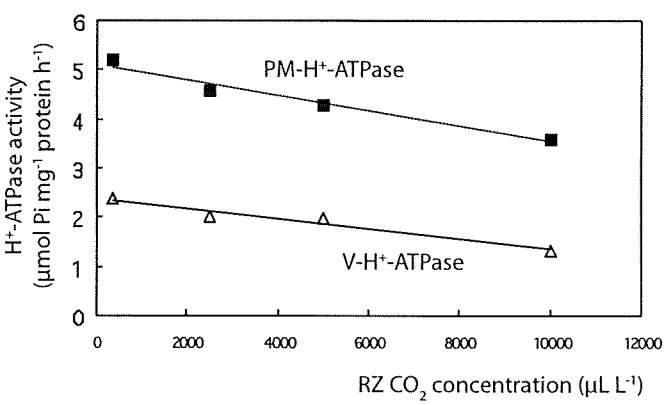

Fig. 4: The relationships between tomato root H^+-ATPase activity and RZ CO_2 concentration. "PM-H^+-ATPase" denotes the relationships between tomato root plasma membrane H^+-ATPase activity and RZ CO_2 (Y=-0.0002X+5.109, r^2=0.967, P=0.0046); "V-H^+-ATPase" denotes the relationships between tomato root tonoplast membrane H^+-ATPase activity and RZ CO_2 concentration. (Y=-0.0001 X+2.370, r^2=0.955, P=0.0075).

Tab. 1: N, P, K, Ca and Mg were analysed in fruits 60 days after the first flower anthesis.

Treatment	mg g^{-1} DW				
	N	P	K	Ca	Mg
370	19.80±0.33 a	10.63±0.60 a	38.35±1.83 a	8.92±0.23 a	8.97±0.08 a
2500	19.57±0.13 a	10.15±0.30 ab	35.03±1.89 b	8.25±0.20 b	8.43±0.16 ab
5000	17.60±1.54 b	9.60±0.22 b	31.75±0.30 c	7.97±0.10 bc	7.73±0.98 b
10000	15.27±0.20 c	8.68±0.08 c	29.43±0.56 c	7.63±0.18 c	6.45±0.30 c

Data are shown as the means of three measurements from different plants ± S.D. Different small letters are significantly different between the treatments at 5% level according to Duncan's multiple comparison.

CO_2 may be due to high RZ CO_2. It could be associated with poor root development at high RZ CO_2, which inevitably reduced plants' ability to uptake certain nutrients. This result implied that the ambient RZ CO_2 maximized the capability of nutrient uptake. These results were similar to the results from our previous study on the netted muskmelon (unpublished). Elevated RZ CO_2 for 60 days decreased root mineral nutrition uptake. This is in agreement with an early study by DOGAR et al. (1980) and YANG et al. (1993) who found that various nutrients are taken by roots such as Zn, Fe, K, P, Mg, and Mn and are subsequently translocated to shoots. However, these physiological processes are inhibited by high concentration of CO_2. Furthermore, K in barley, sorghum, and maize was decreased by HCO_3- treatments between 5 and 20 mM. This has been attributed to their decreased root elongation at high HCO_3^- concentrations (ALHENDAWI et al., 1997). Moreover, the ability of roots to grow and to function in the delivery of inorganic nutrients, water and phytohormones to the shoots and other sinks is essential to plant survival (HORCHANI et al., 2009). The fact that RZ CO_2 had the same effect on N, P, K, Ca and Mg contents in fruits as in roots (Tab. 1) led us conclude that elevated root RZ CO_2 disturbs not only mineral uptake but also transport of ions to aerial organs.

In this research, both PM-H^+ATPase and V-H^+-ATPase activities of RZ CO_2 treatment-plants decreased when compared with the ambient RZ CO_2-treatment plants (Fig. 4). PM-H^+ATPase is responsible for exporting the protons to apoplast while the V-H^+-ATPase is responsible for pumping H^+ into the vacuole and both of them are essential for intracellular pH regulation (PALMGREN, 2001). Reduction of PM-H^+-ATPase and V-H^+-ATPase activities can bring about cytoplasmic acidosis in root cells and retard growth of root tip (DREW, 1997) which was observed in this study (Fig. 4). In addition to the role for modulation of pH in cytoplasm, PM-H^+-ATPase also drives essential processes such as active nutrient transport to supply a driving force for solutes in order to enter the cell (MORSOMME et al., 2000). Accordingly, the reduction of the root nutrient concentration may be the effects of reduced H^+-ATPase in response to prolonged RZ CO_2 enrichment.

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