Short-Term Exposure to Super-Elevated CO₂ Causes Stomatal Opening of Potato

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Short-term effects of super-elevated CO₂ on stomatal aperture of potato (*Solanum tuberosum* L.) were investigated by serial observation of individual stomata of an attached leaf. Potato plantlets cultured *in vitro* from nodal cuttings were acclimatized and used for observation of stomata. Changes in stomatal aperture of attached leaves exposed to 25 000 $\mu\text{mol mol}^{-1}$ CO₂ were observed using a video microscope under an artificial light. The stomatal aperture on adaxial surface enlarged to 1.7 times the size in ambient air at 15-min exposure to super-elevated CO₂ and did not visibly change from 15 to 30 min. The result shows that short-term exposure to super-elevated CO₂ causes stomatal opening of potato under the light condition.

INTRODUCTION

Plant production in Controlled (or Closed) Ecological Life Support Systems (CELSS) is considered to play several significant roles. The objectives of plant production in CELSS are: 1) supply of foods, 2) removal of CO₂ and supply of O₂, and 3) purification of water by water uptake and transpiration. To fully utilize plant functions, optimization of environmental conditions to activate photosynthesis and transpiration is essential. Among the environmental factors in CELSS the effect of gas composition should be examined at extreme ranges. Plant response to CO₂ especially should be investigated at very high concentrations, because CO₂ may elevate to over tens of thousands $\mu\text{mol mol}^{-1}$ in a closed system due to exhaust by residents (Wheeler *et al.*, 1993).

Concentrations of CO₂ have been shown to affect stomatal conductance. Normally, elevated CO₂ induces stomatal closure and as a result reduction of stomatal conductance until the range of several thousand $\mu\text{mol mol}^{-1}$. However, it is reported that potato plants grown under 10 000 $\mu\text{mol mol}^{-1}$ CO₂ showed the higher stomatal conductance than that of potato plants grown at ambient CO₂ concentration (Mackowiak and Wheeler, 1996). In addition, in cucumber and radish, reversal of CO₂-induced decrease of transpiration rates was observed at higher CO₂ concentration, and larger transpiration rates were observed at 20 000 $\mu\text{mol mol}^{-1}$ CO₂ than that at ambient level (Yabuki and Kiyota, 1975). Similar results were reported on cotton and soybean (Pallas, 1965). These results suggest that stomatal opening occurs in such super-elevated levels of CO₂, differing from the effects in elevated CO₂ obtained by studies where the CO₂ range is 1 000 $\mu\text{mol mol}^{-1}$. However, the mechanism is still unknown. In addition, neither has stomatal movement to CO₂ been observed *in vivo* under super-elevated CO₂ conditions nor has the effect of super-elevated CO₂ on stomatal aperture been confirmed.

It is necessary to observe individual stomata directly to investigate accurately the stomatal response to environmental factors (Omasa *et al.*, 1985; Omasa, 1990).

The objective of this study is to investigate the short-term effects of super-elevated CO₂ on the stomatal aperture of potato, which is often selected as a candidate for cultivation in space. We developed a system to observe individual stomata of an attached leaf serially, and measured the changes in stomatal aperture with exposure to super-elevated CO₂.

MATERIALS AND METHODS

Plant materials. Potato (*Solanum tuberosum* L.) plantlets cultured *in vitro* from nodal cuttings were acclimatized and used for stomatal observation. The culture conditions are listed in Table 1. For acclimatization, potato plantlets at 30 d after transplanting of nodal cuttings were transferred into Erlenmeyer flasks (200 mL) with 50 mL of 1/1 000 strength HYPONeX α (Hyponex Japan, Co.) solution. The flasks were set in an environmentally controlled room with temperature of 24°C and relative humidity of 70%. Photosynthetic photon flux density (PPFD) was 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from cool white fluorescent tubes, and the light/dark cycle was 16 h/8 h. The number of holes on the cap of the flasks was gradually increased and the cap was perfectly removed at 7 d after transplanting.

Serial observation of stomatal aperture. Figure 1 shows the schematic layout of the stomatal aperture measuring system. The youngest fully expanded leaf (leaf area: approximately 2 cm²) of a potato plant (fresh weight: approximately 2 g) was set on a plastic cap of the flask in the environmentally controlled room without being detached (Fig. 1-B). The leaf was fixed on the cap's surface by Scotch tape. Using a video microscope (VH-5900, Keyence), the stomata of the leaves were observed under reflected illumination introduced through a fiber

Table 1 Culture conditions for potato nodal cuttings.

Room temperature	22°C
PPFD	100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the upper surface of a culture vessel
Photoperiod	16 h light, 8 h dark
Medium	Murashige and Skoog medium (Murashige and Skoog, 1962) with 30 g L ⁻¹ sucrose and 8 g L ⁻¹ agar
Culture vessel	Test tube with plastic cap

Fig. 1 Schematic layout of the stomatal aperture measuring system.

(A) The system was placed in an environmentally controlled room. (B) Half of the plastic cap was removed and an attached leaf was set on the cap of the flask through the opening of the cap.

Fig. 2 Measurements of stomatal aperture.

(A) The stomatal aperture was measured as the length of the minor axis of the stomatal central pore (central black part in the image), and (B) the black arrow shows the width of the outer ledges (WOL).

cable from a 100 W-halogen lamp. The light intensity at the leaf surface was controlled at PPFD of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Air containing $30\,000 \mu\text{mol mol}^{-1} \text{CO}_2$ provided from a gas cylinder was passed through a bottle with ion-exchanged water for humidification and then blown over the leaf surface via a silicon tube (i.d. 5 mm). The air flow rate was 1 L min^{-1} , and the temperature and humidity of the air were 24°C and 70%, respectively. In this condition, the CO_2 concentration on the surface of the leaf was measured by a gas chromatograph (DC-14A, Shimadzu) with a thermal conductivity detector. The CO_2 concentration was approximately $25\,000 \mu\text{mol mol}^{-1}$. Prior to exposure to super-elevated CO_2 , the air of the environmentally controlled room was blown over the leaf at the same flow rate for 30 min to confirm that the stomatal aperture did not change by the flow of air. The measurements were conducted for 53 stomata of the adaxial surface and 37 stomata of the abaxial surface using several leaves of different plants.

The obtained images were analyzed using computer software (Photoshop, Adobe). The stomatal aperture, which was defined as the length of the minor axis of the stomatal central pore, was measured from the number of pixels. The spatial resolution in the images was $0.8 \mu\text{m/pixel}$. In addition, the length of the minor axis measured from the outer ledges of the stomata (hereafter, width of the outer ledges: WOL, Fig. 2) was used as another index for stomatal aperture, because the epidermal structure was different between both surfaces of leaves and it was difficult to measure stomatal aperture on the abaxial surface in this system.

RESULTS AND DISCUSSION

Figure 3 shows an example of stomatal opening response of potato to super-elevated CO_2 treatment. Short-term exposure to super-elevated CO_2 , 15-min exposure, caused stomata to open more than in ambient air. Figure 4 shows the change in stomatal aperture on the adaxial surface when the degree of the aperture was expressed as the length of the minor axis of the central pore. The stomatal aperture enlarged to 1.7 times the size in ambient air at 15-min exposure and did not visibly change from 15 to 30 min. To investigate the difference in stomatal response between the adaxial and abaxial surfaces, WOL was used as an index for stomatal aperture. Figure 5 shows the changes in WOL of both surfaces with super-elevated CO_2 treatment. Stomatal opening was observed on both surfaces of potato leaves. The effect of stomatal opening by super-elevated CO_2 was pronounced on the adaxial surface. This preference of CO_2 effect agreed with the result described by Yabuki (1985), although the reason is unclear. Also, the mechanism of stomatal opening by super-elevated CO_2 is unclear. Dissolving of CO_2 into epidermal cells such as guard cells may cause acidification in these cells. Acidification in guard cells possibly causes stomatal opening (Jinno and Kuraishi, 1982).

Fig. 3 An example of changes in stomatal aperture with exposure to super-elevated CO₂.

Fig. 4 Effects of super-elevated CO₂ treatment on stomatal aperture on the adaxial surface.

Fig. 5 Effects of super-elevated CO₂ treatment on width of the outer ledges (WOL). WOLs are shown as relative values to the initial values. The average values of initial WOLs on adaxial and abaxial surfaces were 7.9 and 5.2 μm, respectively.

However, Brearley *et al.* (1997) showed that 10 000 μmol mol⁻¹ CO₂ did not cause much decrease in cytosolic pH by experiments using leaf abaxial epidermal stripes of *Vicia faba* L. bathed in CO₂ saturated buffer. The mechanism of stomatal opening at super-elevated CO₂ will be further researched, including accurate pH measurement in guard cells.

In summary, the results obtained from serial observation of potato stomata of attached leaves suggest that short-term exposure to super-elevated CO₂ causes stomatal opening under the light condition.

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<和文抄録>

高濃度 CO₂ によるバレイシヨの気孔開孔

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極めて高い濃度の CO₂ (25 000 μmol mol⁻¹) がバレイシヨの気孔開度を与える影響を調べた。節片から培養したバレイシヨ小植物体を順化し得た幼苗の新葉を、25 000 μmol mol⁻¹ の CO₂ を含んだ空気にさらした時の気孔開度の経時変化をビデオマイクロスコープで、落射照明下において観察した。15 分間、高濃度 CO₂ にさらすことで、向軸面の気孔開度は、通常の空気下に比べ 1.7 倍程度に増加した。このことにより、光照射下において、極めて高い濃度の CO₂ は、バレイシヨの気孔の開孔を促すことが示唆された。