Influence of Short-term Treatment with High CO₂ and N₂ on Ethylene Biosynthesis in Tomato Fruit

Hirofumi Terai, Hironobu Tsuchida, Masashi Mizuno, and Noriyoshi Matsui

Faculty of Agriculture, Kobe University, Rokkodai-cho Nada-ku, Kobe 657, Japan

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Abstract. Tomato fruit were given a short-term (24 h) high CO₂ (80%) or N₂ (100%) treatment and then transferred to air storage at 20 °C. The CO₂ treatment stimulated ACC oxidase activity and ethylene production, whereas the N₂ treatment increased ACC content but did not increase ethylene production. Both CO₂ and N₂ treatments delayed ripening for one day, but fruit ripened normally. Although short-term 80% CO₂ had a stimulating effect, and 100% N₂ had no effect on ethylene production, ripening was delayed slightly by both treatments. Chemical name used: 1-aminocyclopropane-1-carboxylic acid (ACC).

A short-term high CO₂ and/or low O₂ treatment prior to storage has been recommended for maintaining postharvest quality of a few horticultural crops. This is based on the beneficial effects of high CO₂ or low O₂ treatment on respiration of vegetables (Wills et al., 1979), ripening of bananas (Wills et al., 1982), aromatic volatile production by feijoa fruit (Pesis et al., 1991), and shelf life of peaches and nectarines (Lurie and Pesis, 1992). A very high CO₂ and/or low O₂ level can cause stress and induce production of stress ethylene (Hyodo, 1991; Saltveit, 1992), which subsequently hastens senescence. However, Mathooko et al. (1993) reported that CO₂ inhibited ethylene production in excised tissue of tomato and winter squash. Kubo et al. (1990) reported that high CO₂ suppressed ethylene production in intact peach, tomato, and broccoli, but stimulated it in banana. ACC synthase activity and ACC content increased in tomato seedling roots following treatment with N₂ gas (low O₂) (Wang and Arteca, 1992), possibly as a result of stress. If stress caused by N₂ treatment stimulates ethylene production in tomato seedling roots, the high CO₂ or N₂ treatment may affect ACC oxidase activity in tomato fruit, which consequently would have an influence on ethylene production. However, it is unclear if the tomato fruit and seedling roots are affected similarly by stress induced by high CO₂ or N₂ treatment.

Our objective was to determine if a short-term high CO₂ or low O₂ treatment had an effect on the ACC oxidase and ethylene production of ripening fruit. We used 80% CO₂ for the high CO₂ treatment and 100% N₂ for the

anaerobic treatment, and monitored ripening, CO₂ and ethylene production, ACC oxidase activity, and ACC content during ripening to determine the interaction among the physiological responses and ripening.

Materials and Methods

Tomatoes, cv. Zuishu, were grown in a greenhouse at the Kobe Univ. Experimental Farm. Twenty tomato fruit at breaker to turning stage (color chart used in USDA-AMS standard grading system of fresh tomatoes) were placed in a container for each treatment. A humidified gas mixture of 1) 80% CO₂/20% O₂, 2) 100% N₂, or 3) air (control) was passed through the container at a flow rate of ≈20 L·h⁻¹ for 24 h at 20 °C. After treatment, fruit were removed from the containers and allowed to ripen in air at 20 °C. Four individual fruit were sampled from each treatment every day, and ripeness score, CO₂ and ethylene production, ACC oxidase activity, and ACC content were determined. Data presented are means and standard errors for four replications (fruit).

Ripeness was scored as follows: green = 1, breaker = 2, turning = 3, pink = 4, light red = 5, and red = 6.

To determine CO₂ and ethylene production, individual tomato fruit were placed in a 1.4-L container. The containers were sealed for 1 h at 20 °C and the head space gas was analyzed for CO₂ and ethylene concentrations by gas chromatography, using Porapac Q columns with thermal conductivity detector (TCD) and with flame ionization detector (FID), respectively.

The extraction and assay of ACC oxidase were performed according to the method described by Ververidis and John (1991) with slight modifications. Pericarp tissues of tomato fruit were homogenized in liquid nitrogen with mortar and pestle, and 10 g of frozen powdered tissue were suspended in 20 mL of Tris buffer (pH 7.2) containing 10% glycerol.

The homogenate was centrifuged at 20,000 g_n for 15 min, and the supernatant was saved as the crude extract. The ACC oxidase reaction was carried out in a 30-mL test tube containing 2.7 mL of crude extract, 0.1 mL of 900 mM sodium ascorbate, 0.1 mL of 3 mM FeSO₄, and 0.1 mL of 30 mM ACC as a substrate. After incubation for 1.5 and 3 h at 25 °C, a 1-mL sample of head space gas was analyzed for ethylene. ACC was extracted from pericarp tissue with 80% ethanol and assayed as described by Terai (1993).

Results and Discussion

The 80% CO₂ treatment stimulated ethylene production relative to the air control (Fig. 1A). Carbon dioxide production of these fruit was similar to those treated with air except for the high production on the first day after treatment (Fig. 1B), which probably was due to CO₂ diffusing out of the tissue following the high CO₂ treatment. Interestingly, ripening of these fruit was delayed, instead of being en-

Fig. 1. (A) Ethylene and (B) CO₂ production and (C) ripeness score in tomato fruit held in air at 20 °C after short-term treatment with 80% CO₂ (containing 20% O₂) (Δ), 100% N₂ (□), or air (○). The short-term treatment (24 h) was conducted for the fruit at breaker to turning stage in a flow-through system during day 0–1 (horizontal bars shown in figures). Ripeness score (C): green = 1, breaker = 2, turning = 3, pink = 4, light red = 5, and red = 6. Vertical bars represent SE of the mean of four replications.

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Fig. 2. (A) ACC oxidase activity and (B) ACC content in tomato fruit held in air at 20 °C after short-term treatment with 80% CO₂ (containing 20% O₂) (Δ), 100% N₂ (□), or air (○). The short-term treatment (24 h) was conducted for the fruit at breaker to turning stage in a flow-through system during day 0–1 (horizontal bars shown in figures). Vertical bars represent SE of the mean of four replications.

hanced, by the elevated ethylene production (Fig. 1C).

Ethylene production of fruit treated with N₂ was similar to those treated with air (Fig. 1A), and CO₂ production was slightly lower (Fig. 1B) on the first 3 d following treatment than in fruit treated with air. Ripening of these fruit was delayed about 1 d (Fig. 1C), but the fruit ripened normally.

Kubo et al. (1990) reported that ethylene production in tomatoes treated with 60% CO₂ decreased during the 24-h treatment but increased after treatment. Pesis et al. (1994) reported that in 'Golden Delicious' apple, a 95% CO₂ treatment for 24, 36, or 48 h at 20 °C enhanced ethylene production for 2 weeks after treatment. Our results (Fig. 1A) and those of Pesis et al. (1994) suggest that high CO₂ affects the ethylene biosynthetic pathway as does stress conditions, and the effect remains after the fruit are transferred to air.

ACC oxidase activity was stimulated with the 80% CO₂ treatment; the elevated activity was maintained, but not stimulated, by N₂ treatment relative to air treatment (Fig. 2A). Fernandez-Maculet et al. (1993) purified and

characterized ACC oxidase from apple fruit tissue and found that addition of CO₂/HCO₃⁻ activated ACC oxidase *in vitro*. We did not add CO₂/HCO₃⁻ for analysis of ACC oxidase to avoid the confusion of evaluation for CO₂ treatment and CO₂/HCO₃⁻ addition *in vitro*. Mathooko et al. (1993) found that high CO₂ (60%) promoted ACC oxidase activity in excised tomato tissue during treatment, but inhibited activity in winter squash tissues. Kao and Yang (1982) reported that carbon dioxide promoted rapid conversion of ACC to ethylene in detached leaves of rice and tobacco exposed to light, and the promotion was fully reversible. These results indicate that CO₂ increases ACC oxidase activity, but the mechanism of increase may not be identical in tomato fruit, rice and tobacco leaves, and apple fruit.

ACC content increased more rapidly with the 100% N₂ treatment than with air treatment to day 4 in storage, but CO₂ treatment had no effect (Fig. 2B). Increased accumulation of ACC by N₂ treatment may result from activation of ACC synthase because ACC oxidase in the tomato fruit was not depressed by N₂ (Fig. 2A) and conversion of ACC to 1-(malonylamino) cyclopropane-1-carboxylic acid (MACC) increases during ripening (Terai, 1993).

Waterlogging of roots causes petiole epinasty (Kramer, 1951), as does elevated ethylene concentration (Kawase, 1981). Tomato roots synthesized ACC when waterlogged, and ACC was transported to the shoot, where it was converted to ethylene (Bradford and Yang, 1980). Wang and Artega (1992) found that ACC synthase activity in tomato roots increased under low O₂ conditions and that the ACC synthesized in the roots was transported to the leaves. Anaerobic stress under waterlogging led to stimulation of ACC synthase activity, resulting in increased ACC content and promotion of ethylene production. Similarly, short-term N₂ treatment may have caused an anaerobic stress in our experiment, resulting in an increase in ACC content in tomato fruit.

The short-term treatment with 80% CO₂ or 100% N₂ activated the ethylene biosynthetic pathway, possibly because of stress. The fact that this stress activity was retained for several days after the treatment suggests that it is difficult to inhibit the ripening process once it has been initiated.

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