Ground Based Study on Culturing Garlic as a Source of Vegetable Food and Medicine in Space - Growth and Ajoene Accumulation in Garlic Plants Cultured with Different CO₂ Regimes

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Abstract

Life support and effective countermeasures against damaging space radiation for humans on long-duration space missions will be highly dependent on the amount of food and efficient conversion of CO₂/O₂. The cultivation of fresh vegetables enriched in phytomedicine is an alternative option for keeping crews’ health and recycling of CO₂ in a closed environment. A whole garlic plant cultured hydroponically is edible and a rich source of medicinal compound, carbohydrates, proteins and vitamins. Garlic plants were cultured for 60 days at 400 (control), 450, 800 and 900 µmol mol⁻¹ carbon dioxide in controlled environment chambers to study growth and ajoene accumulation. Lighting was provided with fluorescent lamps as a 12 h photoperiod with 450 µmol m⁻² s⁻¹ PPFD. Fresh and dry mass accumulation of each parts of garlic plants were significantly increased by increasing the CO₂ levels from 400 to 900 µmol mol⁻¹. Fresh mass in bulbs, leaves and roots were 28.6, 66.3 and 92.2 g per plant, respectively, and 1.9, 1.8 and 2.0 times, respectively, greater at 900 µmol mol⁻¹ CO₂ than at 400 µmol mol⁻¹ CO₂. Transpiration rate and stomatal conductance were decreased by increasing the CO₂ levels while water use efficiency and relative chlorophyll contents were increased. The concentrations of ajoene accumulation were significantly increased with increasing of CO₂ levels from 400 to 800 µmol mol⁻¹ but no significant increase of ajoene accumulation was observed with increasing CO₂ level from 800 to 900 µmol mol⁻¹. Total ajoene accumulation in bulbs, leaves and roots were 2.3, 2.6 and 2.6 times, respectively, greater at 900 µmol mol⁻¹ CO₂ than at 400 µmol mol⁻¹ CO₂. The results indicate that elevated CO₂ can increase ajoene accumulation as well as biomass production and water-use efficiency in garlic plants. ©2015 Jpn. Soc. Biol. Sci. Space; doi: 10.2187/bss.29.1

Keywords: Ajoene; CO₂; Garlic; Hydroponic culture; Phytomedicine; Space farming

Introduction

Plant production in space have recently been of greater concern as the possibility of realizing manned space flight over a long term increases, because the long term life support of crews in space is greatly dependent on the amounts of food, atmospheric O₂ and clean water produced by plants. Therefore, the Bioregenerative Life Support System (BLSS) including plant production systems with scheduling of crop production, obtaining high yields, effectively converting atmospheric CO₂ to O₂ and purifying water should be established with employing suitable plant species and precisely controlling the environmental condition around plants grown at a high density in a limited space.

In addition, the International Space Station (ISS) has a research focus to investigate human health responses with limited resources, under stress conditions, for long duration missions. The human health risks associated specifically to space and spaceflight include radiation, low gravity, and psychological issues (Horneck et al., 2003). Hornek, et al. (2006) reported a very high probability for the occurrence of intestinal infectious diseases, non-zoonotic bacterial diseases, viral disease, malignant neoplasms, inflammatory diseases of the Central Nervous System (CNS), infections of skin and subcutaneous tissue, cardiovascular disease, crushing injury, and injuries in astronauts during a 180 days mission in space. Stein (2002) reported that oxidative damage is elevated after space flights; while increasing the supply of dietary antioxidants may lessen the severity of post-flight oxidative stress. A diet supplemented with 2% either strawberry or blueberry extracts for 8 weeks before exposure to elevated radiation protected rats from some of the reductions in brain function, this finding suggests that inclusion of antioxidant-rich foods to the diet can increase protection against the deleterious effects of high-energy and charge particles in long-term space flights (Shukitt-Hale et al., 2007). Therefore, the growing of antioxidant-rich fruits and vegetables in space...
Garlic Cultured with Different CO$_2$ Regimes as a Source of Food and Medicine in Space

habitats is of great importance for long-duration missions to increase diet diversity and also to provide potential countermeasures to mitigate risks associated with space radiation (Smith and Zwarts, 2008).

Vegetables are appreciated for their beneficial health effects in humans and underline the importance of nutraceutical properties (Gruda, 2005). Garlic (Allium sativum) is a valuable antioxidant food and medicinal plant that has been introduced by humans into all temperate climates throughout the world. Garlic was used during World War I and II as field dressings for wounds. Recent studies suggest that regular consumption of garlic lowers the risk of developing heart disease and cancer. Garlic consumption has become widely accepted as a dietary food for promoting overall human health (Rybak et al., 2004). In view of the widespread consumption of garlic as fresh plants, as well as supplements, the biological activity of these plants is of great current interest (Block, 1985).

Garlic bulbs are a rich source of carbohydrates (31% on the basis of fresh weight), proteins (5-6%), fat (0.2%) and vitamins (A, B1, B2, B3 and C). Significant levels of phosphorous (3.9-4.6 mg/g), potassium (1.0 mg/g) and calcium (0.5-0.9 mg/g) are present in garlic bulb (Kaufman, et al., 1999).

Ajoene (C$_5$H$_9$OS$_2$) is a sulfur rich metabolite produced by garlic through the conversion of alliin into allicin by an alliinase-induced cleavage. Ajoene is found as a mixture of two isomers, E- and Z-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (Block et al., 1984). Ajoene has anti-fungal (Yoshida et al., 1987), anti-oxidant (Naznin et al., 2010b, Kay et al., 2010), anti-microbial (Naganawa et al., 1996), anti-viral (Walder et al., 1997), anti-tumor (Li et al., 2002) and anti-leukaemia (Hassan, 2004) activities.

The elevated CO$_2$ will promote plant production, because CO$_2$ is generally the sole carbon resource for plant growth and greatest advantages of CO$_2$ enrichment is in the enhancement of photosynthesis (Chen et al., 1997). CO$_2$ enrichment has been shown to increase plant growth, development, and yield of crops, this response is a function of CO$_2$ concentration and duration biomass increment, carbohydrate accumulation, fruit productivity, photosynthetic capacity, particularly under adverse climatic conditions and this would become most apparent in the vegetative growth of young plants (Jaafar, 2006). Plant response to elevated carbon dioxide varies from large increases in growth for some species, to little or no effect on growth for other species (Aoki and Yabuki 1977). There is no report concerning on the effect of CO$_2$ on growth and quality of garlic plants, because garlic has been cultured under field conditions on earth. Therefore, it is imperative to determine how medicinal plant garlic responds on elevated CO$_2$. Exposing medicinal plants to elevated CO$_2$ may give positive response to increase the medicinal properties as well as growth enhancement.

The objective of this study was to evaluate the effect of elevated CO$_2$ on the growth and enrichment of medicinal compound ajoene in garlic plants. A ground based experiment was conducted for obtaining basic knowledge about possibility of garlic production in space.

Materials and methods

Garlic (Allium sativum L., cv. White Roppen) were cultured hydroponically under fluorescent lamps for two months in growth chambers. The hydroponic culture systems were described previously by Naznin et al. (2010a). Briefly, the seed cloves (40 ± 1.3 g/clove) were planted in styrofoam particles on a styrofoam board hanging in each water tank (0.46 × 0.33 × 0.16 m$^3$) as shown in Figure 1 A and B. The bottom of each clove was allowed to contact with the nutrient solution during the root initiation stage. The water surface was decreased by 50 mm to make the space between cloves and the water surface two weeks after planting and maintained the space throughout the growing period. Atmospheric air was supplied using air pumps (Chikara-1500; Niss Co., Tokyo, Japan) at an aeration rate of 9.9 ml s$^{-1}$ in each tank to supply enough O$_2$ for root respiration. The nutrient solution was half strength modified-Hoagland’s solution (A-type recipe of Otsuka House Solution, Otsuka Chemical Co. Ltd., Osaka, Japan). The light source was white fluorescent lamps (FFH32EX-N-H, Matsushita Electric Co., Ltd., Japan). The photosynthetic photon flux density (PPFD) was 450 ± 5.1 µmol m$^{-2}$ s$^{-1}$ and the photoperiod was 12 h$^{1}$ from 6:00 to 18:00. Air temperatures were at 25 ± 2.1/21 ± 2.5˚C (light/dark), relative humidity at 75 ± 4.5/90 ± 5.3% (light/dark), and air current speed at 0.1 ± 0.02 m$^{-1}$.

CO$_2$ regimes: CO$_2$ was supplied from CO$_2$ cylinder and was controlled and monitored by CO$_2$ controller (Fuji Electric Co., Japan). CO$_2$ concentrations were measured every 30 minutes throughout the experimental period. Four CO$_2$ regimes ranging from 400 to 900 µmol mol$^{-1}$ were applied.

The pH and electrical conductivity (EC) of the solution were measured weekly using a pH meter (Model B-212, Horiba Co., Japan) and an EC meter (Model 6-7004-01, Eutech Instruments Co., Singapore). The pH was at 6.1 ± 0.5 and EC at 0.14 ± 0.05 S m$^{-1}$ throughout the experimental period. Garlic plants were harvested 60 days after planting. Fresh mass were determined just after harvesting. Their dry weights were determined after drying at 80˚C for 48 h in an oven. Transpiration rates and leaf conductance were measured with a LI-1600 steady state porometer (LI-COR Inc, USA). Measurements were made at 12:00 to 16:00 in the light period 20 days after planting. Water use efficiency was determined according to Wheeler et al. (1999). In brief, water use efficiency was calculated with total plant biomass divided by total water used. Total water used was determined from total water added to the hydroponic system each day. Relative chlorophyll contents of fully expanded intact leaves were estimated with a portable chlorophyll meter (Model SPAD-502, Konica-Minolta Co., Japan).

Analysis of ajoene: The major sulfur-containing compound alliin and enzyme alliinase are found in different compartments of the garlic clove, and are brought into contact to produce allicin by cutting or crushing the clove. Allicin is very unstable compound,
in a warm environment (40°C to 80°C) it is converted to ajoene (Block, 1992). Ajoene concentrations in plants were determined after the harvest. Two types of ajoene (E- and Z-ajoene) are derived from garlic through the conversion of alliin into alliinol by an alliinase-induced reaction. E-ajoene, a cis (E) isomer is more stable than Z-ajoene, a trans (Z) isomer (Lawson et al., 1991). Determination of E- and Z-ajoene from garlic plants were conducted following the previous method (Naznin et al., 2008). In brief, garlic cloves were cut into 3-4 mm thick slices, and then ground to mix with rice oil using high-flex disperser apparatus (IKA T 18 basic, IKA Co., Japan) mechanically driven at 700 rpm by a drill press. To minimize frictional heating of the sample during the grinding process, the tissue grinder was chilled prior to and during the grinding process with an ice bath. Garlic samples were mixed with oil (0.25 kg L\(^{-1}\)) directly in the disperser apparatus. The mixture was stored at 80˚C for 4 h to allow complete ajoene formation. Ajoene was extracted from ten milliliters samples with ethyl acetate.

Samples were analyzed with SII-HPLC (Model L-6000; Hitachi Co., Ltd., Japan) according to previous method (Naznin et al., 2008). The eluent was n-hexan/2-propanol [85:15 (v/v)] and the flow rate was at 1.0 ml min\(^{-1}\). The eluent was monitored with light at a wavelength of 240 nm. The peak corresponding to Z-ajoene and the peak corresponding to E-ajoene were found at retention times of about 11 min and 18 min, respectively.

Statistical analysis: The statistical analysis was made using analysis of variance (ANOVA). Least significant differences were evaluated by Tukey-Kramer method ($P < 0.05$).

Results

Growth and morphological characteristics

Growth of newly developed garlic in CO\(_2\) treatments was promoted compared with those in the control as shown in Fig. 1. Fresh and dry weights of bulbs, leaves and roots were increased with increasing CO\(_2\) concentrations (Fig. 2. A and B). Fresh mass of bulbs, leaves and roots were 1.9, 1.8 and 2.0 times, respectively, greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\). The dry mass of bulbs, leaves and roots was 1.8, 1.6 and 2.0 times, respectively, greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\).

Water use efficiency (WUE) was 3.5 times higher at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\) (Fig. 3. A). Plants grown at higher CO\(_2\) concentrations also tended to show lower transpiration rates and stomatal conductance (Fig. 3. B). The stomatal conductance at 900 µmol mol\(^{-1}\) CO\(_2\) was 1.6 times lower than that of 400 µmol mol\(^{-1}\) CO\(_2\). The relative chlorophyll content was 1.2 times greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\), indicating the increase of chlorophyll contents in leaves by increasing CO\(_2\) concentration (Fig. 3. D).

Ajoene concentration

Concentrations of E- and Z-ajoene were higher in garlic plants grown under higher CO\(_2\) treatments than those in the control. The concentrations of Z-ajoene in newly developed bulbs, leaves and roots were 1.2, 1.4 and 1.3 times respectively greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\) (Fig. 4. A). E-ajoene concentration in newly developed bulbs, leaves and roots was 1.4, 1.7 and 1.4 times, respectively, greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\) (Fig. 4. B).

Total ajoene contents in whole plants were 2.5 times greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\) (Fig. 5). Total ajoene contents in newly developed bulbs, leaves and roots were 2.3, 2.6 and 2.6 times, respectively, greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\) (Fig. 5). Total ajoene contents in newly developed bulbs, leaves and roots were 2.3, 2.6 and 2.6 times, respectively, greater at 900 µmol mol\(^{-1}\) CO\(_2\) than at 400 µmol mol\(^{-1}\) CO\(_2\).
Garlic Cultured with Different CO₂ Regimes as a Source of Food and Medicine in Space

![Graphs](image)

**Fig. 3.** Effects of CO₂ on water use efficiency (A), transpiration rate (B), stomatal conductance (C) and relative chlorophyll content (D) of garlic plants cultured in a hydroponic system. Water absorption rate was determined from total water added to the hydroponic system each day. Data were the means of ten determination (n = 10). LSD (P < 0.05) was determined according to Tukey-Kramer method.

![Graphs](image)

**Fig. 4.** Effects of CO₂ on accumulation of Z-ajoene (A) and E-ajoene (B) concentrations of garlic plants cultured in a hydroponic system. Data were the means of eight determination (n = 8). LSD (P < 0.05) was determined according to Tukey-Kramer method.

![Graphs](image)

**Fig. 5.** Effects of CO₂ on ajoene content of garlic plants cultured in a hydroponic system. Data were the means of eight determination (n = 8). LSD (P < 0.05) was determined according to Tukey-Kramer method.
Effects of dry mass on ajoene concentration (A) and content (B) of garlic plants cultured in a hydroponic system under different CO₂ regimes. Data were the means of eight determination (n = 8). LSD (P < 0.05) was determined according to Tukey-Kramer method.

μmol mol⁻¹ CO₂ (Fig. 5). Roots produced mostly the same amount of ajoene as the newly developed bulbs.

Total ajoene concentration and content were increased with increasing whole plant dry mass (Fig. 6. A and B). Total ajoene concentration was 1.3 times higher in maximum dry mass of whole plant than that of in minimum dry mass (Fig. 6. A).

Discussion

CO₂ is one of the important factors influencing growth of garlic plants. Fresh and dry mass of garlic plants were significantly increased in higher CO₂ treatments than in the control not only in bulbs but also in leaves and roots (Fig. 2. A and B). Leaves and roots of garlic cultured hydroponically under a relatively low light condition in our experiment were soft and edible. Every part of garlic plant would be a good vegetable foodstuff.

Previous studies showed that plants grown at elevated CO₂ accumulated excessive carbohydrate in leaves (e.g., Kramer 1981). This excessive carbohydrate accumulation in leaves may have caused the increase of fresh and dry mass of the garlic plants grown under higher CO₂ concentration. Wittler and Robb (1964) reported that elevated CO₂ increased in fresh mass of lettuce, cucumbers, and tomatoes. Mauney et al. (1978) revealed that doubling the CO₂ concentration for 12 weeks increased the dry mass of soybean 82%, whereas in sunflower the increase was 60%. Levine and Paré (2009) found that elevated CO₂ and increasing light intensity enhanced biomass accumulation in scallion (Allium fistulosum).

Increase in WUE will be important to reduce the amount of water recycled through the plant production system in the BLSS. WUE was significantly increased in higher CO₂ treatments than in the control. Higher transpiration rates and leaf conductance were noted in the control treatment (400 µmol mol⁻¹ CO₂) than at 900 µmol mol⁻¹ CO₂ treatments (Fig. 3). The CO₂ concentration was increased from 400 to 900 µmol mol⁻¹ thus significantly reducing stomatal conductance and transpirational water loss in garlic plants.

According to Goudriaan and van Laar (1978), the stomatal conductance of corn and bean was reduced by an increasing CO₂ concentration. Stomatal conductance of potato plants was also found to be lower at the elevated CO₂ concentration (Heagle et al. 2003). Adams et al. (2000) noted that a doubling of the atmospheric CO₂ concentration caused consistent reductions in stomatal conductance and transpirational water losses in Andropogon gerardii, thus contributing to significant increases in water-use efficiency.

The relative chlorophyll contents were significantly increased with increasing of CO₂ from 400 to 900 µmol mol⁻¹ (Fig. 3. D). Increase of leaf chlorophyll contents indicates that photosynthesis would be prompted in plants under higher CO₂ concentrations. Griffin, et al. (2001) observed that elevated CO₂ concentration significantly increases the leaf chlorophyll contents.

Concentrations and contents of total ajoene increased with increasing the whole plant dry mass due to the increase in CO₂ concentrations (Fig. 6. A and B). Elevated atmospheric CO₂ concentrations often increase carbohydrates concentrations in plants and thus possibly stimulate secondary metabolism (Booker, 2000). Idso et al. (1985) observed the response of the tropical spider lily (Hymenocallis littoralis) under elevated CO₂ over four growing seasons and found that a 75% increase in the atmospheric CO₂ concentration produced an 8% increase in pancratistatin, an 8% increase in trans-dihydronarciclasine, and a 28% increase in narciclasine that are effective against lymphocytic leukemia and ovary sarcoma. Atmospheric CO₂ enrichment increased the medicinal compound glucosinolate content in broccoli (Schonhof et al., 2007). A tripling of the atmospheric CO₂ concentration produced a modest (7%) increase in antioxidant activity in the leaves and fruits of tomato plants (Madsen, 1971 and 1975). Kim et al. (2003) found that elevated CO₂ increases the concentration of twelve major isoflavones and biomass in soybean. The elevated CO₂ increased the secondary metabolites and antioxidant activity in medicinal herbal plant Labisia pumila (Ibrahim and Jaafar 2011). Previous studies showed that environmental conditions may affect allin contents in garlic (Sterling and Eagling, 2001). Ajoene is produced in large amounts by a stable sulfoxide rearrangement of allin (Ledezma et al., 2006). These indicate environmental conditions affect the ajoene content in garlic. Naznin et al. (2010a) found that,
increased dissolved oxygen concentration in root zone stimulates the ajoene accumulation in garlic plants. The result implied that the increase in secondary metabolites medicinal compound ajoene of garlic plant under elevated CO\textsubscript{2} might be due to enhancement of allin, which is present in intact garlic and precursor of alicin.

Though we did not apply very high CO\textsubscript{2} concentration level in the present study but previous studies revealed that soybean plants grown under very high CO\textsubscript{2} concentrations (>8000 µL L\textsuperscript{-1}) showed reduced growth, lower node number, shorter internodes, smaller leaves and reduced branching but the accumulation of glutathione disulfide and total free sulfhydryls increased significantly compared with those plant cultured under low CO\textsubscript{2} concentrations (344 µL L\textsuperscript{-1}) (Badiani et al., 1993). Wheeler et al. (1999) observed that wheat and potato plants cultured under very high CO\textsubscript{2} concentrations (10000 µmol mol\textsuperscript{-1}) showed increased plant biomass accumulation compared with those plant cultured under low CO\textsubscript{2} concentrations (400 µmol mol\textsuperscript{-1}) and no injurious effects were apparent from the 10000 µmol mol\textsuperscript{-1} treatment. In this study, we employed a hydroponic culture method without any rooting substrates. We need to develop an alternative method suitable for culturing garlic under a microgravity condition in space.

The result of the present study showed the improvement of both crop productivity and crop quality in terms of antioxidant medicinal properties that would result from currently anticipated international space station environmental conditions to increase diet diversity and also to provide potential countermeasures to mitigate risks associated with space radiation. This will form the initial idea to develop production systems for health-promoting quality plants in the future. Garlic containing relatively large amount of medicinal components as well as carbohydrates and other nutritional components is a promising candidate for being used as a healthy foodstuff in space. Higher growth rates and greater ajoene contents of garlic plants must be achieved by a suitable combination of the atmospheric CO\textsubscript{2} concentration and the PPFD level in the space environment.

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