

**EFFECTS OF ELEVATED ATMOSPHERIC CARBON DIOXIDE
CONCENTRATION ON GROWTH, PHOTOSYNTHESIS AND
MORPHOLOGY IN CHINESE YAM**

(大気中の高濃度二酸化炭素がナガイモの生長、光合成および形態に
及ぼす影響に関する研究)

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A DISSERTATION SUBMITTED

BY

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DOCTOR OF PHILOSOPHY

BIOPRODUCTION SCIENCE

THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCES

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**A DISSERTATION SUBMITTED TO
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IN
BIOPRODUCTION SCIENCE**

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CHAPTER 1

General introduction

Yam and its roles

Yam (*Dioscorea* spp.) is a multi-species tuber crop cultivated widely in Africa, Asia, parts of South America, the Caribbean and the South Pacific islands (Asiedu & Sartie, 2010). Yam is among the top ten most consumed foods in the world (FAO, 2010) and plays an important role as a major staple crop for millions of people in tropical and sub-tropical regions (Srivastava et al., 2012) due to its tuber containing high starch content, proteins and micronutrients (Asiedu & Sartie, 2010). Among the various yam species, Chinese yam (*Dioscorea opposita* Thunb), is cultivated in Japan, China, Korea, and Taiwan etc., and is one of the most important root and tuber crops in northern Japan. To feed the growing world population, yam will have an increasingly important role.

Global warming

The climate of the Earth is constantly undergoing changes due to changes in the Earth's orbit, changes in the Sun's intensity, changes in the ocean, volcanic emissions and changes in greenhouse-gas concentrations (Florides & Christodoulides, 2009). The greenhouse gases, including water vapor (H₂O), carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) and fluorinated gases, are the most contributors to climate pattern change. Among greenhouse gases, increased atmospheric CO₂ concentration ([CO₂]) is widely being considered as the main driving factor that causes the phenomenon of global warming. According to the environmental protection agency (EPA), global warming is defined as the increasing in average earth surface temperature. The hazard of

global warming is one of the most important and critical problems of the world (EPA, 2013a,b).

Atmospheric carbon dioxide concentration

CO₂ is the most contributed gas to the greenhouse effect accounting for 80% of the impact. CO₂ and other gases increased from combustion of fossil fuels, agricultural activities, industry, energy use and fertilizers. Comparing with other greenhouse gases like methane or nitrous oxide, CO₂ absorbs less heat per molecule but it's more abundant and it stays in the atmosphere much longer. Over thousands of years in the past, the [CO₂] remained stable under 300 ppm. Since the Industrial Revolution, [CO₂] has increased drastically, to levels of nearly 380 ppm in 2005 and 402 ppm in 2016, and is predicted to reach 550 ppm by 2050 (Inter-government Panel Climate Change [IPCC], 2007). It will continue to increase for the foreseeable future (IPCC, 2013) if no steps are taken to limit the emission of [CO₂] from various sources, particularly from fossil fuels (IPCC, 2001). The increased atmospheric [CO₂] is widely being considered as the main driving factor that causes the phenomenon of global warming (Florides & Christodoulides, 2009). Due to the increase in anthropogenic greenhouse-gas concentrations, particularly increased [CO₂], the associated increase in globally averaged temperatures since the mid-20th century is observed. According to the Fourth Assessment Report (IPCC, 2007), global mean temperature has risen approximately 0.74°C in the last 100 years due to anthropogenic activities. The averaged surface temperature is predicted to rise 0.3–1.7°C by 2081–2100 in comparison with 1986–2005 under low emission and 2.6–4.8°C under high-emission scenarios (IPCC, 2013).

Effect of global warming on yam

Some scientists using past data of change of climatic factors (1971–2009) and yield of yam (1999–2009) in Bayelsa (Nigeria) reported that an increasing trend in temperature and rainfall cause the decreasing trend in yield of yam (Ike, 2012). Other researchers used the crop models such as CROPSYSTVB-Yam (Marcos et al., 2009) and EPIC-Yam (Srivastava et al., 2012) with daily precipitation, maximum and minimum temperature, solar radiation, wind speed and crop characteristics to anticipate the effects of climate change on yam plants. For the EPIC-Yam, the testing showed that the highest increase in temperature and extreme decline in rainfall exhibited a decrease of 33% in yam yield until 2050 under ambient [CO₂] of 350 ppm in West Africa (Srivastava et al., 2012). For CROPSYSTVB-Yam, growth and yield in water yam drastically decreased because of photoperiod induced fast tuber initiation in Central America (Marcos et al., 2011). Raymundo et al. (2014) pointed out the limitation that these models need to be calibrated with modern cultivars under stress conditions (high temperature, heat, drought) and elevated [CO₂]. Understanding the responses of yam to climate change such as increasing [CO₂] and air temperature is very necessary to determine effective strategies in the future. However, no information on the response of Chinese yam to elevated [CO₂] has been available to date.

Effect of elevated [CO₂] on plant growth

The current ambient [CO₂] is considered to be a limiting factor for growth of most agricultural species, especially C₃ crops. Thus, increase in atmospheric [CO₂] will be more beneficial to this group of crops through enhanced photosynthesis and improving transpiration efficiency. Some studies have shown that elevated [CO₂] increases dry matter production in many crops, including rice (Cheng et al., 2009; Roy et al., 2012), potato (Chen & Setter, 2012; Conn & Cochran, 2006), and cassava (Cruz et al., 2014; Rosenthal et al., 2012). Yields also increase under

elevated [CO₂] with productivity increases in the range of 15 to 41% for C₃ crops (IPCC, 2007). However, no reports have appeared on yam response to elevated [CO₂].

Effect of elevated [CO₂] on seed germination

Seed germination and seedling vigour have a marked influence on plant population, plant community dynamics and final yields. Seed germination is a mechanism in which morphological and physiological changes result in activation of the embryo (Miransari & Smith, 2014). Thus, germination process is controlled by a number of mechanisms, including environmental conditions (temperature, light, water etc.). Some researchers showed that plant germination rate under elevated [CO₂] condition increased (Ziska & Bunce, 1993; Edwards et al., 2001) or decreased (Andolo et al., 1996; Quaderi & Reid, 2005; Saha et al., 2015), or even not changed (Thurig et al., 2003). However, in case of Chinese yam, aerial bulbils serve as a means of vegetative reproduction and are largely used for yam production. A bulbil consists of a small bud with short stem and germination of bulbils may differ considerably from seed due to dissimilar anatomical structures. Understanding the effects of elevated [CO₂] on bulbil germination and seedling vigour will be important for seedling establishment and sustainable production of Chinese yam in the future. No attempt of effects of elevated [CO₂] on bulbil germination and seedling vigor has been done to date.

Effect of elevated [CO₂] on plant morphology

Elevated [CO₂] has been reported to have significant effect on plant structure and development (Pritchard et al., 1999). Alterations in plant morphology, anatomy and ultrastructure enable acclimation of plants to elevated [CO₂]. Some authors reported that elevated [CO₂] affects the morphology and anatomy of plant by

increasing thickness of leaf (Radoglou & Jarvis, 1992; Bray & Reid, 2002; Vu et al., 1989; Okasanen et al., 2001); number and width of chloroplasts (Teng et al., 2006; Yang et al., 1997; Thomas & Harvey, 1983), and size and number of starch grains (Hao et al., 2013; Kumar et al., 2013; Wei et al., 2002) but reducing stomatal density, stomatal index of leaves and stomatal conductance (Bunce, 2013; Teng et al., 2006). In case of Chinese yam, the effects of elevated [CO₂] on Chinese yam morphology under different air temperatures were very important, but remain unclear until now.

Objectives of the study

Understanding the responses of Chinese yam to climate change factors such as increasing [CO₂] and air temperature is important to determine effective strategies for yam cultivation in the future. In this study, the general aim was to elucidate the effect of elevated [CO₂] on growth, photosynthesis and morphology in Chinese yam under different conditions. To achieve the aim, several experiments were conducted with the following objectives:

1. To elucidate the effects of elevated [CO₂] on growth and photosynthesis in Chinese yam.
2. To clarify the effects of elevated [CO₂] on bulbil germination and seedling vigour in Chinese yam.
3. To elucidate the effects of elevated [CO₂] on morphology in Chinese yam.

CHAPTER 2

Effects of elevated CO₂ concentration on growth and photosynthesis of Chinese yam

INTRODUCTION

Yams belonging to the *Dioscorea* genus are C₃ plant species that are cultivated in tropical and sub-tropical regions of the world (Coursey & Haynes, 1970). Yam is the third most important tropical root and tuber crop after cassava and sweet potato in west Africa, central America, the Pacific islands, and southeast Asia (Srivastava et al., 2012). Among the various yam species, Chinese yam (*Dioscorea opposita* Thunb.), known as “nagaimo” in Japanese, is cultivated in Japan, China, Korea, and Taiwan etc., and is one of the most important root and tuber crops in northern Japan. To feed the growing world population that is expected to reach 9.7 billion by 2050 (United nation, Department of Economic & Social Affairs, 2015), yam will have an increasingly important role.

The global atmospheric carbon dioxide concentration ([CO₂]) has increased from 300 μmol mol⁻¹ to the present 398 μmol mol⁻¹ (National Oceanic & Atmospheric Administration-Earth System Research Laboratory, 2014). According to recent report (Inter-government Panel on Climate Change [IPCC], 2013), these levels will continue to increase in the foreseeable future. Associated with the [CO₂] increase, the air temperature is predicted to rise 0.3–1.7°C by 2081–2100 and 2.6–4.8°C under high [CO₂] emission scenarios (IPCC, 2013) as a result of global warming. Changes in both [CO₂] and temperature are expected to affect yam production via their effects on many metabolic processes that influence crop growth and yield, both in terms of quantity and quality.

Many studies have evaluated the effects of elevated [CO₂] and high temperature, separately and in combination, at experimental levels in growth

chambers, greenhouses, or field studies. Such studies have mainly focused on cereal crops, especially the main grain cereals such as maize (Holden & Brereton, 2003; Oseni & Masarirambi, 2011), rice (*Oryza sativa* L.) (Cheng et al., 2009; Shimono et al., 2009; Roy et al., 2012), wheat (Nonhebel, 1993; Valizadeh et al., 2014), and sorghum (Prasad et al., 2006; Wall et al., 2001), or grain legumes such as soybean (Kumagai & Sameshima, 2014; Tacarindua et al., 2013) and peanut (Newman et al., 2005). Fewer studies have been conducted on the potential effects of climate change on root and tuber crops such as potato (Chen & Setter, 2012; Craigon et al., 2002; Katny et al., 2005) and cassava (Fernandez et al., 2002; Gleadow et al., 2009). In the case of yam, a small number of statistical analyses on the relationship between climatic data and yam yield in Africa (Ike, 2012; Zakari et al., 2014) as well as simulation studies based on models such as CROPSYSTVB-Yam and EPIC-Yam (Marcos et al., 2011; Srivastava & Gaiser, 2010; Srivastava et al., 2012) have been conducted but no experiments related to the effects of elevated [CO₂] have been performed in yam (Raymundo et al., 2014), including Chinese yam. Therefore, understanding the responses of Chinese yam to climate change factors such as increasing [CO₂] and air temperature is important to determine effective strategies for cultivation in the future.

In studies on plant responses to elevated [CO₂], the source–sink hypothesis is an important consideration. According to this hypothesis, a plant with a low sink capacity will down-regulate acclimation of photosynthesis after long-term exposure to elevated [CO₂] (Ainsworth et al., 2004). Chinese yam is a tuber crop with a large sink organ—the tuber—which is initiated from the early vegetative stage. In contrast, rice is considered as a limited sink plant during the vegetative stage. In this study, Chinese yam and rice were grown under two [CO₂] conditions (ambient and elevated) with different air temperature regimes in summer and autumn, separately. We aimed to determine the effect of elevated [CO₂] at

different air temperature regimes under different seasonal conditions on the growth and photosynthesis of Chinese yam. In addition, our purpose was to clarify the differences in growth and photosynthetic responses to elevated $[\text{CO}_2]$ between Chinese yam (a large sink capacity plant) and rice (a limited sink capacity plant).

MATERIALS AND METHODS

1. Plant materials and seedling preparation

We used two different plant materials: Chinese yam line Enshikei 6, and rice cv. Hitomebore. Seedlings were prepared as follows: yam bulbils were sterilized in 0.5% (v/v) sodium hypochlorite solution for 5 min and then thoroughly washed with water before sowing in small plastic nursery pots filled with commercial soil (containing 320 mg L^{-1} nitrogen (N), 210 mg L^{-1} phosphorus (P_2O_5), and 300 mg L^{-1} potassium (K_2O)). The bulbils were sown on 9 June 2015 for use in summer and autumn experiments. Rice seeds were soaked in 20% (w/v) NaCl solution to eliminate unfilled seeds and then sterilized in 1% (v/v) SPORTAK[®] solution (AGRO-NET; Nissan Chemicals Ltd., Saitama, Japan) for 10 min. The seeds were washed with water and then incubated at 30°C to germinate. The germinated seeds were sown in a seedling tray on 22 June 2015 for summer experiment and on 8 August 2015 for autumn experiment. Chinese yam and rice seedlings were grown under natural temperature, light, and humidity conditions in a greenhouse at Hirosaki University ($40^\circ59'\text{N}$, $140^\circ47'\text{E}$), Hirosaki, Japan.

2. Temperature-gradient chamber and treatments

The two experiments were conducted in temperature-gradient chambers at Tohoku Agricultural Research Center, NARO ($39^\circ74'\text{N}$, $141^\circ13'\text{E}$) in Morioka, Japan. Summer experiment was carried out from 11 July to 3 September, 2015, and autumn experiment was carried out from 23 August to 3 October, 2015. Both

experiments were performed in the same manner, as described below.

In the temperature-gradient chamber, [CO₂] and temperature were controlled independently. Two temperature-gradient chambers were used under two [CO₂] conditions: ambient and elevated (ambient [CO₂] + 200 μmol mol⁻¹ in daytime (0400 to 2030)). Each chamber was a naturally sunlit greenhouse (6 m wide, 30 m long, and 3 m high). Summer and autumn experiments included approximately ambient- and a high-temperature regime. Thus, we were able to test the [CO₂] responsiveness of Chinese yam and rice at ambient and elevated [CO₂] under two temperature conditions (approximately ambient and high). The daytime (0400 to 2030) [CO₂] averaged over the treatment period was 403 ± 10 μmol mol⁻¹ in the ambient [CO₂] plot and 598 ± 10 μmol mol⁻¹ in the elevated [CO₂] plot for summer experiment and 400 ± 9 μmol mol⁻¹ in the ambient [CO₂] plot and 591 ± 26 μmol mol⁻¹ in the elevated [CO₂] plot for autumn experiment. The temperatures averaged over the growing season for the approximately ambient- and high-temperature regimes were respectively 24.1 ± 2.2°C and 29.1°C ± 2.4°C in summer experiment, and 20.2 ± 1.5°C and 24.9°C ± 1.5°C in autumn experiment. Temperatures in the approximately ambient- and high-temperature plots were 0.3°C and 5.3°C higher, respectively, than the outside temperature. The relative humidity in the chambers was similar between summer and autumn experiments. Its mean values reached about 60.6% for approximately ambient-temperature plots, and about 40.4% for high-temperature plots in the chambers. In this study, the difference in relative humidity between the two plots in this study was not big (about 20%) and plant growth is generally much more sensitive to changes in air temperature than in relative humidity. Thus, we set up the treatments as mentioned below. Air temperature, [CO₂] and relative humidity were measured at 5-s intervals and averaged every 1 min, 30 min and 24 h by a datalogger (CR 1000; Campbell Sci. Inc., Logan, UT, USA). The treatments are

abbreviated as follows:

- (1) AA: ambient [CO₂] and approximately ambient air temperature
- (2) EA: elevated [CO₂] and approximately ambient air temperature
- (3) AH: ambient [CO₂] and high air temperature
- (4) EH: elevated [CO₂] and high air temperature

At the start of the experiments, the average Chinese yam seedling height was 22 cm (with five fully expanded leaves) in summer experiment and 49 cm (with 13 expanded leaves) in autumn experiment. Uniform plants were transferred into plastic pipes (50.5 cm long, 10.5 cm inner diameter; one plant/pipe) filled with the same type of soil as that used at the seedling stage. The plants were watered one to three times per week as required. For the rice plants, 2-week-old seedlings at the four- to five-leaf stage were transplanted into Wagner pots (two plants/pot) filled with commercial soil (containing 1.7 g L⁻¹ N, 4.4 g L⁻¹ P₂O₅, and 1.8 g L⁻¹ K₂O). The four treatments were started soon after transplanting Chinese yam and rice seedlings. Rice was flooded during all of these experiments.

3. Plant growth measurement and sampling

Chinese yam and rice plants in summer experiment and autumn experiment were respectively sampled at 55 d and 42 d after the beginning of treatment. For Chinese yam, leaves on each plant were counted and then the vine length was measured. After carefully washing the soil from the roots with running water, we separately sampled leaves, vines, roots, and tubers of individual plants in each treatment. The area of Chinese yam leaves was immediately measured using an automatic leaf area meter (AAM-9; Hayashi Denko Co. Ltd., Tokyo, Japan). Finally, all Chinese yam samples were dried at 80°C for 4 d to constant weight. Dry weight (DW) was measured using an electronic scale (GX 3000; A&D Co., Ltd., Tokyo, Japan). Rice plants were sampled at the same times and in the same manner as that described above for Chinese yam. After counting numbers of

leaves and tillers and measuring plant height, leaf blade area was immediately measured. Leaf blades, shoots without leaf blades, and roots were dried and weighed as described above. For Chinese yam and rice, six individual plants per treatment were used to measure each parameter.

4. Photosynthesis measurements

Photosynthetic gas exchange measurements were conducted on fully expanded leaves at 2–3 d before sampling. For Chinese yam, the measurements were conducted on fully expanded leaves in the middle of the vine because old leaves in the basal part of the vine and young leaves near the tip were not suitable for measuring normal photosynthesis. In rice, 14th and ninth expanded leaves of rice plants were used for measuring photosynthesis in summer experiment and autumn experiment, respectively. The light-saturated photosynthetic rate was measured between 0700 and 1400 using a portable photosynthesis measurement system (LI-6400; Li-Cor, Lincoln, NE, USA). The [CO₂] in the air entering the leaf chamber was adjusted to 400 and 600 $\mu\text{mol mol}^{-1}$ in ambient and elevated [CO₂] plots, respectively. The air temperature in the chamber matched that of the respective regime. The relative humidity of air entering the chamber was adjusted to approximately 60%. The photosynthetic photon flux density inside the chamber was set to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using an internal light source and the leaf temperature was set to 27°C. The measurements were conducted in the center part of a single leaf of three or four individual plants per treatment for Chinese yam and two or three individual plants for rice.

5. Statistical analysis

To test the significance of differences related to [CO₂] levels, temperature regimes, and their interaction, we applied two-way analysis of variance (ANOVA) to the

following data: for Chinese yam, the number of leaves, vine length, leaf area, leaf DW, vine DW, tuber DW, root DW, shoot DW, total DW, and leaf photosynthesis; for rice, the number of leaves and tillers, shoot length, leaf blade area, leaf blade DW, shoot DW, root DW, total DW, and leaf photosynthesis. When ANOVA produced a significant result, Tukey–Kramer’s test for significant differences between means was performed. All statistical analyses were performed with SPSS statistical software (SPSS ver. 24.0; IBM Corp, New York, NY, USA).

RESULTS

1. Growth responses of Chinese yam and rice to elevated [CO₂]

According to Tukey–Kramer’s test, vine length and leaf area in Chinese yam in summer experiment were significantly ($P<0.05$) higher under elevated [CO₂] than under ambient [CO₂] with both temperature regimes (Table 2.1). The number of leaves was also significantly higher in EA than in AA. There was a trend towards more leaves in EH than in AH in summer experiment. In autumn experiment, no significant difference was observed in yam growth between elevated and ambient [CO₂] treatments in terms of number of leaves, vine length, and leaf area under either temperature regime. ANOVA revealed significant effects of both [CO₂] and air temperature in summer experiment and of only temperature in autumn experiment on number of leaves, vine length, and leaf area in Chinese yam (Table 2.1). A [CO₂] × air temperature interaction for leaf area was found in summer experiment.

For rice, the number of leaves was significantly ($P<0.05$) higher in EA than in AA in summer experiment (Table 2.1). There were no significant differences between elevated and ambient [CO₂] in shoot length, number of tillers, and leaf blade area in both temperature regimes in summer experiment. In autumn experiment, the number of leaves, the number of tillers, and leaf blade area were

significantly ($P < 0.05$) higher in EH than in AH. There were no significant differences in the number of leaves, number of tillers, and leaf blade area between AA and EA in autumn experiment. Rice shoot length was not significantly different between ambient [CO₂] and elevated [CO₂] in both temperature regimes in autumn experiment. Significant effects of both [CO₂] and air temperature on the number of leaves in summer experiment and on the number of leaves and tillers and leaf blade area in autumn experiment were found on the basis of the ANOVA results (Table 2.1). Air temperature also affected shoot length in both experiments and the number of tillers in summer experiment.

To understand differences in the contribution of elevated [CO₂] between Chinese yam and rice, we calculated the mean ratio of each growth parameter under elevated vs. ambient [CO₂]. Ratios of the number of leaves under elevated vs. ambient [CO₂] under approximately ambient- and high-temperature regimes, respectively, were 1.29 and 1.54 in summer experiment and 1.38 and 1.06 in autumn experiment in Chinese yam whereas the corresponding values in rice were 1.04 and 1.01 in summer experiment and 1.00 and 1.02 in autumn experiment (Table 2.1). The ratios for vine length under approximately ambient - and high-temperature regimes were respectively 1.49 and 1.86 in summer experiment and 1.11 and 1.05 in autumn experiment in Chinese yam; the corresponding ratios for shoot length in rice were 1.01 and 1.01 in summer experiment and 1.00 and 1.03 in autumn experiment. Leaf area ratios in Chinese yam under approximately ambient- and high-temperature regimes were respectively 1.59 and 1.71 in summer experiment and 1.16 and 0.88 in autumn experiment; in rice, they were 1.01 and 1.03 in summer experiment and 1.15 and 1.29 in autumn experiment (Table 2.1). The ratios of the number of tillers in rice ranged from 1.05 to 1.18 in both experiments.

2. Effects of elevated [CO₂] and temperature on dry matter accumulation

According to Tukey–Kramer’s test, leaf DW, vine DW, and root DW of Chinese yam in summer experiment were significantly ($P<0.05$) higher in EA than in AA (Table 2.2). Leaf DW was also higher in EH than in AH. Chinese yam vine DW, root DW, and tuber DW tended to be higher in EH than in AH in summer experiment. In autumn experiment, Chinese yam tuber DW was significantly ($P<0.05$) higher in EH than in AH, but there was no difference in tuber DW between AA and EA. There were no significant differences in Chinese yam leaf DW, vine DW, and root DW between ambient [CO₂] and elevated [CO₂] under approximately ambient- and high-temperatures. The results of the ANOVA demonstrated the existence of significant effects of [CO₂] and temperature on total plant DW of Chinese yam ($P<0.001$ for all combinations, except for $P<0.01$ for [CO₂] in autumn experiment) in both experiments. No interaction was uncovered between [CO₂] and temperature on Chinese yam total DW (Figure 2.1). Total DW was significantly ($P<0.05$) higher in elevated [CO₂] than in ambient [CO₂] under both temperature regimes in summer experiment and not significantly higher in autumn experiment (Figure 2.1). In general, most Chinese yam DW values were greater in summer experiment than in autumn experiment; the exception was tuber DW (Table 2.2 and Figure 2.1). ANOVA revealed significant effects of both [CO₂] and temperature in summer experiment and of temperature in autumn experiment on Chinese yam leaf DW, vine DW, and root DW, except for tuber DW, which was affected by only [CO₂] in summer experiment and by both factors in autumn experiment (Table 2.2).

In the case of rice, according to Tukey–Kramer’s test, rice root DW and total DW in summer experiment were significantly ($P<0.05$) higher in EH than in AH (Table 2.2 and Figure 2.1). Rice leaf blade DW and shoot DW did not differ between ambient and elevated [CO₂] under either growth temperature regimes in summer experiment. Rice root DW and total DW did not differ significantly

between AA and EA in summer experiment. In autumn experiment, rice leaf blade DW, shoot DW, and total DW were significantly ($P<0.05$) higher in EH than in AH. None of the measured parameters differed significantly between AA and EA in autumn experiment (Table 2.2 and Figure 2.1). Leaf blade DW and shoot DW were significantly affected, according to the ANOVA, by both $[CO_2]$ and temperature in autumn experiment but not in summer experiment (Table 2.2). Significant effects of $[CO_2]$ and temperature on total plant DW in rice ($P<0.001$ for all combinations, except for $P<0.05$ for temperature in summer experiment) were also found in both experiments. An interaction between $[CO_2]$ and temperature was uncovered in rice in autumn experiment (Figure 2.1).

For aboveground parts, the ratios of leaf DW in elevated vs. ambient $[CO_2]$ under approximately ambient- and high-temperature regimes in Chinese yam were respectively 1.61 and 1.76 in summer experiment and 1.31 and 1.07 in autumn experiment (Table 2.2). The corresponding data in rice were 1.07 and 1.07 in summer experiment and 1.16 and 1.36 in autumn experiment. Vine DW ratios in Chinese yam were 1.59 and 1.67 in summer experiment and 1.25 and 1.08 in autumn experiment under approximately ambient- and high-temperature regimes, respectively, while the relative ratios of shoot DW in rice were 1.10 and 1.07 in summer experiment and 1.25 and 1.33 in autumn experiment. For underground parts, the ratios of root DW under approximately ambient- and high-temperature regimes, respectively, were 1.38 and 1.83 in summer experiment and 1.30 and 1.06 in autumn experiment in Chinese yam; the corresponding ratios in rice were 1.03 and 1.31 in summer experiment and 1.17 and 1.23 in autumn experiment (Table 2.2). In addition, tuber DW ratios in Chinese yam were 1.44 and 1.40 in summer experiment and 1.22 and 1.36 in autumn experiment. For total plant DW, the ratios under approximately ambient- and high-temperature regimes in Chinese yam were respectively 1.43 and 1.60 in summer experiment and 1.23 and 1.24 in

autumn experiment, while the corresponding values in rice were 1.16 and 1.21 in summer experiment and 1.22 and 1.40 in autumn experiment (Figure 2.1).

3. Photosynthetic responses of Chinese yam and rice to elevated [CO₂]

According to Tukey–Kramer’s test (Figure 2.2), the single-leaf net photosynthetic rate of Chinese yam was significantly higher under elevated [CO₂] than ambient [CO₂] under both approximately ambient- and high-temperature regimes in summer experiment, but not in autumn experiment. Nevertheless, an increasing trend in the single-leaf net photosynthetic rate of Chinese yam was observed going from ambient to elevated [CO₂] in autumn experiment. For rice, no significant differences in net photosynthetic rate were detected between ambient and elevated [CO₂] in either experiment. ANOVA uncovered significant effects of [CO₂] and temperature on net leaf photosynthesis in Chinese yam ($P < 0.001$ in summer experiment and $P < 0.05$ in autumn experiment) but no significant effects on rice in either experiments (Figure 2.2). However, there is no significant [CO₂] × air temperature interaction on Chinese yam photosynthesis in both experiments.

Ratios of photosynthesis in elevated vs. ambient [CO₂] under approximately ambient- and high-temperature regimes in Chinese yam were respectively 1.73 and 1.39 in summer experiment and 1.36 and 1.47 in autumn experiment; the corresponding values in rice were 1.16 and 0.90 in summer experiment and 1.18 and 0.81 in autumn experiment (Figure 2.2).

DISCUSSION

The experiments in this study were conducted in temperature-gradient chambers. As shown by Okada et al. (2000), both air temperature and vapor pressure deficit increase from the inlet to the outlet in temperature-gradient chambers and these two factors are closely associated. In our experiments, Chinese yams were grown

in approximately ambient- and high-temperature plots in each temperature-gradient chamber. The increase in air temperature resulted in a decrease in relative humidity in the high temperature plots in the chambers. However, Shimizu et al. (1996), who researched the effects of [CO₂] (500 and 1000 ppm) and relative humidity (79% and 37%) on the growth of several plants, reported that there were no obvious interactive effects between CO₂ and relative humidity, and that these environmental factors may affect the growth of plants independently. Therefore, relative humidity would have had a small effect on the experimental results and has no relevance to the conclusions of this study.

Many previous studies of the effects of elevated [CO₂] on the growth of C₃ plants have been published. In these studies, increased [CO₂] has been reported to lead to increased dry matter production in many major food crops, including rice (Baker et al., 1992; Cheng et al., 2009; Roy et al., 2012; Shimono et al., 2008; Ziska et al., 1997), potato (Aien et al., 2014; Chen & Setter, 2012; Conn & Cochran, 2006; Miglietta et al., 1998), and cassava (Cruz et al., 2014; Imai et al., 1984; Rosenthal et al., 2012). To the best of our knowledge, however, no reports have appeared on yam response to elevated [CO₂]. In the present study, the vine length, leaf area, leaf DW and total plant DW of Chinese yam were significantly higher in elevated [CO₂] than in ambient [CO₂] under both approximately ambient- and high-temperature regimes in summer experiment (Table 2.1 and 2.2, Figure 2.1). In addition, the number of leaves, vine DW, and root DW of Chinese yam were significantly higher in EA than in AA (Table 2.1 and 2.2). In autumn experiment, tuber DW was significantly higher in EH than in AH and all other parameters of Chinese yam tended to exhibit higher values under elevated [CO₂] than under ambient [CO₂] (Table 2.1 and 2.2, Figures 2.1). ANOVA results demonstrated the existence of significant effects of [CO₂] and air temperature on total plant DW of Chinese yam in both experiments meanwhile there is no

significant effect of $[\text{CO}_2] \times$ air temperature interaction on Chinese yam total DW in both experiments (Figure 2.1). These results demonstrate that Chinese yam shows positive growth responses to elevated $[\text{CO}_2]$. However, no interaction between $[\text{CO}_2]$ and air temperature on the total DW in Chinese yam was identified in both experiments.

Not only $[\text{CO}_2]$ but also air temperature significantly affected total plant DW in rice in both summer and autumn experiments (Figure 2.1). In both experiments, elevated $[\text{CO}_2]$ clearly increased rice total DW under the high-temperature regime. These results indicate that elevated $[\text{CO}_2]$ and air temperature affect total plant DW in rice as with Chinese yam. In summer experiment, ratios of all growth parameters related to size and weight (Table 2.1 and 2.2, Figure 2.1) in elevated vs. ambient $[\text{CO}_2]$ were higher in Chinese yam than in rice. ANOVA revealed that elevated $[\text{CO}_2]$ more strongly affected most growth parameters except for total DW in Chinese yam than in rice in summer experiment. In this study, solar radiation was higher in summer experiment ($180 \pm 80 \text{ w m}^{-2}$) than in autumn experiment ($150 \pm 61 \text{ w m}^{-2}$). The results consequently suggest that the contribution of elevated $[\text{CO}_2]$ is higher in Chinese yam than that in rice under summer conditions. Additional study is needed to confirm in the future.

The results of some studies have suggested that increasing $[\text{CO}_2]$ will result in increased photosynthesis (Kimball et al., 2002; Vu et al., 1997; Yang et al., 2006). Several recent studies have shown that photosynthesis of C_3 plants increases by 30–50% with doubled $[\text{CO}_2]$ (Ainsworth & Rogers, 2007; Baker et al., 1992). De Costa et al. (2003) observed that leaf net photosynthetic rates of two rice crops were significantly higher in CO_2 -enriched chambers than in ambient-air chambers, namely, 51–75% higher in the *maha* season (October–March) and 22–23% higher in the *yala* season (April–September). Katny et al. (2005) have reported that photosynthesis in potato is increased by 10–40% under elevated $[\text{CO}_2]$. In radish,

net photosynthetic rate has been observed to increase by 46% on average under elevated $[\text{CO}_2]$ (Usuda, 2004). In our study, $[\text{CO}_2]$ and air temperature were found by ANOVA to have significant effects on net photosynthetic rate in both experiments for Chinese yam, but these effects were not significant for rice (Figure 2.2). However, there is no significant effect of $[\text{CO}_2] \times$ air temperature interaction on yam photosynthesis in both experiments as with it on the total DW. The net photosynthetic rate of Chinese yam was higher under elevated $[\text{CO}_2]$ than under ambient $[\text{CO}_2]$ in summer experiment and showed an increasing trend from ambient $[\text{CO}_2]$ to elevated $[\text{CO}_2]$ in autumn experiment. The rates in summer experiment were increased by 73% and 39% in elevated $[\text{CO}_2]$ under approximately ambient- and high-temperature regimes, respectively. In rice under the same experimental conditions, however, no significant differences in net photosynthetic rate were detected between ambient and elevated $[\text{CO}_2]$ in summer and autumn experiments (Figure 2.2). These findings indicate that photosynthesis responds more readily to elevated $[\text{CO}_2]$ in Chinese yam than in rice under the both seasons.

Some studies have shown that plant photosynthetic responses to elevated $[\text{CO}_2]$ may vary depending on the sink–source balance. Ainsworth et al. (2004) tested the source–sink hypothesis of down-regulated photosynthesis under elevated $[\text{CO}_2]$ in field trials with two lines of soybean. They found that plants with a smaller sink capacity showed a more severe photosynthetic down-regulation than those with a larger sink capacity under elevated $[\text{CO}_2]$. Shimono et al. (2010) examined the role of sink size on photosynthetic acclimation under elevated $[\text{CO}_2]$ by removing the panicle in rice, and confirmed that sink-limitation in rice did not prevent photosynthetic down-regulation under long-term elevated $[\text{CO}_2]$. Usuda & Shimogawara (1998) have reported that elevated $[\text{CO}_2]$ increases DW accumulation by 105% in storage roots of radish

and enhances sink capacity. Chen & Setter (2012) have shown that elevated [CO₂] during the potato tuber initiation stage mainly increases DW accumulation in existing sinks (leaves and stems), with cell proliferation in tubers strongly up-regulated. Chinese yam is a root and tuber crop whose tubers have a large sink capacity, whereas the sink capacity of rice is small during the vegetative phase. The results of our research indicate that the effect of elevated [CO₂] on photosynthetic rate is greater in Chinese yam than in rice. Hence, our results are consistent with the source–sink hypothesis.

The information on the effects of elevated [CO₂] at different conditions in Chinese yam that were uncovered in this study will be useful for developing strategies to grow and breed yams suitable for cultivation under different future climatic conditions.

Table 2.1. Effects of elevated CO₂ concentration on the plant growth of Chinese yam and rice

Treatments	Chinese yam			Rice			
	No. of leaves (plant ⁻¹)	Vine length (cm plant ⁻¹)	Leaf area (m ² plant ⁻¹)	No. of leaves (plant ⁻¹)	Shoot length (cm plant ⁻¹)	No. of tillers (plant ⁻¹)	Leaf blade area (m ² plant ⁻¹)
Summer experiment							
AL 24.1°C [†]	107.3 a	230.0 a	0.115 a	13.8 a	102.3 a	28.7 ab	0.295 a
EL 24.1°C [†]	138.7 b (1.29)	343.3 b (1.49)	0.182 b (1.59)	14.4 b (1.04)	103.3 a (1.01)	30.2 a (1.05)	0.299 a (1.01)
AH 29.1°C [†]	37.3 c	111.7 c	0.039 d	14.6 c	114.1 b	24.9 b	0.314 a
EH 29.1°C [†]	57.3 c (1.54)	208.0 a (1.86)	0.067 c (1.71)	14.8 c (1.01)	114.9 b (1.01)	27.5 ab (1.10)	0.323 a (1.03)
ANOVA results							
CO ₂ (C)	***	***	***	***	ns	ns	ns
Temperature (T)	***	***	***	***	***	**	ns
C x T	ns	ns	**	**	ns	ns	ns
Autumn experiment							
AL 20.2°C [†]	18.0 a	64.2 a	0.027 a	10.6 a	67.8 a	18.4 ab	0.071 a
EL 20.2°C [†]	24.8 ab (1.38)	71.3 ab (1.11)	0.031 a (1.16)	10.6 a (1.00)	67.8 a (1.00)	19.6 a (1.07)	0.082 a (1.15)
AH 24.9°C [†]	34.7 b	105.7 b	0.057 b	12.0 b	93.3 b	14.6 c	0.109 b
EH 24.9°C [†]	36.8 b (1.06)	111.2 b (1.05)	0.050 b (0.88)	12.3 c (1.02)	96.4 b (1.03)	17.3 b (1.18)	0.140 c (1.29)
ANOVA results							
CO ₂ (C)	ns	ns	ns	*	ns	**	**
Temperature (T)	**	***	***	***	***	***	***
C x T	ns	ns	ns	ns	ns	ns	ns

Note. Different letters within each plant indicate significant differences at the 5% level (Tukey–Kramer’s test). No. of leaves: Number of leaves, No. of tillers: number of tillers, AL: ambient [CO₂]-low temperature, EL: elevated [CO₂]-low temperature, AH: ambient [CO₂]-high temperature, EH: elevated [CO₂]-high temperature. Numbers in brackets are ratios of values under elevated [CO₂] to those under ambient [CO₂]. [†]: mean air temperature, ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: not significant.

Table 2.2. Effects of elevated CO₂ concentration on the dry weight of Chinese yam and rice

Treatments	Chinese yam				Rice		
	Leaf DW (g plant ⁻¹)	Vine DW (g plant ⁻¹)	Root DW (g plant ⁻¹)	Tuber DW (g plant ⁻¹)	Leaf blade DW (g plant ⁻¹)	Shoot DW (g plant ⁻¹)	Root DW (g plant ⁻¹)
Summer experiment							
AL 24.1°C [†]	7.03 a	2.09 a	1.68 a	3.13 ab	15.1 a	48.7 a	12.75 a
EL 24.1°C [†]	11.31 b (1.61)	3.33 b (1.59)	2.33 b (1.38)	4.49 b (1.44)	16.2 a (1.07)	53.5 a (1.10)	13.16 a (1.03)
AH 29.1°C [†]	2.46 c	0.67 c	0.63 c	2.46 a	14.9 a	47.9 a	8.27 b
EH 29.1°C [†]	4.33 d (1.76)	1.11 c (1.67)	1.15 ac (1.83)	3.43 ab (1.40)	15.8 a (1.07)	51.3 a (1.07)	10.80 a (1.31)
ANOVA results							
CO ₂ (C)	***	***	***	*	ns	ns	**
Temperature (T)	***	***	***	ns	ns	ns	***
C x T	**	*	ns	ns	ns	ns	ns
Autumn experiment							
AL 20.2°C [†]	1.27 a	0.32 a	0.39 a	3.01 a	3.8 a	8.3 a	2.44 a
EL 20.2°C [†]	1.66 ab (1.31)	0.40 ab (1.25)	0.51 ab (1.30)	3.68 a (1.22)	4.4 a (1.16)	10.4 a (1.25)	2.85 ab (1.17)
AH 24.9°C [†]	2.45 b	0.52 b	0.68 ab	3.93 a	5.6 b	15.1 b	3.18 ab
EH 24.9°C [†]	2.61 b (1.07)	0.57 b (1.08)	0.72 b (1.06)	5.34 b (1.36)	7.6 c (1.36)	20.2 c (1.33)	3.91 b (1.23)
ANOVA results							
CO ₂ (C)	ns	ns	ns	**	***	***	ns
Temperature (T)	***	***	**	***	***	***	**
C x T	ns	ns	ns	ns	*	ns	ns

Note. Different letters within each plant indicate significant differences at the 5% level (Tukey–Kramer’s test). DW: dry weight, AL: ambient [CO₂]-low temperature, EL: elevated [CO₂]-low temperature, AH: ambient [CO₂]-high temperature, EH: elevated [CO₂]-high temperature. Numbers in brackets are ratios of values under elevated [CO₂] to those under ambient [CO₂]. †: mean air temperature, ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, ns: not significant.

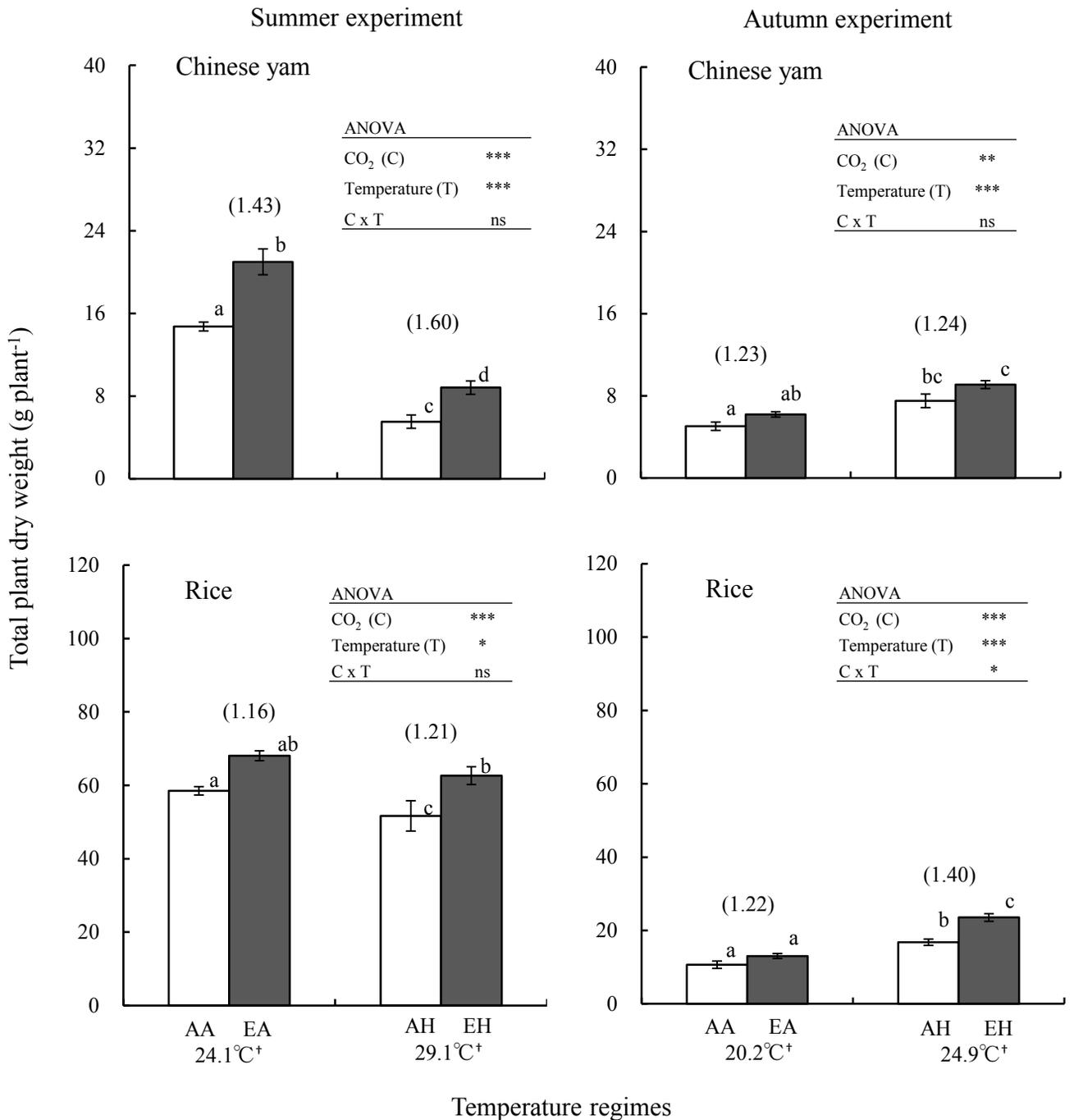


Figure 2.1. Effects of elevated CO₂ concentration on total plant dry weight in Chinese yam and rice.

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test). Bars show mean \pm SE ($n=6$), AA: ambient [CO₂]-approximately ambient temperature, EA: elevated [CO₂]-approximately ambient temperature, AH: ambient [CO₂]-high temperature, EH: elevated [CO₂]-high temperature. Numbers in brackets are ratios of values under elevated [CO₂] to those under ambient [CO₂]. †: mean air temperature, ***: $P<0.001$, *: $P<0.05$, ns: not significant.

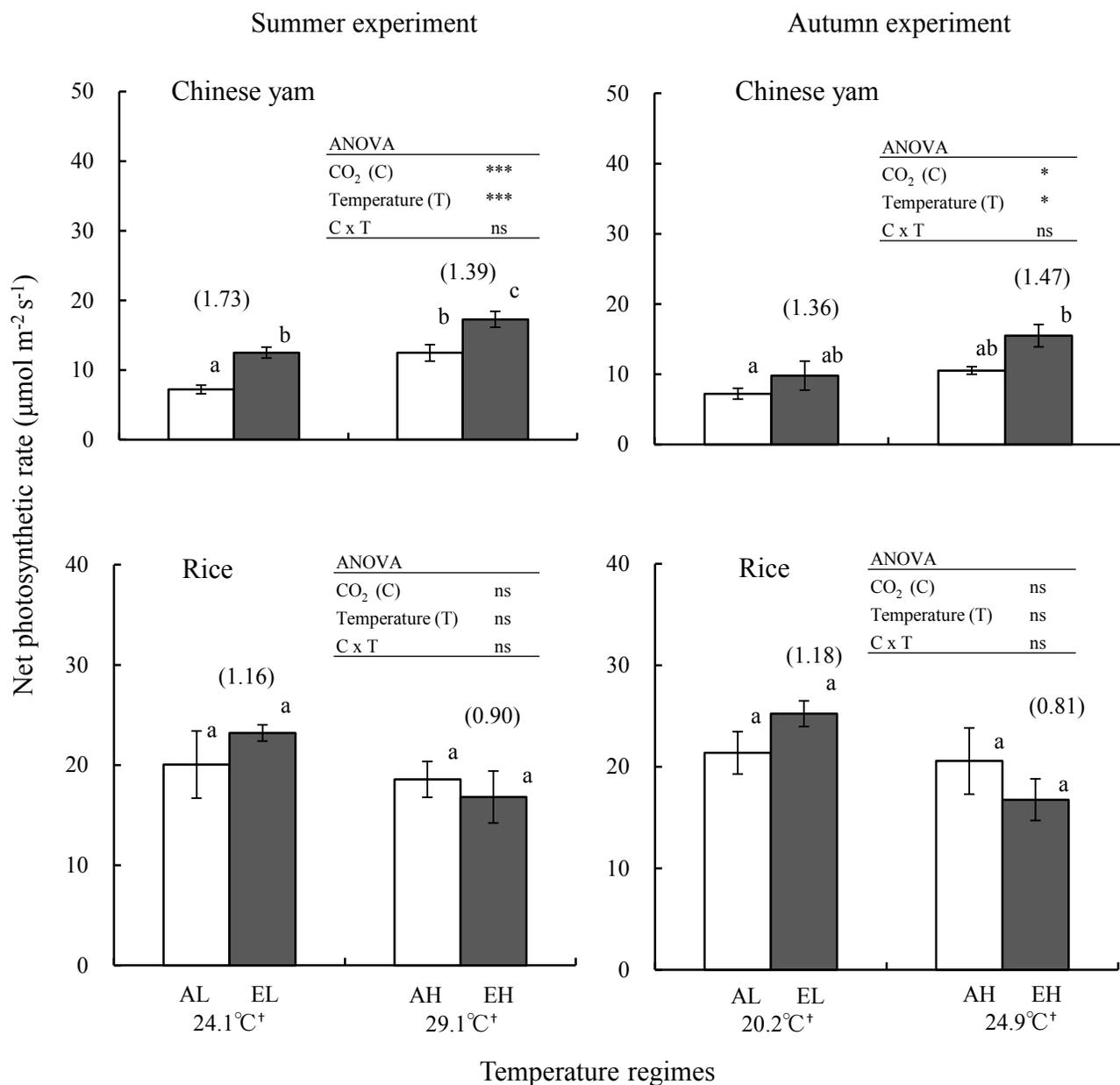


Figure 2.2. Effects of elevated CO₂ concentration on net photosynthetic rates of single leaf in Chinese yam and rice.

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test). Bars show means \pm SE ($n=2-5$), AA: ambient [CO₂]-approximately ambient temperature, EA: elevated [CO₂]-approximately ambient temperature, AH: ambient [CO₂]-high temperature, EH: elevated [CO₂]-high temperature. Numbers in brackets are ratios of values under elevated [CO₂] to those under ambient [CO₂]. [†]: mean air temperature, ***: $P<0.001$, *: $P<0.05$, ns: not significant.

CHAPTER 3

Effects of elevated CO₂ concentration on bulbil germination and early seedling growth in Chinese yam

INTRODUCTION

Yam (*Dioscorea* spp.) tubers contain high levels of carbohydrates and minerals in their tissues (Bhandari et al., 2003) and are an important staple food for millions of people in tropical and sub-tropical regions. Among the various yam species, Chinese yam (*Dioscorea opposita* Thunb.) is widely cultivated in Japan, China, Korea, and Taiwan and is one of the most important root and tuber crops in the northern prefectures of Japan, such as Aomori and Hokkaido.

Atmospheric carbon dioxide concentration ([CO₂]) has increased from 300 $\mu\text{mol mol}^{-1}$ to 402 $\mu\text{mol mol}^{-1}$ (National Oceanic & Atmospheric Administration-Earth System Research Laboratory, 2016) and is likely to continue increasing for the foreseeable future (Intergovernmental Panel on Climate Change [IPCC], 2013). Associated with this [CO₂] increase, the globally averaged surface temperature is predicted to rise 0.3–1.7°C by 2081–2100 in comparison with 1986–2005 under low greenhouse gas emission scenarios and 2.6–4.8°C under high-emission scenarios (IPCC, 2013) as a result of global warming. [CO₂] and air temperature are key variables affecting plant growth, development and function.

Seed germination and seedling vigour play an important role in agriculture production and influence final yield in terms of plant population density. Although many studies have been conducted on the effects of elevated [CO₂] on cereal crops such as rice (Cheng et al., 2009; Roy et al., 2012; Shimono et al., 2009), wheat (Nonhebel, 1993; Valizadeh et al., 2014), and sorghum (Prasad et al., 2006; Wall et al., 2001); grain legumes such as soybean (Kumagai & Sameshima, 2014; Tacarindua et al., 2013) and peanut (Newman et al., 2005); and root and tuber

crops such as potato (Chen & Setter, 2012; Craigon et al., 2002; Katny et al., 2005) and cassava (Fernandez et al., 2002; Gleadow et al., 2009), few studies have considered seed quality in terms of seed germination and early seedling vigour under elevated [CO₂]. In previous studies, seed germination in response to elevated [CO₂] has been reported to decrease in crops like, *Arabidopsis thaliana* (Andalo et al., 1996), chickpea (Saha et al., 2015), soybean, pea, sunflower, pumpkin (Ziska & Bunce, 1993), to show no change in C₃ grassland (Thurig et al., 2003), red kidney bean (Thomas et al., 2009), rice (Chen et al., 2015) and *Pinus taeda* (Way et al., 2010) or to increase in *Plantago lanceolata* (Wulff & Alexander, 1985), *Amaranthus hybridus* L. and *Chenopodium album* L. (Ziska & Bunce, 1993), *Hypochaeris radicata* and *Leontodon saxatilis* (Edwards et al., 2001).

In the case of Chinese yam, aerial bulbils, a vegetative organ, are used for yam production. Walck et al. (2010) reported that, in nature, bulbil germination is controlled through dispersal and ambient temperatures. Our previous study (chapter 2) was the first to show a positive response of growth and photosynthesis to elevated [CO₂] in Chinese yam at the intermediary vegetative stage. However, no attempts have been made to examine the effects of elevated [CO₂] on germination of seed bulbil and growth during the early seedling stage in Chinese yam to date. Under future climate change scenarios, it is likely that plants will be exposed to a combination of both higher [CO₂] and air temperature (Rosenzweig & Hillel, 1998). Therefore, understanding the effects of elevated [CO₂] and air temperature on bulbil germination and seedling vigour will be important for seedling establishment and sustainable production of Chinese yam in the future.

In this study, we hypothesized that elevated [CO₂] may positively affect seed bulbil vigour, including bulbil germination rate and seedling growth characteristics. To test this hypothesis, two Chinese yam lines, which are both current dominant lines in northern part of Japan, were grown under two [CO₂]

conditions (ambient and elevated) under different air temperatures. To our knowledge, this is the first temperature-gradient chamber study that investigated bulbil germination and seeding growth in responses of Chinese yam to elevated [CO₂].

MATERIALS AND METHODS

1. Plant materials

We used two Chinese yam lines: Enshikei 6 and Shojikei, which are widely cultivated in northern Japan, to confirm the response to elevated [CO₂] and air temperature in Chinese yam. The seed bulbils of both Chinese yam lines were harvested in October 2015. After collection, the bulbils were covered with newspaper and placed into a carton box at 4°C until use (from October 2015 to May 2016). For the experiments, seed bulbils of uniform size from each line were selected. The average fresh weight per bulbil was 1.01±0.13 g for Enshikei 6 and 1.83±0.25 g for Shojikei. The selected seed bulbils were then sterilized in 0.5% (v/v) sodium hypochlorite solution for 5 min and then thoroughly washed with water. Directly after sterilization, the seed bulbils were sown in plastic pots on 4 June 2016 and then placed immediately in the temperature-gradient chambers for treatment until 9 July 2016.

2. Temperature-gradient chambers and treatments

The experiments were conducted in temperature-gradient chambers at the Tohoku Agricultural Research Center, NARO (39°74'N, 141°13'E) in Morioka, Japan. [CO₂] and air temperature were controlled separately in each temperature-gradient chamber. Two temperature-gradient chambers were used under two [CO₂] conditions: ambient and elevated (ambient [CO₂] + 200 μmol mol⁻¹ in daytime (0400 to 2030)). Each chamber was a naturally sunlit greenhouse (6 m wide × 30 m long × 3 m high) with an air inlet at one end and exhaust fans at the other end.

The air in the temperature-gradient chamber flowed continuously from the inlet to the exhaust fans. The temperature gradient inside the chamber was continuously maintained along the longitudinal axis by cooling the air with an air conditioner at the inlet end, warming the air by solar radiation or through supplemental heat input (a heater and air ducts) at the outlet end or a combination of solar radiation and supplement heat.

In each temperature-gradient chamber, two treatment plots were set along an air temperature gradient. The first plot had a mean air temperature of $22.2 \pm 1.7^{\circ}\text{C}$ (1.4°C higher than the temperature outside the chamber; hereafter ‘outside temperature’) and the second plot had a mean air temperature of $25.6^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$ (5.2°C higher than the outside temperature). Thus, we were able to test Chinese yam germination and seedling growth at ambient $[\text{CO}_2]$ and elevated $[\text{CO}_2]$ under two different air temperature regimes. The average daytime (0400 to 2030) $[\text{CO}_2]$ over the treatment period was $406 \pm 9 \mu\text{mol mol}^{-1}$ in the ambient $[\text{CO}_2]$ chamber and $603 \pm 22 \mu\text{mol mol}^{-1}$ in the elevated $[\text{CO}_2]$ chamber. The relative humidity was about 58.3% for the approximately ambient-temperature plots and 39.2% and for high-temperature plots in the chambers. The decreasing relative humidity resulted from increasing air temperature and the contributions of these two factors are closely associated with each other in temperature gradient-chambers. In this study, we set up the treatments as below due to small difference (19%) of relative humidity between ambient-temperature plots and high-temperature plots, and air temperature changes generally more sensitive to plants than relative humidity ones. Air temperature, relative humidity and $[\text{CO}_2]$ were measured at 5-s intervals and averaged every 1 min, 30 min and 24 h by a datalogger (CR 1000; Campbell Sci. Inc., Logan, UT, USA).

Both Enshikei 6 and Shojikei were treated in the same manner in the temperature-gradient chambers as follows: three seed bulbils were sown in each

plastic pot (180 mm width × 150 mm height × 130 mm bottom diameter) filled with commercial soil (containing 320 mg L⁻¹ of nitrogen [N], 210 mg L⁻¹ phosphorus [P₂O₅], and 300 mg L⁻¹ of potassium [K₂O]). Five pots were placed in a tray and seven trays were used for each treatment. The trays were rotated at 7-d intervals to minimize the effects of environmental differences. The four treatments were abbreviated as follows:

- (1) AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature)
- (2) EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature)
- (3) AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature)
- (4) EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature)

3. Bulbil germination percentage

The seed bulbil germination percentage was determined as follows: in each treatment, Chinese yam bulbils were divided into seven groups (trays) each with 15 seed bulbils for each Chinese yam line (105 seed bulbils per line). Germination of the bulbils was recorded at 7, 14, 21, 28, 35 days after sowing (DAS). When the bulbil sprouted and emerged above the soil surface, it was considered to have germinated. The number of germinated bulbils in each group and treatment were recorded separately during the monitoring period. The germination percentage was calculated using the following formula:

Germination percentage = $S/T \times 100$, where S is the number of germinated bulbils and T is the total number of bulbils.

4. Sampling and measuring seedling growth parameters

The seedlings of the two Chinese yam lines were sampled at 35 DAS. The number of leaves on each plant was counted and then the vine and root lengths were measured using a ruler. After carefully washing the soil from the roots with running water, we separately sampled the leaves, vines, roots, seed bulbils, and tubers of individual plants in each treatment. Leaf area was immediately measured using an automatic leaf area meter (AAM-9; Hayashi Denko Co. Ltd., Tokyo, Japan). Finally, all samples were dried at 80°C for 4 d to a constant weight. Dry weight (DW) was measured using an electronic scale (GX 3000; A&D Co., Ltd., Tokyo, Japan). Seedling vigour was calculated following Abdul-Baki and Anderson (1973) as:

$$\text{Vigour index I} = \text{Germination \%} \times \text{seedling length (shoot + root)}$$

$$\text{Vigour index II} = \text{Germination \%} \times \text{seedling DW (shoot + root + tuber)}$$

Thirty individual seedlings (in 10 plastic pots) per treatment were randomly selected to measure each growth parameter.

5. Statistical analysis

To test the significance of differences related to [CO₂] levels, air temperature conditions, Chinese yam lines and their interactions, we applied three-way analysis of variance (ANOVA) to the following data: bulbil germination percentage, leaf number, vine length, root length, leaf area, leaf DW, vine DW, tuber DW, root DW, shoot DW, and total DW; and two-way ANOVA to below:aboveground DW ratio and seed bulbil DW. When ANOVA produced a significant result, we performed a Tukey–Kramer’s test for significant differences between means. All statistical analyses were performed with SPSS statistical software (SPSS ver. 24.0; IBM Corp, New York, NY, USA).

RESULTS

1. Effects of elevated CO₂ concentration on bulbil germination

According to the ANOVA results (Table 3.1), no significant effects of elevated [CO₂] on bulbil germination were detected over the whole germination period. However, significant effects of air temperature on the germination were observed at 14 DAS, 28 DAS and at 35 DAS. Significant differences between the Chinese yam lines in terms of the effect of [CO₂] on germination were observed only at 14 DAS; after this point, no significant differences were observed. There were no interactions among [CO₂] concentration, air temperature and line except for [CO₂] × air temperature interaction at 14 DAS.

At first observation (7 DAS), no bulbil germination was observed in any of the plants. The proportion of germinated seedlings increased quickly: at 14 DAS, germination ranged from 32%–51% for Enshikei 6 and 14.4%–35.6% for Shojikei; at 21 DAS, germination ranged from 78.9%–93.4% for Enshikei 6 and 76.6%–90.0% for Shojikei; and at 28 DAS germination ranged from 97.8%–100% for both lines. At each observation, there was no significant difference between AA and EA or between AH and EH in either line according to a Tukey–Kramer’s test (Table 3.1).

2. Effects of elevated CO₂ concentration on seedling growth

According to a Tukey–Kramer’s test (Table 3.2), for Enshikei 6, leaf number, leaf area and root length were significantly higher in EH than in AH and shoot length was greater in EA and EH than in AA and AH, respectively. For Shojikei, shoot length was also greater in EA and EH than in AA and AH, respectively. Although no significant differences were observed, there was an increasing trend in number of leaves and leaf area from AA to EA for both Enshikei 6 and Shojikei, and from AH to EH for Shojikei. The root length of Shojikei was also longer, but not significantly, in EH than in AH.

According to the ANOVA results (Table 3.2), elevated [CO₂], air temperature

and line had significant effects on leaf number, leaf area, shoot length and root length for both Enshikei 6 and Shojikei. However, no interactions among [CO₂], air temperature and yam line were detected for any of these parameters except for air temperature × line on leaf area.

3. Effects of elevated CO₂ concentration on seedling dry weight

A Tukey–Kramer’s test (Table 3.3) showed that, for both yam lines, leaf, vine, shoot, root and belowground DW were significantly higher in EA and EH than in AA and AH, respectively. Tuber DW was also higher in EH than in AH for Enshikei 6. Consequently, total plant DW was noticeably higher in EA and EH than in AA and AH, respectively, for both Chinese yam lines.

According to the ANOVA results (Table 3.3), elevated [CO₂] and air temperature significantly affected the final DW of leaves, vines, shoots, roots, tubers, belowground parts and total plant DW in both lines. There were also significant differences in final DWs according to yam line for all growth parameters except for tuber DW. There were interactions between [CO₂] and air temperature for root DW and belowground DW and between air temperature and line for leaf DW and shoot DW.

4. Effects of elevated CO₂ concentration on below:aboveground dry weight

According to the ANOVA results, elevated [CO₂] significantly affected the ratio of belowground (root and tuber) to aboveground (shoot) DW in both Enshikei 6 and Shojikei, while air temperature affected the below:aboveground DW ratio only in Enshikei 6 (Figure 3.1). No interactions between [CO₂] and air temperature on the ratio in either line were found. According to a Tukey–Kramer’s test, both Enshikei 6 and Shojikei had significantly higher below:aboveground DW ratios in EA and EH than in AA and AH, respectively (Figure 3.1).

5. Effects of elevated CO₂ concentration on early seedling vigour

Elevated [CO₂], high air temperature and yam line significantly affected vigour index I and vigour index II for both yam lines according to the ANOVA results (Table 3.4). However, we did not identify any interactions among these variables.

According to the results of the Tukey–Kramer’s test, vigour index I and vigour index II exhibited the same trend in Enshikei 6; that is, both vigour indexes had significantly higher values in EA and EH than in AA and AH, respectively. For Shojikei, vigour index II values were higher in EA and EH than in AA and AH, respectively; in contrast, the values of vigour index I were clearly higher in EH than in AH, but not significantly higher in EA than in AA (Table 3.4).

6. Effects of elevated CO₂ concentration on planted seed bulbil dry weight

According to the ANOVA results (Figure 3.2), elevated [CO₂] significantly affected seed bulbil DW for both Enshikei 6 and Shojikei, but no effect of air temperature was observed. There were significant interactions between [CO₂] and air temperature for Enshikei 6, but not for Shojikei. As indicated in Figure 2, seed bulbil DW for Enshikei 6 was significantly higher in EH than in AH and somewhat higher in EA than AA, though the difference was not significant. Seed bulbil DW for Shojikei was significantly higher in EA and EH than in AA and AH, respectively.

DISCUSSION

Understanding the influence of elevated [CO₂] on germination is important for the seed production industry under present and future climate conditions. However, the number of studies on the direct effects of elevated [CO₂] on seed germination has been limited, with only nine studies involving 29 species and 37 observations performed by 2014 (Marty & BassiriRad, 2014). The response of seed

germination to [CO₂] in previous studies has varied depending on the plant species studied. Aldalo et al. (1996) reported that elevated [CO₂] decreased seed germination in *Arabidopsis thaliana*. Ziska and Bunce (1993) stated that the germination rates of plants including soybean, pea, sunflower and pumpkin were reduced under enriched [CO₂] conditions. Saha et al. (2015) also concluded that chickpea germination decreased by 45%–47% under elevated [CO₂]. In contrast, Wulff & Alexander (1985) showed that high [CO₂] increased the germination rate of *Plantago lanceolata* L. Ziska & Bunce (1993) also found that elevated [CO₂] increased germination in *Amaranthus hybridus* L. and *Chenopodium album* L. In contrast with these results, recently Thomas et al. (2009) demonstrated that elevated [CO₂] did not affect germination of red kidney bean seeds under two different temperature conditions. Chen et al. (2015) also examined the responses of two rice cultivars to elevated [CO₂] and reported that no clear effects on rice germination were found. The present study indicated that elevated [CO₂] did not affect the bulbil germination rates of either Chinese yam line, though air temperature affected final germination percentage. However, there were no interactions between [CO₂] and air temperature during the germination period except at 14 DAS.

Many previous studies have been published regarding the effects of elevated [CO₂] on the growth of C₃ plants. In these studies, increased [CO₂] reportedly led to increases in dry matter production in many major food crops, including rice (Baker et al., 1992; Cheng et al., 2009; Roy et al., 2012; Shimono et al., 2008; Ziska et al., 1997), potato (Aien et al., 2014; Chen & Setter, 2012; Conn & Cochran, 2006; Miglietta et al., 1998), and cassava (Cruz et al., 2014; Imai et al., 1984; Rosenthal et al., 2012). In chapter 2, we found that Chinese yam showed a positive response to elevated [CO₂] as its photosynthesis rate and plant DW increased with [CO₂] enrichment at the intermediary vegetative stage. The current

study results showed that leaf number, leaf area and root length were higher with elevated [CO₂] than ambient [CO₂] under the high-temperature regimes for Enshikei 6 seedlings. Shoot length was also higher with elevated [CO₂] than ambient [CO₂] under both the approximately ambient- and high-temperature regimes for both Enshikei 6 and Shojikei seedlings. In addition, leaf, vine, shoot, root, belowground DW and total plant DW were significantly higher with elevated [CO₂] than ambient [CO₂] under both air temperature regimes (Tables 3.2 and 3.3). The ANOVA results also showed significant effects of elevated [CO₂] and air temperature on both Chinese yam lines, but no interactions between the two factors. These results indicate that Chinese yam seedlings respond positively to elevated [CO₂] at the early seedling stage in this study.

The below:aboveground ratio depends upon the allocation of photosynthates (Rogers et al., 1996). Many studies have reported that elevated [CO₂] stimulates the growth of both shoots and roots (Conn & Cochran, 2006; Pilumwong et al., 2007; Usuda & Shimogawara, 1998; Ziska et al., 1997). However, the allocation of biomass to shoots and roots in crops under elevated [CO₂] may be different from that under ambient [CO₂] under certain conditions. Variability in the below:aboveground DW ratios found in different studies may result from differences in plant species, development age and other experimental conditions (Rogers et al., 1996). Harmens et al. (2000) reported that elevated [CO₂] altered patterns of the allocation of biomass in *Dactylis glomerata* L. plants only transiently during the early stage of growth. Baxter et al. (1997) showed that elevated [CO₂] increased the root:shoot ratio in *Poa alpine var. vivipara* L. under high concentrations of nitrogen and phosphorous but not under low concentrations. Some previous studies found that elevated [CO₂] increased the allocation of biomass in the tubers and roots of crops such as potatoes (Aien et al., 2014; Chen & Setter, 2012; Conn & Cochran, 2006; Craigon et al., 2002; Miglitta et al., 1998)

and radish (Usuda & Shimogawara, 1998). In a previous study (chapter 2), we also reported that leaf photosynthetic rate responded to elevated $[\text{CO}_2]$ more readily in Chinese yam than in rice because yam has a higher sink capacity than rice at the vegetative stage. In this study, elevated $[\text{CO}_2]$ and air temperature significantly affected aboveground DW and belowground DW in both yam lines (Table 3.4), and both DWs were significantly higher with elevated $[\text{CO}_2]$ than ambient $[\text{CO}_2]$ under both the approximately ambient- and high-temperature regimes. The below:aboveground DW ratios were clearly higher under elevated $[\text{CO}_2]$ than ambient $[\text{CO}_2]$ under both the air temperature regimes in seedlings with very small new tubers of both Chinese yam lines (Figure 3.1). These results indicate that elevated $[\text{CO}_2]$ affected the belowground portion of the plants, particularly the roots, making them stronger from the early stage of growth.

Seedling vigour is one of the most important criteria for determining seed quality and is related to crop growth and yield (Ellis, 1992). Previous studies on dry bean (Sanhewe et al., 1996) and red kidney bean (Thomas et al., 2009) showed no effects of elevated $[\text{CO}_2]$ on early seedling vigour. In contrast, Saha et al. (2015) reported that rising atmospheric $[\text{CO}_2]$ significantly decreased the seedling vigour of chickpea plants (vigour indexes I and II) owing to 45%–47% reductions in germination, although root and shoot lengths and seedling biomass exhibited negligible changes. In the case of Chinese yam, shoot length and seedling DW were significantly higher under elevated $[\text{CO}_2]$ than ambient $[\text{CO}_2]$ (Tables 3.2 and 3.3). The values of vigour index I and II were clearly higher with elevated $[\text{CO}_2]$ (Table 3.2). The vigour values we obtained for Chinese yam were 10 and 30 times higher for vigour indexes I and II, respectively, than those previously obtained in chickpea plants (Saha et al., 2015). In our study, the bulbil germination percentage did not differ between the two $[\text{CO}_2]$ levels and reached approximately 100% in all treatments (Table 3.1). As mentioned above in the

materials section, the seed bulbils of both Chinese yam lines were mature and had been exposed to long-term chilling at 4°C prior to treatment. Thus, the bulbils used in this study were suitable for treatment. That is, we can assume that dormancy did not affect seedling growth in this study. Our results were consistent with those of Wulff and Alexander (1985), who reported that high [CO₂] increased the size of *Plantago lanceolata* L. seedlings.

Bulbils are a means of vegetative reproduction and dispersal for many plants (Walck et al., 2010) including Chinese yam. Okagami (1986) found that bulbils and seeds were similar in terms of their dormancy and germination characteristics. Seed reserves have important effects on early seedling growth and physiology after germination (Kennedy et al., 2004). The major reserves stored within the seed bulbils are mobilized after germination, providing nutrients to support early seedling growth. In a previous study, we found that the net photosynthesis rate of Chinese yam at the vegetative stage was higher under elevated than ambient [CO₂]. Consequently, yam plant DW was also higher with elevated [CO₂] (chapter 2). In the current study, we also found that elevated [CO₂] positively affects Chinese yam growth at the early stage. However, plant growth is the result of contributions from both seed bulbils (specially, the stored reserves) and the photosynthetic process (photosynthates). The results in Figure 2 demonstrate that the DWs of post-treatment seed bulbils were significantly higher with elevated [CO₂] than ambient [CO₂] under both air temperature regimes. This indicates that Chinese yam seedlings used less reserve from seed bulbils under elevated [CO₂] than under ambient [CO₂] conditions. Therefore, elevated [CO₂] is a positive resource for seedling growth in the yams.

Although both air temperature and vapor pressure deficit increased from the inlet to the outlet in temperature-gradient chambers, the parallel relationships between the two factors was observed (Okada et al., 2000). In this study, the

difference in relative humidity between these two plots was not big (about 19%). In addition, plant growth is generally much more sensitive to changes in air temperature than in relative humidity. Some researchers reported that CO₂ and relative humidity may affect the growth of plants independently (Shimizu et al., 1996). Therefore, relative humidity would have had a small effect on the experimental results and has no relevance to the conclusions of this study.

The data in this study will provide important information for the agricultural management strategies, breeding and genetic improvements required to sustain Chinese yam productivity under future climate change.

Table 3.1. Effects of elevated CO₂ concentration on bulbil germination

Chinese yam lines	Treatments		Days after sowing			
			14	21	28	35
Enshikei 6	AA	22.2°C [†]	32.2 a	87.8 a	97.8 a	97.8 a
	EA	22.2°C [†]	38.9 ab	78.9 a	100 a	100 a
	AH	25.6°C [†]	51.1 b	91.1 a	98.9 a	98.9 a
	EH	25.6°C [†]	37.8 ab	93.4 a	100 a	100 a
Shojikei	AA	22.2°C [†]	14.4 a	77.7 a	97.7 a	97.7 a
	EA	22.2°C [†]	22.2 ab	76.6 a	98.8 a	98.8 a
	AH	25.6°C [†]	35.6 b	90.0 a	100 a	100 a
	EH	25.6°C [†]	32.2 ab	83.3 a	100 a	100 a
ANOVA	CO ₂ (C)		ns	ns	ns	ns
	Temperature (T)		*	ns	*	*
	Line (L)		**	ns	ns	ns
	C × T		*	ns	ns	ns
	C × L		ns	ns	ns	ns
	T × L		ns	ns	ns	ns
	C × T × L		ns	ns	ns	ns

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test).

AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature), EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature). [†]: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.

Table 3.2. Effects of elevated CO₂ concentration on number of leaves, leaf area, shoot length and root length

Chinese yam lines	Treatments	No. of leaves (plant ⁻¹)	Leaf area (cm ² plant ⁻¹)	Shoot length (cm plant ⁻¹)	Root length (cm plant ⁻¹)
Enshikei 6	AA 22.2°C [†]	9.67 a	177.38 a	47.63 a	21.92 a
	EA 22.2°C [†]	11.5 a	206.06 a	58.25 b	21.25 a
	AH 25.6°C [†]	16.58 b	308.94 b	73.25 c	21.71 a
	EH 25.6°C [†]	19.54 c	355.49 c	84.75 d	24.88 b
Shojikei	AA 22.2°C [†]	13.75 a	243.8 a	61.29 a	23.17 a
	EA 22.2°C [†]	16.29 ab	282.65 ab	78.29 b	23.00 a
	AH 25.6°C [†]	20.7 bc	336.39 bc	80.75 b	24.71 a
	EH 25.6°C [†]	22.88 c	373.3 c	100.38 c	26.48 a
ANOVA	CO ₂ (C)	**	***	***	*
	Temperature (T)	***	***	***	**
	Line (L)	***	***	***	**
	C × T	ns	ns	ns	ns
	C × L	ns	ns	ns	ns
	T × L	ns	*	ns	ns
	C × T × L	ns	ns	ns	ns

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test).

No. of leaves: number of leaves. AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature), EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature). †: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.

Table 3.3. Effects of elevated CO₂ concentration on dry weight

Chinese yam line	Treatments	Leaf DW (g plant ⁻¹)	Vine DW (g plant ⁻¹)	Shoot DW (g plant ⁻¹)	Root DW (g plant ⁻¹)	Tuber DW (g plant ⁻¹)	Belowground DW (g plant ⁻¹)	Total plant DW (g plant ⁻¹)
Enshikei 6	AA 22.2°C [†]	0.61 a	0.15 a	0.77 a	0.09 a	0.010 a	0.10 a	0.88 a
	EA 22.2°C [†]	0.75 b	0.20 b	0.94 b	0.13 b	0.013 ab	0.14 b	1.06 b
	AH 25.6°C [†]	0.95 c	0.23 b	1.17 c	0.11 ab	0.017 b	0.12 ab	1.30 c
	EH 25.6°C [†]	1.24 d	0.32 c	1.56 d	0.17 c	0.023 c	0.20 c	1.67 d
Shojikei	AA 22.2°C [†]	0.77 a	0.20 a	1.00 a	0.11 a	0.011 a	0.13 a	1.13 a
	EA 22.2°C [†]	1.03 b	0.29 bc	1.32 b	0.17 b	0.016 ab	0.19 b	1.50 bd
	AH 25.6°C [†]	1.04 b	0.28 c	1.32 b	0.13 ab	0.017 b	0.15 ab	1.46 ab
	EH 25.6°C [†]	1.29 c	0.37 bd	1.66 c	0.22 c	0.021b	0.24 c	1.82 d
ANOVA	CO ₂ (C)	***	***	***	***	***	***	***
	Temperature (T)	***	***	***	***	***	***	***
	Line (L)	***	***	***	***	ns	***	***
	C × T	ns	ns	ns	*	ns	*	ns
	C × L	ns	ns	ns	ns	ns	ns	ns
	T × L	*	ns	*	ns	ns	ns	ns
	C × T × L	ns	ns	ns	ns	ns	ns	ns

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test). DW: dry weight. AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature), EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature). †: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.

Table 3.4. Effects of elevated CO₂ concentration on seedling vigour

Chinese yam lines	Treatments	Vigour index I	Vigour index II
Enshikei 6	AA 22.2°C [†]	6918.8 a	88.5 a
	EA 22.2°C [†]	8004.2 b	103.2 b
	AH 25.6°C [†]	9904.2 c	132.0 c
	EH 25.6°C [†]	11397.6 d	165.0 d
Shojikei	AA 22.2°C [†]	8281.9 a	105.8 a
	EA 22.2°C [†]	9426.6 ab	139.2 b
	AH 25.6°C [†]	10590.5 b	146.4 b
	EH 25.6°C [†]	12159.5 c	182.0 c
ANOVA	CO ₂ (C)	***	***
	Temperature (T)	***	***
	Line (L)	***	***
	C × T	ns	ns
	C × L	ns	ns
	T × L	ns	ns
	C × T × L	ns	ns

Note. Vigour index I = germination % × seedling length (shoot + root), vigour index II = germination % × seedling DW (shoot + root + tuber). Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test). AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature (1.4°C above the outside temperature), AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature), EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature (5.2°C above the outside temperature). †: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.

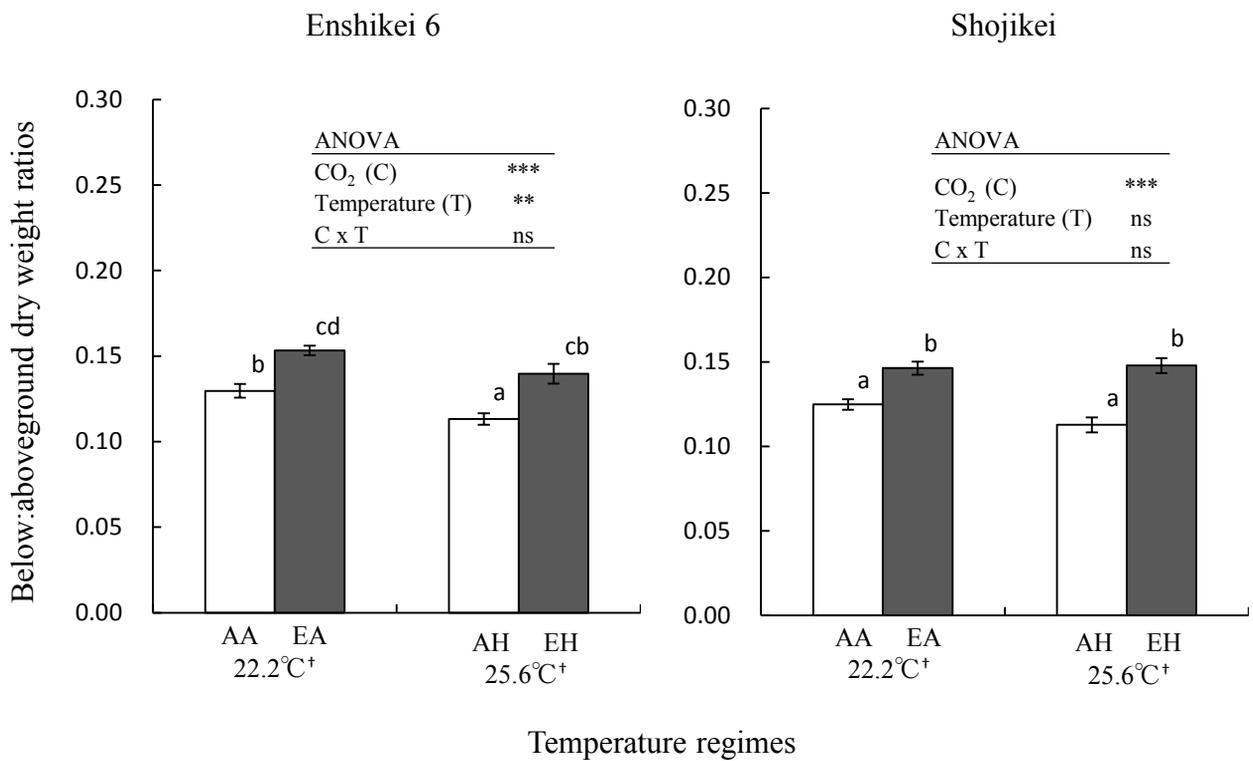


Figure 3.1. Effects of elevated CO₂ concentration on below:aboveground dry weight ratios

Note. Different letters indicate significant difference at the 5% level (Tukey–Kramer’s test).

Bars show mean \pm SE ($n=30$), AA: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and approximately ambient air temperature (1.4° C above the outside temperature), EA: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and approximately ambient air temperature (1.4° C above the outside temperature), AH: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and high air temperature (5.2° C above the outside temperature), EH: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and high air temperature (5.2° C above the outside temperature). †: mean air temperature. ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; ns, not significant.

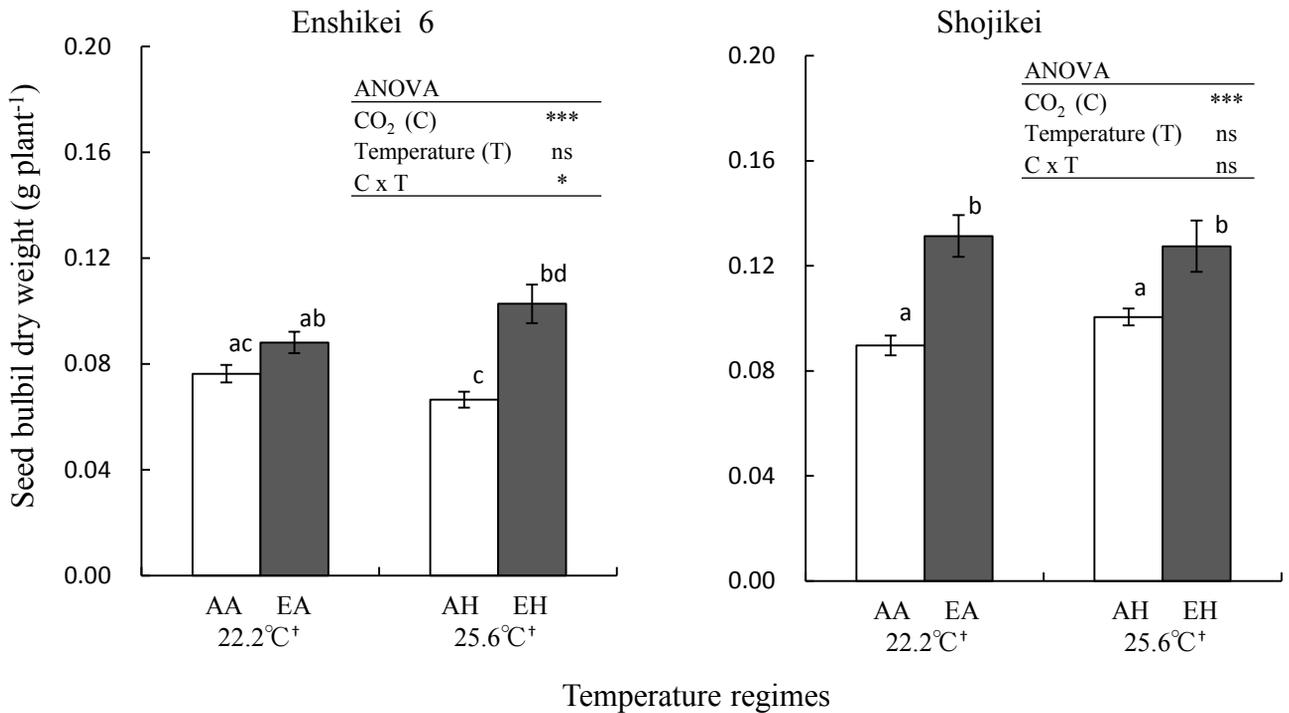


Figure 3.2. Effects of elevated CO₂ concentration on seed bulbils dry weight

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test). Bars show mean \pm SE ($n=30$), AA: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and approximately ambient air temperature (1.4° C above the outside temperature), EA: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and approximately ambient air temperature (1.4° C above the outside temperature), AH: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and high air temperature (5.2° C above the outside temperature), EH: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and high air temperature (5.2° C above the outside temperature). †: mean air temperature. ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; ns, not significant.

CHAPTER 4

Effects of elevated CO₂ concentration on morphology of leaf blades in Chinese yam

INTRODUCTION

Yams (*Dioscorea* spp.) provide a staple food for millions of people and play a role as the third most important root and tuber crop after cassava and sweet potato in West Africa, Central America, the Caribbean, the Pacific Islands and Southeast Asia (Srivastava et al., 2012). Among the various yam species, Chinese yam (*Dioscorea opposita* Thunb.) is largely cultivated in Japan, China, Korea, and Taiwan and is a very important tuber crop in the northern prefectures of Japan, such as Aomori and Hokkaido.

Since the industrial revolution period a couple of centuries ago, the atmospheric carbon dioxide concentration ([CO₂]) has increased from a stable 280 $\mu\text{mol mol}^{-1}$ before the industrial revolution to 400 $\mu\text{mol mol}^{-1}$ for the first time in March 2015, and reached 403.95 $\mu\text{mol mol}^{-1}$ in July 2017 (National Oceanic & Atmospheric Administration (NOAA), 2017). It is predicted to continue increasing in the future (Intergovernmental Panel on Climate Change (IPCC), 2013). In association with the [CO₂] increase, the global air temperature by the end of the 21st century (2081–2100) relative to 1986–2005 is predicted to rise 0.3–1.7°C under low greenhouse gas emission scenarios and 2.6–4.8°C under high-emission scenarios (IPCC, 2013) as a result of global warming. In the latest report, the Global Climate Report – August 2017 (NOAA, 2017), the global air temperature was 0.83°C above the 20th century average of 15.6°C and the third highest August global temperature in the 138-year record. Both [CO₂] and air temperature are key variables affecting plant growth, development and function.

Many studies have shown that elevated [CO₂] can accelerate the growth and

development of some plants (Cheng et al., 2009; Cruz et al., 2014; Koti et al., 2006; Krishnan et al., 2007; Ottman et al., 2001; Roy et al., 2012; Shimono et al., 2008; Zhang et al., 2012), increase the rate of photosynthesis (Aien et al., 2014; Koti et al., 2006; Li et al., 2008; Sekhar et al., 2015; Shimono et al., 2008) and/or decrease the mineral nutrient concentration (Broberg et al., 2017; Luomala et al., 2005; Riikonen et al., 2005; Teng et al., 2006). However, few studies have considered the effects of elevated [CO₂] on leaf ultrastructure, especially the photosynthetic apparatus (Hao et al., 2013; Radoglou & Jarvis, 1992; Teng et al., 2006; Wang et al., 2004).

In the case of Chinese yam, the studies in chapter 2 and 3 on the effects of elevated [CO₂] on growth and photosynthesis showed that Chinese yam responds positively to elevated [CO₂] both at the early growth stage (chapter 3) and at the intermediary vegetative stage (chapter 2). Yam leaf number, leaf area, leaf dry weight (DW) and total plant DW were higher under elevated [CO₂] than under ambient [CO₂]. The photosynthetic rate was also enhanced by elevated [CO₂]. However, no possible mechanism was proposed to explain how yams positively respond to elevated [CO₂] and whether the yam response is related to leaf structure changes under elevated [CO₂]. These aspects are very important for an integrated understanding of the mechanism by which yams show a positive response to elevated [CO₂].

In this study, we hypothesized that elevated [CO₂] affects the leaf internal structure, size and number of stomata and chloroplasts. To test this hypothesis, we observed the morphology of yam leaves collected from two dominant Chinese lines grown under two [CO₂] conditions (ambient and elevated). To our knowledge, this is the first study to investigate yam leaf structure changes in response to elevated [CO₂].

MATERIALS AND METHODS

1. Plant materials and growth conditions

We conducted our experiments in temperature-gradient chambers at the Tohoku Agricultural Research Center, NARO (39°74'N, 141°13'E) in Morioka, Japan. The details of the cultivation method are described in chapter 3. In short, we used two dominant lines of Chinese yam in north Japan: Enshikei 6 and Shojikei. For each line, seed bulbils were sown in plastic pots on 4 June 2016 and then placed immediately in the temperature-gradient chambers for treatment until 9 July 2016. Two temperature-gradient chambers were used under two [CO₂] conditions: ambient and elevated (ambient [CO₂] + 200 μmol mol⁻¹ in daytime (0400 to 2030)). In each temperature-gradient chamber, two treatment plots were set along an air temperature gradient. The first plot had a mean air temperature of 22.2 ± 1.7°C and the second plot had a mean air temperature of 25.6°C ± 1.7°C. The average daytime (0400 to 2030) [CO₂] over the treatment period was 406 ± 9 μmol mol⁻¹ in the ambient [CO₂] chamber and 603 ± 22 μmol mol⁻¹ in the elevated [CO₂] chamber. The relative humidity was about 58.3% and 39.2% for the approximately ambient temperature plots and the high temperature plots, respectively, in the chambers. In this study, we set up the treatments as below because the difference (19%) of relative humidity between the ambient-temperature plots and high-temperature plots was small and plants are generally more sensitive to air temperature changes than relative humidity changes. Air temperature, relative humidity and [CO₂] were measured at 5-s intervals and averaged every 1 min, 30 min and 24 h by a datalogger (CR 1000; Campbell Sci. Inc., Logan, UT, USA).

Both Enshikei 6 and Shojikei were treated in the same manner in the temperature-gradient chambers. The four treatments were abbreviated as follows: (1) AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature.

- (2) EA: elevated $[\text{CO}_2]$ ($603 \mu\text{mol mol}^{-1}$) and approximately ambient air temperature.
- (3) AH: ambient $[\text{CO}_2]$ ($406 \mu\text{mol mol}^{-1}$) and high air temperature.
- (4) EH: elevated $[\text{CO}_2]$ ($603 \mu\text{mol mol}^{-1}$) and high air temperature.

2. Observation of leaf blade tissues

Leaf blade tissues were observed by bright-field optical microscopy as follows. Fully expanded leaves on the middle vines of the yam plants in each treatment were collected on 9 July 2016. Segments of the center parts of the leaf blades were immediately immersed in .05 M sodium phosphate buffer (pH 7.0) containing 2% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde. Then, the samples were washed in .05 M sodium phosphate buffer. The materials were post-fixed in 1% (v/v) osmium tetroxide in .1 M sodium phosphate buffer at 4°C for 10 h and washed in .1 M sodium phosphate buffer and distilled water. After being dehydrated in a graded ethanol series and immersed in propylene oxide, the segments of the leaf blades were embedded in Spurr's resin at 70°C for 24 h. Then, the samples were cut with a glass knife into .7 μm -thick sections using an ultramicrotome (Leica EM UC7, Nussloch, Germany) and stained with .1% (w/v) toluidine blue-O. The sections were observed under a bright-field optical microscope (BX51; Olympus) and photographed.

3. Measurement of leaf blade thickness and counting of chloroplast numbers

From the photographs taken with the bright-field optical microscope, the thickness of the upper (adaxial) epidermis, the thickness of the palisade parenchyma, the thickness of the spongy parenchyma, the thickness of the lower (abaxial) epidermis and the total leaf thickness were determined using the Image J software. The numbers of chloroplasts per palisade cell and per spongy cell in the yam leaves were also counted using the images. Three photographs per leaf blade,

one leaf blade per plant and three to five plants per treatment were used to investigate these parameters. Ten cells from each photograph were randomly selected to determine the number of chloroplasts.

4. Observation of chloroplasts

Chloroplasts were observed by transmission electron microscopy. First, samples were fixed as described above. After being embedded in Spurr's resin at 70°C for 24 h, the samples were cut with a diamond knife into 80 nm-thick sections using the same ultramicrotome. These ultrathin sections were picked up on a 200-mesh copper grid. The grid-mounted sections were stained with aqueous uranyl acetate (4%) for 20 min and then washed in distilled water. The samples were put into lead staining solution (Sigma-Aldrich Co. LLC) for 5 min and washed in distilled water. The stained sections were observed and photographed with a transmission electron microscope (JEOL-JEM 1230, Japan) at 80 kV.

5. Counting of starch grains and measurement of chloroplast profile area and starch grain profile area

Based on the images taken by transmission electron microscopy as mentioned above, the number of starch grains per chloroplast was counted. Additionally, the chloroplast profile area and starch grain area in the palisade and spongy cells were determined using the Image J software. Six to ten chloroplasts per photograph, four to six photographs per leaf blade, one leaf blade per plant and three plants per treatment were investigated.

6. Observation of stomata and measurement of stomatal size and density

Stomata were observed by scanning electron microscopy as follows. Samples were fixed as described above: segments of leaf blades were fixed immediately in .05 M sodium phosphate buffer (pH 7.0) containing 2% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde, and then post-fixed in 1% (v/v) osmium tetroxide

in .1 M sodium phosphate buffer at 4°C for 12 h and washed in .1 M sodium phosphate buffer. The samples were dehydrated in a graded ethanol series, and then immersed in 100% isoamyl acetate at 20°C and dried using a critical point dryer (JCPD-5; JEOL). The dried leaf blade samples were mounted on stubs with conductive carbon tape and coated with platinum using an auto fine coater (JFC-1600; JEOL). The stomata were observed and photographed using a scanning electron microscope (SEM, JSM-7000F; JOEL) at an accelerating voltage of 5 kV. The number of stomata per area unit (mm²) was determined and the stomatal pore length and width were measured using the Image J software. All the stomata in each photograph at a magnification of ×100 were counted for density and four to five stomata per photograph at a magnification of × 500 were measured for size; three photographs per leaf blade, one leaf blade per plant and five plants per treatment were observed.

7. Statistical analysis

To test the significance of differences related to [CO₂] levels, air temperature conditions, Chinese yam lines and their interactions, we applied three-way analysis of variance (ANOVA). When ANOVA produced a significant result, we performed a Tukey–Kramer’s test for significant differences between means. All statistical analyses were performed with the SPSS statistical software (SPSS ver. 24.0; IBM Corp, New York, NY, USA).

RESULTS

1. Effects of elevated CO₂ concentration on internal leaf tissues in Chinese yam

Differences in cell size and number of chloroplasts in the yam leaves between the elevated [CO₂] treatment (Figure 4.1 B, D, F, H) and ambient [CO₂] treatment (Figure 4.1 A, C, E, G) were observed for both Enshikei 6 and Shojikei.

According to a Tukey–Kramer’s test (Table 4.1), for both Enshikei 6 and

Shojikei, the thickness of the palisade layer in the yam leaf blade was significantly higher in EA and EH than in AA and AH, respectively, and the thickness of the whole leaf blade was significantly higher in EA than in AA. Although no significant differences were observed, there was an increasing trend in leaf thickness from AH to EH for both Enshikei 6 and Shojikei. There were no significant differences in the thickness of the adaxial epidermis layer, spongy layer or abaxial epidermis layer between ambient [CO₂] and elevated [CO₂] under either temperature regime.

According to the ANOVA results (Table 4.1), elevated [CO₂] had significant effects on the thickness of the palisade layer, abaxial epidermis layer and whole leaf blade in Chinese yam. In addition, significant effects of air temperature on the thickness of the abaxial epidermis layer and of yam line on the adaxial epidermis thickness and whole leaf blade thickness were detected. There were interactions between air temperature and yam line for adaxial epidermis layer thickness, palisade layer thickness, and spongy layer thickness, and among [CO₂], air temperature and yam line for palisade layer thickness.

2. Effects of elevated CO₂ concentration on chloroplast numbers in Chinese yam leaves

In the Tukey–Kramer’s test (Figure 4.2), the number of chloroplasts per palisade cell in the leaf blades was significantly higher in EA and EH than in AA and AH, respectively, in both Enshikei and Shojikei. In addition, the number of chloroplasts per spongy cell in the leaf blades was higher in EA than in AA for Enshikei 6, and higher in EA and EH than in AA and AH, respectively, for Shojikei. The ANOVA (Figure 4.2) revealed that elevated [CO₂] had significant effects on the numbers of chloroplasts per palisade cell and per spongy cell for both Enshikei 6 and Shojikei. There were no interactions between [CO₂] and air temperature for either yam line.

3. Effects of elevated CO₂ concentration on starch accumulation in chloroplasts in Chinese yam leaves

Differences in the size of starch grains accumulated in the chloroplasts in yam leaves between plants grown under elevated [CO₂] (Figure 4.3 B, D, F and H) and ambient [CO₂] treatment (Figure 4.3 A, C, E and G) were observed in the palisade parenchyma cells for both Enshikei 6 and Shojikei. The starch grains accumulated in spongy cells also showed size differences (no images are shown).

A Tukey–Kramer’s test (Table 4.2) showed that, for both Enshikei 6 and Shojikei, the number of starch grains per chloroplast, starch grain profile area and ratio of starch to chloroplast area in palisade and spongy cells were significantly higher in EA and EH than in AA and AH, respectively. There were no differences in chloroplast profile area in palisade or spongy cells between the ambient [CO₂] treatment and elevated [CO₂] treatment under either air temperature regime.

According to the ANOVA results (Table 4.2), elevated [CO₂] significantly affected the number of starch grains per chloroplast, starch grain profile area and ratio of starch to chloroplast area in both palisade and spongy cells, while air temperature affected the number of starch grains per chloroplast and the ratio of starch to chloroplast area in spongy cells. There were also significant differences in the chloroplast profile area in spongy cells, the starch profile area in palisade cells and the ratio of starch to chloroplast area in palisade cells according to yam line. There were no interactions between [CO₂] and air temperature, [CO₂] and yam line, and air temperature and yam line for any of these parameters except for a [CO₂] × air temperature interaction and a [CO₂] × air temperature × yam line interaction for the number of starch grains per chloroplast.

4. Effects of elevated CO₂ concentration on the density and size of stomata in Chinese yam leaves

Differences in the stomatal density and size on abaxial side of leaf blade in

Chinese yam between plants grown under elevated [CO₂] (Figure 4.4 B, D, F and H) and ambient [CO₂] treatment (Figure 4.4 A, C, E and G) were observed for Enshikei 6 and Shojikei.

The effect of elevated [CO₂] on stomatal density and size was investigated by scanning electron microscopy (Figure 4.4). According to a Tukey–Kramer’s test (Table 4.3), the stomatal density in the leaf blade was clearly higher in EA and EH than in AA and AH, respectively, and stomatal pore length was higher in EA than in AA for both Enshikei 6 and Shojikei. There was no significant difference in stomatal pore length between ambient [CO₂] and elevated [CO₂] under the high temperature regime or in stomatal pore width under either temperature regime.

According to the ANOVA results (Table 4.3), elevated [CO₂] significantly affected the stomatal density and stomatal pore length, while air temperature affected the stomatal width; yam line affected both the stomatal length and width. No interactions among [CO₂], air temperature and yam line for these factors were found except an interaction between [CO₂] and air temperature for stomatal pore length.

DISCUSSION

Leaf thickness is a very important morphological parameter because it has a direct bearing on photosynthesis and water use efficiency (Ashton & Berlyn, 1994; Murthy & Dougherty, 1997). Chinese yam plants exhibited a significant increase in total leaf blade thickness under elevated [CO₂] in this study (Figure 4.1, Table 4.1). This is consistent with previous studies on various crops such as soybean, loblolly pine, and sweet gum (Thomas & Harvey, 1983), *Arabidopsis thaliana* (Teng et al., 2006), *Glycine max* (Vu et al., 1989), and *Phaseolus vulgaris* (Bray & Reid, 2002). However, the causes of increased leaf thickness are not totally consistent. Radoglou and Jarvis (1992) found that the increase of *P. vulgaris* leaf thickness was mainly due to an increase in the spongy parenchyma thickness. Sun

et al. (2011) stated that the increased thickness of potato leaves largely resulted from increases of the thickness of the palisade and spongy tissues, but the rate of spongy tissue thickening was higher than that of the palisade tissue. Vu et al. (1989) reported that elevated [CO₂] caused an increase in soybean leaf thickness due to an increased number of palisade cells. In the case of Chinese yam, we found that the palisade layer thickness of Chinese yam leaf blades was significantly greater under elevated [CO₂] than under ambient [CO₂], and thus the total leaf blade thickness was also noticeably higher in yams grown at elevated [CO₂] than at ambient [CO₂] under the approximately ambient temperature regime. Our results indicate that elevated [CO₂] increased the yam leaf blade thickness by increasing the cell size of palisade tissues.

The chloroplast is the primary photosynthetic organelle in plants (Sharma et al., 2014) and its development influences plant development (Pogson et al., 2015). Thus, the effects of elevated [CO₂] on chloroplasts have been more extensively studied than any other organelle. Teng et al. (2006) reported that the number of chloroplasts per mesophyll cell was significantly higher (17.9%) in the leaves of *A. thaliana* plants grown under elevated [CO₂] than under ambient [CO₂]. Wang et al. (2004) reported a 71% increase in chloroplast number per unit cell area in *Nicotiana sylvestris* leaves under elevated [CO₂] of 730 ppm in a growth chamber. Bockers et al. (1997) also reported that elevated [CO₂] increased the number of chloroplasts in *Marchantia polymorpha*. Similarly, Thomas and Harvey (1983) showed an increase in chloroplast density at high [CO₂] in the leaves of *Zea mays*, *G. max* and *Liquidambar styraciflua*. In Chinese yam, we found that the numbers of chloroplasts per cell in both the palisade and spongy layers were significantly higher in yams grown under elevated [CO₂] compared with ambient [CO₂] (Figure 2). In our previous study (chapter 2), we found that the net photosynthetic rate was higher in Chinese yams grown under elevated [CO₂] than under ambient

[CO₂]. These results suggest that an increase in the number of chloroplasts leads to enhanced photosynthesis under elevated [CO₂].

Some studies on starch grain accumulation in chloroplasts have shown an increase in the number of starch grains per chloroplast and the area per starch grain under elevated [CO₂] (Hao et al., 2013; Kumar et al., 2013; Oksanen et al., 2001; Sun et al., 2011; Teng et al., 2006; Vu et al., 1989; Zhang et al., 2012). For example, Teng et al. (2006) found that the average size and number of starch grains in *A. thaliana* leaf chloroplasts were increased under elevated [CO₂], and that the starch grains in leaves occupied 34.4% of the chloroplast profile under elevated [CO₂], which was significantly higher than under ambient [CO₂]. Additionally, Wang et al. (2004) reported that starch grains occupied as much as 67% of the chloroplast profile under elevated [CO₂] in *N. sylvestris* leaves. Similarly, Hao et al. (2013) observed increases in the number and size of starch grains in chloroplasts in *Isatis indigotica* leaves at elevated [CO₂] compared with ambient [CO₂]. In this study, we observed an increase in the number of starch grains per chloroplast in both palisade and spongy cells, and that the starch profile area was significantly greater in yam leaf chloroplasts at elevated [CO₂] than at ambient [CO₂] (Table 4.2). These results suggest that increased starch grain accumulation in the chloroplast may lead to an increase of leaf photosynthesis under elevated [CO₂] by altering the shape of chloroplasts.

Stomata are the pivotal doors that control the flow of gases between vegetation and the atmosphere (Xu et al., 2016). Therefore, there are several published papers reporting stomatal responses to elevated [CO₂]. Most previous studies on a variety of species such as Scots pine (Lin et al., 2001), *A. thaliana* (Teng et al., 2006), *Lycopersicon esculentum* (Madsen, 1973) and *Olea europaea* (Beerling and Chaloner, 1993) showed a decrease in stomatal density in plants grown under elevated [CO₂]. Woodward (1987) also reported a dramatic (67%)

decrease in stomatal density in the leaves of herbarium specimens as [CO₂] increased from the pre-industrial level of 280 ppm to the ambient level of 340 ppm in 1987. Similarly, Woodward and Kelly (1995) investigated stomatal density in as many as 100 plant species and found a reduction of 14.3% due to elevated [CO₂]. Ainsworth and Rogers (2007) reported a 5% decrease in stomatal density due to elevated [CO₂] from a meta-analysis on stomatal responses. Relatively few studies—on poplar clones (Ceulemans et al., 1995; Tricker et al., 2005) and *Alnus glutinosa* (Poole et al., 2000)—have reported no changes in stomatal density with elevated [CO₂]. However, in this study we found that the stomatal density on the abaxial side of leaves in Chinese yam was significantly higher at elevated [CO₂] than at ambient [CO₂] (Table 4.3, Figure 4.4). This result is consistent with the results of Reid et al. (2003), who observed a higher stomatal density in 15 species exposed to elevated [CO₂] for four years in free air CO₂ enrichment experiments.

The data gathered in this study provide important information and a possible explanation of the mechanism by which Chinese yams responded positively to elevated [CO₂] and yam photosynthesis was enhanced at elevated [CO₂] in chapter 2 and 3.

Table 4.1. Effect of elevated CO₂ concentration on the thickness of the adaxial epidermis, palisade layer, spongy layer, abaxial epidermis and whole leaf blade

Chinese yam lines	Treatments	Adaxial epidermis (μm)	Palisade (μm)	Spongy (μm)	Abaxial epidermis (μm)	Whole leaf blade (μm)
Enshikei 6						
	AA 22.2°C [†]	33.1 a	73.4 a	115.7 a	14.9 a	240.4 a
	EA 22.2°C [†]	34.9 ab	83.9 b	120.3 a	15.7 ab	261.7 b
	AH 25.6°C [†]	38.1 b	77.5 ab	112.9 a	16.9 ab	248.1 ab
	EH 25.6°C [†]	38.1 b	96.3 c	113.3 a	17.0 b	255.7 ab
Shojikei						
	AA 22.2°C [†]	33.8 a	73.4 a	107.6 a	14.8 a	231.4 a
	EA 22.2°C [†]	35.9 a	90.7 c	113.6 a	16.0 a	248.4 b
	AH 25.6°C [†]	32.5 a	75.9 a	111.5 a	15.1 a	241.8 ab
	EH 25.6°C [†]	34.3 a	82.9 b	116.8 a	16.3 a	247.5 ab
ANOVA						
	CO ₂ (C)	ns	***	ns	*	***
	Temperature (T)	ns	ns	ns	**	ns
	Lines (L)	*	ns	ns	ns	**
	C × T	ns	ns	ns	ns	ns
	C × L	ns	ns	ns	ns	ns
	T × L	**	**	*	ns	ns
	C × T × L	ns	**	ns	ns	ns

Note. Different letters indicate significant difference at the 5% level (Tukey–Kramer’s test). AA: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and approximately ambient air temperature, EA: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and approximately ambient air temperature, AH: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and high air temperature, EH: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and high air temperature. †: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.

Table 4.2. Effect of elevated CO₂ concentration on the inner structure of chloroplasts

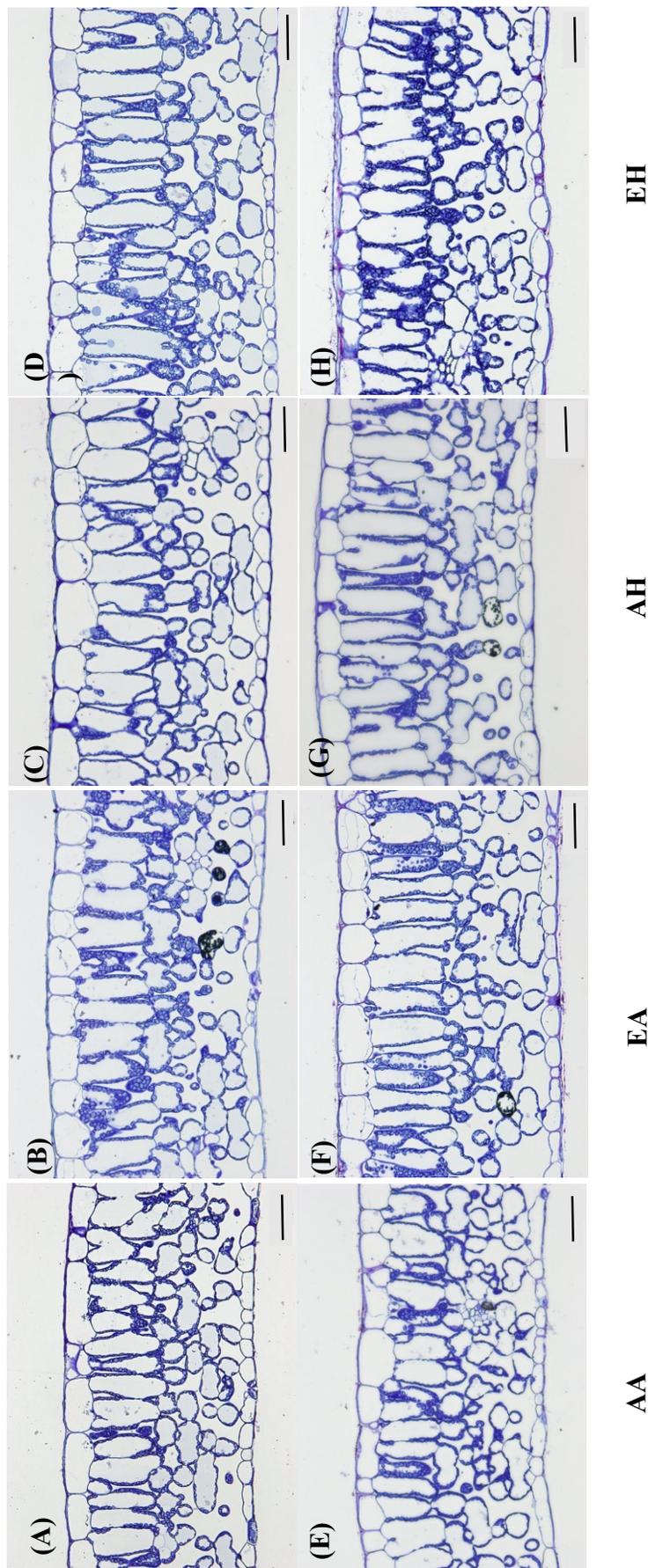
Yam lines	Treatments	Number of starch grain (Chloroplast ⁻¹)	Chloroplast profile area (μm ²)		Starch profile area (μm ²)		Starch to Chloroplast area ratio		
			Palisade	Spongy	Palisade	Spongy	Palisade	Spongy	
Enshikei 6									
	AA	22.2°C [†]	0.9 a	14.2 a	13.9 a	3.9 a	3.2 a	27.4 a	22.4 a
	EA	22.2°C [†]	1.5 c	15.1 a	14.4 a	7.7 b	7.2 b	47.0 b	43.2 b
	AH	25.6°C [†]	1.0 a	14.2 a	13.8 a	3.7 a	3.6 a	26.6 a	29.5 a
	EH	25.6°C [†]	1.2 b	15.9 a	14.7 a	6.7 b	6.3 b	45.1 b	43.9 b
Shojikei									
	AA	22.2°C [†]	1.1 b	12.3 a	11.1 a	3.1 a	3.5 a	22.7 a	24.2 a
	EA	22.2°C [†]	1.4 c	12.9 a	12.9 a	5.1 bc	5.8 b	41.9 b	41.6 b
	AH	25.6°C [†]	0.8 a	13.8 a	12.1 a	3.7 ac	3.4 a	26.9 a	27.5 a
	EH	25.6°C [†]	1.2 cb	13.9 a	13.3 a	5.9 b	5.9 b	40.6 b	45.4 b
ANOVA									
	CO ₂ (C)		***	ns	ns	***	***	***	***
	Temperature (T)		***	ns	ns	ns	ns	ns	*
	Lines (L)		ns	ns	*	**	ns	**	ns
	C × T		*	ns	ns	ns	ns	ns	ns
	C × L		ns	ns	ns	ns	ns	ns	ns
	T × L		ns	ns	ns	ns	ns	ns	ns
	C × T × L		***	ns	ns	ns	ns	ns	ns

Note. Different letters indicate significant difference at the 5% level (Tukey–Kramer’s test). AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature, EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature, AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature, EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature. †: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.

Table 4.3. Effects of elevated CO₂ concentration on stomatal density and size

Chinese yam lines	Treatments	Stomatal density (mm ⁻²)	Stomatal pore length (μm)	Stomatal pore width (μm)
Enshikei 6				
	AA 22.2°C [†]	73.5 a	11.1 a	1.9 a
	EA 22.2°C [†]	85.7 bc	13.2 b	2.1 a
	AH 25.6°C [†]	76.6 ab	11.5 ab	2.2 a
	EH 25.6°C [†]	89.3 c	12.5 ab	2.3 a
Shojikei				
	AA 22.2°C [†]	74.9 a	11.6 a	2.2 a
	EA 22.2°C [†]	92.6 b	13.7 b	2.3 a
	AH 25.6°C [†]	75.1 a	13.1 ab	2.4 ab
	EH 25.6°C [†]	88.8 b	13.2 ab	2.8 b
ANOVA				
	CO ₂ (C)	***	***	ns
	Temperature (T)	ns	ns	*
	Lines (L)	ns	*	**
	C × T	ns	*	ns
	C × L	ns	ns	ns
	T × L	ns	ns	ns
	C × T × L	ns	ns	ns

Note. Different letters indicate significant difference at the 5% level (Tukey–Kramer’s test). AA: ambient [CO₂] (406 μmol mol⁻¹) and approximately ambient air temperature, EA: elevated [CO₂] (603 μmol mol⁻¹) and approximately ambient air temperature, AH: ambient [CO₂] (406 μmol mol⁻¹) and high air temperature, EH: elevated [CO₂] (603 μmol mol⁻¹) and high air temperature. †: mean air temperature. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns, not significant.



Enshikei 6

44 Shojikei

Figure 4.1: Optical micrographs of transverse sections of leaf blades showing the effects of elevated CO₂ concentration on the inner structures.

Note. Chinese yam A–D (for Enshikei 6) and E–H (for Shojikei) respectively grown under AA, EA, AH, EH treatments. AA: ambient [CO₂] (406 μmol mol⁻¹) and approximate ambient-temperature (1.4° C above the outside-temperature), EA: elevated [CO₂] (603 μmol mol⁻¹) and approximate ambient-temperature (1.4° C above the outside-temperature), AH: ambient [CO₂] (406 μmol mol⁻¹) and high temperature (5.2° C above the outside-temperature), EH: elevated [CO₂] (603 μmol mol⁻¹) and high temperature (5.2° C above the outside-temperature). Bars = 50 μm;

Enshikei 6

Shojikei

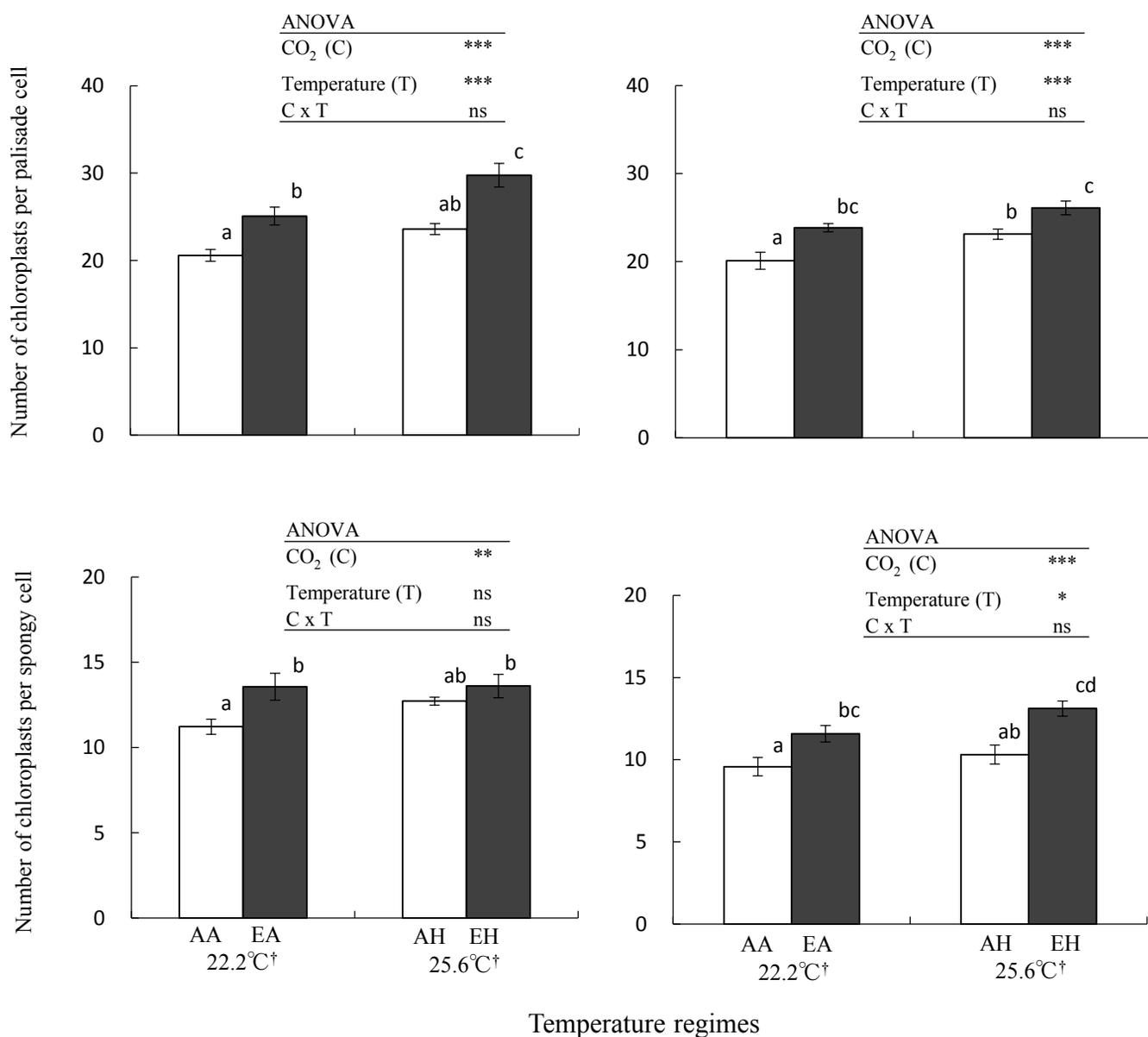


Figure 4.2. Effect of elevated CO₂ concentration on number of chloroplasts per palisade cell and spongy cell

Note. Different letters indicate significant differences at the 5% level (Tukey–Kramer’s test). Bars show mean \pm SE ($n=90$), AA: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and approximate ambient-temperature (1.4° C above the outside-temperature), EA: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and approximate ambient-temperature (1.4° C above the outside-temperature), AH: ambient [CO₂] (406 $\mu\text{mol mol}^{-1}$) and high temperature (5.2° C above the outside-temperature), EH: elevated [CO₂] (603 $\mu\text{mol mol}^{-1}$) and high temperature (5.2° C above the outside-temperature). †: mean air temperature. ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; ns, not significant.

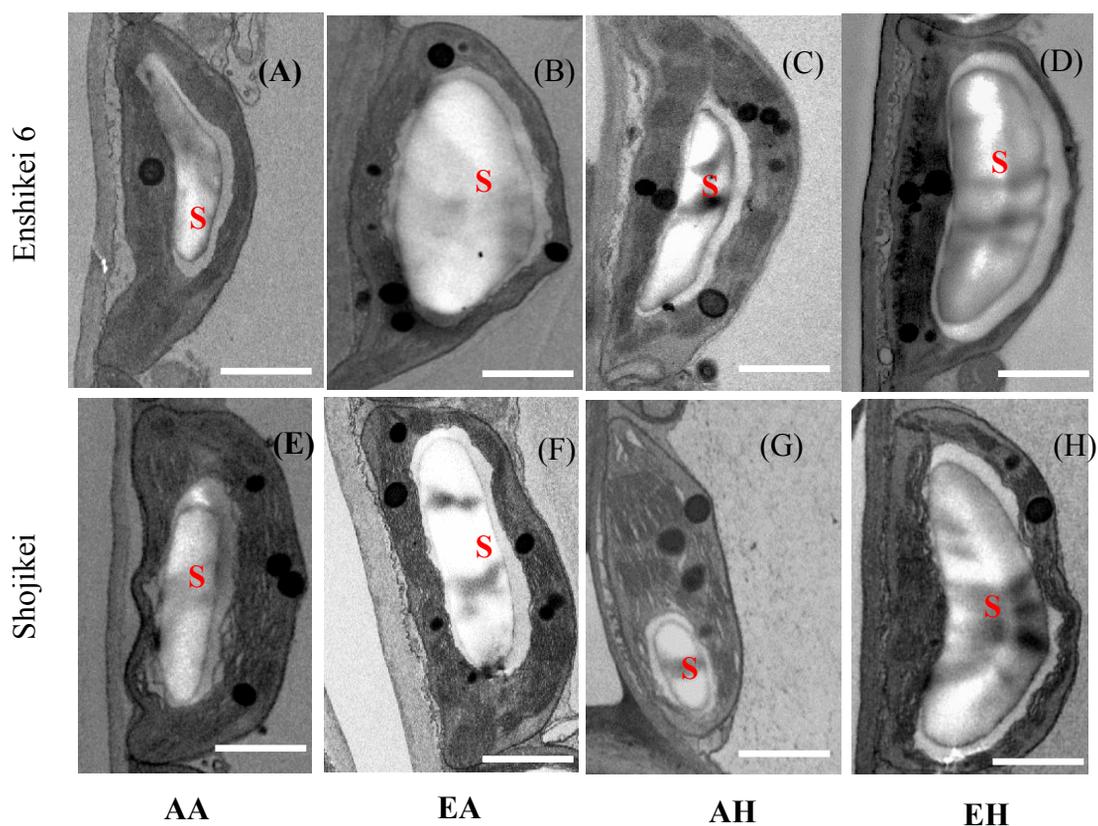
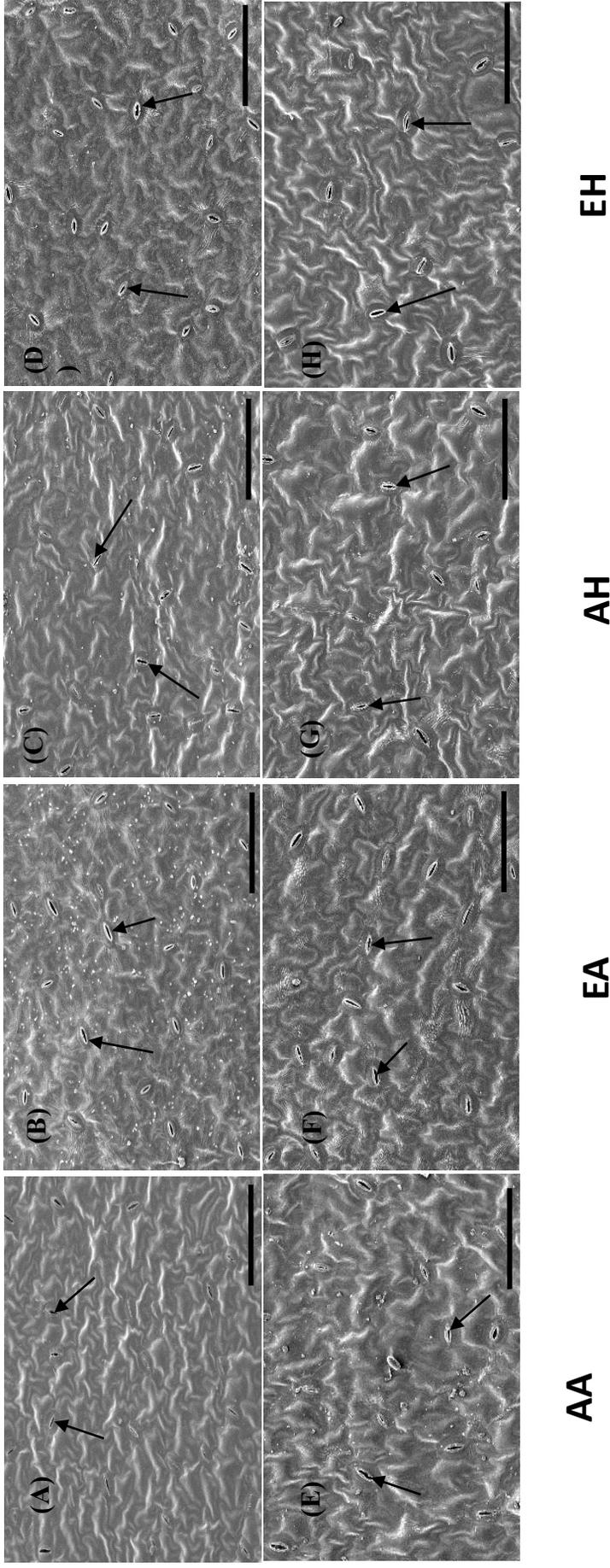


Figure 4.3. Transmission electron micrographs of transverse sections of chloroplast showing the effects of elevated CO_2 concentration on chloroplasts and starch grain in palisade parenchyma cell

Note. Chinese yam A–D (for Enshikei 6) and E–H (for Shojikei) respectively grown under AA, EA, AH, EH treatments. AA: ambient $[\text{CO}_2]$ ($406 \mu\text{mol mol}^{-1}$) and approximate ambient-temperature (1.4°C above the outside-temperature), EA: elevated $[\text{CO}_2]$ ($603 \mu\text{mol mol}^{-1}$) and approximate ambient-temperature (1.4°C above the outside-temperature), AH: ambient $[\text{CO}_2]$ ($406 \mu\text{mol mol}^{-1}$) and high temperature (5.2°C above the outside-temperature), EH: elevated $[\text{CO}_2]$ ($603 \mu\text{mol mol}^{-1}$) and high temperature (5.2°C above the outside-temperature). S: starch grain. Bars = $2 \mu\text{m}$.



Enshikei 6

Shojikei

Figure 4.4. Scanning electron micrographs showing effect of elevated CO_2 concentration on stomatal density

Note. Chinese yam A–D (for Enshikei 6) and E–H (for Shojikei) respectively grown under AA, EA, AH, EH treatments. AA: ambient $[\text{CO}_2]$ ($406 \mu\text{mol mol}^{-1}$) and approximate ambient-temperature (1.4°C above the outside-temperature), EA: elevated $[\text{CO}_2]$ ($603 \mu\text{mol mol}^{-1}$) and approximate ambient-temperature (1.4°C above the outside-temperature), AH: ambient $[\text{CO}_2]$ ($406 \mu\text{mol mol}^{-1}$) and high temperature (5.2°C above the outside-temperature), EH: elevated $[\text{CO}_2]$ ($603 \mu\text{mol mol}^{-1}$) and high temperature (5.2°C above the outside-temperature). Bars = $100 \mu\text{m}$. Arrow(\rightarrow): stomata

CHAPTER 5

General discussion

While many researchers reported the effects of elevated [CO₂] on other plants such as maize (Oseni & Masarirambi, 2011), rice (*Oryza sativa* L.) (Cheng et al., 2009; Roy et al., 2012), wheat (Nonhebel, 1993; Valizadeh et al., 2014), and sorghum (Prasad et al., 2006; Wall et al., 2001), no information related to the effects of elevated [CO₂] on yams has been presented. In the early seedling growth stage (Chapter 3), for Enshikei 6, leaf number, leaf area and root length was higher in elevated [CO₂] than in ambient [CO₂] under high temperature regime; shoot length were greater in elevated [CO₂] than in ambient [CO₂] under both approximately ambient temperature and temperature regimes (Table 3.2). For Shojikei, shoot length was also greater in elevated [CO₂] than in ambient [CO₂] under both temperature regimes, (Table 3.2). In addition, for both Chinese yam lines, leaf DW, vine DW, shoot DW, root DW and below ground DW were significantly higher in elevated [CO₂] than in ambient [CO₂] under both temperature regimes. Tuber DW was also higher in elevated [CO₂] than in ambient [CO₂] under high temperature regime for Enshikei 6. Consequently, total plant DW was clearly higher in elevated [CO₂] than in ambient [CO₂] under both approximately ambient temperature regime and high temperature regime for both Chinese yam lines (Table 3.3). The above:belowground DW ratios were lower in in elevated [CO₂] than in ambient [CO₂] under both temperature regimes (Figure 3.1). These results indicate that Chinese yam respond positively to elevated [CO₂] at the early seedling stage. In the intermediate growth stage (Chapter 2), number of leaves in Chinese yam was higher in elevated [CO₂] than in ambient [CO₂] under approximately ambient temperature regime; vine length and leaf area were also significantly higher in elevated [CO₂] than in ambient [CO₂] under both

approximately ambient temperature regime and high temperature regime in summer experiment (Table 2.1). In the other hand, vine DW and root DW of Chinese yam in summer experiment was higher in elevated [CO₂] than in ambient [CO₂] under approximately ambient temperature regime; yam leaf DW was higher in elevated [CO₂] than in ambient [CO₂] under both temperature regimes in summer experiment (Table 2.2). Chinese yam tuber DW was greater in elevated [CO₂] than in ambient [CO₂] under high temperature regime in autumn experiment (Table 2.2). Total DW was significantly higher in elevated [CO₂] than in ambient [CO₂] under both temperature regimes in summer experiment (Figure 2.1). These results demonstrate that Chinese yam shows positive growth responses to elevated [CO₂] at the intermediate growth stage. In chapter 2 and 3 of this study, our results show that Chinese yam has positive responses to elevated [CO₂] from early seedling stage to intermediate growth stage.

As presented above, elevated [CO₂] increased DW of individual parts of Chinese yam and total plant DW. Understanding the effect of elevated [CO₂] on photosynthesis process and the mechanism by which photosynthetic response to elevated [CO₂] occurred in Chinese yam is very important. As we know, leaf is the organ in which photosynthesis process happens. As mentioned above, the number of leaves and leaf area in Chinese yam were higher in elevated [CO₂] than in ambient [CO₂] (Table 2.1 and Table 3.2). In chapter 4, it was observed that the thickness of palisade cell in leaf blades of Chinese yam was also significantly greater in elevated [CO₂] than in ambient [CO₂] (Figure 4.1; Table 4.1). In addition, the whole leaf blade was thicker in elevated [CO₂] than in ambient [CO₂] under approximately ambient temperature regime (Table 4.1). In the other hand, by investigating the internal of leaf blade structure, I found that the number of chloroplasts both per palisade cell and per spongy cell in leaf blades in Chinese yam were greater in elevated [CO₂] than in ambient [CO₂] (Figure 4.2). Inside a

chloroplast, the number of starch grains was also higher in elevated [CO₂] than in ambient [CO₂]; and starch profile area at both palisade cells and spongy cells was greater in elevated [CO₂] than in ambient [CO₂], although the chloroplast profile area was not changed (Table 4.2). Consequently, starch-to-chloroplast area ratios were clearly higher in elevated [CO₂] than in ambient [CO₂] under both approximately ambient temperature and high temperature regimes (Table 4.2). More over, in abaxial side of leaf blades in Chinese yam, the stomatal density was greater in elevated [CO₂] than in ambient [CO₂] under both temperature regimes; stomatal pore length was also higher in elevated [CO₂] than in ambient [CO₂] under approximately ambient temperature regimes (Table 4.3). All these results show that elevated [CO₂] enhanced photosynthesis process and as our findings in chapter 2, the net photosynthetic rate was higher (from 39% to 73% in summer and from 36% to 47% in autumn) in elevated [CO₂] than in ambient [CO₂] (Figure 2.2). The increase in photosynthesis is considered as one of the possible explanations for increase in DW under elevated [CO₂] in Chinese yam, especially, increase in underground DW and tuber DW. It is really important because Chinese yam is a tuber crop. Some studies have shown that plant photosynthetic responses to elevated [CO₂] may vary depending on the sink–source balance (Ainsworth et al., 2004; Shimono et al., 2010; Usuda & Shimogawara, 1998; Chen & Setter, 2012). In this study (Chapter 2), Chinese yam and rice were grown at the same experimental conditions. Chinese yam is a root and tuber crop whose tubers have a large sink capacity, whereas the sink capacity of rice is small during the vegetative phase. The results of our research indicate that the effect of elevated [CO₂] on photosynthetic rate is greater in Chinese yam than in rice (Figure 2.2). Hence, our results are consistent with the source–sink hypothesis.

The response of seed germination to elevated [CO₂] in previous studies has varied: decreased (Aldalo et al., 1996; Ziska and Bunce, 1993; Saha et al., 2015)

or increased (Wulff & Alexander, 1985; Ziska & Bunce, 1993) or no affected (Thomas et al., 2009; Chen et al., 2015) depending on the plant species studied. In case of Chinese yam, bulbils are used as a means of vegetative reproduction. Bulbils and seeds were similar in terms of their dormancy and germination characteristics (Okagami, 1986). To test the effects of elevated [CO₂] on Chinese yam germination of seed bulbil, two Chinese yam lines – Enshikei 6 and Shojikei, were grown under two [CO₂] conditions (ambient and elevated) in this study (chapter 3). The results indicated that elevated [CO₂] did not affect the bulbil germination rates of either Chinese yam line (Table 3.1). However, after germination, the DW of post-treatment seed bulbils were significantly higher with elevated [CO₂] than under ambient [CO₂] under both air temperature regimes. This indicates that Chinese yam seedlings used less reserve from seed bulbils under elevated [CO₂] than under ambient [CO₂] conditions. Therefore, [CO₂] is a positive resource for seedling growth in Chinese yams.

To our knowledge, this is the first study that has shown (a) effects of elevated CO₂ concentration on growth and photosynthesis in Chinese yam, (b) effects of elevated CO₂ concentration on bulbil germination and seedling vigour in Chinese yam, and (c) effects of elevated CO₂ concentration on morphology in Chinese yam under different conditions. In the future, the climatic conditions will be changed with increasing [CO₂] and air temperature. The information on the effects of elevated [CO₂] at different conditions in Chinese yam that were uncovered in this study will contribute for the yam agricultural management strategies, yam cultivation, breeding and genetic improvements required to sustain Chinese yams productivity under future climatic conditions in northern areas of Japan. Further investigation in elemental components, carbon and nitrogen accumulation in leaves and tubers in Chinese yam should be done to supplement more information about effects of [CO₂] on Chinese yam. Researches to find out the effects of

elevated [CO₂] on the final yield of Chinese yam tuber will be also really necessary.

SUMMARY

The increase in carbon dioxide (CO₂) is widely being considered as the main driving factor that caused the phenomenon of global warming. The effects of elevated atmospheric CO₂ concentration ([CO₂]) on growth of plants such as rice, wheat, soybean, potato etc have been studied but, to date, no experiments related to the effects of elevated [CO₂] have been performed in yam, including Chinese yam. To determine the effective strategies for yam cultivation under the future climatic change, it is important to understand how Chinese yam responds to elevated [CO₂] and by which mechanism the responses occur. This study was carried out with the purpose to elucidate the effects of elevated [CO₂] on growth, photosynthesis and morphology in Chinese yam.

Firstly, to understand the effects of elevated [CO₂] on growth, photosynthesis in Chinese yam, two experiments were conducted in temperature-gradient chambers. Summer experiment was carried out from 11 July to 3 September, 2015 and autumn experiment was carried out from 23 August to 3 October, 2015 with Chinese yam line Enshikei 6 in the chambers. [CO₂] and air temperature were controlled independently in each temperature-gradient chamber. Two temperature-gradient chambers were used under two [CO₂] conditions: ambient (averaged 400 $\mu\text{mol mol}^{-1}$) and elevated (ambient [CO₂] + 200 $\mu\text{mol mol}^{-1}$ in daytime (0400 to 2030), averaged 600 $\mu\text{mol mol}^{-1}$). Each chamber was a naturally sunlit greenhouse. In each temperature-gradient chamber, two treatment plots were set along an air temperature-gradient: approximately ambient-temperature plot and high-temperature plot. Thus, in the experiments, Chinese yam was grown at ambient [CO₂] and elevated [CO₂] under approximately ambient- and high-temperature regimes in summer and autumn, separately. For comparison, rice was also grown under these conditions. Mean air temperatures in the

approximately ambient- and high-temperature plots were respectively 24.1°C and 29.1°C in summer experiment and 20.2°C and 24.9°C in autumn experiment. In summer experiment, Chinese yam vine length, leaf area, leaf dry weight (DW), and total DW were significantly higher under elevated [CO₂] than ambient [CO₂] in both approximately ambient- and high-temperature regimes. Additionally, number of leaves, vine DW, and root DW were significantly higher under elevated [CO₂] than under ambient [CO₂] in the approximately ambient-temperature regime. In autumn experiment, tuber DW was significantly higher under elevated [CO₂] than under ambient [CO₂] in the high-temperature regime. These results demonstrate that yam shows positive growth responses to elevated [CO₂]. Elevated-to-ambient [CO₂] ratios of all growth parameters related to size and weight in summer experiment were higher in Chinese yam than in rice. Analysis of variance revealed that elevated [CO₂] more strongly affected most growth parameters except for total DW in Chinese yam than in rice in summer experiment. The results suggest that the contribution of elevated [CO₂] is higher in Chinese yam than that in rice under summer conditions. Net photosynthetic rate in Chinese yam was significantly higher under elevated [CO₂] than under ambient [CO₂] in both temperature regimes in summer experiment and showed an increasing trend from ambient [CO₂] to elevated [CO₂] in autumn experiment. However, in rice, no significant differences in net photosynthetic rate were detected between ambient [CO₂] and elevated [CO₂] in summer and autumn experiments. These findings indicate that photosynthesis responds more readily to elevated [CO₂] in Chinese yam than in rice.

Secondly, to investigate the effects of elevated [CO₂] on the germination of seed bulbils and the seedling vigour of Chinese yam, two Chinese yam lines, Enshikei 6 and Shojikei, were used in this experiment in the temperature-gradient Chambers. Seed bulbils were sown at 4 June, 2016 and seedlings germinated from

seed bulbils were grown until 9 July, 2016 under two [CO₂] levels, ambient (averaged 400 μmol mol⁻¹) and elevated (ambient + 200 μmol mol⁻¹ in day time (0400 to 2030), averaged 600 μmol mol⁻¹) and two mean air temperature conditions, 22.2°C and 25.6°C. The results showed that elevated [CO₂] did not affect bulbil germination of either Chinese yam lines. During the early growth stage, the DWs of leaves, vines, shoots, roots, belowground parts (roots + tubers) and whole plants were higher under elevated [CO₂] than under ambient [CO₂] for both Chinese yam lines under the approximately ambient- and high-temperature regimes. The values of vigour indexes (index I = germination % × seedling length and index II = germination % × seedling DW) were also higher under elevated [CO₂] than under ambient [CO₂] for both lines. These results indicated that Chinese yam seedlings respond positively to elevated [CO₂] during the early growth stage. The below:aboveground DW ratios were higher under elevated [CO₂] than under ambient [CO₂] in seedlings with very small new tubers for both yam lines, indicating that elevated [CO₂] strongly affected the roots starting in the early growth stage. The DWs of post-treatment seed bulbils were higher with elevated [CO₂] than ambient [CO₂] under both temperature regimes. The results showed that Chinese yam seedling used a smaller amount of the reserves in seed bulbils under elevated [CO₂] than under ambient [CO₂] conditions. Therefore, the results show that elevated [CO₂] is a positive resource for seedling growth in the Chinese yam lines.

Thirdly, to investigate the effects of elevated [CO₂] on leaf morphology of these two Chinese yam lines under the same experiment conditions as described above in the “secondly” section, bright-field optical microscopy and transmission electron microscopy were used to observe the inner structure of leaf blade tissues and scanning electron microscopy was used to observe stomata density and size in yam leaf blade. The palisade layer was thicker under elevated [CO₂] than under

ambient [CO₂] in both temperature regimes, and the whole yam leaf blade was thicker under elevated [CO₂] than under ambient [CO₂] in the approximately ambient temperature regime. The results indicated that elevated [CO₂] increased the yam leaf blade thickness by increasing the cell size of palisade tissues. The numbers of chloroplasts per palisade cell and spongy cell, the number of starch grains per chloroplast, profile area of the starch grain, and starch-to-chloroplast area ratio in both palisade and spongy cells were higher under elevated [CO₂] than under ambient [CO₂] in both temperature regimes. Furthermore, the stomatal density on the abaxial side of the leaf blade in Chinese yam was greater under elevated [CO₂] than under ambient [CO₂] in both temperature regimes, and stomatal-pore length was higher under elevated [CO₂] than under ambient [CO₂] in the approximately ambient temperature regime. These results indicate that elevated [CO₂] positively affects the photosynthetic apparatus and enhances photosynthesis. The results of this study provide important information and a possible explanation for the positive photosynthetic responses of Chinese yam to elevated [CO₂] in our previous study.

To our knowledge, this is the first temperature gradient chamber study that investigated effects of elevated CO₂ concentration on growth and photosynthesis and morphology in Chinese yam. The study shows that elevated [CO₂] increased the thickness of palisade layer and whole leaf blade, numbers of stomata, chloroplasts and starch grains in yam leaves. Consequently, elevated [CO₂] enhanced the net photosynthetic rate. In addition, number of leaves and leaf blade area also increased with elevated [CO₂]. Thus, the results led to increase in yam plant DW, including tuber DW, under elevated [CO₂] than under ambient [CO₂]. The knowledge on the effects of elevated [CO₂] on growth, photosynthesis and morphology in Chinese yam presented in this study will contribute for understanding the characterization of responses to elevated [CO₂] in yam and for

yam production and breeding to make sustainable production in northern Japan under the climate change in the future.

要旨

大気中の二酸化炭素濃度(以下 [CO₂] と記す)の上昇は、地球温暖化を引き起こす主要因として広く認識されている。これまで大気中の高 [CO₂] がナガイモを含むヤマイモ類に及ぼす影響については検証されていなかった。将来の環境変動下でのナガイモ生産に関して予測・検討する上で、ナガイモがどのように高 [CO₂] に対して応答し、どのような機構でその応答がなされるのかを理解することは重要である。そこで、大気中の高 [CO₂] がナガイモの生長、光合成および形態に及ぼす影響を明らかにすることを目的として本研究を実施した。

最初に、高 [CO₂] がナガイモの生長と光合成へ及ぼす影響について調査した。ナガイモ系統園試系 6 を供試し、生長中期の植物体を夏期(2015年7月11日~9月3日)と秋期(2015年8月23日~10月3日)にグラディオトロフチャンバー内で次の4つの処理環境下で自然光を用いてポット栽培した。処理環境は(1)自然[CO₂]区(平均約 400 $\mu\text{mol mol}^{-1}$)+近自然気温区(夏期平均 24.1°C、秋期平均 20.2°C)、(2)高[CO₂]区(平均約 600 $\mu\text{mol mol}^{-1}$ from 4:00 to 20:30)+近自然気温区(夏期平均 24.1°C、秋期平均 20.2°C)、(3)自然[CO₂]区(平均約 400 $\mu\text{mol mol}^{-1}$)+高気温区(夏期平均 29.1°C、秋期平均 24.9°C)、(4)高[CO₂]区(平均約 600 $\mu\text{mol mol}^{-1}$ from 4:00 to 20:30)+高気温区(夏期平均 29.1°C、秋期平均 24.9°C)とした。また、ナガイモの比較材料として水稻品種ひとめぼれも上記と同じ処理条件で栽培した。夏期では、茎長、葉面積、葉乾物重および全乾物重の値は、近自然気温区と高気温区において、自然[CO₂]区よりも高[CO₂]区で有意に高かった。葉数、茎乾物重および根乾物重の値は、近自然気温区で自然[CO₂]区よりも高[CO₂]区で有意に高かった。秋期では、塊茎乾物重が、高気温区で自然[CO₂]区よりも高[CO₂]区で有意に重かった。

これらの結果から、ナガイモが高[CO₂]に対してポジティブな生長反応を示すことが実証された。また、上記全ての生長に関わる調査項目の結果の値において、自然[CO₂]区に対する高[CO₂]の比率は、夏期ではイネよりもナガイモで高かった。更に夏期において高[CO₂]は、全乾物重を除く生長に関わる調査項目でイネよりもナガイモで強くポジティブに影響することが分散分析法により示された。ナガイモの純光合成速度は、夏期の両気温区において自然[CO₂]区よりも高[CO₂]区で有意に高く、秋期でも自然[CO₂]区よりも高[CO₂]区で有意差はないものの高くなる傾向が認められた。一方、イネでは夏期と秋期で自然[CO₂]区と高[CO₂]区との間に有意差は認められなかった。これらの結果は、イネよりもナガイモで光合成が高[CO₂]に対してポジティブな影響を強く受けることを示している。

次に、高[CO₂]がナガイモのむかごの発芽と幼植物体の生長へ及ぼす影響をナガイモ系統園試系 6 と庄司系を用いて調査した。グラディオトロンチャンパー内の次の 4 つの処理環境下で種イモ(むかご)を 2016 年 6 月 4 日にポットに植え付け、発芽により生じた植物体を自然光下で同年 7 月 9 日までポット栽培した。処理環境は(1)自然[CO₂]区(平均約 400 $\mu\text{mol mol}^{-1}$)+近自然気温区(平均 22.2°C)、(2)高[CO₂]区(平均約 600 $\mu\text{mol mol}^{-1}$ from 4:00 to 20:30)+近自然気温区(平均 22.2°C)、(3)自然[CO₂]区(平均約 400 $\mu\text{mol mol}^{-1}$)+高気温区(平均 25.6°C)、(4)高[CO₂]区 (平均約 600 $\mu\text{mol mol}^{-1}$ from 4:00 to 20:30)+高気温区(平均 25.6°C)とした。むかご発芽率においては、近自然気温区と高気温区で自然[CO₂]区と高[CO₂]区との間に有意差は認められなかった。幼植物体では、葉、茎、シュート(葉+茎)、根、地下部(根+塊茎)および全植物体の各乾物重の値が、両気温区において自然[CO₂]区よりも高[CO₂]区で有意に高かった。Vigor indexes (index I = 発芽率(%)) \times 幼植物体長、

index II = 発芽率(%)×幼植物体全乾物重)も、両気温区において自然[CO₂]区よりも高[CO₂]区で有意に高かった。これらの結果から、ナガイモの生長は発芽後の生長初期においても高[CO₂]に対しポジティブに反応することが示された。地下部乾物重に対するシュート乾物重の割合は、自然[CO₂]区よりも高[CO₂]区で有意に低かった。このことから高[CO₂]は生長初期において地下部に強くポジティブな影響をもたらすことが示された。上記処理後の種イモ(むかご)の乾物重は、両気温区において自然[CO₂]区よりも高[CO₂]区で有意に重かった。この結果は、幼植物体による種イモ内貯蔵物質の消費量は自然[CO₂]区よりも高[CO₂]区で少ないことを示し、高濃度のCO₂がナガイモ幼植物体の生長に対してポジティブな資源となることを意味している。

次に、高[CO₂]がナガイモの葉身形態に及ぼす影響を調査するため、前段落で記した処理栽培で得られた植物体の完全展開葉を用い、明視野顕微鏡と透過型電子顕微鏡で葉身の内部構造を、気孔の大きさと存在密度を走査型電子顕微鏡で調査した。葉身を横断した際の柵状組織の厚さは、近自然気温区と高気温区において自然[CO₂]区よりも高[CO₂]区で有意に厚く、これにより葉身の厚さは近自然気温区において有意に厚くなった。これらの結果から、高[CO₂]下では柵状組織が顕著に厚くなる特徴が示された。柵状細胞と海綿状細胞の横断面における各細胞当たりの葉緑体数、葉緑体当たりのデンプン粒数、デンプン粒の面積および葉緑体の面積当たりのデンプン粒の面積の割合、気孔密度の値は、両気温区において自然[CO₂]区よりも高[CO₂]区で有意に高かった。気孔長径は、近自然気温区で自然[CO₂]区よりも高[CO₂]区で有意に長かった。これらのことは、高[CO₂]は光合成に関わる構造的要素に対してポジティブに影響し、光合成速度を高めていると

考えられた。

以上の研究は、大気中の高[CO₂]がナガイモの生長、光合成および形態に及ぼす影響を初めて示したものであり、得られた知見はナガイモのCO₂応答特性を理解し、且つ、将来の気象変動下でのナガイモ生産に関して予測・検討を行う上で価値あるものであると考えられた。

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