

**EFFECTS OF ELEVATED ATMOSPHERIC CARBON DIOXIDE
CONCENTRATION ON GROWTH, PHOTOSYNTHESIS,
MORPHOLOGY AND CORM GERMINATION IN EDDO AT
DIFFERENT AIR TEMPERATURES**

(異なる気温下の大気中の高濃度二酸化炭素がサトイモの成長、
光合成、形態および球茎発芽に及ぼす影響)

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**THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCES
IWATE UNIVERSITY
JAPAN**

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A DISSERTATION SUBMITTED

BY

MD AKIK BIN ZAHER

DOCTOR OF PHILOSOPHY

BIOPRODUCTION SCIENCE

THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCES

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IN
BIOPRODUCTION SCIENCE**

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CHAPTER 1

General introduction

Eddo

Eddo "satoimo" [*Colocasia esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder] is one of root and tuber crops and a member of the taro group. Taro (*Colocasia esculenta* L.) is an herbaceous perennial plant, a member of Araceae family, and is widely cultivated in various parts of the world such as Africa, Asia and Oceania. Taro is one of the most important staple food crops in the Pacific Islands and is also a crop of considerable socio-economic importance in Southeast Asia and the Pacific (Kreike et al., 2004). Worldwide taro production was about 10.6 million tons and the harvested area was about 1.7 million ha in 2018 (FAOSTAT, 2020). Eddo is most widely cultivated among taro crops in Japan. Eddo production was 144,800 tons and the mean yield was 1,260 kg 10a⁻¹ in eddo of Japan in 2018 (e-Stat, 2020). In three prefectures of Tohoku region including Iwate (experimental site of this study), Yamagata and Fukushima prefectures that are the northern area of Japan, the production was 4,496 tons (3.1% in Japan) and mean yield was lower (803 kg 10a⁻¹) in 2018 (e-Stat, 2020). The minimum temperature that can germination for the seed corms is 15°C, and the optimum temperature of growth is 25-30°C while the growth can be completely arrested below 5°C in eddo (Hoshikawa, 1980). Therefore, the northern area, which is in lower mean air temperature and shorter suitable cultivation period, has disadvantage in the viewpoint of temperature condition for eddo cultivation in Japan.

Atmospheric carbon dioxide concentration and global warming

The global atmospheric carbon dioxide concentration ([CO₂]) is increasing with an average of 1.3 μmol mol⁻¹ per year in last 40 years of the 20th century (1960-2000), while in the

last 19 years of the 21st century (2001-2019) it increasing at the rate of 2.2 $\mu\text{mol mol}^{-1}$ per year (NOAA, 2020). Recently, it is very likely to rise up to 409 $\mu\text{mol mol}^{-1}$ in 2019 (NOAA, 2020) and it will continue to increase for the foreseeable future (IPCC, 2013) without any obligatory steps such as limit the emission of $[\text{CO}_2]$ from various sources mainly the burning of fossil fuels (Höök & Tang, 2013). Increasing in greenhouse gases that includes carbon dioxide (CO_2) is associated with the cumulative increase of global average temperature. The global average surface temperature rose 0.6 to 0.9 degrees Celsius (1.1 to 1.6° F) between 1906 and 2005, and the rate of temperature increase has nearly doubled in the last 50 years (NASA, 2020). The $[\text{CO}_2]$ is increasing year after year by the burning of fossil fuels, and it enhances the natural greenhouse effect that ultimately leading the warmer planet (Anderson et al., 2016). The greenhouse gases, including CO_2 , water vapor (H_2O), methane (CH_4), nitrous oxide (N_2O), and other fluorinated gases, are major contributes to gradual heating of Earth's surface.

Effects of elevated $[\text{CO}_2]$ on plant growth at different air temperatures

Previous many studies have focused on the combined effects of elevated $[\text{CO}_2]$ and temperature for many major crop species such as rice (Baker et al., 1992; Cheng et al., 2009; Jing et al., 2016; Nakagawa & Horie. 2000; Roy et al., 2012; Shimono et al., 2008), maize (Mina et al., 2019; Ruiz-Vera et al., 2015), wheat (Cai et al., 2016; Ottman et al., 2012), soybean (Palacios et al., 2019), cassava (Imai et al., 1984) and peanut (Bannayan et al., 2009; Newman et al., 2005). Recent ambient $[\text{CO}_2]$ is considered to be a negative factor for growth of most agricultural species, especially C_3 crops. Some studies reported that elevated $[\text{CO}_2]$ conditions caused the increased plant growth and their respective yields (Kimball 1983; Kimball et al. 2002; Long et al. 2004). It is possible to increase the biomass and yield of C_3 plants, and the average yield of some C_3 grain crops can be surpassed

around 19% by FACE (Kimball 2016). Plant photosynthesis can be accelerated by the elevated $[\text{CO}_2]$ due to the carboxylation rate of Rubisco increases and the oxygenation of Ribulose-1,5-bisphosphate competitively inhibits (Drake et al., 1997). However, an initial increasing of photosynthesis can subsequently be partially or fully down regulated during long-term exposure to elevated $[\text{CO}_2]$ (Stitt, 1991). Long (1991) showed that stimulation of photosynthesis by elevated $[\text{CO}_2]$ can be higher under warmer conditions. Usuda and Shimogawara (1998) have reported that elevated $[\text{CO}_2]$ increases dry weight (DW) accumulation by 105% in storage roots of radish and enhances sink capacity. Eddo corms are observable from the early vegetative stage. Eddo has a large unlimited sink capacity in the corms, whereas cereals such as rice do not in underground parts. From the above information, the corms may be benefit for eddo when responding to elevated $[\text{CO}_2]$. The warmer temperatures at the experimental site of this study in northern Japan should enhance eddo production and may produce a better $[\text{CO}_2]$ response in eddo compared to rice. In this study, we hypothesized that eddo may have a greater response to elevated $[\text{CO}_2]$ than rice and that elevated $[\text{CO}_2]$ would positively affect eddo growth and biomass during the early to intermediate vegetative stages, especially under warmer conditions in comparison with the ambient conditions at our experimental site. Till to date, there is not any reports have been published in eddo regarding on response to elevated $[\text{CO}_2]$.

Effect of elevated $[\text{CO}_2]$ on morphology of leaf blades at different air temperatures

Elevated $[\text{CO}_2]$ has significant effects on plant growth and development (Pritchard et al., 1999). Elevated $[\text{CO}_2]$ affects the morphology and anatomy of plants by increasing thickness of leaf (Bray & Reid, 2002; Okasanen et al., 2001; Radoglou & Javis, 1992; Vu et al., 1989); number and width of chloroplast (Teng et al., 2006); number of starch grains (Hao et al., 2013; Kumar et al., 2013); reducing stomatal density, stomatal index of leaves

and stomatal conductance (Bunce, 2013; Teng et al., 2006). Körner & Larcher (1988) reported that the rate of cell division is tightly regulated by temperature and the number of cell divisions involved in the formation of a new leaf is drastically reduced in low temperature. Therefore, information on the effects of elevated [CO₂] and temperature on leaf anatomical characteristics of plants is important for understanding the reaction of plant growth. However, the effects of elevated [CO₂] on eddo-morphology under different air temperature remain unclear.

Effect of elevated [CO₂] on germinations of seed and storage vegetative organs at different air temperatures

Seed germination plays an important role in agriculture production and influence final yield in terms of plant production density. Enhanced germination rate by the increased [CO₂] was reported in *Plantago lanceolata* (Wulff and Alexander 1985), while elevated [CO₂] did not affect in the germination rate of *Abutilon theophrasti*, *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, and *Setaria faberii* (Garbutt et al. 1990). In contrast, some researchers showed that plant germination rate was decreased under elevated [CO₂] condition (Andolo et al., 1996, Saha et al., 2015). Steckel et al. (2004) reported that optimal temperatures for maximum germination were greater than 20°C for nine *Amaranthus* species, except prostrate pigweed.

There are few studies on elevated [CO₂] for the germination of the root and tuberous crops (storage vegetative organs). Thinh et al. (2017b) indicated that elevated [CO₂] did not affects the bulbil germination rates of either Chinese yam line, though air temperature affected final germination percentages. Eddo is generally propagated by the corm which is a vegetative organ of enlarged short stem. Hoshikawa (1980) showed that the optimum temperature of growth is 25-30°C and the minimum temperature that can germination for

the seed corms is 15°C in eddo. Understanding the effects of elevated [CO₂] on corm germination and seedling growth will be important for seedling establishment and sustainable production of eddo in near future. However, there is not any investigation on corm germination and seedling growth using the elevated [CO₂] under different air temperatures.

Objectives of the study

The source-sink hypothesis is an important consideration for studies on plant responses to elevated [CO₂]. Eddo tubers have a large sink capacity whereas rice is considered as limited sink plants. In addition, eddo production can be even enhanced by warmer temperature condition. However, I do not know positive or negative, and superior or not. Based on the above information, I hypothesized that in chapter two eddo may respond to elevated [CO₂] better than rice and that elevated [CO₂] positively affects eddo growth and biomass during the early-to-intermediate vegetative growth stage, especially at temperatures greater than the ambient temperature at our experimental site. In chapter three, it was hypothesized that elevated [CO₂] positively or negatively affects the leaf internal structure, and the morphology of stomata and chloroplasts. In chapter four, it was hypothesized that elevated [CO₂] affects or does not affect germination rate and seedling growth responses. Therefore, this study purpose is to clarify characteristics of response to elevated [CO₂] in eddo under two different air temperatures from germination to intermediate vegetative growth stage with using rice and soybean as comparative materials at the experimental site at Iwate prefecture in Japan. To achieve the general aim, several experiments were conducted in chapter 2 to 4 with each following objective:

Chapter 2: Effects of elevated atmospheric CO₂ concentration on growth and

photosynthesis in eddo at two different air temperatures.

Objective is to elucidate the effects of elevated $[\text{CO}_2]$ on the growth, photosynthesis in eddo and at two different air temperatures.

Chapter 3: Effects of elevated atmospheric CO_2 concentration on the leaf blades morphology in eddo at two different air temperatures

Objective is to elucidate the effects of elevated $[\text{CO}_2]$ on the leaf blades morphology in eddo at two different air temperatures.

Chapter 4: Effects of elevated atmospheric CO_2 concentration on the corm germination and early seedling growth in eddo at two different air temperatures

Objective is to clarify the effects of elevated $[\text{CO}_2]$ on the corm germination and early seedling growth in eddo at two different air temperatures.

CHAPTER 2

Effects of elevated atmospheric CO₂ concentration on growth and photosynthesis in eddo at two different air temperatures

INTRODUCTION

The atmospheric carbon dioxide concentration ([CO₂]) has increased from a stable 280 $\mu\text{mol mol}^{-1}$ before the industrial revolution to 409 $\mu\text{mol mol}^{-1}$ in 2019 (NOAA, 2020). The [CO₂] has increased by an average of 1.3 $\mu\text{mol mol}^{-1}$ per year over the past 40 years (1960–2000), and recent estimates indicate that the average [CO₂] has risen by 2.2 $\mu\text{mol mol}^{-1}$ per year from 2001 to 2019 (NOAA, 2020). Thus, various studies have investigated the effects of elevated [CO₂] on plants in open-top chambers, growth chambers, greenhouses, and fields with free-air CO₂ enrichment (FACE) systems. Many of these studies revealed that elevated [CO₂] conditions positively affect plant growth and yield (Kimball 1983; Kimball et al. 2002; Long et al. 2004). Kimball (2016) reported that it is possible to increase the biomass and yield of C₃ species and that the average yield of some C₃ grain crops can increase by approximately 19% using a FACE system.

Photosynthesis can be accelerated by elevated [CO₂] because the carboxylation rate of RuBisCo increases and the oxygenation of ribulose-1,5-bisphosphate is competitively inhibited (Drake et al., 1997). An initial increase in photosynthesis can subsequently be partially or fully down-regulated during a long-term exposure to elevated [CO₂] (Stitt, 1991). Photosynthetic regulation partly depends on the balance between the substrate for photosynthesis (such as CO₂) and the sink capacity (Paul & Foyer, 2001). Plants with large vegetative sinks are stimulated by elevated [CO₂] more than plants with shorter-lived reproductive sinks (Arp, 1991; De Temmerman et al., 2007; Miglietta et al., 1998). Potato has a large carbohydrate sink in the form of tubers that have apoplastic phloem for loading

sucrose, which leads to an increased yield under elevated [CO₂] (De Temmerman et al., 2002; Farrar and Williams, 1991; Schapendonk et al., 2000). Thus, there is a hypothesis that sink constraints limit the CO₂ fertilization effect, and root and tuber crops may have a more significant response to elevated [CO₂] than other plant types because their sink size is often unlimited.

Carbon dioxide is a significant contributor to global warming. The global surface temperature will likely rise by 1.5°C between 2030 and 2052 even if stringent measures are imposed to limit greenhouse gas emissions (IPCC, 2018). Therefore, it is imperative that the effects of increasing [CO₂] and temperature on future crop growth and agricultural productivity are investigated. Previous studies have focused on the combined effects of elevated [CO₂] and temperature on many major crop species such as rice (Baker et al., 1992; Cheng et al., 2009; Jing et al., 2016; Nakagawa & Horie, 2000; Roy et al., 2012; Shimono et al., 2008), maize (Mina et al., 2019; Ruiz-Vera et al., 2015), wheat (Cai et al., 2016; Ottman et al., 2012), soybean (Palacios et al., 2019), cassava (Imai et al., 1984), and peanut (Bannayan et al., 2009; Newman et al., 2005). In rice, doubling the [CO₂] increases the whole plant dry weight (DW) more at high air temperatures [33/26°C (day/night)] than at low air temperatures [28/21°C (day/night)] during the vegetative growth stage (Imai et al., 1985). However, a recent review of the responses of various crops to [CO₂] revealed both positive and negative interactions between [CO₂] and temperature on crop yield and growth in FACE systems (Kimball, 2016). Kimball (2016) suggested that increasing the temperature alone can stimulate or slow plant growth depending on whether a plant is currently exposed to an environmental temperature that is below or above its optimum temperature for growth.

Eddo [*Colocasia esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder] is a root and tuber crop belonging to the taro family (Araceae). Taro is cultivated in various

regions worldwide, including in Africa, Asia, and Oceania. Its corms are highly valuable as a staple food. Thus, the demand for this crop is likely to continue to increase as the global human population increases. In 2018, taro production was about 10.6 million tons worldwide, and the harvested area was about 1.7 million ha (FAOSTAT, 2020). Eddo is the most widely cultivated taro crop in Japan. In 2018, eddo production in Japan was 144,800 tons, with a mean yield of $1,260 \text{ kg } 10\text{a}^{-1}$ (e-Stat, 2020). More specifically, the eddo production in 2018 in Iwate (experimental site of this study), Yamagata, and Fukushima prefectures in the Tohoku region of northern Japan was 4,496 tons (3.1% of the total production in Japan), with a mean yield of $803 \text{ kg } 10\text{a}^{-1}$ (e-Stat, 2020). The minimum temperature needed for the eddo seed corms to germinate is 15°C and the optimum temperature for growth is $25\text{--}30^{\circ}\text{C}$, whereas eddo plants cease to grow at temperatures below 5°C (Hoshikawa, 1980). Cultivating eddo in northern Japan can be difficult because of the relatively low mean air temperature and short cultivation period at suitable temperatures.

Eddo corms are detectable from the early vegetative stage. Eddo has an unlimited sink capacity in the corms, whereas the underground parts of cereals, such as rice, do not. Accordingly, the corms may positively contribute to eddo responses to elevated $[\text{CO}_2]$. Increased temperatures at the experimental site of this study in northern Japan should enhance eddo production and result in better responses to $[\text{CO}_2]$ compared with the corresponding responses in rice. In this study, it was hypothesized that eddo may respond to elevated $[\text{CO}_2]$ better than rice and that elevated $[\text{CO}_2]$ positively affects eddo growth and biomass during the early-to-intermediate vegetative growth stage, especially at temperatures greater than the ambient temperature at our experimental site. To test these hypotheses, we used two different types of crops, eddo and rice, and investigated the responses to elevated $[\text{CO}_2]$ at different air temperatures during the early-to-intermediate

vegetative growth stage. We used temperature gradient chambers (TGCs) to examine the effects of elevated [CO₂] on eddo and rice plant growth, biomass, the soil plant analysis development (SPAD) value, stomatal conductance (g_s), and the net photosynthetic rate (P_N) of leaf blades at two different air temperatures.

MATERIALS AND METHODS

2.1. Study sites and treatment conditions

I evaluated the effects of elevated [CO₂] at two different air temperatures in two TGCs from June 30 to August 4, 2018 and from June 15 to July 20, 2019 at the Tohoku Agriculture Research Center, The National Agriculture and Food Research Organization (NARO) (39°74'N, 141°13'E) in Morioka, Japan.

The TGCs were sunlit greenhouses (6 m wide × 30 m long × 3 m high). Fans were used to ensure the air in the TGCs flowed continuously from the inlet to the exhaust fans. A temperature gradient was continuously maintained along the longitudinal axis by cooling the air with an air conditioner at the inlet end and/or by warming the air with solar radiation or a supplemental heat input (an air heater and ducts) at the outlet end. The [CO₂] in one TGC was kept at ambient levels, whereas that in the other TGC was kept at about 200 $\mu\text{mol mol}^{-1}$ above the ambient levels by injecting pure CO₂ (the [CO₂] exceeded 99.95%; HOKURYO Co., Ltd., Iwate, Japan) at the air inlet between 03:30 and 19:30. The CO₂ emission rate was set proportional to the air exhaust rate. An infrared gas analyzer (LI-820; LI-COR, Lincoln, NE, USA) was used to monitor the [CO₂] based on the air collected from about 2 m above the ground in the middle of each TGC. The average daytime (04:00 to 20:00) [CO₂] over the treatment period was $416 \pm 12 \mu\text{mol mol}^{-1}$ (data are presented here and subsequently as the mean \pm standard deviation) in 2018 and $415 \pm 8 \mu\text{mol mol}^{-1}$

in 2019 in the ambient [CO₂] TGC, whereas it was $616 \pm 13 \mu\text{mol mol}^{-1}$ in 2018 and $609 \pm 8 \mu\text{mol mol}^{-1}$ in 2019 in the elevated [CO₂] TGC.

The air temperature and relative humidity were monitored at each location using Pt 100 resistance hygrothermographs with an aspirated double-tube radiation-shield. Five hygrothermographs were set at approximately even intervals along the longitudinal axis of each TGC, and the height of the hygrothermographs was gradually increased so that it was consistent with the maximum plant height of rice. Two plots were set along an air temperature gradient in each TGC. The air temperature and relative humidity of each plot were calculated based on their gradients in each TGC. In the ambient [CO₂] TGC, the low air temperature plot (approximately the same as the ambient air temperature) had an average air temperature of $23.4^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ in 2018 and $19.0^{\circ}\text{C} \pm 1.6^{\circ}\text{C}$ in 2019, whereas the high air temperature plot had an average air temperature of $27.7^{\circ}\text{C} \pm 2.6^{\circ}\text{C}$ in 2018 and $23.1^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$ in 2019. In the elevated [CO₂] TGC, the low air temperature plot had an average air temperature of $23.5^{\circ}\text{C} \pm 2.6^{\circ}\text{C}$ in 2018 and $19.1^{\circ}\text{C} \pm 1.6^{\circ}\text{C}$ in 2019, whereas the high air temperature plot had an average air temperature of $27.9^{\circ}\text{C} \pm 2.7^{\circ}\text{C}$ in 2018 and $23.2^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$ in 2019. The natural air temperatures outside of the TGCs during the treatment periods were $24.2^{\circ}\text{C} \pm 3.4^{\circ}\text{C}$ in 2018 and $19.7^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ in 2019.

In the ambient [CO₂] chamber, the relative humidity was $76\% \pm 8\%$ and $77\% \pm 8\%$ in the low air temperature plot and $40\% \pm 4\%$ and $49\% \pm 8\%$ in the high air temperature plot in 2018 and 2019, respectively. In the elevated [CO₂] chamber, the relative humidity was $70\% \pm 8\%$ and $75\% \pm 11\%$ in the low air temperature plot and $45\% \pm 5\%$ and $48\% \pm 5\%$ in the high air temperature plot in 2018 and 2019, respectively. Solar radiation was measured using sunlight sensors (LP02; TAIYO KEIKI, Tokyo, Japan), which were positioned approximately 5 m above the ground beside the TGCs. Total light transmission of outer cover films (F-CLEANTM clear; AGC Green-Tech Co., Ltd., Tokyo, Japan) for

TGCs is 94%. The average daytime solar radiation during the treatment period was $193 \text{ Wm}^{-2} \text{ day}^{-1}$ in 2018 and $181 \text{ Wm}^{-2} \text{ day}^{-1}$ in 2019. The $[\text{CO}_2]$, air temperature, relative humidity, and solar radiation were recorded at 5 s intervals and the averages for 1 min intervals were calculated by a data logger (CR 1000; Campbell Sci. Inc., Logan, UT, USA.).

Plants are generally more sensitive to air temperature changes than to relative humidity changes. Thus, the four treatments in this study were as follows:

- a) AL: ambient $[\text{CO}_2]$ and low air temperature;
- b) EL: elevated $[\text{CO}_2]$ and low air temperature;
- c) AH: ambient $[\text{CO}_2]$ and high air temperature;
- d) EH: elevated $[\text{CO}_2]$ and high air temperature.

2.2 Plant materials and cultural practices

Eddo cultivar Aichiwase, which is one of the major cultivars in Japan, was analyzed in this study because of a lack of widely cultivated cultivars in the Tohoku region. Additionally, we were interested in how common eddo varieties may respond to future conditions in northern Japan resulting from global warming (i.e., elevated $[\text{CO}_2]$ and high temperatures). Rice cultivar Hitomebore, which is widely cultivated in northern Japan, was also analyzed in this study. Eddo seed corms of a uniform size were selected, with an average fresh weight per corm of $71 \pm 6 \text{ g}$ and $78 \pm 7 \text{ g}$ in 2018 and 2019, respectively. The selected seed corms were sterilized in 0.5% (v/v) sodium hypochlorite solution for 5 min and then washed with water for 30 min. The corms were sown in small plastic pots (13 cm height \times 12 cm internal diameter) filled with commercial soil (containing 0.32 g L^{-1} nitrogen, 0.21 g L^{-1} phosphorus, and 0.30 g L^{-1} potassium) (Ikubyobaiyodo; TAKII & Co., Ltd., Kyoto, Japan). The seed corms were covered by 3-4 cm of soil on May 25 for the 2018 experiment and May 16 for the 2019 experiment. Rice seeds were soaked for 10 min in 20% (v/v)

sodium chloride solution to eliminate unfilled seeds and then sterilized with a fungicide containing Procloraz (SPORTAK®; Nissan Chemicals Co. Ltd., Saitama, Japan). The seeds were submerged in water at 20°C for 3 days and then at 30°C for 1 day. The germinated seeds were sown in a seedling tray on June 21 for the 2018 experiment and June 6 for the 2019 experiment. The eddo and rice seedlings were grown in a greenhouse with opened windows at Hirosaki University (40°59'N, 140°47'E), Hirosaki, Japan.

Treatments were initiated in the TGCs when the plants reached the early vegetative growth stage and lasted for 5 weeks, at which point the plants were in the intermediate vegetative growth stage. Just before the treatments in the TGCs, two young eddo plants at the 3- to 4-leaf stage in 2018 and at the 2- to 2.5-leaf stage in 2019 were transplanted into a plastic pot (35 cm height × 32 cm internal diameter) filled with the same soil used in the seedling period. The lateral buds had not yet extended when the plants were transplanted. The corms were covered by about 6 cm of soil. The plants were suitably watered three to five times every week during the treatment period. At 9 days after sowing, the rice seedlings (the plant age in leaf number was three- to three point two) were transplanted into 1/5000a Wagner's pots (19 cm height × 16 cm internal diameter) filled with commercial soil (containing 1.7 g L⁻¹ nitrogen, 4.4 g L⁻¹ phosphorus, and 1.8 g L⁻¹ potassium) (Agrobaido; Kanumasangyo Co. Ltd., Tochigi, Japan), with two seedlings per pot, before starting the treatments in the TGCs. The rice seedlings were flooded during the treatment period. For both eddo and rice plants, each pot was rotated and their positions were changed weekly to minimize the effects of environmental differences.

2.3 Growth and dry weight measurements

Regarding eddo, an apical bud germinates from a seed corm and develops into the main plant part. Lateral buds also germinate and develop into the lateral plant parts. Therefore,

an eddo plant consists of a main plant part and some lateral plant parts. In this study, 9–10 eddo plants were analyzed per treatment. The number of leaves and number of lateral plants in an eddo plant were individually counted, and then the plant height was measured 1 day before the final treatment day. The same eddo plants were collected from pots on the final treatment day, after which the roots were carefully washed with tap water to remove soil. The leaf blades, petioles, corms, and roots were collected separately for each treatment. The eddo leaf blades and a scale were photographed with a digital camera. The leaf area was measured using the Image J software (1.52a; Wayne Rasband, National Institutes of Health, MD, USA). Eddo plants were dried at 80°C for 6 days before measuring the DW. Additionally, 10 rice plants were analyzed per treatment. The number of leaves on the main stem part as well as the plant height and number of tillers were determined 1 day before the final treatment day. Rice plants were sampled at the same time and in the same manner as the eddo plants. The rice leaf blade area was measured using an automatic leaf area meter (AAM-9; Hayashi Denko Co. Ltd., Tokyo, Japan). Analyses were completed with the 9–10 eddo plants and the 10 rice plants collected for each treatment.

2.4 SPAD, stomatal conductance, and net photosynthetic rate measurements

For each treatment, we randomly selected five eddo and rice plants from among the analyzed plants described above to measure the g_s and P_N at the central part of the youngest fully expanded leaves on the eddo main plant and rice main stem at 2–3 days before the final treatment day in 2018 and 2019. The measurements were conducted between 07:00 and 14:00 using a portable photosynthesis measurement system (LI-6400; LI-COR, Lincoln, NE, USA). The $[CO_2]$ in the air entering the leaf chamber cuvette was adjusted to 400 and 600 $\mu\text{mol mol}^{-1}$ for the ambient and elevated $[CO_2]$ plots, respectively. The relative humidity of the air entering the cuvette was adjusted to about 60%. In the cuvette,

the photosynthetic photon flux density was set to $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ using the internal blue and red LED light source and the block temperature was adjusted to 27°C . After the g_s and P_N were measured, the SPAD value was determined using a SPAD meter (SPAD-502, Minolta, Tokyo, Japan). The measurements were completed using a single leaf blade from the main parts of five individual eddo and rice plants per treatment.

2.5 Statistical analysis

I conducted an analysis of variance (ANOVA) with a split-plot design to determine the significance of the effects of $[\text{CO}_2]$ and temperature on eddo and rice growth. The main plot was $[\text{CO}_2]$ and the subplot was air temperature, with years (2018 and 2019) treated as replications (random effect). The natural logarithms of the mean value ratios in elevated vs ambient $[\text{CO}_2]$ for the total DW and P_N were used for the ANOVA. In the present study, the statistical significance was evaluated at $\alpha < 0.1$ since replicate number was limited. All statistical analyses were conducted using the general linear model procedure of the SPSS statistical software (version 24.0; IBM, New York, NY, USA).

RESULTS

1. Growth parameters

The number of leaves, leaf area and plant height growth parameters in eddo were significantly ($P < 0.05$) increased by elevated $[\text{CO}_2]$ (Table 2.1). High air temperature had also positive significant ($P < 0.05$) effect on the number of leaves, leaf area and number of lateral plants in eddo (Table 2.1). In rice, the number of leaves ($P < 0.01$) and number of tillers ($P < 0.1$) were significantly increased by elevated $[\text{CO}_2]$, but the all growth parameters were significantly ($P < 0.1$) increased by high temperature (Table 2.1). Combination between $[\text{CO}_2]$ and temperature had significant effects on increasing the

number of lateral plants ($P < 0.05$) in eddo, but there were not significant $[\text{CO}_2] \times$ temperature interactions for the all growth parameters in rice (Table 2.1).

2. Dry weights

The DWs of leaf blade, petiole, root and above ground part in eddo and the DWs of leaf blade, shoot and above ground part in rice were significantly ($P < 0.1$) increased by elevated $[\text{CO}_2]$ (Table 2.2 and 2.3). The all DW parameters were significantly ($P < 0.1$) increased by high temperature in rice but in only corm DW of eddo (Tables 2.2 and 2.3). All DW parameters of eddo were not increased by the effects of combination between $[\text{CO}_2]$ and temperature (Table 2.2). In rice, leaf blade DW parameter was significantly ($P < 0.1$) increased by the effects of combination between $[\text{CO}_2]$ and temperature (Table 2.3).

Total DWs of eddo ($P < 0.1$) and rice ($P < 0.01$) were significantly increased under the elevated $[\text{CO}_2]$ (Figure 2.1). The total DW was also significantly ($P < 0.01$) increased by high temperature in rice, but not in eddo. A combination between $[\text{CO}_2]$ and temperature showed significant ($P < 0.05$) effects on increasing the total DW in rice but did not in eddo (Figure 2.1). The mean value ratios in elevated vs. ambient $[\text{CO}_2]$ under low and high temperatures were, respectively, 1.26 and 1.30 in eddo and 1.17 and 1.26 in rice (Table 2.4). The ratio was significantly ($P < 0.05$) higher in eddo than in rice under the both temperature regimes and was also significantly ($P < 0.05$) higher in the high temperature regime than in the low temperature regime (Table 2.4).

3. SPAD value, stomatal conductance, and net photosynthetic rate

The SPAD values of eddo was significantly ($P < 0.01$) increased under the effects of elevated $[\text{CO}_2]$ (Figure 2.2). There were no significant interactions between $[\text{CO}_2]$ and temperature for the SPAD values in eddo (Figure 2.2). But in rice, there were no significant

effects of [CO₂] and temperature, and their combinations (Figure 2.2). All the EH treatments showed the highest mean values of SPAD in eddo and rice (Figure 2.2). Elevated [CO₂] and temperature, and their combinations had no effects on the g_s in eddo and rice (Figure 2.3).

P_N was significantly ($P < 0.1$) increased by elevated [CO₂] in eddo and rice (Figure 2.4). High temperature had significantly ($P < 0.05$) positive effects on P_N in eddo, but not in rice. The effects of CO₂ were similar in both temperatures levels as evidenced by the non-significant interaction between CO₂ and temperature (Figure 2.4). The mean ratios of the P_N in elevated [CO₂] vs. in ambient [CO₂] under low- and high- temperatures were, respectively, 1.32 and 1.38 in eddo (Table 2.4). The corresponding data of the P_N were, respectively, 1.28 and 1.18 in rice (Table 2.4). There were no significant differences of the ratios between eddo and rice, and between the both temperature regimes (Table 2.4).

DISCUSSION

Previous studies showed that elevated [CO₂] had substantial impacts on plants growth and biomass. Saminathan et al. (2019) reported that the number of storage roots, and the DWs of leaf, root, stem and total biomass were significantly higher under the elevated [CO₂] condition in sweet potato. Similarly, Aien et al. (2014) showed that elevated [CO₂] exposure significantly increased number of tubers per plant and total dry matter production in two potato cultivars. In case of this study, many parameters related with the growth and DW in eddo and rice were significantly increased by elevated [CO₂] (Tables 2.1, 2.2 and 2.3). Therefore, to the best of our knowledge, this is the first report of the positive effects of elevated [CO₂] on eddo growth and biomass during the early-to-intermediate vegetative growth stage. The total DW of rice plants increased significantly ($P < 0.01$) in response to elevated [CO₂], whereas the total DW of eddo plants tended to increase ($P < 0.1$) under

elevated [CO₂] conditions (Figure 2.1). However, we also determined that the increasing ratio of the total DW in elevated vs ambient [CO₂] was higher for eddo than for rice (Table 4). The result suggests that elevated [CO₂] increases the biomass of eddo plants more than the biomass of rice plants under the analyzed experimental conditions. This finding may support the hypothesis that plants with a large sink capacity, such as root and tuber crops, are more responsive to elevated [CO₂] than plants with a limited sink capacity. The number of leaves and the leaf area of eddo plants increased significantly following an exposure to elevated [CO₂], whereas the leaf area of rice plants did not significantly increase in response to elevated [CO₂] (Table 1). Thus, the leaf area as well as the number of leaves may contribute to the higher increasing rate of biomass in eddo under elevated [CO₂] conditions.

Various results have been reported for the combined effects of elevated [CO₂] and temperature on biomass and/or yield of rice, wheat, maize, soybean, and C₃ and C₄ grasses (Kimbell, 2016). In cassava, a root and tuber crop, the number of leaves, leaf area, and stem length as well as the leaf blade, petiole, stem, root, and whole plant DWs under elevated [CO₂] conditions are significantly higher at high air temperatures [33/26°C (day/night)] than at low air temperatures [28/21°C (day/night)] (Imai et al., 1984). In the current study, the mean values for the analyzed growth and biomass parameters for eddo and rice under elevated [CO₂] conditions were higher at the high air temperature than at the low air temperature, regardless of significance (Tables 1, 2 and 3). Additionally, under elevated [CO₂], the total DW of rice increased significantly at the high air temperature (Figure 2.1). In contrast, under elevated [CO₂], the eddo DW was not significantly increased by the exposure to the high air temperature (Figure 2.1). This may be explained by the notable difference in plant growth between 2018 and 2019, which was primarily due to the difference in the average air temperature and solar radiation during the treatment

period between the 2 years. Accordingly, these differences between years likely affected the ANOVA results. Additional investigations will need to be conducted to verify the hypothesis that the positive effects of elevated [CO₂] on the eddo biomass are enhanced by warm conditions at our experimental site.

The leaf chlorophyll contents of garlic (*Allium sativum* L., cv. White Roppen) reportedly increase significantly with increasing CO₂ concentrations (Naznin et al., 2015). SPAD value is one of the indicators of chlorophyll content. In an earlier study on rice, the simultaneous increase in [CO₂] and air temperature led to a 14% increase in the SPAD value, relative to the average value in the open field (Figueiredo et al., 2015). In the current study, the SPAD value significantly increased in response to elevated [CO₂] for eddo, but not for rice (Figure 2.2). Upreti et al. (2002) reported that an exposure to high CO₂ levels decreases the g_s , which affects the photosynthetic rate. Similarly, the g_s of eddo and rice exhibited a decreasing trend under elevated [CO₂] conditions at the low and high air temperatures, although the changes were not significant (Figure 2.3). Regarding eddo, we propose that the increase in the chlorophyll content may be one of the factors influencing the tendency of P_N ($P < 0.1$) to increase under elevated [CO₂] conditions at both temperatures (Figure 2.2 and 2.4).

In this study, the positive effects of elevated [CO₂] on eddo plants during the early-to-intermediate vegetative growth stage were revealed by analyzing plant growth, biomass, SPAD values, and P_N (a positive tendency). The actual mean values of the number of lateral plants and corm DW in eddo in 2018 and 2019 were higher under elevated [CO₂] than under ambient [CO₂] and both values were the highest in elevated [CO₂] conditions under the high air temperature, although not significantly so (Tables 1 and 2). Regarding eddo, increases in the number of lateral plant parts are associated with increases in the number of corms. The tendency for eddo plants to respond positively to elevated [CO₂] in

the early-to-intermediate vegetative growth stage might contribute to the final eddo yield. However, Sage et al. (1989) indicated that a long-term exposure to elevated [CO₂] affects the CO₂ response of photosynthesis and that the patterns of acclimation vary among species in the following three ways: (a) the initial CO₂ response is unaffected, but the photosynthetic rate at high CO₂ levels increases (*Solanum tuberosum*); (b) the initial CO₂ response decreases, but the CO₂-saturated photosynthetic rate is only slightly affected (*Chenopodium album* and *Phaseolus vulgaris*); (c) both the initial CO₂ response and the CO₂-saturated photosynthetic rate decrease (*Brassica oleracea* and *Solanum melongena*). Future studies on the effects of a long-term exposure to elevated [CO₂] on eddo plants may lead to a more comprehensive characterization of the acclimation of photosynthesis and plant yield. This study firstly provided information related with elevated [CO₂] response of eddo at two different air temperatures with using TGCs.

Table 2.1. Effects of elevated CO₂ concentration ([CO₂]) on plant growth in eddo and rice at two different air temperatures.

Treatments	Eddo				Rice			
	No. of leaves	Leaf area	Plant height	No. of lateral plants	No. of leaves in a main stem	Leaf area	Plant height	No. of tillers
	(plant ⁻¹)	(cm ² plant ⁻¹)	(cm plant ⁻¹)	(plant ⁻¹)	(plant ⁻¹)	(cm ² plant ⁻¹)	(cm plant ⁻¹)	(plant ⁻¹)
2018								
AL	10.5	3233.2	31.3	2.3	11.4	1431.5	74.4	27.3
EL	12.6	3671.4	39.9	2.9	12.1	1530.2	76.5	30.2
AH	11.3	4032.3	34.2	2.6	12.1	1638.8	81.5	29.7
EH	13.9	5619.3	40.1	3.8	13.1	1693.4	84.5	33.5
2019								
AL	8.5	2577.8	26.4	1.8	10.1	1002.7	51.1	22.3
EL	10.7	3352.8	33.1	2.0	10.9	1108.8	56.0	25.4
AH	9.6	3466.9	29.9	1.9	11.2	1420.6	65.6	25.3
EH	12.4	4456.6	37.0	2.9	12.1	1684.7	72.7	30.4
Mean of 2018 and 2019								
AL	9.5	2888.2	28.7	2.1	10.7	1217.7	62.7	24.8
EL	11.6	3512.1	36.5	2.4	11.5	1319.5	66.2	27.8
AH	10.4	3734.7	31.9	2.3	11.6	1529.7	73.5	27.5
EH	13.1	5007.3	38.5	3.3	12.6	1689.1	78.6	32.0
ANOVA								
CO ₂ (C)	**	**	**	ns	***	ns	ns	*
Temperature (T)	**	**	ns	**	**	*	*	**
Year (Y)	**	*	**	ns	***	ns	*	*
C x T	ns	ns	ns	**	ns	ns	ns	ns
C x Y	ns	ns	ns	ns	ns	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

Table 2.2. Effects of elevated CO₂ concentration ([CO₂]) on dry weight (DW) of eddo at two different air temperatures.

Treatments	Leaf blade DW (g plant ⁻¹)	Petiole DW (g plant ⁻¹)	Corm DW (g plant ⁻¹)	Root DW (g plant ⁻¹)	Above ground DW (g plant ⁻¹)	Below ground DW (g plant ⁻¹)
2018						
AL	12.1	16.8	10.4	12.6	28.9	22.9
EL	14.2	18.6	14.5	16.4	32.8	30.9
AH	12.4	15.2	11.4	12.7	27.6	24.1
EH	14.3	20.3	15.4	15.6	34.6	30.9
2019						
AL	8.2	8.1	5.6	6.6	16.3	12.2
EL	9.6	11.0	7.6	8.6	20.6	16.2
AH	9.7	10.8	6.9	8.3	20.5	15.2
EH	12.5	14.0	9.4	11.7	26.4	21.1
Mean of 2018 and 2019						
AL	10.1	12.2	7.9	9.4	22.3	17.3
EL	11.9	14.8	11.1	12.5	26.7	23.5
AH	11.0	12.8	9.0	10.4	23.8	19.4
EH	13.3	17.0	12.2	13.5	30.3	25.7
ANOVA						
CO ₂ (C)	**	**	ns	*	**	ns
Temperature (T)	ns	ns	**	ns	ns	ns
Year (Y)	***	**	ns	**	**	*
C × T	ns	ns	ns	ns	ns	ns
C × Y	ns	ns	*	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

Table 2.3. Effects of elevated CO₂ concentration ([CO₂]) on dry weight (DW) of rice at two different air temperatures.

Treatments	Leaf blade DW (g plant ⁻¹)	Shoot DW (g plant ⁻¹)	Root DW (g plant ⁻¹)	Above ground DW (g plant ⁻¹)
2018				
AL	5.1	9.3	4.7	14.4
EL	5.7	11.5	5.0	17.2
AH	6.4	11.1	5.5	17.5
EH	7.5	13.4	6.9	21.0
2019				
AL	3.6	6.1	4.2	9.7
EL	3.8	6.9	5.7	10.7
AH	4.5	7.1	5.4	11.6
EH	5.9	10.1	6.3	16.0
Mean of 2018 and 2019				
AL	4.3	7.7	4.4	12.0
EL	4.8	9.2	5.3	14.0
AH	5.4	9.1	5.5	14.6
EH	6.7	11.8	6.6	18.6
ANOVA				
CO ₂ (C)	**	**	ns	**
Temperature (T)	***	**	*	**
Year (Y)	**	**	ns	**
C × T	*	ns	ns	ns
C × Y	ns	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

Table 2.4. Effects of elevated CO₂ concentration ([CO₂]) on mean value ratios of total dry weight (DW) and net photosynthetic rate in elevated vs. ambient [CO₂] in eddo and rice at two different air temperatures.

	Total DW	Net photosynthetic rate
2018		
Eddo		
Low temperature	1.23	1.35
High temperature	1.27	1.31
Rice		
Low temperature	1.16	1.40
High temperature	1.21	1.19
2019		
Eddo		
Low temperature	1.29	1.29
High temperature	1.33	1.46
Rice		
Low temperature	1.18	1.16
High temperature	1.31	1.17
Mean of 2018 and 2019		
Eddo		
Low temperature	1.26	1.32
High temperature	1.30	1.38
Rice		
Low temperature	1.17	1.28
High temperature	1.26	1.18
ANOVA		
Crop (Cr)	**	ns
Temperature (T)	*	ns
Year (Y)	**	ns
Cr x T	ns	ns
Cr x Y	ns	ns

The natural logarithms of the mean value ratios in elevated vs. ambient [CO₂] were used for ANOVA. Statistically significant effects are indicated **: P < 0.05, *: P < 0.1, ns: not significant

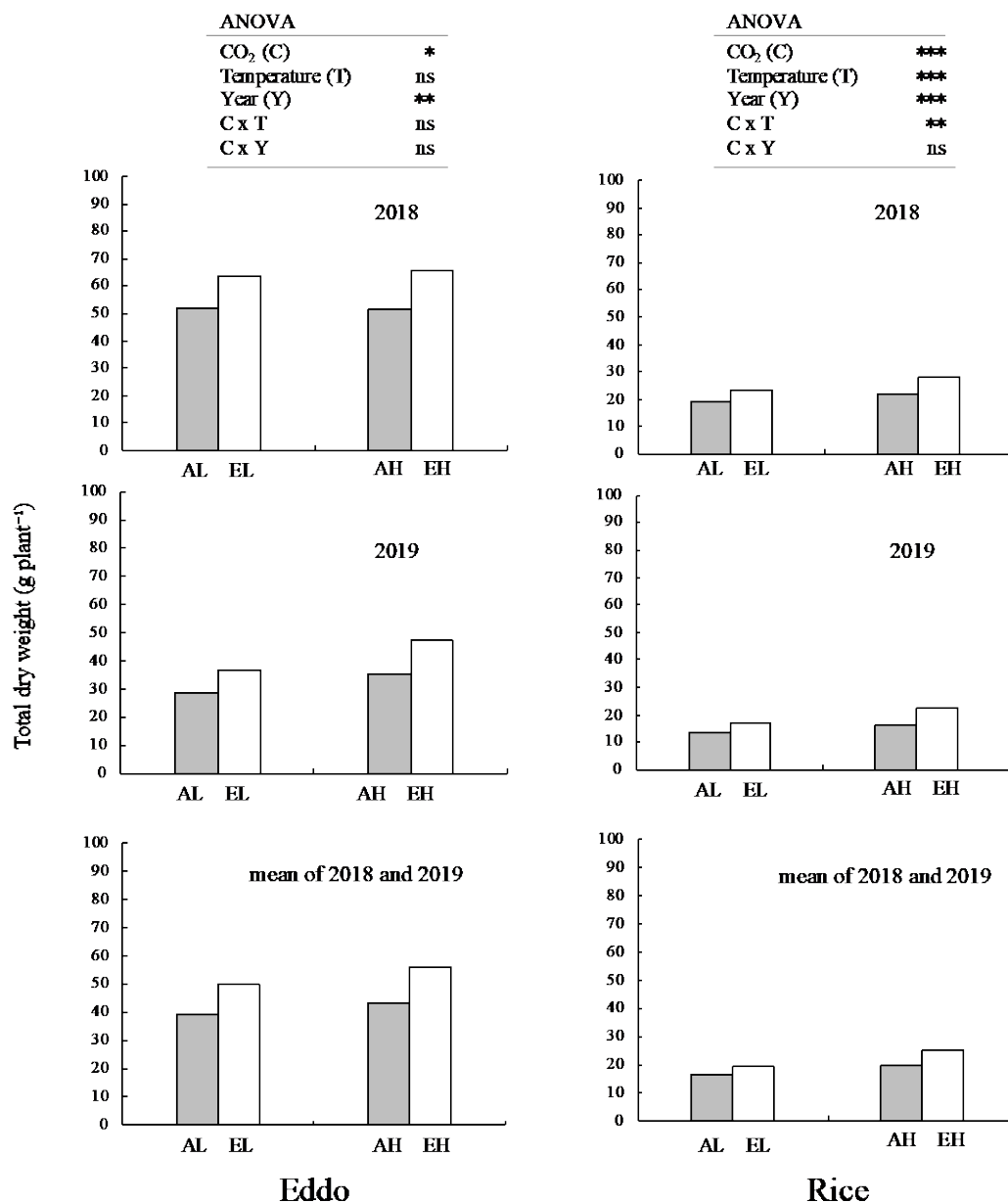


Figure 2.1. Effects of elevated CO₂ concentration ([CO₂]) on total dry weight in eddo and rice at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

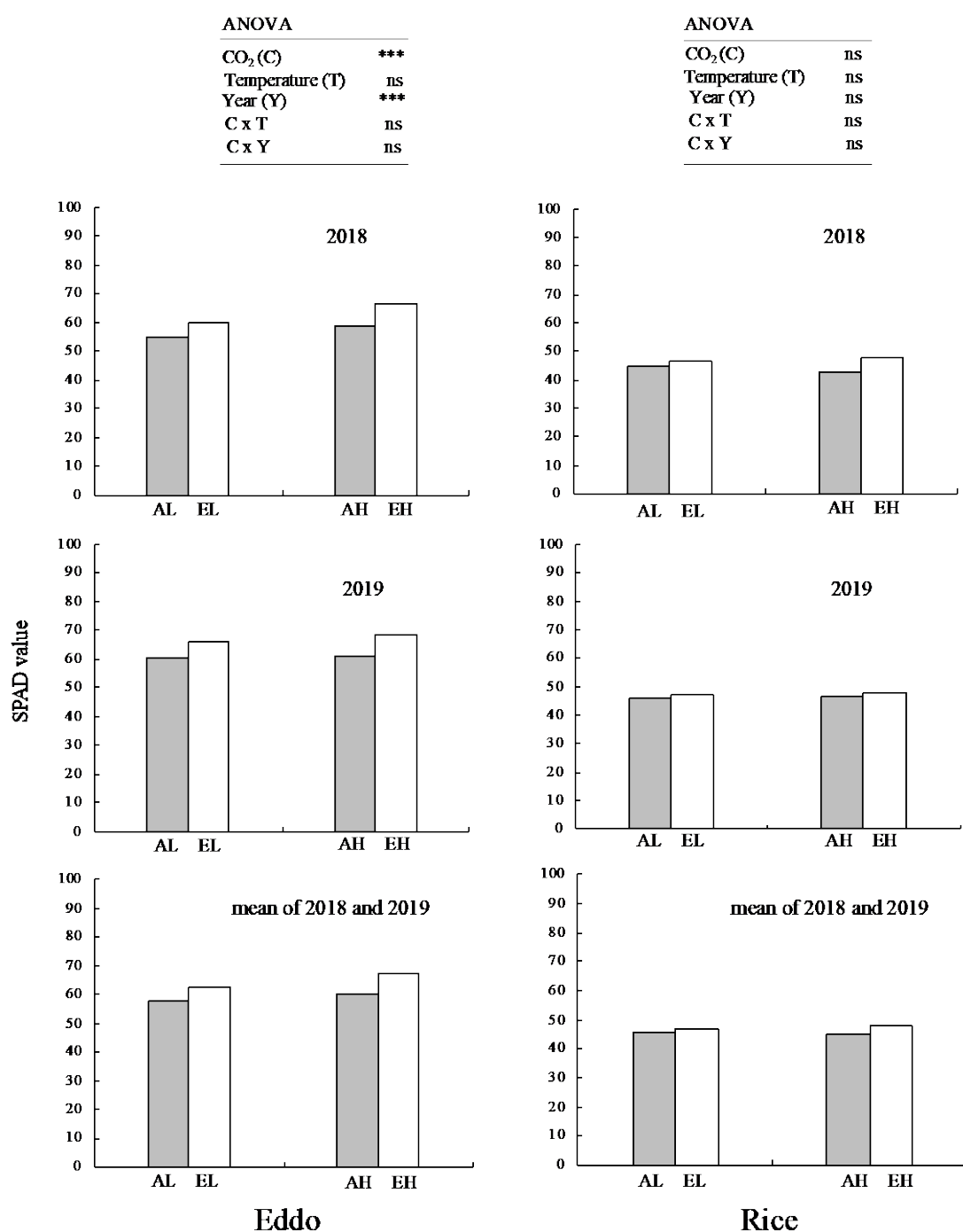


Figure 2.2. Effects of elevated CO₂ concentration ([CO₂]) on SPAD value of a single leaf blade in eddo and rice at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, ns: not significant.

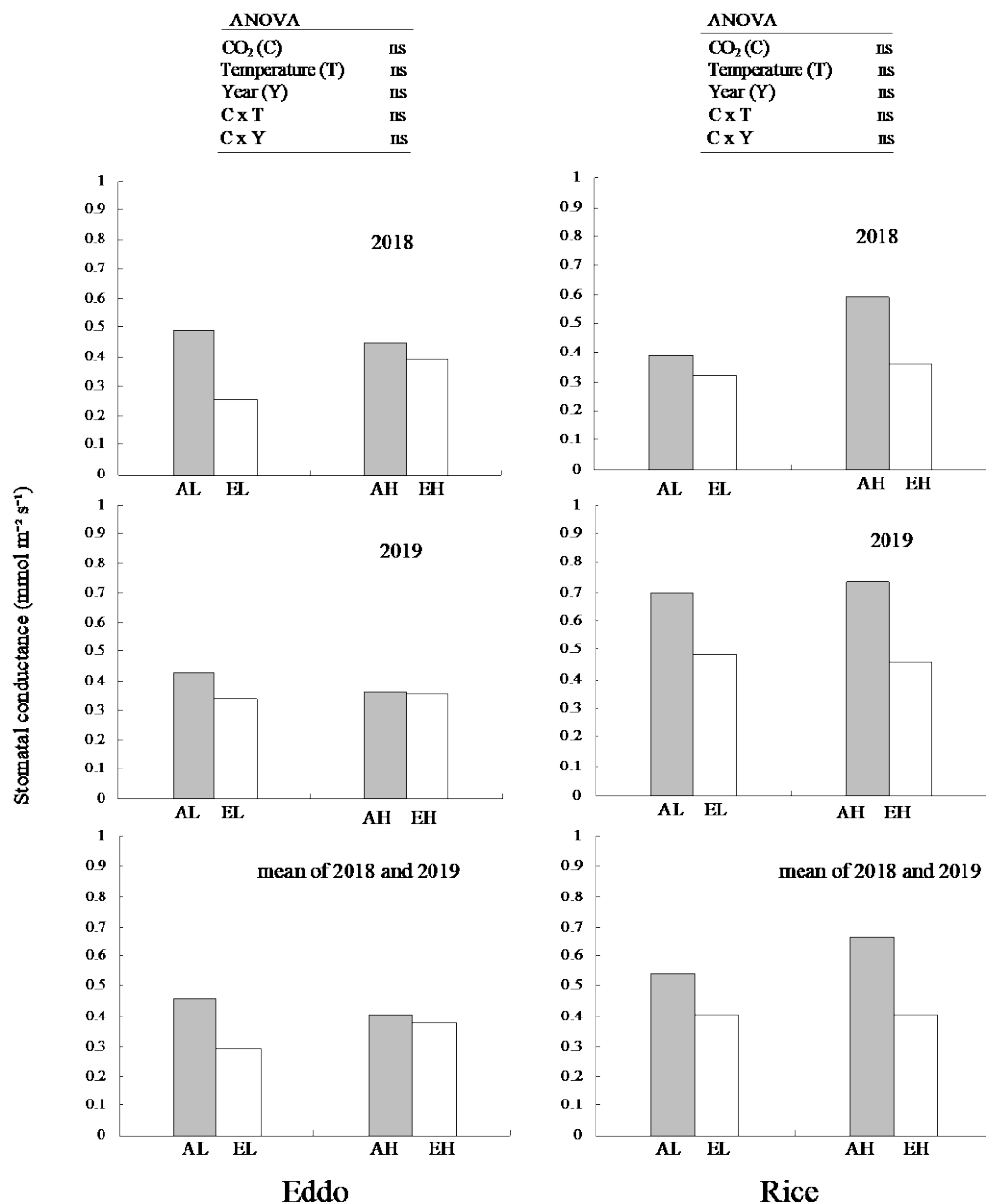


Figure 2.3. Effects of elevated CO₂ concentration ([CO₂]) on stomatal conductance of a single leaf blade in eddo and rice at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ns: not significant.

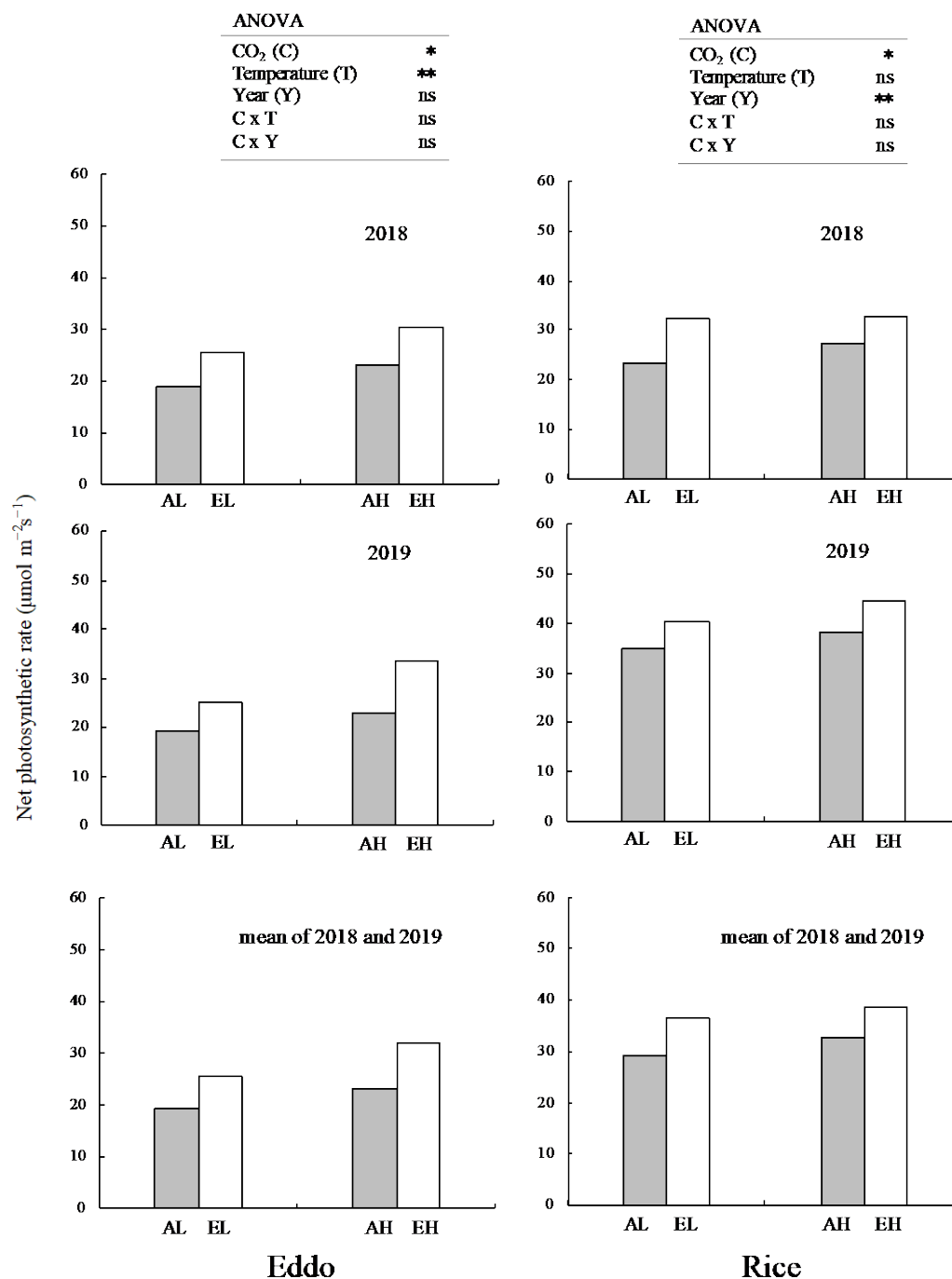


Figure 2.4. Effects of elevated CO₂ concentration ([CO₂]) on net photosynthetic rate of a single leaf blade in eddo and rice at two different air temperatures. AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated **: P < 0.05, *: P < 0.1, ns: not significant.

CHAPTER 3

Effects of elevated atmospheric CO₂ concentration on leaf blades morphology in eddo at two different air temperatures

INTRODUCTION

The atmospheric CO₂ concentration ([CO₂]) is increasing year on year as we burn fossil fuels, which enhances the natural greenhouse effect and warms the planet. It is predicted to continue increasing in the future (IPCC, 2013). If [CO₂] increases, the global air temperature by the end of the 21st century is predicted to rise (IPCC, 2013) as a result of global warming.

The effects of elevated [CO₂] on leaf morphology under different temperatures have been reported in many studies (Croonenborghs et al., 2009; Luomala et al., 2005; Thanh et al., 2018). Elevated [CO₂] stimulates cell division and cell expansion (Ferris et al., 2001; Ferris & Taylor, 1994) which cause thicker leaves (Yin, 2002) with higher numbers of cells or, cell layers and/or, larger cells (Masle, 2000; Radoglou & Jarvis, 1992). Alterations of the relative volumes in intercellular air spaces (Masle, 2000; Oksanen et al., 2001) occur by the elevated [CO₂], and it can also able to change the palisade and spongy mesophyll and vascular elements (Engloner et al., 2003; Lin et al., 2001; Pritchard et al., 1997). Anatomical changes in the mesophyll and vascular elements can alter resistance for [CO₂] diffusion by affecting the gas exchange. This alteration can influence water and assimilate transport that lead to produce extra carbon at elevated [CO₂] (Luomala et al., 2005). Körner & Larcher (1988) reported that the rate of cell division is tightly regulated by temperature and the number of cell divisions involved in the formation of a new leaf is drastically reduced in low temperature.

Stomata mediate CO₂ and water exchange for photosynthesis and transpiration. The changes in the stomatal aperture (length and width of guard cell and stoma) did not show

a clear pattern in response to higher temperature under CO₂ enrichment, which suggests that it is a genotypic character in rose (Pandey et al., 2007). Pandey et al. (2007) reported that significant increases in stomatal density was recorded in plants grown of rose at high temperature over control with CO₂ enrichment. Uprety et al. (2002) reported that the CO₂ enrichment brought about significant reduction in the stomatal density but increases stomata length and width on adaxial side of Pusa Basmati-1, P-677 and P-834 in rice cultivars. Plants grown under elevated [CO₂] decreases stomatal density in *Arabidopsis thaliana* ecotypes and many other species (Hetherington & Woodward, 2003; Woodward, Lake & Quick, 2002), while a meta-analysis of stomatal density responses to elevated [CO₂] showed a little evidence for significant stomatal density reduction (Ainsworth & Rogers, 2007). Therefore, information on the effects of both elevated [CO₂] and temperature on morphology of leaf blade characteristics of plants is important for understanding the reaction of plant growth.

In case of eddo and rice, chapter 2's study demonstrated that positive responses to elevated [CO₂] were shown on the growth and biomass in the early to intermediate vegetative growth stage. Additionally, the total DW and their increasing ratio is more sensitively increased by elevated [CO₂] in eddo than in rice. The number of leaves and leaf area in eddo was significantly increased by elevated [CO₂] while rice leaf area was not. But it is not possible the other leaf morphological factors were proposed to explain how the total DW is more sensitively increased by elevated [CO₂] and its increasing ratio in elevated [CO₂] vs ambient [CO₂] is higher in eddo than in rice. Hence, it is essential to clarify the effects of elevated [CO₂] on leaf morphology in detail.

In this chapter, depend on leaf morphology information under elevated [CO₂] and air temperatures, I aimed at identifying the influences of these climatic factors and their interaction on the leaf internal structure and the morphology of stomata and chloroplast.

Therefore, it is hypothesized that elevated [CO₂] affects the leaf internal structure, and the morphology of stomata and chloroplasts under warmer condition. To test this hypothesis, I observed the leaf morphology of eddo and rice to elevated [CO₂] under two different temperatures. To my knowledge, this is the first study to investigate eddo leaf structure changes at the tissue and subcellular levels in response to elevated [CO₂] under two different temperatures.

MATERIALS AND METHODS

1. Plant materials and growth conditions

This experiment was conducted in temperature-gradient chambers at the Tohoku Agricultural Research Center, NARO (39°74'N, 141°13'E) in Morioka, Japan in the same manner of chapter 2. The details of the cultivation method are described in our previous study chapter 2. In short, we used two dominant cultivars, eddo: Aichiwase, and rice: Hitomebore. The treatment to evaluate the effects of elevated [CO₂] was carried out in the early to intermediate vegetative stage for two time in two temperature-gradient chambers (TGCs) from from June 30 to August 4, 2018 and from June 15 to July 20, 2019.

The TGCs were sunlit greenhouses (6 m wide × 30 m long × 3 m high). Two temperature-gradient chambers were used under two [CO₂] conditions: ambient and elevated (ambient [CO₂] + 200 μmol mol⁻¹ in the daytime (04:00 to 20:00)). In each temperature-gradient chamber, two treatment plots were set along an air temperature gradient. The CO₂ emission rate was set proportional to the air exhaust rate. An infrared gas analyzer (LI-820; LI-COR, Lincoln, NE, USA) was used to monitor the [CO₂] based on the air collected from about 2 m above the ground in the middle of each TGC. The [CO₂], air temperature and relative humidity were recorded at 5 s intervals and the averages for 1 min intervals were calculated by a data logger (CR 1000; Campbell Sci. Inc., Logan,

UT, USA). The average day-time (03:30 to 19:30) [CO₂] over the treatment period were $416 \pm 12 \mu\text{mol mol}^{-1}$ in 2018 and $415 \pm 8 \mu\text{mol mol}^{-1}$ in 2019 in the ambient chamber, and $616 \pm 13 \mu\text{mol mol}^{-1}$ in 2018 and $609 \pm 8 \mu\text{mol mol}^{-1}$ in 2019 in the elevated chamber.

The air temperature and relative humidity were monitored at each location using Pt 100 resistance hygrothermographs with an aspirated double-tube radiation-shield. Five hygrothermographs were set at approximately even intervals along the longitudinal axis of each TGC, and the height of the hygrothermographs was gradually increased so that it was consistent with the maximum plant height. Two plots were set along an air temperature gradient in each TGC. The air temperature and relative humidity of each plot were calculated based on their gradients in each TGC. In the ambient [CO₂] chamber, the low air temperature plot had average air temperature of $23.4^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ in 2018 and $19.0^{\circ}\text{C} \pm 1.6^{\circ}\text{C}$ in 2019, and the high air temperature plot had an average air temperature of $27.7^{\circ}\text{C} \pm 2.6^{\circ}\text{C}$ in 2018 and $23.1^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$ in 2019. In the elevated [CO₂] chamber, the low air temperature plot had average air temperature of $23.5^{\circ}\text{C} \pm 2.6^{\circ}\text{C}$ in 2018 and $19.1^{\circ}\text{C} \pm 1.6^{\circ}\text{C}$ in 2019, and the high air temperature plot had average air temperature of $27.9^{\circ}\text{C} \pm 2.7^{\circ}\text{C}$ in 2018 and $23.2^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$ in 2019.

In the ambient [CO₂] chamber, the relative humidity was $76 \pm 8\%$ and $77 \pm 8\%$ in the low air temperature plot, and $40 \pm 4\%$ and $49 \pm 8\%$ in the higher air temperature plot in 2018 and 2019, respectively. In elevated [CO₂] chamber, the relative humidity was $70 \pm 8\%$ and $75 \pm 11\%$ at low air temperature plot and $45 \pm 5\%$ and $48 \pm 5\%$ in the high air temperature plot in 2018 and 2019, respectively. Solar radiation was measured using sunlight sensors (LP02; TAIYO KEIKI, Tokyo, Japan), which were positioned approximately 5 m above the ground beside the TGCs. Total light transmission of outer cover films (F-CLEANTM clear; AGC Green-Tech Co., Ltd., Tokyo, Japan) for TGCs is 94%. Average day time solar radiation was $193 \text{ Wm}^{-2} \text{ d}^{-1}$ in 2018 and $181 \text{ Wm}^{-2} \text{ d}^{-1}$ in

2019 during the treatment period. Just before the treatment, eddo and rice plants transferred into plastic pots (35 cm height \times 32 cm internal diameter) filled with the same type of soil that used in the seedling period. The plants were suitably watered three to five times every week. In this study, the four treatments were abbreviated as follows:

- a) AL: ambient [CO₂] and low air temperature
- b) EL: elevated [CO₂] and low air temperature
- c) AH: ambient [CO₂] and high air temperature
- d) EH: elevated [CO₂] and high air temperature

For morphological observations, the center parts of the youngest fully expanded leaves in main plants of the eddo and rice in each treatment were collected between 08:00 and 11:00 on the final day of treatment.

2. Observation and measurement of inner tissues of leaf blades

The inner tissues of leaf blades were observed by a bright-field optical microscope as follows. Segments of the center parts of the leaf blades were immediately immersed in 0.05 M sodium phosphate buffer (pH 7.2) containing 2% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde. Then, the samples were washed in 0.05 M sodium phosphate buffer. The materials were post-fixed in 1% (v/v) osmium tetroxide in 0.1 M sodium phosphate buffer at 4°C for 10 h and washed in 0.1 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 0.7 μ m-thick sections using an ultramicrotome (EM UC7; Leica, Germany) and stained with 0.1% (w/v) toluidine blue-O. The sections were observed under a bright-field optical microscope (BX51; Olympus, Japan) and photographed.

From the photographs taken with the bright-field optical microscope, the thickness of

the adaxial epidermis, palisade parenchyma, spongy parenchyma, abaxial epidermis, mesophyll (palisade and spongy cells) cells and whole leaf blade thickness in eddo, and mesophyll cells in rice were determined using the Image J software. Additionally, in rice there were no palisade and sponge cells separately in mesophyll layer. Three photographs per leaf blade; one leaf blade per plant and three plants per treatment were used to investigate these parameters. Totally, eight cells from each photograph were randomly selected to determine the number of chloroplasts.

3. Observation of chloroplasts and starch grains and measurement of the number of chloroplasts and the number of starch grains

Chloroplasts and starch grains were observed by a scanning electron microscope (SEM). First, samples were fixed as described above. Then, 0.7- μ m-thick samples embedded Spurr's resin were stained by EM stainer (Nisshin EM, Japan) and incubated for 30 min and rinsed by distill water. After drying at 60 °C, the samples were stained by lead stain solution (SIGMA-ALDRICH, USA) and keep for 5 min and washed in distilled water. Finally, the stained samples were drying at 60 °C and then coated by carbon with a carbon coater (VC-100, Vacuum Device, Japan). The stained sections were observed and photographed with a SEM at an accelerating voltage of 5 kV.

Based on the images taken by a SEM, the number of chloroplasts per mesophyll cell and the number of chloroplasts per unit profile area of mesophyll cell and the number of starch grains per chloroplast in the palisade and spongy cells in eddo and the mesophyll cells in rice were counted by using the Image J software. Three to four photographs per leaf blade, one leaf blade per plant and three plants per treatment were investigated

4. Observation of stomata density and measurement of stomatal pore length and width

Stomatal density, stomatal pore length and stomatal pore width were measured on the abaxial and adaxial sides of leaf blades using scanning electron micrographs. The leaf blade segments of eddo and rice were fixed immediately in 0.05 M sodium phosphate buffer (pH 7.2) containing 2% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde, and post-fixed in 1% (v/v) osmium tetroxide in 0.1 M sodium phosphate buffer at 4°C for 10 h and washed in 0.1 M sodium phosphate buffer. After washing with 0.1 M sodium phosphate buffer, the leaf segments were dehydrated in a graded ethanol series, immersed in 100% t-butyl alcohol, and then dried using a t-BuOH freeze dryer in 2018 (VFD-21S, Vacuum Device, Japan). The leaf segments were also immersed in 100% isoamyl acetate at 20°C and dried using a critical point dryer in 2019 (JCPD-5; JEOL, Japan). The dried leaf segments were mounted on stubs with conductive carbon tape and coated with platinum using an auto fine coater (JFC-1600, JEOL, Japan). Stomata on the adaxial and abaxial sides of the leaf blade were then observed using a SEM (JSM-7000, JOEL, Japan) at an accelerating voltage of 5 kV. The number of stomata per unit area (mm^{-2}) was investigated, and the stomatal pore length and stomatal pore width were measured with the Image J software. Six photographs per leaf blade, one leaf blade per plant, and three plants per treatment were observed for stomatal density, and all the stomata in each photograph ($150 \times 200 \mu\text{m}$ in size) were counted for eddo and rice. Three to five stomata per photograph for eddo and five to ten stomata per photograph for rice were measured for pore length and width. Eight photographs were randomly taken, and six photographs per leaf blade, one leaf blade per plant, and three plants per treatment were used for measuring the stomatal pore length and width of eddo and rice.

5. Statistical analysis

To test the significance of the effects of growth [CO₂] and temperature on each crop, we conducted an analysis of variance (ANOVA) applying a split-plot design; the main plot was [CO₂] and the subplot was air temperature, with years (2018 and 2019) treated as replications (random effect). In the present study, the statistical significance was evaluated at $\alpha < 0.1$ since replicate number was limited. All statistical analyses were conducted using the general linear model procedure of the SPSS statistical software (SPSS ver. 24.0; IBM Corp, USA).

RESULTS

1. Effects of elevated CO₂ concentration on inner tissues of leaf blades in eddo and rice at two different air temperatures

With a blight-field optical microscope, differences in thickness of internal tissues of leaf blades in transverse sections of eddo (Figure 3.1) and rice (Figure 3.2) were observed in the ambient [CO₂] and elevated [CO₂] treatments at two different air temperatures.

In eddo, the thicknesses of the palisade layer, spongy layer and whole leaf blade were significantly ($P < 0.1$) increased by elevated [CO₂] (Table 3.1). In addition, temperature, year and the [CO₂] \times temperature interaction showed significantly ($P < 0.1$) positive effects on the thicknesses of palisade layer and whole leaf blade (Table 3.1).

In rice, the thicknesses of the mesophyll layer and whole leaf blade were significantly ($P < 0.1$) increased by elevated [CO₂] (Table 3.2). Year also had significant ($P < 0.1$) effects on all parameters related with the leaf blade thickness in rice (Table 3.2).

2. Effects of elevated CO₂ concentration on the number of chloroplasts and the number of starch grains in leaf blades of eddo and rice at two different air temperatures

Differences in the number of chloroplasts and the number of starch grains were observed

by scanning electron microscopy with semi-ultrathin sections in leaves transverse sections of the both eddo (Figures 3.3 and 3.4) and rice (Figure 3.5) in the ambient [CO₂] and elevated [CO₂] treatments at two different air temperatures.

In eddo, the number of chloroplasts per mesophyll cell in palisade layer, number of chloroplasts per unit profile area of mesophyll cell in both palisade and spongy layers and number of starch grains per chloroplast were significantly ($P < 0.1$) increased by elevated [CO₂] (Table 3.3). The all parameters related with the number of chloroplast and starch grains were significantly ($P < 0.1$) increased by high temperature (Table 3.3). The [CO₂] \times temperature interactions showed significant ($P < 0.05$) effects on the number of chloroplasts per mesophyll cell in both palisade and spongy layers and on the number of starch grains in the both layers (Table 3.3). Number of chloroplasts per mesophyll cell in spongy layer was significantly ($P < 0.1$) increased by the [CO₂] \times year interactions (Table 3.3).

In rice, the number of chloroplasts per unit profile area of mesophyll cell and the number of starch grains per chloroplast in mesophyll layer were significantly ($P < 0.1$) increased by elevated [CO₂] and high temperature, separately (Table 3.4).

3. Effects of elevated CO₂ concentration on stomatal morphology in leaf blades of eddo and rice at two different air temperatures

In this study, stomata were observed with a SEM on the both adaxial and abaxial sides of leaf blades in eddo and rice (Figures 3.6 and 3.7).

In eddo, stomatal density on the abaxial side ($P < 0.1$), stomatal pore lengths on the adaxial ($P < 0.1$) and abaxial ($P < 0.05$) sides were significantly increased by elevated [CO₂] (Table 3.5). The stomatal density on the adaxial ($P < 0.01$) and abaxial ($P < 0.05$) sides, stomatal pore length on the abaxial ($P < 0.01$) side, and stomatal pore widths on the

adaxial ($P < 0.05$) and abaxial ($P < 0.01$) sides were also significantly increased by high temperature in eddo (Table 3.5). Year showed no effect on the stomatal parameters in eddo, except for stomatal pore length on the abaxial side (Table 3.5). There were no significantly positive $[\text{CO}_2] \times \text{temperature}$ interactions for all parameters in eddo (Table 3.5).

In rice, the stomatal pore length on the adaxial side and the pore width on the abaxial side were significantly ($P < 0.05$) increased by elevated $[\text{CO}_2]$ (Table 3.6). The stomatal density ($P < 0.01$) on the adaxial side, stomatal pore lengths ($P < 0.1$) and widths ($P < 0.05$) on the both sides were also significantly increased by the effect of high temperature in rice (Table 3.6). Year showed a significant ($P < 0.1$) effect on the stomatal pore length of the adaxial side in rice (Table 3.6). The $[\text{CO}_2] \times \text{temperature}$ interactions had significant positive effects on the stomatal density ($P < 0.05$) of the adaxial side, stomatal pore lengths ($P < 0.1$) of the both sides, and stomatal pore width ($P < 0.1$) of the abaxial side in rice (Table 3.6).

DISCUSSION

Leaves are considered as one of the important plant parts of a plant, because it has direct bearing on photosynthesis and water use efficiency. The response variability of the anatomical characteristics of the leaves to elevated air temperature and $[\text{CO}_2]$ appears to be high (Han et al., 2007). Doubling the atmospheric $[\text{CO}_2]$ stimulated growth for *Guzmania* 'Hilda' shown by an increase in leaf thickness (Croonenborghs et al., 2009). Johns and Hughes (2002) found that leaf thickness increased under elevated $[\text{CO}_2]$ in the high temperature treatment. In this experiment, the results indicated that the whole leaf blade thickness positively increased under elevated $[\text{CO}_2]$ condition in eddo and in rice (Tables 3.1 and 3.2). The whole leaf thickness of eddo also was increased by the high temperature and the $[\text{CO}_2] \times \text{temperature}$ interaction (Table 3.1), whereas the same

parameter in rice did not have significant effects (Table 3.2). Therefore, these results in this study suggest that the whole leaf blade thickness positively responds to elevated [CO₂] under the high air temperature in eddo, but the thickness does not in rice.

In aspen clones, elevated CO₂ increased leaf thickness by increasing spongy mesophyll layer thickness (Oksanen et al., 2001). Radoglou and Jarvis (1992) found that the increase of *Phaseolus vulgaris* leaf thickness was mainly due to an increase in the spongy parenchyma thickness. Thinh et al. (2018) indicated that elevated [CO₂] increased the Chinese yam leaf blade thickness by increasing the thickness of palisade tissue layer. In this study, the thicknesses of both palisade and spongy layers in eddo leaf blade and the thickness of the mesophyll layer in rice leaf blade were significantly greater under elevated [CO₂] whereas the thicknesses of epidermis layers were not (Table 3.1 and 3.2). These results indicated that elevated [CO₂] contributes to increase the thickness of whole leaf blade by the increasing thicknesses of the both mesophyll tissues (i.e., palisade and spongy) in eddo, and by the increasing thickness of the mesophyll tissue in rice.

Plants absorbed light energy drive photosynthetic electron transport through the thylakoid membranes of the chloroplasts (Yamori et al., 2011). Chloroplasts are indispensable for plant development; not only by performing photosynthesis and thus rendering the plant photoautotrophic, but also for biochemical processes (which in some instances can also take place in other plastids types), such as the synthesis of pigments, lipids, and plant hormones and sensing environmental stimuli (Pogson et al., 2015). Thus, the chloroplast has been more important than the other organelles for the particular study. Thomas and Harvey (1983) showed an increase in chloroplast density at high [CO₂] in the leaves of *Zea mays*, *Glycine max* and *Liquidambar styraciflua*. Similarly, Teng et al. (2006) reported that the number of chloroplasts per mesophyll cell was significantly higher in the leaves of *Arabidopsis thaliana* plants grown under elevated [CO₂]. Thinh et al. (2018)

found that the number of chloroplasts per cell was significantly higher in both the palisade and spongy layers but the number of chloroplasts per unit cell profile area was higher only in the palisade layer in Chinese yam grown in elevated [CO₂] under high air temperature condition. In this study, the number of chloroplasts per mesophyll cell in the palisade layer, and the number of chloroplast per unit profile area of mesophyll cell in both layers were higher in elevated [CO₂] than in ambient [CO₂] in eddo (Table 3.3), whereas only the number of chloroplasts per unit profile area of mesophyll cell was higher in rice (Table 3.4). The results suggest that elevated [CO₂] contributes to increase the number of chloroplasts in both eddo and rice. However, the [CO₂] × temperature interactions had significant effects on the number of chloroplasts per mesophyll cell in both palisade and spongy layers in eddo (Table 3.3). On the other hand, the [CO₂] × temperature interaction had no significant effects on the number of chloroplasts per mesophyll cell and number of chloroplasts per unit profile area of mesophyll cell in rice (Table 3.4). Thus, the results in this study suggest that the number of chloroplasts per mesophyll cell is more sensitive in eddo than in rice in elevated [CO₂] under high air temperature condition. Wang et al. (2004) found a concomitant increase in chloroplast number and photosynthesis in *Nicotiana sylvestris* and a close correlation between them. They concluded that the higher rate of photosynthesis was the result of an adjustment of the photosynthetic apparatus, including chloroplast numbers, under higher [CO₂] conditions. Chapter 2's study showed that the mean net photosynthetic rates of 2018 and 2019 were increase in elevated [CO₂] than in ambient [CO₂] in eddo and rice. Therefore, these results suggest that the increase in the number of chloroplasts per mesophyll cell would be one of the factors underlying the increase of photosynthesis under elevated [CO₂] condition.

Changes in starch grains in chloroplasts in elevated [CO₂] depend on many factors such as species, cultivar, developmental age, leaf position and duration of CO₂ exposure

(Sharma et al., 2014). Hao et al. (2013) observed increases in the number of starch grains in chloroplasts in *Isatis indigotica* leaves at elevated [CO₂] compared with ambient [CO₂]. Similarly, Thinh et al. (2018) showed an increase in the number of starch grains per chloroplast in both palisade and spongy cells in leave chloroplasts of Chinese yam in elevated [CO₂] condition. This result agrees with results that the number of starch grains per chloroplast increased both layers in eddo and rice in elevated [CO₂] (Tables 3 and 4). Wolfe et al. (1998) reviewed that the increased starch accumulation in chloroplasts in elevated CO₂ may act as a mechanism for storing carbon and thereby expand sink capacities. Thinh et al. (2017a, 2018) suggested that the increased starch grain accumulation in the chloroplast in elevated [CO₂] may be caused by an increase in the leaf photosynthetic rate in elevated [CO₂]. In this study, the increasing starch grain accumulation in chloroplast in elevated [CO₂] is possibly caused by the increase in the photosynthetic rate in eddo and rice.

Many previous studies (Beerling & Chaloner, 1993; Lin et al., 2001; Madsen, 1973; Teng et al., 2006) have shown a decrease in stomatal density in plant species grown under elevated [CO₂]. Reid et al. (2003) observed a higher stomatal density in 15 species (*Bothriochloa ischaemum*, *Bromus japonicas*, *Convolvulus equitans*, *Eriogonum trichopes*, *Larrea tridentata*, *Lepidium lasiocarpum*, *Liquidambar styraciflua*, *Lonicera japonica*, *Parthenocissus quinquefolia*, *Paspalum pubiflorum*, *Pinus taeda*, *Polygonatum biflorum*, *Solanum dimidiatum*, *Solidago canadensis*, and *Sorghum halepense*) exposed to elevated [CO₂] for 4 years under free air CO₂ enrichment conditions. In this study, stomatal density on the abaxial side was also significantly increased by elevated [CO₂] in eddo (Table 3.5) and that is consistent with the results of Chinese yam exposed under elevated [CO₂] (Thinh et al., 2018). As described above, the response of stomatal density to elevated [CO₂] may depends on a species specificity. In this study, stomatal pore length was significantly

increased by elevated $[\text{CO}_2]$ on the adaxial and abaxial sides in eddo and on adaxial side in rice (Tables 3.5 and 3.6). On the other hand, there was not significant difference of stomatal pore width of leaf blades between ambient $[\text{CO}_2]$ and elevated $[\text{CO}_2]$ on both sides in eddo and on the adaxial side in rice (Tables 3.5 and 3.6). From these data, while the stomatal density of abaxial side increases to in elevated $[\text{CO}_2]$ in eddo, whereas the response of stomatal pore size to elevated $[\text{CO}_2]$ in eddo and rice was not clearly demonstrated. It is generally known that stomatal density and stomatal pore size are important factors for stomatal conductance (Franks & Beerling, 2009). In chapter 2, the stomatal conductance showed a decreasing trend under elevated $[\text{CO}_2]$ in the both crops. So, the relationships between stomatal morphology and stomatal conductance will be needed to investigate more for eddo.

In chapter 2, it was shown that photosynthesis and biomass were increased by elevated $[\text{CO}_2]$, and the elevated $[\text{CO}_2]$ increases the biomass of eddo plants more than the biomass of rice under the analyzed experimental conditions. Therefore, I discussed how the morphological and physiological factors are related with the response of photosynthesis and biomass to elevated $[\text{CO}_2]$ in eddo and rice as below. According to both results of chapter 2 and this chapter, the number of leaves, leaf area, SPAD values, the thickness of the whole leaf blade, number of chloroplasts per mesophyll cell in palisade layer, number of chloroplasts per unit profile area of mesophyll cell both palisade and sponge layers, and stomata density would contribute to increase the photosynthesis and biomass under the elevated $[\text{CO}_2]$ condition in eddo. On the other hands, the number of leaves, the thickness of the whole leaf blade, and number of chloroplasts per unit profile area of mesophyll would contribute to increase photosynthesis and biomass under the elevated $[\text{CO}_2]$ condition in rice. Comparing these results, it was concluded that the higher sensitiveness to elevated $[\text{CO}_2]$ of leaf area, SPAD values and stomata density possibly contribute to the

higher increasing rate of biomass in eddo under this experimental condition.

Table 3.1. Effect of elevated CO₂ concentration ([CO₂]) on the thickness of the adaxial epidermis, palisade layer, spongy layer, abaxial epidermis and whole leaf blade in eddo at two different air temperatures.

Treatments	Adaxial epidermis (µm)	Palisade layer (µm)	Spongy layer (µm)	Abaxial epidermis (µm)	Whole leaf blade (µm)
2018					
AL	24.7	136.9	173.2	23.7	366.6
EL	25.5	163.2	185.5	24.6	405.4
AH	25.7	152.2	180.4	24.2	393.7
EH	25.2	168.3	186.0	25.0	411.3
2019					
AL	25.4	145.9	174.0	24.7	372.9
EL	26.3	176.7	193.6	24.8	423.8
AH	26.0	164.3	196.4	24.5	422.7
EH	27.2	172.3	198.5	24.9	428.0
Mean of 2018 and 2019					
AL	25.0	141.4	173.6	24.2	369.8
EL	25.9	170.0	189.6	24.7	414.6
AH	25.8	158.3	188.4	24.3	408.2
EH	26.2	170.3	192.3	25.0	419.7
ANOVA					
CO ₂ (C)	ns	**	*	ns	***
Temperature (T)	ns	*	ns	ns	*
Year (Y)	ns	*	*	ns	**
C × T	ns	*	ns	ns	*
C × Y	ns	ns	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant

Table 3.2. Effect of elevated CO₂ concentration ([CO₂]) on the thickness of the adaxial epidermis, mesophyll layer, abaxial epidermis and whole leaf blade in rice at two different air temperatures.

Treatments	Adaxial epidermis (μm)	Mesophyll layer (μm)	Abaxial epidermis (μm)	Whole leaf blade (μm)
2018				
AL	7.6	87.1	7.2	101.9
EL	7.7	104.0	7.6	115.1
AH	7.7	93.0	7.4	108.3
EH	8.0	97.8	7.6	114.5
2019				
AL	8.3	96.8	7.8	106.2
EL	8.9	114.7	8.3	128.5
AH	8.7	110.5	8.4	123.2
EH	8.7	116.0	8.3	127.4
Mean of 2018 and 2019				
AL	7.9	92.0	7.5	104.1
EL	8.3	109.3	7.9	121.8
AH	8.2	101.9	7.9	115.7
EH	8.3	106.9	8.0	121.0
ANOVA				
CO ₂ (C)	ns	**	ns	*
Temperature (T)	ns	ns	ns	ns
Year (Y)	*	**	**	*
C \times T	ns	ns	ns	ns
C \times Y	ns	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated **: P < 0.05, *: P < 0.1, ns: not significant

Table 3.3. Effect of elevated CO₂ concentration ([CO₂]) on the number of chloroplasts per mesophyll cell, per unit profile area of mesophyll cells and number of starch grains in eddo at two different air temperatures.

Treatments	Number of chloroplasts per mesophyll cell		Number of chloroplasts per unit profile area of mesophyll cell (mm ⁻²)		Number of starch grains per chloroplast
	Palisade	Spongy	Palisade	Spongy	Palisade + spongy
2018					
AL	9.2	5.0	7737.4	1681.5	1.0
EL	11.4	5.9	8393.9	1844.4	1.3
AH	11.1	5.8	8197.0	1825.9	1.2
EH	11.8	5.8	8601.0	1933.3	1.3
2019					
AL	10.2	5.2	7479.8	1533.3	1.1
EL	12.3	6.8	8096.0	1766.7	1.4
AH	12.4	6.3	7732.3	1863.0	1.3
EH	12.7	6.8	8222.2	1859.3	1.3
Mean of 2018 and 2019					
AL	9.7	5.1	7608.6	1607.4	1.0
EL	11.9	6.3	8244.9	1805.6	1.4
AH	11.8	6.1	7964.6	1844.4	1.3
EH	12.2	6.3	8411.6	1896.3	1.3
ANOVA					
CO ₂ (C)	*	ns	**	*	**
Temperature (T)	**	**	**	*	*
Year (Y)	*	ns	**	*	*
C × T	**	**	ns	ns	**
C × Y	ns	*	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated **: P < 0.05, *: P < 0.1, ns: not significant

Table 3.4. Effect of elevated CO₂ concentration ([CO₂]) on the number of chloroplasts per mesophyll cell, per unit profile area of mesophyll cells and number of starch grains in rice at two different air temperatures.

Treatments	Number of chloroplasts per mesophyll cell	Number of chloroplasts per unit profile area of mesophyll cell (mm ⁻²)	Number of starch grains per chloroplast
2018			
AL	6.8	2489.9	2.5
EL	7.1	2626.8	3.0
AH	7.2	2575.8	2.7
EH	7.8	2671.8	3.1
2019			
AL	7.1	2565.7	2.2
EL	7.2	2659.6	2.8
AH	7.1	2626.3	2.8
EH	7.6	2712.1	3.2
Mean of 2018 and 2019			
AL	6.9	2527.8	2.3
EL	7.2	2642.9	2.9
AH	7.2	2601.0	2.8
EH	7.7	2691.9	3.1
ANOVA			
CO ₂ (C)	ns	*	**
Temperature (T)	ns	**	*
Year (Y)	ns	ns	ns
C × T	ns	ns	ns
C × Y	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated **: P < 0.05, *: P < 0.1, ns: not significant

Table 3.5. Effects of elevated CO₂ concentration ([CO₂]) concentration on stomatal morphology of leaf blades in eddo at two different air temperatures.

Treatments	Stomatal density		Stomatal pore length		Stomatal pore width	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
	(mm ⁻²)	(mm ⁻²)	(μm)	(μm)	(μm)	(μm)
2018						
AA	17.0	20.1	14.1	13.2	3.5	3.3
EA	18.6	25.7	14.9	14.8	3.5	3.4
AH	22.6	25.9	14.8	14.3	3.6	3.6
EH	25.3	33.1	14.9	14.9	3.7	3.6
2019						
AA	24.3	23.7	14.0	13.0	3.4	3.3
EA	28.9	27.0	14.8	14.4	3.6	3.5
AH	29.9	25.4	14.5	14.1	3.5	3.6
EH	38.2	32.3	14.7	14.8	3.7	3.7
Mean of 2018 and 2019						
AL	20.7	21.9	14.1	13.1	3.4	3.3
EL	23.7	26.3	14.8	14.6	3.5	3.5
AH	26.2	25.7	14.6	14.2	3.5	3.6
EH	31.8	32.7	14.8	14.8	3.7	3.6
ANOVA						
CO ₂ (C)	ns	*	*	**	ns	ns
Temperature (T)	***	***	ns	***	*	***
Year (Y)	ns	ns	ns	*	ns	ns
C × T	ns	ns	ns	ns	ns	ns
C × Y	***	ns	ns	ns	ns	ns

AA: ambient [CO₂] and low air temperature, EA: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

Table 3.6. Effects of elevated CO₂ concentration ([CO₂]) on stomatal morphology of leaf blades in rice at two different air temperatures.

Treatments	Stomatal density		Stomatal pore length		Stomatal pore width	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
	(mm ⁻²)	(mm ⁻²)	(μm)	(μm)	(μm)	(μm)
2018						
AL	70.0	58.9	14.1	13.5	2.5	2.3
EL	76.9	66.6	14.9	14.3	2.6	2.6
AH	72.1	64.7	14.6	14.1	2.6	2.7
EH	86.2	66.9	15.1	14.4	2.7	2.8
2019						
AL	81.2	69.6	14.2	14.3	2.5	2.4
EL	82.4	68.1	15.3	14.7	2.6	2.7
AH	84.0	64.7	15.1	14.8	2.5	2.7
EH	90.4	69.6	15.2	15.0	2.7	2.8
Mean of 2018 and 2019						
AL	75.6	64.2	14.1	13.9	2.6	2.3
EL	79.7	67.3	15.1	14.5	2.6	2.6
AH	78.1	64.7	14.9	14.5	2.5	2.7
EH	88.3	68.2	15.1	14.7	2.7	2.8
ANOVA						
CO ₂ (C)	ns	ns	**	ns	ns	**
Temperature (T)	***	ns	*	**	**	**
Year (Y)	ns	ns	*	ns	ns	ns
C × T	**	ns	*	**	ns	*
C × Y	**	ns	ns	ns	*	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

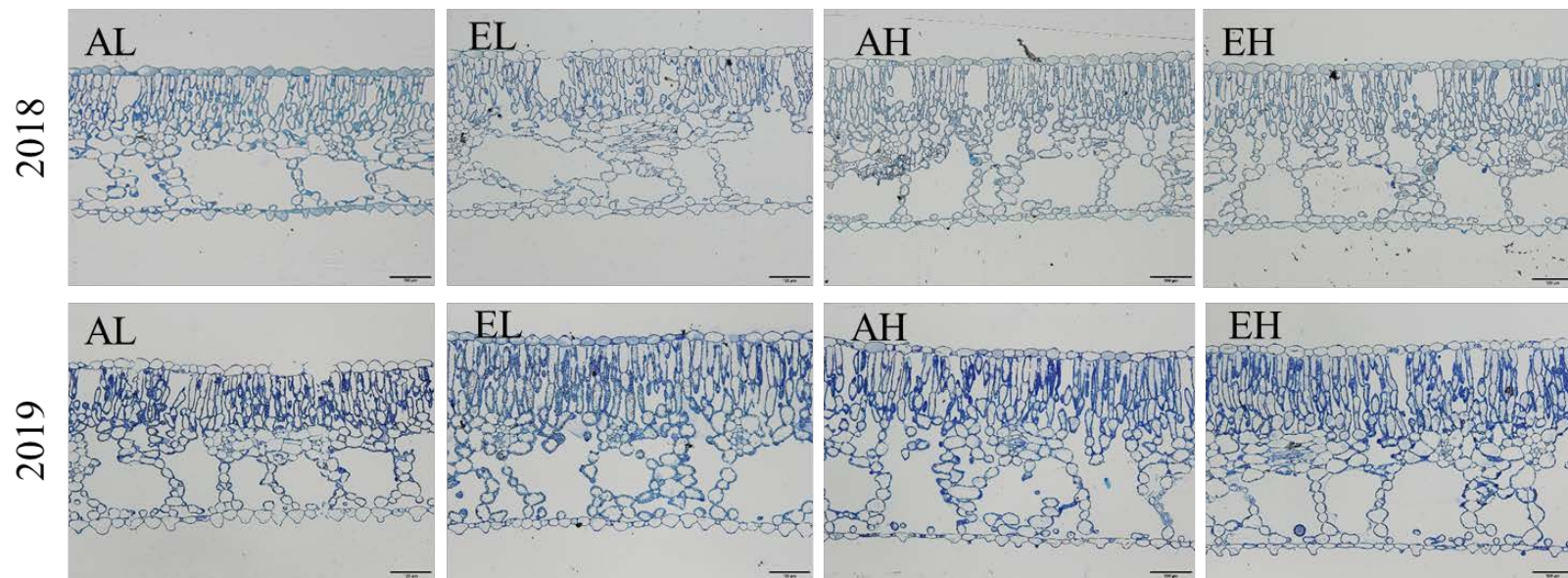


Figure 3.1. Optical micrographs of transverse sections showing the effects of elevated CO_2 concentration ($[\text{CO}_2]$) on the inner structures of leaf blades in eddo at two different air temperatures.

AL: ambient $[\text{CO}_2]$ and low air temperature, EL: elevated $[\text{CO}_2]$ and low air temperature, AH: ambient $[\text{CO}_2]$ and high air temperature, EH: elevated $[\text{CO}_2]$ and high air temperature. Black bars = 10 μm .

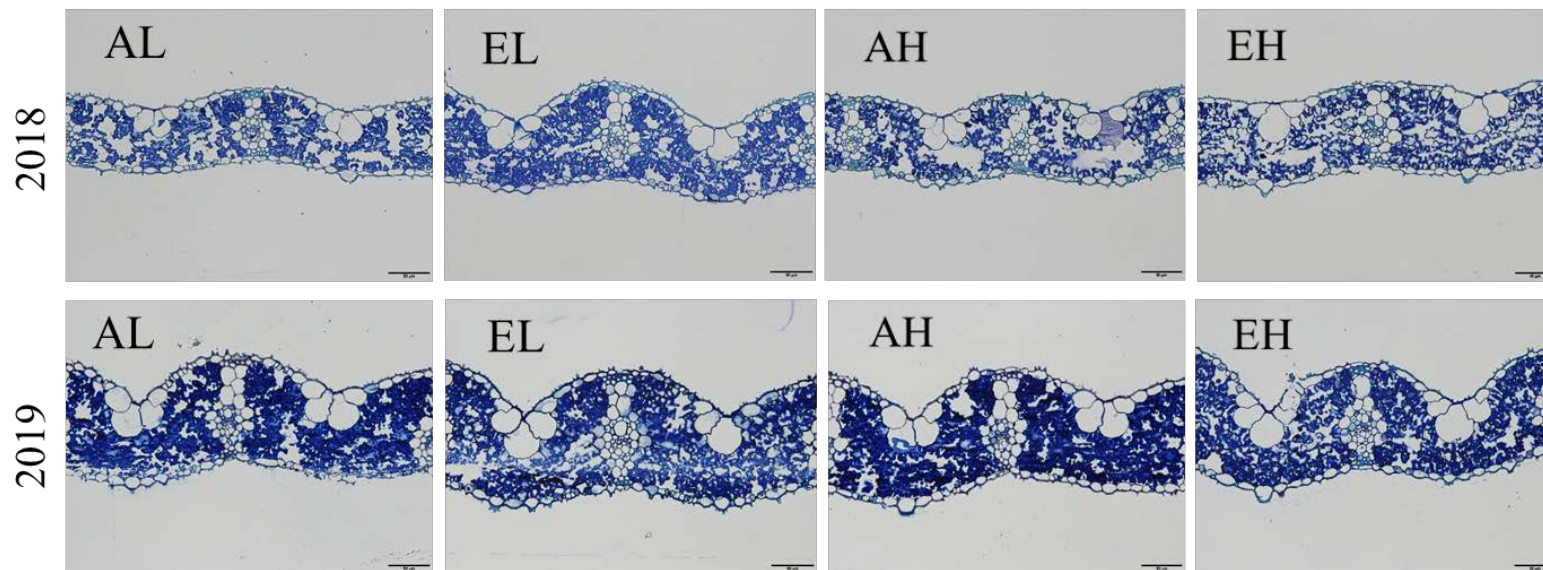


Figure 3.2. Optical micrographs of transverse sections showing the effects of elevated CO₂ concentration ([CO₂]) on the inner structures of leaf blades in rice at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Black bars = 10 μm.

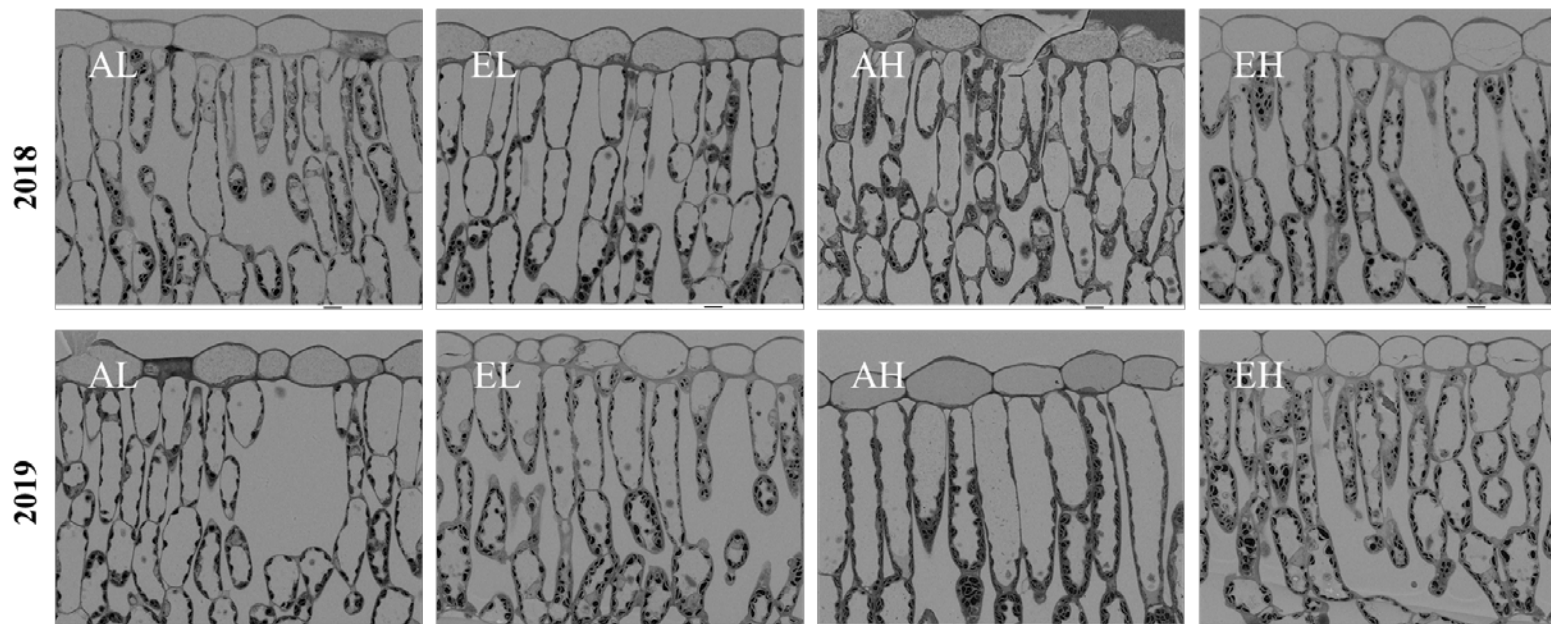


Figure 3.3. Scanning electron micrographs showing the effects of elevated CO₂ concentration ([CO₂]) on number of chloroplasts and starch grains in palisade layer of leaf blades in eddo at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Black bars = 10 μm.

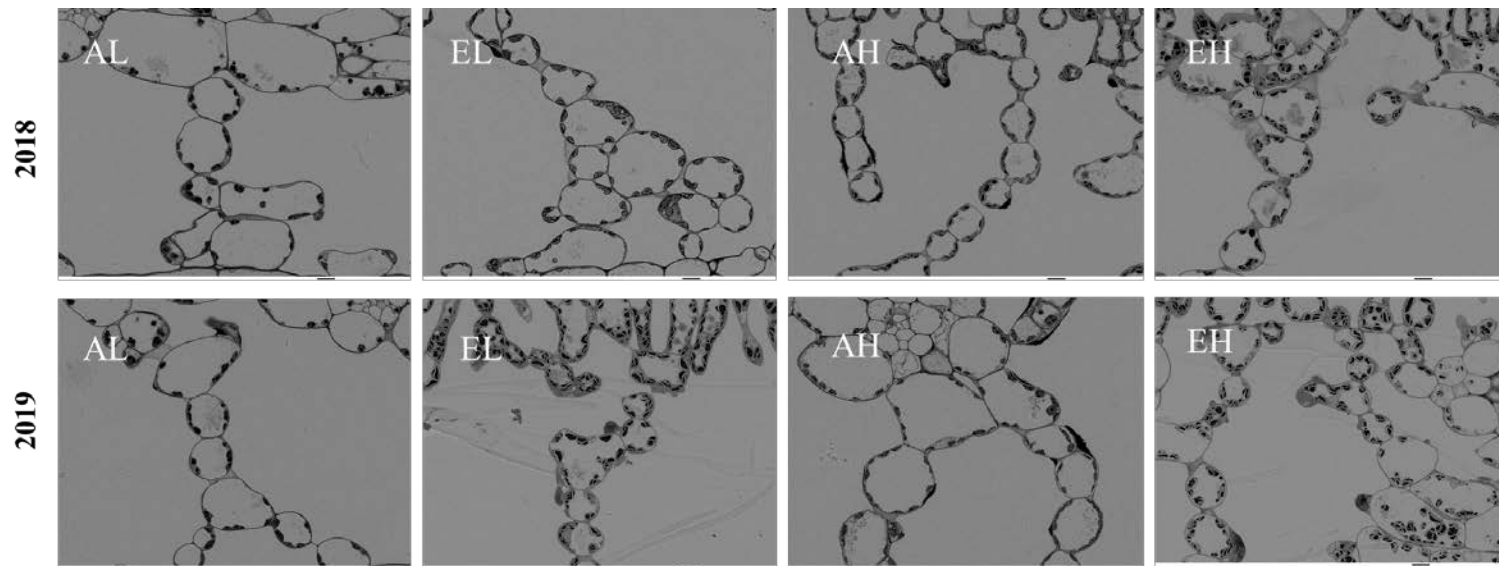


Figure 3.4. Scanning electron micrographs showing the effects of elevated CO₂ concentration ([CO₂]) on number of chloroplasts and starch grains in spongy layer of leaf blades in eddo at two different air temperatures. AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Black bars = 10 μm.

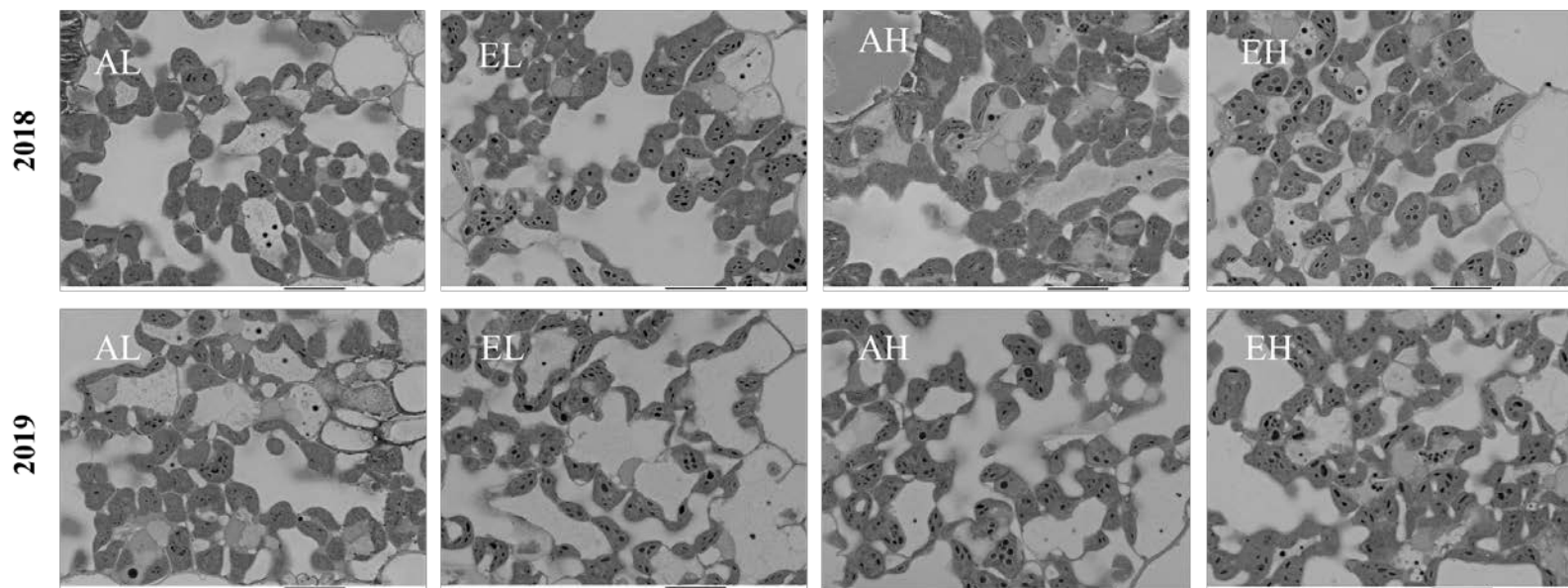
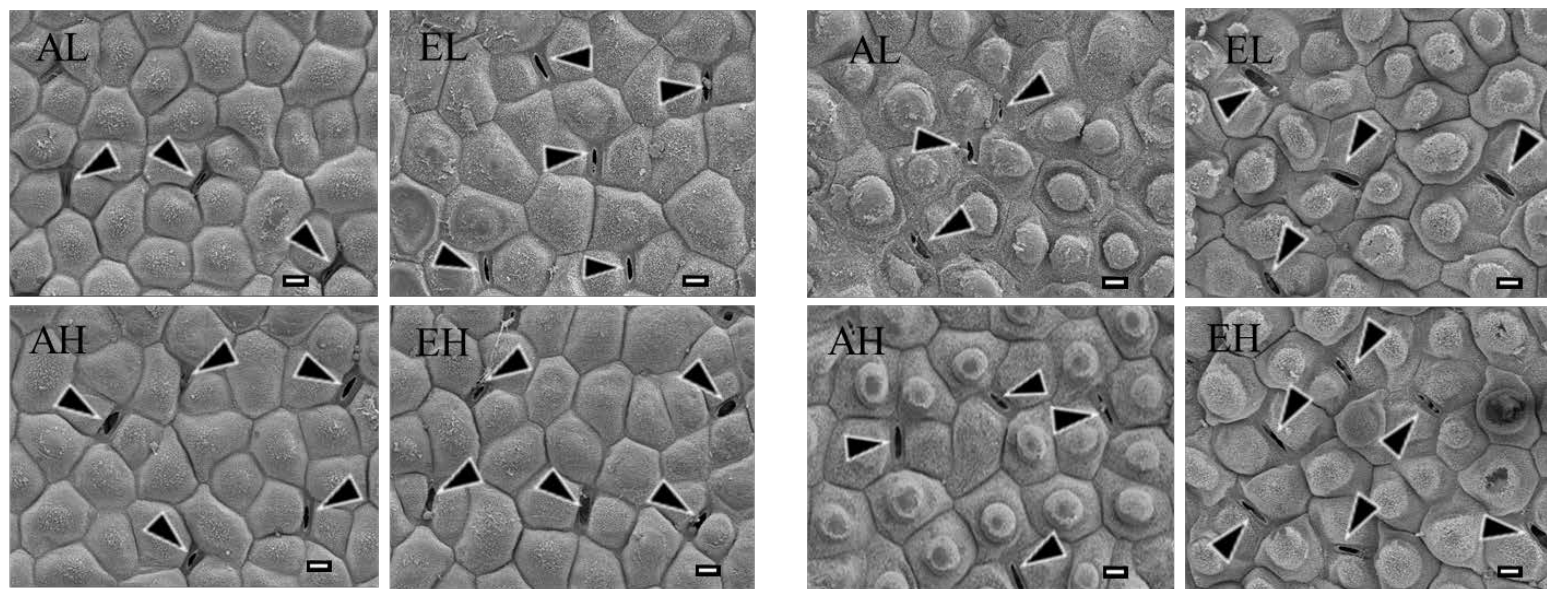


Figure 3.5. Scanning electron micrographs showing the effects of elevated CO₂ concentration ([CO₂]) on number of chloroplasts and starch grains in mesophyll layer of leaf blades in rice at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Black bars = 10 μm.

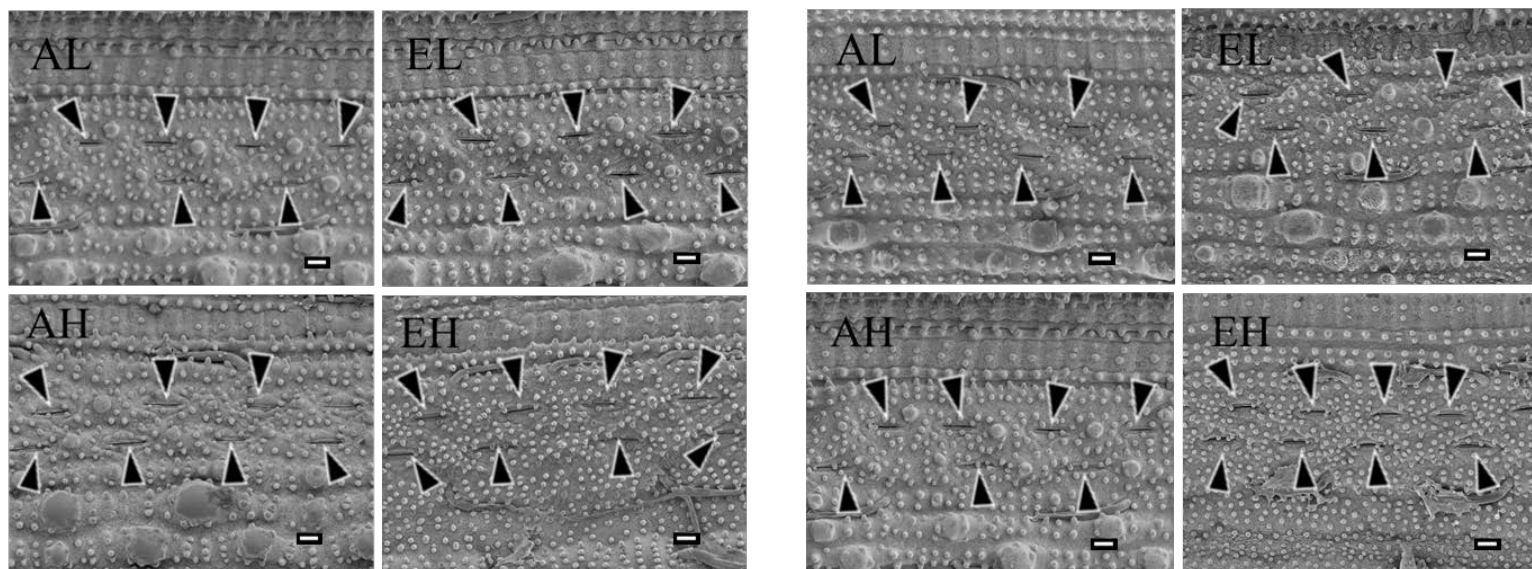


Adaxial side

Abaxial side

Figure 3.6. Scanning electron micrographs showing the effects of elevated CO_2 concentration ($[\text{CO}_2]$) on stomatal morphology on adaxial and abaxial sides of leaf blades in eddo at two different air temperatures.

AL: ambient $[\text{CO}_2]$ and low air temperature, EL: elevated $[\text{CO}_2]$ and low air temperature, AH: ambient $[\text{CO}_2]$ and high air temperature, EH: elevated $[\text{CO}_2]$ and high air temperature. White bars = 10 μm . Arrowheads: stomata. The samples of experiment 2019 were used for these images.



Adaxial side

Abaxial side

Figure 3.7. Scanning electron micrographs showing the effects of elevated CO₂ concentration ([CO₂]) on stomatal morphology on adaxial and abaxial sides of leaf blades in rice at two different air temperatures.

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. White bars = 10 μm. Arrowheads: stomata. The samples of experiment 2019 were used for these images.

CHAPTER 4

Effects of elevated atmospheric CO₂ concentration on the corn germination and early seedling growth in eddo at two different air temperatures

INTRODUCTION

Global atmospheric CO₂ concentration ([CO₂]) has continuously increased from a preindustrial level of 280 $\mu\text{mol mol}^{-1}$, and recently global monthly mean [CO₂] has increased from 410 $\mu\text{mol mol}^{-1}$ in June 2019 to 412 $\mu\text{mol mol}^{-1}$ in June 2020 (NOAA, 2020). Temperature is an important driving factor that influencing crop development rate (Kamkar et al., 2008). Under the 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). Naturally, crop will need to germinate and growth in early seedling stage after the germination under the higher [CO₂] and air temperature in the future.

Seed germination is considered as one of the sensitive stages in the life cycle of plants because it plays a major role in determining the final plant density. Factors such as water, air and temperature are all essential for the influence on germination and seedling emergence. Although many studies have focused on the combined effects of elevated [CO₂] and temperature on the growth in some plant species such as rice (Baker et al., 1992; Cheng et al., 2009; Jing et al., 2016; Nakagawa & Horie. 2000; Roy et al., 2012; Shimono et al., 2008), maize (Mina et al., 2019; Ruiz-Vera et al., 2015), wheat (Cai et al., 2016; Ottman et al., 2012), soybean (Palacios et al., 2019), Chinese yam (Thinh et al., 2017a, 2017b), cassava (Imai et al., 1984) and peanut (Bannayan et al., 2009; Newman et al., 2005), studies related with seed quality in terms of seed germination in elevated [CO₂] under different temperatures were limited. In case of plant seeds, Saha et al. (2015) concluded that under elevated [CO₂]

condition chickpea germination rate decreased by 45– 47%. In contrast, Edwards et al. (2001) showed that elevated [CO₂] increased the seed germination rate of *Hypochoeris radicata* and *Leontodon saxatilis*. On the other hand, in cases of seed corms and seed tubers which are vegetative organ, there is very few information of effects of elevated [CO₂] on germination. Thinh et al. (2017b) indicated that elevated [CO₂] had no effect on the bulbil germination rates of two Chinese yam lines, though air temperature positively affected germination percentage rate. Eddo [*Colocasia esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder], a member of the taro group, is an important edible crop in the Araceae family and is widely cultivated in Asia. Eddo corms are a vegetative organ and usually plant into fields as seed corms for eddo production.

In Chapter 2, it was shown the first to show a positive response of growth, dry weight (DW) and photosynthesis to elevated [CO₂] in eddo at the early to intermediate vegetative stage. However, no attempts have been made to examine the effects of elevated [CO₂] on corm germination and growth during the early seedling stage in eddo. The minimum temperature that can germination for the seed corms is 15°C, and the optimum temperature of growth is 25-30°C while the growth can be completely arrested below 5°C in eddo (Hoshikawa, 1980). Therefore, understanding the effects of elevated [CO₂] on corm germination, seedling growth and seedling biomass will be important for plant establishment and sustainable production of eddo under different air temperature in the future.

Seed corms are a vegetative organ to use for eddo production in eddo. But soybean seed is normal seed for use soybean production. In addition, I use green soybean line and cultivar as targets for comparison in normal seed vs corm seed. Maybe, there is no or few information about effect of elevated [CO₂] on green soybean. Based on the above information in this chapter, it can be made hypothesis whether elevated [CO₂] positively or negatively affects the

germination rate. In addition, I also want to clarify the differences in seedling growth responses to elevated [CO₂] between eddo cultivars and soybean materials. To test this hypothesis, two different types of crops, eddo and soybean, were used. To my knowledge, this is the first temperature-gradient chamber (TGCs) study that investigated corm and seed germination and seedling growth in responses of eddo to elevated [CO₂] under two different temperature.

MATERIALS AND METHODS

1. Plant materials

In this chapter, two eddo cultivars Aichiwase and Dotare, and soybean line Kemame and cultivar Hiden were used to compare the response to elevated [CO₂] under two different air temperatures in two different crops. For the experiments, uniform size eddo seed corms each cultivar were selected. The selected uniform eddo corms 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 corms were sown in plastic containers (28 cm wide × 70 cm long × 25 cm high) and then placed immediately in the chambers for treatment on 14 June 2019 to until 19 July 2019 in Fujisaki satellite field (40°39'N, 140°29'E), Hirosaki University, Aomori, Japan, and 15 June 2019 to until 20 July 2019 at the Tohoku Agricultural Research Center, NARO (39°74'N, 141°13'E) in Morioka, Japan. For soybean, fresh uniform and creak less seeds from each cultivars were sown for first experiment on 15 to 29 June, 2019 and the second experiment 29 June to 13 July, 2019 in (39°74'N, 141°13'E) in Morioka, NARO.

2. Temperature-gradient chambers and treatments in Morioka

The treatment to evaluate the effects of elevated [CO₂] was carried out in in two TGCs at

Morioka. The $[\text{CO}_2]$ and air temperature were controlled separately in each temperature-gradient chamber. In Morioka, two temperature-gradient chambers were used under two $[\text{CO}_2]$ conditions: ambient and elevated (ambient $[\text{CO}_2] + 200 \mu\text{mol mol}^{-1}$).

In each TGC, two treatment plots were set along an air temperature gradient. For experiment of eddo, in the ambient $[\text{CO}_2]$ chamber, low air temperature plot had average air temperature of $19.0^\circ\text{C} \pm 1.6^\circ\text{C}$, and the high air temperature plot had an average air temperature of $23.1^\circ\text{C} \pm 1.7^\circ\text{C}$. In the elevated $[\text{CO}_2]$ chamber, the low temperature plot had average air temperature of $19.1^\circ\text{C} \pm 1.6^\circ\text{C}$, and the high air temperature plot had average air temperature of $23.2^\circ\text{C} \pm 1.7^\circ\text{C}$. In the ambient $[\text{CO}_2]$ chamber, the relative humidity was $77 \pm 8\%$ in the low air temperature plot, and $49 \pm 8\%$ in the high air temperature plot, respectively. In elevated $[\text{CO}_2]$ chamber, the relative humidity was $75 \pm 11\%$ at the low air temperature plot and $48 \pm 5\%$ in the high air temperature plot, respectively. Average day time solar radiation was $181 \text{ Wm}^{-2} \text{ d}^{-1}$ during the treatment period. Thus, I was able to test germination and seedling growth at ambient $[\text{CO}_2]$ and elevated $[\text{CO}_2]$ under two different air temperature regimes. The average daytime $[\text{CO}_2]$ over the treatment period was $415 \pm 8 \mu\text{mol mol}^{-1}$ in the ambient $[\text{CO}_2]$ chamber and $609 \pm 8 \mu\text{mol mol}^{-1}$ in the elevated $[\text{CO}_2]$ chamber.

For first treatment (experiment-1) of soybean, in the ambient $[\text{CO}_2]$ chamber, low air temperature plot had average air temperature of $19.0^\circ\text{C} \pm 1.6^\circ\text{C}$, and the high air temperature plot had an average air temperature of $23.1^\circ\text{C} \pm 1.6^\circ\text{C}$. In the elevated $[\text{CO}_2]$ chamber, the low temperature plot had average air temperature of $19.1^\circ\text{C} \pm 1.6^\circ\text{C}$, and the high air temperature plot had average air temperature of $23.2^\circ\text{C} \pm 1.7^\circ\text{C}$. In the ambient $[\text{CO}_2]$ chamber, the relative humidity was $77 \pm 12\%$ in the low air temperature plot, and $41 \pm 5\%$ in the high air temperature plot, respectively. In elevated $[\text{CO}_2]$ chamber, the relative humidity was $73 \pm 11\%$ at the low

air temperature plot and $46 \pm 6\%$ in the high air temperature plot, respectively. Average day time solar radiation was $160 \text{ Wm}^{-2} \text{ d}^{-1}$ during the treatment period. Thus, we were able to test germination and seedling growth at ambient $[\text{CO}_2]$ and elevated $[\text{CO}_2]$ under two different air temperature regimes. The average daytime $[\text{CO}_2]$ over the treatment period was $418 \pm 5 \mu\text{mol mol}^{-1}$ in the ambient $[\text{CO}_2]$ chamber and $612 \pm 6 \mu\text{mol mol}^{-1}$ in the elevated $[\text{CO}_2]$ chamber.

In second treatment (experiment-2) of soybean, in the ambient $[\text{CO}_2]$ chamber, low air temperature plot had average air temperature of $19.0^\circ\text{C} \pm 1.1^\circ\text{C}$, and the high air temperature plot had an average air temperature of $23.1^\circ\text{C} \pm 1.1^\circ\text{C}$. In the elevated $[\text{CO}_2]$ chamber, the low temperature plot had average air temperature of $19.1^\circ\text{C} \pm 1.1^\circ\text{C}$, and the high air temperature plot had average air temperature of $23.2^\circ\text{C} \pm 1.1^\circ\text{C}$. In the ambient $[\text{CO}_2]$ chamber, the relative humidity was $76 \pm 9\%$ in the low air temperature plot, and $41 \pm 4\%$ in the high air temperature plot, respectively. In elevated $[\text{CO}_2]$ chamber, the relative humidity was $72 \pm 9\%$ at the low air temperature plot and $45 \pm 5\%$ in the high air temperature plot, respectively. Average day time solar radiation was $173 \text{ Wm}^{-2} \text{ d}^{-1}$ during the treatment period. Thus, I was able to test germination and seedling growth at ambient $[\text{CO}_2]$ and elevated $[\text{CO}_2]$ under two different air temperature regimes. The average daytime $[\text{CO}_2]$ over the treatment period was $412 \pm 8 \mu\text{mol mol}^{-1}$ in the ambient $[\text{CO}_2]$ chamber and $606 \pm 8 \mu\text{mol mol}^{-1}$ in the elevated $[\text{CO}_2]$ chamber.

Air temperature and $[\text{CO}_2]$ were measured at 5-s intervals and the averages for 1 min intervals were calculated by a data logger (CR 1000; Campbell Sci. Inc., Logan, UT, USA). In this study, the treatments were set up as described below because plants are generally more sensitive to air temperature changes than relative humidity changes. The four treatments were abbreviated as follows:

a) AL: ambient $[\text{CO}_2]$ and low air temperature

- b) EL: elevated [CO₂] and low air temperature
- c) AH: ambient [CO₂] and high air temperature
- d) EH: elevated [CO₂] and high air temperature.

3. Temperature-gradient chambers and treatments in Fujisaki

The treatment to evaluate the effects of elevated [CO₂] was carried out in two chambers at the Fujisaki. At Fujisaki field, two chambers were used under three condition separately,

- a) Control chamber: Inside of this chamber is managed to maintain the same atmospheric condition as the outside, by not being covered with the plastic film at the front, back and lateral sides and thus always being open. The chamber was a naturally sunlit greenhouse (17 m wide × 20 m long × 5 m high).
- b) Elevated [CO₂] and high temperature chamber: A pipe chamber with the same size and structure as higher by 300 $\mu\text{mol mol}^{-1}$ compare with the outside, while the humidity of the inside is managed to be almost the same as the outside by enough ventilation. At the same time, air conditioner runs to keep the inside air temperature to be always higher by 3°C than the outside air temperature. For this purpose, one kerosene-burning CO₂ supplier and two heat pumps are equipped in addition to the equipment's of high temperature chamber.

In each chamber, one treatment plot was set. The control chamber had average air temperature of $18.6^{\circ}\text{C} \pm 3.7^{\circ}\text{C}$. In the elevated [CO₂] and high air temperature chamber had average air temperature of $21.6^{\circ}\text{C} \pm 3.9^{\circ}\text{C}$. The average daytime [CO₂] over the treatment period was $422 \pm 25 \mu\text{mol mol}^{-1}$ in the control chamber, and $736 \pm 205 \mu\text{mol mol}^{-1}$ in the elevated [CO₂] and high air temperature chamber. In the control chamber, the relative humidity was $81 \pm 14\%$. In the elevated [CO₂] and high air temperature chamber, the relative humidity was $76 \pm 15\%$ in the. Air temperature and [CO₂] were measured at 5-s intervals and the

averages for 10 min intervals were calculated by a data logger (CR 1000; Campbell Sci. Inc., USA). The three treatments were abbreviated as follows:

- a) AL: ambient [CO₂] and low air temperature
- b) EH: elevated [CO₂] and high air temperature.

4. Measurement of germination percentage of eddo seed corm and soybean seed

The corm and seed germination percentages were determined as follows: in each treatment, eddo corms were divided into six groups (two containers) each with 15 seed corms for each eddo cultivar (30 seed corms per cultivar). Germination of the corms was recorded at 12, 15, 19, 22, 26, 29 days after sowing (DAS). In soybean, seeds were divided into sixteen groups (two containers) each with 56 seeds for each soybean material (112 seeds per cultivar). Germination of the seed was recorded at 8, 12, 15 days after sowing (DAS). When the corm and seed sprouted and emerged above the soil surface, it was considered to have germinated. The number of germinated corm and seed in each group and treatment were recorded separately during the monitoring period. The germination percentage was calculated using the following formula:

$$\text{Germination percentage} = S/T \times 100,$$

where S is the number of germinated seed corms or seed and T is the total number of seed corms or seed.

5. Sampling and measuring seedling growth parameters

In eddo, the seedlings of the two eddo cultivars were sampled at 7 days after germination each seed corm. The number of leaves on each plant was counted and then the shoot length was measured using a ruler. After carefully washing the soil from the roots with running tap water,

first the number of roots was counted and then the shoot (with leaf), seed corms of individual plants in each treatment were separately sampled. In soybean, after carefully washing the soil from the roots with running tap water, the stem, shoot and root lengths were measured using a ruler. Finally, all samples were dried at 80°C for 4 d to a constant weight and measured dry weight (DW) separately and then the total DW was measured with using an electronic scale (GX 3000; A&D., Tokyo, Japan). In case of soybean, twenty individual seedlings (in two plastic containers per cultivar) per treatment were randomly selected to measure for the seedling growth and DW parameters.

6. Statistical analysis

For soybean, to test the significance of the main and interaction effects of experimental factors, an analysis of variance (ANOVA) was performed. In the split-plot design main plot was [CO₂], the subplot was air temperature and interaction of two factors, which was replicated twice. In the present study, the statistical significance was evaluated at $\alpha < 0.1$ since replicate number was limited. All statistical analyses were conducted using the general linear model procedure of the SPSS statistical software (SPSS ver. 24.0; IBM, New York, NY, USA). However, in eddo significance tests for the all parameters were not analysed, only mean data were presented here because the statistic replication is not possible by different treatments between Morioka and Fujisaki in split-plot design. In the scatter plot, the correlation and regression for different conditions were presented.

RESULTS

1. Effects of elevated CO₂ concentration on germination of seed corm in eddo and seed in soybean at two different air temperatures

In eddo, at first, the effects of elevated [CO₂] and temperature on germination were observed at 9 DAS, but there was no germinated corm in almost all of the plants. The germination percentage increased as the day passed from 15 DAS to 29 DAS (Table 4.1). Finally, the range of germination percentage was 83.3–90.0% for Aichiwase and 76.7–86.7% for Dotare in the experiment at Morioka at 29 DAS (Table 4.1). In the experiment at Fujisaki, the range of germination percentage was 93.3% in Aichiwase and 70.0–83.3% in Dotare (Table 4.1). At each observation of final germination percentage at 29 DAS, Aichiwase germination percentage was higher in elevated [CO₂] under low air temperature than under high air temperature but Dotare germination percentage is higher in elevated [CO₂] under high air temperature than under low air temperature in Morioka (Table 4.1). However, in Fujisaki, Aichiwase germination percentage was higher in ambient [CO₂] under low air temperature and it was also higher in elevated [CO₂] under high air temperature, but Dotare germination percentage is higher in elevated [CO₂] under low air temperature than under high air temperature (Table 4.1). In eddo, Aichiwase had a negative correlation ($R^2=0.674$) between the germination percentage and temperature in ambient [CO₂] condition, but Dotare did not have the high correlation (Figure 4.1). However, in elevated [CO₂] condition, both eddo cultivars did not have high correlation between the germination percentage and temperature (Figure 4.1).

In soybean, according to the ANOVA results (Table 4.2), significantly ($P < 0.05$) negative effects of elevated [CO₂] on seed germination were detected at 8 DAS. There was also significant ($P < 0.05$) interaction between two experiments at 8 DAS (Table 4.2). The range of germination percentage at 15 DAS was 83.9–94.6% in Hiden and 87.5–98.2% in Kemame in experiment-1, and 85.7–98.2% in Hiden and 92.9–98.2% in Kemame in experiment-2 (Table 4.2). However, there were no significant effects of elevated [CO₂], temperature and their

interactions on the seed germination at 12 and 15 DAS (Table 4.2).

2. Effects of elevated CO₂ concentration on seedling growth at two different air temperatures

According to the average growth data in Aichiwase in experiment at Morioka, the number of leaves was the higher AL and AH treatments than in EL and EH treatments, respectively. The shoot length in AH treatment and number of roots in AL treatment were the highest in Aichiwase, whereas the highest mean values of the all parameters were observed in EH treatments for Dotare at Morioka (Table 4.1). At Fujisaki, the mean value of the number of roots in EH treatment was higher but shoot length in AL treatment was higher in Aichiwase. The mean values of all parameters in Dotare were higher in EH treatment than in AL treatment (Table 4.1).

According to the ANOVA results in soybean (Table 4.3), there were no significant effects of elevated [CO₂] and temperature on the stem length and root length, but only temperature had significant ($P < 0.05$) effects on shoot length in both soybean materials. Plant materials had also significant ($P < 0.1$) effects root length, but both experiments had also significant ($P < 0.1$) effects on stem and shoot length in both soybean materials (Table 4.3).

3. Effects of elevated CO₂ concentration on seedling dry weight at two different air temperatures

According to the eddo average DW values in experiment at Morioka (Table 4.4), DWs of shoot in AH treatment, root in AH treatment, total plant in AH treatment and seed corm in EL treatment were the highest values among the treatments in Aichiwase, respectively. In Dotare, mean values of shoots, roots, total plant and seed corm DW in EH treatment were the highest values among the treatments, respectively. In the experiment at Fujisaki, EH treatment in eddo

showed higher mean values on the shoots, roots and total DWs in Aichiwase, except for seed corm DWs. In Dotare, EH treatment showed higher mean values on the roots, total DWs, (Table 4.4). Total plant DW of Aichiwase showed a positive correlation ($R^2=0.819$) with temperatures in ambient $[CO_2]$ but there was no clear relationship in the elevated $[CO_2]$ in Aichiwase (Figure 4.2). However, total plant DW of Dotare showed a positive correlation with temperature in ambient $[CO_2]$ condition ($R^2=0.659$) and in elevated $[CO_2]$ condition ($R^2=0.783$) (Figure 4.2).

In both soybean materials, ANOVA uncovered no significant effects of elevated $[CO_2]$ on all DW parameters (Table 4.5). However, high temperature had significant ($P < 0.1$ or 0.01) effects on almost all DW parameters in both soybean materials (Table 4.5). Materials had also significant effects on the stem DW and shoot DW (Table 4.5). However, there was no $[CO_2] \times$ temperature interactions on all DW parameters (Table 4.5).

DISCUSSION

Earth's temperature is increasing globally with the increased $[CO_2]$, and it is important to determine the likely effects of elevated $[CO_2]$ and air temperature on seed germination, seed and crop production under present and future climate conditions. However, limited number of studies have been reported on the direct effects of elevated $[CO_2]$ on seed germination under different air temperatures. Particularly, there is only one journal thesis (Thin et al., 2017b) about the effects of elevated $[CO_2]$ on the germination of vegetation organ such as seed corms and seed tubers. Total germination percentage were stimulated by elevated $[CO_2]$ under high temperature in seeds of maternal lines of *Plantago lanceolata* (Wulff & Alexander, 1985). Similarly, doubling the CO_2 concentration resulted in an increase in the final percentage of

seed germination, for *Medicago sativa*, *Amaranthus hybridus* and *Chenopodium album* (Ziska & Bunce, 1993). In contrast with these results, elevated [CO₂] did not influence seed emergence percentage in red kidney bean either at low or high temperatures (Thomas et al., 2009). On the other hand, in case of seed tuber which is vegetative organ, Thinh et al. (2017b) indicated that elevated [CO₂] did not affect the bulbil germination percentage of two Chinese yam lines, although air temperature positively affected the final germination percentage. In two eddo cultivars of the experiment at Morioka in this study, the mean values of germination percentage were higher in elevated [CO₂] than in ambient [CO₂] at both low- and high-temperatures. The results suggested that the germination percentage of seed corms in eddo showed an increasing trend in elevated [CO₂] in Morioka. The mean values of germination percentage were the highest in elevated [CO₂] under high temperature in Dotare in Morioka (Table 4.1). Therefore, effects of elevated [CO₂] might be grater under the high temperature than the lower temperature in Dotare cultivar. On the other hand, in soybean, elevated [CO₂] and temperature had no significant effects on the seed germination percentage, separately (Table 4.2). In the future, more studies are needed to confirm about the effects of elevated [CO₂] on the seed corms germination at two different air temperature with the repeated testing at the same place under similar conditions.

White birch (*Betula pendula* Roth) has a limited ability to increase its biomass and secondary compounds simultaneously in both leaves and stems when the seedlings were grown under elevated CO₂ and temperature (Kuokkanen et al., 2001). The stimulatory effect of CO₂ was the greatest on root and stem DW, which doubled. The total plant DW increased by 77% at early growth of mangosteen (*Garcinia mangostana* L.) (Downton et al. 1990). Kuokkanen et al. (2004) indicated that elevated [CO₂] and temperature increased the plant

height and shoot biomass of silver birch (*Betula pendula* Roth) seedlings. In case of tuber crop, Thinh et al. (2017b) showed that the leaf number, leaf area and root length were higher to elevated [CO₂] under the high temperature regimes in seedlings of Chinese yam line Enshikei 6. Kuokkanen et al. (2001) suggested that increasing either temperature or CO₂ alone may affect herbivory more than a combination of increased CO₂ and temperature. In chapter two, the results also demonstrate that positive responses to elevated [CO₂] are shown on the growth and biomass in the early to intermediate vegetative growth stage in eddo as well as in rice. The results of current study demonstrated that the number of leaves, shoot length and the number of roots, and all most all DWs are clearly the highest in elevated [CO₂] under high air temperature in Dotare (Tables 4.1 and 4.4). These all mean data of Dotare are higher in elevated [CO₂] than in ambient [CO₂] under high temperature at two sites (Tables 4.1 and 4.4). But, effects of elevated [CO₂] and temperature are not clear on seedling growth and DW parameters in Achiwase (Table 4.1 and 4.4). Additionally, Dotare also showed a positive correlation between total DW and temperature in elevated [CO₂] ($R^2=0.783$) and ambient [CO₂] ($R^2=0.659$) condition (Figure 4.2). So, it is suggested that Dotare seedlings may has positive sensitivity to elevated [CO₂] under high temperature, and there is a great difference of the response to elevated [CO₂] between Achiwase and Dotare. In case of soybean materials, elevated [CO₂] had no effects on the growth and DW parameters. However, more studies are also needed to confirm about the effects of elevated [CO₂] on the seedling growth and DW for eddo at two different air temperatures with the repeated testing at the same place under similar conditions in the future.

Table 4.1. Effects of elevated CO₂ concentration ([CO₂]) on the germination percentage of seed corm, number of leaves, shoot length and number of roots in eddo at two different air temperatures.

Germ., number of leaves, shoot length and number of roots in eudc at two different air temperatures.							
Cultivars	Treatments	Germination Percentage (%)			No. of leaves (plant ⁻¹)	Shoot length (plant ⁻¹)	No. of roots (plant ⁻¹)
		15 DAS	22 DAS	29 DAS			
Morioka							
Aichiwase	AL	53.3	80.0	86.7	1.2	6.8	19.2
	EL	63.3	90.0	90.0	1.0	6.0	16.5
	AH	60.0	83.3	83.3	1.2	7.2	18.3
	EH	80.0	86.7	86.7	1.1	6.4	17.5
Dotare							
Dotare	AL	50.0	76.7	76.7	1.5	10.2	35.5
	EL	60.0	80.0	80.0	1.3	6.0	27.0
	AH	60.0	76.7	76.7	1.6	8.1	29.2
	EH	66.7	83.3	86.7	2.0	11.2	37.0
Fujisaki							
Aichiwase	AL	44.0	90.0	93.3	1.3	5.2	15.1
	EH	46.7	90.0	93.3	1.3	4.8	19.0
Dotare							
Dotare	AL	25.0	80.0	83.3	1.0	5.3	20.3
	EH	33.3	70.0	70.0	1.4	7.4	23.6

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature.

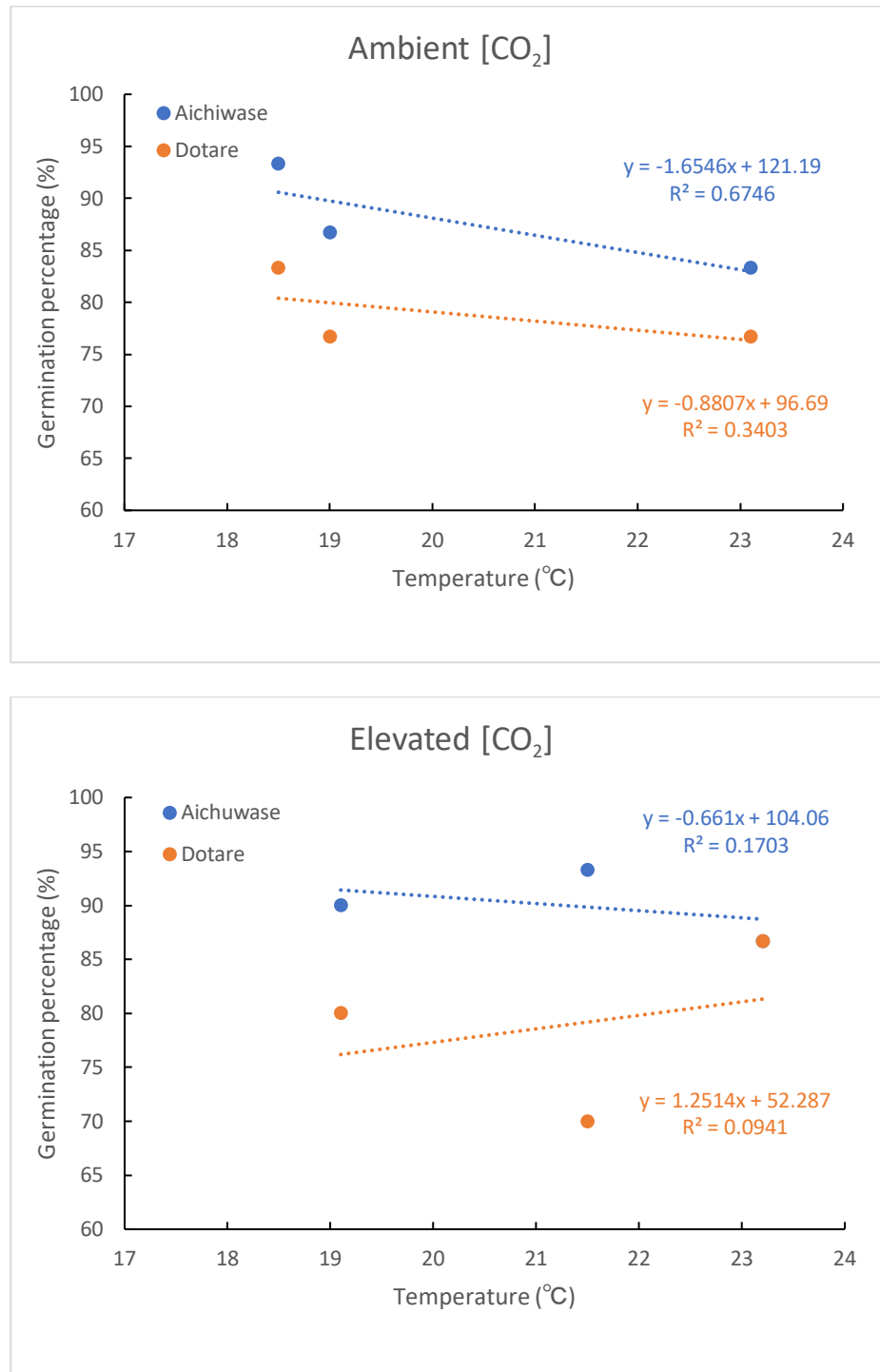


Figure 4.1. Relationships between [CO₂] and air temperatures on germination in eddo.

Table 4.2. Effects of elevated CO₂ concentration ([CO₂]) on the germination percentage of soybean seed at two different air temperatures.

Materials	Treatments	Seed germination percentage (%)		
		8 DAS	12 DAS	15 DAS
Experiment-1				
Hiden	AL	78.6	92.9	94.6
	EL	76.8	91.1	92.9
	AH	75.0	83.9	83.9
	EH	67.8	82.1	83.9
Kemame	AL	71.4	85.7	89.3
	EL	67.8	92.9	94.6
	AH	91.1	94.6	98.2
	EH	75.1	78.6	87.5
Experiment-2				
Hiden	AL	96.4	96.4	98.2
	EL	82.1	82.1	85.7
	AH	94.6	94.6	94.6
	EH	87.5	87.5	87.5
Kemame	AL	94.6	94.6	94.6
	EL	98.2	98.2	98.2
	AH	94.6	94.6	94.6
	EH	92.9	92.9	92.9
ANOVA	CO ₂ (C)	**	ns	ns
	Temperature (T)	ns	ns	ns
	Materials (M)	ns	ns	ns
	Experiment (E)	**	ns	ns
	C × T	ns	ns	ns
	C × M	ns	ns	ns
	C × E	ns	ns	ns
	C × T × M	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated **: P < 0.05, ns: not significant.

Table 4.3. Effects of elevated CO₂ concentration ([CO₂]) on stem length, shoot length and root length in soybean at two different air temperatures.

Materials	Treatments	Stem length (cm plant ⁻¹)	Shoot length (cm plant ⁻¹)	Root length (cm plant ⁻¹)
Experiment-1				
Hiden	AL	10.7	15.2	11.8
	EL	10.6	15.1	12.0
	AH	11.3	15.9	11.2
	EH	10.2	14.2	12.1
Kemame	AL	10.2	13.8	11.2
	EL	9.6	13.3	11.2
	AH	13.0	17.3	11.5
	EH	10.7	13.6	8.5
Experiment-2				
Hiden	AL	11.3	16.9	13.7
	EL	14.3	15.7	14.9
	AH	13.0	19.1	10.9
	EH	11.1	17.9	16.9
Kemame	AL	14.1	17.0	10.6
	EL	9.9	16.9	9.4
	AH	13.5	19.7	14.2
	EH	13.6	18.6	10.2
ANOVA	CO ₂ (C)	ns	ns	ns
	Temperature (T)	ns	**	ns
	Materials (M)	ns	ns	*
	Experiment (E)	*	*	ns
	C × T	ns	ns	ns
	C × M	ns	ns	*
	C × E	ns	ns	ns
	C × T × M	*	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated **: P < 0.05, *: P < 0.1, ns: not significant.

Table 4.4. Effects of elevated CO₂ concentration ([CO₂]) on shoot DW, root DW, total DW and corm DW in eddo at two different air temperatures.

Corn DW in cobs at two different air temperatures.					
		Shoot	Root	Total	Seed corm
Cultivars	Treatments	(g plant ⁻¹)	(g plant ⁻¹)	(g plant ⁻¹)	(g plant ⁻¹)
Morioka					
Aichiwase	AL	0.30	0.12	0.39	14.50
	EL	0.25	0.13	0.35	14.81
	AH	0.31	0.15	0.42	14.25
	EH	0.27	0.14	0.40	13.61
Dotare					
Aichiwase	AL	0.31	0.12	0.44	5.61
	EL	0.28	0.18	0.43	5.89
	AH	0.26	0.18	0.46	5.77
	EH	0.44	0.20	0.64	6.38
Fujisaki					
Aichiwase	AL	0.29	0.08	0.36	15.12
	EH	0.33	0.20	0.45	12.43
Dotare					
Aichiwase	AL	0.30	0.10	0.40	6.73
	EH	0.28	0.22	0.46	5.75

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature.

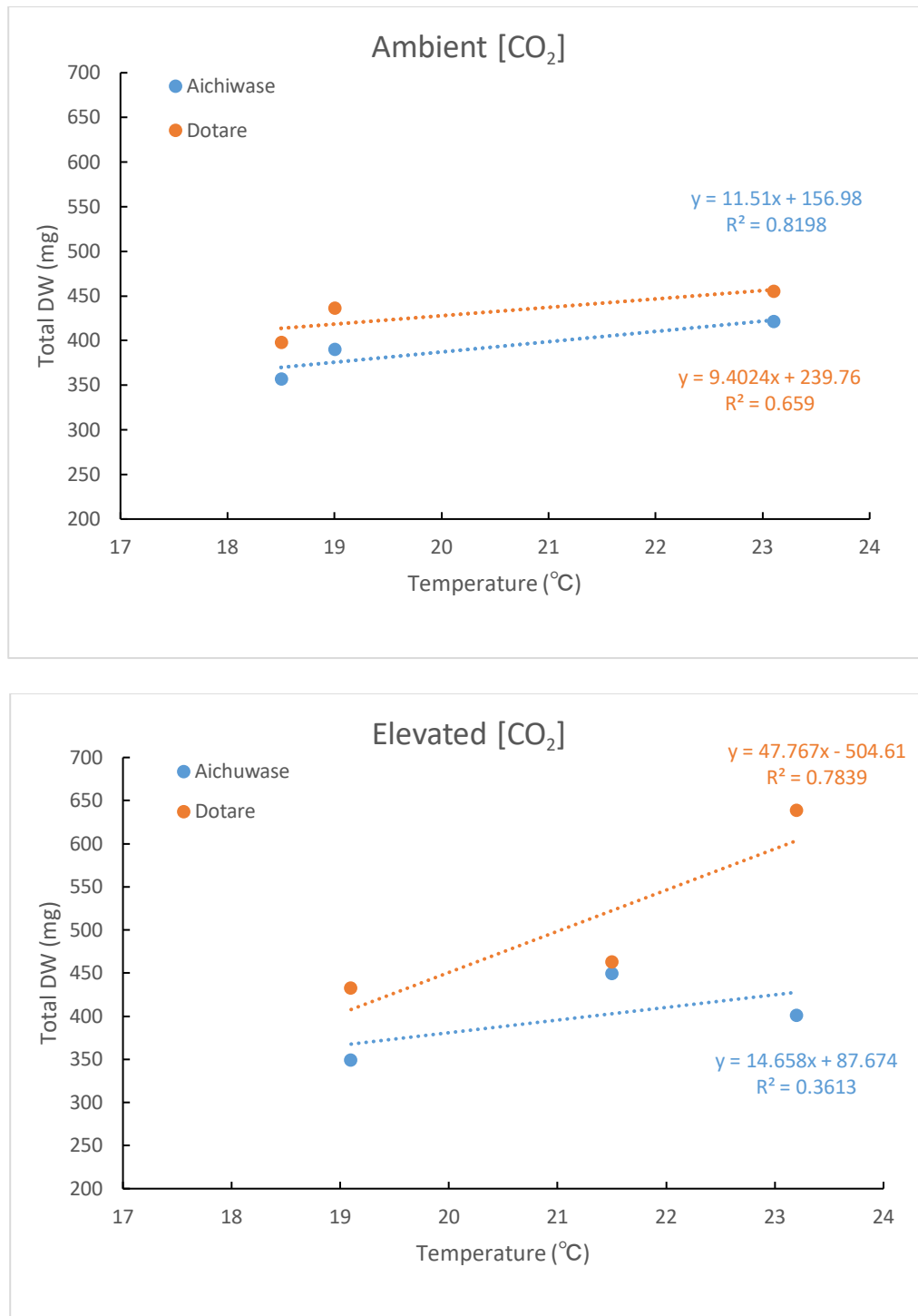


Figure 4.2. Relationships between [CO₂] and air temperatures on total plant DW in eddo.

Table 4.5. Effects of elevated CO₂ concentration ([CO₂]) on leaf DW (without cotyledon), stem DW, shoot DW (without cotyledon), root DW and total DW (without cotyledon) in soybean at two different air temperatures.

Materials	Treatments	Leaf (g plant ⁻¹)	Stem (g plant ⁻¹)	Shoot (g plant ⁻¹)	Cotyledon (g plant ⁻¹)	Root (g plant ⁻¹)	Total (g plant ⁻¹)
Experiment-1							
Hiden	AL	0.21	0.09	0.30	0.13	0.07	0.37
	EL	0.18	0.11	0.29	0.14	0.07	0.36
	AH	0.27	0.12	0.39	0.12	0.08	0.47
	EH	0.24	0.11	0.35	0.16	0.06	0.41
Kemame	AL	0.15	0.10	0.25	0.16	0.11	0.36
	EL	0.15	0.09	0.24	0.15	0.09	0.34
	AH	0.23	0.11	0.34	0.13	0.08	0.42
	EH	0.23	0.10	0.33	0.13	0.07	0.40
Experiment-2							
Hiden	AL	0.17	0.10	0.27	0.18	0.05	0.32
	EL	0.19	0.10	0.29	0.15	0.06	0.35
	AH	0.30	0.12	0.41	0.12	0.08	0.49
	EH	0.29	0.11	0.41	0.15	0.07	0.47
Kemame	AL	0.20	0.09	0.29	0.14	0.06	0.34
	EL	0.18	0.09	0.27	0.17	0.07	0.34
	AH	0.28	0.11	0.38	0.14	0.07	0.45
	EH	0.27	0.10	0.37	0.14	0.07	0.44
ANOVA							
	CO ₂ (C)	ns	ns	ns	ns	ns	ns
	Temperature (T)	***	***	***	*	ns	***
	Materials (M)	ns	**	**	ns	ns	ns
	Experiment (E)	ns	ns	ns	ns	ns	ns
	C × T	ns	ns	ns	ns	ns	ns
	C × M	ns	ns	ns	ns	ns	ns
	C × E	ns	ns	ns	ns	ns	ns
	C × T × M	ns	ns	ns	ns	ns	ns

AL: ambient [CO₂] and low air temperature, EL: elevated [CO₂] and low air temperature, AH: ambient [CO₂] and high air temperature, EH: elevated [CO₂] and high air temperature. Statistically significant effects are indicated ***: P < 0.01, **: P < 0.05, *: P < 0.1, ns: not significant.

CHAPTER 5

GENERAL DISCUSSION

Many papers have reported results from such combined effects of elevated [CO₂] and temperature on other plants like as rice (Cheng et al., 2010; Roy et al., 2012), maize (Dhakhwa et al., 1997; Qiao et al., 2019), wheat (Tan et al., 2018), soybean (Jin et al., 2017), and Chinese yam (Thinh et al., 2017a, 2017b & 2018). However, the information related to the effects of elevated [CO₂] under different temperatures has not been published on taro group including eddo.

The responses to elevated [CO₂] under different air temperatures in eddo compared with rice in the early to intermediate growth stage were investigated in chapter 2. As a result, it was revealed that some biomass parameters in both eddo and rice significantly increased at elevated [CO₂] with low and high air temperatures. It was also shown that photosynthesis was increased by elevated [CO₂]. The increasing ratios of total DW was significantly greater in eddo than in rice. The results suggest that an exposure to elevated [CO₂] more increases in eddo biomass than in rice biomass. Therefore, the results can support the hypothesis that the large sink capacity, such as root and tuber crops (i.e. eddo), may have a greater response to elevated [CO₂] than low sink capacity plant such as cereal crops (i.e. rice). According to the results in this chapter, it is concluded that the increased leaf area and SPAD value (chlorophyll content) may contribute to the higher increasing ratio of biomass in eddo plants to elevated [CO₂].

In chapter 3, effects of elevated [CO₂] on leaf blades morphology in eddo at two different air temperatures. The whole leaf blade thickness and number of chloroplasts per unit profile area of mesophyll cell in eddo and rice were greater under elevated [CO₂]. Considering both results of chapter 2 and chapter 3, the number of leaves, leaf area, SPAD values, the thickness of the whole leaf blade, number of chloroplasts per mesophyll cell in

palisade layer, number of chloroplasts per unit profile area of both palisade and sponge layers, and stomata density would contribute to increase the photosynthesis and biomass in eddo. On the other hands, the number of leaves, the thickness of the whole leaf blade, and number of chloroplasts per unit profile area in mesophyll would contribute to increase photosynthesis and biomass in rice. Comparing these results, it was concluded that the higher sensitiveness to elevated $[\text{CO}_2]$ of leaf area, SPAD values and stomata density possibly contribute to the higher increasing rate of biomass in eddo under elevated $[\text{CO}_2]$ conditions.

Additionally, in chapter 4, the results indicated that elevated $[\text{CO}_2]$ would be effects on the germination percentage of eddo both cultivars, but soybean materials did not. Additionally, it was shown in chapter 2 that, the values of corm DW and the number of lateral plants in eddo in 2018 and 2019 are higher in elevated $[\text{CO}_2]$ than in ambient $[\text{CO}_2]$ and the both values were the highest in elevated $[\text{CO}_2]$ under high temperature regimes, although that is not significantly. In eddo, if the number of lateral plants increases, the number of corms also naturally increases. These tendency of positive responses in early to intermediate vegetative growth stage might contribute to final production of eddo in the future. To our knowledge, this study firstly provided information related with elevated $[\text{CO}_2]$ response of eddo at two different air temperatures.

In the study, there were large differences in the data of growth and DW between 2018 and 2019, because the average air temperature and solar radiation were higher in 2018 than in 2019 during the treatment period in the gradient chambers. Therefore, the almost all of data in growth and DW were significantly affected by year in eddo and rice. However, effects of the treatments showed a similar tendency between the two year's experiments.

The experiments in this study were performed in TGCs. Eddo plants were grown under low- and high- temperature regimes in each temperature-gradient chamber. Naturally, when the temperature increases, the relative humidity decreases in the chambers. Shimizu et al. (1996) reported that the interactive effects between CO₂ and relative humidity on the growth and the transpiration were insignificantly observed in several C₃ plants and a C₄ (corn) plant. The magnitude of the response of stomatal conductance to changes in [CO₂] varies with humidity, and that the humidity effect can be quite localized (Bunce, 1998). In this study, almost all of data were higher in the high temperature regime (40-49% relative humidity) than in the low temperature regime (70-77% relative humidity). The stomatal conductance was not remarkably difference between the low and high temperature regimes in 2018 and 2019 in this experiment. However, effects of relative humidity on eddo growth in elevated [CO₂] are also needed to investigate in detail in the future. In addition, Sage et al. (1989) indicated that a long-term exposure to elevated [CO₂] affects the CO₂ response of photosynthesis and that the patterns of acclimation vary among species. Therefore, I also need further intensive studies on the effects of long-term exposure to elevated [CO₂] on eddo plants. That may lead to a more comprehensive characterization of the acclimation of photosynthesis and plant yield. Some environmental conditions (i.e. soil temperature, and difference in temperature between day and night) in pot experiments of TGCs and field experiments are possibly different. FACE tests with customary cultivation are also needed in the future.

This study is one of case studies but shows first information related with elevated [CO₂] response of eddo at different air temperatures with using TGCs. These knowledges will be valuable case information for speculating of eddo response to the future's environment and for making the strategy of eddo cultivation in the future.

SUMMARY

Agriculture is being done under increasing atmospheric CO₂ concentration ([CO₂]) and temperature in tandem at present. It is important for the future agricultural development to understand the responsiveness of crops to elevated [CO₂] at different air temperatures. However, the responses of taro plant species, including eddo, to [CO₂] at different air temperatures remain largely unknown. To determine the effective strategies for eddo cultivation under the future climatic change, it is important to understand how eddo responds to elevated [CO₂] at different air temperature and what kind of mechanisms occurs the responses. There is a hypothesis that sink constraints limit the CO₂ fertilization effect, and root and tuber crops may have a more significant response to elevated [CO₂] than the other plant types because their sink size is often unlimited. Eddo has a large sink capacity in the corms. Therefore, this study was carried out with the purpose to clarify the characterizations of effects of elevated [CO₂] on growth, photosynthesis, leaf blades morphology and corm germination in eddo at two different air temperatures in comparison to rice and soybean.

In chapter 2, the responses of plant growth and photosynthesis in eddo and rice plants to elevated [CO₂] (ambient [CO₂] + 200 $\mu\text{mol mol}^{-1}$) were investigated at low and high temperatures during the early-to-intermediate growth stage in temperature gradient chambers (TGCs) in 2018 and 2019 at The National Agriculture and Food Research Organization (NARO) at Morioka city. In this experiment, the dry weights (DWs) of the eddo leaf blades, petioles, roots, and above-ground parts as well as the DWs of the rice leaf blades, leaf sheaths and stems, above-ground parts, and whole plants significantly increased under elevated [CO₂]. The number of leaves, leaf area, and SPAD value of eddo significantly increased under elevated [CO₂], whereas the leaf area and SPAD value of rice did not. The net photosynthetic rates of eddo and rice increase under elevated [CO₂]. The

increasing ratio of the total DW in elevated vs ambient [CO₂] was significantly higher in eddo than in rice. The result indicates that elevated [CO₂] increases the eddo biomass more than the rice biomass, possibly because of the increasing eddo leaf area and SPAD value induced by elevated [CO₂]. Thus, this finding may support the hypothesis that plants with a large sink capacity, such as root and tuber crops, are more responsive to elevated [CO₂] than the other plants with a limited sink capacity. The total DW of rice increased significantly in response to the high air temperature under elevated [CO₂] conditions. However, the effect of [CO₂] on the total DW of eddo was similar at both temperatures, reflecting the non-significant interaction between [CO₂] and temperature.

In chapter 3, the effects of elevated [CO₂] on leaf blades morphology of eddo at two different air temperatures to compare with rice were investigated in the same experiment conditions as described above the paragraph. Both bright-field optical microscopy and scanning electron microscopy were used to investigate the inner structure of leaf blade tissues and, stomatal density and size in eddo and rice. In eddo, the thickness of the whole leaf blade, number of chloroplasts per mesophyll cell in palisade layer, number of chloroplasts per unit profile area of mesophyll at both palisade and sponge layers, and stomata density were higher in elevated [CO₂] than in ambient [CO₂]. On the other hands, the thickness of the whole leaf blade, and the number of chloroplasts per unit profile area of mesophyll were higher in elevated [CO₂] than in ambient [CO₂] in rice. Considering both the results of chapter 2 and 3, it was concluded that the higher sensitiveness to elevated [CO₂] of the leaf area, SPAD values and stomata density may contribute to the higher increasing rate of biomass in eddo than in rice under the elevated [CO₂] conditions.

In chapter 4, the effects of elevated [CO₂] on the seed corm germination in two eddo cultivars and seed germination in two soybean materials, and on the seedling growth of eddo and soybean were investigated at different temperatures. For eddo, the experiment

was carried out in chambers at Fujisaki field of Hirosaki University and in TGCs of NARO at Morioka in 2019. The treatments in TGCs were conducted under two [CO₂] conditions: ambient (average 400 $\mu\text{mol mol}^{-1}$) and elevated [CO₂] (ambient [CO₂] + 200 $\mu\text{mol mol}^{-1}$) with low- and high-temperature regimes. The treatments in chambers of Fujisaki field were used under two conditions in a control chamber (ambient [CO₂] with low temperature) and an elevated [CO₂] and high temperature chamber (ambient [CO₂] + 300 $\mu\text{mol mol}^{-1}$ with high temperature), separately. The results suggest that the germination percentage of seed corms in eddo cultivars showed an increasing trend under elevated [CO₂] conditions in the treatments of TGCs. In addition, it was suggested in this chapter that the seedlings of Dotare may have the positive sensitivity to elevated [CO₂] under high temperature. On the other hand, elevated [CO₂] had no significant effects on the seed germination percentage, and the growth and DW of the seedlings in soybean.

It was shown in the chapter 2 that the actual mean values of corm DW and the number of lateral plants in eddo in 2018 and 2019 were higher under elevated [CO₂] than under ambient [CO₂] and both values were the highest in elevated [CO₂] conditions under the high air temperature, although not significantly so. Regarding eddo, increases in the number of lateral plant parts are associated with increases in the number of corms. The tendency for eddo plants to respond positively to elevated [CO₂] in the early-to-intermediate vegetative growth stage might contribute to the final eddo yield in northern Japan. This study is one of case studies but shows first information related with elevated [CO₂] response of eddo at different air temperatures. These knowledges will be valuable case information for speculating of eddo response to the future's environment and for making the strategy of eddo cultivation in the future.

要旨

現在、農業生産は、大気中の二酸化炭素濃度(以下 $[\text{CO}_2]$ と記す)と気温の上昇する中で行われている。従って、異なる気温下での高 $[\text{CO}_2]$ に対する作物の反応性について理解することは、将来の農業の発展に対して重要である。しかしながら、サトイモを含むタロイモ類全体における高 $[\text{CO}_2]$ に対する反応性についてはよく分かっていない。将来の気候変動下においてサトイモ栽培に対する効果的な戦略を考えるために、サトイモが異なる気温下で高 $[\text{CO}_2]$ にどのように反応するのか、そしてその反応がどのようなメカニズムで起きるのかを理解することが重要である。近年、 $[\text{CO}_2]$ のポジティブな効果は、植物のシンク能を制限することで抑制され、大きなシンク能を有するイモ類は高 $[\text{CO}_2]$ に対してはポジティブな反応性を強く示すとの仮説がある。サトイモは球茎において大きなシンク容量を有する。これらのことから本研究では、異なる気温下でのサトイモの成長、光合成、葉身の形態および球茎の発芽における高 $[\text{CO}_2]$ の影響の特徴をイネやダイズと比較し、明らかにすることを目的として実施した。

本論文の第2章では、初期から中期までの栄養成長期のサトイモとイネにおける近自然気温(以下、低気温と記す)と高気温の条件下での自然 $[\text{CO}_2]$ と高 $[\text{CO}_2]$ (自然 $[\text{CO}_2] + 200 \mu\text{mol mol}^{-1}$, 4:00 to 20:00) に対する生長と光合成の各反応について、グラディオオトロンチャンバー(以下 TGC と記す)を用いて 2018 年と 2019 年に調査した。この試験では、サトイモ品種愛知早生と水稻品種ひとめぼれを用いた。その結果、サトイモにおける葉身、葉柄、根、地上部の各乾物重とイネにおける葉身、葉鞘+茎、地上部および植物全体の各乾物重は、高 $[\text{CO}_2]$ 区で有意に増加した。また、サトイモの葉数、葉面積および SPAD 値は高 $[\text{CO}_2]$ 区で有意

に増加したが、イネの葉面積と SPAD 値は高 [CO₂] 区で有意に増加しなかった。純光合成速度も、サトイモとイネにおいて高 [CO₂] 区で増加した。自然 [CO₂] 区に対する高 [CO₂] 区の植物体の全乾物重の増加率は、イモよりもサトイモで有意に高かった。この結果は、高 [CO₂] はイネよりもサトイモでよりバイオマスを増加させる可能性を示し、これは高 [CO₂] 下のサトイモの葉面積や SPAD 値の有意な増加が起因していることが示唆された。この知見は、イモ類などの大きなシンク容量を有する植物が、シンク容量が小さな植物と比べて高 [CO₂] に対してより強く反応するとする仮説を支持するものであると考えられた。また、高 [CO₂] 下でのイネ植物体の全乾物重は、低温区よりも高温区で有意に増加した。一方、サトイモ植物体の全乾物重に対する高 [CO₂] の影響は、今回の試験では両気温区との間で有意な差が認められなかった。

第 3 章では、高 [CO₂] が低温区と高温区でサトイモとイネの葉身の形態に及ぼす影響について前章と同様の植物・処理条件で調査した。この調査では、明視野顕微鏡と走査型電子顕微鏡を用いた。サトイモでは、葉身の厚さ、柵状細胞当たりの葉緑体数、柵状組織と海綿状組織の各単位面積当たりの葉緑体数および背軸面の気孔密度が高 [CO₂] の影響により増加した。一方、イネでは、葉身の厚さ、葉肉組織の単位面積当たりの葉緑体数が高 [CO₂] の影響により増加した。第 2 章と第 3 章の結果を考慮すると、サトイモにおける葉面積、SPAD 値(クロロフィル含有量)および気孔密度における高 [CO₂] に対するポジティブな反応が、高 [CO₂] 下でバイオマスの増加率がイネよりもサトイモで高くなったことに貢献している可能性が示された。

第4章では、低温下と高温下での高 $[\text{CO}_2]$ がサトイモ球茎とダイズ種子の発芽率および両作物の幼植物体の生長に及ぼす影響について調査した。サトイモでは品種愛知早生と品種土垂を、ダイズでは青森県在来のエダマメ「毛豆」と品種秘伝を供試した。サトイモの試験を弘前大学農学生命科学部の藤崎農場のチャンバーと上記と同じTGCsで2019年に行った。また、ダイズの試験を同TGCsで2019年に2回実施した。TGCsでは上記と同様の自然 $[\text{CO}_2]$ と高 $[\text{CO}_2]$ 条件を用い、それぞれ低温区と高温区を設けて処理した。藤崎農場の処理では、自然 $[\text{CO}_2]$ + 低温および高 $[\text{CO}_2]$ (自然 $[\text{CO}_2]$ + $300 \mu\text{mol mol}^{-1}$) + 高温の2つの区を設けた。その結果、TGCsの処理では2つサトイモ品種において球茎の発芽率は高 $[\text{CO}_2]$ 下の低温区と高温区で共に高くなる傾向が示された。一方、ダイズ種子の発芽率では高 $[\text{CO}_2]$ 処理による有意な影響は認められなかった。サトイモ幼植物体の全乾物重は、土垂では高 $[\text{CO}_2]$ 下の高温区で有意に増加したが、愛知早生では認められなかった。ダイズの幼植物体では、高 $[\text{CO}_2]$ は種子発芽率と幼植物体の全乾物重に有意に影響を及ぼさなかった。

また第2章では、サトイモの乾物重と側生の子イモ数は、有意ではなかったものの自然 $[\text{CO}_2]$ 区より高 $[\text{CO}_2]$ 区で高く、どちらの平均値も高 $[\text{CO}_2]$ 下の高温区で最も高いことが2か年の試験において認められた。このような初期から中期までの成長期における塊茎の $[\text{CO}_2]$ と温度に対する数量的な増加傾向は、北日本において最終的なサトイモの収量増加に結びつくかもしれない。本研究では、異なる温度条件下における高 $[\text{CO}_2]$ 対するサトイモの成長、光合成、形態および球茎の発芽の影響に関する知見を初めて報告した。これらの知見は、将来

の環境に対するサトイモの反応を予測し、サトイモ栽培の戦略を検討する上で有意義な事例的情報になると考えられた。

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