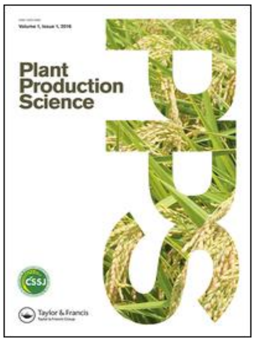

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Effect of Altitude on the Response of Net Photosynthetic Rate to Carbon Dioxide Increase by Spring Wheat

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Abstract: The partial pressure of CO_2 in air decreases with the increase in altitude. Therefore, increase in molar concentration of CO_2 is smaller at higher altitudes than at lower altitudes for increases in molar fraction of CO_2. This study aimed to predict the effect of global CO_2 increase on net photosynthetic rate of spring wheat (*Triticum aestivum* L.) at high altitudes. The net photosynthetic rate of spring wheat grown in Lhasa (3688 m above sea level), China, was compared with that of the same cultivar grown in Sapporo (15 m above sea level), Japan. At the current level of CO_2, it was significantly lower in Lhasa than in Sapporo, and stomatal conductance, chlorophyll content (SPAD value) and apparent quantum yield were similar in both locations. The interaction of CO_2 level and altitude was suggested; the amount of increase in net photosynthetic rate caused by increase in CO_2 was smaller at high altitudes than at low altitudes. Lower CO_2 partial pressure at higher altitude could explain the difference in net photosynthetic rate between altitudes, and the interaction of CO_2 level and altitude.

Key words: Altitude, CO_2 increase, CO_2 partial pressure, Net photosynthetic rate, Tibetan plateau, Wheat.

The partial pressure of CO_2 in air decreases with increase in altitude, and its effect on photosynthesis has been of interest to plant physiologists and ecologists (Billings et al., 1961; Körner and Diemer, 1987; Friend et al., 1989; Terashima et al., 1995; Bowman et al., 1999; Sakata and Yokoi, 2002; Kumar et al., 2005). Friend et al. (1989) measured the net photosynthetic rate (P_n) in *Vaccinium myrtillus* L. and *Nardus strica* L. along altitudinal gradients between 200 m and 1100 m. P_n increased in both species with the increase in altitude, probably because of the increase in leaf nitrogen per unit leaf area. Bowman et al. (1999), however, observed similar levels of P_n in populations of *Frasera speciosa* grown between 1800 m and 3500 m, and they considered that the increase in internal conductance of leaves at higher altitude results in maintenance of similar P_n among populations. Kumar et al. (2005) also measured photosynthetic parameters for the same varieties of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) in fields at elevations of 1300 m and 4200 m, and they found no difference in P_n between altitudes.

Atmospheric CO_2 levels are predicted to rise from the current 380 μmol mol^{-1} to 460–560 μmol mol^{-1} by year 2050 (IPCC 2007). Their effect on plant growth, including P_n, has been investigated in many studies, such as by Körner and Arnone (1992), Berryman et al. (1994), Amthor (2001) and Ainsworth and Long (2005). For example, field experiments showed that an increase in CO_2 from 350–380 to 680–700 μmol mol^{-1} increases P_n by 30–50% in spring wheat (Mulholland et al., 1997; Van Oijen et al., 1999). In the review of free-air CO_2 enrichment experiments using crops and natural vegetation, Ainsworth et al. (2005) also reported that an increase in CO_2 increases P_n by around 30% on the average of all plants tested. Most of those studies, however, examined in low-altitude regions, and few studies considered the effect of CO_2 increase on plant growth in high altitude regions.

Körner et al. (1987) estimated the response of P_n to the increase in CO_2 concentration in natural vegetation at different altitudes. Although P_n at ambient CO_2 level (335 μmol mol^{-1}) was similar at a low (600 m) and high elevation (2600 m), the estimated increase in P_n was 21%

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Abbreviations: A_g, gross photosynthetic rate; DAS, days after sowing; K, potassium; N, nitrogen; OTC, open-top chamber; P, phosphorus; P_n, net photosynthetic rate; P_{nmax}, maximum net photosynthetic rate; PPFD, photosynthetic photon flux density; rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; V_c, RuBP carboxylation rate; V_o, RuBP oxygenation rate.

at a low elevation and 31 % at a high elevation, when CO_2 concentration increased to 435 μmol mol^{-1}. When populations grown at different altitudes are compared, these estimations of altitudinal effect on photosynthesis would be the result of the combined effects of environmental conditions and plant adaptations to the environment. Körner et al. (1987) compared different species in the same family. In this case, the estimation was affected by morphological adaptation of each species, as well as by CO_2 partial pressure. Also, the estimation was dependent on a short time response curve of P_n to an increase in CO_2 concentration. Photosynthetic acclimation was reported for various C_3 plants (Sage et al., 1989; Habash et al., 1995; Sharma-Natu et al., 1997; Sicher and Bunce, 1997; Pozo et al. 2005).

Terashima et al. (1995) predicted the effects of low air pressure on gross photosynthetic rate (A_g) using the theoretical model for A_g of rubisco. A_g can be calculated from the maximum rate of RuBP carboxylation (V_{cmax}), the maximum rate of RuBP oxygenation (V_{omax}), Michaelis constants for CO_2 and O_2 (K_c and K_o, respectively), and the concentration of CO_2 and O_2 in mesophyll cells (C and O, respectively) (Farquhar et al., 1980; Terashima et al., 1995). V_{cmax}, V_{cmax}, K_c and K_o depend on temperature. C and O depend on the temperature and partial pressure of CO_2 and O_2, respectively, in the intercellular spaces. Therefore, when the temperature is the same in two locations at different altitudes, V_{cmax}, V_{omax}, K_c and K_o are independent of altitude and C and O depend on altitude. The prediction indicated that the amount of increase in A_g with a given increase in molar concentration of CO_2 (in moles CO_2 per cubic meter) was independent of altitude. The A_g for a given molar concentration of CO_2, however, was consistently higher at higher altitudes than at lower altitudes due to the reduced O_2 inhibition at higher altitudes with lower atmospheric pressure (Terashima et al., 1995). On the other hand, the increase in A_g with a given mole fraction of CO_2 (in moles CO_2 per mole) was lower at higher altitudes than at lower altitudes suggesting interaction between global CO_2 increase and altitude. The A_g for a given mole fraction of CO_2 was lower at higher altitudes than at lower altitudes.

This study aimed to test the predictions about the effects of altitudes and global CO_2 increase on P_n reported by Terashima et al. (1995). To test long-term, rather than short-term, response of crop growth to high CO_2 concentrations, we erected open-top chambers (OTCs) at high altitudes and grew wheat crops under ambient and increased CO_2 concentrations. To analyze the altitudinal difference in P_n, the same wheat cultivar was grown at a low altitude using growth-chambers. Wheat was also cultivated in an open field under an ambient CO_2 concentration at the low altitude to compare plants grown in growth-chambers at an ambient CO_2 concentration.

Materials and Methods

The spring wheat cultivar 3u90, widely cultivated in Lhasa on the Tibetan plateau, China, was used in an OTC experiment in Lhasa, and growth-chamber and open field experiments in Sapporo, Japan. A growth-chamber was used to cultivate wheat plants at Sapporo under the CO_2 partial pressure at Lhasa, where the CO_2 concentration was lower than the current CO_2 concentration in Sapporo.

1. Experimental conditions

(1) OTC experiment in Lhasa

Field experiments were done at the Lhasa Plateau Ecological Research Station (29°N, 91°E, 3688 m above sea level) of the Chinese Academy of Sciences, China, in 2001. The experiment was done in an open field (Open-field) and in OTCs at two levels of CO_2, i.e., one OTC with ambient levels of CO_2 (OTC-Ambient) and one OTC with increased levels of CO_2 (OTC-Increased), in three replicates arranged in a randomized complete block design. Six OTCs (each 3 m×3 m, 2 m height; consisted of aluminum frames and polyethylene wall) were constructed for the two treatments. CO_2 for the increased levels was supplied from liquefied petroleum gas-firing equipment (CG-253S2G, Nepon, Japan) and was injected into a blower that supplied 1800 m^3 h^{-1} air (approximately 2500 μmol CO_2 mol^{-1}) through plastic pipes placed about 15 cm above the canopy. The CO_2 level was increased from 16 days after sowing (DAS) (19 May 2001) for a 13-hour day (0500-1800 h solar time) until the day before the final harvest (2 October 2001).

Long-term gas detector tubes (GASTEC, Japan) did not detect carbon monoxide (measuring range 0.4–400 μmol mol^{-1}), nitrogen dioxide (0.1–30 μmol mol^{-1}) and sulfur dioxide (0.2–100 μmol mol^{-1}) in the air directly from the gas-firing equipment. Hydrocarbons, including ethylene, were not measured. CO_2 levels and air temperature above the crop canopy were measured four times before heading between 0800-1600 by using a portable open gas-exchange system (LI-6400, LI-COR, USA). The mean mole fraction of CO_2 was 375±S.D. 7, 384±S.D. 4 and 584±S.D. 81 μmol mol^{-1} in Open-field, OTC-Ambient and OTC-Increased, respectively, which was around 10.0, 10.2 and 15.6 mmol m^{-3}, respectively. The air temperature was highest in OTC-Increased (26.3±S.D. 3.2°C), followed by OTC-Ambient (25.4±S.D. 3.2°C) and in Open-field (24.3±S.D. 3.3°C). The difference between treatments was caused by warm air from the gas-firing equipment and the chamber effect. Mean, lowest and highest daily average air temperature from sowing to heading was 13.6, 9.0 and 17.8°C, respectively, in open field.

Seeds were sown on 3 May 2001 at 550 seeds per m^2. Ears emerged around 18 July 2001 (76 DAS). In the same way as used by local farmers, nitrogen (N), phosphorus (P)

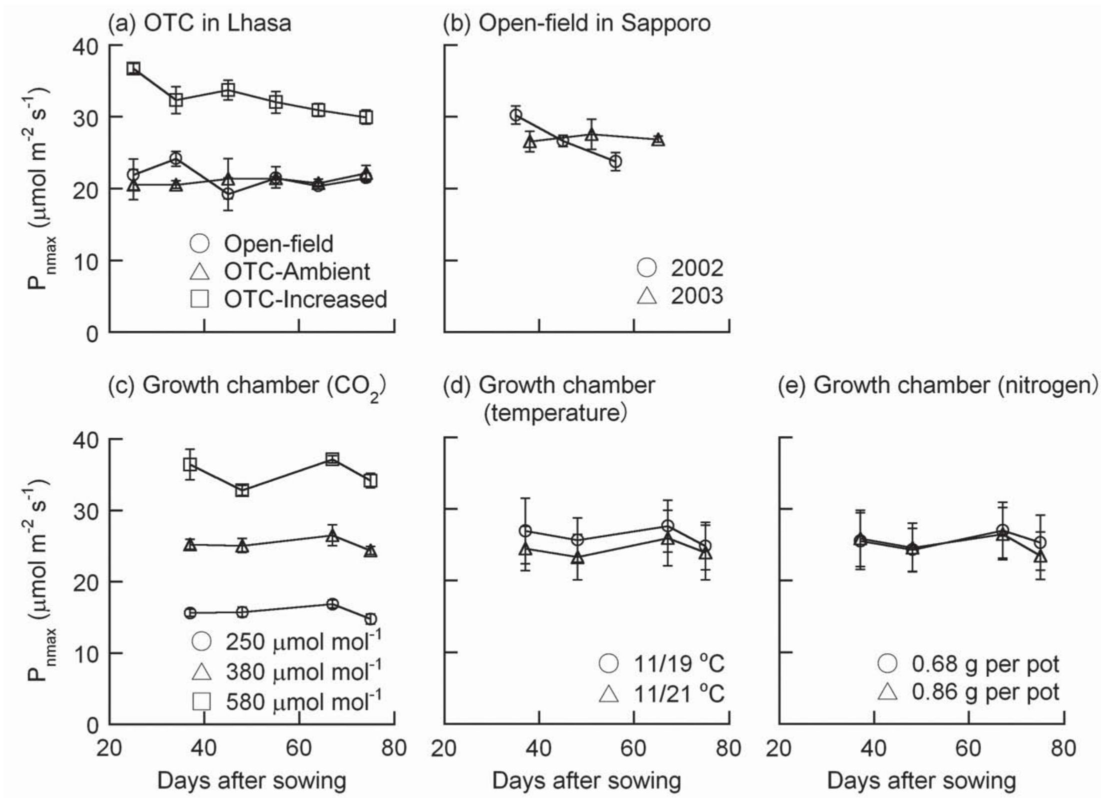

Fig. 1. Change in maximum net photosynthetic rate (P_{nmax}) of spring wheat before heading. (a) Open-top chamber (OTC) experiment in Lhasa. (b) Open-field experiment in Sapporo. (c) Main effect of CO_2 level in the growth-chamber experiment. (d) Main effect of temperature in the growth-chamber experiment. (e) Main effect of nitrogen doses in the growth-chamber experiment. Each point shows the mean (±standard error) of 3-6 replications. OTC-Ambient, open-top chamber with ambient levels of CO_2; OTC-Increased, open-top chamber with increased levels of CO_2.

and potassium (K) were applied at 40.0, 7.9 and 9.1 kg ha^{-1}, respectively, at sowing, and at 35.0, 2.6 and 3.3 kg ha^{-1}, respectively, at heading. Sheep manure was also applied at 10 t ha^{-1} at sowing. The crop was irrigated when needed.

(2) Growth-chamber experiment in Sapporo

Wheat was grown in pots (16 cm diameter, 20 cm height) in a glasshouse at the Field Science Center for Northern Biosphere of Hokkaido University, Sapporo (15 m above sea level). Nine seeds were sown per pot and the plants were thinned to three plants per pot when the second leaf emerged. The pots were transferred to the growth-chambers (KG50-HLA, Koito, Japan) at 10 DAS. Pots were filled with Andosol soil, which was mixed with 0.50 g of N, 0.26 g of P and 0.42 g of K per pot at sowing. At 32 DAS a dose of 0.16 g of P and 0.30 g of K per pot was applied. The chamber was illuminated by using white fluorescent tubes during 13-hour photoperiod (day). The photosynthetic photon flux density (PPFD) at the canopy level was about 500 μmol m^{-2} s^{-1} and the relative humidity in the chambers was about 80%. Gaseous CO_2 (purity 99.5%) was injected into the chambers to control CO_2 concentration. To control CO_2 concentration below ambient level, ambient air was injected after trapping CO_2 with soda lime. Ears emerged around 76 DAS.

The following treatments were used in factorial combinations after transferring the plants to the growth-chambers.

(i) CO_2 levels (during the day): 250, 380 and 580 μmol mol^{-1}; actual day means achieved were 246, 394 and 587 μmol mol^{-1}, respectively, which was around 10.4, 15.8 and 24.1 mmol m^{-3}, respectively. To represent molar concentration of CO_2 in OTC-Ambient and OTC-Increased in Lhasa, we used 250 and 380 μmol mol^{-1}, respectively, in Sapporo.

(ii) Temperature: 11/19°C and 11/21°C (night/day maximum and minimum temperature cycle).

(iii) Nitrogen: low (0.18 g per pot at 32 DAS i.e. 0.68 g per pot during growth) and high (0.36 g per pot at 32 DAS i.e. 0.86 g per pot during growth).

Thus 12 (3×2×2) treatment combinations were used without chamber replications. The temperature and photoperiod levels maintained in the growth-chambers were equivalent to those of the seasonal averages in Lhasa.

Table 1. Maximum net photosynthetic rate (P_{nmax}), stomatal conductance and chlorophyll content (SPAD value) of spring wheat in the open-top chamber (OTC) experiment in Lhasa.

Treatment	P_{nmax} (μmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	SPAD value
Open-field	21.5±0.67 b	0.44±0.022 a	46.3±1.24 b
OTC-Ambient	21.2±0.26 b	0.42±0.035 a	46.8±1.95 b
OTC-Increased	31.2±1.60 a	0.39±0.022 a	50.5±1.10 a
Statistical effect			
Treatment	*	NS	*
Date (D)	NS	NS	*
Treatment×D	NS	NS	NS

Each value represents the mean±standard error (n=6). Date is included in the model as a continuous variable. Statistical significance of treatment-, date- and their interaction-effect is indicated as * ($P<0.05$). Values with the same letters were not significantly different from each other at $P<0.05$ (Tukey HSD). OTC-Ambient, open-top chamber with ambient levels of CO_2; OTC-Increased, open-top chamber with increased levels of CO_2.

(3) Open field experiment in Sapporo

Details of the open field experiment (Open-field) in Sapporo were previously reported (Fujimura et al., 2009). Briefly, the experiment was done in 2002 and 2003 at the Experimental Farms of Field Science Center for Northern Biosphere of Hokkaido University (43°N, 141°E, 15 m above sea level). Seeds were sown on 23 Apr 2002 and 28 Apr 2003 at 450 seeds per m^2. N, P and K were applied at 54, 39.3 and 37.4 kg ha^{-1}, respectively, at sowing. Ears emerged around 27 June (65 DAS) and 4 July (67 DAS) in 2002 and 2003, respectively. The mean, lowest and highest daily average air temperatures from sowing to heading were 13.8°C, 7.9°C and 21.6°C, respectively, in 2002, and 14.3°C, 6.1°C and 20.8°C, respectively, in 2003.

2. Measurements

Maximum P_n (P_{nmax}) and stomatal conductance were measured at PPFD 1600 μmol m^{-2} s^{-1} for the uppermost fully expanded leaf before heading by using the LI-6400. Two leaves were measured for each plot. Measurements were conducted under each growth CO_2 concentration. The leaf temperature and relative humidity was maintained at 20–25°C (actual value achieved was 19.1–26.8°C) and 50–60% (actual value achieved was 43–66%), respectively. The light response curve of P_n was measured. The slope of linear part of light response curve at PPFD 0 to 150 μmol m^{-2} s^{-1} was used to estimate the apparent quantum yield. The light response curve of P_n was measured when P_{nmax} was measured, except in the OTC experiment when the light response curve of P_n was measured at 74 DAS, i.e., the last P_{nmax} measuring date. The P_{nmax} of leaves measured was used to determine the chlorophyll content (SPAD value) using SPAD-502 (Konica Minolta Sensing, Japan).

3. Statistical analysis

In the field experiment in Lhasa and the growth-chamber experiment in Sapporo, a repeated-measures analysis of variance was used to test for the main effects of treatments and measuring date, and their interaction on P_{nmax}, stomatal conductance and SPAD value. To evaluate the apparent quantum yield, data at coefficient of determination less than 0.97 were excluded. Data of the same treatment were pooled and the apparent quantum yield was calculated. The P_{nmax} and apparent quantum yield under different growth conditions were determined by regression analysis for CO_2 concentration and photosynthetic photon flux, respectively, with growth conditions treated as a dummy variable.

Result

P_{nmax} measured at PPFD 1600 μmol m^{-2} s^{-1} was higher in OTC-Increased than in Open-field and OTC-Ambient, and the difference between the latter two were not significant in Lhasa (Fig. 1, Table 1). The date and treatment×date interaction effects were not significant. In the growth-chamber experiment, the effects of CO_2 concentration and temperature were significant, but the difference between treatments was much larger for CO_2 concentration than for temperature (Table 2). The effect of date was significant, but the treatment×date interaction effects were not significant. P_{nmax} measured at the current level of CO_2 was similar in Open-field and the growth-chamber in Sapporo. The ambient level of CO_2, P_{nmax} was 19% lower in Lhasa than in Sapporo ($P<0.05$ by t-test).

The values of P_{nmax} from all experiments in Lhasa and Sapporo were plotted against molar concentration of CO_2 in the air and mole fraction of CO_2 in the air to determine if an increase in CO_2 at these two locations at different altitudes had a different effect on P_{nmax} (Fig. 2). Linear relationships were observed between CO_2 and P_{nmax} in the

Table 2. Maximum net photosynthetic rate (P_{nmax}), stomatal conductance and chlorophyll content (SPAD value) of spring wheat in Sapporo.

Treatment		P_{nmax} (μmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	SPAD value
Open-field		27.0±0.85	0.51±0.043	45.6±2.06
Growth-chamber				
CO_2 (μmol mol^{-1})	250	15.7±0.43 c	0.35±0.034 a	61.8±1.79 a
	380	25.3±0.45 b	0.34±0.011 a	63.2±2.03 a
	580	35.1±1.00 a	0.33±0.036 a	62.3±1.41 a
Temperature (°C)	11/19	26.3±0.63	0.37±0.019	61.9±1.77
	11/21	24.5±0.57	0.32±0.036	63.0±1.42
Nitrogen (g per pot)	0.68	25.6±0.56	0.36±0.024	62.1±1.59
	0.86	25.2±0.68	0.33±0.017	62.8±1.55
Statistical effect				
Growth-chamber				
CO_2		*	NS	NS
Temperature (T)		*	NS	NS
Nitrogen (N)		NS	NS	NS
Date (D)		*	NS	*
CO_2×D		NS	NS	*
T×D		NS	NS	NS
N×D		NS	NS	NS

Each value represents mean±standard error (n=6 for the Open-field experiment and n=4 for the growth-chamber experiment). Data of the Open-field experiment were pooled across two years. Date is included in the model as a continuous variable. Statistical significance of treatments-, date- and their interaction-effect is indicated as * ($P<0.05$). Values with the same letters were not significantly different from each other within CO_2 treatment in the growth-chamber experiment at $P<0.05$ (Tukey HSD).

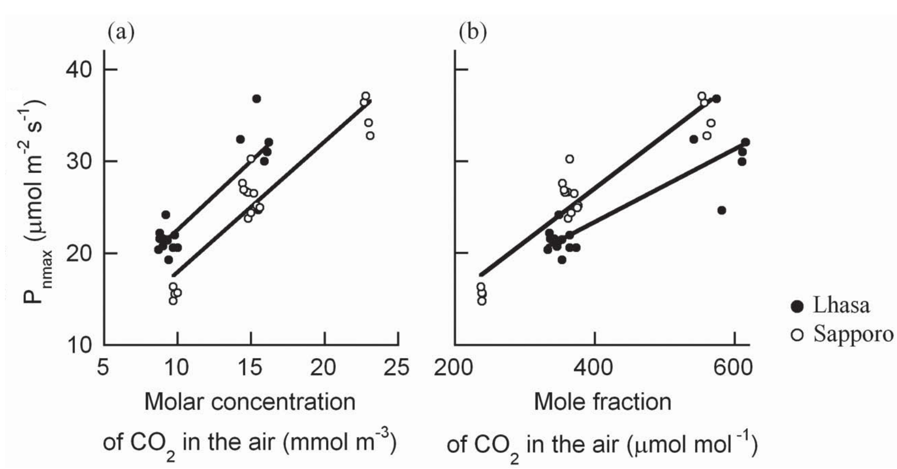

Fig. 2. Effect of CO_2 concentration in the air on maximum net photosynthetic rate (P_{nmax}) of spring wheat before heading. (a) Effect of molar concentration of CO_2 in the air (in moles CO_2 per cubic meter) on P_{nmax}. Regression equation was $y=1.5x+7.6$ ($R^2=0.88$, $P<0.01$) for Lhasa and $y=1.4x+3.8$ ($R^2=0.94$, $P<0.01$) for Sapporo. (b) Effect of mole fraction of CO_2 in the air (in moles CO_2 per mole) on P_{nmax}. Regression equation was $y=0.040x+7.6$ ($R^2=0.88$, $P<0.01$) for Lhasa and $y=0.058x+3.8$ ($R^2=0.94$, $P<0.01$) for Sapporo. Each point shows the values in Fig. 1(a), (b) and (c).

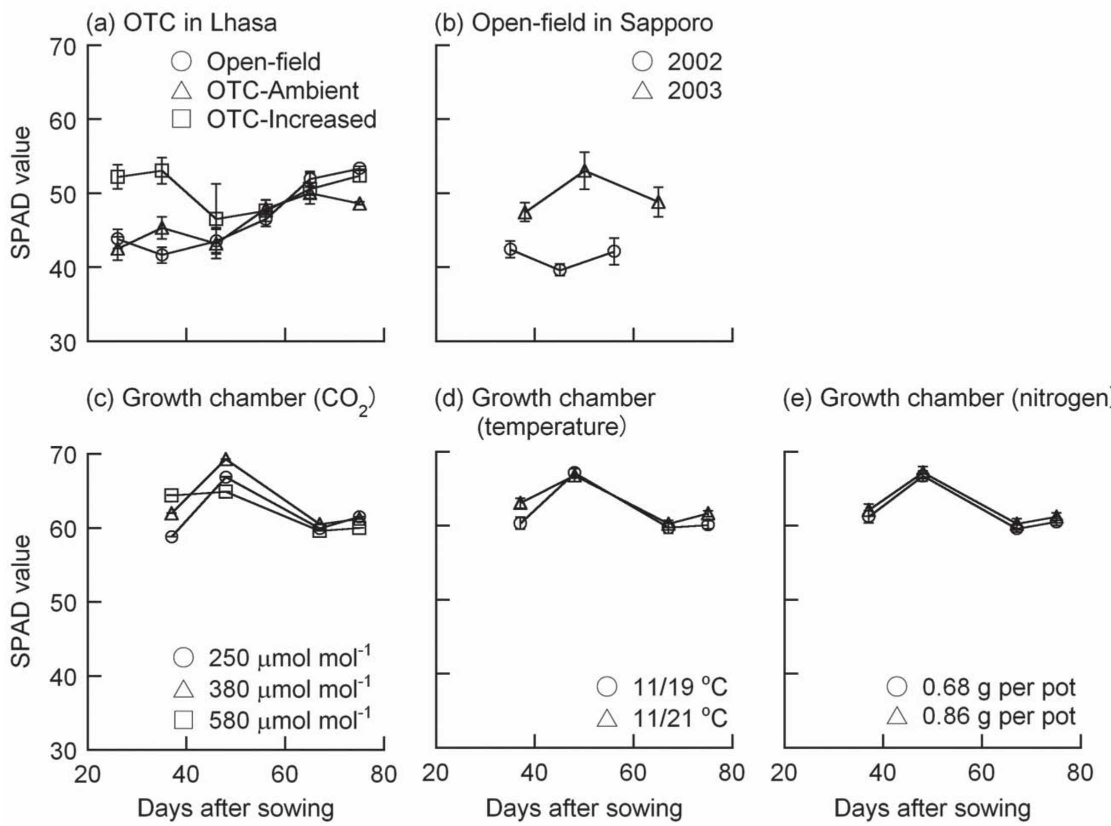

Fig. 3. Change in chlorophyll content (SPAD value) of spring wheat before heading. (a) Open-top chamber (OTC) experiment in Lhasa. (b) Open-field experiment in Sapporo. (c) Main effect of CO_2 level in the growth-chamber experiment. (d) Main effect of temperature in the growth-chamber experiment. (e) Main effect of nitrogen doses in the growth-chamber experiment. Each point shows the mean (± standard error) of 3-6 replications. OTC-Ambient, open-top chamber with ambient levels of CO_2; OTC-Increased, open-top chamber with increased levels of CO_2.

range of this study. Regression equations showed similar slopes for the effect of molar concentration of CO_2 on P_{nmax} in Lhasa and Sapporo ($P=0.75$), but P_{nmax} for a given molar concentration of CO_2 was higher in Lhasa than in Sapporo. However, the slope of P_{nmax} against mole fraction of CO_2 was significantly steeper in Sapporo than in Lhasa ($P<0.05$).

No significant effect of treatments on stomatal conductance was observed in the OTC and growth-chamber experiments. The interaction effects of treatment and date were also not significant. Stomatal conductance measured at the current level of CO_2 showed no significant difference between Lhasa (0.43 mol m^{-2} s^{-1}) and Sapporo (0.44 mol m^{-2} s^{-1}) ($P=0.84$).

The SPAD value was slightly higher in OTC-Increased than in Open-field and OTC-Ambient, and the difference between the latter two was not significant. The effect of date was significant and the SPAD values varied between 40 and 55 (Fig. 3). In the growth-chamber experiment, the main effects of CO_2 level, temperature and nitrogen doses were not significant. The effect of date was significant and the SPAD values varied between 55 and 70. The interaction effect of date and CO_2 level was significant. The SPAD value in all treatments of the OTC experiment was similar to that of the Open-field experiment in Sapporo, but the SPAD value in the growth-chamber experiment tended to be higher than that in the field experiments in both Lhasa and Sapporo.

The initial slopes of light response curves (apparent quantum yield) was not affected by treatments in the OTC experiment ($P=0.41$) (Table 3). In the growth-chamber experiment, it significantly increased with the increase in CO_2 ($P<0.05$). The effects of temperature and nitrogen doses were not significant ($P=0.90$ and 0.81, respectively). The apparent quantum yield at the current level of CO_2 did not show any difference between the location at different altitudes ($P=0.13$).

Discussion

Wheat plants grown in OTCs and growth-chambers simulated well the P_{nmax} of wheat plants grown in open field in this study, in agreement with previous studies on the OTC effect (Mulholland et al., 1997; Van Oijen et al., 1999), in which P_{nmax} did not show any difference between outside and inside OTCs at the ambient CO_2 concentration.

The values of P_{nmax} were plotted against CO_2 concentration

Table 3. Apparent quantum yield of spring wheat in different growth conditions.

Location and treatment		Apparent quantum yield (μmol CO_2 μmol^{-1} photon)	Standard error
Lhasa			
Open-field		0.0570	0.0021
OTC-Ambient		0.0538	0.0026
OTC-Increased		0.0593	0.0027
Sapporo			
Open-field		0.0571	0.0024
Growth-chamber			
CO_2 (μmol mol^{-1})	250	0.0537	0.0018
	380	0.0625	0.0016
	580	0.0687	0.0013
Temperature (°C)	11/19	0.0625	0.0015
	11/21	0.0622	0.0016
Nitrogen (g per pot)	0.68	0.0626	0.0017
	0.86	0.0621	0.0015

Data of the Open-field experiment in Sapporo were pooled across two years. OTC-Ambient, open-top chamber with ambient levels of CO_2; OTC-Increased, open-top chamber with increased levels of CO_2.

in the air not against intercellular CO_2 concentration in this study. Since there was no significant difference in stomatal conductance between two locations, the relationship between P_{nmax} and CO_2 concentration in the air would be similar to that between P_{nmax} and intercellular CO_2 concentration. Regression equations of P_{nmax} against molar concentration of CO_2 in the air showed similar slopes in Lhasa and Sapporo and P_{nmax} for a given molar concentration of CO_2 in the air was higher in Lhasa than in Sapporo. This was agreement with the prediction by the theoretical model for A_g of rubisco (Terashima et al., 1995). Terashima et al. (1995) predicted that the amount of increase in A_g with a given increase in molar concentration of CO_2 was independent of altitude. The prediction, however, indicated that the A_g for a given molar concentration of CO_2 was consistently higher at higher altitudes than at lower altitudes due to the reduced O_2 inhibition at higher altitudes (Terashima et al., 1995).

The regression equations of P_{nmax} against mole fraction of CO_2 showed significantly steeper slope in Sapporo than in Lhasa suggesting an interaction between CO_2 level and altitude. The theoretical model for A_g of rubisco (Terashima et al., 1995) predicted lower slope of A_g against mole fraction of CO_2 at higher altitudes than at lower altitudes, which was in agreement with the results of this study. The difference in slopes of P_{nmax} against mole fraction of CO_2 between Lhasa and Sapporo was explained by lower air pressure in Lhasa. The relationship between mole fraction and molar concentration of CO_2 depends on air pressure. The increase in molar concentration of CO_2 is smaller at high altitudes than at low altitudes for a given increase in mole fraction. Because the slopes of P_{nmax} against molar concentration of CO_2 were similar in both locations, we expected that the same increase in mole fraction of CO_2 resulted in a lower increase in P_{nmax} in Lhasa than in Sapporo.

P_{nmax} for each mole fraction of CO_2 in the air was consistently lower in Lhasa than in Sapporo, probably due to the difference in air pressure at the two altitudes. Both RuBP carboxylation rate (V_c) and oxygenation rate (V_o) decreased with the increase in elevation because of lower air pressure at the higher altitude (Terashima et al., 1995). Because the absolute value of V_c is larger than that of V_o, the reduction with altitude increase is greater for V_c than for V_o. As a result, P_{nmax} would be lower at a high altitude than at a low altitude.

Contrary to the results of this study, Kumar et al. (2005) reported no significant difference in P_n of wheat and barley between altitudes 1300 m and 4200 m above sea level. They suggested that higher efficiency of carbon uptake at higher altitude resulted in similar P_n at both altitudes. However, stomatal conductance was significantly lower at lower altitude than at higher altitude (Kumar et. al., 2005). The values of stomatal conductance at low altitude were 0.14–0.17 mol m^{-2} s^{-1}, which were relatively lower compared with the values in this study at 0.21–0.69 mol m^{-2} s^{-1} and other studies at 0.1–1 mol m^{-2} s^{-1} (Reynolds et al., 2000; Martínez-Carrasco et al., 2005). This suggests environmental stresses were at low altitude in the experiment by Kumar et al. (2005), leading to lower stomatal conductance and P_n at low altitude than at high altitude. In this study, the values of stomatal conductance in all experiments were similar to other studies (Reynolds et al., 2000; Martínez-Carrasco et al., 2005), and stomatal conductance showed no difference between altitudes.

Chlorophyll content of leaf could affect P_n, and the significant positive relationship between chlorophyll content of leaf and SPAD value was reported (Monje and Bugbee, 1992). In the present study, SPAD value was almost the same in the field experiments in Lhasa and Sapporo. Although there was difference in SPAD value between the Lhasa experiment and the growth-chamber experiment, the values in the Lhasa experiment were similar level reported in other studies (Mulholland et al., 1997; Yang et al., 2002; Tahir et al., 2005), suggesting that chlorophyll content was not the limiting factor for P_n in the Lhasa experiment.

CO_2 increase influences P_n by changes in apparent quantum yield (Ku and Edwards, 1978; Farquhar et al., 1980). In this study, apparent quantum yield slightly increased with the increase in CO_2, but a significant difference was not detected in the OTC experiment. On the other hand, the apparent quantum yield increased with the increase in CO_2 in the growth-chamber experiment, indicating that photon use efficiency was increased with the increase in CO_2. The different response of apparent quantum yield to CO_2 increase may have partly caused the interaction effect of CO_2 level and altitude on P_{nmax}. No difference was detected between locations in apparent quantum yield at the current level of CO_2. Open-field in Lhasa and Open-field in Sapporo showed similar value of apparent quantum yield, but there was difference in the P_{nmax} between locations. Therefore, apparent quantum yield did not explain the difference in P_{nmax} between locations.

In this study, a growth-chamber was used to cultivate wheat plants under the CO_2 partial pressure under high altitude at low altitude conditions. As a result, P_n measured in the field experiment was directly compared with P_n measured in the growth-chamber. The response of P_{nmax} to CO_2 concentration at a high altitude was evaluated at two CO_2 concentrations. The predictions in this study will be followed up by studies with plants grown under three or more CO_2 concentrations in the same growth facility at different altitudes.

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