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 CO₂ in air decreases with the increase in altitude. Therefore, increase in molar concentration of CO₂ is smaller at higher altitudes than at lower altitudes for increases in molar fraction of CO₂. This study aimed to predict the effect of global CO₂ 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 CO₂, 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 CO₂ level and altitude was suggested; the amount of increase in net photosynthetic rate caused by increase in CO₂ was smaller at high altitudes than at low altitudes. Lower CO₂ partial pressure at higher altitude could explain the difference in net photosynthetic rate between altitudes, and the interaction of CO₂ level and altitude.

Key words: Altitude, CO₂ increase, CO₂ partial pressure, Net photosynthetic rate, Tibetan plateau, Wheat.

The partial pressure of CO₂ 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 (Pn) in Vaccinium myrtillus L. and Nardus stricta L. along altitudinal gradients between 200 m and 1100 m. Pn 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 Pn 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 Pn 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 Pn between altitudes.

Atmospheric CO₂ 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 Pn, has been investigated in many studies, such as by Körner and Arnone (1992), Berryman et al. (1994), Ainsworth (2001) and Ainsworth and Long (2005). For example, field experiments showed that an increase in CO₂ from 350–380 to 680–700 μmol mol⁻¹ increases Pn by 30–50% in spring wheat (Mulholland et al., 1997; Van Oijen et al., 1999). In the review of free-air CO₂ enrichment experiments using crops and natural vegetation, Ainsworth et al. (2005) also reported that an increase in CO₂ increases Pn 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 CO₂ increase on plant growth in high altitude regions.

Körner et al. (1987) estimated the response of Pn to the increase in CO₂ concentration in natural vegetation at different altitudes. Although Pn at ambient CO₂ level (335 μmol mol⁻¹) was similar at a low (600 m) and high elevation (2500 m), the estimated increase in Pn was 21%
at a low elevation and 31% at a high elevation, when CO₂ 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 CO₂ partial pressure. Also, the estimation was dependent on a short time response curve of Pₚ to an increase in CO₂ concentration. Photosynthetic acclimation was reported for various C₃ plants (Sage et al., 1989; Habash et al., 1995; Sharma-Natu et al., 1997; Sicher and Bunce, 1997; Pozo et al., 2005).

Teraslama et al. (1995) predicted the effects of low air pressure on gross photosynthetic rate (A₀) using the theoretical model for A₀ of rubisco. A₀ can be calculated from the maximum rate of RuBP carboxylation (Vₗₚₚ₀), the maximum rate of RuBP oxygenation (Vₗₚₐ₀), Michaelis constants for CO₂ and O₂ (Kₗ and Kₐ, respectively), and the concentration of CO₂ and O₂ in mesophyll cells (C₀ and O₀, respectively) (Farquhar et al., 1980; Teraslama et al., 1995). Vₗₚₚ₀, Vₗₚₐ₀, Kₗ and Kₐ depend on temperature. C₀ and O₀ depend on the temperature and partial pressure of CO₂ and O₂, respectively, in the intercellular spaces. Therefore, when the temperature is the same in two locations at different altitudes, Vₗₚₚ₀, Vₗₚₐ₀, Kₗ and Kₐ are independent of altitude and C₀ and O₀ depend on altitude. The prediction indicated that the amount of increase in A₀ with a given increase in molar concentration of CO₂ (in moles CO₂ per cubic meter) was independent of altitude. A₀ for a given molar concentration of CO₂, however, was consistently higher at higher altitudes than at lower altitudes due to the reduced O₂ inhibition at higher altitudes with lower atmospheric pressure (Teraslama et al., 1995). On the other hand, the increase in A₀ with a given mole fraction of CO₂ (in moles CO₂ per mole) was lower at higher altitudes than at lower altitudes suggesting interaction between global CO₂ increase and altitude. The A₀ for a given mole fraction of CO₂ was lower at higher altitudes than at lower altitudes.

This study aimed to test the predictions about the effects of altitudes and global CO₂ increase on Pₚ reported by Teraslama et al. (1995). To test long-term, rather than short-term, response of crop growth to high CO₂ concentrations, we erected open-top chambers (OTCs) at high altitudes and grew wheat crops under ambient and increased CO₂ concentrations. To analyze the altitudinal difference in Pₚ, the same wheat cultivar was grown at a low altitude using growth-chambers. Wheat was also cultivated in an open field under an ambient CO₂ concentration at the low altitude to compare plants grown in growth-chambers at an ambient CO₂ 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 CO₂ partial pressure at Lhasa, where the CO₂ concentration was lower than the current CO₂ 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 OTGs at two levels of CO₂, i.e., one OTC with ambient levels of CO₂ (OTC Ambient) and one OTC with increased levels of CO₂ (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. CO₂ for the increased levels was supplied from liquefied petroleum gas-firing equipment (CG-255S2G, Nepon, Japan) and was injected into a blower that supplied 1800 m³ h⁻¹ air (approximately 2500 μmol CO₂ mol⁻¹) through plastic pipes placed about 15 cm above the canopy. The CO₂ 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. CO₂ 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 CO₂ 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 15.6, 9.0 and 17.8°C, respectively, in open field.

Seeds were sown on 3 May 2001 at 550 seeds per m². Ears emerged around 18 July 2001 (76 DAS). In the same way as used by local farmers, nitrogen (N), phosphorus (P)
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.

### 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 \(\mu\)mol 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.

1. CO\(_2\) levels (during the day): 250, 380 and 580 \(\mu\)mol mol\(^{-1}\); actual day means achieved were 246, 394 and 587 \(\mu\)mol 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 \(\mu\)mol mol\(^{-1}\), respectively, in Sapporo.

2. Temperature: 11/19ºC and 11/21ºC (night/day maximum and minimum temperature cycle).

3. 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 \((3 \times 2 \times 2)\) 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.
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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 (43ºN, 141ºE, 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 13.8ºC, 7.9ºC and 21.6ºC, respectively, in 2002, and 14.3ºC, 6.1ºC and 20.8ºC, respectively, in 2003.

2. Measurements
Maximum $P_n$ ($P_{\text{max}}$) and stomatal conductance were measured at PPFD 1600 $\mu$mol 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 CO2 concentration. The leaf temperature and relative humidity was maintained at 20–25ºC (actual value achieved was 19.1–26.8ºC) and 50–60% (actual value achieved was 49–66%), respectively. The light response curve of $P_n$ was measured. The slope of linear part of light response curve at PPFD 0 to 150 $\mu$mol m$^{-2}$ s$^{-1}$ was used to estimate the apparent quantum yield. The light response curve of $P_n$ was measured when $P_{\text{max}}$ was measured, except in the OTC experiment when the light response curve of $P_n$ was measured at 74 DAS, i.e., the last $P_{\text{max}}$ measuring date. The $P_{\text{max}}$ 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 $P_{\text{max}}$, 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 $P_{\text{max}}$ and apparent quantum yield under different growth conditions were determined by regression analysis for CO2 concentration and photosynthetic photon flux, respectively, with growth conditions treated as a dummy variable.

Result
$P_{\text{max}}$ measured at PPFD 1600 $\mu$mol 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 CO2 concentration and temperature were significant, but the difference between treatments was much larger for CO2 concentration than for temperature (Table 2). The effect of date was significant, but the treatment × date interaction effects were not significant. $P_{\text{max}}$ measured at the current level of CO2 was similar in Open-field and the growth-chamber in Sapporo. The ambient level of CO2, $P_{\text{max}}$ was 19% lower in Lhasa than in Sapporo (P < 0.05 by t-test).

The values of $P_{\text{max}}$ from all experiments in Lhasa and Sapporo were plotted against molar concentration of CO2 in the air and mole fraction of CO2 in the air to determine if an increase in CO2 at these two locations at different altitudes had a different effect on Pn (Fig. 2). Linear relationships were observed between CO2 and $P_{\text{max}}$ in the

| Treatment        | P$_{\text{max}}$ (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    |

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 CO2; OTC-Increased, open-top chamber with increased levels of CO2.
Table 2. Maximum net photosynthetic rate (P_{max}), stomatal conductance and chlorophyll content (SPAD value) of spring wheat in Sapporo.

| Treatment          | P_{max} (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 ± 0.96|
| Growth-chamber CO2 |                               |                                          |            |
| 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              | 24.3 ± 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

| Factor       | CO2 | Temperature (T) | Nitrogen (N) | Date (D) | CO2 × D | T × D | N × D |
|--------------|-----|-----------------|--------------|----------|---------|------|------|
| Statistical  |     |                 |              |          |         |      |      |
| effect       | *   | NS              | NS           | NS       | NS      | NS   | NS   |
| Growth-chamber CO2 | *   | NS              | NS           | NS       | NS      | *    | NS   |
| Date (D)     |     |                 |              |          |         |      |      |
| CO2 × D      |     |                 |              |          |         |      |      |
| T × D        |     |                 |              |          |         |      |      |
| N × D        |     |                 |              |          |         |      |      |

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 CO2 treatment in the growth-chamber experiment at P<0.05 (Tukey HSD).

Fig. 2. Effect of CO2 concentration in the air on maximum net photosynthetic rate (P_{max}) of spring wheat before heading. (a) Effect of molar concentration of CO2 in the air (in moles CO2 per cubic meter) on P_{max}. 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 CO2 in the air (in moles CO2 per mole) on P_{max}. 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).
Regression equations showed similar slopes for the effect of molar concentration of CO₂ on \( P_{\text{max}} \) in Lhasa and Sapporo \((P = 0.75)\), but \( P_{\text{max}} \) for a given molar concentration of CO₂ was higher in Lhasa than in Sapporo. However, the slope of \( P_{\text{max}} \) against mole fraction of CO₂ 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 CO₂ showed no significant difference between Lhasa \((0.43 \text{ mol m}^{-2} \text{s}^{-1})\) and Sapporo \((0.44 \text{ mol m}^{-2} \text{s}^{-1})\) \((P = 0.84)\).

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 \((P = 0.05)\). In the growth-chamber experiment, the main effects of CO₂ 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 CO₂ 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 \((P = 0.41)\) \((P = 0.05)\). In the growth-chamber experiment, it significantly increased with the increase in CO₂ \((P = 0.05)\). The effects of temperature and nitrogen doses were not significant \((P = 0.90 \text{ and } 0.81, \text{ respectively})\). The apparent quantum yield at the current level of CO₂ 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 \( P_{\text{max}} \) of wheat plants grown in open field in this study, in agreement with previous studies on the OTC effect \((P = 0.05)\) \((P = 0.90 \text{ and } 0.81, \text{ respectively})\). The apparent quantum yield at the current level of CO₂ did not show any difference between the location at different altitudes \((P = 0.13)\).
in the air not against intercellular CO₂ concentration in
this study. Since there was no significant difference in
stomatal conductance between two locations, the
relationship between Pₙmax and CO₂ concentration in
the air would be similar to that between Pₙmax and intercellular
CO₂ concentration. Regression equations of Pₙmax against
molar concentration of CO₂ in the air showed similar
slopes in Lhasa and Sapporo and Pₙmax for a given molar
concentration of CO₂ in the air was higher in Lhasa than
in Sapporo. This was agreement with the prediction by the
theoretical model for Aₙ of rubisco (Terashima et al.,
1995). Terashima et al. (1995) predicted that the amount
of increase in Aₙ with a given increase in molar
concentration of CO₂ was independent of altitude. The
prediction, however, indicated that the Aₙ for a given molar
concentration of CO₂ was consistently higher at higher
altitudes than at lower altitudes due to the reduced O₂
inhibition at higher altitudes (Terashima et al., 1995).

The regression equations of Pₙmax against mole fraction of CO₂ showed significantly steeper slope in Sapporo than
in Lhasa suggesting an interaction between CO₂ level and altitude. The theoretical model for Aₙ of rubisco (Terashima et al., 1995) predicted lower slope of Aₙ against mole fraction of CO₂ at higher altitudes than at lower altitudes, which was in agreement with the results of this study. The difference in slopes of Pₙmax against mole fraction of CO₂ between Lhasa and Sapporo was explained by lower air pressure in Lhasa. The relationship between mole fraction and molar concentration of CO₂ depends on air pressure. The increase in molar concentration of CO₂ is smaller at high altitudes than at low altitudes for a given
increase in mole fraction. Because the slopes of Pₙmax
against molar concentration of CO₂ were similar in both
locations, we expected that the same increase in mole
fraction of CO₂ resulted in a lower increase in Pₙmax in
Lhasa than in Sapporo.

Pₙmax for each mole fraction of CO₂ 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 (Vₖ) and oxygenation rate (Vₒ)
deceased with the increase in elevation because of lower
air pressure at the higher altitude (Terashima et al., 1995).
Because the absolute value of Vₖ is larger than that of Vₒ,
the reduction with altitude increase is greater for Vₖ
than for Vₒ. As a result, Pₙmax 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 Pₙ 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 Pₙ 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 Pₙ at low altitude than at high
altitude. In this study, the values of stomatal conductance

| Location and treatment | Apparent quantum yield (μmol CO₂ 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         |                                               |               |
| CO₂ (μmol mol⁻¹)       |                                               |               |
| 250                    | 0.0537                                        | 0.0018        |
| 380                    | 0.0625                                        | 0.0016        |
| 580                    | 0.0697                                        | 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        |

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

Data of the Open-field experiment in Sapporo were pooled across two years. OTC-Ambient, open-top
chamber with ambient levels of CO₂. OTC-Increased, open-top chamber with increased levels of CO₂.
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 $P_{\text{max}}$. 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 $P_{\text{max}}$ between locations. Therefore, apparent quantum yield did not explain the difference in $P_{\text{max}}$ 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 $P_n$ in the growth-chamber. The response of $P_{\text{max}}$ 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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