Aspects of CO_2 Uptake in the Crassulacean Acid Metabolism Orchid *Phalaenopsis*

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Abstract

Phalaenopsis* and its hybrids are the most important orchid pot plant commercially in the world now. Research on photosynthesis gives us practical and useful information for improving cultivation. Although conventional gas-exchange technique has some limitations in the research of a crassulacean acid metabolism plant (CAM), we investigated CO_2 uptake in *Phalaenopsis*. CO_2 uptake at night (Phase 1) changed with temperature. Maximum CO_2 uptake was observed around 20°C. CO_2 absorption at night increased in proportion to CO_2 concentration. Rate of CO_2 absorption was higher at 20°C than at 25°C. CO_2 uptake in the late afternoon (Phase 4) showed a maximum around 20°C. CO_2 absorption in Phase 4 increased in proportion to CO_2 concentration but stomata conductance decreased under high CO_2 levels. CO_2 absorption response curve to light intensity in Phase 4 was not saturated till 500 umol·m^{-2}·s^{-1} PPFD when CO_2 level was 2000 ppm, and then the stomata conductance showed very low values. Under various water and humidity condition when *Phalaenopsis* was irrigated weekly, CO_2 uptake in Phase 1 was the largest at one day before watering, but that of Phase 4 was the largest at one day after watering. Total CO_2 uptake in all Phases was stimulated the most at 70% relative humidity and suppressed drastically at 30% relative humidity. These results indicated that higher relative humidity is likely the most important factor for high CO_2 absorption in *Phalaenopsis*.

INTRODUCTION

Flower beauty, rapid growth, easy to control flowering, and other features make *Phalaenopsis* production advantageous. Because of

these features *Phalaenopsis* production among orchid pot plant in Japan is the largest at present. But many problems must still be solved.

Photosynthetic ability is an important basis for the growth of all green plants. To clarify the optimal culture condition of a green plant, investigation of the photosynthetic characteristics is a conventional and a convenient method. Photosynthetic ability can be estimated by measuring CO_2 absorption of a plant within a short time. In C3 plants, the optimal light intensity and the optimal temperature for CO_2 absorption are considered to be the optimal environmental condition for growth. Photosynthetic characters of C3 orchids were investigated to ascertain culture conditions (Johnson, 1992; Kako et al., 1979a, b; Miura, 1981).

In *Phalaenopsis*, which performs CAM, there were many investigations to clarify optimal environmental condition for CO_2 absorption (Guo and Lee, 2006; Ichihashi, 1997). However, no direct light- and temperature-photosynthetic curve was ascertained. Unlike C3 plants, CO_2 absorption of CAM plants occurs mainly at night (Phase 1) and partly in the late afternoon (Phase 4). In CAM plants, CO_2 fixed and accumulated as malic acid during the previous night is assimilated into sugars the following day during Phase 3.

The optimal temperature for CO_2 absorption in Phase 1 was 20^{o}C (Kano and Naitoh, 2001; Ota et al., 1991), but photosynthesis in Phase 3 measured by O_2 electrode was the highest at 30^{o}C (Ota et al., 2001). Plant growth was also better under a constant temperature of 30^{o}C rather than 20^{o}C (Kubota and Yoneda, 1990). *Phalaenopsis* grew better under higher day and night temperature of 30/25^{o}C than that at 25/20^{o}C (Kaziwara et al., 1992; 1993). There is a gap between optimal temperature for CO_2 absorption at night and for growth and for O_2 release at daytime.

Light condition is another main environmental factor for the photosynthesis and growth of *Phalaenopsis*. In *Phalaenopsis,* favorable light conditions of daytime had been estimated by measuring CO_2 absorption at night in Phase 1. Light condition at daytime affected night CO_2 absorption. Night CO_2 absorption saturated when small and young plant received about 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (PPF; Ota et al., 1991), 15 klx light (Kawamitsu et al., 1995) or 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF (Lootens and Heursel, 1998) at daytime. However, CO_2 absorption by larger plants was promoted by much higher light

intensities. Leaves at lower positions receive week and diffused light. Overall CO_2 absorption by large plants at night (Phase 1) and during Phase 4 increased when they received high light intensity (677 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during day time, because CO_2 absorption in the lower leaves was enhanced under higher light intensity (Suto, 1993). These results indicate that the saturation points for single leaf and multiple leaves plant differs because of self shading (Lin and Hsu, 2004).

The light saturation point in Phase 3, measured by O_2 electrode using a leaf disc, was much higher than that mentioned above and it was around 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Ota et al., 2001). The reason of this difference is not clear.

CO_2 level in the ambient atmosphere is another factor affecting CO_2 absorption by a leaf. CO_2 exchange rate was saturated at about 700 ppm both in Phase 1 and Phase 4 (Kawamitu et al., 1995). Initial growth rate of young *Phalaenopsis* increased as the CO_2 level increased (Endo and Ikusima, 1997a). Flower production and the quality were improved also by CO_2 enrichment at 700 ppm (Endo and Ikusima, 1997b). These reports indicate that CO_2 enrichment favorably affects *Phalaenopsis* growth.

CAM plants exhibit higher potential for limited water supply. In *Phalaenopsis* production, weekly irrigation is a common practice. However, CO_2 uptake declined at 10 days after irrigation (Ota et al., 1991). Beside drought, higher levels of mineral ions in nutrient solutions limited photosynthesis (Cui et al., 2004). Water potential is an important factor of CO_2 uptake in *Phalaenopsis*.

Lower relative humidity (RH) caused dehydration of plants but resulted in no severe damage to the photosynthetic system in *Phalaenopsis* (Su et al., 2001). Relative water content and photosynthetic efficiency (Fv/Fm) of plantlets in vitro were affected significantly by RH during acclimatization (Jeon et al., 2006).

In this investigation, effects of temperature, light intensity, CO_2 concentration and RH for CO_2 uptake were investigated to reveal factors that limit CO_2 uptake and to clarify the optimal culture condition for *Phalaenopsis*.

MATERIALS AND METHODS

Phalaenopsis White Dream ‘MM74’, with six leaves and a maximum length of 25 cm in leaf spread, were potted in 14-cm diameter

unglazed clay pots; *Phalaenopsis* Miki, 6 leaves with a maximum leaf spread of 20 cm potted in 10.5-cm diameter plastic pots and *Doritaenopsis* Cinderella Beauty, 4 or 5 leaves with a maximum leaf spread of 17.3 cm potted in 9-cm diameter plastic pots with New Zealand sphagnum moss were purchased from a local grower and served as materials for CO_2 measurement. Hyponex, a commercial fertilizer (6 N:10 P_2O_5:5 K_2O, Hyponex, Japan) diluted to 2000 fold, was used for irrigation every time when the sphagnum moss had dried up and irrigated with leaching.

Plants were transferred, more than 1 week before measurements, to a walk-in growth chamber (KG-50HLA, Koito) at a constant 25°C and 70% RH under about 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD provided by florescent lamps (FPR96EX-N/A, Matushita) at light period（17:00-10:00）and at 20°C and 70% RH at dark period（10:00-17:00). For the convenience of measurement, the photoperiod was reversed.

Photosynthetic characteristics were measured by a portable photosynthesis analyzer (CIRAS, PP System). Measurements were made by holding the uppermost fully expanded leaf with a leaf cuvette in a growth chamber (BIOTRON LPH-200-RDSCT, Nippon Ika Co.). The light intensity of the chamber was 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF in light condition provided by florescent lamps (FL 20SS N/18, Toshiba). The temperature of light period （17:00-10:00） was 25°C and that of dark period was 22°C （10:00-17:00）. RH was kept at 70%. The condition of the leaf cuvette for measurements was changed accordingly.

RESULTS AND DISCUSSION

Daily Outline of CO_2 Absorption

Phalaenopsis leaves showed CO_2 absorption patterns of a CAM plant in 24-hour measurements (Fig. 1). CO_2 was absorbed at night (Phase 1), just after day break (Phase 2) and in the late afternoon (Phase 4). CO_2 uptake and stomata conductance showed similar changes in the same measurement, but the absorption patterns differed among each measurement (Fig. 1A, 1B). The correlation values (r) between stomata conductance and CO_2 absorption rates were 0.944 and 0.752, respectively, in measurements shown in Fig. 1A and 1B.

In Fig. 1A and 1B, the conditions of measurements were the same, but CO_2 absorption rates at late light period increased rapidly in 1B but stayed low in 1A. CO_2 absorption rates at night also stayed

higher in 1B but lower in 1A. CO_2 absorption rates during the dark period were higher in Fig. 1B than 1A. CO_2 absorption rates at the early light period (Phase 2) decreased rapidly and reached almost zero within 1.5 hours (Phase 3).

Phalaenopsis showed CAM photosynthesis but unexpected factor affected daily CO_2 uptake. It was suspected that water condition affected CO_2 uptake of Phases 4 and 1, but it was not identified in this experiment.

Nocturnal Aspect of CO_2 Absorption

CO_2 uptake during the dark period (Phase 1) was affected markedly by temperature (Fig. 2). The absorption rates were the highest at 20°C followed by 15°C. The rates lowered at temperatures higher than 20°C and dropped to nearly zero at 35°C. Stomata conductance also showed a similar trend.

Results from other studies had indicated that CO_2 absorption at night was stimulated by temperature of about 20°C and suppressed by temperatures higher than 25°C and 15°C or lower (Kano and Naitoh, 2001; Ota et al., 1991). Inhibition of CO_2 absorption at higher night temperatures as a result of stomata closure is typical for CAM plants (Osmond, 1978). In *Phalaenopsis*, closure of stomata under higher temperature to reduce transpiration also seems to be the main reason of decreased CO_2 uptake.

CO_2 enrichment has been a major interest of *Phalaenopsis* growers to promote growth and the utility is well known. CO_2 concentration affected the absorption in Phase 1. CO_2 uptake increased significantly as the ambient CO_2 concentration increased to 1500 ppm. At 500 ppm, CO_2 absorption continued to increase for about 13 hours and then plateaued. However, at 1000 or 1500 ppm, the absorption continued to increase for more than 13 hours before leveling off (Fig. 3).

CO_2 uptake was saturated or slowed down about 13 hours in this experiment. This agrees with the results of Kawamitu et al. (1995). CO_2 enrichment for 13 hours at night seems to be effective and practical. CO_2 concentration affected the absorption of CO_2. Initial growth rate increased according to increased CO_2 level of 438, 700 and 1000 ppm (Endo and Ikusima, 1997a). Flower production and the quality were improved also by CO_2 enrichment (Endo and Ikusima, 1997b). In this experiment, CO_2 absorption was promoted corresponding CO_2 levels and

the limit was not clear. Kawamitu et al. (1995) suggested using 700 ppm CO_2 and 13 hours of exposure as a guideline for CO_2 enrichment at 20°C. Under higher levels of CO_2 than 700 ppm, shorter dark periods than 13 hours may be acceptable.

Besides CO_2 concentration, CO_2 absorption during Phase 1 was affected by temperature, too. The rate of CO_2 uptake was larger at 20°C than at 25°C. The correlation value (r^2) between CO_2 uptake rate and CO_2 concentration were 0.889 and 0.827 at 20°C and 25°C, respectively (Fig. 4). Stomata conductance and CO_2 level showed no clear relationship but consistently higher at 20°C than at 25°C (Data not shown).

Diurnal Aspect of CO_2 Absorption in Phase 3

No significant CO_2 uptake was observed in Phase 3, but differences in CO_2 uptake or release among various temperatures was observed (Fig. 5). At 15°C, there was low level of CO_2 absorption but no absorption was observed at 20 and 25°C. At 30 and 35°C, release of CO_2 was observed. Stomata conductance was low and no significant difference was observed but the value was highest at 20°C. The aspect of stomata conductance curve was similar to that of Phase 1. Stoma opening seems to be partially controlled by temperature.

Phase 3 is specific to CAM plants. There is no clear CO_2 uptake but photosynthetic carbon fixation is active in Phase 3. Measured by O_2 electrode, *Phalaenopsis* released O_2 in Phase 3 and showed the maximum at 35°C. For O_2 release, higher temperature than 20°C and higher light levels than 600 $umol \cdot m^{-2} \cdot s^{-1}$ were preferred (Ota et al., 2001). Transpiration is not the limiting factor of photosynthesis in Phase 3 because of stomata closure. CO_2 supply is not a limiting factor either, because CO_2 is supplied by decarboxylation of malic acid being accumulated the previous night. Photosynthesis in Phase 3 is free from being affected by both water and CO_2 deficiency. This may be the main reason for the higher optimum temperature and light intensity in Phase 3.

Diurnal Aspect of CO_2 Absorption in Phase 4

CO_2 uptake in Phase 4 fluctuated and was affected significantly by temperature. The uptake rates were highest at 20°C. The rates decreased drastically at 25 and 30 °C and reached zero at 35°C. Stomata

conductance showed a trend similar to CO_2 uptake (Fig. 6), but the values were lower than that in Phase 1.

Phase 4 is transient and does not last long. Phase 4 lasted longer under long day condition (Suto, 1993). In the current experiment, long day condition of 17 hours was adopted to secure measurements in Phase 4. The aspects of CO_2 uptake and temperature curve were similar to Phase 1 (Fig. 2). This suggests that opening and closure of *Phalaenopsis* stomata may be controlled mainly by temperature.

CO_2 concentration and CO_2 uptake response curve is shown in Fig. 7. CO_2 uptake increased linearly with the increase of CO_2 concentration. However, stomata conductance increased up to 177 ppm and then declined when CO_2 level increased further. At 1819 ppm the highest CO_2 uptake was observed without stomata opening. Higher CO_2 levels brought higher rates of CO_2 uptake, but CO_2 levels and stomata opening showed no correlation (r=-0.07). CO_2 uptake was not inhibited by stomata closure under higher levels of CO_2.

Light intensity and CO_2 uptake response curve was made at 2000 ppm CO_2 levels and is shown in Fig. 8. CO_2 uptake rate was high and increased till 800 $umol \cdot m^{-2} \cdot s^{-1}$ PPFD. No significant increase in CO_2 uptake was observed beyond 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The stomata conductance in Phase 4 was very low or near zero at all light levels. This was likely the response to super high CO_2 level than to the light intensity.

CO_2 uptake and stomata conductance in Phase 4 showed no correspondence. Although stomata conductance was very low, higher CO_2 uptake was observed. Under higher levels of CO_2, much CO_2 could be absorbed without stomata opening and higher rate of CO_2 assimilation was attained under higher levels of light. This suggests the effectiveness of CO_2 enrichment without stomata opening. Low RH, high light level and high temperature might be acceptable under high CO_2 levels.

The big gap of light saturation points and optimal temperature between different measurement of CO_2 uptake and O_2 release seems to be reflected by limitation of CO_2 supply. Under condition where CO_2 supplies are not limiting, higher light and higher temperature seem to be preferred. Limitation of CO_2 uptake seems to restrict optimal temperature to 20^oC and optimal light levels to 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Ota et al., 1991) or 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF (Lootens and Heursel, 1998).

Water Condition in Pots and Relative Humidity (RH)

To clarify the effects of water condition, measurements of CO_2 uptake in various water conditions in pots and RH was made. CO_2 uptake was monitored one day before irrigation, just after irrigation and one day after irrigation of weekly irrigation under 30%, 50% or 70% relative humidity.

CO_2 uptake rate was affected the most by RH. Under 70% RH, CO_2 uptake was enhanced the most in all water conditions in Phases 1, 2, and 4. CO_2 uptake rates differed among various Phases. The rates of CO_2 uptake in Phase 1 were the highest followed by Phase 2 or Phase 4 (Fig. 9).

The amount of CO_2 uptake in each Phase showed the same trend of being stimulated the most by RH. Under 70% RH, CO_2 uptake was the highest in all water conditions. The amount of CO_2 uptake in Phase 1 was the greatest followed by Phase 4. Water condition of pots affected CO_2 uptake in Phase 4 significantly. CO_2 uptake in Phase 4 was stimulated the most at one day after irrigation but the total amount of CO_2 uptake was the largest at one day before irrigation (Fig. 10).

In *Phalaenopsis*, CO_2 is absorbed the most in Phase 1. CO_2 absorption in Phase 1 was affected by both RH and water condition in a pot. The best condition for higher CO_2 uptake was higher humidity in the dark period at one day before of weekly irrigation. Although CO_2 absorption in Phase 4 was affected by RH and irrigation, but the amount of CO_2 absorbed was not close to that in Phase 1.

The results of these experiments show that there seems to be some choices to promote CO_2 uptake in practical cultivation. In Phase 1, high RH is a practical and an economical method to promote CO_2 uptake. In lower RH, CO_2 enrichment may be used to increase CO_2 uptake. However, there is a limitation of CO_2 enrichment in Phase 1. Excessive CO_2 enrichment dose not make sense because there is a limitation of cell capacity where CO_2 can be stored. In Phase 4, CO_2 is absorbed and fixed directly. There seems to be less limitation of cell capacity. In Phase 4, higher RH is also recommendable for promotion of CO_2 uptake. When RH is low in Phase 4, CO_2 enrichment will increase CO_2 uptake.

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Figures

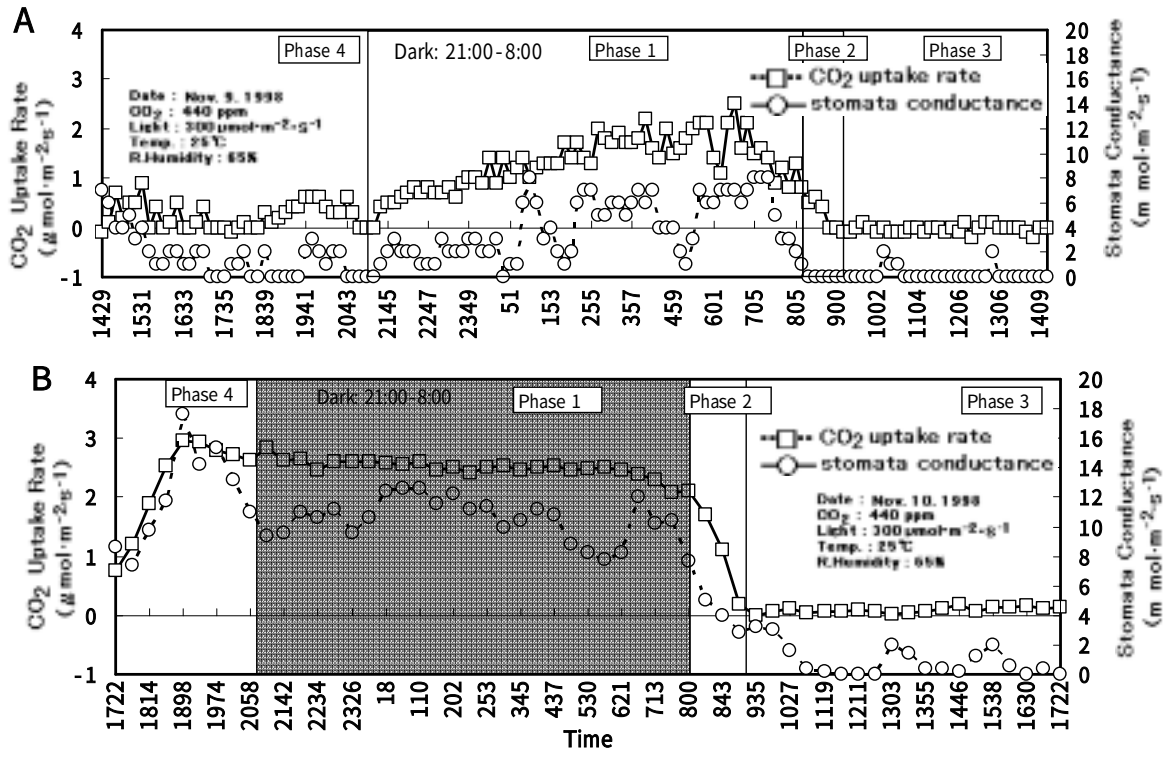

Fig. 1. Aspects of CO_2 uptake and stomata conductance throughout 24 hours. Measurements were made on the same plants of *Phal*. White Dream 'MM-74' successively.

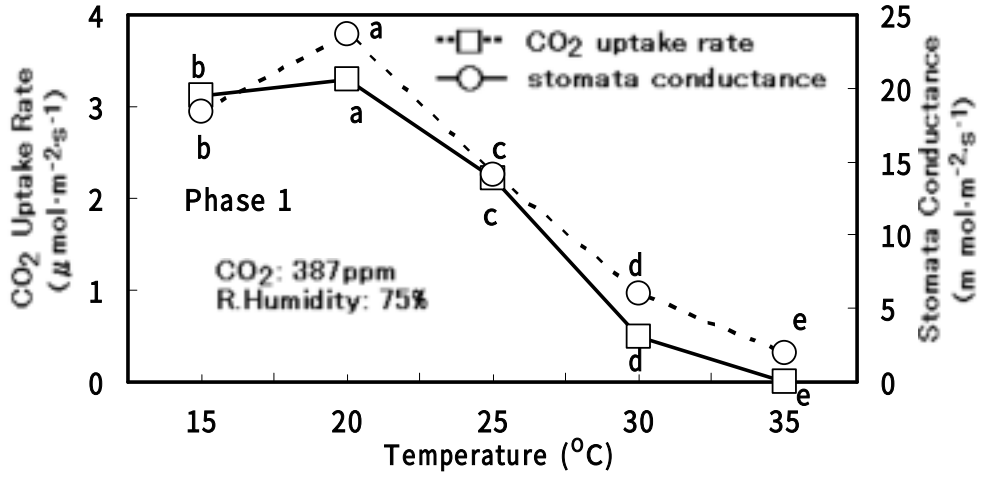

Fig. 2. CO_2 uptake rate and stomata conductance in Phase 1 at various temperatures. Measurements were made 5 times on *Phal*. White Dream 'MM-74'. Different letters indicate significant difference at 1% by DMRT.

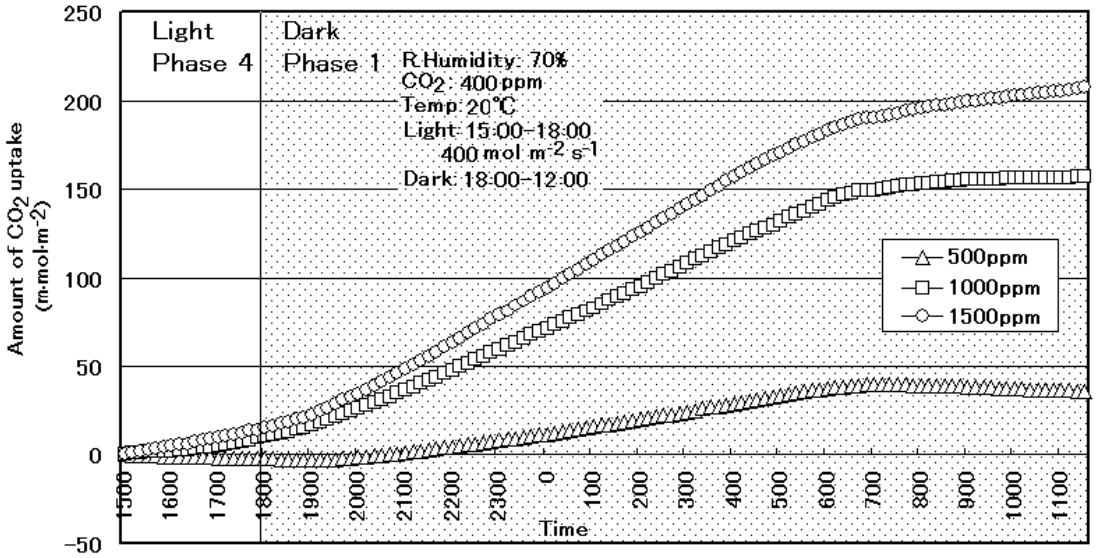

Fig. 3. CO_2 uptake under various CO_2 concentrations during Phase 1. Each data point represents the average of 3 measurements of *Doritaenopsis* Cinderella Beauty plants at 500, 1000 or 1500ppm CO_2 levels. All data after 16:00 indicate significant difference at 5 % by DMRT.

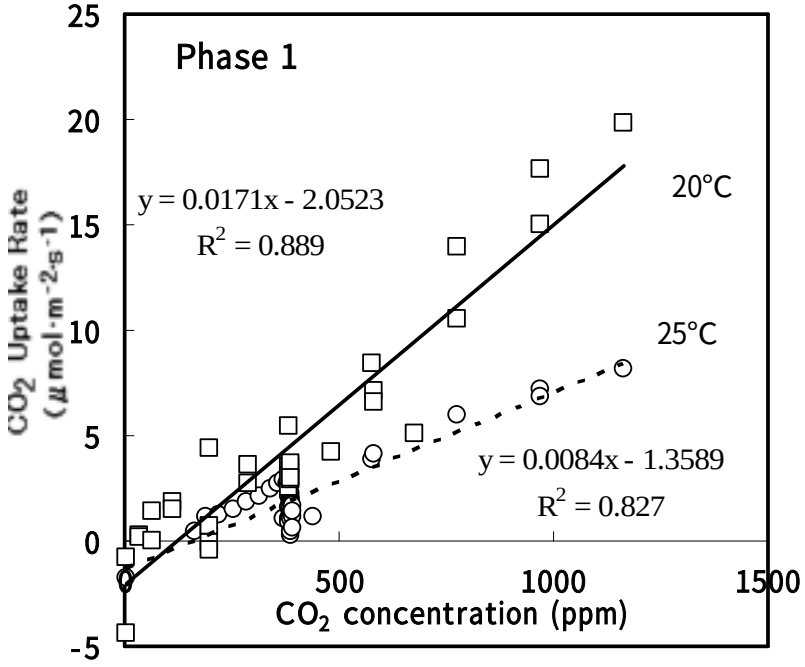

Fig. 4. CO_2 uptake at 20 and 25°C at various CO_2 levels during Phase 1. Data were collected from different measurements of *Phal.* White Dream 'MM-74' at 20°C (□) or 25°C(○).

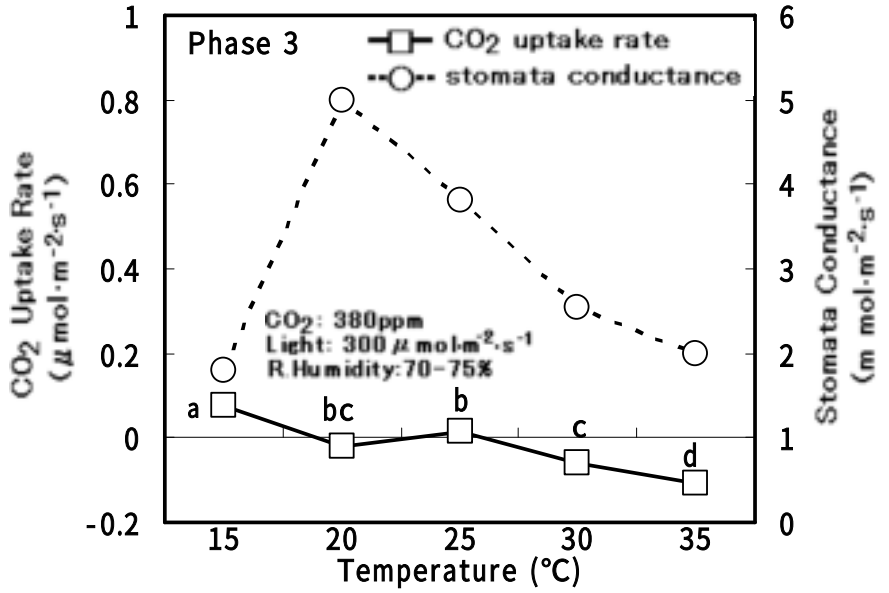

Fig. 5. CO_2 uptake rate and stomata conductance in Phase 3 at several temperatures. Each data point represents an average of 4 measurements of *Phal.* White Dream 'MM-74'. Different letters indicate significant difference at 5% by DMRT. Stomata conductance shows no significant difference.

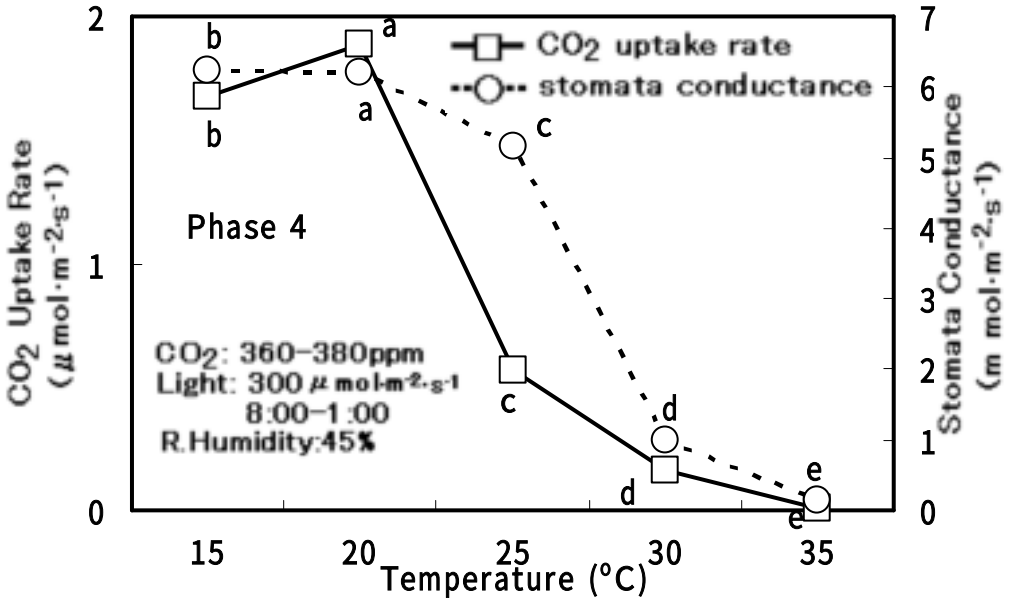

Fig. 6. CO_2 uptake rate and stomata conductance in Phase 4 at several temperatures. Measurements were made at least 14 times on *Phal.* White Dream 'MM-74'. Different letters indicate significant difference at 1% levels by DMRT.

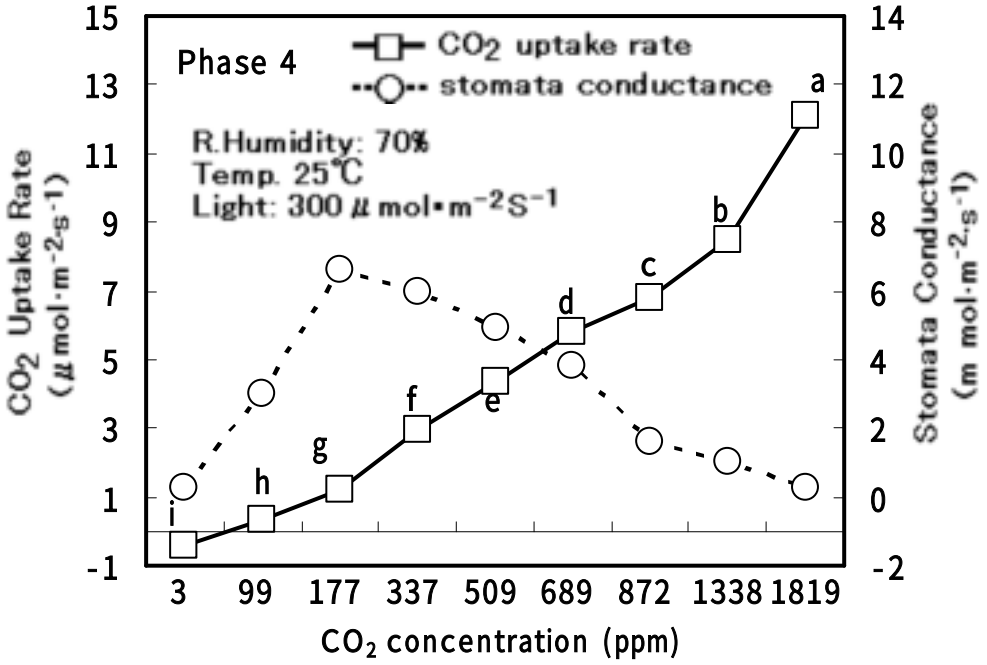

Fig. 7. CO_2 uptake rate and stomata conductance in Phase 4 under various CO_2 concentration. Each data represents the average of 4 measurements on *Phal*. White Dream 'MM-74'. Different letters indicate significant difference at 1% levels by DMRT. Stomata conductance shows no significant difference.

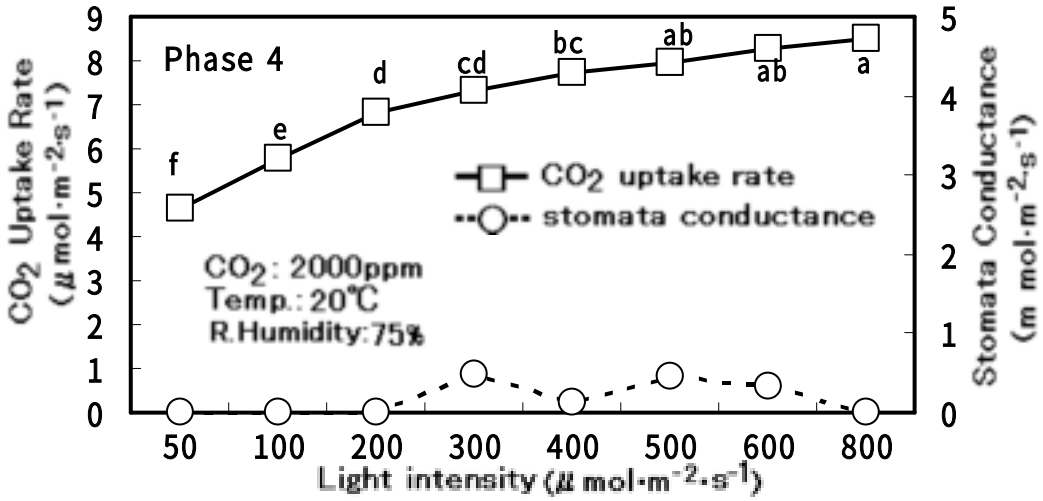

Fig. 8. CO_2 uptake and stomata conductance in Phase 4 under various light intensities. Each data point represents the average of 10 measurements on *Phal*. White Dream 'MM-74'. Different letters indicate significant difference at 1% levels by DMRT. Stomata conductance shows no significant difference.

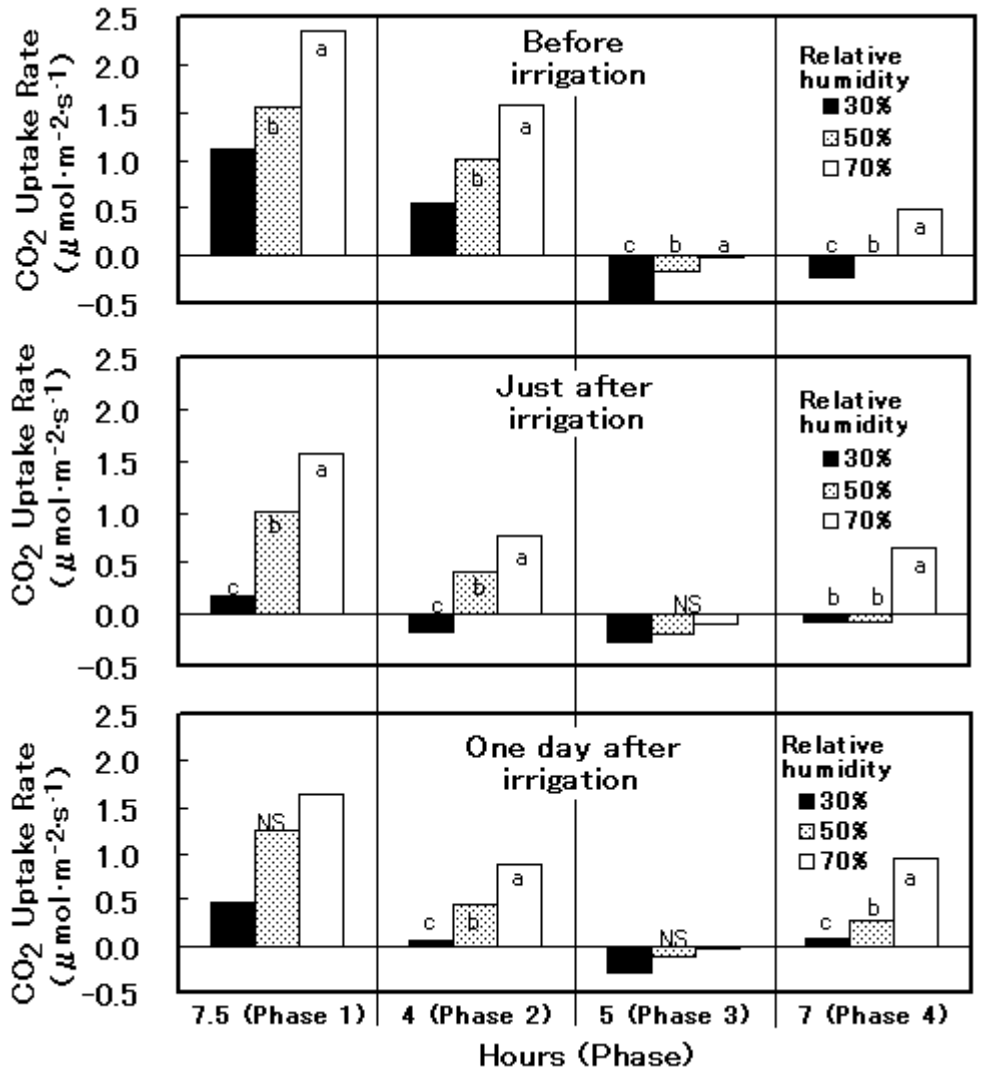

Fig. 9. Effects of different water conditions and relative humidity on CO_2 uptake rate in each period. Data represent the average of five measurements on *Phal.* Miki. Different letters indicate significant difference at 5% levels by DMRT.

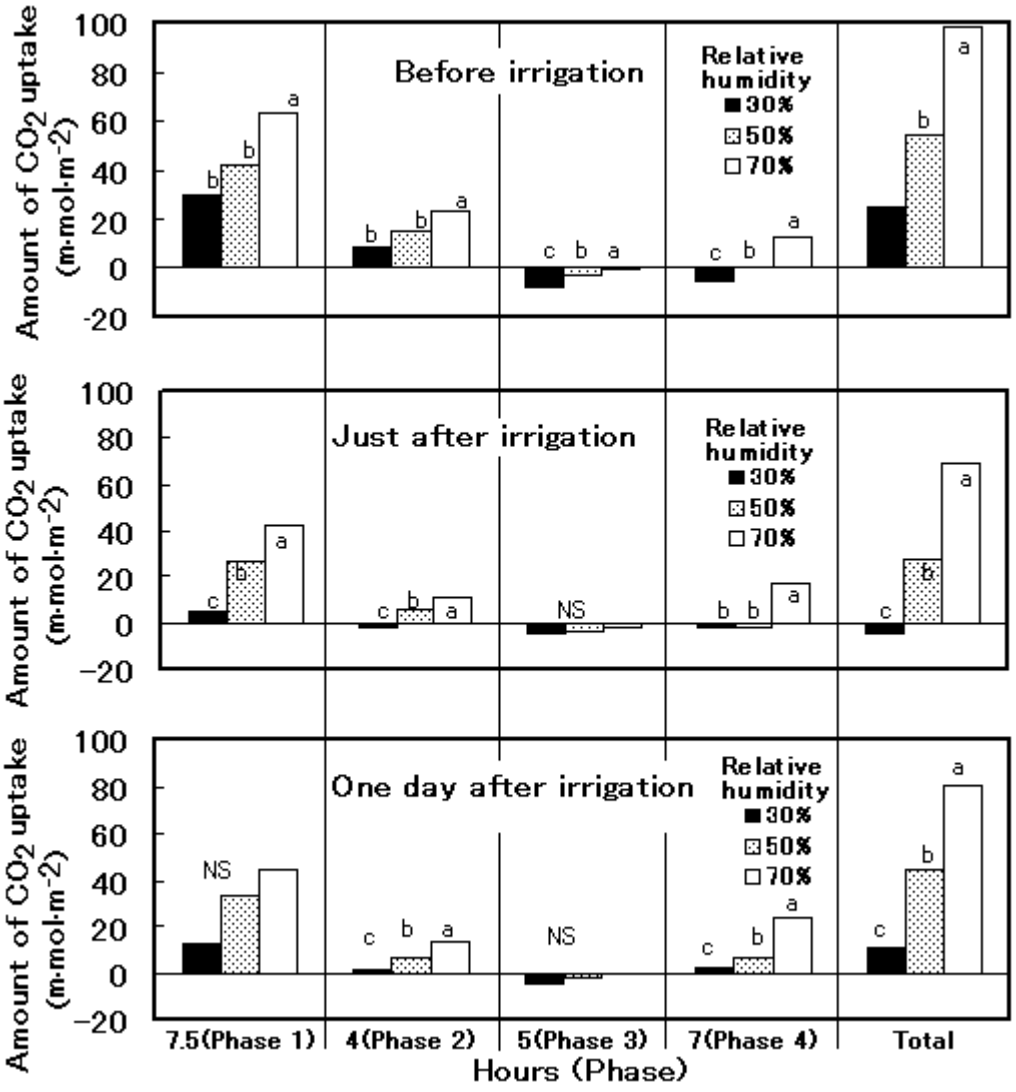

Fig. 10. Effects of different water condition and relative humidity on amount of CO_2 uptake in each period. Data represent the average of five measurements on *Phal.* Miki. Different letters indicate significant difference at 5% levels by DMRT.