PHOTOSYNTHETICA 43 (2): 299-302, 2005

BRIEF COMMUNICATION

Comparison of photosynthetic activity of *Orychophragmus violaceus* and oil-seed rape

Y.Y. WU*, X.M. WU, P.P. LI, Y.G. ZHAO, X.T. LI, and X.Z. ZHAO

The Institute of the Agricultural Equipment Engineering, Jiangsu University, Zhenjiang, Jiangsu, 212013, China

Abstract

Orychophragmus violaceus, *Brassica campestris* cv. Chuanyou No.8, and *Brassica juncea* cv. Luzhousileng diurnal changes of net photosynthetic rate (P_N) and activities of carbonic anhydrase (CA) of leaves were studied. One uni-modal curve occurred at the diurnal changes of P_N in *O. violaceus*, but bimodal curves were found in *B. campestris* and *B. juncea*. Thus photosynthetic midday depression was not found in *O. violaceus* but in both *Brassica* species. Midday depression of P_N in *O. violaceus* was not related to high temperature or low humidity at midday but to the activity of CA.

Additional key words: *Brassica*; carbonic anhydrase; net photosynthetic rate; photosynthetic midday depression.

Orychophragmus violaceus, which belongs to *Brassicaceae,* is an annual or biennial wild plant. It is adaptable to karst and has a great economic worth and medical value (Wu 1997, 2002). Diurnal changes of net photosynthetic rate (P_N) in various plant species are studied frequently (cf. Shirke and Pathre 2003). Nevertheless, photosynthetic characteristics of *O. violaceus* have not been yet recorded. We compared photosynthetic characteristics of this species and compared them with those of two species of rape.

The experiment was conducted in the greenhouse of Agriculture Ecology Laboratory of Jiangsu University. *Orychophragmus violaceus* (L.) O.E. Schulz, *Brassica campestris* L. cv. Chuanyou No.8, and *Brassica juncea* (L.) Czern. *et* Coss. cv. Luzhousileng were sown in October 2002, then transplanted into the greenhouse in November, cultivated in soil of medium fertility, and watered regularly. P_N of single attached leaves at flower-pod stage was measured in March 2003 using the portable photosynthesis measurement system *LI-6400* (*LI-COR Corp.*, Lincoln, NE, USA). Leaf temperature [°C], photosynthetically active radiation, PAR [µmol m^{-2} s^{-1}], and air CO_2 concentration, C_i [cm^3 m^{-3}] were determined in parallel. The activities of carbonic anhydrase (CA; EC 4.2.1.1) were measured in 0.5 g samples of leaves. The leaves were quickly frozen in liquid N_2, ground into powder with a mill, then homogenized with 3 cm^3 of extraction buffer (10 mM veronal buffer with 50 mM 2-mercaptoethanol, pH 8.2). The homogenate was centrifuged at 10 000×*g* for 5 min. CA activity in the above 0.2–1.0 cm^3 supernatants was electrochemically determined by measuring the time required for the pH drop from 8.2 to 7.2 in 15 cm^3 of ice-cold barbital buffer (20 mM, pH 8.30) with 10 cm^3 of ice-cold CO_2-saturated distilled H_2O. One unit (WA-unit, Wilbur and Anderson 1948) of activity was defined as: WA = $t_0/t - 1$, where t_0 and t are the time taken, respectively, in the enzyme-free buffer (control) and the buffer containing enzyme sample.

On 26 March 2003, the environmental factors in the greenhouse, such as PAR, temperature, and relative humidity (RH), changed regularly within one day (Fig. 1). The diurnal change curves of P_N and g_s of *O. violaceus* of this day were bell-shaped, uni-modal, while those of *B. campestris* and *B. juncea* were bimodal (Fig. 2*A*, *B*). The peak values of P_N and g_s in *O. violaceus* appeared between 09:00 and 10:00, and some declines but no vales occurred between 13:00 and 14:00, *i.e*. there was no obvious "photosynthetic midday depression". But P_N of *B. campestris* and *B. juncea* kept dropping at about 11:00, and the phenomenon of "photosynthetic midday

Received 1 October 2004, *accepted* 6 January 2005.
*Corresponding author; fax: (+86)511-8797158, e-mail: yanyouwu@ujs.edu.cn
Abbreviations: CA – carbonic anhydrase; *E* – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; RH – relative humidity; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; WUE – water use efficiency.

depression" appeared in these rapes.

The diurnal change curves of *E* of the three species were bell-shaped. *E* was the highest during 12:00 to 13:00 and one very small bottom lasted for a short time at about 14:00 (Fig. 2*C*). From 11:00 to 13:00, *E* of *O. violaceus* was obviously slower than that of both *Brassica* species. The curves of water use efficiency (WUE) of all species were similar. WUE decreased quickly from 07:00 to 10:30; after 10:30 it was reduced extremely slowly, then increased slowly at about 15:20, and at last decreased again at 17:00. No corresponding relation between WUE and *E* was found (Fig. 2*D*). After

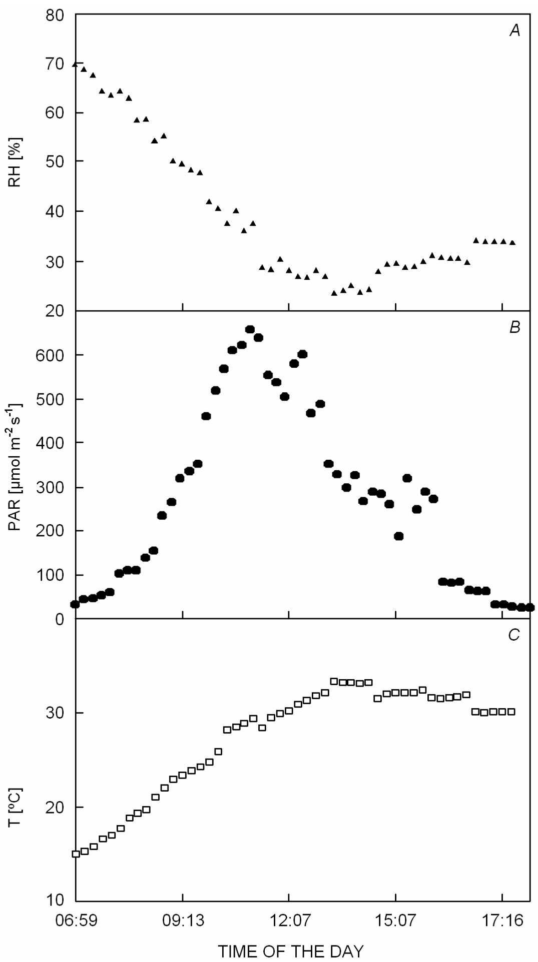

Fig. 1. The diurnal changes of air relative humidity, RH (*A*), photosynthetically active radiation, PAR (*B*), and air temperature, T (*C*).

09:00, WUE of *O. violaceus* was higher than that of the *Brassica* species.

Since all three tested species are C_3 plants belonging to *Cruciferae*, why was WUE of *O. violaceus* the highest, and why did the "photosynthetic midday depression" not occur in this species? The depression has often been ascribed to stronger irradiance, lower humidity, and higher temperature at noon (Pardo *et al.* 1995, Palanisamy 1996, Singh *et al.* 1996). The phenomenon did not occur in *O. violaceus* as a result of stronger irradiance, lower humidity, and higher temperature. Hence this species has a better mechanism to adapt to adverse circumstance at noon and this mechanism is related to higher WUE. The determination of the activities of CA offered some clues to the explanation of "photosynthetic midday depression" phenomenon (Fig. 2*E*). The activity of CA of *O. violaceus* was obviously higher than that of *Brassica* species. The activity of CA of *O. violaceus* increased fast, and those of *Brassica* species did change from 09:00 to 12:00 only a little.

CA, a zinc-containing metalloenzyme that catalyzes the reversible conversion of CO_2 to bicarbonate, is widely distributed in animals, plants, archea, and eubacteria, where it is involved in ion exchange, acid-base balance, carboxylation/decarboxylation reactions, and inorganic carbon diffusion between the cell and its environment as well as within the cell (Badger and Price 1994, Sasaki *et al.* 1998, Kaplan and Reinhold 1999). CA regulates the availability of CO_2 to RuBPCO by catalyzing the reversible hydration of CO_2. The greater CA activity leads to greater velocity of transforming bicarbonate into H_2O and CO_2. So there was more water and CO_2 in *O. violaceus* than in *Brassica* species, which compensated for the shortage of water and CO_2 under high evaporation and low g_s between 09:00 and 12:00. Therefore, P_N decreased insignificantly and midday depression did not occur.

An inhibition of CA activity in ethoxyzolamide infiltrated C_3 plant leaf pieces resulted in 80–90 % inhibition of P_N at low CO_2 concentrations (Williams *et al.* 1996). A significant correlation between CA activity and P_N was found in *B. juncea* (Ahmad *et al.* 2001, Hayat *et al.* 2000, 2001). The above analysis suggests that *O. violaceus* has a greater CA activity at midday for the sake of higher WUE, and no photosynthetic midday depression between 09:00 and 12:00. The plants grown in karst areas are often physiologically devoid of water. This mechanism of *O. violaceus* offers theoretical foundation for explaining its adaptation to karst (Wu 1997).

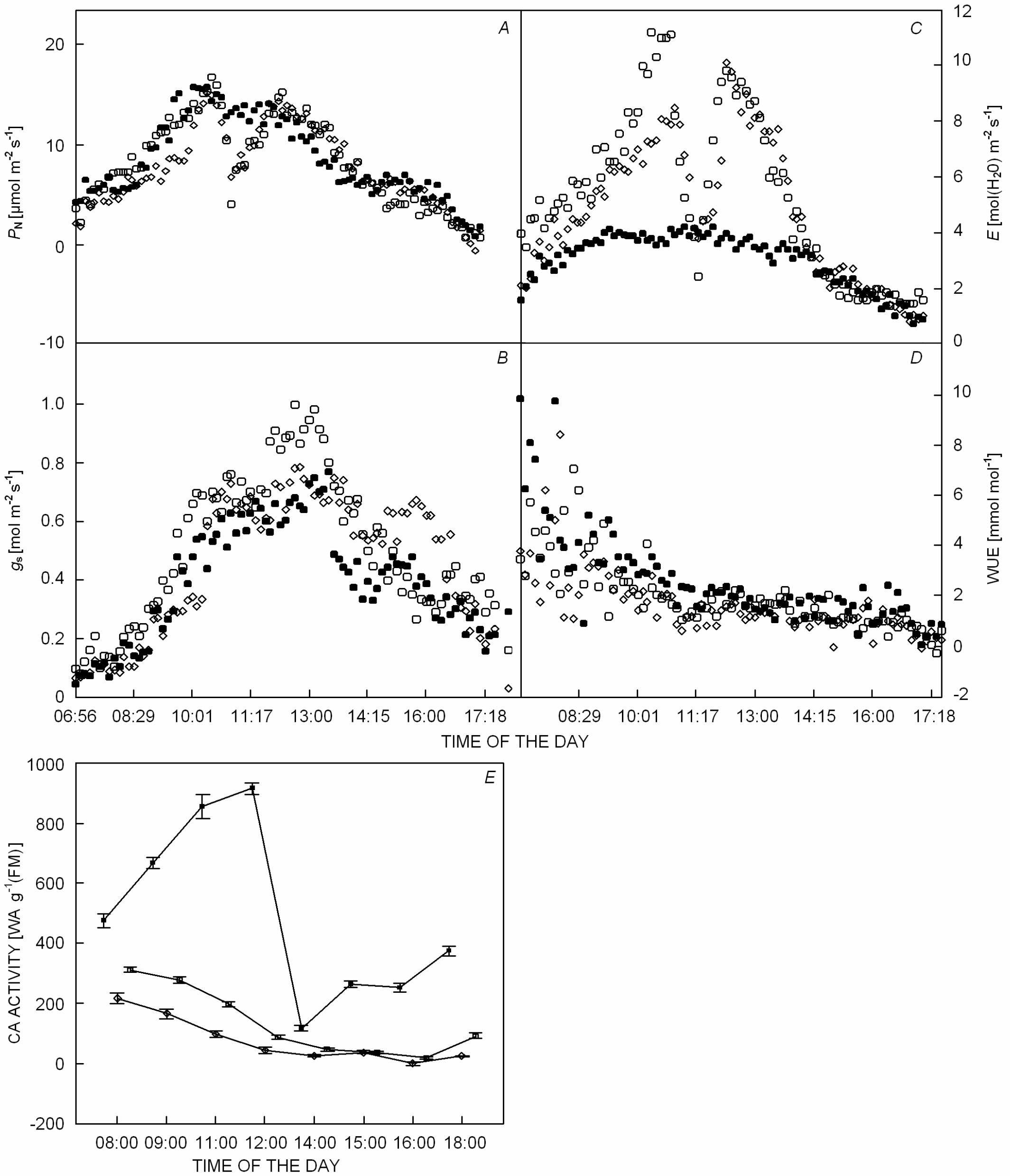

Fig. 2. The diurnal changes of net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), transpiration rate, *E* (*C*), water use efficiency, WUE (*D*), and carbonic anhydrase (CA) activity (*E*) in ■ *Orychophragmus violaceus*, ◊ *Brassica juncea*, and □ *Brassica campestris*. In part *E*, means ± SE.

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